A meta-analysis of genetic parameter estimates for milk and serum minerals in dairy cows

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SUPPLEMENTARY FILE

Materials and methods

Most meta-analyses do not use the published correlation estimate itself because it usually does not have a normal distribution. Rather, the published correlation is converted to the Fisher's *Z* scale, and all analyses are performed using the transformed values. The results, such as the estimated parameter and its confidence interval, would then be converted back to correlations for presentation (Borenstein *et al.*, 2009). The approximate normal scale based on Fisher's *Z* transformation (Steel and Torrie, 1960; Borenstein *et al.*, 2009) is as follows:

$$Z_{ij} = 0.5\left[\ln\left(1+r_{g_{ij}}\right)-\ln\left(1-r_{g_{ij}}\right)\right]$$

where r_{gij} is the published genetic correlation estimate for the i^{th} trait in the j^{th} article. To return to the original scale, the following equation (Borenstein *et al.*, 2009) was used:

$$r^{*}_{g_{ij}} = \frac{e^{2Z_{ij}}-1}{e^{2Z_{ij}}+1}$$

where $r^{*}_{g_{ij}}$ is the re-transformed genetic correlation for the i^{th} trait in the j^{th} article and Z_{ij} is the Fisher's *Z* transformation.

The 95% lower and upper limits for the estimated parameter would be computed respectively for each trait as follows:

$$LL_{\bar{\theta}} = \bar{\theta} - 1.96 \times SE_{\bar{\theta}} \quad \text{and} \quad UL_{\bar{\theta}} = \bar{\theta} + 1.96 \times SE_{\bar{\theta}}$$

where $SE_{\bar{\theta}}$ is the predicted standard error for the estimated parameter $\bar{\theta}$, given by:

$$SE_{\bar{\theta}} = \sqrt{\frac{1}{\sum_{j=1}^{k} w_j}}$$

Publication bias

Egger's linear regression asymmetry was used to examine the presence of publication bias. When significant (P<0.10) bias was detected; the trim-and-fill method (Duval and Tweedie, 2000) was applied to find the number of missing studies (Sales, 2011). Also, funnel plots were used to present asymmetry. This technique indicates the symmetric distribution of effect sizes around the true effect size if it is assumed that no publication bias exists, that is, the most extreme results have not been published. Once the number of missing observations is estimated, estimated missing values are included to recalculate a weighted mean effect size and its variance. When heterogeneity (Q test, P<0.10) was detected for the parameters analyzed, testing for the occurrence of possible publication bias is inappropriate as it may lead to false-positive claims (Ioannidis and Trikalinos, 2007; Sales, 2011).

Discussion

A strong and popular tool to merge findings from various studies is meta-analysis. This technique helps to decide in different scopes. The definition of objectives in this study and generally the wide variability among genetic parameter estimates from different studies showed the essentiality of considering the random-effects model. In the field of animal breeding and genetics, it is necessary to conduct a meta-analysis based on a random-effects model due to the interest in making inferences at the population level (de Oliveira *et al.*, 2017; Ghavi Hossein-Zadeh, 2021). A random-effects model provides outputs that can be generalized (Sutton *et al.*, 2000; Safari *et al.*, 2005).

Sodium is an essential macro-mineral that has been indicated to be a significant factor in milk production (Spek *et al.*, 2013), and is disappeared through milk, urine, saliva, and feces (Denholm *et al.*, 2019). Milk phosphorus presents in numerous forms (e.g., phospholipids, colloidal Ca phosphate, and casein phosphoserines), all of which are known to indicate great genetic variation (Heck *et al.*, 2008). Therefore, a portion of the genetic variation in milk phosphorus can be justified by its casein phosphoserine residues. Milk magnesium presents chiefly as citrate, phosphate, and free ions. Only 35% of magnesium is attached to casein micelles (Gaucheron, 2005); thus, the number of casein phosphoserines might not be so influential in assessing the milk magnesium variation. The milk calcium secretion is a very complicated event with a great variety of forms, including casein-bound Ca, colloidal Ca phosphate, Ca citrate, and free ionized Ca (Neville *et al.*, 1995). Most of Ca (nearly 65%) is connected with casein micelles (Neville *et al.*, 1995). Therefore, the number of casein phosphoserines in milk may determine Ca concentrations and maybe also Zn concentrations because the major part of Zn is also attached to casein micelles (Neville *et al.*, 1995). Large influences of dietary Se concentration on its content in milk have been reported (Haug *et al.*, 2007; Phipps *et al.*, 2008). Also, the Se content of soil impacts the Se content in plants which are applied as roughages. Furthermore, it is observed that Se content in milk can be enhanced by increasing Se content in the fertilizer that is used in grassland (van Hulzen *et al.*, 2009). Wiking *et al.* (2008) reported that the Zn content of bovine milk is significantly influenced by the dietary intake of fat. Fat transfer from diet to milk eases the transfer of Zn from diet to milk (van Hulzen *et al.*, 2009).

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Table S1. The list of studies included in the database for conducting this meta-analysis

Reference	Country	Breed	Method of analysis
Bonfatti et al. (2017)	Italy	Italian Simmental	REML
Buitenhuis et al. (2015)	Denmark	Danish Holstein and Jersey	REML
Costa et al. (2019)	Italy	Holstein	REML
Denholm et al. (2019)	United Kingdom	Holstein	REML
Govignon-Gion et al. (2015)	France	Holstein, Montbéliarde, and Normande	REML
Sanchez et al. (2018)	France	Montbéliarde	REML
Soyeurt et al. (2012)	Belgium	Holstein	REML
Toffanin et al. (2015)	Italy	Holstein	REML
Tsiamadis et al. (2016)	Greece	Holstein	REML
van Hulzen et al. (2009)	Netherlands	Holstein	REML
Visentin et al. (2019)	Italy	Holstein	REML
Zaalberg et al. (2021)	Denmark	Danish Holstein and Jersey	Bayesian

Table S2. Results from statistical tests to evaluate publication bias and the trim-and-fill method to correct funnel plot asymmetry in mean heritability estimates of minerals that did not present heterogeneity

Trait	Egger's test p-value	Trim-and-fill method		
		Missing	Mean	95% CI
Se_m	0.783	0	0.171	0.076-0.266
Zn_m	0.437	2	0.337	0.213-0.461
Fe_m	0.553	0	0.013	0.000-0.067

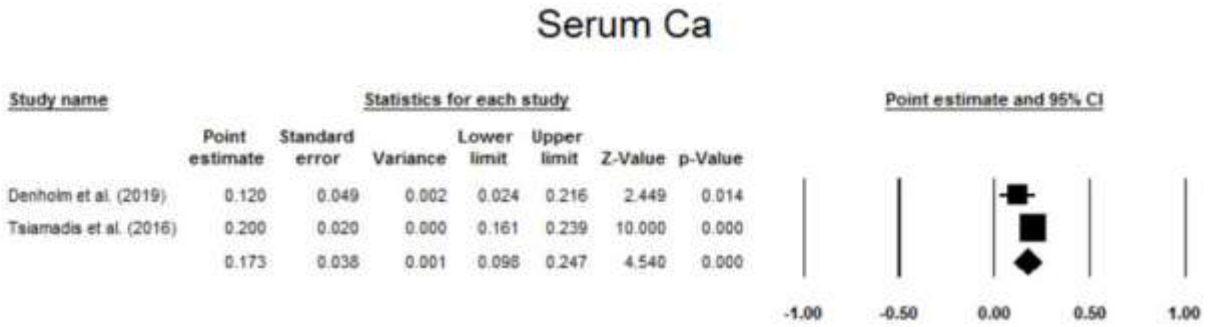
"m" subscript indicated the concentrations of the minerals in milk.

Missing: Number of missing studies.

Table S3. Results from statistical tests to evaluate publication bias and the trim-and-fill method to correct funnel plot asymmetry in mean genetic correlation estimate between milk calcium and phosphorus

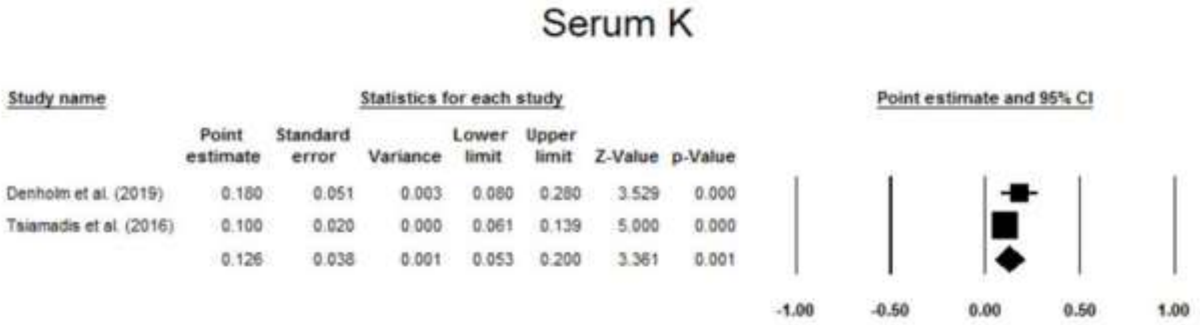
Egger's test p-value	Trim-and-fill method		
	Missing	Mean	95% CI
0.279	2	0.430	0.275-0.563

Missing: Number of missing studies.



Serum Ca

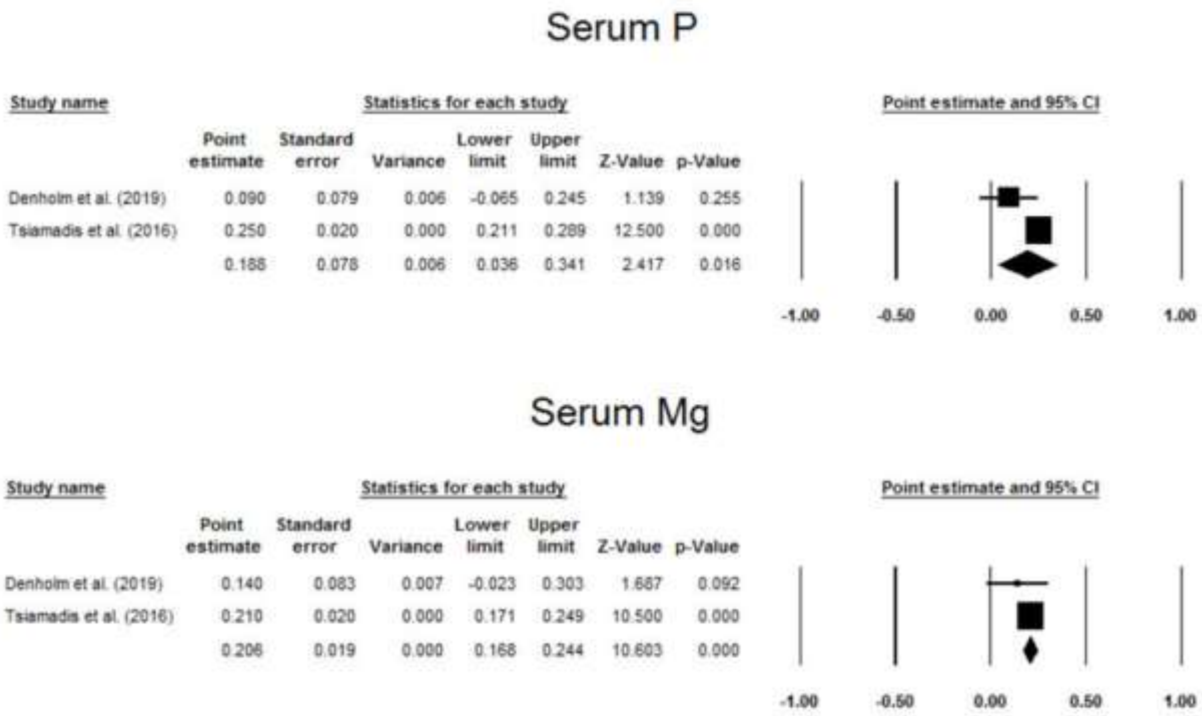
Study name	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	0.120	0.049	0.002	0.024	0.216	2.449	0.014
Tsiamadis et al. (2016)	0.200	0.020	0.000	0.161	0.239	10.000	0.000
	0.173	0.038	0.001	0.098	0.247	4.540	0.000



Serum K

Study name	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	0.180	0.051	0.003	0.080	0.280	3.529	0.000
Tsiamadis et al. (2016)	0.100	0.020	0.000	0.061	0.139	5.000	0.000
	0.126	0.038	0.001	0.053	0.200	3.361	0.001

Fig. S1. The forest plots of individual studies and the overall outcome for heritability estimates of serum calcium and potassium in dairy cows. Detailed information is provided in Fig. 1.


Serum P

Study name	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	0.090	0.079	0.006	-0.065	0.245	1.139	0.255
Tsiamadis et al. (2016)	0.250	0.020	0.000	0.211	0.289	12.500	0.000
	0.188	0.078	0.006	0.036	0.341	2.417	0.016

Serum Mg

Study name	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	0.140	0.083	0.007	-0.023	0.303	1.687	0.092
Tsiamadis et al. (2016)	0.210	0.020	0.000	0.171	0.249	10.500	0.000
	0.206	0.019	0.000	0.168	0.244	10.603	0.000

Fig. S2. The forest plots of individual studies and the overall outcome for heritability estimates of serum phosphorus and magnesium in dairy cows. Detailed information is provided in Fig. 1.

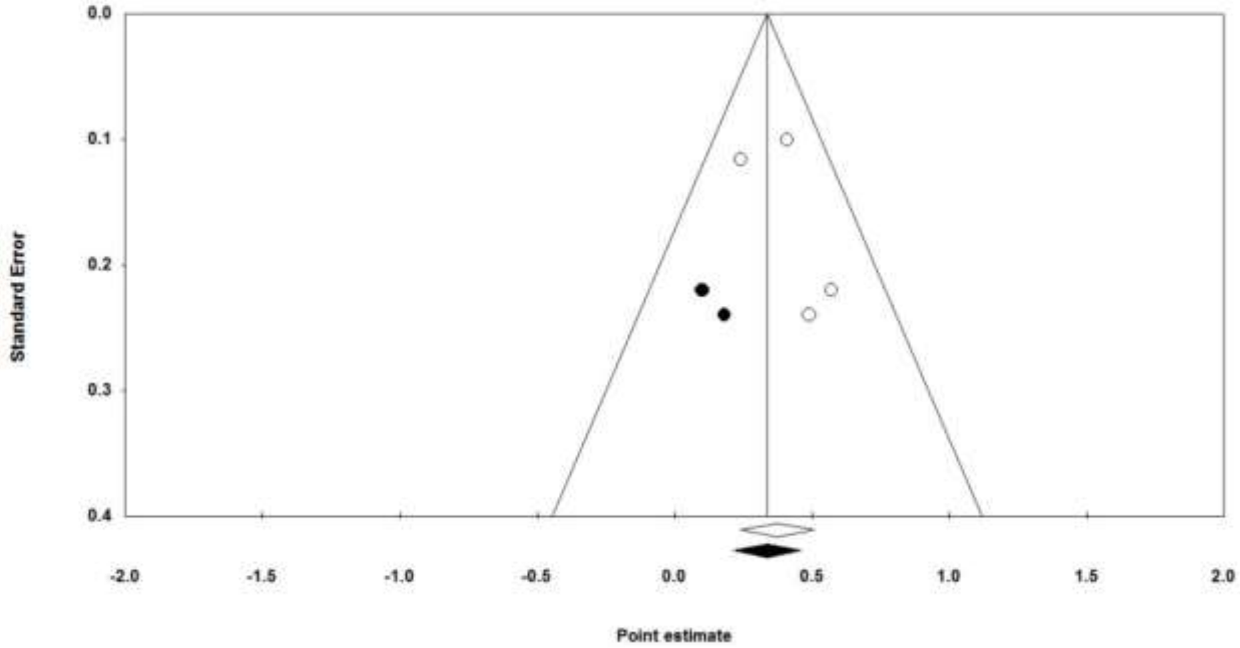


Fig. S3. Funnel plot of mean heritability estimates for milk zinc. Detailed information is provided in Fig. 5.

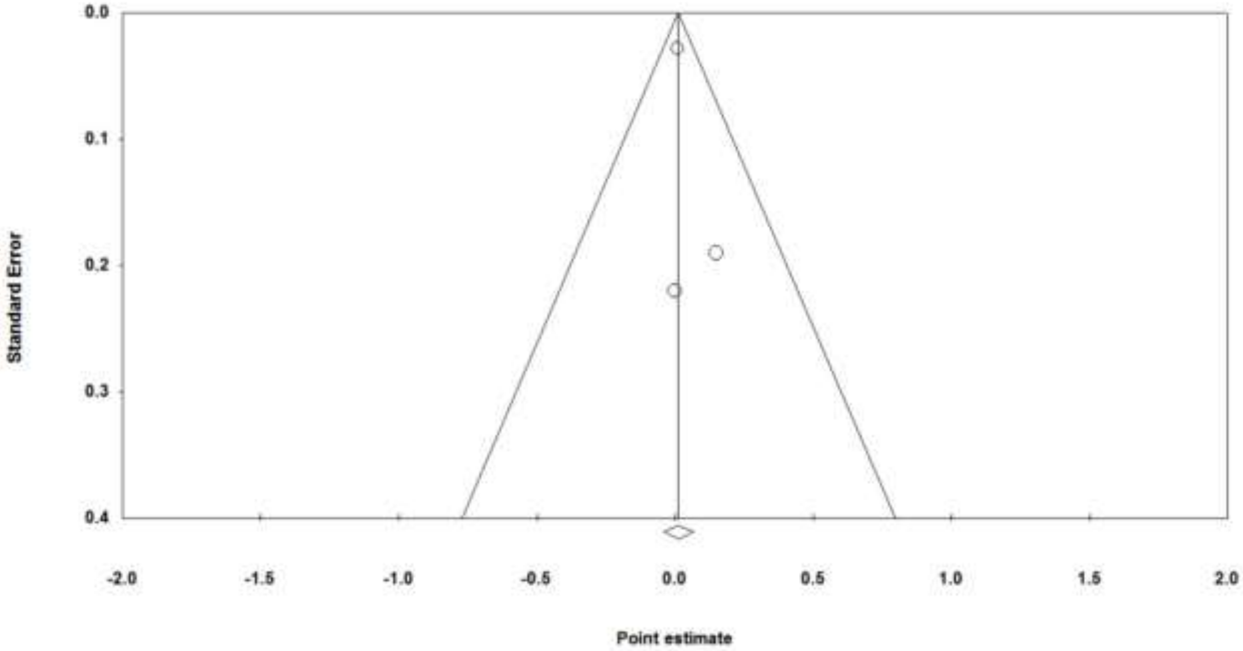
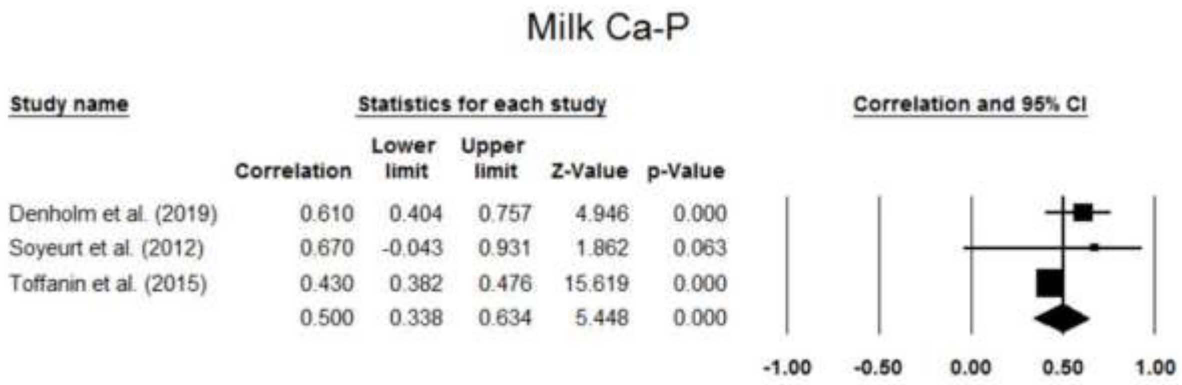
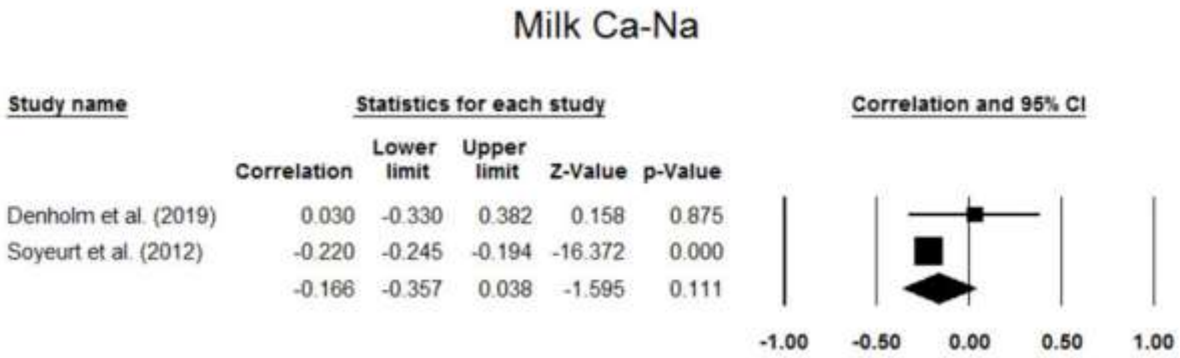


Fig. S4. Funnel plot of mean heritability estimates for milk iron. Detailed information is provided in Fig. 5.



Milk Ca-P

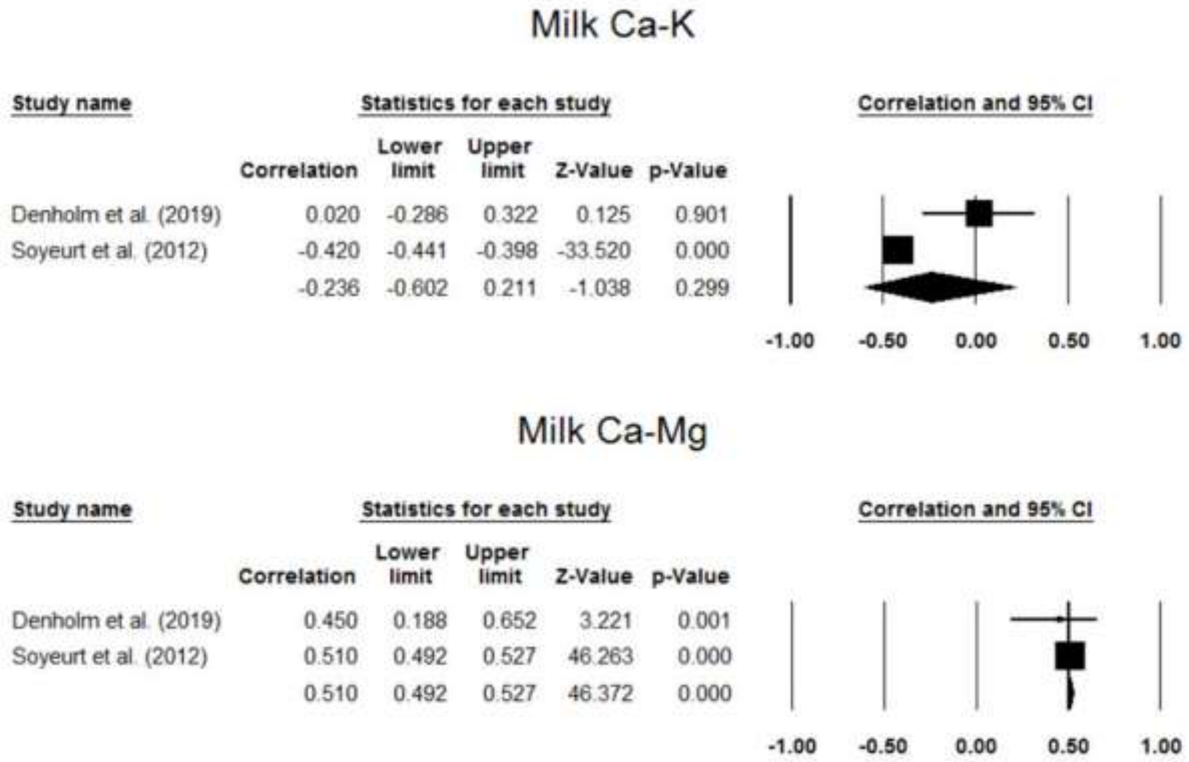
Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	0.610	0.404	0.757	4.946	0.000
Soyeurt et al. (2012)	0.670	-0.043	0.931	1.862	0.063
Toffanin et al. (2015)	0.430	0.382	0.476	15.619	0.000
	0.500	0.338	0.634	5.448	0.000



Milk Ca-Na

Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	0.030	-0.330	0.382	0.158	0.875
Soyeurt et al. (2012)	-0.220	-0.245	-0.194	-16.372	0.000
	-0.166	-0.357	0.038	-1.595	0.111

Fig. S5. The forest plots of individual studies and the overall outcome for genetic correlation estimates between milk calcium with milk phosphorus and sodium in dairy cows. Detailed information is provided in Fig. 1.



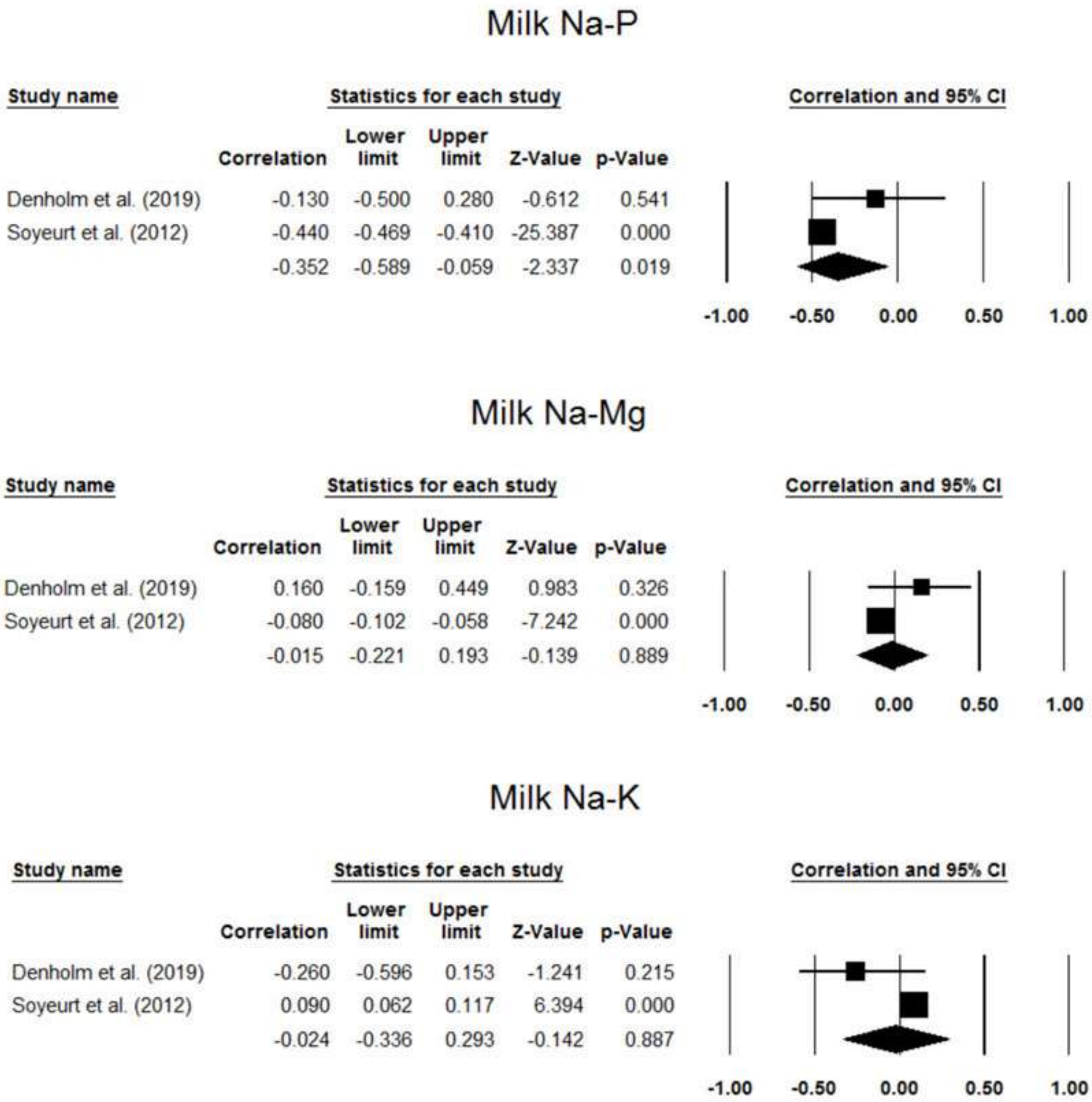
Milk Ca-K

Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	0.020	-0.286	0.322	0.125	0.901
Soyeurt et al. (2012)	-0.420	-0.441	-0.398	-33.520	0.000
	-0.236	-0.602	0.211	-1.038	0.299

Milk Ca-Mg

Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	0.450	0.188	0.652	3.221	0.001
Soyeurt et al. (2012)	0.510	0.492	0.527	46.263	0.000
	0.510	0.492	0.527	46.372	0.000

Fig. S6. The forest plots of individual studies and the overall outcome for genetic correlation estimates between milk calcium with milk potassium and magnesium in dairy cows. Detailed information is provided in Fig. 1.



Milk Na-P

Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	-0.130	-0.500	0.280	-0.612	0.541
Soyeurt et al. (2012)	-0.440	-0.469	-0.410	-25.387	0.000
	-0.352	-0.589	-0.059	-2.337	0.019

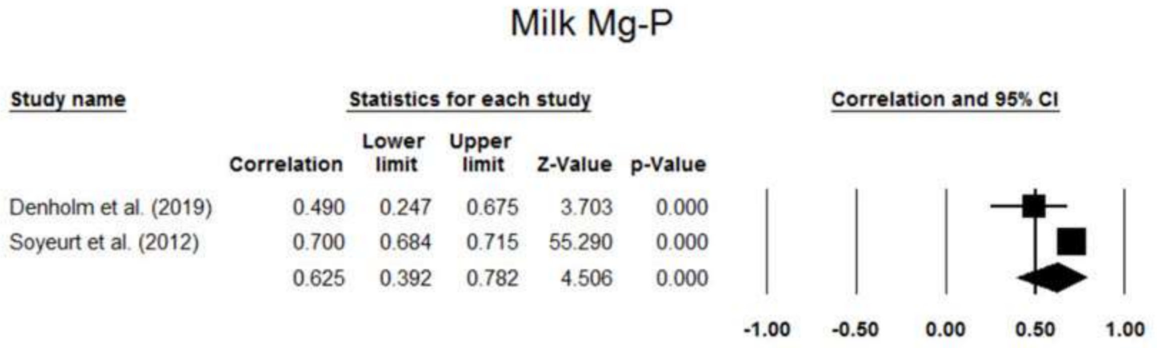
Milk Na-Mg

Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	0.160	-0.159	0.449	0.983	0.326
Soyeurt et al. (2012)	-0.080	-0.102	-0.058	-7.242	0.000
	-0.015	-0.221	0.193	-0.139	0.889

Milk Na-K

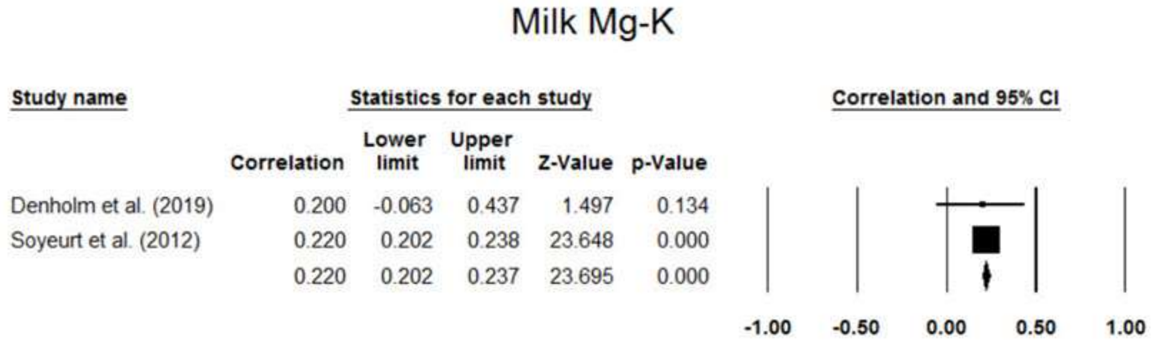
Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	-0.260	-0.596	0.153	-1.241	0.215
Soyeurt et al. (2012)	0.090	0.062	0.117	6.394	0.000
	-0.024	-0.336	0.293	-0.142	0.887

Fig. S7. The forest plots of individual studies and the overall outcome for genetic correlation estimates between milk sodium with milk phosphorus, magnesium, and potassium in dairy cows. Detailed information is provided in Fig. 1.



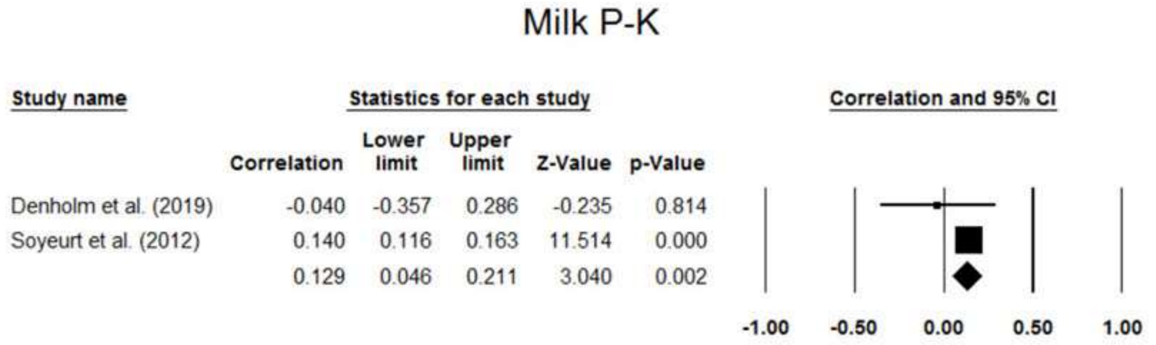
Milk Mg-P

Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	0.490	0.247	0.675	3.703	0.000
Soyeurt et al. (2012)	0.700	0.684	0.715	55.290	0.000
	0.625	0.392	0.782	4.506	0.000



Milk Mg-K

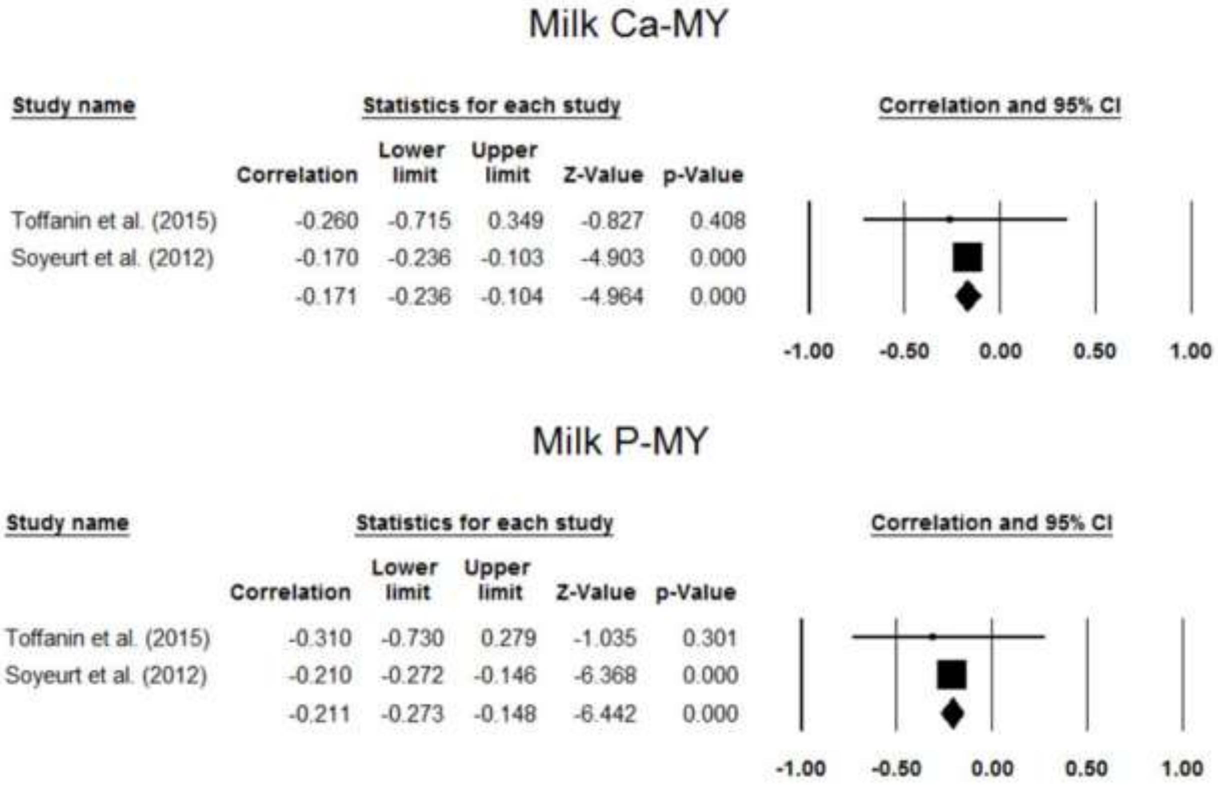
Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	0.200	-0.063	0.437	1.497	0.134
Soyeurt et al. (2012)	0.220	0.202	0.238	23.648	0.000
	0.220	0.202	0.237	23.695	0.000



Milk P-K

Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Denholm et al. (2019)	-0.040	-0.357	0.286	-0.235	0.814
Soyeurt et al. (2012)	0.140	0.116	0.163	11.514	0.000
	0.129	0.046	0.211	3.040	0.002

Fig. S8. The forest plots of individual studies and the overall outcome for genetic correlation estimates between milk magnesium with milk phosphorus and potassium, and between milk phosphorus with milk potassium in dairy cows. Detailed information is provided in Fig. 1.



Milk Ca-MY

Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Toffanin et al. (2015)	-0.260	-0.715	0.349	-0.827	0.408
Soyeurt et al. (2012)	-0.170	-0.236	-0.103	-4.903	0.000
	-0.171	-0.236	-0.104	-4.964	0.000

Milk P-MY

Study name	Correlation	Lower limit	Upper limit	Z-Value	p-Value
Toffanin et al. (2015)	-0.310	-0.730	0.279	-1.035	0.301
Soyeurt et al. (2012)	-0.210	-0.272	-0.146	-6.368	0.000
	-0.211	-0.273	-0.148	-6.442	0.000

Fig. S9. The forest plots of individual studies and the overall outcome for genetic correlation estimates between milk calcium and phosphorus with milk yield in dairy cows. Detailed information is provided in Fig. 1.

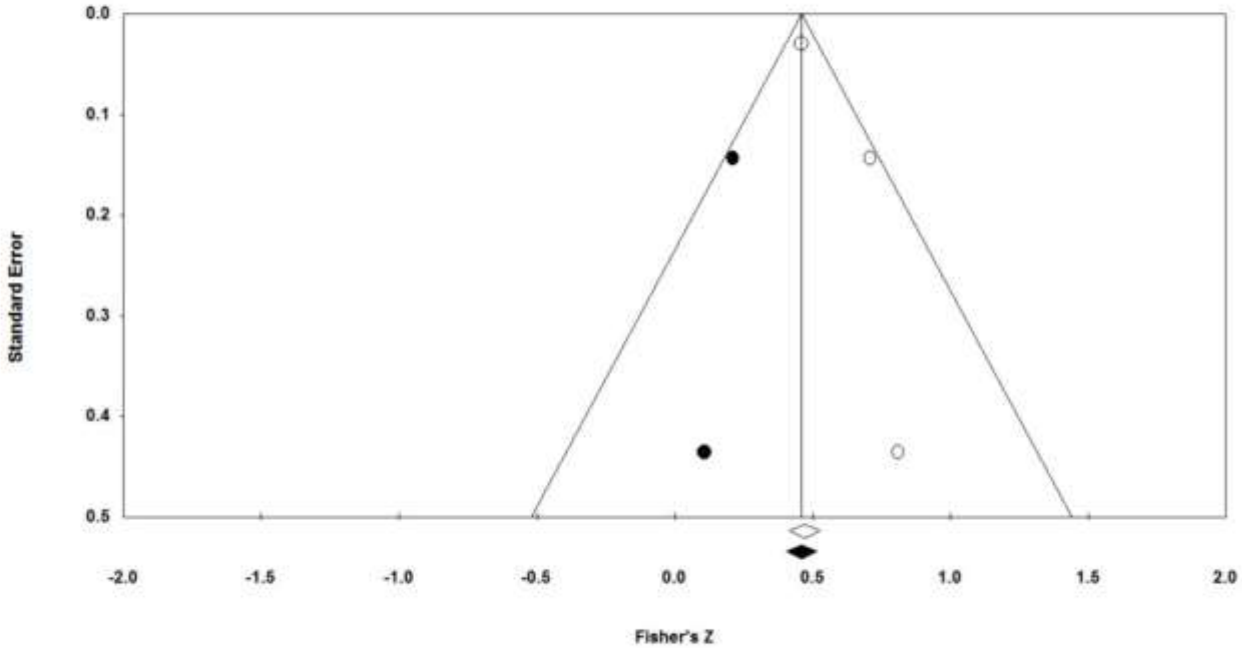


Fig. S10. Funnel plot of Fisher's Z for the genetic correlations between milk calcium and phosphorus in dairy cows. Detailed information is provided in Fig. 5.