STUDY ON THE INFLUENCE OF SOME NON-GENETIC FACTORS ON THE PHENOTYPIC CORRELATIONS BETWEEN MILK PRODUCTION TRAITS

M. Ivancia*, A. Ciobanu, G. Nacu, R.-F. Amariei

"Ion Ionescu de la Brad" Iasi University of Life Sciences, Romania

Abstract

Milk and dairy products are a major source of energy, protein and micronutrients in the human diet, and milk yield, fat and protein content determine the payment value of the raw. Milk yield and its component yields are strongly, positively correlated, while milk yield is often weakly and negatively correlated with fat and protein. Beyond the size of these correlations, phenotype (P) results from genotype (G), environment (E) and their interaction ($P = G + E + G \times E$); herd (farm) and production year are two of the most accessible proxies for this environmental component, known to materially change both trait levels and heritability estimates. For Holstein herds in Iași County, no study has yet formally tested whether farm and year affect milk yield, composition and their correlations. This study therefore used two-way ANOVA to test the effect of herd, year and their interaction on milk yield and composition, and estimated the phenotypic correlations between these traits, separating stable, breed-level relationships from those driven by non-genetic, farm- and year-specific factors.

Key words: *milk yield, phenotypic correlation, non-genetic factors, herd and year effect, Holstein cattle*

INTRODUCTION

Milk and dairy products are a major source of energy, protein and micronutrients in the human diet, and their nutritional and health-related roles are well documented in the scientific literature [1]. For the dairy industry, milk yield together with its main quality attributes, fat and protein content, determine both the payment value of the raw material and its technological suitability for processing. Improving milk yield without deteriorating composition, or vice versa, therefore remains one of the central objectives of cattle breeding programmes worldwide.

Numerous studies have shown that milk yield and its component yields (fat yield, protein yield) are strongly and positively correlated, both phenotypically and genetically, while milk yield tends to be weakly and often negatively correlated with the percentage content of these components [2, 3, 4]. This pattern, generally interpreted as a dilution effect of higher water/milk volume on solute concentration, has practical implications for selection: intensive selection for yield alone carries a risk of eroding fat and protein percentages unless composition traits are included in the breeding objective [5, 6].

A question that is at least as important for practical breeding and management as the magnitude of these correlations is what actually generates the observed phenotypic variation in milk yield and composition in the first place, and to what extent this variation, and the correlation structure built on it, are attributable to the animal's genetic merit versus to non-genetic, environmental causes. In quantitative genetics terms, an individual's phenotype (P) results from the combined action of genotype (G), environment (E) and

* Corresponding author: mihaela.ivancia@iuls.ro
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the genotype-by-environment interaction (G×E): P = G + E + G×E. Herd (farm) and production year are two of the most accessible and, at the same time, most consequential proxies for "environment" available in on-farm data, since they jointly summarize feeding regime, housing, milking routine, herd health status, reproductive management and climatic conditions specific to a given place and a given twelve-month period. Castillo-Juarez et al. [2] demonstrated that genetic parameters for milk production and composition traits differ measurably between low- and high-production herd environments in primiparous Holstein cows, i.e., a genotype-by-herd-environment interaction, while Van Niekerk et al. [7] showed, using fixed and random regression models on nearly 700,000 South African Holstein test-day records, that herd and herd×calving-year×season effects materially improve model fit and that heritability estimates themselves differ between production systems. Martinez-Castillero et al. [8] and Denholm et al. [9] further showed, using recursive and multivariate mixed-effects animal models with herd, parity and year-season as explicit fixed or random effects, that both the genetic and the permanent-environmental (non-genetic) components of the covariance between milk yield, composition and other traits (fertility, mineral content) are non-negligible and can even act in different directions.

Herd, year, season, parity and management practices have repeatedly been shown to significantly affect both the level and the covariance structure of milk traits in Holstein populations from very different production systems, including Turkey [10], Serbia [11], Ukraine [12, 13], the Czech Republic [14, 15], Iran [16], Russia [17, 18], Croatia [19] and Brazil [20, 21, 22, 23, 24, 25]. Weather-related variables such as ambient temperature, relative humidity and the temperature-humidity index have also been reported to correlate significantly, although inconsistently in direction, with both yield and composition traits across years [13, 14, 16], while farm-level management practices, including milking frequency, feeding strategy, hygiene and udder health status, further modify the yield-composition relationship [26, 27, 28, 29, 30, 31, 32]. Gorelik et al. [18] additionally showed that the sign and magnitude of the yield-fat percentage correlation can differ not only between herds but even between paternal (sire) lines and successive lactations within the same herd, underlining how fine-grained non-genetic and structural factors can be.

For Holstein populations raised in Romania, and in Iași County in particular, comparable phenotypic data remain scarce. A recent study conducted at the Dancu research and development farm, one of the two herds used in the present work, reported that milk yield and composition were closely related to seasonal and management factors rather than to genetic origin alone [33], while regional data on Holstein-Friesian populations from the Moldavian area confirmed the classic strong positive correlation between milk yield and component yields, together with a weak negative correlation between yield and component percentages [3]. However, none of these regional studies has directly tested, with a formal statistical model, whether farm and production year significantly affect milk yield and composition traits, nor has any of them compared the stability of the phenotypic correlations between two commercial farms operating under different management regimes over several consecutive years.

The objective of the present study was therefore twofold: (i) to formally test, by two-way analysis of variance, the effect of herd (farm), year and their interaction on milk yield and on the yields and percentages of fat and protein in two Holstein herds from Iași County, Dancu and Polena, over 2020-2022; and (ii) to estimate the

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phenotypic (Pearson) correlations between milk yield and these composition traits in the same herd-year subgroups, in order to determine which of these relationships behave as robust, breed-level characteristics and which are instead driven by the non-genetic, farm- and year-specific factors identified in the first objective. Taken together, these two analyses were designed to give a coherent, statistically grounded picture of how much of the variation, and of the trait-to-trait relationships, observed in real commercial milk-recording data is attributable to non-genetic causes tied to the farm and the year of production, information that is directly relevant to local breeding and herd-management decisions.

MATERIAL AND METHOD

The biological material consisted of individual lactation records of Holstein cows raised on two commercial dairy farms located in Iași County, Romania: Dancu and Polena. Records were available for three consecutive years, 2020, 2021 and 2022. For each cow and year, the following traits were recorded from official milk recording data: milk yield (kg), fat yield (kg), fat content (%), protein yield (kg) and protein content (%). After the exclusion of incomplete records and non-individual entries (summary/aggregate rows), the final data set comprised 952 valid lactation records distributed as follows: Dancu, 278 (2020), 253 (2021) and 237 (2022) records (768 in total), and Polena, 63 (2020), 69 (2021) and 52 (2022) records (184 in total).

Data were processed in Python 3 (pandas and NumPy libraries) for cleaning, merging and descriptive statistics. For each herd-year subgroup, the mean and standard deviation were computed for all five traits.

To formally test the effect of the non-genetic factors of interest, a two-way, fixed-effects analysis of variance (ANOVA) was fitted for each trait (milk yield, fat yield, fat %, protein yield, protein %), according to the linear model:

$$Y_{ijk} = \mu + Farm_i + Year_j + (Farm \times Year)_{ij} + e_{ijk}$$

where Y_{ijk} is the observed value of the trait for cow k in farm i and year j, μ is the overall mean, Farm_i (i = Dancu, Polena) and $Year_j$ (j = 2020, 2021, 2022) are the fixed effects of herd and production year, $(Farm \times Year)_{ij}$ is their interaction, and e_ijk is the random residual. Because the design was unbalanced (unequal numbers of cows per herd-year subgroup), effects were tested using Type III-equivalent general linear hypotheses computed on the exact cell means and their sampling variances (herd and year effects were tested as unweighted contrasts among the six herd-year cell means, following the general linear model approach used for unbalanced factorial designs); statistical significance was declared at $p<0.05$. Partial eta-squared (η^2_p) was computed for each effect as a measure of effect size (proportion of variance explained after accounting for the other model terms).

Pearson correlation coefficients (r) and their associated two-tailed significance levels (p) were calculated separately for each of the six herd-year subgroups (SciPy stats module), for the following trait pairs considered most relevant to breeding and milk-quality decisions: milk yield-fat yield, milk yield-fat percentage, milk yield-protein yield, milk yield-protein percentage, fat percentage-protein percentage, and fat yield-protein yield. Correlations were classified as statistically significant at $p<0.05$, and further denoted as $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***) throughout the results. Charts were produced in Python (Matplotlib library); tables and descriptive statistics were cross-checked against the correlation matrices already available in the farm data set, with an exact match confirming the reliability of both data sources.

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RESULTS

Descriptive statistics for milk yield and composition traits, by herd and year, are presented in Table 1 and illustrated in Figure 1. Mean milk yield ranged from 6,580.7 kg (Polena, 2021) to 8,117.8 kg (Dancu, 2022). The Dancu herd showed a consistent upward trend in milk yield across the three years (7,313 to 7,492 to 8,118 kg), whereas the Polena herd showed a marked decrease from 2020 to 2021 (7,726 to 6,581 kg) followed by a partial recovery in 2022 (6,969 kg). Fat content averaged between 4.04% and 4.17%, and protein content between 3.37% and 3.51%, across all herd-year subgroups, values consistent with those reported for Holstein populations under standard management [3, 33].

To move beyond this descriptive comparison and formally test whether the differences observed between farms and years are statistically meaningful rather than sampling noise, a two-way ANOVA (Farm × Year) was fitted for each trait; the results are presented in Table 2.

Table 1. Descriptive statistics (mean ± SD) for milk yield and composition traits, by herd and year

Herd	Year	n	Milk yield (kg)	Fat yield (kg)	Fat (%)	Protein yield (kg)	Protein (%)
Dancu	2020	278	7313.1 ± 2367.3	297.4 ± 96.1	4.07 ± 0.08	257.2 ± 84.5	3.51 ± 0.11
Dancu	2021	253	7491.7 ± 2199.2	312.3 ± 91.6	4.17 ± 0.16	252.7 ± 74.6	3.37 ± 0.12
Dancu	2022	237	8117.8 ± 2425.5	336.4 ± 101.1	4.14 ± 0.16	279.0 ± 83.6	3.44 ± 0.11
Polena	2020	63	7725.5 ± 1696.4	311.2 ± 66.4	4.04 ± 0.12	268.0 ± 61.2	3.46 ± 0.11
Polena	2021	69	6580.7 ± 1684.6	273.8 ± 69.3	4.16 ± 0.18	222.4 ± 56.6	3.39 ± 0.15
Polena	2022	52	6969.2 ± 1284.0	287.6 ± 54.5	4.12 ± 0.19	239.4 ± 45.2	3.43 ± 0.13

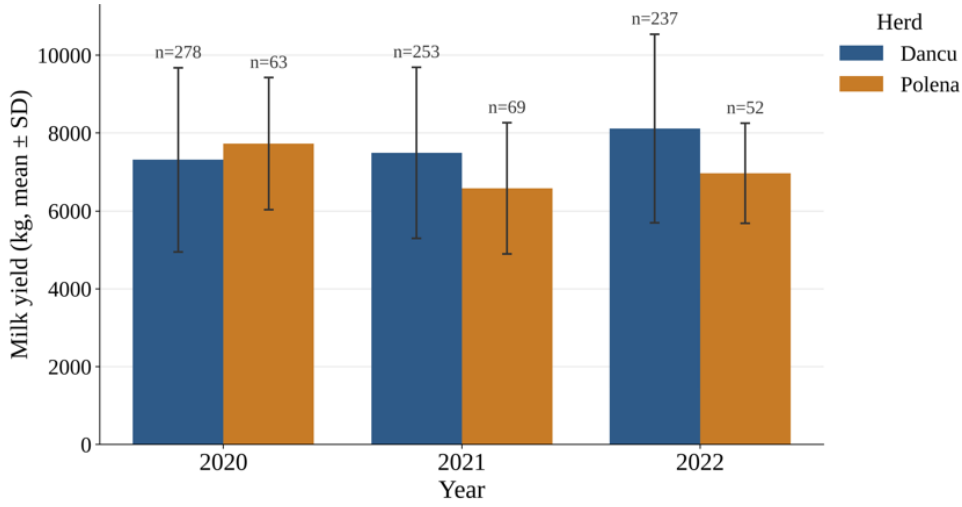


Fig. 1 – Milk yield annual dynamics in Dancu and Polena farms

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Farm, year and their interaction were all statistically significant sources of variation for milk yield (Farm: F=9.06, p=0.003; Year: F=3.45, p=0.032; Farm×Year: F=7.15, p=0.001) and for protein yield (Farm: F=9.69, p=0.002; Year: F=6.52, p=0.002; Farm×Year: F=5.98, p=0.003), while fat yield was significantly affected by farm and by the farm×year interaction, but not by year alone (Farm: F=10.59, p=0.001; Year: F=2.14, p=0.118; Farm×Year: F=6.70, p=0.001). This pattern, and in particular the highly significant farm×year interaction for all three yield traits, statistically confirms what Figure 2 shows visually: the two herds did not simply differ by a constant offset but diverged and re-converged differently over the three years (Dancu increasing steadily, Polena falling sharply in 2021 before a partial recovery in 2022), which is only possible if farm-specific, year-varying management or nutritional conditions, rather than a fixed, additive herd effect, were acting on the animals.

Table 2. Two-way ANOVA (Farm × Year) for milk yield and composition traits

Trait	Farm	Year	Farm × Year	η^2 (Farm/Year/F×Y)
Milk yield (kg)	F(1,946)=9.06, p=0.003**	F(2,946)=3.45, p=0.032*	F(2,946)=7.15, p<0.001***	0.010 / 0.007 / 0.015
Fat yield (kg)	F(1,946)=10.59, p=0.001**	F(2,946)=2.14, p=0.118 ns	F(2,946)=6.70, p=0.001**	0.011 / 0.005 / 0.014
Fat (%)	F(1,946)=2.64, p=0.104 ns	F(2,946)=35.31, p<0.001***	F(2,946)=0.17, p=0.840 ns	0.003 / 0.070 / 0.000
Protein yield (kg)	F(1,946)=9.69, p=0.002**	F(2,946)=6.52, p=0.002**	F(2,946)=5.98, p=0.003**	0.010 / 0.014 / 0.013
Protein (%)	F(1,946)=1.58, p=0.209 ns	F(2,946)=43.90, p<0.001***	F(2,946)=3.87, p=0.021*	0.002 / 0.085 / 0.008

*Note: two-way fixed-effects ANOVA (Y = μ + Farm + Year + Farm×Year + e), Type III-equivalent general linear hypothesis tests on unbalanced cell means; *** p<0.001, ** p<0.01, * p<0.05, ns = not significant. η^2 = partial eta-squared (effect size) for Farm, Year and Farm×Year, respectively*

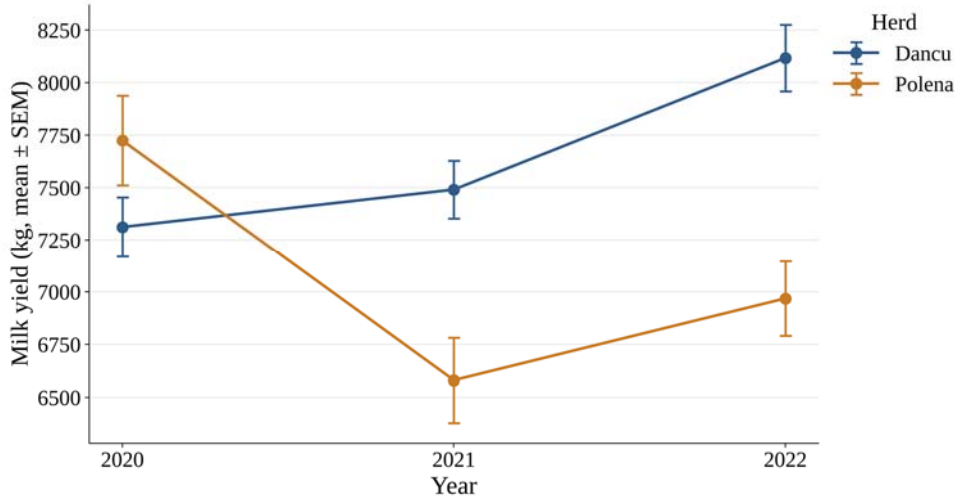


Fig. 2. Interaction plot: mean milk yield (±SEM) by year, per herd

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A markedly different pattern was found for the percentage-based composition traits. Fat percentage and protein percentage were both very highly significantly affected by year (fat %: F=35.31, p<0.001; protein %: F=43.90, p<0.001) but not significantly by farm alone (fat %: F=2.64, p=0.104; protein %: F=1.58, p=0.209), and the farm×year interaction was significant for protein % (F=3.87, p=0.021) but not for fat % (F=0.17, p=0.840). This is illustrated in Figure 3, which shows fat and protein percentage pooled across the two herds for each year: both traits shifted from 2020 to 2021-2022 in a broadly parallel manner in the two herds, consistent with a factor common to the whole county or region in a given year (e.g., feed quality/availability, climatic conditions during the growing season, or region-wide changes in ration formulation) rather than with a farm-specific management practice. Effect sizes (partial η^2) were modest for all effects (0.002-0.085), as expected given the large amount of individual, cow-level variability typical of production data, but the year effect on fat % (η^2_p=0.070) and protein % (η^2_p=0.085) was considerably larger than any farm-related effect on these two traits ($\eta^2_p \leq 0.003$), reinforcing the distinction between yield traits (farm- and interaction-sensitive) and percentage traits (predominantly year-sensitive) in this data set.

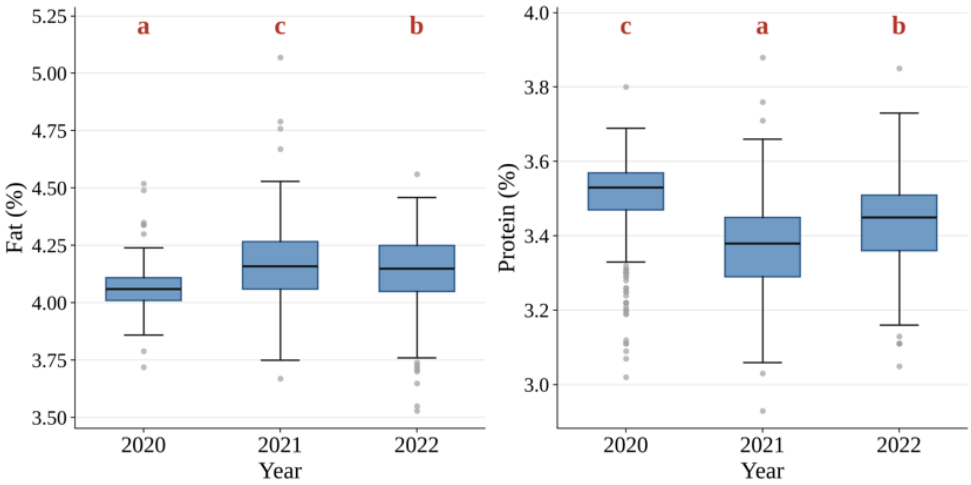


Fig. 3. Fat (%) and protein (%) by year, pooled across both herds, illustrating the significant year effect common to both farms (p<0.001 for both traits)

Table 3. Pearson correlation coefficients between milk yield and composition traits, by herd and year

Herd	Year	n	Milk– Fat kg	Milk– Fat %	Milk– Prot kg	Milk– Prot %	Fat %– Prot %	Fat kg– Prot kg
Dancu	2020	278	0.999***	-0.118*	0.997***	0.158**	0.394***	0.996***
Dancu	2021	253	0.992***	-0.116 ns	0.993***	0.014 ns	0.355***	0.990***
Dancu	2022	237	0.991***	0.067 ns	0.995***	0.033 ns	0.314***	0.990***
Polena	2020	63	0.993***	-0.448***	0.991***	0.199 ns	-0.063 ns	0.984***
Polena	2021	69	0.986***	-0.046 ns	0.985***	-0.162 ns	0.456***	0.985***
Polena	2022	52	0.971***	0.050 ns	0.978***	0.043 ns	0.682***	0.982***

*Note: values are Pearson correlation coefficients (r); *** p<0.001, ** p<0.01, * p<0.05, ns = not significant (p≥0.05). kg = kilograms; % = percentage content.*

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In contrast, correlations between milk yield and the percentage content of fat or protein were weak, inconsistent in sign, and largely non-significant across the six subgroups (fat%: r=-0.448 to 0.067; protein%: r=-0.162 to 0.199), with only the Polena 2020 subgroup (milk-fat%, r=-0.448, p<0.001) and the Dancu 2020 subgroup (milk-protein%, r=0.158, p<0.01) reaching significance. This pattern is compatible with a dilution effect of yield on solute concentration and mirrors the negative or near-zero phenotypic correlations between yield and composition percentages reported for other Holstein and crossbred populations [2, 21, 15], while also showing that the exact magnitude and even the sign of this relationship can differ between herds in the same region and between successive years within the same herd, a finding consistent with reports that environmental and management conditions modulate the yield-composition relationship [10, 11, 13].

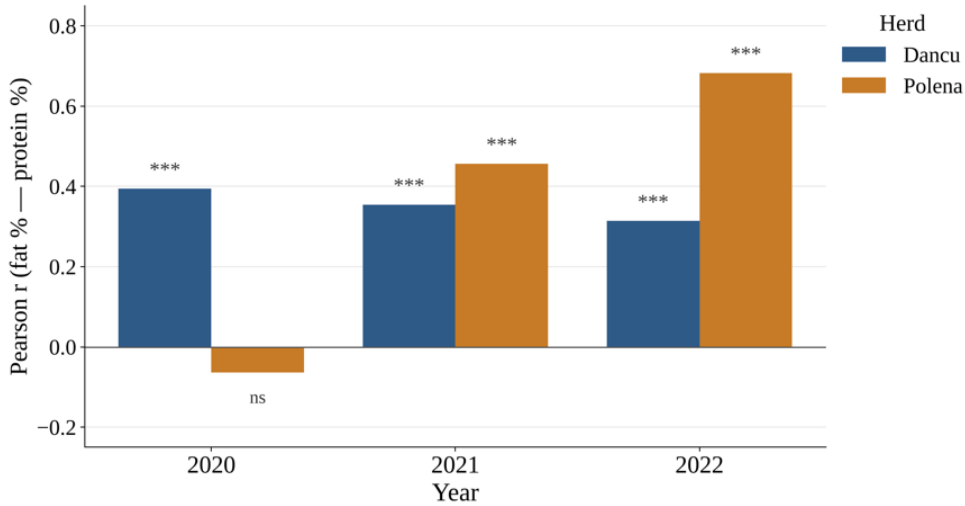


Fig. 4. Pearson correlation coefficient between fat % and protein % by herd and year, showing herd/year instability (*** p<0.001, ns = not significant)

The correlation between fat and protein percentage (Figure 4) proved to be the most herd- and year-dependent relationship examined. It was moderate and highly significant in Dancu across all three years (r=0.314-0.394, p<0.001), but ranged in Polena from a non-significant, near-zero value in 2020 (r=-0.063, p=0.621) to a strong, highly significant positive correlation in 2022 (r=0.682, p<0.001), passing through an intermediate value in 2021 (r=0.456, p<0.001). Since fat and protein percentages are both influenced by energy balance, diet composition and stage of lactation, such marked instability across years within the same, smaller herd (Polena, n=52-69 per year) suggests a stronger sensitivity to nutritional and management conditions in that herd, an interpretation consistent with studies reporting that feed management, herd size and seasonal/thermal conditions significantly affect the joint variation of fat and protein content in dairy cows [29, 16, 14, 20], and with the finding of Gorelik et al. [18] that the yield-fat percentage correlation itself differed by sire line and by parity within a single Russian Holstein herd. By comparison, fat yield and protein yield were, as expected, very strongly and consistently correlated in all herd-year subgroups (r=0.982-0.996, p<0.001), since both are largely driven by the shared milk-volume component [35, 6].

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DISCUSSIONS

Taken together, the ANOVA and correlation results converge on the same, internally consistent picture: (a) yield-based correlations (milk-fat yield, milk-protein yield, fat yield-protein yield) were uniformly very strong, highly significant and essentially identical across herds and years, and can therefore be regarded as stable, breed-characteristic relationships, even though the absolute levels of the underlying yield traits are themselves significantly shaped by farm, year and their interaction; and (b) percentage-based correlations (milk-fat%, milk-protein%, and especially fat%-protein%), which were weak to moderate, inconsistent in sign and magnitude, and strongly dependent on herd and year, mirror the strong, predominantly year-driven, non-genetic component already identified by the ANOVA for the percentage traits themselves. In other words, the same non-genetic factors, farm and year, that were shown by the ANOVA to significantly shift the level of milk yield and composition traits are also, plausibly, responsible for the instability of the phenotypic correlations built on those traits [10, 11, 12, 13, 14, 30]. This distinction is directly relevant to breeding and management decisions at farm level: selection or management changes aimed at increasing milk yield can be expected to reliably increase fat and protein yield in absolute terms, since this relationship is stable regardless of farm or year, but the realised level of fat and protein percentage, and the covariance between the two percentages, cannot be predicted from breed-level averages alone and should instead be monitored locally, on a herd- and year-specific basis, as also emphasised for other Holstein populations raised under variable environmental and management conditions [37, 32, 38].

These findings are also consistent with the broader pattern described for fertility and longevity traits, whose genetic and phenotypic associations with milk yield have likewise been shown to change over time, across production systems, and even, in a recursive-model framework, along the causal pathway linking herd and year-season effects to the genetic component of the trait covariance [39, 40, 41, 8, 9], and with evidence that body and udder conformation traits correlate only moderately with milk yield and are themselves influenced by management and nutritional status [42]. Overall, the results reinforce the view that, while the biological link between milk volume and its component yields is essentially fixed at the breed level, both the absolute level of milk yield and, even more so, the quality-related, percentage-based traits of milk remain sensitive to the specific farm and the specific year of production, and should be interpreted, and selected upon, in a herd- and year-specific context rather than assumed to be constant across production systems.

CONCLUSIONS

The two-way ANOVA performed on 952 individual lactation records demonstrated that farm (herd), production year and their interaction are statistically significant, non-genetic sources of variation for milk yield and its component yields (fat, protein, in kg), with a particularly strong farm×year interaction ($p \leq 0.003$ for all three yield traits), reflecting the fact that the two herds studied did not differ by a constant amount but diverged and partly re-converged differently from year to year. Fat and protein percentage, in contrast, were shaped predominantly by production year, common to both herds, rather than by farm identity itself, pointing to a region- or period-wide non-genetic influence, most likely related to feed quality, ration formulation or climatic conditions in a given year, rather than to farm-specific management alone. Built on this statistically confirmed non-genetic structure, the phenotypic correlations between milk yield and its component yields (fat and protein, in kg) were consistently very strong ($r > 0.97$) and highly significant in both herds and in all

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three years, showing that this relationship is a robust, largely breed-level characteristic unaffected by farm or year, whereas correlations between milk yield and the percentage content of fat and protein, and particularly the correlation between fat and protein percentage, were weak to moderate, inconsistent in sign, and strongly dependent on the same herd and year factors shown by the ANOVA to significantly affect the traits themselves, ranging from statistically non-significant to strong and highly significant for the same trait pair in different herd-year subgroups. Taken together, these two complementary analyses give a coherent, quantitatively grounded answer to how non-genetic, farm- and year-related factors shape milk production performance in the studied populations: they act mainly through a farm×year-specific pathway on absolute yield, and mainly through a shared year effect on composition percentage, while leaving the yield-to-component-yield relationship essentially untouched. From a practical breeding and management perspective, the study indicates that selection or management strategies targeting milk yield can be expected to proportionally raise fat and protein yield regardless of farm or year, but their impact on fat and protein percentage should be monitored and interpreted separately for each farm and production year, rather than extrapolated from breed-level literature values. These data, derived from real commercial-farm records in Iași County, provide an updated, regionally relevant and statistically substantiated reference point for dairy cattle breeding and management decisions in similar production systems, and establish a phenotypic basis that could be extended in future work with pedigree information to disentangle the genetic and non-genetic components of these relationships more formally.

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