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Genomics of ultrasound-derived phenotypes and liver metabolic status during the transition period in Holstein cattle

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Introduction

During the transition from late gestation to early lactation, Holstein cows experience a rapid shift in nutrient partitioning, followed by metabolic and endocrine adjustments that support the onset of milk production (Gross and Bruckmaier, 2019). This period often results in negative energy balance, driving adipose mobilization and elevating circulating non-esterified fatty acids (NEFA), which can overwhelm hepatic oxidation and very-low-density lipoprotein export capacity. Consequently, triglycerides accumulate in the liver, increasing the risk of hepatic lipidosis and often concurrent ketosis, with downstream effects on health, immune function, fertility, and productivity (Bobe et al., 2004). Liver biopsy is the diagnostic gold standard, but its invasive nature limits routine uses and large-scale phenotyping. Therefore, ultrasound (US) has been proposed as a practical, non-invasive approach to characterize liver alterations during the transition period (Giannuzzi et al., 2021). However, the magnitude of genetic variation in US-derived liver traits, as well as their genetic associations with milk production and blood biomarkers of metabolic dysfunction, remain poorly characterized. Therefore, the objectives of this study were to: (i) estimate the heritability and repeatability of ultrasound-derived liver traits, including predicted liver triacylglycerol (pTAG) content, liver depth (LD), portal vein area (PVA), and portal vein depth (PVD), across the transition period; (ii) estimate genetic and permanent environmental correlations between these traits and key blood biomarkers and milk traits indicative of energy balance, inflammation, and liver function.

Materials & Methods

Dataset. A total of 231 Holstein–Friesian cows from one commercial herd were evaluated at -60, -15, +5, +30, and +60 d relative to calving. At each time point, transcutaneous liver ultrasonography was performed by a single trained operator (right 10th intercostal space; MyLab OneVET with linear probe), with images stored as uncompressed DICOM; one representative image per cow per time point was analyzed. Liver depth, PVA, and PVD were obtained in MyLab Desk, texture features were extracted in MaZda (v4.6), and pTAG was computed using the published regression (Banzato et al., 2016). After morning milking and before feeding, 5 mL of jugular blood (lithium-heparin) was chilled, centrifuged within 2 h (3,500×g, 16 min, 6°C), and plasma stored at −20°C. Milk yield and fat/protein were recorded on sampling days.

Twenty-nine plasma biomarkers (energy, liver function, inflammation, oxidative stress, and minerals) were quantified on an ILAB 650 as previously described (Mezzetti et al., 2025). The final dataset comprised ~1,155 ultrasound/blood records and 950 milk and body records, including body weight (BW) and BCS (1–5 scale).

Genomic information. A total of 231 Holstein cows were genotyped using the GeneSeek Genomic Profiler (GGP) Bovine 100K SNP array. After excluding non-autosomal markers and

markers with GenCall score < 0.6, genotype quality control removed SNPs with MAF < 0.05, Hardy–Weinberg disequilibrium ($P \leq 1\times10^{-5}$), and call rate < 0.90 for SNPs and samples. No samples exhibited call rate lower than 0.90. Data from 231 animals and 75,759 SNPs were available for subsequent analyses.

Genetic analysis. Genetic variance components and heritability of liver-status traits were estimated using a repeatability animal model fitted with single-trait genomic BLUP (GBLUP) as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wpe} + \mathbf{e} \quad (1)$$

where $\mathbf{y}$ is the vector of US liver measurements; $\mathbf{b}$ is the vector of fixed effects, including sampling time point, parity (classes), and calving season, $\mathbf{a}$ is the additive genetic effect [$\mathbf{a} \sim N(0, \mathbf{G}\sigma_a^2)$], $\mathbf{pe}$ permanent environmental effect of the cow [$\mathbf{pe} \sim N(0, \mathbf{I}\sigma_{pe}^2)$] and $\mathbf{e}$ is the residual [$\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$]. $\mathbf{X}$, $\mathbf{Z}$ and $\mathbf{W}$ are the incidence matrices linking $\mathbf{y}$ to $\mathbf{b}$, $\mathbf{a}$, and $\mathbf{pe}$; and $\mathbf{G}$ is the genomic relationship matrix (VanRaden, 2008). Genetic correlations between liver traits and (i) production traits and (ii) metabolic indicators were estimated using bivariate GBLUP models by extending (1) to:

$$\begin{bmatrix}\mathbf{y_1}\\ \mathbf{y_2}\end{bmatrix} = \begin{bmatrix}\boldsymbol{X_1} & \mathbf{0}\\ \mathbf{0} & \boldsymbol{X_2}\end{bmatrix}\begin{bmatrix}\mathbf{b_1}\\ \mathbf{b_2}\end{bmatrix} + \begin{bmatrix}\boldsymbol{Z_1} & \mathbf{0}\\ \mathbf{0} & \boldsymbol{Z_2}\end{bmatrix}\begin{bmatrix}\mathbf{a_1}\\ \mathbf{a_2}\end{bmatrix} + \begin{bmatrix}\boldsymbol{W_1} & \mathbf{0}\\ \mathbf{0} & \boldsymbol{W_2}\end{bmatrix}\begin{bmatrix}\boldsymbol{pe_1}\\ \mathbf{pe_2}\end{bmatrix} + \begin{bmatrix}\mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix}, \quad (2)$$

Model inference was performed in gibbsf90+ (Aguilar et al., 2018) using Gibbs sampling (500,000 iterations, 100,000 burn-in, thinning = 10), yielding 40,000 posterior samples for parameter estimation. Convergence was evaluated using standard diagnostic procedures.

Heritability was calculated as: $h^2 = \sigma_a^2/(\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)$ and repeatability as: $t = (\sigma_a^2 + \sigma_{pe}^2)/(\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)$, where σ_a^2 is the additive genetic variance, σ_{pe}^2 the permanent environmental variance, and σ_e^2 the residual variance.

Results

Genetic analyses indicated that ultrasound-derived liver traits exhibit exploitable additive genetic variation, with posterior heritability estimates being low to moderate for pTAG(0.15 ± 0.061) and LD (0.17 ± 0.014), and moderate for portal vein traits (PVA = 0.26 ± 0.065; PVD = 0.34 ± 0.075). The repeatability estimates indicate moderate temporal consistency of ultrasound-derived liver traits across the period from pre–dry-off (−60 d) to postpartum (+60 d), with values of 0.30 ± 0.071 for pTAG, 0.33 ± 0.037 for PVA, 0.33 ± 0.020 for LD, and 0.47 ± 0.036 for PVD.

Table 1. Posterior means of additive genetic variance (σ_a^2), permanent environmental variance (σ_{pe}^2), residual variance (σ_e^2), heritability (h^2), and repeatability (t) for ultrasound-derived liver traits in Holstein cows.

Item	pTAG[1] (mg/g)	PVA (mm^2)	PVD (mm)	LD (mm)
σ_a^2	15.43 ± 10.91	17791.00 ± 2646.50	38.26 ± 10.37	249.21 ± 39.34
σ_{pe}^2	20.74 ± 12.78	6244.20 ± 1507.20	23.60 ± 9.39	359.02 ± 47.89
σ_e^2	85.93 ± 13.85	48673.00 ± 2467.40	70.49 ± 3.58	1245.80 ± 101.55
h^2	0.15 ± 0.06	0.26 ± 0.06	0.34 ± 0.07	0.17 ± 0.01
t	0.30 ± 0.07	0.33 ± 0.03	0.47 ± 0.03	0.33 ± 0.02

[1]pTAG: predicted liver triacylglycerol; PVA: portal vein area; PVD: portal vein depth and LD: liver depth.

Ultrasound traits exhibited moderate-to-high genetic and permanent environmental correlations with key biomarkers and inflammatory indicators (Fig. 1). For energy-related indicators, pTAG showed a strong additive genetic correlation with BHB (r_g = 0.75 ± 0.09) and permanent environmental correlation with cholesterol (r_{pe} = -0.69 ± 0.15). LD was strongly associated with NEFA (r_g = 0.64 ± 0.09; r_{pe} = 0.53 ± 0.15). PVA showed moderate permanent environmental

correlations negative with BHB (r_{pe} = -0.59 ± 0.13) and positive with glucose (r_{pe} = 0.58 ± 0.13), whereas PVD showed a negative correlation with urea (r_{pe} = -0.74 ± 0.15).

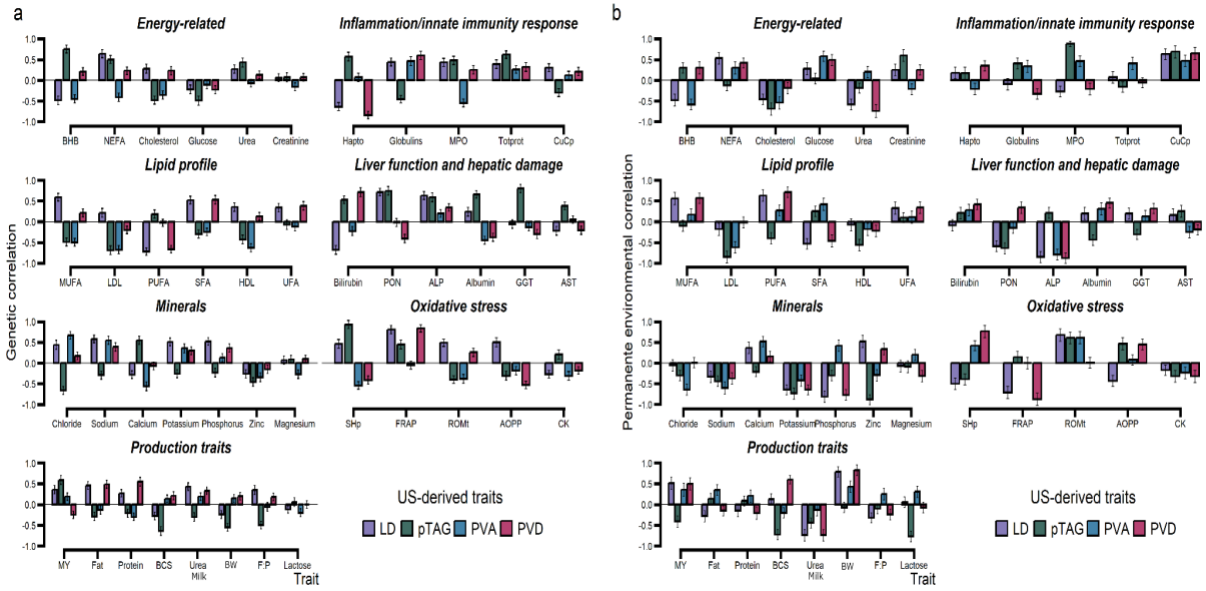

Figure 1. Posterior mean genetic (a) and permanent environmental (b) correlations between ultrasound-derived liver traits, namely liver depth (LD), predicted liver triacylglycerol (pTAG), portal vein area (PVA), and portal vein depth and liver depth (PVD) and blood/milk indicators grouped as energy, inflammation, lipid profile, liver function/damage, minerals, oxidative stress, and production. Bars show correlation estimates with standard deviation (SD).

For inflammation/innate immunity, the highest correlation was for PVD and LD with haptoglobin (r_g = -0.84 ± 0.09 and -0.64 ± 0.10, respectively) and for pTAG with myeloperoxidase (MPO) and ceruloplasmin (CuCp) (r_{pe} = 0.88 ± 0.07 and 0.69 ± 0.14, respectively). Lipid profiles showed high genetic correlations for pTAG and low-density lipoprotein (r_g = -0.69 ± 0.11; r_{pe} = -0.85 ± 0.14), and for LD and PVD and PUFA (r_g = -0.72 ± 0.09 and -0.67 ± 0.10). For liver function/damage, the strongest genetic signals were pTAG–GGT (r_g = 0.80 ± 0.10), LD– Paraoxonase (PON) (r_g = 0.71 ± 0.09), and PVD–bilirubin (r_g = 0.71 ± 0.11), while ALP showed consistently negative permanent environmental correlation with LD, PVA and PVD (r_{pe} = -0.79 to -0.88).

Mineral associations were driven by strong negative permanent environmental correlations, particularly pTAG–zinc (r_{pe} = -0.88 ± 0.12) and LD–phosphorus (r_{pe} = -0.81 ± 0.13), while chloride showed positive genetic relationships with PVA (rg = 0.67 ± 0.09) and pTAG (rg = -0.66 ± 0.09). Oxidative stress showed marked sign discordance for FRAP, with LD and PVD showing positive genetic correlation (rg = 0.81 – 0.84) but negative permanent environmental correlations (r_{pe} = -0.71 to -0.87).

Discussion

Ultrasound-derived liver phenotypes show promise as indicators of transition health, combining exploitable additive genetic variance with repeatable cow-specific differences from −60 to +60 d relative to calving. Portal vein traits, especially PVD, showed higher heritability and repeatability, consistent with stable between-cow differences in portal–hepatic nutrient delivery relevant to metabolic adaptation in early lactation (Bobe et al., 2004). On the other hand, lower heritability for pTAG likely reflects transient physiological variations during negative energy balance, further amplified by the prediction noise inherent to image-based estimates (Banzato et al., 2016). The correlation pattern further supports biological relevance. The strong additive

genetic correlation between pTAG and BHB suggests shared genetic drivers of hepatic lipid accumulation and ketosis susceptibility, consistent with transition physiology in which adipose mobilization elevates NEFA, promotes ketogenesis and increases hepatic TAG (Bobe et al., 2004). Associations between LD and NEFA further indicate that ultrasound-derived phenotypes capture lipid mobilization, whereas negative correlations between pTAG and LDL are consistent with impaired hepatic lipid export when triacylglycerol accumulates in the liver (Bobe et al., 2004; Trevisi et al., 2012). Associations with haptoglobin and MPO further link these phenotypes to inflammatory status, which is closely coupled with metabolic stress and compromised liver function around calving (Sordillo et al., 2009; Trevisi et al., 2012). Associations with PON are expected given its hepatic origin and sensitivity to oxidative stress (Sordillo et al., 2009). Opposite directions of the FRAP associations at the genetic (rg) versus cow-level (rpe) components suggest that genetic predisposition may differ from short-term environmental influences, implying that selection and management may affect distinct aspects of antioxidant regulation. These findings should be interpreted as exploratory due to the single-herd design and moderate sample size; therefore, multi-herd replication is needed to confirm the magnitude of correlations and to assess trait stability across different management conditions. Overall, portal vein traits (PVA and PVD) appear to be the most robust candidates for selection and monitoring, whereas pTAG provides complementary information on energy–inflammation dynamics during the transition period.

Conclusions

Portal vein ultrasound traits, particularly PVD, exhibited the highest heritability and repeatability, supporting their potential as robust phenotypes for both genetic selection and transition-period monitoring. Their moderate-to-high correlations with indicators of energy balance, lipid metabolism, inflammation, liver function, mineral status, and oxidative stress suggest that these measures capture key biological pathways underlying metabolic resilience. Together, these results contribute to a deeper biological understanding of complex traits by integrating multiple complementary phenotypes, thereby improving the characterization of metabolic resilience and adaptive responses during the transition period.

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