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Effects of transport age (14 d vs. 28 d of age) on blood total cholesterol, insulin and IGF-1 concentrations of veal calves

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ABSTRACT

The main aim of the current study was to find biomarkers of health in calves transported at different ages. The selected blood parameters were total cholesterol, insulin and IGF-1 and the longitudinal study investigated whether or not these concentrations were different between calves that were transported from the dairy farm to the veal farm at 14 d or 28 d of age. Relationships between these blood variables and health characteristics of veal calves were investigated. In a 34-wk study period, a total of 683 calves originating from 13 Dutch dairy farms were transported at an age of 14 or 28 d to 8 Dutch veal farms. Calves were blood sampled the first wk after birth (mean and SD: 4.4 ± 2.1 d), a day before transport (mean and SD: 25.8 ± 7.3 d) and in wk 2 post-transport (mean and SD: 36.7 ± 12.2 d). In these samples, insulin, IGF-1 and total cholesterol were determined and analyzed with a linear mixed model (**LMM**). Individual medical treatments were recorded from birth until the day of transport at the dairy farm, and from the moment of arrival at the veal farm until slaughter, and analyzed as a binary response variable (calf treated or not) with a generalized linear mixed model. Fecal (calf with or without loose or liquid manure) and navel (calves with or without swollen and inflamed navel) scores measured during a single visit in wk 2 post-transport were also analyzed as binary response variables, whereas carcass weights at slaughter age were analyzed with a LMM. Cholesterol, insulin and IGF-1 were included as covariates in the previous models to test their relationships with the likelihood of calves being medically treated, fecal and navel scores, and carcass weights. One day before transport 28 d-old calves had higher blood cholesterol ($\Delta = 0.40$ mmol/l) and IGF-1 ($\Delta = 53.6$ ng/ml) concentrations, and evidence of higher insulin ($\Delta = 12.2$ μU/ml) compared with 14 d-old calves. In wk 2 post-transport, 28-d old calves had higher blood IGF-1 ($\Delta = 21.1$ ng/ml), with evidence of higher insulin ($\Delta = 12.2$ μU/ml) concentrations compared with 14-d old calves. Cholesterol concentration measured one day before transport and in wk 2 post-transport had a positive relationship with carcass weight at slaughter ($\beta = 4.8$ and 7.7 kg/mmol/l, respectively). Blood cholesterol concentration in wk 2 post-transport was negatively associated with the fecal score measured at the same sampling moment ($\beta = -0.55$/mmol/l), with the likelihood of a calf of being treated with antibiotics ($\beta = -0.36$/mmol/l) and other medicines ($\beta = -0.45$/mmol/l) at the veal farm. Blood IGF-1 concentration in wk 2 post-transport was negatively associated with the likelihood of a calf of being treated with antibiotics and other medicines (both $\beta = -0.01$/ng/ml) at the veal farm, and with fecal score recorded in wk 2 post-transport ($\beta = -0.004$/ng/ml). When looking at the blood indicators, it appeared that calves transported at 28 d of age were more developed compared with 14 d old calves, thus transport at an older age might be more beneficial for the animals. It can be concluded that both blood cholesterol and IGF-1 concentrations seemed to be valuable biomarkers of health and energy availability in veal calves.
Keywords: veal calf, transport age, cholesterol, insulin, IGF-1

INTRODUCTION

Dairy calves destined to veal production in the Netherlands are usually transported from several dairy farms (in or outside the Netherlands) to a veal farm with an in-between stop at a collection center (Marcato et al., 2022a). Particularly calves are transported at a minimum age of 14 d in the Netherlands. Commingling of calves during transport increases the exposure to pathogens and their young age increases the susceptibility to infectious diseases (Wilcox et al., 2013; Roadknight et

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al., 2021), which is a major concern for veal farmers in the first weeks post-transport. Marcato et al. (2022b) showed that an average of 36% of calves had loose or liquid manure in wk 2 post-transport and Pardon et al. (2015) reported that a total of 61% of calves developed bovine respiratory disease in the first 18 d at the veal farm. The high morbidity often results in greater use of antibiotic treatments early after transport (Schnyder et al., 2019; Antonis et al., 2022), which is one of the major problems in the veal sector. A potential approach to identify calves with a high risk profile for diseases upon arrival at the veal farm is the use of blood biomarkers. A biomarker can provide information about the current health status or future risk of disease of a calf (Pletcher and Pignone, 2011; Marcato et al., 2018; Goetz et al., 2021). Marcato et al. (2022a) showed, for example, that a higher serum IgG at wk 1 after birth is associated with a lower likelihood for a calf being treated with antibiotics and medicines at the veal farm. Cholesterol is a novel biomarker and higher concentrations upon arrival at the veal farm are associated with a lower hazard of morbidity and mortality of calves (Renaud et al., 2018; Goetz et al., 2021). Cholesterol has also been suggested as a marker of colostrum intake, since cholesterol concentration is much higher in colostrum than in milk replacer (Ontsouka et al., 2016). Insulin and IGF-1 are 2 other potential biomarkers related to nutrition (e.g., high-intensity vs. low intensity colostrum diet, or milk replacer diet) on the dairy farm and related to the development of the gastrointestinal tract (**GIT**) and growth of calves (Hammon and Blum, 1997; Hammon et al., 2000; Ontsouka et al., 2016). The latter studies are, however, conducted in dairy calves and there is no information yet on veal calves. Thus, more information on these 3 biomarkers could provide insights into management of veal calves at the dairy farms. Additionally, they might be suitable biomarkers for susceptibility to diseases of calves when entering the veal farm. Blood cholesterol, insulin and IGF-1 concentrations are increasing with age (Hammon and Blum, 1997; Ontsouka et al., 2016) and consequently, they might be relevant indicators of robustness and development of calves transported at an older age compared with younger calves (Piccione et al., 2010; Marcato et al., 2022a,b). The impact of transport age on cholesterol has been evaluated recently (Goetz et al., 2023), but the impact on insulin and IGF-1 concentrations is lacking. Moreover, effects of specific transport age of 14 and 28 d have never been evaluated for all 3 biomarkers, thus the current study investigates these aspects. The first aim of the study was to investigate whether or not blood cholesterol, insulin and IGF-1 concentrations are different between calves transported from the dairy farm to the veal farm at different ages and to investigate how the concentrations are changing over time. This will provide knowledge on these parameters and will help to understand whether or not calves transported at 28 d of age are more robust than calves transported at 14 d of age as indicated in previous studies (Marcato et al., 2022a,b). The second aim of the study was to investigate any association between these blood indicators and measures of health and performance at the veal farm. The hypothesis was that calves transported at 28 d of age have higher concentrations of cholesterol, insulin and IGF-1 in plasma compared with calves transported at 14 d, and that high values of these indicators can positively relate to a lower prevalence of clinical problems at the veal farm.

MATERIALS AND METHODS

Experimental design

The longitudinal experiment was executed between March 2019 and May 2020 and was approved by the Central Committee on Animal Experiments (The Hague, the Netherlands; approval number 2017.D-0029). A detailed description of the experimental design can be found in Marcato et al. (2022a). A total of 683 calves, born on 13 Dutch dairy farms, were recruited in the experiment. Farmers participated in the experiment on a voluntary basis. At each farm, calves were transported at an age of 14 or 28 d to one of 8 selected veal farms. On the basis of an experimental schedule the calves born on all dairy farms in the first 2 weeks into the trial were assigned to the 28 d transport, whereas calves born in wk 3 and 4 were assigned to the 14 d transport. Thus depending on the week the calves were monitored, calves were assigned to a specific transport group. At each transport day (at the end of this 4-week timeframe), calves of both age groups were transported from the dairy farms of origin directly to the same veal farm and they endured a short transport duration (<6 h). This scheme was repeated over time and calves born in a different timeframe were transported to a different veal farm. We stopped to recruit calves into the experiment when the last veal farm was filled with calves. After the first 3 wks post-transport at the veal farm (average herd size of 1,065 calves) during which calves were individually housed on a slatted floor, the calves were housed in group pens (5 or 6 calves per pen) with a space allowance of 1.8 m^2/calf. At the veal farm calves received similar diets, all belonging to the same affiliated veal company (i.e., milk replacer, Denkavit B.V., for a 3-phase feeding program, and a 2-phase feeding program for the solid feed, consisting of a mixture of concentrates and straw). All veal farms were based on an all-in, all-out system, where the experimental calves were mixed with other calves and were treated in the same way as the non-experimental calves. Moreover, veal farmers were blinded to the background of calves and the age of calves at slaughter was on average 210.2 ± 10.7 d.

Sample size calculation

For the calculation of the number of experimental units a power analysis was conducted. The number of calves allocated to each age group (>300 animals) was based on calculations on experimental units reported by previous studies (Engel et al., 2016; Marcato et al., 2020) and the full details on the power calculations of the current study are described in Marcato et al. (2022a). Overall the power calculations led to a sample size of 235 per age group, 470 in total when a fully balanced design would be assumed. To be able to deal with an unbalanced design we aimed for a surplus of animals.

Collection of blood samples and analyses

Blood samples were collected from calves (n = 683) within 1 wk after birth, 1 d before transport at 14 or 28 d of age, and in wk 2 after arrival at the veal farm. Blood (10 mL) was collected from the jugular vein of calves into EDTA vacutainer tubes (Vacuette, Greiner BioOne). Samples were stored at room temperature for a few hours until centrifugation (3,000 × g for 15 min at 4°C), and then plasma was decanted and stored at −20°C until analysis. Concentrations of insulin and IGF-1 in were measured by radioimmunoassay (RIA) as described earlier by Vicari et al. (2008). Inter- and intra-assay coefficients of variation (CVs) for insulin and IGF-1 were <10%. Total cholesterol concentration in plasma was measured using a commercially available enzymatic kit (Cholesterol FS 1.1350 99 10 021, from DiaSys Diagnostic Systems GmbH) with an autoanalyzer (Cobas Mira, Roche). The intra-assay CV for cholesterol was 2.3%, whereas there was no inter-assay CV as all samples were analyzed with the same kit.

Relationship between blood parameters and measures of health and performance

Measures of health and performance of calves at the veal farm were recorded at an animal-individual level by researchers with the aim to investigate their relationships with blood indicators. Clinical health parameters included fecal and navel scores in wk 2 after arrival of calves at the veal farm. These parameters were scored as binary response variables, as described in detail by Marcato et al. (2022b). Fecal score was measured as a calf with (score 1) or without loose or liquid manure (score 0) and navel score was measured as calves with (score 1) or without (score 0) swollen and inflamed navel. Individual use of antibiotics and other medicines was recorded at both the dairy and veal farm and included the following data: (1) whether or not a calf was treated with antibiotics or other medical treatments (this latter category referred to products such as anti-inflammatories, multivitamins, and anticoccidial drugs); (2) whether single or repeated antibiotic/medical treatments were applied; and (3) age at which treatments were applied. Moreover, carcass weights provided at slaughter were also analyzed in relationship to blood parameters.

Additional information on calf and dam characteristics

Information on characteristics of calves (body weight (**BW**) at birth, breed, and sex) and their dams (parity and ease of birth) were recorded at the dairy farm. Breed of calves included 3 main categories: Holstein Friesian (**HF**), HF × Belgian Blue (**BB**), and other crossbred calves. This latter category included different types of crossbreds such as Fleckvieh × HF, Black Angus × HF, Montbeliarde × HF and others. More details can be found in Marcato et al. (2022a,b).

Statistical analyses

Before the analyses the distribution of calves across (experimental) factors was checked to allow for an unbiased estimation of all main effects. As reported in Marcato et al. (2022a,b) the different levels of other factors, including sex, parity of dam, ease of birth, and breed were sufficiently (almost equally) represented within each transport age group. A detailed overview of all the models used to analyze the data is provided in Table 1. Concentrations of insulin, IGF-1 and total cholesterol were analyzed as continuous variables with a linear mixed model (LMM). Components of variance were estimated with REML, using the MIXED procedure from SAS 9.4 (SAS Institute Inc.). Residuals were checked for normality and homogeneity of variance, and variables were log-transformed when needed. Approximate F-tests (Kenward and Roger, 1997) were used for fixed effects. Subsequently, pairwise comparisons were done with using Tukey's studentized range test (HSD) to control for type 1 error inflation. Two-way interactions between fixed effects were included in the model and these interactions were considered not statistically significant when $P > 0.05$. Carcass weights were analyzed with LMM, whereas clinical health problems and individual medical treatments were expressed as binary variables and analyzed with a generalized linear mixed model (**GLMM**), utilizing a logit link function and the Bernoulli variance as an "error" variance. Inference was by penalized quasi-likelihood (**PQL**, which is equivalent to the use of pseudo-likelihood), with SAS procedure GLIMMIX. To test relationships between blood indicators and measures of health and performance, blood variables were added as covariates in these models.

RESULTS

Descriptive statistics

The number of calves allocated to 14-d and 28-d treatment groups were 363 and 320, respectively. A total of 85 ± 13 (average ± SD) calves were transported to each veal farm. Out of 683 calves, 508 were bulls and 175 were heifers. A total of 246 calves were HF × BB crossbreds, 235 calves were HF, and 202 calves were crossbreds other than HF × BB. The total number of

Table 1. Mixed models used per response variable included in the current longitudinal study assessing effects of transport age (14 or 28 d), calf and cow characteristics on blood biomarkers, health and performance measures. The study included calves (n = 683) born on 13 dairy farms and transported to 8 veal farms

Model	Response variables	LMM[1] or GLMM[2]	Covariate	Fixed effects	Random effects
Model 1	Blood indicators (insulin, IGF-1 or total cholesterol) measured in wk 1 after birth	LMM	Birthweight	• Sex of calf (bull or heifer) • Breed of calf (Holstein Friesian or Holstein Friesian x Belgian Blue or other crossbreds) • Parity of the dam (1, 2, 3, 4–10) • Ease of birth (0 = unassisted birth or 1 = assistance during birth)	• Dairy farm
Model 2	Blood indicators (insulin, IGF-1 or total cholesterol) measured one day before transport	LMM	Birthweight	• Sex of calf (bull or heifer) • Breed of calf (Holstein Friesian or Holstein Friesian x Belgian Blue or other crossbreds) • Transport age of calf (14 d or 28 d) • Parity of the dam (1, 2, 3, 4–10) • Ease of birth (0 = unassisted birth or 1 = assistance during birth)	• Dairy farm
Model 3	Blood indicators (insulin, IGF-1 or total cholesterol) measured in wk 2 post-transport	LMM	—	• Sex of calf (bull or heifer) • Breed of calf (Holstein Friesian or Holstein Friesian x Belgian Blue or other crossbreds) • Transport age of calf (14 d or 28 d) • Parity of the dam (1, 2, 3, 4–10) • Ease of birth (0 = unassisted birth or 1 = assistance during birth)	• Dairy farm • Veal farm • Dairy farm × Veal farm • Transport
Model 4	Health parameters (wk 2 at the veal farm)	GLMM	Blood indicators (insulin, IGF-1 or total cholesterol)	• Sex of calf (bull or heifer) • Breed of calf (Holstein Friesian or Holstein Friesian x Belgian Blue or other crossbreds) • Transport age of calf (14 d or 28 d) • Parity of the dam (1, 2, 3, 4–10) • Ease of birth (0 = unassisted birth or 1 = assistance during birth)	• Dairy farm • Veal farm • Dairy farm × Veal farm • Transport
Model 5	Probability of a calf of being treated with antibiotics or other medicines (0 = calf not treated with any antibiotics or other medicines; 1 = calf treated at least once with antibiotics or other medicines during their rearing period) at both dairy farm and veal farm	GLMM	Blood indicators (insulin, IGF-1 or total cholesterol)	• Sex of calf (bull or heifer) • Breed of calf (Holstein Friesian or Holstein Friesian x Belgian Blue or other crossbreds) • Transport age of calf (14 d or 28 d) • Parity of the dam (1, 2, 3, 4–10) • Ease of birth (0 = unassisted birth or 1 = assistance during birth)	• Dairy farm • Veal farm • Dairy farm × Veal farm • Transport

Continued

Table 1 (Continued). Mixed models used per response variable included in the current longitudinal study assessing effects of transport age (14 or 28 d), calf and cow characteristics on blood biomarkers, health and performance measures. The study included calves (n = 683) born on 13 dairy farms and transported to 8 veal farms

Model	Response variables	LMM[1] or GLMM[2]	Covariate	Fixed effects	Random effects
Model 6	Probability of a calf of being treated with antibiotics or other medicines (0 = calf not treated with any antibiotics or other medicines; 1 = calf treated at least once with antibiotics or other medicines during their rearing period) at the dairy farm	GLMM	Blood indicators (insulin, IGF-1 or total cholesterol)	• Sex of calf (bull or heifer) • Breed of calf (Holstein Friesian or Holstein Friesian x Belgian Blue or other crossbreds) • Transport age of calf (14 d or 28 d) • Parity of the dam (1, 2, 3, 4–10) • Ease of birth (0 = unassisted birth or 1 = assistance during birth)	• Dairy farm
Model 7	Carcass weight	LMM	Blood indicators (insulin, IGF-1 or total cholesterol)	• Sex of calf (bull or heifer) • Breed of calf (Holstein Friesian or Holstein Friesian x Belgian Blue or other crossbreds) • Transport age of calf (14 d or 28 d) • Parity of the dam (1, 2, 3, 4–10) • Ease of birth (0 = unassisted birth or 1 = assistance during birth)	• Dairy farm • Veal farm • Dairy farm × Veal farm • Transport

[1]LMM = linear mixed model; [2]GLMM = generalized linear mixed model.

calves born from first-parity cows was 90, whereas a total number of 165 was born from second-parity cows, 151 from third-parity cows, and 252 from cows with parity 4 or higher. Preventive medicines, such as vaccines, were not used on calves and their dams. No vaccination was given to calves at the veal farm.

Effects of transport age

One day before transport, 28 d-old calves had a greater blood cholesterol ($\Delta = 0.40$ mmol/l; $P < 0.01$) and IGF-1 ($\Delta = 53.6$ ng/ml; $P < 0.01$) concentration, and a greater insulin ($\Delta = 12.2$ μU/ml; $P = 0.05$) concentration compared with 14 d-old calves (Table 2). In wk 2 post-transport, 28-d old calves had a greater blood IGF-1 ($\Delta = 21.1$ ng/ml; $P < 0.01$) and evidence of greater insulin ($\Delta = 12.2$ μU/ml; $P = 0.06$) concentration compared with 14-d old calves (Table 3).

Effects of breed

The wk after birth, crossbreds other than HF × BB had a lower insulin concentration ($\Delta = -33.5$ μU/ml on average; $P = 0.02$) compared with HF × BB crossbreds and HF calves (Table 4). Crossbred calves had lower IGF-1 ($\Delta = -15.7$ ng/ml on average; $P = 0.04$) concentrations compared with HF calves (Table 4). The day before transport, HF calves had a lower total cholesterol concentration compared with crossbred calves ($\Delta = -0.51$ mmol/l on average; $P < 0.01$; Table 2). In wk 2 post-transport, crossbreds other than HF × BB had lower concentrations of insulin ($\Delta = -24.0$ μU/ml on average; $P = 0.03$) and IGF-1 ($\Delta = -24.2$ ng/ml on average; $P < 0.01$) compared with HF × BB crossbreds and HF calves (Table 3).

Effects of transport age × breed

The interaction transport age × breed had an effect only on cholesterol concentrations measured in wk 2 post-transport (Table 5; $P < 0.01$). HF × BB crossbreds transported at 14-d of age had the highest cholesterol concentrations in their blood compared with all the other calves. HF calves transported at either 14-d or 28-d had the lowest cholesterol concentrations compared with the other crossbreds of both transport ages. Moreover, cholesterol concentrations of both HF × BB and HF calves were higher at a transport age of 14-d compared with 28-d of age, whereas cholesterol concentration of other crossbreds was higher when calves were transported at 28-d of age compared with 14-d of age (Table 5). All the other tested interactions were not significant.

Effects of sex

One wk after birth, bull calves had a lower blood IGF-1 concentration ($\Delta = -13.1$ ng/ml) compared with heifers ($P = 0.01$; Table 4). In wk 2 post-transport, there was evidence of a lower blood cholesterol concentration in bull calves compared with heifers (Table 3; $P = 0.07$).

Table 2. Effects of calf transport age (14 d vs. 28 d), sex and breed on blood concentrations of insulin, IGF-1 and total cholesterol in calves one day before transport to the veal farm (Lsmeans ± SEM). The study included calves born on 13 dairy farms and transported to 8 veal farms

	Transport age				Sex				Breed[2]				
Parameter	14 d	28 d	SEM[1]	*P*-value	Bull	Heifer	SEM	*P*-value	HF	HF × BB	O	SEM	*P*-value
N. of calves	362	319			507	174			234	246	201		
Insulin (µU/ml)	58.1[a]	70.3[b]	5.0	0.05	66.7	55.3	4.7	0.60	79.0	57.7	53.7	5.9	0.10
IGF-1 (ng/ml)	143.6[a]	197.2[b]	10.4	<0.01	174.0	166.8	10.6	0.27	170.3	168.7	172.2	12.0	0.95
Total cholesterol (mmol/l)	2.60[a]	3.00[b]	0.12	<0.01	2.75	2.85	0.12	0.23	2.46[a]	2.94[b]	3.00[b]	0.14	<0.01

[1]SEM = pooled standard error of the mean; [2]Breed: HF = Holstein Friesian, HF × BB = Holstein Friesian × Belgian Blue crossbreds, O = other crossbreds.

a, b Lsmeans within a factor and line lacking a common superscript differ ($P \leq 0.05$).

Table 3. Effects of transport age (14 d vs. 28 d), sex and breed on concentrations of insulin, IGF-1 and total cholesterol measured in blood of calves at wk 2 post-transport (Lsmeans ± SEM). The study included calves born on 13 dairy farms and transported to 8 veal farms

	Transport age		SEM[1]	*P*-value	Sex		SEM	*P*-value	Breed[2]				
Parameter	14 d	28 d			Bull	Heifer			HF	HF × BB	O	SEM	*P*-value
N. of calves	362	319			507	174			234	246	201		
Insulin (µU/ml)	59.2	71.4	7.02	0.06	61.4	75.1	7.5	0.14	78.3[b]	65.9[ab]	48.1[a]	8.0	0.03
IGF-1 (ng/ml)	108.9[a]	130.0[b]	6.7	<0.01	119.5	119.4	6.8	0.99	131.4[a]	123.7[a]	103.3[b]	7.3	<0.01
Total cholesterol (mmol/l)[2]	2.47	2.42	0.11	0.43	2.39	2.51	0.11	0.07	2.24[a]	2.58[b]	2.52[b]	0.12	<0.01

[1]SEM = pooled standard error of the mean; [2]Breed: HF = Holstein Friesian, HF × BB = Holstein Friesian × Belgian Blue crossbreds, O = other crossbreds.

[2]Table 5 showed the interaction between transport age and breed for total cholesterol.

a, b Lsmeans within a factor and line lacking a common superscript differ ($P \leq 0.05$).

Table 4. Effects of calf sex and breed, parity of the dam and ease of birth on concentrations of insulin, IGF-1 and total cholesterol measured in blood of calves in wk 1 after birth at the dairy farm (LSmeans ± SEM). The study included calves born on 13 dairy farms

	Sex				Breed[2]					Parity						Ease of birth[3]			
Parameter	Bull	Heifer	SEM[1]	*P*-value	HF	HF × BB	O	SEM	*P*-value	1	2	3	4–10	SEM	*P*-value	Unassisted	Assisted	SEM	*P*-value
N. of calves	507	174			234	246	201			87	166	151	252			525	153		
Insulin (μU/ml)	55.3	58.6	5.3	0.31	70.1[a]	62.1[a]	32.6[b]	5.0	0.02	62.4	53.5	62.9	44.7	7.1	0.23	56.2	55.4	4.8	0.57
IGF-1 (ng/ml)	124.8[a]	137.9[b]	6.2	0.01	141.8[b]	129.7[a]	122.5[a]	7.4	0.04	130.8	125.6	139.3	129.8	6.9	0.10	135.1	127.6	6.2	0.13
Total cholesterol (mmol/l)	1.52	1.54	0.05	0.72	1.51	1.60	1.50	0.06	0.20	1.55	1.52	1.51	1.55	0.06	0.84	1.58	1.49	0.06	0.07

[1]SEM = pooled standard error of the mean; [2]Breed: HF = Holstein Friesian, HF × BB = Holstein Friesian × Belgian Blue crossbreds, O = other crossbreds; [3]Ease of birth: 0 = unassisted; 1 = assisted by the farmer.

a, b Lsmeans within a factor and line lacking a common superscript differ ($P \leq 0.05$).

Effects of parity of the dam and calving ease

In wk 2 post-transport, there was evidence of lower blood cholesterol concentrations of calves born from first and second parity cows compared with calves born from cows with higher parity ($P = 0.07$; Table 6). Parity had no significant effect on insulin, IGF-1, and cholesterol concentrations at the other sampling moments (Table 4 and 7). The first wk after birth, there was evidence of a lower blood cholesterol concentration in calves born with assistance of the farmer compared with calves with unassisted birth (Table 4). In wk 2 post-transport, calves with assistance at birth had a lower IGF-1 concentration ($\Delta = -10.0$ ng/ml) in their blood compared with calves born without assistance (Table 6).

Relationships among analyzed parameters

Table 8 shows the regression coefficients and significant *P*-values of relationships between variables analyzed in the current study. Cholesterol concentration at one day before transport and in wk 2 post-transport had a positive relationship with carcass weight ($P < 0.01$). Cholesterol concentration in wk 2 post-transport was negatively associated with the fecal score measured at the same sampling moment ($P < 0.01$), and with the likelihood of a calf of being treated with antibiotics ($P = 0.01$) and medicines other than antibiotics ($P < 0.01$) at the veal farm. Evidence of a negative relationship between cholesterol concentration at wk 1 after birth and fecal score at wk 2 post-transport was found ($P = 0.09$). IGF-1 concentration at wk 2 post-transport was negatively associated with the likelihood of a calf of being treated with antibiotics ($P < 0.01$) and medicines other than antibiotics ($P < 0.01$) at the veal farm, and with fecal score at wk 2 post-transport ($P = 0.02$). A negative relationship was also found between IGF-1 concentration at wk 1 after birth and the day before transport with the likelihood of a calf being treated with antibiotics ($P = 0.03$) and medicines other than antibiotics ($P < 0.01$) at the veal farm. Insulin concentration measured in the first wk after birth and one day before transport were negatively associated with fecal score ($P = 0.01$) and navel score ($P = 0.03$) at wk 2 post-transport.

DISCUSSION

Effects of transport age

The main hypothesis of the current study was that concentrations of cholesterol, insulin, and IGF-1 were higher in blood of calves transported at 28 d of age

Table 5. Effects of the interaction between transport age and breed on cholesterol measured in blood of calves in wk 2 post-transport. (Lsmeans ± SEM[1]). The study included calves born on 13 dairy farms and transported to 8 veal farms

	Transport age x breed[2]							
Parameter	(14-d) HF	(14-d) HF × BB	(14-d) O	(28-d) HF	(28-d) HF × BB	(28-d) O	SEM	P-value
Total cholesterol (mmol/l)	2.33[a]	2.75[b]	2.41[a]	2.14[c]	2.41[ad]	2.63[bd]	0.13	<0.01

[1]SEM = pooled standard error of the mean; [2]Breed: HF = Holstein Friesian, HF × BB = Holstein Friesian × Belgian Blue crossbreds, O = other crossbreds.

a, b, c, d Lsmeans within a factor and line lacking a common superscript differ ($P \leq 0.05$).

Table 6. Effects of parity of the dam and ease of birth on concentrations of insulin, IGF-1 and total cholesterol measured in plasma of calves in wk 2 post-transport (Lsmeans ± SEM). Calves were transported at 14 or 28 d of age from 13 dairy farms to 8 veal farms

	Parity						Ease of birth			
Parameter	1	2	3	4–10	SEM[1]	*P*-value	Unassisted	Assisted	SEM	*P*-value
N. of calves	87	166	151	252			525	153		
Insulin (μU/ml)	45.5	67.6	67.4	71.4	9.7	0.39	69.3	50.4	6.2	0.27
IGF-1 (ng/ml)	120.0	114.5	123.5	119.9	7.4	0.41	124.5[a]	114.5[b]	6.8	0.03
Total cholesterol (mmol/l)	2.35	2.39	2.51	2.54	0.11	0.07	2.44	2.45	0.10	0.90

[1]SEM = pooled standard error of the mean.

a, b Lsmeans within a factor and line lacking a common superscript differ ($P \leq 0.05$).

compared with calves transported at 14 d of age. Our results confirmed this hypothesis, with 28 d old calves having higher IGF-1 and showing evidence of higher insulin concentrations on both the day before transport and in wk 2 post-transport. Previous studies reported that higher concentrations of these blood parameters represent the available energy in the organism and hence they are related to development of the gastrointestinal tract (GIT) of calves and to their growth (Ontsouka et al., 2016). Aref et al. (2016) reported that IGF-1 is responsible for the regulation of both skeletal and muscle development in growing calves and the concentrations of IGF-1 are higher in calves with higher body weight (Smith at al., 2002). This is also applicable to the 28 d old calves as was shown by our previous study (Marcato et al. 2022b) as they were almost 12 kg heavier at transport age than 14 d old calves. Moreover, the evidence of higher insulin concentration in 28 d calves is also age-related (Bach et al., 2013; MacPherson et al., 2019), and it is especially linked to the on-farm plane of nutrition (e.g., high-intensity vs. low intensity colostrum diet, or milk replacer diet) (Hammon et al., 2000; Terré et al., 2009; Ontsouka et al., 2016). In the current study, older calves had likely a higher feed intake compared with younger calves (especially on the dairy farms, where in most cases they received ad libitum milk replacers), which stimulated the production of insulin. Thus, the measurement of both insulin and IGF-1 the day prior transport might be useful to assess level of nutrition and the developmental stage of the calves, which are important conditions to prepare calves for transport to the veal farm. Moreover, concentrations of insulin and IGF-1 measured the day before transport in 28 d group of calves were higher (Δ = 11 μU/ml and Δ = 88 ng/ml, respectively) than these concentrations at wk 2 at the veal farm in calves transported at 14 d of age. Calves of both transport groups were then at the same age. This suggests the negative carry-over effect of early life transport and/or the impact of receiving a

Table 7. Effects of parity of the dam and ease of birth on concentrations of insulin, IGF-1 and total cholesterol measured in plasma of calves one day before transport to the veal farm (Lsmeans ± SEM). Calves were transported at 14 or 28 d of age from 13 dairy farms to 8 veal farms

	Parity						Ease of birth			
Parameter	1	2	3	4–10	SEM[1]	*P*-value	Unassisted	Assisted	SEM	*P*-value
N. of calves	87	166	151	252			525	153		
Insulin (μU/ml)	82.9	70.3	63.9	50.8	7.9	0.13	59.7	77.4	6.0	0.65
IGF-1 (ng/ml)	175.9	166.4	172.1	167.2	11.2	0.66	169.1	171.7	10.6	0.68
Total cholesterol (mmol/l)	2.70	2.81	2.82	2.86	0.13	0.66	2.77	2.83	0.12	0.48

[1]SEM = pooled standard error of the mean.

Table 8. Regression coefficients (β) and statistical significance of relationships between different variables measured in the study assessing effects of transport age (14 or 28 d) on blood biomarkers, health and performance measures. The study included calves (n = 683) born on 13 dairy farms and transported to 8 veal farms

Response variable	Explanatory variable	Regression coefficient (β)	*P*-value
Carcass weight (kg)	Total cholesterol_one day before transport (mmol/l)	4.80	<0.01
Fecal score_wk 2 at the veal farm	Total cholesterol_wk 1 after birth (mmol/l)	0.28	0.09
Individual treatments with antibiotics at the veal farm	Total cholesterol_ wk 2 at the veal farm (mmol/l)	- 0.36	0.01
Carcass weight (kg)	Total cholesterol_ wk 2 at the veal farm (mmol/l)	7.74	<0.01
Fecal score_wk 2 at the veal farm	Total cholesterol_ wk 2 at the veal farm (mmol/l)	- 0.55	<0.01
Individual medical treatments other than antibiotics at the veal farm	Total cholesterol_ wk 2 at the veal farm (mmol/l)	- 0.45	<0.01
Carcass weight (kg)	IGF-1_one day before transport (ng/ml)	0.06	<0.01
Individual medical treatments other than antibiotics at the veal farm	IGF-1_one day before transport (ng/ml)	−0.005	<0.01
Individual treatments with antibiotics at the veal farm	IGF-1_wk 1 after birth (ng/ml)	−0.005	0.03
Carcass weight (kg)	IGF-1_ wk 1 after birth (ng/ml)	0.05	0.03
Individual treatments with antibiotics at the veal farm	IGF-1_ wk 2 at the veal farm (ng/ml)	−0.01	<0.01
Carcass weight (kg)	IGF-1_ wk 2 at the veal farm	0.14	<0.01
Fecal score_wk 2 at the veal farm	IGF-1_ wk 2 at the veal farm (ng/ml)	−0.004	0.02
Individual medical treatments other than antibiotics at the veal farm	IGF-1_ wk 2 at the veal farm (ng/ml)	−0.01	<0.01
Navel score_wk 2 at the veal farm	Insulin_one day before transport (μU/ml)	0.002	0.04
Fecal score_wk 2 at the veal farm	Insulin_ wk 1 after birth (μU/ml)	0.003	0.01
Carcass weight (kg)	Insulin_ wk 2 at the veal farm (μU/ml)	0.02	<0.01
Fecal score_wk 2 at the veal farm	Insulin_ wk 2 at the veal farm (μU/ml)	−0.002	0.09

different dietary scheme at the veal farm in comparison to the one at the dairy farm on these metabolites.

In the current study calves were directly transported from the dairy farms to veal farms and they endured a short transport duration. This means that effects of the main stressors for calves (the stop at a collection center and a long transport duration) were not considered in this research. A follow-up research would be relevant to investigate if the carry-over effect of early life transport would be the same under circumstances including bigger stressors. Additionally, another aspect that might need further investigation is the confounding effect of age and BW. Separating effects of transport age and BW is almost impossible, because BW is a function of age, thus results might be related to a higher BW of calves and not only transport age per se, because both of these factors may be associated with underlying characteristics related to the development of the calf.

Cholesterol is usually described as a biomarker of colostrum intake (Herosimczyk et al., 2013; Pisoni et al., 2023) and it plays an important role in GIT signaling and development, regulating plasma lipid concentration and absorption (Thurnhofer and Hauser, 1990), and promoting intestinal lactase activity (Goetz et al., 2021), which are crucial functions especially in neonatal calves (Ontsouka et al., 2016). Higher cholesterol concentrations are also associated with older calves (Goetz et al., 2021; Ferronato et al., 2022), which explain the higher level of cholesterol in 28 d calves the day before transport. Our results might also be due to the higher volume of milk replacer fed to older calves on the dairy farm, as reported by Renaud and Pardon (2022). Cholesterol has been shown to be a relevant biomarker of health in veal calves and a predictor of morbidity and early and late mortality. Calves with low concentrations of cholesterol upon arrival at the veal farm were at greater risk to die not only in the first 21 d (hazard ratio of 0.28 for every 1 mmol/l increase in cholesterol; Renaud et al., 2018), but also at 78 d post-transport (hazard ratio of 0.572 for every 1 mmol/l increase in cholesterol across the 78-d period; Goetz et al., 2021) compared with calves with higher levels. Since calves transported at 28 d of age had higher pre-transport cholesterol concentration compared with 14 d old calves, it might be assumed that they were more robust. Moreover, the higher concentration of IGF-1 and insulin in these animals the day before transport in combination with their higher body weight (Marcato et al., 2022b) are other metabolic traits, suggesting a better development of older animals, which might be more favorable for transport and for coping with the different rearing conditions at the veal farm.

Effects of breed

The results of the current study showed differences in blood IGF-1 and insulin concentrations between calf breeds, which can be attributed to the different metabolic types of breeds (Bellmann et al., 2004). Previous studies have investigated the genetic basis for GH, IGF-1, and insulin in both dairy and beef cattle (Stick et al., 1998; Snijders et al., 2001; Lucy et al., 2009). On the one hand, beef cattle breeds had more frequent GH pulses, which were associated with lower concentrations of both insulin and IGF-1 to promote lipolytic effect and direct nutrients to the muscles for protein synthesis (Bellmann et al., 2004). Thus, the genetic selection of beef cattle has been directed in favor of muscle development (Clinquart et al., 1995). On the other hand, in dairy breeds GH and high insulin concentration were positively related to high IGF-1 concentrations, which promoted the uptake of nutrients, especially glucose and acetate in the peripheral tissues, and the accumulation of fat depots (Bellmann et al., 2004). These findings are in line with the results of the current study, although there was a difference in IGF-1 and insulin response among different types of crossbred calves. In fact, crossbreds other than HF × BB had always the lowest amount of insulin and IGF-1. These differences are difficult to explain as, to our knowledge, there are no studies focusing on these specific crossbreds in calves. However, data of the same study (Marcato et al., 2022b) showed that crossbreds other than HF × BB had a higher likelihood of being treated with antibiotics and other medicines at the veal farm, suggesting a lower robustness compared with HF × BB calves. Thus, the lowest IGF-1 concentrations in blood of crossbreds other than HF × BB might point in the same direction since lower concentrations of IGF-1 in the current study were related with higher likelihood of a calf of being treated with both antibiotics and other medicines at the veal farm.

Differences in blood cholesterol among calves' breeds is not well documented and most of the work focuses on dairy cows (Barlowska et al., 2011; Alameen et al., 2014). Blood cholesterol can rise after ingestion of a higher volume of colostrum or milk replacer (Kühne et al., 2000), and it is positively associated with body weight and age (Ontsouka et al., 2016). In the current study crossbred calves were heavier than HF calves (e.g., $\Delta = 3.6$ kg more on average on the day before transport (Marcato et al., 2022b)), and this could explain the difference in cholesterol concentrations in their blood. Variability in milk/meat type genes within different breeds might also explain the difference in cholesterol concentrations measured in wk 2 post-transport in both age groups. Overall, crossbred calves in the current study comprehended a wide range of calves including, for example, Fleckvieh × HF, Black Angus × HF, Montbeliarde × HF. Thus, the wide variation of breeds used for the crossbreds could explain the deviating metabolic and performance responses of these animals in comparison to HF × BB calves.

Effects of sex

Sex-related hormones have an effect on body growth rate and GH secretions in cattle (Ardiyanti et al., 2009). Male cattle are usually characterized by a greater rate of body growth and GH and IGF-1 blood concentrations than female cattle (Plouzek and Trenkle, 1991; Gatford et al., 1998; Brandt et al., 2007). This is in contrast with our results, showing that, in the first wk after birth, heifers had a higher IGF-1 concentration in their blood compared with bull calves. This difference might be due to the nutrition received at the dairy farm (although we did not recorded information on the feeding scheme at calf level and thus it remains an hypothesis). In fact, in the first days after birth, some dairy farmers participating to the experiment were still evaluating whether or not to keep these heifers on the farm and thus they might have fed them a higher amount of milk replacers compared with bull calves, that were considered as surplus calves immediately.

Effects of parity of the dam and calving ease

Parity can influence the pattern of changes in metabolic hormones and metabolites before and after calving in dairy cows (Ferreira et al., 2021; Walter et al., 2022). Primiparous cows are in a different metabolic state compared with multiparous cows, because they require nutrients to sustain both their own growth and the development of their calf (Coffey et al., 2006). For this reason, in late gestation blood cholesterol concentrations are reported to be lower in primiparous cows compared with multiparous cows (Walter et al., 2022). Total cholesterol content in colostrum and milk after calving can also be negatively affected by parity (Strzalkowska et al., 2010). Thus, the tendency toward lower cholesterol level in both blood and milk of first parity cows might have led also to a lower cholesterol concentration in the blood of their calves compared with calves born from older cows. The same study (Marcato et al., 2022a,b) showed that calves born from first parity cows had a lower birth weight, a lower body weight at slaughter and a lower blood IgG concentration compared with calves born from higher parity cows. More research is needed to assess the impact of dam management (e.g., maternal nutrition during pregnancy and dry period) on characteristics of the offspring to optimize rearing of calves on the dairy and/or veal farm according to parity of the dam.

Calving ease is known to affect post-natal blood metabolites, including cholesterol, in calves (Barrier et at, 2013). Civelek et al. (2008) indicated that calves with a difficult birth (dystocia) had increased concentrations of cholesterol in their blood after birth. This is in contrast to the current study, which showed a tendency toward lower cholesterol in calves with an assisted birth in comparison with calves without assistance at birth. These results might be due to less vigorous calves after a longer delivery, resulting perhaps in a lower colostrum/ cholesterol intake. However, these observations were conducted one wk after birth and not directly in newborn calves. Another aspect to consider is the difference in definition of calving ease between the studies, which might explain the contrast. In fact, in the current paper, calving ease was defined as any assistance of the farmer during birth of calves, whereas most of the other studies mentioned dystocia and thus whether or not the cow encountered difficulty in giving birth. Overall, the current findings seem to be in line with other results of the same study (Marcato et al., 2022 a,b), showing that calving ease does not have huge effects on long-term health and performance of veal calves.

Relationships among analyzed parameters

Cholesterol has been recently used as a biomarker of health and performance in veal calves (Renaud et al., 2018; Goetz et al., 2021). Renaud et al. (2018) showed that calves that died in the first 21 d post-transport had lower cholesterol concentrations upon arrival at the veal farm compared with calves that survived (1.44 vs. 1.79 mmol/l). Goetz et al. (2021) similarly showed a lower post-transport cholesterol concentration in calves that died in the first 78 d at the veal farm compared with calves that survived (1.76 vs. 1.96 mmol/l). Moreover, Goetz et al. (2021) showed that calves treated with a combination of antibiotic and non-steroidal anti-inflammatory agents during the first 28 d post-transport had a lower cholesterol concentration in their blood upon arrival at the veal farm (1.93 vs. 2.02 mmol/l) compared with non-treated calves. In accordance to these studies, our results showed that lower cholesterol concentrations in wk 2 post-transport were associated with a higher likelihood of a calf of being treated with both antibiotics and medicines other than antibiotics at the veal farm. Moreover, calves showing signs of pasty and loose manure 2 wks post-transport had lower cholesterol concentrations in the first wk after birth and in wk 2 post-transport. Additionally, higher cholesterol was associated with a higher carcass weight of calves at slaughter. Collectively, these results reinforce the idea that cholesterol might be a valuable biomarker of morbidity and performance in veal calves, and that high colostrum intake after birth is extremely important for a good robustness of calves.

Several studies (Ontsouka et al., 2016; MacPherson et al., 2019) explained the relationships between IGF-1, insulin and growth and development of calves in the first wks at the dairy farm. The current study is the first one to investigate long-term relationships between IGF-1 and insulin and measures of performance in veal calves. Interestingly our results showed that, besides cholesterol, IGF-1 might be useful as a biomarker of health as higher IGF-1 concentrations were linked to a lower likelihood of a calf of being treated with both antibiotics and medicines other than antibiotics at the veal farm, and with an improved fecal score. Further research is necessary to understand the results on insulin. Contrary to our expectations, a higher insulin concentration was associated with a worse fecal and navel score of calves. At the moment, no good explanation is found for these relationships and it remains unclear whether or not insulin might be a useful biomarker.

CONCLUSIONS

The current study supports the concept that 28-d old calves were more developed and robust compared with 14-d old calves, thus transport at an older age might be beneficial. Moreover, both blood cholesterol and IGF-1 concentrations seemed to be valuable biomarkers of health and performance during the rearing period, which could be used for practical implementations, such as profiling of calves in different risk categories upon arrival at the veal farm.

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