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Residual feed intake is related with metabolic and inflammatory response during the pre-weaning period in Italian Simmental calves

Giulia Ferronato,[1] Luca Cattaneo,[2]* Annalisa Amato,[3] Andrea Minuti,[2] Juan J. Loor,[4] Erminio Trevisi,[2] Carmelo Cavallo,[3] George Attard,[5] Luigi Liotta,[3] and Vincenzo Lopreiato[3]

[1] Department of Civil Engineering, Architecture, Environment, Land Planning and Mathematics (DICATAM), Università degli Studi di Brescia, 25121 Brescia, Italy.
[2] Department of Animal Science, Food and Nutrition (DIANA), Faculty of Agricultural, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy
[3] Department of Veterinary Sciences, Università di Messina, 98168 Messina, Italy
[4] Mammalian NutriPhysioGenomics, Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, 61801 Urbana, IL, USA
[5] Department of Rural Sciences and Food Systems, University of Malta, 2080 Msida, Malta

ABSTRACT

Residual Feed Intake (**RFI**) is defined as the difference between measured and predicted intake. Understanding its biological regulators could benefit farm profit margins. The most-efficient animals (**M-Eff**) have observed intake smaller than predicted resulting in negative RFI, whereas the least-efficient (**L-Eff**) animals have positive RFI. Hence, this observational study aimed at retrospectively comparing the blood immunometabolic profile in calves with divergent RFI during the preweaning period. Twenty-two Italian Simmental calves were monitored from birth through 60 d of age. Calves received 3 L of colostrum from their respective dams. From 2 to 53 d of age, calves were fed a milk replacer twice daily, whereas from 54 to 60 d (i.e., weaning) calves were stepped down to only one meal in the morning. Calves had *ad libitum* access to concentrate and intakes were recorded daily. The measurement of BW and blood samples were performed at 0, 1, 7, 14, 21, 28, 35, 45, 54, and 60 d of age. Calves were ranked and categorized as M-Eff or L-Eff according to the median RFI value. Median RFI was –0.06 and 0.04 kg of DMI/d for M-Eff and L-Eff, respectively. No evidence for group differences was noted for colostrum and plasma IgG concentrations. Although growth rate was not different, as expected, [0.67 kg/d (95% CI = 0.57 – 0.76) for both L-Eff and M-Eff) throughout the entire pre-weaning period (0–60 d), starter intake was greater in L-Eff compared with M-Eff calves (+36%). Overall, M-Eff calves had a greater gain-to-feed ratio compared with L-Eff calves (+16%). Plasma ceruloplasmin, myeloperoxidase, and reactive oxygen metabolites concentrations were greater in L-Eff compared with M-Eff calves. Compared with L-Eff, M-Eff calves had an overall greater plasma concentration of globulin, and γ-glutamyl transferase (indicating a better colostrum uptake) and Zn at 1 d. Retinol and urea were overall greater in L-Eff. The improved efficiency in nutrient utilization observed in M-Eff was paired with a lower grade of oxidative stress and systemic inflammation. L-Eff may have had greater energy expenditure to support the activation of the immune system.
Keywords: Residual feed intake, Growth performance, Metabolic profile, Simmental calves

INTRODUCTION

Residual feed intake (**RFI**) is commonly used to evaluate feed efficiency because of its independence from other productive parameters (Koch et al., 1963; Veerkamp et al., 1995; Dekkers et al., 2010). The concept of RFI is based on the difference between the expected DMI for a given production trait and the observed DMI (Herd and Arthur, 2009). Being independent of the production traits of interest, RFI accounts for variations in the energy requirements for basic metabolic processes. Although it can have a genetic basis, several factors can contribute to the large individual variability observed among the animals. The physiological basis of RFI falls into processes that contribute to feed intake, feed digestion, metabolism, physical activity, and thermoregulation (Herd and Arthur, 2009). Animals with positive RFI are considered inefficient while animals with negative RFI are considered efficient (Martin et al., 2021). Animals with negative RFI have usually reduced DMI without compromising growth performance, resulting in lower feed costs (Elolimy et al., 2018a). Considering the importance of this index, the correlation of RFI with specific phenotypes has been investigated to under-

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*Corresponding author: Luca Cattaneo, luca.cattaneo@unicatt.it

stand its genetic heritability and assess the possibility of including an RFI index in genetic selection programs (Hoque and Suzuki, 2009; Freetly et al., 2020).

To date, however, most of the studies available in the literature are focused on mature dairy cows from early to mid-lactation (Potts et al., 2017; Elolimy et al., 2022; Flay et al., 2019),young heifers and beef (Nkrumah et al., 2007; Kelly et al., 2010; McDonnell et al., 2016). These studies investigated the possible relationships between RFI and feeding behavior, growth performance, metabolic profile, or methane emissions. To our knowledge, only one study investigated the effect of divergent RFI in dairy calves, focusing on hindgut microbiome and metabolome (Elolimy et al., 2020), but no studies aimed at detecting the relationship between RFI, immunometabolic profile, and performance in neonatal and preweaning calves. Moreover, differences in RFI measured in early life can persist into the first lactation, despite being reduced (Macdonald et al., 2014). Therefore, this study aimed at comparing the immunometabolism of Italian Simmental calves with divergent feed efficiency, to discover possible differences in productive traits associated with metabolic and inflammation. Italian Simmental is a dual-purpose breed widely farmed in marginal and mountainous areas of Italy due to its ability to adapt to suboptimal farming and breeding conditions. The most-efficient (**M-Eff**) or least-efficient (**L-Eff**) calves, retrospectively classified using RFI, were compared during the preweaning period.

MATERIALS AND METHODS

The experimental protocol was approved by Ethical Animal Care and Use Committee of the Magna Graecia University of Catanzaro (Protocol No. 271/2017). The research was performed in a commercial dairy farm and the farmer consented to and was compliant with the purposes and methods of the research.

Animal Management

Twenty-two Italian Simmental calves (heifers, n = 14; bulls, n = 8) were monitored in this observational study. Immediately after birth, newborn calves were separated from their dams. All calves were cleaned and had the navel disinfected with oxytetracycline hydrochloride (Neo Spray Caf Aerosol; Gellini S.p.a., Aprilia, Italy). Calves were weighed and fed 3 L of their dam's colostrum by nipple bottle (within 4 h from birth). If voluntary colostrum intake did not reach the 3 L required, calves were fed with an esophageal feeder (Speedy Drencher XL, Agri-Zoo San Marino srl, Domagnano, San Marino). Afterward, animals received 4 feedings of their dam's transition milk (3 L at each feeding) over 2 d (i.e., at 12, 24, 36, and 48 h after birth). From 3 to 53 d of age, all calves received twice daily (08:00 and 16:00) 2 L of milk replacer (4 L/d) at a rate of 200 g/L of water (**MR**; 21.5% protein and 18% fat; Elvor, Maen Roch, France). From 54 to 60 d of age, calves received only one meal (2 L/d) in the morning and then weaned at 60 d. Refusals of MR were recorded at each meal.

From 4 d, calf starter (DM: 87.87%, starch: 24.38%, CP: 17.81%, fat: 2.47%, NDF: 33.22%, ADF: 23.18%, ADL: 6.85%, and ash: 8.77%, all referred to DM basis; Vitamin A: 13,000 IU/kg, D3: 1,300 IU/kg, E: 35 mg/kg, B1: 10 mg/kg, B2: 5.5 mg/kg, B6: 4 mg/kg, B12: 0.08 mg/kg, choline chloride: 67.5 mg/kg; Dietovit Excellence, SIVAM Spa, Casalpusterlengo, Lodi, Italy) was offered *ad libitum* once every morning after MR feeding. Fed and refused starter were recorded daily. All calves were monitored for fecal score using a 1 to 5 scale (score 1 being normal and 5 being watery). Moreover, no vaccinations were performed during the study period.

Sampling and Analysis

Body weight (BW) and heart girth (HG) were measured at birth, then at 7, 14, 21, 28, 35, 45, 54, and 60 d of age, before the morning MR meal and solid feeds distribution. Calves were weighed using a calibrated calves scale (Calf scale 1–2–3 Animal scales, BOSCHE Weighing Systems, Damme, Germany) and HG was measured as the minimal circumference around the body immediately behind the front shoulder. Average daily gain (**ADG**) was calculated as partial (between 2 subsequent measurements of BW) and total (between each measurement and birth BW).

Blood samples were collected by jugular venipuncture into heparinized tubes (BD Vacutainer; BD and Co., Franklin Lakes, NJ) at d 1 (24 ± 2 h from first colostrum intake) and before the morning milk meal at 7, 14, 21, 28, 35, 45, 54, and 60 d of age. Tubes were immediately cooled and, once arrived in the laboratory, they were centrifuged at 3500 × g for 15 min at 4°C. Plasma was harvested and stored at –20°C for further analyses. Plasma biomarkers, except for vitamins, were analyzed at 37°C by a clinical automated analyzer (ILAB 650; Instrumental Laboratory – Werfen, Bedford, MA), as described in Lopreiato et al. (2021) and Morittu et al. (2021). Briefly, metabolites assessed were total protein, globulin, γ-glutamyl transferase (**GGT**), glucose, BHB, nonesterified fatty acids (**NEFA**), fructosamine, creatinine, urea, albumin, paraoxonase, cholesterol, alkaline phosphatase, aspartate-aminotransferase (**AST**), bilirubin, haptoglobin, ceruloplasmin, Zn, myeloperoxi-

dase, total reactive oxygen metabolites (**ROM**), ferric reducing antioxidant power (**FRAP**), Na, Ca, Mg, K, and P. Plasma retinol and tocopherol were extracted with hexane and analyzed by reverse-phase HPLC using Spherisorb ODS-2, 3 μm, in a 150 × 4.6 mm column (Alltech, Deerfield, IL), with a UV detector set at 325 (for retinol) and 290 (for tocopherol) and 80:20 methanol: tetrahydrofuran as the mobile phase.

Colostrum and plasma IgG (at 24 h after first colostrum intake) were assessed by a commercial RID assay (Bovine IgG ID-Ring test, IDBiotech, ImmunoDiffusion Biotechnologies SARL, Issoire, France) according to the manufacturer's instructions. In addition, IgG data from both colostrum and calf plasma were used to calculate the apparent efficiency of IgG absorption (AEA) as [plasma IgG (g/L) x (kg of birth BW x 0.089) / IgG intake (g)] x 100 (Quigley et al., 1998).

Residual feed intake calculation

Predicted DMI was calculated using the PROC MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). An RFI coefficient was calculated for each calf throughout the entire preweaning period (from birth to 60 d of age), and assumed to represent the residuals from a multiple regression model regressing the combined DMI of starter and milk replacer on ADG and mid-test metabolic BW (MMW, BW at 28 d of age $^{0.75}$): predicted DMI = β0 + (β1 × ADG) + (β2 × MMW) + E, where β0 is the y-intercept, β1 is the partial regression coefficient of ADG, β2 is the partial regression coefficient of MMW, and E is the error term. The RFI coefficient (kg DMI/d) for each calf was then calculated as the difference between actual and predicted DMI (Elolimy et al., 2020).

All calves were retrospectively ranked by RFI, and divided into 2 groups based on the median RFI value: (1) L-Eff group with unfavorable (i.e., more positive) RFI coefficients (n = 11; 7 heifers and 4 bulls), (2) M-Eff group with a desirable (i.e., more negative) RFI (n = 11; 7 heifers and 4 bulls).

Statistical Analysis

Before analysis, the normality of distributions was checked (UNIVARIATE procedure of SAS), and non-normally distributed variables were log-transformed. Data with a single measure were subjected to ANOVA (GLM procedure of SAS). Data with multiple observations were analyzed with repeated measures mixed models (GLIMMIX procedure of SAS) with the compound symmetry covariance structure, considering the day as the repeated measure. The models included the fixed effects of the RFI group (RFI; L-Eff vs. M-Eff), days of age (Day), their interaction (RFI × Day), and the random effect of the calf nested within the RFI group. Data are presented in tables and figures as LSM ± 95% CI. Pairwise comparisons were carried out using the LSD test of SAS. Differences were considered significant when $P \leq 0.05$.

RESULTS

The distribution and overall RFI coefficients for L-Eff and M-Eff are shown in Figure 1. The median RFI coefficient for M-Eff calves was –0.06 kg DMI/d (ranging from –0.21 to –0.002 kg DMI/d), whereas for L-Eff calves was 0.04 (ranging from 0.02 to 0.31 kg DMI/d). Calves enrolled in the present study were not affected by any severe health disorder and no antibiotic treatment was performed during the study period.

Passive Immunity, Intake and Growth

The IgG concentration in the colostrum delivered at birth was not significantly different between groups ($P = 0.59$; Table 1). Plasma IgG concentration and AEA were not associated with RFI ($P = 0.16$ and $P = 0.49$, respectively).

There was no difference between groups in MR intake (Table 2), but starter intake was greater in L-Eff compared with M-Eff (RFI, $P < 0.03$; Figure 2). Similarly, ME intake from MR did not differ, whereas starter and total ME intakes were greater in L-Eff (RFI, $P < 0.03$ and 0.02, respectively). Growth (i.e., both BW and HG) and ADG were not associated with RFI or the interaction RFIxDay (Table 2). However, the gain-to-feed ratio was overall lower in L-Eff compared with M-Eff [0.61 (95% CI: 0.55–0.67) vs. 0.71 (95% CI: 0.65–0.77); RFI: $P = 0.03$; Table 2].

Plasma biomarkers

The least squares means (**LSM**) of plasma biomarkers throughout the study in M-Eff and L-Eff are reported in Table 3. Most of the plasma biomarkers investigated (except for haptoglobin, FRAP, and Na) changed with time ($P < 0.01$; Table 3). Glucose concentration was greater in L-Eff calves compared with M-Eff the day after birth [8.25 (95% CI: 7.74–8.76) vs. 6.89 (95% CI: 6.40–7.38) mmol/L, respectively; $P < 0.01$; Figure 3A]. Compared with M-Eff, L-Eff group had overall greater plasma urea [3.45 (95% CI: 3.04–3.87) vs. 2.79 (95% CI: 2.37–3.21) mmol/L, respectively; RFI, $P = 0.04$].

Plasma GGT was greater in M-Eff at 1 d compared with L-Eff [1948.7 (95% CI: 1752.3–2145.1) vs. 1277.5 (95% CI: 1072.2–1485.7) U/L, respectively; RFIxDay, $P = 0.01$; Figure 3D]. Overall, L-Eff group had greater

Table 1. LSM and 95% CI for colostrum and plasma IgG, and apparent efficiency of colostrum IgG absorption in the least-efficient (L-Eff, n = 11) or the most-efficient (M-Eff, n = 11) Italian Simmental calves

	Group				
Items	L-EFF	95% CI	M-EFF	95% CI	*P*-value
IgG, g/L					
Colostrum	101.7	72.1–131.2	112.9	86.5–139.4	0.59
Plasma of calves	16.9	12.4–21.4	22.2	16.9–27.6	0.16
AEA[1], %	28.0	21.6–34.4	31.3	24.4–37.7	0.49

[1]Apparent efficiency of colostrum IgG absorption.

ROM concentration compared with M-Eff [8.89 (95% CI: 7.72–10.07) vs. 6.62 (95% CI: 5.45–7.79) mg of H_2O_2/dL, respectively; RFI, $P < 0.01$; Table 3]. L-Eff group had also greater myeloperoxidase concentration [321.7 (95% CI: 279.3–364.1) vs. 216.5 (95% CI: 174.1–258.9) U/L; RFI, $P < 0.01$; Table 3]. Overall, plasma retinol was greater in L-Eff compared with M-Eff calves [21.35 (95% CI: 19.07–23.65) vs. 16.88 (95% CI: 14.56–19.19) μg/mL, respectively; RFI, $P = 0.01$; Table 3]. Ceruloplasmin was greater in L-Eff compared with M-Eff [1.75 (95% CI: 1.50–1.99) vs. 1.14 (95% CI: 0.89–1.39) μmol/L, respectively; RFI, $P < 0.01$; Table 3].

Among minerals, Zn was overall greater in M-Eff compared with L-Eff [28.6 (95% CI: 23.70–33.51) vs. 21.1 (95% CI: 16.16–25.96) μmol/L; RFI, $P = 0.04$; Table 3]. Differences between groups reduced after 45 d of age (RFI × Day, $P = 0.04$; Figure 4). No evidence for group differences were detected for the other plasma biomarkers investigated.

DISCUSSION

Feed efficiency in early life is a relevant aspect of profitable farm systems due to the high costs of this phase (Connor et al., 2012) and its influence on future performance. The knowledge of genetic and metabolic basis of divergent feed efficiency could enhance management and performances in this phase. In this context, RFI is a widely used index because, being independent of BW and growth, it allows to investigate other factors contributing to efficiency. Variations in plasma metabolites have been investigated to detect physiological biomarkers correlated with RFI values

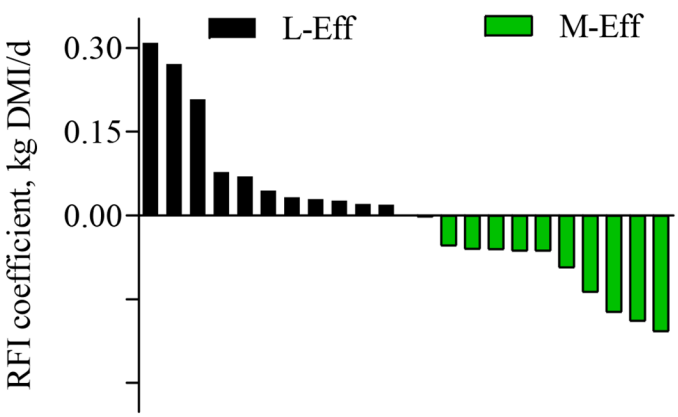

Figure 1. Residual feed intake (RFI) for each calf in the least-efficient (L-Eff, n = 11) or most-efficient (M-Eff, n = 11) Italian Simmental calves during the preweaning period.

Table 2. LSM and 95% CI of MR, starter, and total feed intake (in terms of DM and ME), BW, hearth girth, ADG, and gain-to-feed ratio in the least-efficient (L-Eff, n = 11) or the most-efficient (M-Eff, n = 11) Italian Simmental calves

	Group				*P*-value[1]		
Item	L-Eff	95% CI	M-Eff	95% CI	RFI	Day	RFI × Day
BW, kg	63.27	58.78–67.76	63.80	59.30–68.29	0.87	<0.01	0.96
Heart girth, cm	85.15	83.15–87.15	86.02	84.02–88.02	0.55	<0.01	0.86
ADG, kg/d	0.67	0.57–0.76	0.67	0.57–0.76	0.98	<0.01	0.36
MR intake, kg of DM/d	0.70	0.68–0.71	0.70	0.69–0.71	0.64	<0.01	0.37
Starter intake, kg of DM/d	0.47[a]	0.36–0.58	0.29[b]	0.17–0.39	0.03	<0.01	0.94
Total DMI, kg/d	1.16[a]	0.67–1.65	0.98[b]	0.49–1.47	0.02	<0.01	0.65
ME intake from MR, Mcal/d	3.18	3.12–3.23	3.19	3.14–3.24	0.64	<0.01	0.37
ME intake from starter, Mcal/d	1.16[a]	0.88–1.42	0.70[b]	0.43–0.97	0.03	<0.01	0.94
Total ME intake, Mcal/d	4.33[a]	4.09–4.59	3.88[b]	3.62–4.13	0.02	<0.01	0.61
Gain-to-feed ratio[2]	0.61[b]	0.55–0.67	0.71[a]	0.65–0.77	0.03	<0.01	0.85

[1]*P*-values of the main effects: Residual Feed Intake (RFI), days of age (Day), and their interaction (RFI × Day).

[2]Ratio between BW gain (kg) and DMI of MR and starter (kg).

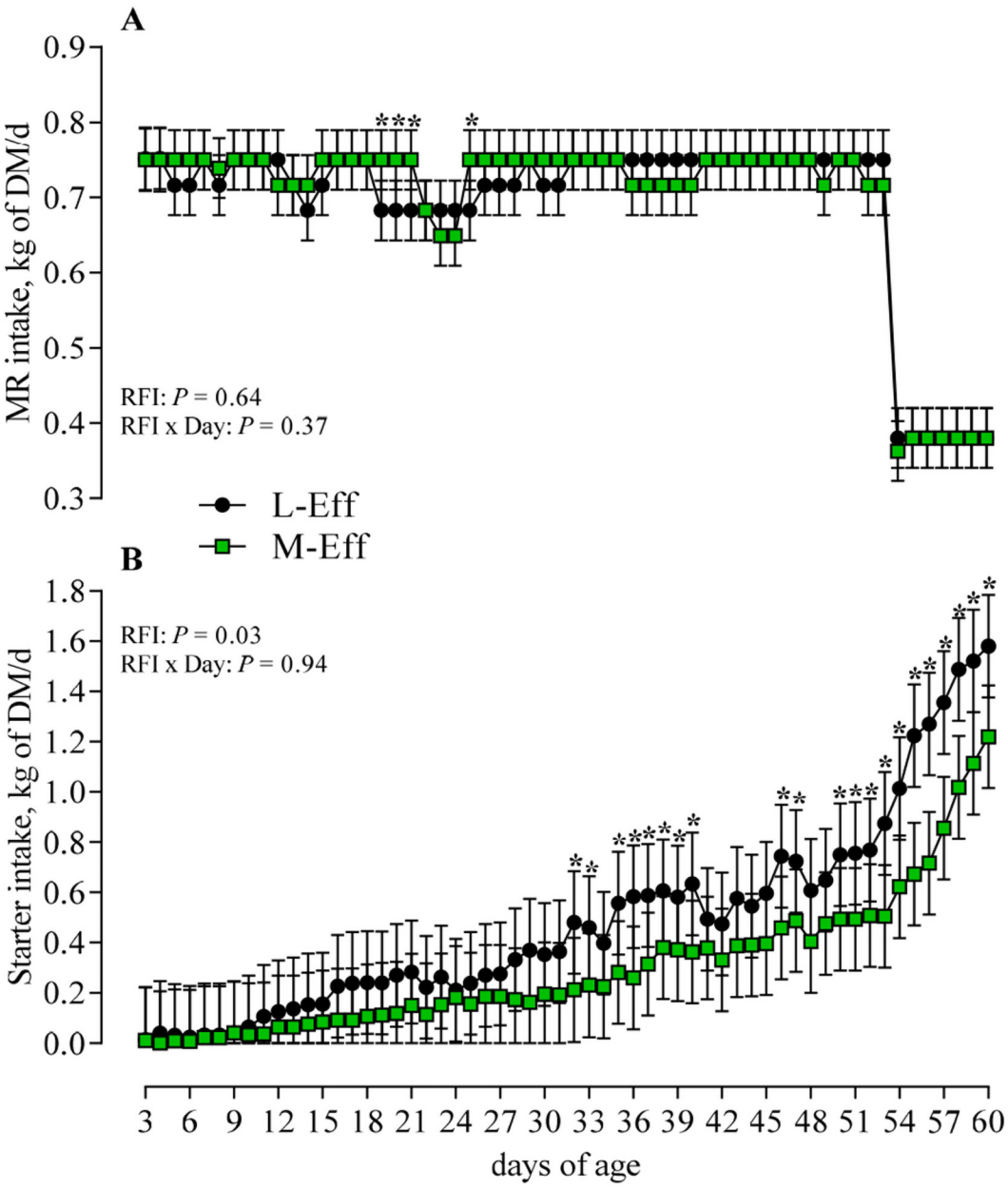

Figure 2. Milk replacer (MR; A) and calf starter intakes (B) in the first 60 d of age in Italian Simmental calves categorized as the least-efficient (L-Eff, n = 11) or most-efficient (M-Eff, n = 11) according to their residual feed intake (RFI). Data are presented as LSM ± 95% CI. Asterisks indicate significant differences between groups at each time point ($P \leq 0.05$).

(Kelly et al., 2010; Weikard et al., 2010; Gonaro et al., 2014), but studies focusing on pre-weaning calves are lacking. The present study, carried out in a commercial farm, retrospectively focused on differences related to the inflammometabolic profile of Italian Simmental calves, since no study has been carried out yet during the pre-weaning period in this breed widely farmed for milk production in Europe.

The L-Eff calves in the present study had an overall greater DMI (+18%) that, paired with the similar growth performance (as expected when animals are classified based on RFI), resulted in a worse feed conversion efficiency compared with the M-Eff. Although responses might differ between Holstein and Italian Simmental, our results are in line with those by Elolimy et al. (2020) in Holstein calves. Of interest, milk replacer intake was similar but there was a large difference in starter intake (+62%), leading also to greater plasma retinol concentration throughout the entire period in L-Eff calves. The greater plasma retinol was

likely because of the starter intake difference, which, together with milk replacer, is the only dietary source of retinol.

In the present study, L-Eff calves had greater plasma urea concentration than M-Eff, in line with previous results in beef bulls and heifers (Fitzsimons et al., 2013; Foroutan et al., 2020). Plasma urea in calves is related to ruminal fermentable protein intake and rumen protein degradation, because dietary protein is degraded into ammonia by rumen microbes, which is absorbed by the epithelium and converted to urea (Welboren et al., 2019). Overall, the greater urea concentrations in L-Eff calves could reflect both the greater starter intake and an increase of muscle proteolysis upon the immune system activation (Carroll et al., 2021). Muscle proteolysis was previously suggested as provider of AA to support gluconeogenesis upon the activation of immune system (Wannemacher et al., 1980; Horst et al., 2021). Thus, the carbon skeletons from the deamination of AA are utilized for glucose synthesis, whereas the amino groups enter ureagenesis. Based on this, in animal models with induced immune activation, blood urea concentration consistently increases (especially in monogastrics). Thus, 2 factors likely determined the greater plasma urea concentration in L-Eff calves: the increased muscle proteolysis induced by a greater degree of immune and inflammatory responses (i.e., greater ceruloplasmin, myeloperoxidase, ROM, and lower Zn concentrations) and the greater starter intake, since the growth rates of L- and M-Eff calves were similar.

Moreover, the M-Eff group tended to have greater plasma concentration of creatinine. Greater blood creatinine is usually observed in animals with low RFI (Lawrence et al., 2011; Paula et al., 2013). Creatinine concentration is influenced by muscle creatinine concentration and muscle mass changes, which, in animals

Table 3. LSM and 95% CI of plasma biomarkers during the pre-weaning period (0–60 d) in least-efficient (L-EFF, n = 11) or most-efficient (M-EFF, n = 11) Italian Simmental calves

	Group				*P*-value[2]		
Biomarker[1]	L-Eff	95% CI	M-Eff	95% CI	RFI	Day	RFI × Day
Transfer of passive immunity							
Total protein, g/L	60.55	57.66–63.43	63.87	60.98–66.76	0.12	<0.01	0.66
Globulin, g/L	29.61	26.97–32.27	33.05	30.40–35.71	0.08	<0.01	0.61
GGT, U/L	225.25	125.2–325.3	345.34	244.9–445.7	0.11	<0.01	0.01
Energy metabolism							
Glucose, mmol/L	5.54	5.31–5.77	5.53	5.30–5.76	0.94	<0.01	<0.01
BHB, mmol/L	0.16	0.14–0.18	0.14	0.12–0.17	0.34	<0.01	0.69
NEFA, mmol/L	0.23	0.21–0.26	0.25	0.22–0.28	0.37	<0.01	0.29
Fructosamine, µmol/L	299.97	289.1–310.8	308.78	297.9–319.6	0.27	<0.01	0.87
Creatinine, mmol/L	102.22	96.71–107.7	109.36	103.8–114.9	0.08	<0.01	0.22
Urea, mmol/L	3.45	3.04–3.87	2.79	2.37–3.21	0.04	<0.01	0.58
Retinol, µg/mL	21.35	19.07–23.65	16.88	14.56–19.19	0.01	<0.01	0.12
Liver functionality							
Albumin, g/L	30.93	30.10–31.78	30.81	29.98–31.65	0.84	<0.01	0.61
Paraoxonase, U/mL	59.15	52.23–66.07	55.45	48.52–62.39	0.47	<0.01	0.37
Cholesterol, mmol/L	2.70	2.48–2.93	2.72	2.50–2.94	0.93	<0.01	0.33
Alkaline phosphatase, U/L	298.79	249.2–348.4	340.15	290.4–389.9	0.26	<0.01	0.25
AST, U/L	82.44	51.9–112.9	55.41	24.89–85.92	0.23	<0.01	0.19
Bilirubin, µmol/L	4.93	4.21–5.67	5.47	4.73–6.20	0.32	<0.01	0.77
Inflammatory response							
Haptoglobin, g/L	0.31	0.27–0.36	0.28	0.24–0.33	0.39	0.09	0.40
Ceruloplasmin, µmol/L	1.75	1.50–1.99	1.14	0.89–1.39	<0.01	<0.01	0.19
Zn, µmol/L	21.06	16.16–25.96	28.61	23.70–33.51	0.04	<0.01	0.04
Myeloperoxidase, U/L	321.69	279.3–364.1	216.46	174.1–258.9	<0.01	<0.01	0.10
Oxidative stress and antioxidant status							
ROM, mg of H_2O_2/dL	8.89	7.72–10.07	6.62	5.45–7.79	0.01	<0.01	0.09
FRAP, µmol/L	192.17	171.1–213.2	206.93	185.7–228.1	0.35	0.51	0.41
Tocopherol, µg/mL	2.25	1.85–2.64	2.31	1.91–2.71	0.82	<0.01	0.84
Minerals							
Na, mmol/L	144.4	143.6–145.2	145.4	144.5–146.2	0.14	0.51	0.84
Ca, mmol/L	2.59	2.54–2.66	2.62	2.56–2.68	0.56	<0.01	0.81
Mg, mmol/L	0.90	0.88–0.93	0.89	0.87–0.92	0.58	<0.01	0.41
K, mmol/L	5.17	5.03–5.31	5.05	4.90–5.20	0.27	<0.01	0.03
P, mmol/L	3.29	3.15–3.43	3.24	3.10–3.37	0.55	<0.01	0.31

[1]NEFA = nonesterified fatty acids; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; ROM = reactive oxygen metabolites; FRAP = ferric-reducing ability of plasma.

[2]*P*-values of the main effects: Residual Feed Intake (RFI), days of age (Day), and their interaction (RFI × Day).

with normal kidney function, directly affects the plasma and urine concentrations (Megahed et al., 2019). Considering the similar growth observed in the 2 divergent groups, it may be hypothesized a different body composition, with M-Eff having lower fat deposition and greater muscle mass, as a consequence of increased protein anabolism (Uemura et al., 2014).

Among the other analytes related to protein metabolism, globulin showed a greater concentration in M-Eff calves, which significantly decreased over time. Within globulins class, there are the positive acute phase proteins mainly synthesized by the liver (Eckersall, 2008), being markers of inflammatory status (Bionaz et al., 2007; Bertoni et al., 2008), and Ig. Circulating globulins in the first days of life completely depends on passive transfer of Ig, that were only numerically greater in the plasma of M-Eff calves. Although no significant

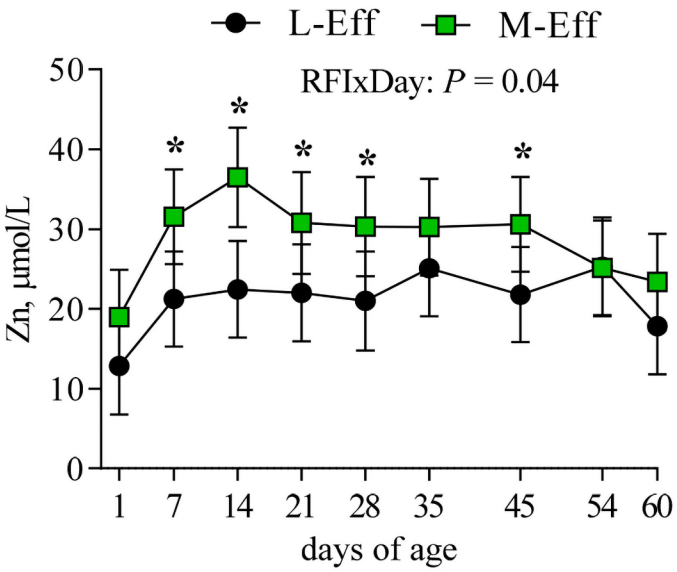

Figure 4. Plasma concentrations of Zn from birth to 60 d of age (weaning) in Italian Simmental calves categorized as the least-efficient (L-Eff, n = 11) or most-efficient (M-Eff, n = 11) according to their residual feed intake (RFI). Data are presented as LSM ± 95% CI. Asterisks indicate significant differences between groups at each time point ($P \leq 0.05$).

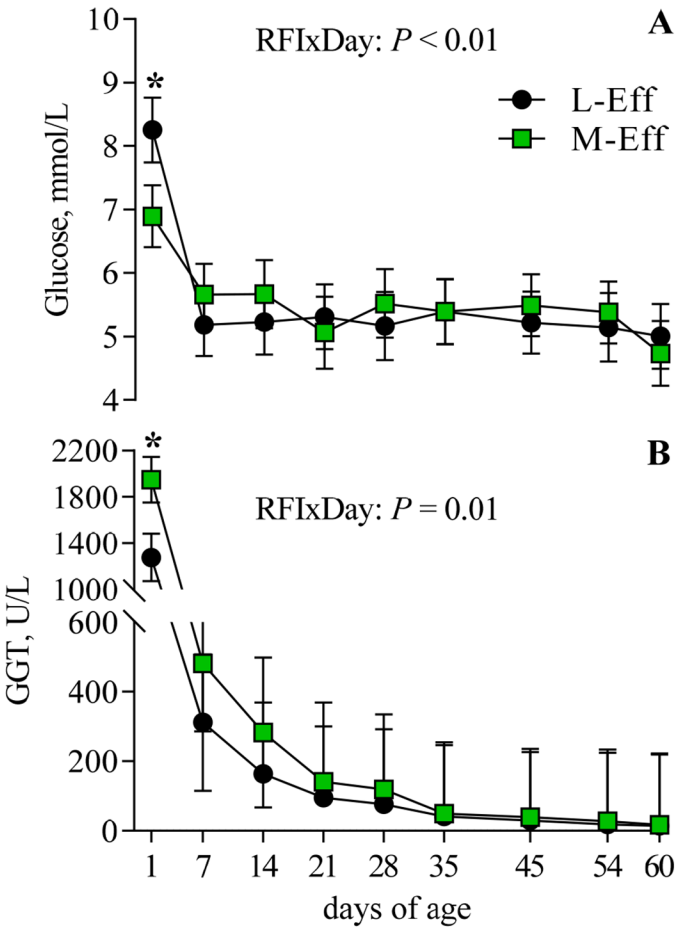

Figure 3. Plasma concentrations of glucose (A) and γ-glutamyl transferase (GGT; B) from birth to 60 d of age (weaning) in Italian Simmental calves categorized as the least-efficient (L-Eff, n = 11) or most-efficient (M-Eff, n = 11) according to their residual feed intake (RFI). Data are presented as LSM ± 95% CI. Asterisks indicate significant differences between groups at each time point ($P \leq 0.05$).

difference was observed for blood IgG concentration and AEA at 24 h, also the differences in plasma GGT observed 24 h after colostrum intake could suggest a better Ig absorption ability in M-Eff calves. The measurement of serum GGT concentrations has been considered a potential marker for the assessment of passive immunity transfer or at least for IgG absorption efficiency in neonatal calves (Pisoni et al., 2023), since GGT is absorbed in the small intestine of the calf via the same nonselective passage that is used by IgG (Parish et al., 1997). We cannot fully confirm our hypothesis and studies with a larger number of subjects are needed. Indeed, the small sample size represents a limitation of the present study since we were not able to detect as significant differences between groups that were large and could have been biologically meaningful (i.e., plasma IgG and AEA). Nevertheless, despite the lack of significance in the direct markers of colostrum absorption, the metabolic profile might indicate that, in the present study, one of the driving factors of the divergent RFI might be the transfer of passive immunity. Similar results have been recently obtained in terms of ADG by Sutter et al. (2023).

Interestingly, another factor contributing to the differences in RFI observed herein was the inflammatory and immune status. Calves with high RFI (L-Eff) had greater activity of ceruloplasmin and myeloperoxidase, and concentration of ROM, coupled with lower Zn concentration. Ceruloplasmin is one of the main

positive acute phase proteins, which increases in case of inflammation. Consistently, Zn is involved in gut barrier integrity maintenance and immune response, and it modulates oxidative stress. Zn plasma concentration decreases in inflammatory conditions (Rink and Kirchner, 2000; Trevisi et al., 2015). Therefore, L-Eff calves might have experienced a more severe degree of systemic inflammation in a critical phase of their life. Moreover, ROM and myeloperoxidase, which are biomarkers related to oxidative stress, had greater concentrations in L-Eff. These greater values in L-Eff may reflect a more stressful condition associated with an altered inflammatory status (Lykkesfeldt and Svendsen, 2007), in agreement with Zn concentration and ceruloplasmin activity.

CONCLUSIONS

Improving feed efficiency to achieve a better nutrient utilization is an important goal of modern livestock production systems. The use of RFI is a useful way to identify the most efficient animals on the farm and maximize profit margins. To our knowledge, no previous studies were carried out to investigate the association between RFI and immunometabolism in dairy calves. Our study showed that, despite consuming less starter than L-Eff, M-Eff calves achieved similar growth. This improved efficiency in nutrient utilization was paired with a lower grade of oxidative stress and systemic inflammation. In fact, L-Eff likely had greater energy expenditure to support the activation of the immune system. However, further investigations on future animal performance up to the first lactation and involving a greater number of animals are needed to better comprehend the inclusion of RFI into selection indices.

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ORCIDS

Giulia Ferronato https://orcid.org/0000-0001-9153-8540
Luca Cattaneo https://orcid.org/0000-0001-6027-7536
Annalisa Amato https://orcid.org/0009-0009-0591-4366
Andrea Minuti https://orcid.org/0000-0002-0617-6571
Juan J. Loor https://orcid.org/0000-0003-1586-4365
Erminio Trevisi https://orcid.org/0000-0003-1644-1911
Luigi Liotta https://orcid.org/0000-0002-3242-1817
Vincenzo Lopreiato https://orcid.org/0000-0001-6965-7340