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Changes in plasma UCP-1, leptin, lipids, and pro-inflammatory interleukins in calves from birth to weaning

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A R T I C L E I N F O

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A B S T R A C T

The neonatal period is crucial for calf development, particularly for immune acquisition through colostrum intake, this study aimed to assess the energy metabolism and inflammatory response. Ten Italian Simmental calves were monitored from birth to 60 days of age, with blood samples taken at birth (0 d) to weaning. Plasma concentrations of UCP-1, leptin, TNF-α, IL-1β, IL-6, lipids, triglycerides, and total cholesterol were measured.

Results showed significant dynamic changes ($P < 0.05$) in all parameters and showing an increasing trend from birth to the 60 d of age in investigated calves. The plasma leptin levels were positively correlated with the concentration of UCP-1 ($r = 0.37$, $P = 0.0003$), total lipids ($r = 0.47$, $P < 0.0001$), triglycerides ($r = 0.53$, P < 0.0001), total cholesterol ($r = 0.38$, $P = 0.0002$), and negatively correlated with TNF-α ($r = -0.24$, P 0.02). UCP-1 was positively correlated with the levels of total lipids ($r = 0.31$, $P = 0.003$), triglycerides ($r = 0.29$, $P = 0.005$), and IL-1β (r = 0.29, P = 0.005) in calves throughout the monitoring period. IL-6 values positively correlated with total lipids ($r = 0.36$; $P = 0.0004$), triglycerides ($r = 0.37$; $P = 0.0003$), and total cholesterol ($r = 0.21$; $P = 0.04$) in calves throughout the monitoring period.

These findings suggest that lipid metabolism and inflammatory responses undergo significant changes as calves adapt to the neonatal phase and transition to solid food, with nutritional shifts playing a key role in metabolic and immune system development.

1. Introduction

The newborn calf must face a period of adjustment to extrauterine life, adapting to a completely new environment with a high pathogen load, to thermoregulate its body, and then to switch from pre-ruminant to ruminant (Arfuso et al., 2023). The colostrum intake in the first 24 h of life (better if it is assumed with 12 h) becomes indispensable for the offspring as, thanks to its biological compounds, it helps newborn calf to face the new extrauterine life and to start the cascade of reactions guiding the physiological adaptation of the newborn in the new environment. As a matter of facts, colostrum is rich in nutrients, and other precious elements including immune and growth factors IgA, IgM, IgG, IGF-1, lactoferrin and lysozyme which endow the organism with transient immunity and stimulate gut maturation (Yang et al., 2015). Considering that calves has to face several challenges during the neonatal period, like a significant amount of stress, their immunological status undergo to relatively relevant simuli within the first 3 weeks of life, when harmful pathogens could colonize the digestive tract (Meganck et al., 2014). This coincides with the need to transition from passive to active immunity (Hulbert and Moisá, 2016). While the neonatal period is a stressful time for the offspring, the physiological adaptations that the newborn makes lead to the proper maturation of various systems of the organism, such as the immune and digestive systems. During weaning, the main source of nutrients is shifted from milk to solid feed. At the same time, the composition of the rumen changes, particularly with respect to the bacteria that are the main contributors (Hao et al., 2021; Meale et al., 2016). Adipose tissue has the main function of maintaining the body's energy balance by storing lipids and to provide energy to the body when needed (Jayaprakash et al., 2016). In addition to the above, being an endocrine organ, it produces hormones, peptides and adipokines. One of the most important being leptin, which is capable of playing a role in energy homeostasis at the

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central level (Flier, 1995; Zhang et al., 1994). Leptin is considered an adipokine recognized as a hunger signal, in fact a decrease in its levels results in neurohumoral and behavioral changes, trying to preserve energy reserves for vital functions in mammals (Fernández-Riejos et al., 2010). In non-ruminants and ruminants, an increase in leptin can lead to an increase in energy expenditure, which causes an expression of mitochondrial uncoupling protein 1 (UCP1), thus an increase in lipolysis and fatty acid oxidation (Brondani et al., 2012; Giacobino, 2002; Busiello et al., 2015). About this adipokine, research has focused on understanding the complex network linking metabolism, nutrition, reproduction, and especially inflammation and immune functions (Popovic et al., 2002). Indeed, it appears that leptin can modulate the immunological status of animals by stimulating humoral and cellular immune responses. Studies in humans and rodents, shown that adipokine can exerts a pro-inflammatory effect by activating pro-inflammatory cells, promoting T-helper 1 response, and mediating the production of pro-inflammatory interleukins such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-2, and IL-6 (Fernández-Riejos et al., 2010; Pérez-Pérez et al., 2020).

The role of UCP-1 in lipid metabolism is still unclear. Mitochondrial uncoupling in adipose contributes to the control of lipid metabolism and adiposity, UCP-1, a membrane transporter, plays an important role in non-tremendous thermogenesis (Pan et al., 2019). It promotes inner mitochondrial membrane proton conduction and uncouples ATP synthesis from cellular respiration and consumes lipids to generate heat. Lipid metabolism is thus stimulated to provide the necessary substrates for thermogenesis. Despite the extensive research that has been conducted in the field of neonatal physiology in calves, there are still significant gaps in our understanding of some of the biological mechanisms that enable neonatal adaptation in metabolic and immunological processes. To the authors' knowledge, a clear understanding of how leptin can modulate the production of inflammatory cytokines is lacking. In addition, the links of leptin, UCP-1 on lipid and inflammatory metabolism in particular during the neonatal phase and weaning are still unclear and have not yet been studied. In view of the above considerations and in order to renew the current knowledge on this field and to provide new insights ones, the present study aimed to investigate both the changes in the plasma concentration of the main markers of energetic balance (i.e. leptin, UCP1, total lipids triglycerides and total cholesterol), and markers of inflammation (i.e. IL-1β, IL-6, and TNF-α) in calves from birth to weaning. Furthermore, this study was also carried out to assess the potential relationship among lipid metabolism and inflammation markers in calves in response to the physiological adaptation processes characterizing the neonatal period and the weaning phase.

2. Materials and methods

2.1. Animal management and experimental design

The trial was carried out in accordance with Italian laws on animal experimentation (DL n. 26, 04/03/2014) and received an institutional approval from the Ethical Animal Care and Use Committee of the Magna Graecia University of Catanzaro (Protocol No. 302-5/5/2017). Furthermore, the protocol of animal husbandry and experimentation was reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal experiments. In particular, agreeing to the Directive 2010/63/EU for animal experiment, blood sampling procedures have been carried out according to the Section III of the cited Directive (Examples of different types of procedure assigned to each of the severity categories on the basis of factors related to the type of the procedure) point (b). The blood sampling was performed by trained veterinarian, while the owners were present. Moreover, the farm owner was previously informed and the consent for animal use was obtained in compliance with the purposes and methods of the research.

During winter season, from a commercial dairy farm in Southern Italy (Calabria) a total of 10 newborn Italian Simmental calves (5 heifers and 5 bulls) were enrolled in the study. Within 4 h after birth, calves were weighed and then transferred to individual straw-bedded pens (2.8 m long x 1.80 m wide). At birth, calves were cleaned and had the navel disinfected with oxytetracycline hydrochloride (Neo Spray Caf Aerosol; Gellini S.p.a., Aprilia, Italy). All calves were fed 3 L of their dam's colostrum by nipple bottle (within 4 h from birth). If voluntary colostrum intake had not reached the 3 L required, calves were fed with an esophageal feeder (Speedy Drencher XL, Agri-Zoo San Marino srl, Domagnano, San Marino). Afterwards, animals received 4 feedings of their dam's transition milk (3 L at each feeding) over 2 days (at 12, 24, 36, and 48 h after birth).

Calves received milk-based milk replacer (MR; 21.5 % protein and 18 % fat; Elvor, Maen Roch, France) twice daily (08:00 and 16:00) at a rate of 135 g/ L of water. All calves received 6 L/d from 3 to 53 d of age, whereas from 54 to 60 d of age (weaning) calves were stepped down at one meal of 3 L/d in the morning. Fed and refused MR was recorded for morning and evening meal.

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; Dietovit Excellence, SIVAM Spa, Casalpusterlengo, Lodi, Italy) was offered from 4 d of age once every morning after MR feeding for ad libitum intake. Newly fed and refused calf starter was recorded daily. Calves' health status was evaluated daily, and feces were scored daily in the morning before the milk feeding using a 1 to 5 scale (score 1 being normal and 5 being watery). Calves were weighed after birth before colostrum intake (0), and then, in the morning before MR meal and solid feeds distribution, at days 1, 7, 15, 21, 28, 45, 54 and 60 of age (Fig. 1). Calves enrolled in the study did not present any acute health disorders during the entire experimental period. Additionally, no vaccinations therapy and antibiotic treatments were applied during experimental period in the enrolled calves.

2.2. Blood sample collection and laboratory analysis

From each calf, blood samples were collected by jugular venipuncture into 9-ml lithium heparin vacutainer test tubes (Vacutest Kima srl, Arzergrande, PD, Italy) after birth before colostrum intake (0), and then, in the morning before MR meal and solid feeds distribution, at days 1, 7, 15, 21, 28, 45, 54 and 60 of age. After collection, all lithium-heparin tubes were immediately cooled in a water-ice bath, once arrived in the lab were centrifuged at 1900*g* for 16 min at 4 °C in order to obtain plasma that was aliquoted and stored at −20 °C until analysis. The plasma samples were analyzed whithin 1 month upon storage.

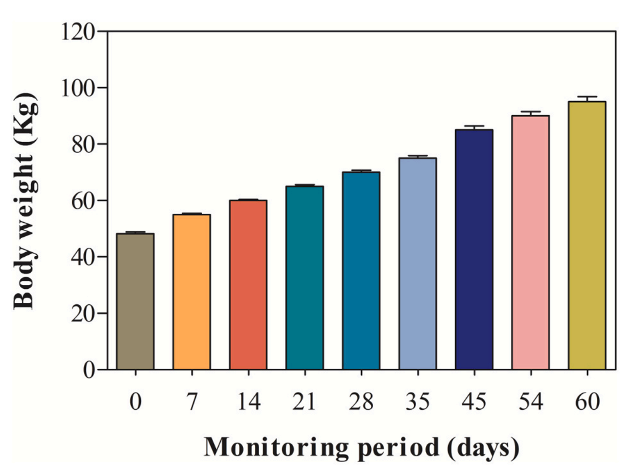

Fig. 1. Mean values ± standard error of the mean (±SEM) of body weight measured in newborn calves after birth, before colostrum intake (0), and at days 1, 7, 15, 21, 28, 45, 54 and 60 of age.

Specifically, the plasma concentration of UCP1, leptin, TNF-α, IL-1β and IL-6 and was assessed using ELISA kits specific for bovine species (Bovine UCP1 Elisa kit, MyBioSource, Inc. San Diego, California, USA, catalog number MBS2602914; Bovine Leptin Elisa kit, MyBioSource, Inc. San Diego, California, USA, catalog number MBS703026; Bovine Tumor Necrosis Factor α (TNF-α) Elisa kit, MyBioSource, Inc. San Diego, California, USA, catalog number MBS2802340; Bovine Interleukin 1 beta (IL-1β) Elisa kit, MyBioSource, Inc. San Diego, California, USA, catalog number MBS2609338; Bovine Interleukin 6 (IL-6) Elisa kit, MyBioSource, Inc. San Diego, California, USA, catalog number MBS2023586) by means of a microtiter plate reader (EZ Read 400 ELISA, Biochrom, Cambridge, United Kingdom). The sensitivity as well as the intra- and the inter-assay coefficients of variation of each ELISA test were reported in Table 1. All calibrators and samples were run in duplicate and samples exhibited parallel displacement to the standard curve for both ELISA analyses. The plasma concentration of fats (i.e. total lipids, tryglicerides and total cholesterol) was assessed using commercially available kits (total lipids, Spinreact, Ctra. Santa Coloma, Spain, Catalog number 1001270; triglycerides, BioSystems, Barcelona, Spain, catalog number 11528; total cholesterol, BioSystems, Barcelona, Spain, catalog number 11506) by means of an automated analyzer UV spectrophotometer (model Slim SEAC, Florence, Italy).

2.3. Statistical analysis

The normal distribution of the data was checked by application of the Shapiro-Wilk test. The data were found to be normally distributed ($P > 0.05$) and were subjected to one-way analysis of variance (ANOVA) for repeated measures in order to assess whether the investigated parameters were affected by age of calves. Bonferroni's multiple comparison test was applied for post-hoc mean comparison. Pearson's correlation analysis was applied to evaluate the relationship between the concentration of UCP1 and/or leptin with respect to all other parameters tested (TNF-α, IL-1β, IL-6, total lipids, triglycerides, total cholesterol) throughout experimental period and at each considered time point (days 0–60). The same correlation analysis was conducted regarding TNF-α, IL-1β, IL-6 versus lipid indices (total lipids, triglycerides and total cholesterol). To confirm these relationships and to determine the degree of correlation, a linear regression model ($y = a + bx$) was applied. P values <0.05 were considered statistically significant. Data were analyzed with Prism v. 9.00 software (Graphpad Software Ltd., San Diego, CA, USA, 2020).

3. Results

The results obtained in this study are expressed as mean values ± standard error of the mean (±SEM). All plasma biomarkers were affected by age of calves ($P < 0.05$). Specifically, calves showed an increase in UCP1 concentration at 60 days compared to birth (0 d) ($P < 0.05$, Fig. 2). Compared with the weaning time (60 days), lower leptin concentration was found at birth and at 1 day of age (24 h after colostrum intake) ($P < 0.05$, Fig. 2). Regarding the lipid markers, the concentration of total lipids, triglycerides and total cholesterol gradually increased from day 0 until the end of the monitoring period (60 days) ($P < 0.05$, Fig. 3). Regarding the interleukins, calves showed higher TNF-α values at 1 day compared to all other time points ($P < 0.05$, Fig. 4), whereas higher IL-1β concentration was found at 60 days compared to 0, and days 7, 15, 21, 28, 45, and 54 ($P < 0.05$ Fig. 4). Lowest values of IL-6 were found at 0 compared to all other time points ($P < 0.05$) and at 7 compared to 1, 21 and 28 days ($P < 0.05$, Fig. 4). Considering the entire period (Supplementary Table 1), the leptin concentration showed a positive correlation with the values of UCP-1, total lipids, triglycerides, and total cholesterol, whereas it was negatively correlated with TNF-α ($P < 0.05$; Fig. 5). The values of UCP1 were positively correlated with the concentration of total lipids, triglycerides and IL-1β in calves ($P < 0.05$; Table 1); Moreover, IL-6 values positively correlated with total lipids, triglycerides, and total cholesterol ($P < 0.05$; Fig. 5). According to the correlation analysis performed among investigated parameters in calves at each time point (Supplementary Table 2), the values of UCP-1 were correlated with IL-1β at day 0 ($r = -0.70$, $P = 0.025$) and at day 60 ($r = 0.66$, $P = 0.04$), and with leptin at day 45 ($r = 0.77$, $P = 0.009$). Leptin concentration was correlated with IL-6 ($r = -0.67$, $P = 0.035$) and with TNF- α ($r = 0.78$, $P = 0.008$) at 60 days, and with IL-1β at 35 days ($r = 0.76$, $P = 0.010$). The values of triglycerides were correlated with TNF-α at day 0 ($r = 0.69$, $P = 0.027$), with IL-1β at 7 days ($r = 0.66$, $P = 0.037$), and with IL-6 at 45 days ($r = -0.71$, $P = 0.023$).

Table 1
Summary of detection limits, intra- and inter-assay variations of each ELISA test used to asses the concentration of leptin, UCP1, TNF-α, IL-1β, and IL-6 in investigated calves.

	Detection limit	Intra-assay Variation	Inter-assay Variation
UCP-1 (ng/mL)	0.05	<8 %	<12 %
Leptin (ng/mL)	3.12	<15 %	<15 %
TNF-α (ng/mL)	20.8	<8 %	<12 %
IL-1β (pg/mL)	5	<8 %	<12 %
IL-6 (pg/mL)	0.49	<10 %	<12 %

4. Discussion

The current study aimed to investigate the dynamic change of some markers related to energy metabolism and inflammation in calves from birth to weaning. Although many studies have studied aspects in calves to the authors knowledge, there is limited evidence about the potential link between energetic and inflammatory response of calf during neonatal period and around weaning. According to the results gathered in the current study, high leptin levels were found at weaning (60 days of age). This trend could be due to the role of leptin in postnatal life; as a matter of facts, white adipose cells, in fact, produce leptin, an adipokine that exerts an effect on the hypothalamic nuclei by influencing the processes of food absorption and energy expenditure (Block et al., 2003; Blum et al., 2005). An increase in plasma leptin concentrations in calves at two months of age may be due to increase body fat content thanks to milk intake. Investigations on pre-ruminant cattle demonstrate there is a positive relationship between circulating leptin and fat content (Wegner et al., 2001). From 54 to 60 days, the animals partly change their diet, the calves receiving 3 L of reconstituted milk, increasing their ingestion levels of concentrate. This affects the energy level due to the drastic reduction in diet density by relying solely on concentrate ingestion. This finding agrees with the current investigation in which a positive correlation was found between leptin and total cholesterol, triglycerides, total lipids. It is well known that the release of leptin occurring in proportion to the amount of body fat reflecting the content of triglycerides and lipid deposits (Kulcsár et al., 2005). Subsequently, leptin stimulates the depletion of triglycerides in white adipose tissue, resulting in free fatty acids release (Chilliard et al., 2005). The total lipids, triglycerides and total cholesterol values showed a gradual increase in calves from birth up to day 60. These results are in agreement with other studies conducted on calves (Arfuso et al., 2017; Kühne et al., 2000) and justified by fats intake through milk. Noteworthy leptin concentration was positively correlated with UCP-1 values in calves throughout monitoring period. The plasma concentration of leptin stimulates adipose tissue and influences the expression of UCP-1 protein, which in turn influences lipid concentration by promoting increased lipolysis and fatty acid oxidation. In particular, this occurs in brown adipose tissue (BAT), in which the mitochondria are equipped with the UCP-1 (man, rodents, non-ruminants, ruminants). UCP-1 acts by short-circuiting the electron transport chain and dissipating the mitochondrial membrane potential in the form of heat. This process makes BAT a key tissue in the regulation of energy balance and calorie consumption (Asano et al., 2013; Porter, 2017). The results showed a high level of UCP-1 at 60 days compared to

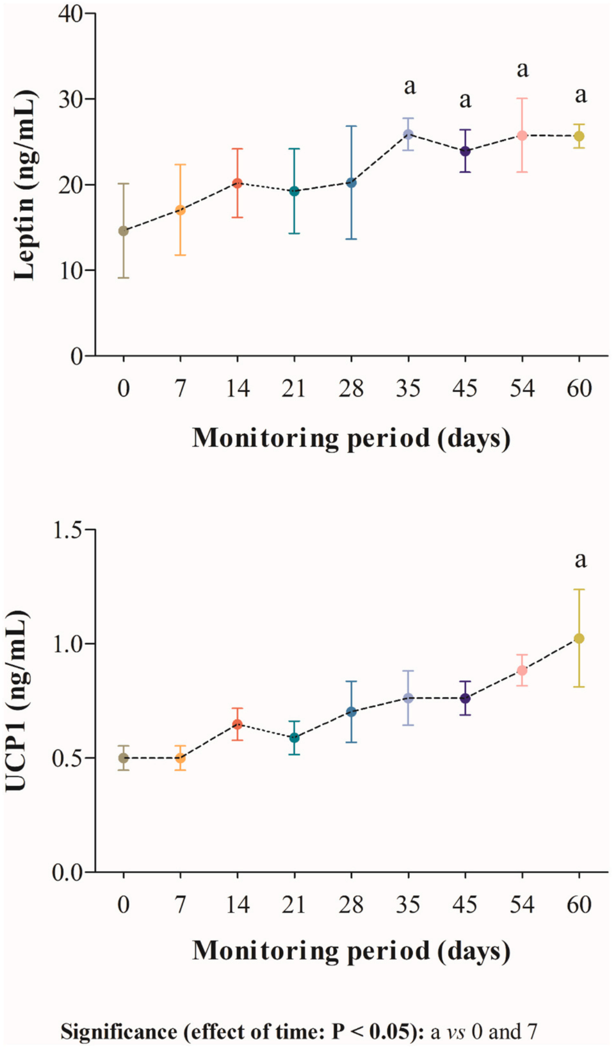

Fig. 2. Mean values ± standard error of the mean (±SEM) of plasma leptin and uncoupling protein 1 (UCP1) measured in newborn calves after birth, before colostrum intake (0), and at days 1, 7, 15, 21, 28, 45, 54 and 60 of age, together with the relative statistical significances.

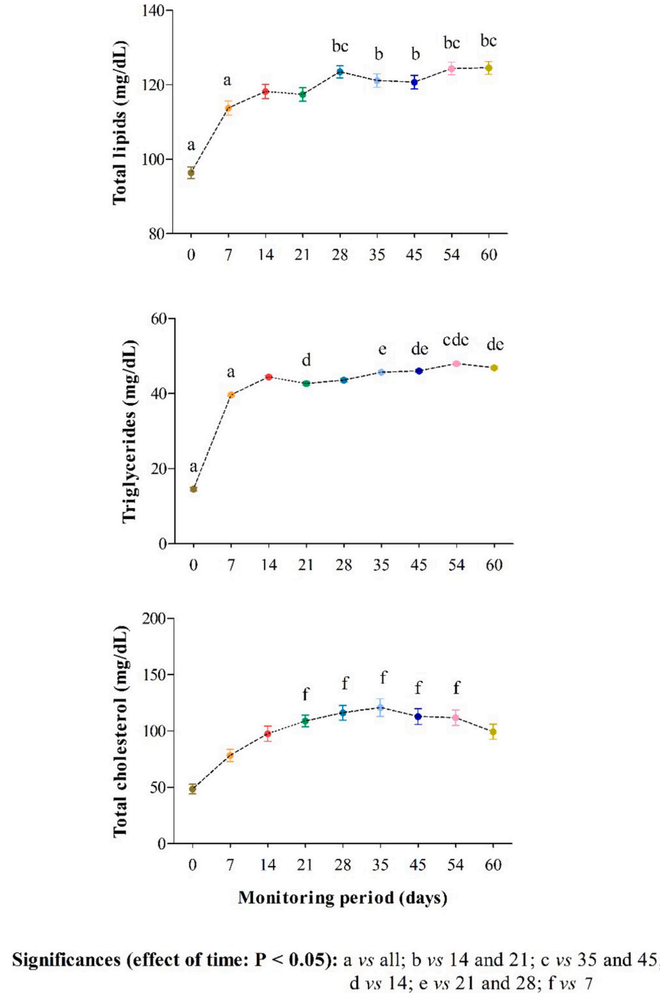

Fig. 3. Mean values ± standard error of the mean (±SEM) of plasma total lipids, triglycerides and total cholesterol measured in newborn calves after birth, before colostrum intake (0), and at days 1, 7, 15, 21, 28, 45, 54 and 60 of age, together with the relative statistical significances.

zero. In newborn mammals, white adipocytes regulate energy distribution. Brown adipocytes, on the other hand, are responsible for regulating body temperature through the activation of UCP1-dependent thermogenesis (Fuju et al., 2024). Its activation ensures a significant increase in heat production, which is particularly important for the calf's adaptation to the extra-uterine environment. UCP-1 levels also vary due to maternal nutrition and as reported above, an increase in food/feed intake leads to an increase in UCP-1 levels (Stephenson et al., 2001; Arfuso et al., 2021). Regarding inflammatory markers herein investigated the results showed higher concentration of TNF-α in calves at day 1 and of IL-1β at day 60 than the other time points. Lower values of IL-6 were found in calves before colostrum intake at 7 days of life then the other time points. Bovine sinepithelial-chorionic placenta prevents immunoglobulins from being transferred to the calf during pregnancy, hence in fact, the immune system of newborns is considered to be functionally immature, and the immune components will be acquired via colostrum by passive transfer (Yang et al., 2015). Therefore, the colostrum feeding in a timely manner is important to safeguard calves during the neonatal period (Turini et al., 2020; Weaver et al., 2000). A previous study by ELISA techniques detected by various cytokines in both colostrum and milk with higher concentration in colostrum (Peetsalu et al., 2022). It should be noted that the levels of 6 that have been rising for 21 days could be attributable to the fact that in this phase the adaptive immune system begins to become functional. In fact, precisely during this period we have a shift between neutrophils and lymphocytes where the latter increase (Arfuso et al., 2023). Noteworthy, the results obtained in the current study highlighted the interaction between markers on inflammation and lipid metabolism as suggested by the correlation test. In particular leptin was negatively correlated with TNF-α in calves throughout the monitoring period. As already mentioned, leptin is an adipokine and therefore plays a role in T-cell mediated immunity. This was also understood through the use of ob/ob mice displaying lymphoid atrophy. Instead, following administration of exogenous leptin, it normalized the population of lymphocytes and monocytes, also causing a reduced induction of TNF-α (Faggioni et al., 2000, 2001). A positive correlation was found between UCP-1 and IL-1β. This could be related to the change in the calf's diet which influences the expression levels of UCP-1 in the subcutaneous white adipose tissue (WAT), increasing as a result of higher protein and energy density (Asano et al., 2013). IL-1β interferes with thermogenesis by causing oxidative stress and mitochondrial dysfunction that manifests itself through the production of reactive oxygen species (ROS) and a decrease in SOD enzyme activity (Okla et al., 2017). The study also showed a positive correlation between IL-6 and total cholesterol, triglycerides, total lipids. As it is well known, IL-6 is transferred via colostrum and it is involved in the innate immune response and thus in general protection,

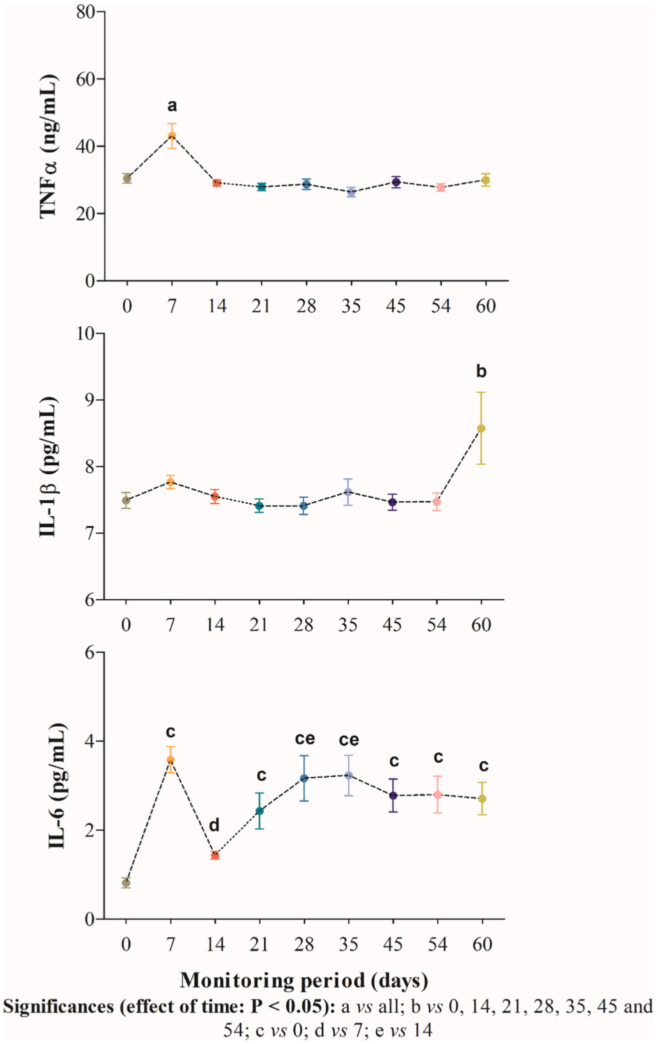

Fig. 4. Mean values ± standard error of the mean (±SEM) of plasma Tumor Necrosis Factor α (TNF-α), interleukin- (IL-)1β and IL-6 and measured in newborn calves after birth, before colostrum intake (0), and at days 1, 7, 15, 21, 28, 45, 54 and 60 of age, together with the relative statistical significances.

which is important in the calf during the neonatal period (Arfuso et al., 2023). An increase in inflammation, is certainly caused by the stressful phase that calves are going through in order to get used to extrauterine life. These conditions of inflammation could induce changes in lipid metabolism (Kushibiki et al., 2009). Studies (Guckian, 1973; Kaufman, 1976) have shown that inflammatory reaction there is not only an increase in hepatic lipid synthesis but also a change in the metabolism of circulating lipoproteins.

When considering the correlation among investigated parameters in calves at each time point (i.e. 0–60 days), the results showed that UCP-1 was correlated with IL-1β, and triglycerides was correlated with TNF-α at day 0. These correlations may be justified by the adaptive conditions of the calf needed to successfully adatpt to extra-uterine life. Indeed. they must begin to trigger the mechanisms of thermoregulation and lipid metabolism. Maintaining body temperature is essential for survival and is closely related to the need for energy reserves and energy production (Piccione et al., 2010; Goanta et al., 2010). The calf's adaptation also concerns its need to protect itself from pathogens, which are immunologically naive and therefore need to receive interleukins from the placenta (Arfuso et al., 2023). The correlations found between IL-1β and triglycerides at days 7, and between leptin and IL-1β at days 35 may be related to changes in inflammation and lipid metabolism due to the body's adaptive drive from the neonatal period to weaning when diet shift colostrum-milk-forage of calves (Palczynski et al., 2020). Moreover, UCP-1 values were correlated with leptin values at days 45; at days 60, the UCP-1 values were correlated with IL-1β levels, whereas, the leptin concentration was correlated with TNF-α and IL-6 values. These relationships may be due to the delicate phase of weaning in dairy calves which have to face numerous challenges. In this regard, the animal will have to refine its adaptive digestion strategies typical of polygastrics such as rumen development and ruminal microbial population. In this regard, following the start of feed intake, calves may produce butyric acid which is a major product of rumen fermentation of rapidly fermented carbohydrates such as sugars and starches that are abundant in feed. This may affect lipid metabolism and this adaptive drive may affect the animal's production of inflammatory mediators (Niwińska et al., 2017; Wu et al., 2024). However, a limitation of this study could be the lacking informations on ruminal activity and characteristics; furthermore. it must be taken into account that, performing the correlation analysis per time point, the number of animals decrease as well as factors, including environmental variables and diet, could influence the trend as well as the correlation analysis coefficients, as suggested by Simpson's paradox; therefore, this advocates further investigations on a greater number of animals in order to confirm the hypotheses which have been put forward in the current survey.

5. Conclusions

The current study highlighted that lipid metabolism and inflammatory response of calves dynamically change during neonatal period up to weaning. Moreover, leptin, UCP-1 and the investigated pro-inflammatory interleukins seem to work in concert within the energetic metabolism adaptation of calves throughout neonatal period and weaning. Though the findings obtained in the current study highlighted the dynamic changes in metabolic and inflammatory markers during early life, with clear correlations between leptin, UCP-1, lipid metabolism, and inflammatory cytokines, the small sample size herein investigated ($n = 10$ calves) could represent a limitation of the study, therefore, further investigations on a larger animals' group are advocated to better characterize the relationship between lipid metabolism and inflammatory responses in calves during the neonatal and weaning periods. Recognizing and understanding this cross talk is critical to developing nutritional and management strategies that optimize calf health and reduce the risks associated with inflammatory and metabolic disorders during critical growth stages.

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CRediT authorship contribution statement

Francesca Arfuso: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Federica Arrigo:** Writing – original draft, Software, Methodology, Formal analysis. **Vincenzo Lopreiato:** Investigation, Data curation. **Maria Rizzo:** Software, Data curation. **Luigi Liotta:** Validation, Supervision. **Claudia Giannetto:** Writing – review & editing, Project administration. **Giuseppe Piccione:** Writing – review & editing, Validation, Supervision, Project administration.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

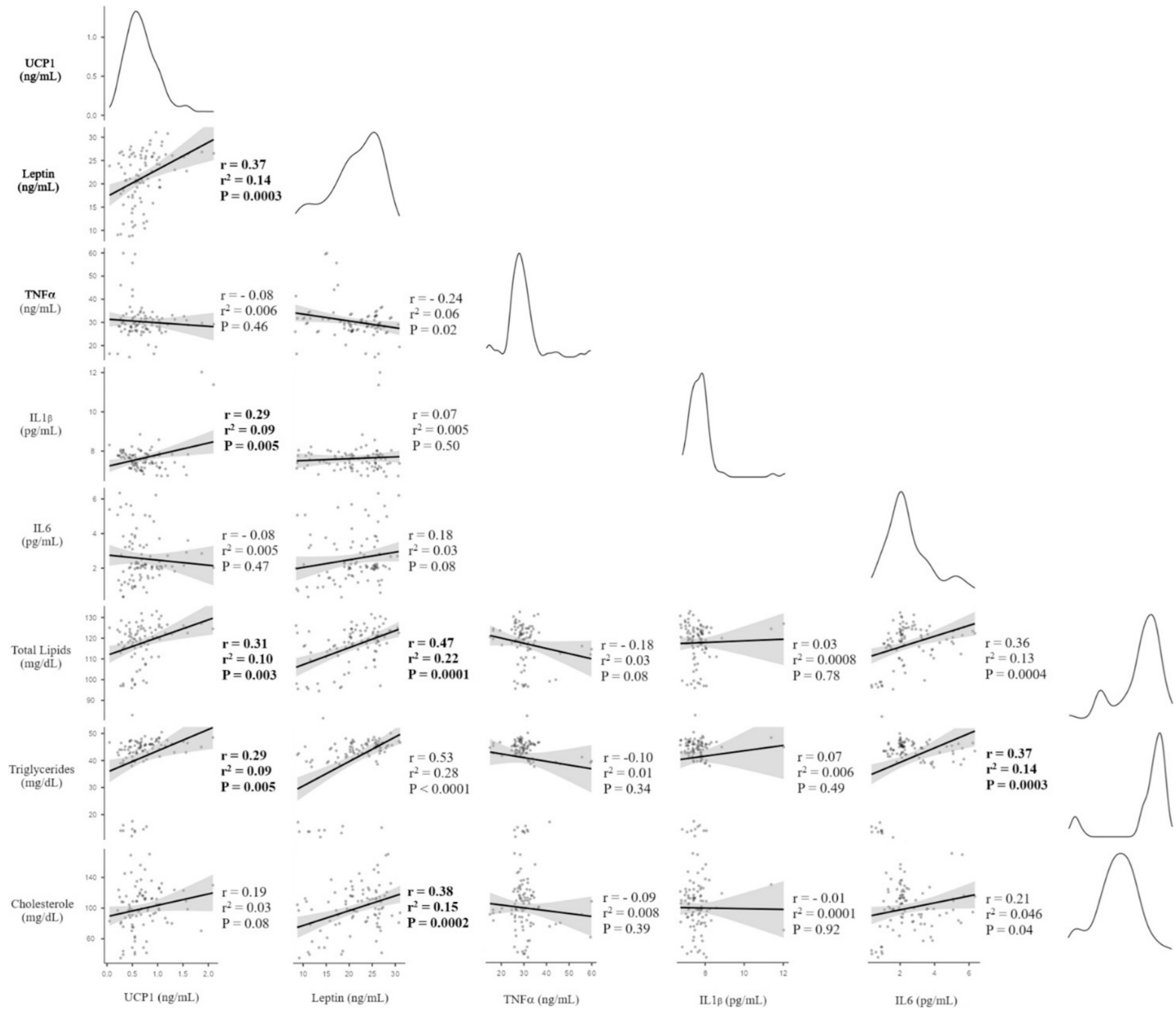

Fig. 5. Coefficients of correlation among the plasma concentration of leptin and/or UCP1 and the plasma levels of total lipids, triglycerides, total cholesterol, TNF-α, IL-1β, and IL-6 measured in newborn calves after birth, before colostrum intake (0), and at days 1, 7, 15, 21, 28, 45, 54 and 60 of age. *P* values <0.05 were considered statistically significant. Significant values have been highlighted in bold.

the work reported in this paper.

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Further-reading

Goanță, A.-M., 2025. A review of the adaptation of the newborn calf to its environment n.d.