Long-term development of the oxidative status in growing Holstein calves depending on early postnatal feeding with transition milk or milk replacer

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CONTENTS

ABBREVIATIONS

Abbreviation	Meaning	Abbreviation	Meaning
ADG	Average daily gain	IVF	In vitro fertilization
AI	Artificial insemination	ME	Metabolizable energy
AOPP	Advanced oxidation protein products	Mg	Magnesium
BCS	Body condition score	miRNA	MicroRNA
BFT	Back fat thickness	MR	Milk replacer
BW	Body weight	mRNA	Messenger RNA
Ca	Calcium	O	Oxygen
DM	Dry matter	OS	Oligosaccharides
dROM	Derivatives of reactive oxygen metabolites	OSi	Oxidative status index
FA	Fatty acids	P	Phosphorus
Fe	Iron	ROM	Reactive oxygen metabolites
FPT	Failed passive transfer	SCC	Somatic cell count
FRAP	Ferric reducing ability of plasma	SL	Sialyloligosaccharides
GIT	Gastrointestinal tract	TBARS	Thiobarbituratic acid reactive substances
GSH-Px	Glutathione peroxidase activity	TGF	Transforming growth factor
Hp	Haptoglobin	TMR	Total mixed ration
Ig	Immunoglobulins	TP	Total protein
IGF	Insulin like growth factor	TRANS	Transition milk
IgG, IgM, IgA	Immunoglobulin G, M, A	WM	Whole milk

I. INTRODUCTION

The health of a calf during the rearing period has a significant effect on the age at first calving and with that the beginning of its usability as dairy cow (WALTNER-TOEWS et al., 1986; GODDEN et al., 2019). Calves that are healthy, do not need many or any treatment, and develop good in the first month of their life get pregnant earlier and thus enter into lactation earlier (TAUTENHAHN et al., 2020; HOEDEMAKER, 2021). An important prerequisite for calf health is the sufficient supply of colostrum, i.e., the first milk of the dam *post partum* (GODDEN et al., 2019). Apart from nutrients, it contains immunoglobulins (Ig) (KORHONEN et al., 2000), which are the basis for the first, passive immunity of the calf, until their own active immune system develops in the first weeks of life (HASSIG et al., 2007). A few hours *post natum* the ability to absorb these Ig in the gut declines (called "gut closure"; WEAVER et al. (2000)). If calves do not receive enough Ig in the first days, this leads to development issues, losses in performance and earlier disposals (NOCEK et al., 1984). The knowledge about these correlations between the importance of colostrum and the development and health of calves has been around for over 100 years (EHRLICH, 1892; HOWE, 1921) and improved over the years to develop the right management (GODDEN et al., 2019). The components of colostrum to additionally aid the calf in its development do not vanish abruptly after colostrum, but do so slowly in the first five days after parturition until the composition of mature whole milk (WM) is reached (BLUM & HAMMON, 2000a). The milk during this time is thus called transition milk (TRANS) and prohibited to be sold as normal milk according to regulation EC 853/2004 Annex III Section IX of the European Union. Historically, the content of Ig has been perceived as the most important factor of colostrum. As the transport of Ig from the gut lumen into circulation is not possible after 24 h *post natum*, the potential of TRANS in its importance as feed source for calves has been less studied as compared to colostrum. To this day it is still not known to which extend TRANS feeding affects the calf and standard protocols for a correct TRANS feeding regimen are not yet established.

One factor that could be influenced by early-life nutrition is the development of the oxidative status. The oxidative status describes the balance between oxidants produced during normal metabolism and the antioxidative defense mechanisms against them. It is known that the calves` antioxidative system is not yet fully developed after birth but does so in the first days of their life (GAÁL et al., 2006). Additionally, the exposure to an

oxygen-rich environment after birth poses a challenge for the metabolism of the calf and leads to the production of reactive oxygen metabolites (ROM) (CUERVO et al., 2021). These are highly reactive oxygens, leading to oxidative damage in proteins and lipids, if they cannot be reduced by the antioxidative defense. The redox balance of the colostrum fed further influences the oxidative status, as colostrum naturally contains a high concentration of ROM (ABUELO et al., 2019). This risk could be lessened by the feeding of TRANS the following days as it is known that TRANS contains more antioxidative potential than WM or milk replacer (MR) (KANKOFER & LIPKO-PRZYBYLSKA, 2008; SOBERON et al., 2012b). Compared to calves fed MR, TRANS fed calves consume higher amounts of antioxidants that could potentially improve their antioxidative status and promote growth and health (SOBERON et al., 2012b; ABUELO et al., 2014).

If the oxidative status is disrupted in favor of prooxidants, either by an accelerated production of oxidants or a compromised antioxidative system, it can lead to damage in proteins, lipids and DNA (SIES et al., 2017). This situation, called oxidative stress, may result in metabolic disorders and diseases, if it persists for longer periods of time (SORDILLO & RAPHAEL, 2013). Oxidative stress in newborn calves compromises the functions of their lymphocytes (CUERVO et al., 2021) and plays an important role in the pathogenesis of various diseases. Additionally, ROM can inactivate steroidogenic enzymes and thus oxidative stress can impair reproduction (MILLER et al., 1993). To this day there is no study about the development of the oxidative status of growing heifers after weaning. The knowledge about the mechanisms in adaption during the development of the metabolism and behind oxidative stress in diseases can help to prevent damage caused by these. The oxidative status is complex and heavily influenced by the metabolism (MILLER et al., 1993). As the metabolism is still developing after weaning, the gap in the knowledge about the oxidative status between weaning and first lactation is substantial. To understand the influence early calf nutrition has on later performance, comprehensive knowledge about physiological mechanisms and the metabolic programming are necessary.

The aim of this thesis was thus to

1) evaluate the long-term effects of a five-day transition milk feeding in German Holstein calves on growth performance and health during the rearing period and into their first lactation,
2) closing the knowledge gap about the systemic oxidative status of growing heifers from birth until their first lactation.

II. LITERATURE REVIEW

1. Colostrum and Transition Milk

In contrast to transition milk, colostrum has been the focus of calf research for many decades. All the basic knowledge we have about the importance of colostrum for the calf, the main components and in which way they effect the calf are based on publications from almost 100 years ago. With that the best practices and guides for a good colostrum management have been established. In contrast, transition milk is a more recent topic and there are still big gaps in our knowledge about its importance for the calf as compared to colostrum.

The following chapters give an overview about the long history of colostrum research and the knowledge we gained from that for today's practices. They will further explain the differences between colostrum and transition milk and establish, what is known about transition milk feeding to calves in particular.

1.1. History of Colostrum Research

Late 19th century: Globulins found in colostrum

Much of today's knowledge about the importance and composition of colostrum are based on research done in the last centuries. The first publication about globulins in milk can be found in the years 1885 and 1888 (CROWTHER & RAISTRICK, 1916). In 1892, EHRLICH found that these are maternal antibodies which are transported to the newborn via colostrum and termed the immunity acquired thereby "passive" in contrast to the "active" immunity achieved after an infection (EHRLICH, 1892). It was discovered that these globulins make up not more than 0.005% of total content in mature whole milk (WM), but 18 - 20% in colostrum and are rapidly vanishing within 48 hours after parturition (ENGEL & DENNEMARK., 1912; CROWTHER & RAISTRICK, 1916). In the latter study they found that these globulins are the only proteins found in colostrum identical to the one found in the dams' serum, therefore most likely transported from the blood of the dam into colostrum (CROWTHER & RAISTRICK, 1916).

In 1921, HOWE discovered that calves are born without any proteins precipitable in their blood but do have them after colostrum ingestion. Additionally, by comparing these results to calves fed whole milk, they found neglectable amounts of protein in

the blood of these WM-fed calves. They concluded that the globulins found in the calves' blood need to come from colostrum or must be formed after some time. Until this point, the role of colostrum was solely based on the enhanced nutritional value posed to the newborn, as studies in dogs, goats and guinea pigs had shown that development could be achieved without receiving colostrum, albeit goats showed better development when fed colostrum (HOHLFELD, 1907). We do know today that the placentation type of ungulates prevents the transport of larger molecules from the dam to the foetus and thus the offspring are born with low blood levels of these (KRESSIN, 2019b). They are dependent on the uptake of colostrum to achieve passive immunity with the immunoglobulins (Ig) found therein (further discussed in Chapter II.1.2.). In contrast, in dogs and guinea pigs, larger molecules get transported through the placenta in utero and neonates are born with a passive immune system provided during gestation (KRESSIN, 2019b).

1920s: The role of globulins in calves

RAGSDALE and BRODY (1923) concluded from the previous findings, that the globulins found in colostrum, as well as antibodies synthesized in the dam after infectious diseases, pass from the blood to the mammary gland. They further surmised that due to missing globulins in calves before colostrum ingestion but the abundance of them thereafter, that these globulins and "immune bodies" can be absorbed from the colostrum by the calf. This led them to the notion that colostrum is vital to provide the calf with the necessary protection due to its own lack of defence mechanisms (RAGSDALE & BRODY, 1923).

One year prior, this was shown by SMITH and LITTLE (1922a), when they found that calves deprived of colostrum were more susceptible for bacterial infection and thus died or had to be euthanized. They noted that the bacteria isolated from the colostrum-deprived calves were ubiquitous and harmless for the calf later in life "when the protective functions of the calf have begun to operate and accumulate energy" (SMITH & LITTLE, 1922a). As an alternative for colostrum, since tuberculosis transmission through infected colostrum was feared at the time, they suggested cow serum (SMITH & LITTLE, 1922b). Oral admission was found to be more effective in replacing colostrum than injection, as they noted that the protection of the digestive tract seems of importance for survival of the calf (SMITH & LITTLE, 1922b). Alternatively, pasteurization of colostrum in a water bath at 60 °C for 30 minutes would inactivate bacteria found in the colostrum (RAGSDALE & BRODY, 1923). In 1923, SMITH &

LITTLE showed that uptake of the globulins and immune bodies of colostrum and serum administered orally only occurs in the first hours of life and colostrum administration should happen as soon as possible. These findings still form the basis of the recommendations for good colostrum management today. The best practice for calf health is sufficient, clean, high-quality colostrum provided as soon as possible after birth to improve the calf's performance (GODDEN et al., 2019).

Early 1940s: Vitamins in colostrum

In the following years, research about other components of colostrum compared to WM began (SHOPE & GOWEN, 1928; HENRY & KON, 1937; KUIKEN & PEARSON, 1949). The main focus was put on vitamins, mainly vitamin A, as it was believed to be the solution against scours (STEWART & MCCALLUM, 1938); even more important than colostrum itself (LUNDQUIST & PHILIPS, 1942). Similarly to the globulins discovered previously, vitamin A was found to be low in calves after birth and increased with colostrum uptake, but also falling again with the transition to WM feeding (MOORE & BERRY, 1944). The reason for this was the high vitamin A content found in colostrum, which declined in the first three milkings after parturition and the fact, that vitamin A appeared to be not passable *in utero* to the calf (HANSEN et al., 1946). Similar patterns were seen for the tocopherol (vitamin E) content in colostrum and WM (PARRISH et al., 1947).

Late 1940s: Different practices of colostrum feeding

After the second world war, research regarding bovine colostrum and the effects on calves developed in broader directions. One focus was the effect of extended colostrum feeding. SUTTON and KAESER (1946) tested an extended colostrum feeding for three or seven days. They found no differences in health with the longer colostrum feeding, but better vitamin supply and better weight gain, albeit not significant. In a different approach they tested the intermitted feeding of surplus colostrum to calves, replacing whole milk feedings with colostrum if available (KAESER & SUTTON, 1948). Calves in this group, compared to calves fed WM the whole time, showed better weight gain, had a better physical appearance and higher vitamin concentrations in their blood. A possible explanation for this was found in different studies. SMITH (1948) found that globulins make up to 50 - 60% of protein concentrations in colostrum, which was much higher than the percentage found in maternal blood. Additionally, Ig consumed via colostrum were different to those found later in life in the blood of calves, with the colostrum Ig slowly declining and the endogenic Ig

gradually appearing (SMITH & HOLM, 1948). Thus, by feeding colostrum to the calves, the Ig of it not only provides additional nutritive value but also further benefits for the immune system.

Both studies also noted that the milk in the first days *post partum* tended to be similar to colostrum, albeit with slight changes towards the composition of WM. This finding was further confirmed by PARRISH et al. (1950). They analyzed the changes in composition of colostrum in the first days after calving; specific gravity, solids-non-fat, and total protein are decreasing, lactose, ash and total solids increasing. They found the main source of energy in colostrum to be the protein, whereas in WM it was mainly from fat and lactose (PARRISH et al., 1950). These publications can be seen as the first mention of transition milk and the start to focus on the milk closer to calving and its effects on the calf in further research.

1950s: Globulin uptake in the newborn calf

Another focus of research was the mechanism of the uptake of Ig by calves. Firstly it was discovered that the absorption of IgG, one of the Ig found in colostrum, only occurs in the first 24 h of life in calves (MCGIRR, 1947; DEUTSCH & SMITH, 1957). Additionally, they found that the absorption appears to be happening via the lymphatic system (COMLINE et al., 1951) and be unspecfic, with larger molecules found mostly in the lymph, whereas smaller molecules (like albumin) can also be found in the blood (BALFOUR & COMLINE, 1959). As uptake mechanism, pinocytosis was determined and the decreasing ability of uptake *post natum* was explained by the replacement of the fetal epithelium by adult cells, which are unable of this mechanism (EL-NAGEH, 1967). To date, this is essentially still all we know about the mechanisms of Ig absorption from colostrum in the calf (WEAVER et al., 2000).

1970s: Optimizing colostrum management

After World War II, more milk was used for human nutrition rather than calf rearing and therefore the need for milk replacer (MR) rose. WILLIAMS (1950) outlined the requirements of contents for these milk replacements, based on dried skim milk. All these requirements were based on the prerequisite that calves were fed colostrum for the first five days of life. With this finding it became more important to amplify the quality of colostrum, the uptake of Ig by the calf, and the durability of colostrum (YU et al., 1976; POLZIN et al., 1977; FOLEY et al., 1978; FALLON, 1979; HOFFMANN et al., 1979; JENNY et al., 1984).

An important series of publication, still cited today, to summarize the feeding of colostrum to calves and the uptake of Ig was published in 1979 by STOTT et al. They found that the uptake of Ig progressively declines after 12 h and the mean closure was near 24 h *post natum* (STOTT et al., 1979a). Feeding colostrum earlier lead to earlier closure and with a slight delay in feeding, gut closure was also slightly delayed (STOTT et al., 1979a). Additionally, the beginning of closure was dependent on the amount of colostrum ingested, with the best intake of Ig reached with 2 L of colostrum (STOTT et al., 1979d). With increased age, Ig could still be taken up in the gut, but would not be further transported into the blood circulation (STOTT et al., 1979c). STOTT et al. (1979b) discussed the possibility of suckling as an improvement for Ig uptake. They found higher Ig absorption rates in suckled calves, but came to the conclusion that most likely there are some other components, like hormones, in colostrum which are influential and lost through freezing in the pooled colostrum fed to the control calves rather than an effect of suckling (STOTT et al., 1979b).

1980s: Vaccination via colostrum

Another discovery was made in the 80s, when experiments showed that by vaccinating the dams, antibodies are transferred to the colostrum and can prevent infection of calves with these diseases (FERRER & PIPER, 1981; SNODGRASS et al., 1982; SAIF et al., 1983; CASTRUCCI et al., 1984; FAYER et al., 1989). This practice is used to this day to enhance colostrum quality and to enhance passive antibody titers against common pathogens, typically *E. Coli*, rotavirus or coronavirus (GODDEN et al., 2019)

1.2. Gut Closure

"Gut closure" is a term to describe the reduced uptake of large molecules in the gut after birth in neonates. To understand the importance of this mechanism for the newborn calf, it is important to understand its *in utero* development. The blood-placenta barrier in cattle is not permeable for larger molecules like Ig. Cows have a synepitheliochorial placenta, a special form of an epitheliochorial placenta, where the chorion cells of the fetal trophectoderm only fuse with the maternal endometrial epithelium (KRESSIN, 2019b). The substance transfer between fetus and dam therefore depends mainly on diffusion, preventing the passage of most macromolecules (KRESSIN, 2019a). As a result, calves are born agammaglobulinemic.

In addition, the ability of the calf to synthesize Ig in the first days of life is limited. Passive immunity is only achieved via the uptake of antibodies in colostrum, until their immune system is fully developed (HASSIG et al., 2007).

To get the maximum out of the consumed colostrum, Ig transfer into the blood is through the enterocytes by transcytosis without regulation (MEALE et al., 2017). The neonatal enterocytes in the small intestine have the ability to absorb macromolecules unselected by pinocytosis (WEAVER et al., 2000). In the enterocytes, endosomes are formed, containing the absorbed macromolecules. The endosomes are transported into the Peyer`s Plaques and enter the circular system through the *Ductus thoracicus* (WEAVER et al., 2000). This passive transfer stops once the fetal-type enterocytes are replaced by the adult-type enterocytes, as they lack the ability to endocytose intact proteins (BUSH & STALEY, 1980; MEALE et al., 2017).

The permeability of the gut is almost completely stopped 24 h *post natum*, a decline is seen already 12 h *post natum* (STOTT et al., 1979a). The best results of Ig uptake are achieved in the first hours after birth (GODDEN et al., 2019). Early colostrum feeding can shorten the absorption timeframe by 2 to 3 h (STOTT et al., 1979a), delaying colostrum feeding can only shortly postpone the closure up to 36 h (FISCHER et al., 2018). The factors regulating the mechanism of gut closure still remain unknown and need further research (FISCHER et al., 2018). It was shown that heat stressed calves have higher rates of jejunal apoptosis and also a more rapid gut closure (AHMED et al., 2021). Additionally bacterial contamination of colostrum also reduces the Ig uptake. It is believed that the bacteria bind free Ig in the gut lumen or block the uptake of macromolecules (JAMES et al., 1981).

The key factors to ensure a sufficient uptake of especially Ig by the calf are therefore not only a quick colostrum feeding, but also the volume and quality to provide the necessary amount of Ig until the immune system of the calf is fully developed (GODDEN et al., 2019). Failed passive transfer (FPT), e.g. an insufficient supply of colostrum to the calf, is defined as a serum IgG concentration of less than 10 g/L in the serum of the calf after colostrum intake (GODDEN et al., 2019). FPT is related to increased morbidity and mortality rates, as well as more enteric diseases and shows effects on production and future growth (CUTTANCE et al., 2018).

1.3. Contents of Colostrum and Transition Milk

Colostrogenesis and lactogenesis

Colostrogenesis starts several weeks before calving, when a mixture of lacteal secretions and constituents of blood serum accumulate in the mammary gland. It is mainly controlled by the hormone prolactin and stops at parturition (BRUCKMAIER et al., 2022). Starting approximately 40 days before calving, the composition of mammary secretions begins to change (HARE, 2023). Up to 500 g of IgG per week are transported from the circulation of the dam into its mammary gland at this time (BRANDON et al., 1971). The accumulation of bioactive components from the circulation of the dam functions via specific receptors at the plasma membrane to capture them from the extracellular fluid and an Fc-specific receptor at mammary epithelial cells to internalize them via transcytosis (BARRINGTON et al., 2001). With the increase of lactose secretion 5 to 10 days before calving, milk yield starts to increase and concentrations of Ig, hormones and enzymes decrease due to dilution effects (HARE, 2023). The composition of colostrum and therefore the quality as nutrient and Ig source for the calf in detail is highly variable, depending on lactation number, breed, pre-partum nutrition and length of the dry period of the dam (MANN et al., 2016; DENHOLM et al., 2018). Generally, colostrum yield and composition do have low to moderate heritability. Brown-Swiss cows are known to have lesser density compared to Holstein; Angus are characterized by lower levels of dry-matter and higher level of lactose in their colostrum (PUPPEL et al., 2019). In contrast, Holstein x Simmental crossbred cows are reported to have higher dry matter, fat and lactose contents in their colostrum than Holstein pure breeds (PUPPEL et al., 2019). Another factor influencing the composition of colostrum is the duration of the dry period: the shorter the dry period, the higher are the lactose levels and the lower are fat and protein content (SOUFLERI et al., 2021). Multiparous cows additionally produce colostrum with greater dry matter and higher Ig levels than primiparous cows (BORCHARDT et al., 2022). The cause for this is seen in the greater mammary gland development and the increased antigenic exposure (PUPPEL et al., 2019).

The tight junctions in the mammary gland are not completely closed in the colostral phase, therefore blood components can be found in the milk the days after parturition (BRUCKMAIER et al., 2022). The concentrations of components of colostrum decline slowly with each milking after birth, until composition of WM is reached. The milk in between is called “transition milk” (TRANS) and in the literature different timeframes of TRANS can be found, depending on the components focused on in the respective

study. GODDEN et al. (2019) defined TRANS as the first six milkings, i.e. first three days after colostrum, VAN SOEST et al. (2020) defined it as the second to fourth milking after calving. According to Annex III Section IX of regulation 853/2004 of the European Union, TRANS is to be considered the milk up to five days *post partum.* As the latter has the greatest practical significance for the feeding practices of the farmers concerned, this period is considered in this thesis.

Colostrum is an important energy source for the newborn, as calves are born with very limited energy reserves, only about 3% of body weight (BW) of the newborn are fat (MORRILL et al., 2012). Therefore, the calf is dependent on the energy provided by colostrum (MORRILL et al., 2012). Apart from fat, that is higher in colostrum than in WM, the main energy source in colostrum are the proteins, whereas later in life it is the lactose. Additionally, colostrum is a source of essential amino acids and vitamins, as well as minerals. The biggest difference to WM is the large variety in antimicrobial and immune modulatory components found in colostrum (BLUM & HAMMON, 2000b).

Proteins

Many different proteins can be found in colostrum, divided into two groups: whey proteins and casein, both providing nutrients and bioactive properties. The predominant proteins found in colostrum are immunoglobulins (Ig), with IgG making 85 - 90%, IgA 5% and IgM 7% of the total Ig amount in colostrum (GODDEN et al., 2019). A good colostrum is considered a milking with ≥ 50 g/L IgG (MCGRATH et al., 2016). IgA and IgM are mostly produced in the plasma cells of the mammary gland, whereas IgG, IgG_1 in particular, are transported out of the blood of the dam via a receptor (FcRn) (BRUCKMAIER et al., 2022). The expressing of this receptor stops with the onset of lactation, probably due to increasing prolactin concentrations (BARRINGTON et al., 1997). The main function of Ig is to detect pathogens and to protect calves against them. IgG is involved in various mechanisms, mainly bacterial opsonization and binding of pathogens to inactivate them. IgM is the first immunoglobulin to appear in B-Lymphocytes and it identifies and destroys bacteria as the principal agglutinating antibody (LOPEZ & HEINRICHS, 2022). IgA prevents the attachment of pathogens in the intestine and protects the mucosal membranes (LOPEZ & HEINRICHS, 2022). The half-life of colostrum delivered Ig in the circulation of the calf is at around 28.5 days (MURPHY et al., 2014). In WM only 1 – 2% of the proteins are immunoglobulins (KORHONEN et al., 2000), which equals around

0.7 - 1.0 mg/mL Ig (LARSON et al., 1980). The transport of Ig begins 5 weeks before parturition, peaks at 1 to 3 d before, and decreases with each milking at a rate of around 3.7% for each subsequent hour after (MORIN et al., 2010).

Casein is the predominant phosphoprotein found in colostrum, accounting for about 75% of proteins in milk and cheese (PLAYFORD & WEISER, 2021). It is usually formed as colloidal casein micelles and apart from nutrition, it is involved in the transport of minerals, in particular calcium, and trace elements, and can also reduce proteolytic degradation in the gut, acting as an enzyme-inhibiting protein (PLAYFORD & WEISER, 2021; SATS et al., 2024). Additionally it also seems to function as a immune-modulator (MIGLIORE-SAMOUR & JOLLÈS, 1988). In colostrum casein micelles are slightly bigger than in WM (227 nm; SATS et al. (2024)). The size in WM is varying from 150 to 230 nm. Concentrations of casein are declining in the milkings after parturition (6.35% in colostrum, 4.84% in milking 4), but seen as percentage of total protein, the casein content is increasing (42.5% in colostrum, 54.4% in milking 4; SATS et al. (2024)).

Another protein found in colostrum is lactoferrin, an iron-binding glycoprotein (SÁNCHEZ et al., 1988). It has protective functions for the mammary gland as well as the neonate, as it can inhibit bacteria by chelating iron and functions bacteriostatic (ROBBLEE et al., 2003). Additionally lactoferrin plays a critical role in the transport of iron from sites of absorption to iron requiring cells (ZHANG et al., 2011). It is found in the highest concentrations in the first milking after parturition, decreases sharply in the days thereafter and with a slower decline until final WM values are reached at the third week *post partum* (SÁNCHEZ et al., 1988). SÁNCHEZ et al. (1988) measured concentrations of 0.83 mg/mL in colostrum and 0.09 mg/mL in WM. BLUM and HAMMON (2000a) measured 1.84 g/L in colostrum with a similar sharp decline and 0.36 g/L at day 4 *post partum*.

Serum albumin is transported from the blood into the mammary gland, similar to immunoglobulins (GODDEN et al., 2019). It plays a role in the transport of molecules and is found in higher concentrations in high quality colostrum (KAÇAR & BATMAZ, 2023). Albumin concentration in calves increase in the first week of life and is dependent on colostrum intake (BLUM & HAMMON, 2000a). Concentrations reported previously are at around 1.21 mg/mL in colostrum with a sharp decline in the milkings thereafter and leveling off after milking 4 (LEVIEUX & OLLIER, 1999).

Carbohydrates

In contrast to the aforementioned proteins, the concentrations of lactose are low in colostrum (around 2.5%), less than in WM (around 5%, FISCHER-TLUSTOS et al. (2020)). Lactose is one of the main energy sources for the calf during the milk feeding period while also stimulating functions of the spinal cord and the brains' nerve cells (PUPPEL et al., 2019; SILVA et al., 2024). As lactose is responsible for about 50% of the osmotic pressure of milk, the production leads to the movement of water into the milk (MCGRATH et al., 2016). Lower concentrations of lactose therefore lead to a more viscous milk, with very low water concentrations (BLECK et al., 2009). In colostrum the lactose concentrations are at around 2.4 % or 3.02 g/100g (CONTARINI et al., 2014; FISCHER-TLUSTOS et al., 2020) and increase quickly in the following days after parturition, reaching concentrations of around 4.8 % or > 4.58 g/100g within seven days *post partum* (CONTARINI et al., 2014; MCGRATH et al., 2016; FISCHER-TLUSTOS et al., 2020). The density of colostrum thus is usually at around 1.048 g/mL^{-1}, whereas WM is at around 1.029 g/mL^{-1} (MADSEN et al., 2004). Other carbohydrates to be found in colostrum and milk are oligosaccharides. They are divided into two classes, neutral and acidic, with the latter being the most abundant class in colostrum (GOPAL & GILL, 2000). Predominating in colostrum are 3'Sialylactose, 6'Dialylactose and Disialyllactose (FISCHER-TLUSTOS et al., 2020). They prevent pathogen adhesion to the intestinal epithelium and may enhance the uptake of IgG (FISCHER-TLUSTOS et al., 2020). The concentrations for all found oligosaccharides in bovine milk are highest in colostrum and decreasing in different speeds afterwards (FISCHER-TLUSTOS et al., 2020). 3'Sialyloligosaccharides (SL) and 6'SL are decreasing rapidly until milking 4 and leveling off afterwards (0.853 ± 0.262 mg/mL to 0.098 ± 0.01 mg/mL for 3'SL and 0.141 ± 0.062 mg/mL to 0.045 mg/mL; NAKAMURA et al. (2003)), while 6'Sialyllactose is already on a low level after milking 2 (FISCHER-TLUSTOS et al., 2020). Disialyllactose is only found in high concentrations in colostrum (FISCHER-TLUSTOS et al., 2020).

Fats and lipids

The next complex of macronutrients found in colostrum are fats and lipids. Colostrum contains a variety of different fatty acids, with a higher concentration of unsaturated fatty acids compared to saturated. This may be due the alteration of the microflora in the dam due to changes in the feeding regime and the mobilization of body fat prior to parturition (ELFSTRAND et al., 2002). The variety of fatty acids found in colostrum

is known for the beneficial effects on development and health (O'CALLAGHAN et al., 2020). For example, linoleic acid (C18:2n6c) and α-linolenic acid (C18:3n3) are the most abundant ω-3 and ω-6 fatty acids in milk (O'CALLAGHAN et al., 2020). They are essential fatty acids and improve gut development with higher villi and higher enrichment of the gut microbiota (RAMIRO-CORTIJO et al., 2020). With that they are effecting average daily gain and feed efficiency in calves (O'CALLAGHAN et al., 2020). Palmitic acid play a role in the establishing of the gut microbiome and have a slight influence of the appetitive response in neonates (WILMS et al., 2022). Docosahexaenoic acid and arachidonic acid are dominating parts of the membrane lipids found in the brain (SVENNERHOLM, 1968). Accelerated intake of these through milk leads to increased brain growth and cognitive development in newborns (HELLAND et al., 2008). As precursor for *inter alia* cytokines and eicosanoids, they also have anti-inflammatory function (CALDER, 2010). Colostrum typically has a higher fat content than WM (around 6 to 7% in colostrum), reaching normal levels of around 3.8 to 4% at days 4 to 5 *post partum* (CONTARINI et al., 2014; FISCHER-TLUSTOS et al., 2020). Linked with this change in fat content, fat composition changes in this time, too (MCGRATH et al., 2016). For example, linolic acid and linolenic acid are both highest in colostrum and decreasing in concentrations until day 3 (O'CALLAGHAN et al., 2020); palmitic acid are 16% higher in colostrum than in WM, decreasing slowly over the first days after parturition (WILMS et al., 2022).

The phospholipids mostly found in colostrum are glycerophospholipids, glycerolipids, and sphingolipids (CONTARINI & POVOLO, 2013). They are mainly located in the milk fat globule membrane and have health benefits, such as protection against gastrointestinal infections (CONTARINI et al., 2014). Membrane lipids can function as receptor for pathogens, preventing infections with Helicobacter, *E. coli* or Campylobacter (SPRONG et al., 2002). The lipid status in calves is not only dependant on ingested amount of fat, but colostrum digestion in general (BLUM & HAMMON, 2000a).

Cholosterol is essential for the development of the calf, as it is an integral part of the cell membrane. It affects the content of other lipids, esp. sphingomyelin and as the precursor of steroid hormones, is responsible for pattering and development of the nervous system (CONTARINI et al., 2014). Free cholesterol is very high in colostrum and reaching levels of WM 5 days after parturition (CONTARINI et al., 2014).

Vitamins and minerals

Calves are born with very low levels of vitamin E and A and therefor require colostrum intake (ZANKER et al., 2000). Vitamin E is a radical scavenger and can protect molecules from peroxidative damage (QUIGLEY & DREWRY, 1998). The result is a tocopheryl oxyradical that is relatively stable and can be recovered into tocopherol by the reduction with ascorbic acid. The concentration of vitamin E in colostrum is dependent on the vitamin E supplementation of the dam during colostrogenesis (QUIGLEY & DREWRY, 1998). ß-Carotene is the dietary precursor of vitamin A and is transferred in high concentrations to the neonate via colostrum, as they have very low plasma ß-carotene concentrations and can already utilize it to synthesise vitamin A (ZANKER et al., 2000). The concentration of these vitamins in colostrum depends on the dietary supply and can be managed through supplementation in the dry period (WEISS et al., 1990). Additionally, colostrum contains high concentrations of essential minerals, like calcium (Ca), phosphorus (P), natrium (Na) and magnesium (Mg) (TSIOULPAS et al., 2007), but the blood concentrations of these are not significantly affected by the amount of colostrum fed or the time of feeding in calves (BLUM & HAMMON, 2000a). They are all very high in colostrum (54.2 mM Ca, 52.8 mM P, 12.1 mM Mg) and dropping off rapidly to milking 2 (39.6 mM Ca, 40.7 mM P, 8.3 mM Mg) and staying on this level for the following weeks, only reaching normal levels at d 30 in lactation (TSIOULPAS et al., 2007).

Bioactive factors

Besides these different macro- and micronutrients, colostrum also contains an abundancy of different bioactive factors, like hormones, enzymes and growth factors, further aiming to improve the calf's growth or immune response.

An important developmental step in the maturation of the gastrointestinal tract (GIT) of newborn calves is the replacement of foetal enterocytes with the ability of pinocytosis with adult enterocytes in the first day of life after colostrum ingestion (MEALE et al., 2017). Major growth factors found in colostrum are transforming growth factor (TGF) and insulin-like growth factors (IGF) (ELFSTRAND et al., 2002). They control cell division, differentiation and apoptosis, as well as stimulating growth and development of the gastrointestinal tract (ELFSTRAND et al., 2002). Growth factors enhance the proliferation rate of cells in the gastrointestinal tract and therefore enhance absorption rates (BLUM & BAUMRUCKER, 2008). TGF plays an important role in embryogenesis as well as formation of bone and cartilage and proliferation of

cells (POULIOT & GAUTHIER, 2006). The most abundant form of TGF is TGF-ß2 with up to 90%, second is TGF-ß1 (PAKKANEN, 1998). The concentration of TGF-ß2 is related to IgA, IgG1 and IgM contents in colostrum, maybe due to positive stimulating effects of TGF-ß2 on the secretion of these into the milk. IGF-1 is found in large quantities in colostrum (PYO et al., 2020) and is one of the two most abundant growth factors (with IGF-2) with a concentration of around 50-2000 µg/L. It stimulates enterocyte and myocyte proliferation and reduces apoptosis rates in the gastrointestinal tract and is seen as the key enzyme in early-life gastrointestinal development (GODDEN, 2008). Growth hormone is the single most important hormone for *post natal* growth, as it induces the synthesis and release of IGF-1 (ELFSTRAND et al., 2002). Insulin-like-growth factor-I (IGF-I) concentrations are highest in colostrum and decline by around 60% to the second milking and declining further during the following days; in the tenth milking only 3% of the original IGF-I concentrations can be found in milk (BLUM & HAMMON, 2000a; TORTADÈS et al., 2023b). IGF-II, TGF and growth hormone can only be found in significant amounts in colostrum (BLUM & HAMMON, 2000a).

Another group of bioactive components found in colostrum is influencing the immune system. The immune system works with two different pathways, the innate and the adaptive immunity. In colostrum, multiple constituents influencing both pathways can be found. One important factor affecting the immune system of the newborn calves are the above-described immunoglobulins. Other factors are cytokines. Cytokines are small proteins that act in an autocrine or paracrine manner by binding to specific cellular receptors (SACERDOTE et al., 2013). The main cytokines in colostrum are interleukins, tumor necrosis factor and interferon gamma (SACERDOTE et al., 2013). They can regulate a wide range of immune responses in the innate and adaptive immune system. They are responsible for attracting neonatal lymphocytes in the gut to promote their maturation (HÜLSEBUSCH, 2018). As part of the innate immune system, the largest proportion of leukocytes in cattle are macrophages, lymphocytes (B-Cells, T-Cells, killer cells), and neutrophils (REBER et al., 2005). They enter the tissue of the calf after ingestion of colostrum and can modify the immune response and enhance the initial protection of the newborn calf against cellular infection (DONOVAN et al., 2007). In contrast to the consumption of immunoglobulins, studies showed, that leukocytes alone do not offer sufficient immune protection for the calf and lacking consumption of leukocytes only has a small effect on infection rates

(GODDEN et al., 2019). Macrophage concentrations do not differ between colostrum, TRANS and WM (CHANDLER et al., 2023). Lymphocytes have the smallest share of leukocytes and increase more than 2-fold from colostrum to WM (CHANDLER et al., 2023).

Colostrum not only contains beneficial contents for the calf but is also a source of prooxidants. Due to the high concentration of immune cells, that use reactive oxygen metabolites (ROM) generating mechanisms to kill bacteria, as well as the activity of the xanthinoxidase and lactoperoxidase, concentrations of ROM are high in colostrum (KANKOFER & LIPKO-PRZYBYLSKA, 2008). Additionally colostrum contains unsaturated fatty acids that are prone to oxidation (PRZYBYLSKA et al., 2007). However, antioxidative enzymes can also be found in colostrum, such as superoxide dismutase, catalase, and glutathione peroxidase (KANKOFER & LIPKO-PRZYBYLSKA, 2008). The redox balance of colostrum, i.e., the ratio between prooxidants and antioxidants, influences the passive immune transfer and the oxidative status of the calf (ABUELO et al., 2014). While the levels of prooxidants found in colostrum are maintained in TRANS and WM (KANKOFER & LIPKO-PRZYBYLSKA, 2008; ALBERA & KANKOFER, 2011), the antioxidative capacity increases in TRANS in the first 24 - 48 h after parturition (KANKOFER & LIPKO-PRZYBYLSKA, 2008; ALBERA & KANKOFER, 2011).

The most recently detected functional element of colostrum to be investigated are exosomes (ADMYRE et al., 2007). These are small extracellular vesicles, carrying proteins, RNA and miRNA. The proteins found in exosomes are potentially regulating immune response and growth (SAMUEL et al., 2017). The miRNA, small, non-coding RNA, also have immune modulatory functions (SUN et al., 2013). These exosomes are suspected to be taken up in the intestine of the calf, and their cargo then repacked and released into the blood system (KIRCHNER et al., 2020), but the exact kinetics of this mechanism are not known. The cargo of exosomes in colostrum contains proteins and RNAs with immune modulatory effects (SAMUEL et al., 2017), whereas in high lactation mostly proteins for milk synthesis can be found (REINHARDT et al., 2012). The *in vivo* importance of the exosomes and their cargo for the development of the calf are still unknown (VAN HESE et al., 2020). Extracellular vesicles decline in their concentrations from colostrum to WM, but the changes in the TRANS period are not known (SANTORO et al., 2023).

1.4. Effects of Transition Milk Feeding

As colostrum is much energy denser than WM and contains many factors to influence calf development on top of nutrition, the theory arose that feeding TRANS could also be beneficial for the calf. Due to gut closure, it is unclear to which extend the calf can utilize these beneficial components in the later days of life. A few authors dealt with this question in recent years and came to varying results.

Feeding TRANS for 4 days at 1.9 L per meal, compared to MR, resulted in better weight gains until weaning at day 56 (VAN SOEST et al., 2020). Similar results were seen by KARGAR et al. (2021). They replaced 6 L of waste milk (non-saleable milk, containing residues of antibiotics or other drugs) partially with TRANS (0.5, 1 or 2 L/d) for 21 days and had higher weight gains in calves fed the highest rate of TRANS. In contrast, DA SILVA et al. (2023) found no differences in the body weight of their calves over eight weeks. They fed 4 L TRANS per day over 3 days and compared this to the same level of WM feeding.

Health results in TRANS studies showed similarly differing results. CONNEELY et al. (2014) fed calves 2 L of TRANS twice daily for two or four feedings after colostrum intake and compared these groups to WM fed calves. They found no effect of TRANS feeding on the serum IgG concentrations in their calves, but lower odds ratios for a worse eye/ear or nasal score than the WM fed calves.

Contrary to this study, VAN SOEST et al. (2020) found no differences in eye, ear or fecal scores in their calves, but lower haptoglobin concentrations in the serum, whereas KARGAR et al. (2021) found fewer cases of diarrhea in their TRANS fed calves during the differential feeding period, but not afterwards. The same group also found that replacing some of WM with colostrum, mimicking TRANS, decreased the occurrence of diarrhoea, fever and pneumonia (KARGAR et al., 2020). In accordance with that, ZOLOVA et al. (2022) showed, that compared to WM, TRANS fed calves were at a lower risk for an infection with *Cryptosporidium* spp. They fed 4 L of TRANS or WM for two weeks and detected oocytes in the calves' feces after this period. Samples taken from TRANS fed calves were less often positive for *Cryptosporidium* spp. (ZOLOVA et al., 2022).

The most effects of TRANS feeding were seen in gut development and energy homeostasis of the calves. YANG et al. (2015) showed that feeding TRANS instead of colostrum had similar effects on efficiency of absorption, but improved antioxidative capacities, villi length and density compared to WM feeding. In their study they fed calves either 7 L of colostrum, TRANS or WM starting immediately

after birth until d 8. Similar results were seen by VAN SOEST et al. (2022). TRANS calves had larger villi in all small-intestinal sections, thicker submucosal layer and a bigger mucosal surface area in ileum and duodenum compared to calves fed MR. They also found indications for increased cell proliferation in TRANS calves. The findings on the villi length confirmed previous findings by BLUM (2006) and PYO et al. (2020). In the latter study, colostrum, WM and a mixture of colostrum and WM (to mimic TRANS) were compared for 3 days at 5% of BW given twice daily. TRANS fed calves showed greater villi heights in duodenum, jejunum and ileum than the WM fed group, with no differences to the colostrum fed group. Both groups also had a greater mucosal surface area in the proximal jejunum and ileum and TRANS calves also had a greater surface area in the distal jejunum (PYO et al., 2020). Additionally, INABU et al. (2019) compared colostrum, WM and a mixture of colostrum and WM (to mimic TRANS) feeding for 3 days at 5% of BW of the calves twice daily. They showed increased plasma glucagon-like-peptide 2 concentrations on d 3 in the colostrum-fed and the TRANS-fed calves, albeit lower in TRANS. This could indicate an enhanced nutrient absorption from the intestine.

DA SILVA et al. (2023) found lower lactate level in TRANS calves, indicating a better energy homeostasis. This benefit of TRANS could also been seen with the results of STEINHOFF-WAGNER et al. (2014). They showed higher glucose absorption rates by a stimulation of mucosal growth and increasing absorptive activity in the small intestine with TRANS feeding.

In further confirmation that the main effect of TRANS is seen in the gut, BAHADORI-MOGHADDAM et al. (2023), in the companion trial to KARGAR et al. (2021), showed marginal effects of TRANS on the serum metabolome of the calves, with only two metabolites showing significant differences: Ceramides were higher and phosphatidylserine lower. They conclude that most of the non-nutritive components in TRANS are not absorbed into the bloodstream and effects of its feeding therefore is limited to the local gut microbiome and growth and development of the intestinal epithelium (BAHADORI-MOGHADDAM et al., 2023).

TORTADÈS et al. (2023a) showed positive effects of TRANS feeding after a period of fasting, like a transport, compared to feeding MR. Feeding TRANS (or colostrum) for 4 or 10 days after a fasting period showed benefits on intestinal cells and helped to restore the intestinal absorptive function as well as improve gut immune protection.

Overall, these studies show that feeding TRANS can have positive effects on growth performance and health of the calves (CONNEELY et al., 2014; VAN SOEST et al., 2020; KARGAR et al., 2021). The most prominent effect achieved by TRANS feeding appears to be a predominantly local effect on the gastrointestinal tract (GIT) of the calves (PYO et al., 2020; VAN SOEST et al., 2022; ZOLOVA et al., 2022; BAHADORI-MOGHADDAM et al., 2023; TORTADÈS et al., 2023b).

2. Oxidative Status

The redox homeostasis of cells is maintained by the production of reactive oxygen species and the antioxidants to counteract them. Reactive oxygen species or reactive oxygen metabolites (ROM) are free radicals which are produced continuously during normal metabolism. They are not always harmful. Superoxide (O_2^-) and hydrogen peroxide (H_2O_2) for example are involved in the chemistry of several enzymes and used by phagocytic cells to kill bacteria (MILLER et al., 1993). Under normal conditions, the antioxidative system is capable to antagonize these prooxidants by delaying or inhibiting the oxidation of oxidizable substrates. Stress and pathogenesis of diseases can lead to an accelerated production of ROM (KAUSHAL et al., 2019). Activated macrophages and neutrophils lead to a higher production of ROM and its derivates, called the oxidative burst. If this production exceeds the capabilities to safely be removed by the antioxidants, ROM can cause direct and indirect damage in abstracting electrons from molecules resulting in a chain reaction (MILLER et al., 1993), which can lead to disruptions in the cell membrane and damage to lipids, proteins and DNA. This process, if sustained for a longer period, is known as oxidative stress (SIES et al., 2017) and can lead to further inflammatory processes and diseases (KAUSHAL et al., 2019). It is per definition a disbalance between prooxidants and antioxidants in favor of the former and therefore only measurable through its two components, the ROM and antioxidants in the system and the ratio between them (ABUELO et al., 2014; SIES et al., 2017). Apart from ROM, other reactive species play a role in redox biology and therefore OS. These include reactive nitrogen species, reactive sulfur species, reactive carbonyl species, and reactive selenium species (SIES et al., 2017). As they make up only a small side of prooxidants, they will not be discussed further in this thesis.

2.1. Reactive Oxygen Metabolites

Formation of reactive oxygen metabolites

The common allotrope of elemental oxygen on Earth is dioxygen. O_2, is stable and undergoes a stepwise reduction process in normal metabolism. It has two unpaired electrons with parallel spins, enabling it to accept electrons from donors. The redox reaction with transfer of electrons from one species to another is a vital part in a living system, as it is the chain reaction of oxygen from air used for energy synthesis via adenosine triphosphate (GULCIN, 2020). In a complete, four-step reduction, oxygen can accept four electrons, resulting in the formation of water:

$$O_2 + e^- + H^+ \rightarrow O_2^- + e^- + H^+ \rightarrow O_2^{2-} + e^- + H^+ \rightarrow OH\cdot + e^- + H^+ \rightarrow H_2O$$

Most consumed oxygen gets fully reduced to H_2O in the terminal cytochrome of the oxygen transport chain in the mitochondria (GULCIN, 2020). If the reduction is only partial, the result is an oxygen molecule with one or more unpaired electrons, like superoxide anion (O_2^-) or hydroxyl (OH^-) and hydrogen peroxide (H_2O_2, Figure 1) (BRIVIBA et al., 2008; KAUSHAL et al., 2019). These free radicals are molecules with at least one unpaired electron and therefore highly unstable, also called reactive oxygen metabolites (ROM). Characteristics of ROM are their short life, and the damage caused to the surrounding tissue (GULCIN, 2020). They can react with surrounding molecules by electron donation, electron acceptance, reducing radicals or oxidizing radicals (CAROCHO & FERREIRA, 2013).

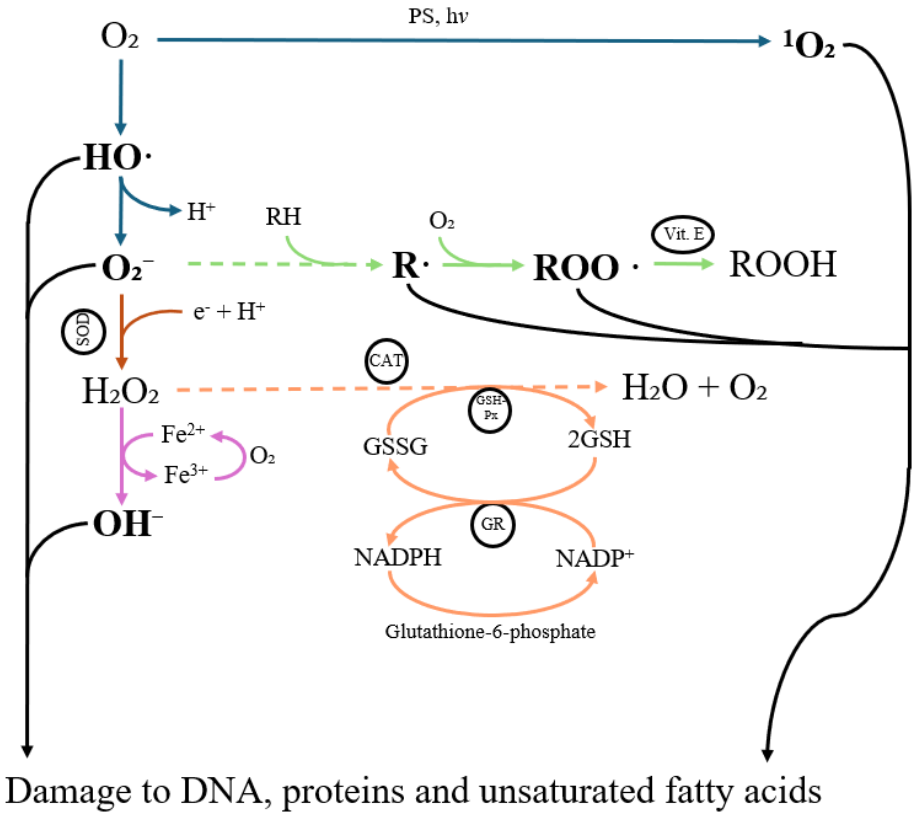

Figure 1: Production of reactive oxygen metabolites (ROM) and antioxidative defense mechanisms. Red arrows show the Haber-Weis reaction, where SOD dismutate O_2^- to H_2O_2; pink arrows show the Fenton reaction, where the reaction of H_2O_2 with metal ions yields the highly reactive OH^-; green arrows show the process of peroxidation to free radicals and orange arrows the antioxidative reaction of GSH-Px with its recovery by glutathione reductase with use of NADPH. SOD: Superoxide dismutase enzyme, CAT: catalase enzyme, GSH: Glutathione, GSSG: Glutathione disulfide, GR: glutathione reductase, GSH-Px: gluathione peroxidase, $NADP^+$/NADPH: nicotinamide adenine dinucleotide phosphate PS: exogenous or endogenous photosensitizer, hv: light. R: free radical (lipid or protein), ROO: peroxylradical (lipid or protein). Modified from BRIVIBA et al. (2008); KAUSHAL et al. (2019); GULCIN (2020).

Apart from the synthesis in the mitochondria, ROM are also a product of several enzyme reactions. NADPH oxidase, aldehyde oxidase or xanthine oxidase transfer electrons from their respective substrate to O_2, leading to the production of O_2^- (Figure 1, HALLIWELL and GUTTERIDGE (2015)). Additionally many cytochrome P-450 enzymes that are involved in production of cholesterol, or steroid hormones, lead to the production of ROM (MILLER et al., 1993). External noxae like UV radiation, environmental pollution, ozone or drugs can also lead to tissue damage and thus cause the production of ROM (Figure 1, BRIVIBA et al. (2008)).

Effects of ROM

Under a normal physiological redox equilibrium, ROM function as important regulators for signal transduction pathways in biological cellular responses, such as cell proliferation, growth factor signaling, immune responses and autophagy (MILLER et al., 1993; KAUSHAL et al., 2019). The ROM play an important role in immune response; phagocytic cells synthesize in their defense against pathogens large amounts of O_2^- (GULCIN, 2020). The redox status of the cell participates in the modulation of transcriptional factors. High concentrations of ROM can lead to the activation of these factors, who in turn regulate the expression of cytokines and other mediators of inflammatory response (ASEHNOUNE et al., 2004). Additionally ROM concentrations modulate autophagy in cells as a protective measurement to eliminate damaged macromolecules and to provide cytoprotection (KAUSHAL et al., 2019). Nonetheless all forms of ROM are, as stated by the name, highly reactive and, in case of a massive overproduction or insufficient reduction, are aggressive oxidants. Depending on the location of ROM in the cell, the damage caused by them can vary. Oxidation of proteins can alter their function and affect productivity, transcription factors and other regulatory factors (INUPAKUTIKA et al., 2016). Similar effects can be seen with the oxidation of lipids or DNA, leading to further chain reactions and disrupting the physiological cell metabolism and damaging the cell membrane (MILLER et al., 1993). Oxidative modification in proteins can be at the level of a specific amino acid, of free radical-mediated peptide cleavage, and of formation of protein cross-links due to a reaction with lipid peroxidation products (CAROCHO & FERREIRA, 2013). Damage to DNA can be in form of modification of all bases, frame shifts, strand breaks, DNA-protein cross-links, and chromosomal arrangements (CAROCHO & FERREIRA, 2013). Lipid peroxidation is more likely in polyunsaturated fatty acids than saturated fatty acids or monounsaturated fatty acids, as the radical abstracts a hydrogen atom from a methylene carbon on a side chain (CAROCHO & FERREIRA, 2013). These can also stimulate free radical chain reactions, subsequently further damaging the cell and finally lead to diseases (KAUSHAL et al., 2019).

Measurement of ROM

Due to their short lifespan, measurement of ROM is difficult and usually derivates are identified. A definite assessment of these measurements still poses some challenges, as they can be identified and repaired by the body and additionally these molecules can still react further (BRIVIBA et al., 2008). A possible way to asses oxidants is by the detection of ROM (dROM) test, which estimates the concentrations of hydroperoxides in blood by monitoring the formation of radical cations formed in the reaction of alkoxy and peroxy radicals derived from hydroxy peroxide with an additive (ALBERTI et al., 2000). Additionally, several methods are available to assess the damage to the different molecules caused by ROM. Damage to DNA can be measured by measuring the created bonds or etheno-adducts of DNA bases or nucleosides or the comet assay, but sensitivity and reproducibility of these methods are still relatively low (BRIVIBA et al., 2008; GONZALEZ-HUNT et al., 2018). The most common damage to proteins due to oxidative stress is the insertion of a carbonyl group, these protein carbonyls are often used as marker of oxidation either in a Western blot or with fluorescence probes (BARAIBAR et al., 2013). The main setback in these methods is the reproducibility and accuracy due to precipitation of lipids, resulting in a overestimation of the advanced oxidation protein products (AOPP) (HANASAND et al., 2012). The oxidative damage of lipids results in hundreds of products (BRIVIBA et al., 2008). The chosen method for their assessment depends on the sample type, the targeted biomarker and the objective (NIKI, 2014). Most commonly used is the thiobarbituric acid-reactive substances (TBARS) assay, measuring the concentrations of malondialdehyde (MDA) in a sample (GUTTERIDGE & QUINLAN, 1983). MDA is one of the end products of lipid peroxidation (GUTTERIDGE & QUINLAN, 1983).

2.2. Antioxidants

HALLIWELL and GUTTERIDGE (1995) defined antioxidants as “any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate”. The main antioxidant defense is provided by enzymes or low-molecular-mass-components and can be either endogenous or exogenous (Figure 2, SIES et al. (2017)). The mechanisms by which the antioxidants defense works can be divided into different principles. These are (1) prevention, (2) interception, and (3) repair of the oxidant (SIES et al., 2017). Preventive antioxidants are either enzymes that avoid the release of radicals or ones who change the metabolite into a less harmful product and thus lower the risk for

further damage (SIES et al., 2017). Interceptive antioxidants disrupt the chain reactions of peroxidation by directly counteracting the oxidant by chemical reduction (SIES et al., 2017). The last group of antioxidants are those that repair damage caused by oxidants or recover other antioxidants to a working form (SIES et al., 2017).

In addition, antioxidants can be sorted in to two categories: primary antioxidants, which directly scavenge free radicals, or secondary antioxidants which operate by a variety of mechanisms and do not directly scavenge a free radical (GULCIN, 2020).

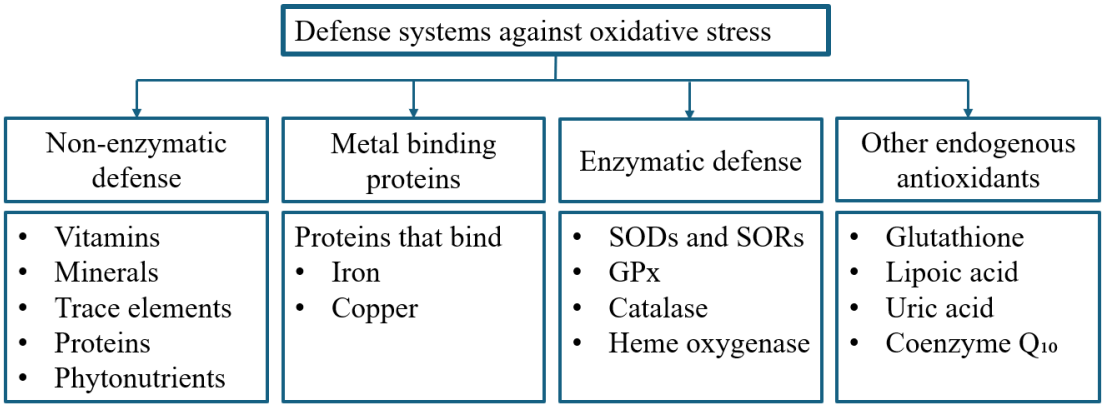

Figure 2: Categorization of antioxidative defense mechanisms in mammalian cells. Antioxidants can be classified into four categories: non-enzymatic defense, metal binding proteins, enzymatic defense and other endogenous antioxidants. GPX: Glutathione peroxidase, SOD: Superoxide dismutase, SOR: Superoxide radical. Modified from PONNAMPALAM et al. (2022)

Endogenous antioxidants

Glutathione-peroxidase (GSH-Px or GPx) is the most abundant enzymatic antioxidant which can remove O_2^- and H_2O_2 before they approach the Fenton reaction (Figure 1, 2). The reduction of the peroxide is accompanied by the oxidation of the reduced glutathione to glutathione disulfide and can be regenerated by the glutathione reductase with nicotinamide adenine dinucleotide phosphate ($NADPH_2$) and glucose-6-phosphate (MILLER et al., 1993; LOSADA-BARREIRO et al., 2022).

Another important enzymatic antioxidant is catalase. Catalase converts H_2O_2 to H_2O and molecular oxygen (Figure 1, 2, CAROCHO and FERREIRA (2013); (LOSADA-BARREIRO et al., 2022)). One molecule of catalase alone converts up to 6 billion molecules of H_2O_2 and its efficiency has been linked to various diseases (CAROCHO & FERREIRA, 2013; LOSADA-BARREIRO et al., 2022).

The last primary enzymatic antioxidant is superoxide dismutase (SOD). It is one of the most powerful intracellular antioxidants and reduces O_2^- to the slightly less reactive H_2O_2 and O_2 (Figure 1, 2, CAROCHO and FERREIRA (2013)). The activity of this

enzyme is dependent on different metallic cofactors (Fe, Zn, Cu and Mn), varying with the subcellular distribution (LOSADA-BARREIRO et al., 2022).

Other endogenous antioxidants (meaning they can be produced by cells) are mostly proteins with a thiol or phenolic group who can easily scavenge ROM. Glutathione, a intracellular thiol, cofactor of GSH-Px, has chelating properties and can regenerate vitamin C and E by donating hydrogen or an electron (CAROCHO & FERREIRA, 2013; LOSADA-BARREIRO et al., 2022). Coenzyme Q10 is present in all eukaryotic cells and membranes and is an important part in the respiratory chain (Figure 2). As an antioxidant it prevents the formation of peroxyl radicals and can regenerate other antioxidants.

Exogenous antioxidants

Exogenous antioxidants are not produced in the cell but have to be ingested through the diet and can be divided into three subgroups: polyphenols, some vitamins and derivatives (FULORIA et al., 2021). They function mostly as an electron donor to free radicals to neutralize them. Flavonoids are the most abundant natural antioxidants and can act as reducing agents, metal chelators and can activate antioxidant enzymes (CAROCHO & FERREIRA, 2013; FULORIA et al., 2021). Vitamin A is a carotenoid produced in the liver, from its ingested precursor carotene, and has the ability to combine with peroxyl radicals before they propagate peroxidation of lipids (CAROCHO & FERREIRA, 2013). Vitamin C can scavenge superoxide radical anions, hydrogen peroxide, hydroxyl radicals, singlet oxygen and reactive nitrogen oxide and can also regenerate vitamin E from its tocopheroxyl radical form to an intermediate form (CAROCHO & FERREIRA, 2013; PONNAMPALAM et al., 2022). In high concentrations it can also act as a pro-oxidant. Vitamin E donates phenolic hydrogen to peroxyl radicals to halt the chain reaction of lipid peroxidation mainly of membrane fatty acids as it is lipid-soluble (CAROCHO & FERREIRA, 2013; PONNAMPALAM et al., 2022). Metals function predominantly as prooxidants in a reaction with oxygen, creating ROM. On the other hand, they also are essential for the antioxidative system as cofactors in enzymes and proteins, e. g. selenium as a central part of GSH-Px, and in the regeneration of other antioxidants like vitamin C (PONNAMPALAM et al., 2022).

Measurement of antioxidants

Due to the great variety of antioxidative systems in the metabolism and the small proportion of them being actually accessible for measurement, a statement about the overall antioxidative status is not possible (BRIVIBA et al., 2008). The best solution is to use multiple methods, targeting different components of the antioxidative system (CAROCHO & FERREIRA, 2013). Most of the currently used methods employ the same principal of a colored racial and the monitoring of the reduction or scavenging of the radical in the sample by a spectrometer (SIES et al., 2017; GULCIN, 2020). For example the FRAP assay (ferric reducing ability of plasma) measures the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), resulting in an increase of absorbance in the spectrometer at 593 nm (CHEN et al., 2003). This method measures the ability of electron transfer in a sample and gives an overview of the total antioxidant capacity in combination with other methods (GULCIN, 2020).

2.3. Oxidative Status in Cattle

Oxidative status during the transition period

The most researched period in the oxidative status of cattle is the transition period in dairy cows. The transition period is typically defined as the last three weeks of the dry period, to three weeks after parturition. In this time the cow switches from not producing milk as involution and preparation for lactation to the high demands of milk production (GRUMMER, 1995). This period confronts the dairy cow with several metabolic and endocrine changes (GRUMMER, 1995) and leads to insulin resistance and usually reduced feed intake during this time (KOSTER & OPSOMER, 2013). In combination with the higher energy demand due to the beginning milk production, this leads to a negative energy balance, lipolysis and weight loss, as well as hypocalcemia and a reduced immune function in the cow (KOSTER & OPSOMER, 2013). This is the time where most of the diseases in cows are seen, including retained placenta, metritis, mastitis, ketosis or a displaced abomasum (SORDILLO & RAPHAEL, 2013).

The preparation for the onset of lactation already starts a few weeks before calving. With the changes in the metabolic activity a decrease in the concentrations of ROM with a simultaneous increase in antioxidant activity can be seen (BERNABUCCI et al., 2005; CASTILLO et al., 2005). After calving, this balance shifts as the concentrations of ROM increase with the now highly enhanced metabolic activity while antioxidant defenses get depleted (BERNABUCCI et al., 2005; KONVIČNÁ et al., 2015). Additionally, the liver function is typically reduced in the early *post partum*

period. This might also explain the lower antioxidant capacities, as albumin is synthesized in the liver and linked with antioxidative capacities (CASTILLO et al., 2005). The energy reduction *ante partum* additionally results in an increased fat mobilization and with that an increased concentration of lipid peroxides in the peripartum period (CASTILLO et al., 2005; KONVIČNÁ et al., 2015).

Oxidative status during lactation

In healthy cows, antioxidant parameters peak after calving, indicating that they can cope efficiently with the disbalance and protect themselves against oxidative stress (CASTILLO et al., 2005). During lactation, ROM concentrations remain relatively high, due to the high energy demands of milk production, whereas antioxidants decline because of their depletion with milk and consumption to counteract ROM production (ABUELO et al., 2016). To reliably assess the amount of oxidative stress in a lactating cow, ABUELO et al. (2013) proposed the estimation of the oxidative stress index (OSi) as a ratio between prooxidants and antioxidants. Changes in this arbitrary index suggest a disbalance in the redox balance and a risk for oxidative stress. With this index, they saw an oxidative challenge in cows after parturition, but also during peak lactation (ABUELO et al., 2013). In contrast, with the abrupt cessation of milking at dry off to allow the udder a regeneration period for the following lactation, the OSi showed a balanced redox status in the cows as well as lower levels of lipoperoxide production (CASTILLO et al., 2006; PUTMAN et al., 2018). ROM production increased at dry off, but so did the antioxidant potential (PUTMAN et al., 2018).

Factors influencing the oxidative status

One of the factors associated with the oxidative status in transition cows is the body condition score (BCS). Cows with a higher BCS favor lipid mobilization after calving and therefore also lose more weight after calving. The non-esterified fatty acids mobilized are used as energy source through gluconeogenesis as most of the glucose is used for milk production at the beginning of lactation (KOSTER & OPSOMER, 2013). In over-conditioned cows, the mobilization of fatty acids can exceed the capabilities of hepatocytes and lead to the accumulation of triglycerides in the liver, resulting in a so called fatty liver (SORDILLO & RAPHAEL, 2013). With an imbalance in the oxidative status, the liver function can be further decreased due to higher concentrations of oxidants that need to be removed (BERNABUCCI et al., 2005). Another factor influencing the oxidative status during the peripartum period is the number of lactations. URH et al. (2019) showed that cows in their first or second

lactation are faced with an increased oxidative status and thus risk for oxidative stress when compared to cows with more lactations. Signs for oxidative stress can also be seen in cows with acute mastitis. Infection in the udder leads to an accumulation of lymphocytes, macrophages and neutrophils and their defense mechanisms lead to increased production of ROM in the milk. Additionally, the antioxidative activity of milk is lower in infected cows (ANDREI et al., 2016). However, the systemic impact on the cow is not yet fully researched (IBRAHIM et al., 2016; ZHANG et al., 2022). Heat stress also leads to an increased oxidant challenge. Erythrocyte SOD and GSH-Px activity, as well as lipid peroxidation (measured with the TBARS method), are higher in cows faced with heat stress (BERNABUCCI et al., 2002; TANAKA et al., 2011). Left displaced abomasum also leads to an imbalance in the redox status due to the inflammatory response and tissue damage that lead to the production of free radicals (FIORE et al., 2019).

Strategies to manage the oxidative status in cows

Some antioxidants can be synthesized by the body, other need to be administered orally. But relying solely on naturally produced antioxidants from the body or feedstuff puts cows at risk of deficiency, especially in times of increased demands. Supplementation of vitamins and trace elements during the transition period and into peak lactation proved to improve the antioxidant status of the cow to minimize the effects of prooxidants (ABUELO et al., 2015). The supplementation of e.g. vitamin E or selenium also proved to impact the capabilities of bovine neutrophils and macrophage and control intercellular ROM accumulation (SORDILLO & AITKEN, 2009). This can then reduce the severity of diseases like mastitis, metritis or ketosis (ABUELO et al., 2016). Supplementing cows with vitamin E can reduce the risk of clinical mastitis, mostly against environmental pathogens (ABUELO et al., 2015). Additionally both vitamin E and selenium supplementation can reduce the somatic cell count in milk, a sign for subclinical mastitis (ABUELO et al., 2015). Oxidative stress seems to play a key role in the improper release of the placental membranes, and cows with endometriosis show significantly lower blood levels of Ca, Cu, Mg and Zn (ABUELO et al., 2015). Supplementing these minerals in the transition period can reduce the bacterial load in these cows, but the mechanisms are not yet fully understood (ABUELO et al., 2015).

2.4. Oxidative Status in Calves

Early life development of the oxidative status

The first challenge for the oxidative balance of the newborn calves happens immediately after birth. The calf changes from oxygen supply via the blood of the dam to oxygen intake via breathing by themselves which leads to an increased production of ROM. GAÁL et al. (2006) showed additionally that the antioxidative system starts to develop in the first days of life. The combination of high ROM production and rudimental antioxidative defense puts the calf at a high risk for oxidative stress and its consequences after birth. A healthy calf is usually able to cope with this challenge (GAÁL et al., 2006). However, colostrum contains not only antioxidants, but also ROM in higher concentrations (KANKOFER & LIPKO-PRZYBYLSKA, 2008). With the consumption of colostrum, these ROM and antioxidants are also absorbed and the redox balance of the newborn is further influenced (ABUELO et al., 2014). The concentrations of ROM decrease in the following days and components of the antioxidative system increase (GAÁL et al., 2006; ABUELO et al., 2014). In studies with WM feeding, a steady increase in the antioxidant capacity could be noted in the first month of life until weaning (ALBERA & KANKOFER, 2011; RANADE et al., 2014). In contrast, another study showed no increase of the antioxidative capacity in MR-fed calves until weaning (ABUELO et al., 2014). The reason for this is unknown, a possible explanation is the lower antioxidative potential of MR compared to WM (ABUELO et al., 2019). With its higher content in saturated fatty acids and the different amino acid composition, WM has a higher antioxidative activity than most MR, in addition to a higher concentration of vitamins, that are often not supplemented in MR (SOBERON et al., 2012a). These additional antioxidants consumed by the calves fed WM could be the reason for the increased antioxidative capacity and it is therefore recommended to supplement MR with further antioxidants (ABUELO et al., 2019). However the oxidative status in calves is not influenced by the amount of milk replacer they get fed during the rearing period (SEIBT et al., 2021). The development of the oxidative marker are parallel in calves fed 5.7 L/d or 10 L/d. Additionally, the majority of studies also reported no effects of antioxidative supplementation on average daily gain (ADG) and BW development in growing calves (CARLSON & ABUELO, 2024). Further research is needed to establish the mechanisms behind the development of the oxidative system in growing calves and the effect further supplementation could have on their performance.

Oxidative status during weaning

Another challenge for every calf is the process of weaning. Weaning induces an accumulation of ROM (MAJLESI et al., 2021), most likely due to the increased mobilization of body fat reserves during this period (RANADE et al., 2014). Reports whether this increase in ROM also results in oxidative stress as it exceeds antioxidant capacities are differing (RANADE et al., 2014; MAJLESI et al., 2021) and this is probably linked to the age at weaning and solid food already consumed at this time (RANADE et al., 2014). RANADE et al. (2014) found lower antioxidative potential in their calves during weeks 6 to 12 of their life, the typical timeframe for weaning in conventional rearing systems. With weaning in this time, an additional stressor is added to the weak oxidative system and could exacerbate the disease risk for the calves (RANADE et al., 2014). Shifting the weaning age to a later point in life, when the antioxidative capacities are higher, could lessen the risk for oxidative stress. Furthermore, a slow decrease in milk allowance and an early supplementation with solid feed might help the calves to meet their nutritional requirements during weaning, as they would already be accustomed to solid feed. Supplementing calves with parenteral minerals and vitamins could also stabilize the oxidative status during the transition time (MATTIOLI et al., 2020). As there are no further studies measuring the oxidative status of calves during weaning, further research is needed to evaluate the physiological development of the oxidative status at that time and possible preventive measures that can be taken.

Factors influencing the oxidative status

The oxidative status of calves is already influenced at conception. Production of the calf via artificial insemination (AI), in vitro fertilization (IVF) or cloning, showed different effects on the redox balance of calves (DANTAS et al., 2020). Calves produced by IVF and cloning showed increased production of ROM compared to AI calves after birth (DANTAS et al., 2020). IVF calves showed greater enzymatic activity to adapt to this increased production of ROM (DANTAS et al., 2020) in contrast to cloned calves. The higher concentrations of ROM may be correlated to the greater rates of mortality, mainly due to respiratory diseases seen in IVF and cloned calves (DANTAS et al., 2020).

Another factor impairing the oxidative status of the calf in the first month of life is metabolic stress of the dam during late gestation (LING et al., 2018). Calves exposed to high maternal lipid mobilization show altered gene expression related to the

oxidative metabolism (LING et al., 2018). Supplementing the dam during this period with different substrates, like methionine or fatty acids can influence the oxidative status of the newborn and prevent oxidative stress (JACOMETO et al., 2016; LIERMANN et al., 2021).

At birth, dystocia, e.g. abnormalities at calving, lead to increased lipid peroxidation in affected calves (VANNUCCHI et al., 2019). GSH-Px activity is increased, with its exhaustion shortly after (VANNUCCHI et al., 2019). Due to the stressful situation and thus rise in adrenaline and glucocorticoids, ROM production in these calves is increased and antioxidant defenses still deficient, which puts them at a higher risk for oxidative stress (KANDEMIR et al., 2016). Oxidative stress in newborn calves can compromise functions of their lymphocytes and increases the risk for infections (CUERVO et al., 2021). During infections with diarrhea pathogens, macrophages are activated to eliminate these pathogens. The activity of macrophages produces ROM and can lead to oxidative damage, as it is seen in calves infected with *E. Coli* or Coronavirus (AYDIN et al., 2022). In calves with bronchopneumonia, markers for oxidative stress are increased (AL-QUDAH, 2009). Additionally, in calves with chronic bronchopneumonia, the activity of antioxidative enzymes is decreased, further disbalancing the oxidative system (AL-QUDAH, 2009). In contrast, calves with acute bronchopneumonia show increasing activity of antioxidant markers (AL-QUDAH, 2009). This highlights the enhanced risk for damage in the lungs of chronically affected animals due to the apparent depletion of the antioxidative system (AL-QUDAH, 2009). To lessen the damage done by excessive ROM production in cases of illness, supplementation of vitamins or trace elements can improve immunity in calves (BITTAR et al., 2018; MATTIOLI et al., 2020).

III. Transition milk - Effect on feeding behavior, development, and oxidative status

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Long-term effects of transition milk feeding on feed intake, feeding behavior, development, and oxidative status in Holstein calves

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Long-term effects of transition milk feeding on feed intake, growth performance, feeding behavior, and oxidative status of Holstein calves

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ABSTRACT

This study investigated the long-term effects of feeding 5-d transition milk (TRANS) compared with milk replacer (MR) on parameters including intake, growth, feeding behavior, and oxidative stress. Fifty Holstein calves (30 females and 20 males) were fed 12 L/d of either TRANS or MR for the first 5 d after an initial colostrum feeding of 3.5 L. Thereafter, all calves were fed with 12 L of MR/d (140 g/L) and were gradually weaned starting in wk 8 until wk 14. Throughout the 14 wk, the calves had unrestricted access to concentrate (up to 9.8 kg/calf per day), hay, and water. After weaning all heifers were fed a TMR for young cows. Oxidative status was assessed in blood samples from birth to first insemination. Parameters assessed included the ferric-reducing ability of plasma (FRAP) for antioxidant capacity and the concentration of reactive oxygen metabolites via detection of reactive oxygen metabolites (dROM) assay. In addition, the activity of glutathione peroxidase (GSHPx) and oxidative damage in the form of lipid peroxidation as thiobarbituric acid-reactive substances (TBARS) and as advanced oxidation protein products (AOPP) were measured. An oxidative stress index was calculated: dROM/FRAP × 100. Total protein concentration was also quantified via Bradford assay. The only significant difference in feeding behavior between the 2 treatment groups was a higher concentrate intake by the TRANS calves during the weaning phase. Body weight and ADG did not differ significantly between the TRANS and MR groups. The TRANS calves showed a trend for fewer cases of health disorders. Markers of oxidative status, including TBARS, AOPP, GSHPx, FRAP, and reactive oxygen metabolites, showed no treatment effects but varied significantly over time. Of note, the oxidative stress index, as ratio between pro- and antioxidants in both groups, peaked during weaning and then returned to baseline, suggesting an effective response to this transition phase. Overall, the results indicate that feeding TRANS during the first 5 d of life had no long-term effect on the parameters studied as compared with MR feeding under the present rearing conditions. These results provide insight into the changes of oxidative status with age and confirm that the relatively high milk feeding level, with slow and late weaning, enables calves to adapt well to solely solid feed.

Key words: calf, colostrum, health metrics, antioxidant capacity

INTRODUCTION

Bovine colostrum is a nutrient-rich substance that provides passive immunity through immunoglobulins and contains various bioactive compounds such as cytokines, lactoferrin, and hormones that support the development of the calf's gastrointestinal tract (Hammon et al., 2000; Blum and Baumrucker, 2008). In addition to colostrum feeding, the National Academies of Science, Engineering, and Medicine (NASEM, 2021) recommend the administration of transition milk as an important aspect of early feeding management in calf rearing. Transition milk is defined as milk collected after milking colostrum, between the second and sixth milking following parturition (Godden, 2008). According to European Union regulations (European Council, 2004), transition milk is milk produced up to 5 d postpartum and may not be sold. The concentrations of immunoglobulins and bioactive compounds in transition milk gradually decrease compared with colostrum and eventually reach a composition similar to that of mature milk (Inabu et al., 2019; Fahey et al., 2020; Fischer-Tlustos et al., 2020). Studies have shown increased weight gain over the whole rearing period, when 5.7 L of transition milk were fed in the first 3 d compared with milk replacer (**MR**; Van Soest et al., 2020) or when 6 L of whole milk (**WM**) is partially replaced by transition milk over 21 d

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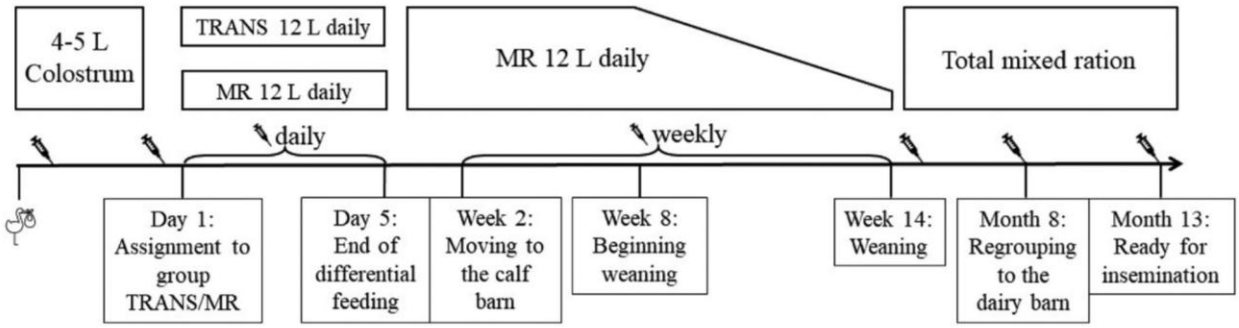

Figure 1. Feeding regimen and blood sampling schedule for calves throughout the rearing period.

(Kargar et al., 2021). In addition, feeding 4 L of transition milk over 2 d reduces the likelihood of poorer eye/ear or nose scores (Conneely et al., 2014) over a 56-d rearing period, and feeding 5.7 L/d reduces the likelihood of poorer fecal scores (Van Soest et al., 2022). In addition, feeding 4 L/d transition milk for 2 wk reduces the risk of *Cryptosporidium* spp. infection in this time (Zolova et al., 2022). Previous research has also shown that transition milk feeding supports the calf's developing gastrointestinal tract by providing essential nutrients, growth factors, and bioactive compounds that stimulate the development of the intestinal epithelium (Pyo et al., 2020; Van Soest et al., 2022).

The beneficial effects of transition milk can be attributed to several factors, including additional nutrient intake compared with WM or MR. Transition milk has a 4-fold greater protein content than WM, mainly due to higher IgG and casein contents. It also has a higher fat content (6.7% average in colostrum, 3.6% in WM) and greater concentrations of vitamins A, E, and B_{12}, and selenium (Godden et al., 2019). Additionally, transition milk provides greater concentrations of insulin-like growth factor-1 and glucagon-like peptide-2, which promote maturation of the small intestine (Pyo et al., 2020). Calves fed transition milk also show improved glucose uptake (Steinhoff-Wagner et al., 2014) and lower lactate levels (da Silva et al., 2023), indicating a benefit for maintaining energy homeostasis. In addition, transition milk supports the local intestinal immune system due to its content of antibodies and bioactive compounds (Schalich et al., 2021).

Exposure to an oxygen-rich environment after birth is associated with an increase in the production of reactive oxygen species (**ROS**; Abuelo et al., 2019; Carlson and Abuelo, 2024). At this early stage, calves' antioxidant mechanisms are underdeveloped (Albera and Kankofer, 2011; Ranade et al., 2014), leading to an imbalance between pro-oxidants and antioxidants that can cause oxidative stress when ROS levels exceed the capacity of antioxidant systems (Cuervo et al., 2021). Colostrum naturally contains ROS as a by-product of its defense mechanisms against microorganisms and lipids that are prone to peroxidation (Abuelo et al., 2019). The consumption of transition milk by calves after colostrum intake has been shown to improve the balance between antioxidants and pro-oxidants, as evidenced by an increase in antioxidant content and a decrease in the content of pro-oxidants such as reactive oxygen metabolites (**ROM**; Kankofer and Lipko-Przybylska, 2008; Abuelo et al., 2014, 2019). Feeding transition milk to calves in the first days of life provides important antioxidants that help to regulate their redox balance and reduce oxidative stress. Compared with calves fed MR, calves fed transition milk (**TRANS**) consume higher levels of antioxidants, potentially improving their oxidative status and promoting growth and health during the rearing phase (Soberon et al., 2012b; Abuelo et al., 2014).

Early calf nutrition plays a crucial role in laying the foundations for later development (Soberon et al., 2012a). The concept of lactocrine programming states that bioactive, non-nutritive factors from milk are passed from dams to suckling offspring, influence postnatal development, and have long-term consequences that extend into maturity (Bagnell and Bartol, 2019).

We therefore hypothesized that feeding TRANS in the first 5 d of life will provide calves with not only higher nutritional value but also bioactive compounds that may improve their oxidative status, growth performance, and overall health compared with calves fed MR, well beyond the time of transition milk feeding.

We aimed to investigate feeding behavior, growth performance, and oxidative marker in calves fed TRANS or MR to determine whether the early benefits of transition milk may translate into long-term improvements in calf development and health after weaning. Additionally, this study provides insights into the development of the systemic oxidative status of calves as they progress into

Table 1. Composition and ingredients of milk replacer, pelleted calf concentrate and total mixed ration

Item (% of DM)	Milk replacer[1]	Concentrate[2]	TMR[3]
Crude protein	22.5	10.0	17
Crude fat	25.0	4.0	—
Crude fiber	0.0	6.7	17
Ash	7.0	7.2	—
Ca	1.1	1.0	0.17
P	0.7	0.6	0.26
Na	0.4	0.25	0.02
Lysin	2.0	1.0	—
Methionine	0.75	0.4	—

[1]Milk replacer ingredients: 50.0% skim milk powder, vegetable oil, whey powder, whey powder (partly desugared).

[2]Concentrate ingredients: 35% maize, 18.7% soy extraction meal, 13.0% beet molasses pulp, 7.6% rapeseed extraction meal, 7.5% wheat, 4.9% barley, 4.8% linseed, 3.5% beet molasses, 3.2% wheat gluten, 3.2% wheat bran, 1.2% calcium carbonate, 0.3% natrium chloride, 0.06% magnesium oxide.

[3]Total mixed ration (TMR) ingredients: 38.88% grass (second cut), 35.49% corn silage, 9.05% straw, 7.32% concentrate meal mix, 0.37% mineral mix.

reproductive maturity, by measuring oxidative markers up to first insemination

MATERIALS AND METHODS

The current study was conducted at the Educational and Research Centre for Animal Husbandry, Hofgut Neumühle, Münchweiler a. d. Alsenz, Germany, following the guidelines of the German Law for Animal Welfare by permission of the local authority in charge (G 21-20-049; Landesuntersuchungsamt Rheinland-Pfalz, Koblenz, Germany).

Animals, Diets, Feeding, and Management

This study followed 50 Holstein-Friesian calves (30 females, 20 males) from birth to the postweaning period (males) and to first insemination (females), respectively. The dams were housed in calving pens with deep straw bedding and fed a dry cow TMR with free access to water. Calving was monitored with Moocall sensors (Moocall, Dublin, Ireland) to ensure that blood could be collected before colostrum intake. Cows were milked after calving, and calves were included in the study once at least 3.5 L of colostrum had been milked. The quality of the colostrum was determined using a specific gravity spindle and a digital Brix refractometer. The calves were given 3.5 L of colostrum by bottle within the first 2 h after birth, as shown in Figure 1. If necessary, the remaining amount was administered via an esophageal tube to ensure a total intake of up to 4 L (n = 29).

After colostrum feeding, each calf received 7 mL of iron suspension by oral administration (Ursoferran 150 mg/mL, Serumwerk Bernburg AG, Bernburg, Germany), and the navels were disinfected with a 10% povidone-iodine solution (Vet-Sept Solution, aniMedica GmbH, Senden-Bösensell, Germany). Birth weight was measured, subtracting the amount of colostrum ingested, and the calves were moved to individual pens padded with straw, where they were offered hay and water ad libitum. After 11.5 h, the calves were given 1 to 1.5 L of colostrum by bottle a second time. If the colostrum yield from the first milking was insufficient, a second milking was carried out (n = 8), or high-quality colostrum that had previously been stored at −20°C was fed (n = 8).

The calves were then divided into 2 different feeding groups according to their sex and the parity of their dam. Both groups consisted of 15 female and 10 male calves, with 10 calves born to primiparous cows in the TRANS group and 7 in the MR group.

Both groups had similar birth weights (mean ± SD), with TRANS-fed calves averaging 42.4 ± 4.70 kg and MR-fed calves averaging 42.8 ± 2.89 kg. Each group consumed approximately the same amount of colostrum, with both averaging around 4.6 L. In the TRANS group, 13 calves, and in the MR group, 14 calves, were fed using an esophageal tube. The time to first colostrum intake after birth (mean ± SD) was similar between the groups, with TRANS calves taking an average of 45.6 ± 4.76 min and MR calves taking 44.9 ± 3.25 min. The Brix values of the colostrum (mean ± SD) were consistent across both groups, measuring 26.5 ± 3.84% for TRANS and 26.9 ± 4.34% for MR. Serum protein concentrations, determined by refractometry after colostrum intake, indicated adequate intakes for all calves, without significant differences between the groups (6.49 ± 0.78 g/dL for TRANS and 6.51 ± 0.90 g/dL for MR [mean ± SD]).

The TRANS group received 6 L of fresh transition milk twice daily from their respective dam via a teat bucket during the first 5 d of life (Figure 1; Table 1).

The MR group received the same amount of MR twice daily (140 g/L, Milkivit Sweet Rubin, Trouw Nutrition Deutschland GmbH, Burgheim, Germany; see Table 1). Compositions of colostrum and transition milk are shown in Table 2. After 10 feedings, all calves received 6 L of MR twice daily at 0600 and 1600 h. The intake per meal was documented by weighing the bucket before and after feeding to calculate consumption.

On d 14 (±3 d [mean ± SD]), the calves were moved to a group housing system (up to 10 calves per pen) in an open straw pen. An automated feeding system (Vario Kombi, Förster Technik, Engen, Germany) provided MR and pelleted starter concentrate (CombiKorn Elite Start Press, Raiffeisen Waren-Zentrale Rhein-Main eG, Cologne, Germany; Table 1) according to a predetermined feeding schedule. Starting in wk 8, the daily MR allocation was gradually reduced from a maximum of

Table 2. Composition of colostrum and transition milk

Item	Colostrum	D 1	D 2	D 3	D 4	D 5
Protein[1] (g/L)	84.0	52.8	48.9	60.0	60.1	57.5
Fat[2] (g/L)	54.4	53.6	77.0	63.6	65.9	59.5
Lactose[3] (g/L)	27.4	46.8	48.7	50.1	50.9	52.6
ME[4] (Mcal/L)	0.99	0.91	0.98	1.05	1.07	0.99

[1]Protein was measured with the Bradford assay (Bradford, 1976).

[2]Fat content was measured using the Mojonnier method (Kleyn et al., 1988).

[3]Lactose concentrations were measured at the Research Unit Nutritional Physiology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, using the protocol of Görs et al. (2009), modified for bovine milk (Uken et al., 2021).

[4]Calculated according to the equation from NRC (2001): [0.057 × Crude protein (%) + 0.092 × Fat (%) + 0.0395 × Lactose (%)] × 0.97 × 0.96.

12 L until complete weaning in wk 14 (d 98 ± 2). The starter concentrate was offered ad libitum (i.e., maximal 9.8 kg/calf per day). The automated system recorded the individual intake of MR and starter concentrate per visit and day. The system also recorded data on rewarded and unrewarded visits, drinking speed, and interruptions (break-offs) during MR intake. Hay and water were offered ad libitum throughout the study. After weaning (wk 14), calves were given free access to hay, water, and TMR. At around 8 mo of age, the female calves were moved to group housing in the dairy barn and continued to receive the same TMR as in the calf barn. The first insemination was performed when the heifers had reached a minimum BW of 400 kg and a minimum age of 13 mo and showed standing estrus.

Body weight was measured weekly using a mobile electronic scale. After weaning, the female calves were weighed monthly. Average daily gain was calculated by dividing the weight gain between 2 measurements by the days between each weighing.

Weekly health examinations during the preweaning and weaning periods (14 wk) were performed by veterinarians. Vital signs were assessed and scored according to an established protocol on the research farm (Seibt et al., 2021; Ghaffari et al., 2022; Golbeck et al., 2023). Body posture of the calf was scored from 1 to 4, where 1 = lively; 2 = slightly subdued, standing; 3 = subdued, chest position; and 4 = subdued, side position. Cardiovascular examination included heart rate, episcleral vessel filling and sharpness, skin turgor, and color of the mucosa in the mouth. Scores were given from 1 to 4, with 1 = normal, 2 = slightly abnormal, 3 = moderately abnormal, and 4 = highly abnormal. For assessment of respiratory health, the complete examination included examination of nasal and ocular discharge, ear tilt, cough, and upper and lower respiratory tract auscultation. Scores were given from 0 to 3, where 0 = normal, 1 = slightly aggravated, 2 = mildly aggravated, and 3 = highly aggravated. Abdominal examination included checks of abdominal rigidity, scored from 1 to 3 (1 = soft, 2 = slightly tense, 3 = highly tensed), and abdominal splatter or ringing (1 = negative on both sides, 2 = positive on the left side, 3 = positive on the right side). The navel was checked for inflammation (1 = yes, 2 = no). Fecal consistency was scored from 1 to 4, where 1 = pasty, 2 = thinly mushy, 3 = soupy, 4 = watery. These scores were then categorized and summarized into 3 health classifications: no observed disorders, a single noncritical disorder with no other signs of disease, or the presence of confirmed disease or a rectal temperature above 40°C. In addition, trained personnel monitored the calves daily. A veterinarian was consulted in case of any abnormalities. If necessary, calves were treated according to a standardized protocol (Table 3), and all health events were documented.

Calves were vaccinated against bovine respiratory syncytial virus and parainfluenza 3 (Rispoval 2, Zoetis, Berlin, Germany) as well as *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, and *Trichophyton sarkisovii* (Insol Trichophyton, Boehringer Ingelheim, Ingelheim, Germany), *Coxiella burnetii* (Coxevac, Ceva Tiergesundheit, Düsseldorf, Germany), and blue tongue disease (Syvazul BTV, Laboratorios Syva, León, Spain). Disbudding was performed, if necessary, in wk 4 of life.

Blood Sampling

Blood samples were collected throughout the study via jugular venipuncture into 9 mL serum tubes (Beckton Dickinson AG, Allschwil, Germany). The first sample (d 0) was taken 30 min after birth; that is, before the first colostrum feeding. The second sample (d 1) was taken 12 h after birth, following complete colostrum intake (Figure 1). During the treatment phase (d 2–5), blood samples were collected daily between 0700 and 0900 h. Subsequently, from wk 2 to 14, blood samples were drawn weekly between 0930 and 1130 h. A further blood sample was taken in wk 16, 2 wk after weaning. Female calves were sampled again 24 h after regrouping from the calf barn to the heifer group in the dairy cow barn, at around 8 mo of age. Another sample was taken when

Table 3. Definition of diagnosis or findings and standard therapies registered and administered

Diagnosis	Score	Definition[1]	Therapy
Respiratory tract infection			
	Slight	Slightly to moderately altered breathing, increased respiratory rate, GC slightly reduced	If necessary antibiosis
	Moderate	Moderately altered breathing, GC slightly or moderately reduced, T >39.5°C	5 d antibiosis, if necessary antiphlogism
	Severe	Moderately to severely altered breathing, GC moderately reduced, T >39.5°C	5 d antibiosis, 5 d antiphlogism
Navel infection			
	Omphalitis simplex	Outer navel hardened, increasingly warm, painful on palpation, exudate can be massaged out, GC slightly reduced, T >39.5°C	5 d antibiosis, antiphlogism[2]
	Omphalophlebitis/ -orachitis/ -arteritis	Intra-abdominal umbilical structures palpable, joints increasingly filled, GC moderately or severely reduced	5 d high-dose antibiosis, antiphlogism,[2] if necessary surgery
Umbilical hernia		No prolapse of intestines, GC undisturbed	None
		Contents of the hernia sac can be fully repositioned, GC undisturbed	Progress monitoring, repositioning of the hernia sac contents
		Prolapse of intestines, not completely reducible, GC impaired	Initially symptomatic treatment, follow-up, if necessary surgery
Diarrhea			
	Slight	Feces pulpy or soupy, GC undisturbed	Offer electrolyte drinks[3]
	Moderate	Feces soupy or watery, GC undisturbed, GC slightly to moderately reduced	Offer electrolyte drinks,[3] if necessary antiphlogism[2]
	Severe	Feces soupy, GC slightly to moderately reduced, T >39.5°C	Electrolytes,[3] antiphlogism,[2] if necessary i.v. fluids
Colic			
	Slight	Mild colic symptoms, slightly tense abdomen	If necessary antiphlogism[2]
	Moderate	Moderate colic symptoms	1× Metamizol[4] i.v., follow-up, if necessary antibiosis; further antiphlogism,[2] if abomasal displacement: rolling or surgery
	Severe	Moderate to severe colic symptoms, GC highly reduced, PA negative; PA positive, suspicion of ileus	5 d antibiosis, Metamizol[4] until condition improves, i.v. depending on the circulatory condition; rolling or surgery
Fever			
	Slight	>39.5 °C, <40°C	If necessary antiphlogism[2]
	Moderate	>40°C, <41°C	Antiphlogism,[2] if necessary antibiosis
	Severe	>41°C	Metamizol[4] i.v. until condition improves, if necessary 5 d antibiosis

[1]T = temperature; GC = general condition; PA = percussion auscultation.
[2]0.5 mg/kg BW of Meloxidyl, 20 mg/mL meloxicam (Ceva Tiergesundheit).
[3]2 L (Rehydion; Ceva).
[4]Metamizol WDT, 500 mg/mL, 30 mg/kg BW.

reaching the criteria for first insemination (13 mo of age, at least 400 kg BW).

Serum was prepared according to standard protocols. In brief, serum samples were allowed to clot for at least 30 min at room temperature before centrifugation at 1,730 × *g* for 15 min at 20°C. The EDTA samples were centrifuged as soon as possible at 4°C for 20 min at 1,850 × *g*. Following centrifugation, serum aliquots were stored at −20°C and plasma aliquots at −80°C in the research facility. The samples were then transported on dry ice to the University of Bonn (Bonn, Germany) for further analyses, where they were kept at their storage temperatures until analysis. The total protein concentration in serum was determined using an optical refractometer (RHC-200/AD, HHTC, Heidelberg, Germany).

Haptoglobin (**Hp**), adiponectin, and leptin in serum of the female calves were measured using in-house-developed ELISA (Hiss et al., 2004; Sauerwein et al., 2004; Mielenz et al., 2013). The mean intra- and interassay CV were 9.5% and 11.9% in Hp, 9.21% and 11.8% in adiponectin, and 8.3% and 11.0% in leptin.

Assessment of Oxidative Status Indicators in Serum

Reactive oxidative species in serum were measured using detection of ROM (**dROM**) test, which measures hydroperoxides, breakdown products in the reaction with N,N-diethylparaphenylenediamine (Alberti et al., 2000). The values are expressed as H_2O_2 equivalents. The mean intra-assay CV was 1.68% and the mean in-

terassay 1.77%. The antioxidant capacity of the serum was determined using the ferric-reducing capability of plasma (**FRAP**) assay. This assay measures the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by antioxidants, resulting in an increase in absorbance at 593 nm (Chen et al., 2003). The mean intra-assay CV was 1.63%, and the mean interassay CV was 1.78%. The values are given in μmol Fe^{2+}/L. The oxidative stress index (**OSi**) in serum was calculated as the ratio of dROM/FRAP × 100. Glutathione peroxidase activity (**GSHPx**) in serum was measured spectrometrically. One unit corresponds to the amount of enzyme catalyzing the oxidation of 1 m*M* nicotinamide adenine dinucleotide phosphate per minute at 25°C and pH 7.0 (Paglia and Valentine, 1967). The mean intra-assay CV was 3.77% and the mean interassay 4.53%. The intensity of lipid peroxidation was measured using the thiobarbituric acid-reactive substances assay (**TBARS**; Gutteridge and Quinlan, 1983), with a mean intra-assay CV of 7.07% and interassay of 6.52% in serum. The results are expressed in nanomolar (n*M*) of malonyldialdehyde, which served as the standard. To assess the degree of oxidant-mediated protein damage, advanced oxidation protein products (**AOPP**) were measured (Witko-Sarsat et al., 1996). Total protein concentration in serum was measured by the Bradford method using BSA as a standard (Bradford, 1976). The mean intra-assay CV was 2.62%, and the mean interassay CV was 2.86%, indicating good assay performance.

Statistical Analysis

To estimate the appropriate sample size (Morris, 1999; Hintze, 2008), a pre-study power analysis was performed based on previously reported values for feed intake, BW, and ADG (DeVries and Keyserlingk, 2009; Zhang et al., 2010; Miller-Cushon and DeVries, 2011). Assuming a significance level (α) of 0.05 and a discriminatory power of 0.8, the analysis resulted in a sample size of 30 calves for growth performance. Before analysis, the normal distribution of all residuals was checked by testing the Shapiro-Wilk statistic and the homogeneity of variance with the Levene test using the UNIVARIATE procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). If the data were not normally distributed, the data were $\log_{10}$-transformed. Data were analyzed using PROC MIXED from SAS with time (age) as a repeated measures ANOVA. This model included the fixed effects of treatment (feeding), time (day, week, or month), treatment-time interaction, calf's sex, and dam's parity, with individual calves as random effects. Calf sex and dam parity were excluded if they were found to be insignificant. Calf sex was excluded for all calculations after weaning, as well as for the oxidative markers, acute-phase proteins, and adipokines before weaning. Dam parity was excluded for BW and ADG and oxidative marker before and after weaning as well as for feeding behavior. Brix value of the colostrum was excluded from all calculations, as it was not significant. Different variance-covariance structures (autoregressive type 1, compound symmetry, and Toeplitz) were tested to determine the best-fitting model. Based on the Akaike information criterion and Bayesian information criterion, an autoregressive type 1 co-structure was selected as the best fit for BW, ADG, and feed intake. For the markers of oxidative status, a Toeplitz covariance structure and for feeding behavior, a compound symmetry covariance structure were selected as the best fit. Tukey-Kramer adjustment was used for post-hoc tests to assess the significance of the observed group × time interaction. Student's *t*-test was used to compare the developmental data at individual time points. In addition, the chi-squared test was used to analyze the health data. The data on BW, ADG, and oxidative status collected during the preweaning period represent the measurements of all 50 calves (30 females and 20 males). In contrast, acute-phase-protein, adipocytokines, and postweaning data for all parameters were limited to the 30 female calves (15 per group). In the analysis of feed intake data, 2 female calves from the TRANS group and 2 male calves from the MR group were excluded due to technical errors in recording. Data are presented as means ± SEM. The graphs were created using GraphPad Prism version 5.02 (GraphPad Software, Boston, MA). The threshold of significance was set at $P \leq 0.05$; trends were declared at $0.05 < P \leq 0.10$.

RESULTS

Milk and MR Intake

The intake of milk and MR did not differ between the treatments during the entire rearing period (Figure 2a, b; Table 4). In the first week of life, both treatment groups had high intakes and maintained this level throughout the trial, with a slight decrease around d 14, when they were moved to the automatic feeder. Starter feed intake was not influenced by treatment or by the interaction of treatment and time during the rearing period (Figure 2c; Table 4). Before weaning, negligible amounts of starter feed were consumed in both treatment groups. With the onset of weaning, starter intake increased rapidly; on d 98, the last day of MR feeding, TRANS calves consumed 4.37 ± 0.46 kg of starter feed and MR calves consumed 4.18 ± 0.52 kg.

Calves fed the TRANS diet had a significantly higher ME intake than calves fed the MR diet from birth until being fully weaned (Figure 2d; Table 4). This finding was most evident during the treatment feeding phase, which comprised the first 5 d (Figure 2d). Notably, both

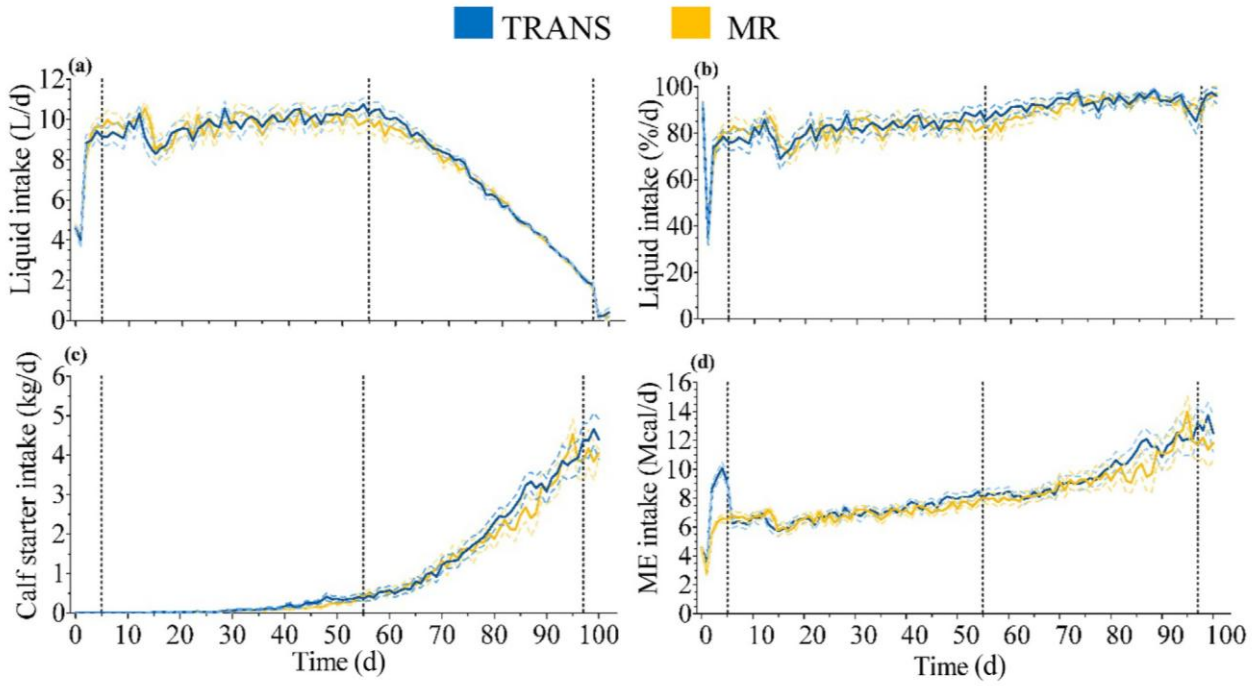

Figure 2. Intake of transition milk or milk replacer (L/d, panel a; % daily intake, b), as well as starter milk intake (kg/d, c) and total ME intake (Mcal/d, d) from d 0 to d 100 of life. The first vertical line marks the end of differential feeding, the second the beginning of gradual weaning, and the third the end of the weaning process. Solid lines represent mean values for each group; dotted lines indicate the SE, reflecting the variability around the mean.

groups showed an upward trend in ME intake during the weaning phase (Figure 2d).

Feeding Behavior

Neither the drinking speed nor the daily frequency of rewarded or unrewarded feeding visits, nor the number of break-offs observed per day were influenced by the treatments (Table 4). As the calves grew older, their drinking speed increased, and the frequency of break-offs and the number of rewarded visits to the feeder decreased with the age of the calves (time, $P < 0.01$; Figure 3). The number of unrewarded visits to the feeder remained relatively constant before weaning and then increased as the calves underwent the gradual weaning process (time, $P < 0.01$; Figure 3).

Body Weight and ADG

Body weight and ADG were not influenced by the treatment group, nor by the interaction of time and group (Figure 4a, b; Table 4). Body weight was affected by time ($P < 0.01$; Table 4) as was ADG until weaning. After weaning, ADG was not affected by treatment, time, or their interaction. Both treatment groups doubled their birth weight in wk 7. The average BW at weaning was 144 ± 14.1 kg in TRANS and 141 ± 13.3 kg in MR calves. In none of the treatment groups was weaning associated with weight loss. The ADG decreased from wk 2 to 3 in both treatments and increased afterward throughout the whole trial period. The TRANS-fed calves showed a trend for higher ADG than MR calves in wk 9 (1.13 ± 0.08 kg in TRANS and 0.84 ± 0.08 kg in MR, $P = 0.09$) and a higher gain in wk 11 (1.18 ± 0.06 kg in TRANS and 0.97 ± 0.06 kg in MR, $P = 0.02$). Afterward, no further differences were observed. After weaning, ADG decreased; the lowest ADG after weaning was observed in TRANS calves at mo 6 (0.64 ± 0.26 kg/d) and in MR calves at mo 15 (0.4 ± 0.21 kg/d).

Health Data

Health disturbances noticed in the weekly checks or during the staff checks until weaning tended to occur more often in MR calves than in TRANS calves (Tables 5 and 6). In total, 67 cases of disorders were observed in 32 of 50 calves during the rearing period. Of these, 19 were in MR calves and 13 were in TRANS calves ($P = 0.08$).

Table 4. Intake, feeding behaviors, and growth performance of calves fed milk replacer (MR) or transition milk (TRANS) over 5 d; preweaning data included 50 calves (male and female), and postweaning data included 30 female calves

	Treatment			*P*-value		
Item	TRANS	MR	SEM	Group (G)	Time (T)	G × T
Intake						
Liquid intake (L/d)						
Treatment phase (d 1–5)	8.07	8.17	0.26	—	—	—
Preweaning (d 6–55)	9.81	9.79	0.06	—	—	—
During weaning (d 56–98)	6.09	5.94	0.10	—	—	—
Birth to weaning (d 1–98)	8.01	7.94	0.07	0.44	<0.01	0.94
Liquid intake (%/d)						
Treatment phase (d 1–5)	67.82	68.06	2.14	—	—	—
Preweaning (d 6–55)	82.03	81.65	0.53	—	—	—
During weaning (d 56–98)	93.15	91.73	0.41	—	—	—
Birth to weaning (d 1–98)	86.38	85.57	0.37	0.57	<0.01	0.99
Starter intake (kg/d)						
Preweaning (d 6–55)	0.12	0.09	0.01	—	—	—
During weaning (d 56–98)	2.16	2.03	0.06	—	—	—
Birth to weaning (d 1–98)	1.02	0.95	0.03	0.36	<0.01	0.96
Total ME intake (Mcal/d)						
Treatment phase (d 1–5)	8.17	5.63	0.19	—	—	—
Preweaning (d 6–55)	7.02	6.92	0.03	—	—	—
During weaning (d 56–98)	10.10	9.67	0.09	—	—	—
Birth to weaning (d 1–98)	8.43	8.05	0.05	0.01	<0.01	0.00
Feeding behaviors						
Break-offs (no./d)						
Preweaning (d 14–55)	1.15	1.05	0.03	—	—	—
During weaning (d 56–98)	0.29	0.33	0.02	—	—	—
Birth to weaning (d 14–98)	2.69	3.32	0.13	0.54	<0.01	0.69
Rewarded visits (no./d)						
Preweaning (d 14–55)	5.55	5.17	0.05	—	—	—
During weaning (d 56–98)	4.47	4.30	0.04	—	—	—
Birth to weaning (d 14–98)	6.98	7.06	0.08	0.13	<0.01	0.99
Unrewarded visits (no./d)						
Preweaning (d 14–55)	4.57	4.68	0.10	—	—	—
During weaning (d 56–98)	8.20	7.70	0.14	—	—	—
Birth to weaning (d 14–98)	6.08	5.31	0.17	0.28	<0.01	0.09
Drinking speed (mL/min)						
Preweaning (d 14–55)	523.7	543.4	3.27	—	—	—
During weaning (d 56–98)	690.6	682.6	3.21	—	—	—
Birth to weaning (d 14–98)	570.3	575.4	4.71	0.32	<0.01	0.59
Growth metrics						
BW (kg)						
Preweaning (wk 8)	93.04	91.84	1.30	—	—	—
During weaning (wk 14)	143.4	138.7	2.01	—	—	—
Birth to weaning (d 0–wk 14)	95.42	92.98	1.58	0.24	<0.01	0.54
Postweaning (mo 4–17)	331.3	331.0	5.67	0.94	0.01	0.74
Total ADG (kg/d)						
Preweaning (wk 8)	0.97	1.06	0.05	—	—	—
During weaning (wk 14)	1.30	1.34	0.07	—	—	—
Birth to weaning (d 0–wk 14)	1.06	1.02	0.43	0.45	<0.01	0.16
Postweaning (mo 4–17)	0.89	0.87	0.03	0.83	0.11	0.65
BW change (kg/period)						
Preweaning (d 0–wk 8)	50.62	49.08	1.14	0.51	—	—
During weaning (wk 8–14)	50.34	46.90	1.19	0.15	—	—
Birth to weaning (d 0–wk 14)	100.96	95.98	1.74	0.15	—	—

Of these calves, 11 MR and 4 TRANS calves were sick more than once ($P = 0.12$); 18 calves required treatment (11 MR, 7 TRANS, $P = 0.24$), with 13 requiring antibiotic treatment (8 MR, 5 TRANS, $P = 0.33$). Diarrhea and respiratory diseases were the most prevalent health disorders. Most cases occurred in wk 1 and 4 in both treatment groups.

The Hp concentrations (Figure 6a) were influenced by time ($P \leq 0.01$) but not by treatment or the interaction of treatment group and time. Concentrations remained

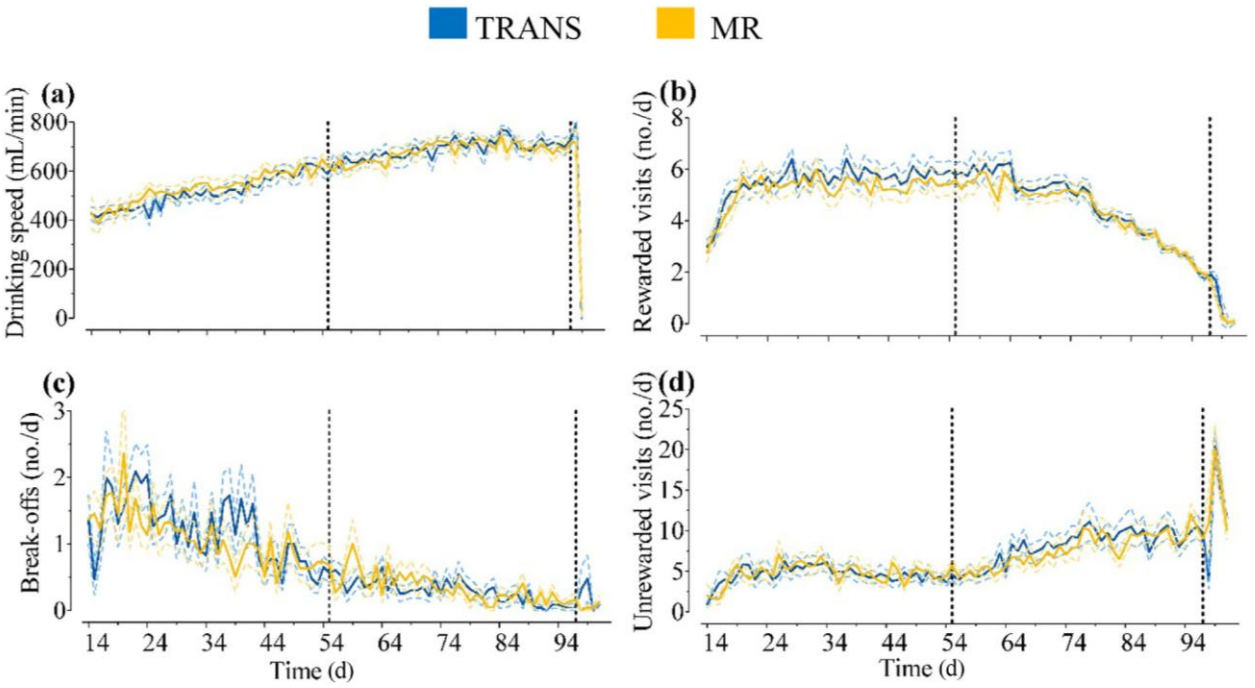

Figure 3. Drinking speed (a), rewarded visits per day (b), break-offs per day (c), and unrewarded visits per day (d). Rewarded visits are considered visits with "entitlement" (the calf receives its full portion of MR designated for that visit); unrewarded visits are visits to the feeding station without entitlement (either because whole entitlement for that day is consumed or the break period of 2 h after each visit is not yet expired). Break-offs are considered ending of the visit without the complete consumption of the 2.5 L allowed per visit. The first vertical line marks the beginning of gradual weaning, and the second designates the end of the weaning process. Solid lines represent mean values for each group; dotted lines indicate the SE, reflecting the variability around the mean.

relatively constant throughout the preweaning period and increased during the weaning period.

Indicators of Oxidative Status

The dROM values (Table 7; Figure 5a) fluctuated over time but were not affected by treatment, although a trend existed, suggesting an interaction between treatment and time. Concentrations were below the detection limit immediately after birth, increased after colostrum intake, and remained relatively stable during the rearing period. After weaning, dROM concentrations in mo 8 were lowest when the young heifers were brought into the dairy barn and then increased again.

The FRAP values (Table 7; Figure 5b) were not affected by treatment nor by the interaction of treatment and time. Both treatment groups showed a similar development during the whole experimental period. The highest values were measured after birth and decreased over time. After weaning, the values remained at the same level as before weaning.

The OSi (Table 7; Figure 5c) was not affected by treatment nor by the interaction of treatment and time. It was lowest after colostrum intake and increased thereafter. In TRANS calves, OSi decreased after d 3, but it remained at the same level in MR calves. In both treatment groups values increased at the start of weaning. After weaning, the lowest value was recorded at mo 8 and increased again in mo 13.

The GSHPx activity (Table 7; Figure 5d) was affected by treatment, time, and the interaction thereof. It was highest in both treatment groups after birth. The TRANS calves had a decrease from 0.44 ± 0.06 to 0.36 ± 0.05 U/g protein after colostrum intake, whereas MR calves had an increase from 0.41 U/g protein ± 0.09 to 0.43 ± 0.09 U/g protein ($P < 0.01$). The GSHPx activity decreased in both groups until wk 6 and then increased again in both groups. After weaning, GSHPx activity was not affected by treatment, time, or the interaction thereof, and the values remained at the same level as before weaning.

The preweaning TBARS concentrations (Table 7; Figure 5e) were affected by time and the interaction of treatment and time, but not by treatment. After weaning, treatment, time, and their interaction were significant. The TBARS values were highest in MR calves after birth and decreased thereafter. The TRANS calves had

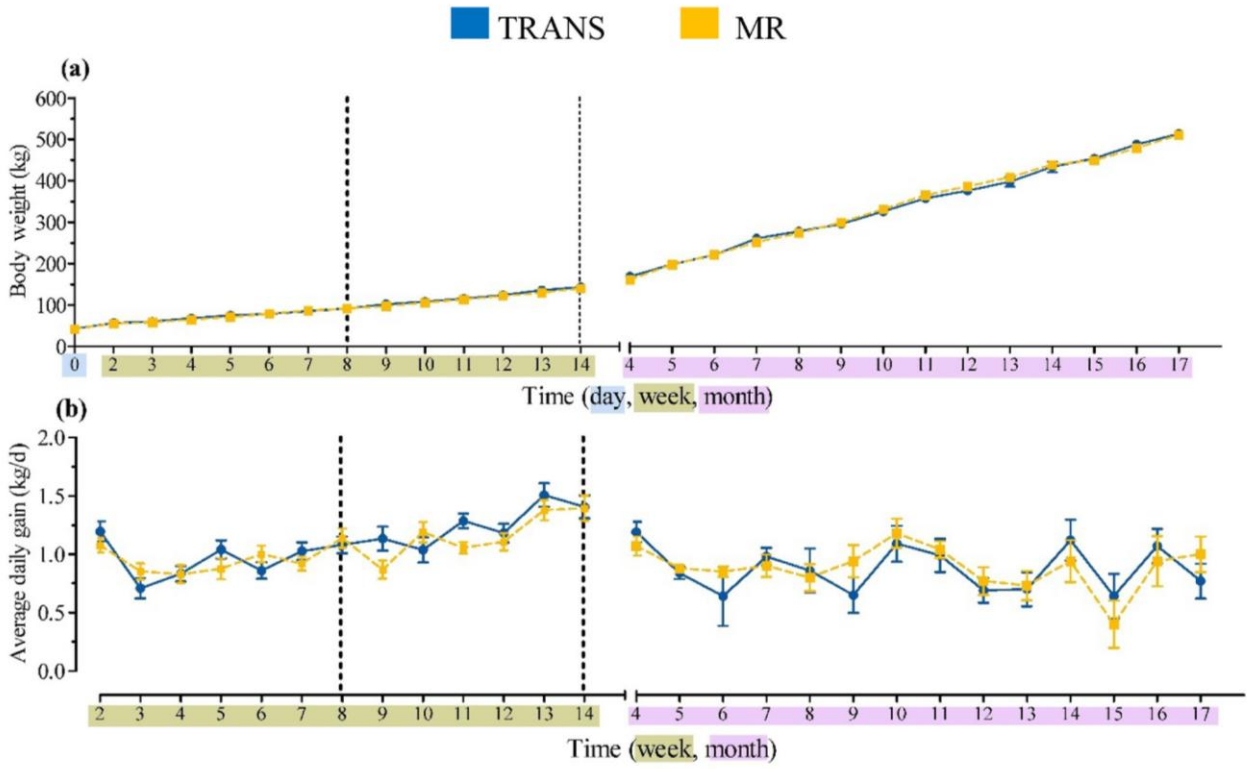

Figure 4. Body weight (a) and average daily gain (b) development of TRANS and MR calves from birth until wk 14 and mo 4 until mo 17. Data from d 0 until wk 14 include 50 male and female calves, data after weaning, from mo 4 to mo 17 include 30 female calves. The first vertical line marks the beginning of weaning, the second the end of weaning. Data are presented as mean ± SE.

lower TBARS levels, which increased after colostrum intake. During the treatment feeding (5 d), the MR calves showed temporarily increased TBARS concentrations compared with TRANS calves. These levels plateaued until wk 3, and then both treatment groups showed similar, gradually decreasing TBARS levels. After weaning, the TBARS levels increased in both treatments until mo 8, followed by a decrease until mo 13.

The preweaning AOPP concentrations (Table 7; Figure 5f) were affected by time, but neither by treatment nor the interaction of treatment and time. After weaning none of the fixed factors were significant. The AOPP levels were lowest after birth and increased in both groups during the first days of life, with a higher peak in the TRANS calves at d 5. Afterward, values decreased slowly over time in both groups.

The total protein concentration (Table 7; Figure 5g) before weaning was not affected by treatment, but by time and the interaction of treatment and time. After weaning, the concentration was affected by treatment and time, but not by their interaction. Both treatment groups had the lowest serum protein concentrations immediately after birth (at 37.4 ± 0.65 g/L in TRANS calves and 38.2 ± 0.92 g/L in MR calves) and an increase in concentration after colostrum intake (46.0 ± 0.78 g/L in TRANS calves and 48.0 ± 0.9 g/L in MR calves). In the MR calves, this value decreased with the start of MR feeding (d 2), whereas in the TRANS calves a further increase occurred on d 2 until the protein concentration also began to decrease. Both treatment groups had similar protein concentrations throughout the rearing period, which increased at the start of weaning in wk 9. This increase continued after weaning, with the highest concentration measured at mo 13 (50.0 ± 1.28 g/L in TRANS and 52.8 ± 1.17 g/L in MR calves).

Adipokines

The adiponectin concentrations (Table 8; Figure 6b) were influenced by time ($P \leq 0.01$) and treatment over time ($P = 0.01$). Values were lowest after birth and increased after colostrum intake. They remained at the same level in the preweaning period and increased during the weaning period. The concentrations of leptin (Table 8;

Table 5. Number of health events observed in calves fed milk replacer (MR) or transition milk (TRANS) over 5 d during 98 d of rearing

	Treatment				
Item	TRANS (n)	MR (n)	OR[1]	95% CI	*P*-value
Total number of sick calves	52% (13/25)	76% (19/25)	0.34	0.10, 1.14	0.08
Thereof, sick twice or more	16% (4/25)	44% (11/25)	0.34	0.09, 1.30	0.12
Total number of medicated calves	28% (7/25)	48% (12/25)	0.50	0.15, 1.60	0.24
Thereof, treated twice or more	4% (1/25)	20% 5/25	0.31	0.03, 3.16	0.30
Total number of antibiotic-treated calves	20% (5/25)	32% (8/25)	0.53	0.15, 1.93	0.33
Thereof, twice or more	0% (0/25)	8% (2/25)	0.18	0.01, 4.04	0.15
Total number of calves with diarrhea	28% 7/25	48% (12/25)	0.64	0.10, 4.21	0.64
Total number of calves with respiratory illness	20% (5/25)	52% (13/25)	0.34	0.10, 1.31	0.12
Total number of calves with other sickness	24% (6/25)	52% (13/25)	0.60	0.10, 2.50	0.48

[1]OR = odds ratio.

Figure 6c) were affected by treatment ($P = 0.03$) and time ($P < 0.01$), but not by their interaction. Concentrations increased with colostrum intake and decreased thereafter, remaining at the same level after wk 3.

DISCUSSION

Previous studies have investigated the effects of transition milk on the growth performance and health of dairy calves in the preweaning period (Van Soest et al., 2020, 2022; Zwierzchowski et al., 2020; Kargar et al., 2021; Zolova et al., 2022); however, the long-term effects of transition milk feeding are still insufficiently known. This study fills this knowledge gap by investigating the long-term influence of transition milk feeding on intake, growth performance, feeding behavior, and oxidative status of dairy calves.

Growth Performance and Feeding Behavior

In the current study, BW did not differ between the treatment groups before or after weaning. Average daily gain differed slightly before weaning, with higher values in the TRANS group, whereas no difference was detectable between treatments after weaning. It is difficult to compare the results of other studies with the results of our study, as different feeding regimens were used. So far, the benefits of transition milk feeding have not been tested at a high feeding level. Van Soest et al. (2020) fed transition milk for 3 d, after which MR was fed for 56 d at a rate of 5.7 L/d. Kargar et al. (2021) fed calves a mixture of transition milk and pasteurized waste milk, sometimes replacing 6 L of waste milk with 0, 0.5, 1, or 2 L of transition milk. Due to the lower energy intake, previous studies also reported lower weight gains than we saw in our calves (Van Soest et al., 2020; Kargar et al., 2021). An important factor discussed for the differences in weight gain found by these authors was the higher feed efficiency and higher energy and nutrient intake of transition milk calves (Van Soest et al., 2020; Kargar et al., 2021). We assume that, due to the overall higher energy and nutrient intake in our study, the needs of the MR calves were better met, and the differences were therefore less pronounced.

Our results show that the calves were able to adapt to the reduced amount of milk by consuming more solid feed during the slow and late weaning, and that their energy intake was not affected during this time. Comparing our results with the studies that had a shorter weaning period (Eckert et al., 2015; step-down weaning from 8 L/d to 4 L/d at 5 or 7 wk) or an earlier weaning (Sweeney et al., 2010; at d 41, gradually or abrupt), all studies found low starter feed intake and decreasing energy intake during weaning, as well as a decrease in ADG. The digestive system of calves is not yet fully developed after birth; their diet is based on milk, and with the development of the forestomach system, solid feed is also introduced (Baldwin et al., 2004). Our study showed that energy intake increased during weaning, as the intake of starter increased rapidly while the amount of MR was gradually reduced. The increase in energy intake during the weaning period was 6% higher

Table 6. Results of 348 weekly health checks with rectal temperature >39.5°C, or abnormal scores for general appearance[1] (>1), fecal consistency[2] (>2), or breathing sounds[3] (>2) of calves fed milk replacer (MR) or transition milk (TRANS) from d 0 to wk 14

	Number of observations with abnormal scores				
Item	TRANS	MR	OR	95% CI	*P*-value
Rectal temperature	2	9	0.22	0.05, 1.04	0.04
Body posture score	1	5	0.20	0.02, 1.70	0.10
Fecal score	22	13	1.70	0.87, 3.53	0.11
Breathing sounds score	11	10	1.11	0.47, 2.65	0.81

[1]General appearance scores: 1 = lively; 2 = slightly subdued; 3 = subdued, chest position; 4 = subdued, side position.

[2]Fecal consistency scores: 1 = pasty, 2 = thinly mushy, 3 = soupy, 4 = watery.

[3]Breathing sound scores: 0 = none, 1 = slightly aggravated, 2 = moderate aggravated, 3 = severe aggravated.

in TRANS calves than in MR calves. According to Van Soest et al. (2022), a possible explanation for the greater starter intake in TRANS calves could be better gut development and thus easier adaptation to the weaning process. This could also be an explanation for the partially better ADG of TRANS calves in the weaning phase in the present study. As we did not collect data of gut development, we cannot prove this theory with certainty. Further research would be needed to show that the results of Van Soest et al. (2022) are an explanation for the differences observed in our calves.

Health Outcomes

Transition milk feeding had some effects on calf health, with TRANS calves tending to have fewer health impairments compared with MR calves. Previous studies have shown mixed results regarding the benefits of transition milk on calf health. Whereas Conneely et al. (2014) only found an effect of feeding transition milk for 2 or 3 d compared with whole (mature) milk (WM) feeding on eye/ear or nasal scores, Kargar et al. (2021) found health benefits when feeding transition milk for 14 d. The odds ratios of abnormal fecal scores or abnormal respiratory scores were higher in the WM-fed calves than in the groups receiving supplemental colostrum. The authors attributed these differences to the effects of the bioactive compounds in colostrum on the intestinal immune system and local protection against pathogens. The duration of feeding was 14 d, compared with 5 d in our study, so the local protection and strengthening of the intestinal immune system may have lasted longer in their calves and could explain our different results. Comparing our results to a similarly designed study (Van Soest et al., 2020) where calves were fed either 5.7 L of MR or transition milk for 3 d after birth and then MR until weaning at d 56, the other study found that the calves were not affected by treatment. In that study, daily assessment of calves in the first 21 d of life showed no effect of the treatment on eye/ear scores, but only on fecal scores. Given the small sample size in this trial, and 27 of 694 weekly checks indicating adverse health events, it is very difficult to detect significant differences in these parameters. Larger samples would be required to see whether the current numerical differences can be substantiated. The frequent and intensive health checks in this study probably allowed for a more sensitive detection of abnormalities, compared with typical farm conditions. This increased sensitivity may have resulted in a greater frequency of detected health problems that might go undetected or that would be detected only later when becoming more obvious under normal farm conditions. It is therefore important to consider that the relatively high portion of calves with health disturbances detected in our study might have been due to the rigorous diagnostic approach rather than a herd problem. This context is crucial for the accurate interpretation of the results in relation to real farming practices.

Haptoglobin is an acute-phase protein that binds free hemoglobin and has an anti-inflammatory effect by inhibiting the chemotaxis and phagocytosis of inflammatory cells. It is a key protein in the nonspecific immune response in cattle (Klenner, 2013; Baumgärtner and Schmidt, 2020). Twenty-six extreme outliers with high Hp concentrations were detected in our calves (SD <2; Z standardization within each time point). Of these outliers, 3 could be associated with clinical findings at this time point.

Oxidative Status

Another aim of this study was to investigate the oxidative status of the calves. To date, no study has investigated the effect of transition milk on the oxidative status of calves, nor has there been any monitoring of oxidative stress levels from birth to first insemination.

Table 7. Oxidative status of calves fed milk replacer (MR) or transition milk (TRANS) over 5 d; preweaning data included 50 calves (male and female), and postweaning data included 30 female calves

	Treatment			*P*-value		
Item	TRANS	MR	SEM	Group (G)	Time (T)	G × T
TBARS (n*M*)						
Treatment phase (d 1–5)	393.3	405.4	7.32	—	—	—
Preweaning (wk 2–8)	235.9	249.2	4.68	—	—	—
During weaning (wk 8–14)	213.3	202.3	3.05	—	—	—
Birth to weaning (d 0–wk 14)	278.8	283.4	4.04	0.62	<0.01	0.01
Postweaning (wk 16–mo 13)	273.3	233.1	9.26	0.01	<0.01	<0.01
AOPP (µmol/L)						
Treatment phase (d 1–5)	3.39	2.67	0.1	—	—	—
Preweaning (wk 2–8)	1.9	1.92	0.05	—	—	—
During weaning (wk 8–14)	1.65	1.57	0.03	—	—	—
Birth to weaning (d 0–wk 14)	2.16	1.95	0.04	0.5	<0.01	0.23
Postweaning (wk 16–mo 13)	1.2	1.1	0.03	0.3	0.63	0.72
GSHPx (U/g protein)						
Treatment phase (d 1–5)	0.37	0.39	0.006	—	—	—
Preweaning (wk 2–8)	0.26	0.29	0.004	—	—	—
During weaning (wk 8–14)	0.28	0.29	0.004	—	—	—
Birth to weaning (d 0–14)	0.30	0.33	1.029	0.02	<0.01	<0.01
Postweaning (wk 16–mo 13)	0.307	0.302	0.01	0.65	0.16	0.27
FRAP (µ*M* Fe^{2+}/L)						
Treatment phase (d 1–5)	315.7	305.9	4.04	—	—	—
Preweaning (wk 2–8)	254.5	257.5	3.00	—	—	—
During weaning (wk 8–14)	247.2	251.9	3.09	—	—	—
Birth to weaning (d 0–wk 14)	271.7	270.9	2.14	0.92	<0.01	0.87
Postweaning (wk 16–mo 13)	239.8	243.6	4.76	0.81	<0.01	0.93
dROM (µg H_2O_2 eq./L)						
Treatment phase (d 1–5)	44.6	46.8	1.85	—	—	—
Preweaning (wk 2–8)	47.3	55.3	1.81	—	—	—
During weaning (wk 8–14)	49.1	51.2	1.64	—	—	—
Birth to weaning (d 0–wk 14)	47.1	51.6	0.003	0.41	<0.01	0.04
Postweaning (wk 16–mo 13)	51.6	49.0	2.50	0.53	<0.01	0.65
OSi (arbitrary unit)						
Treatment phase (d 1–5)	13.3	13.9	0.71	—	—	—
Preweaning (wk 2–8)	19.9	23.0	0.85	—	—	—
During weaning (wk 8–14)	21.7	21.2	0.80	—	—	—
Birth to weaning (d 0–wk 14)	18.2	19.7	0.48	0.63	<0.01	0.25
Postweaning (wk 16–mo 13)	23.0	21.6	1.50	0.55	<0.01	0.84
Total protein (g/L)						
Treatment phase (d 1–5)	43.8	43.6	0.32	—	—	—
Preweaning (wk 2–8)	44.0	43.2	0.23	—	—	—
During weaning (wk 8–14)	44.4	45.1	0.25	—	—	—
Birth to weaning (d 0–wk 14)	44.1	43.9	0.15	0.76	<0.01	<0.01
Postweaning (wk 16–mo 13)	47.7	49.9	0.54	0.07	<0.01	0.59

Our results provide a good insight into the developing antioxidant system of calves in adulthood. Oxidative stress appears to be a key mediator in various diseases (Ranjan et al., 2006; Lykkesfeldt and Svendsen, 2007; Sordillo and Aitken, 2009; Cuervo et al., 2021, Fu et al., 2024), and therefore knowledge of the oxidative status of animals could open ways to improve calf performance and health of calves.

Concentrations of ROM in newborn calves have been used to assess oxidative status, but results have been inconsistent (Abuelo et al., 2014; Ranade et al., 2014). For example, Gaál et al. (2006) reported greater ROM concentrations in calves before colostrum intake compared with after, which is in contrast to our results. The rapid changes in ROM concentrations after birth observed by Ranade et al. (2014) suggest that the discrepancies between Gaál et al. (2006) and our study may be due to different sampling times and the rapid increase in ROM concentrations in neonatal blood. In our study, calves fed TRANS showed a tendency toward a slightly faster decline in ROM concentrations compared with calves fed MR, possibly due to the lower concentrations of antioxidants in MR (Soberon et al., 2012b). During weaning, an increase in ROM levels was observed, indicating the need for metabolic adaptation, which is also reflected in an increase in oxidative stress (Róth, 1997).

After weaning, heifers deviated less from the mean ROM of the group. Because ROM are a normal by-product of endogenous metabolism, a high deviation before weaning could indicate greater variability in develop-

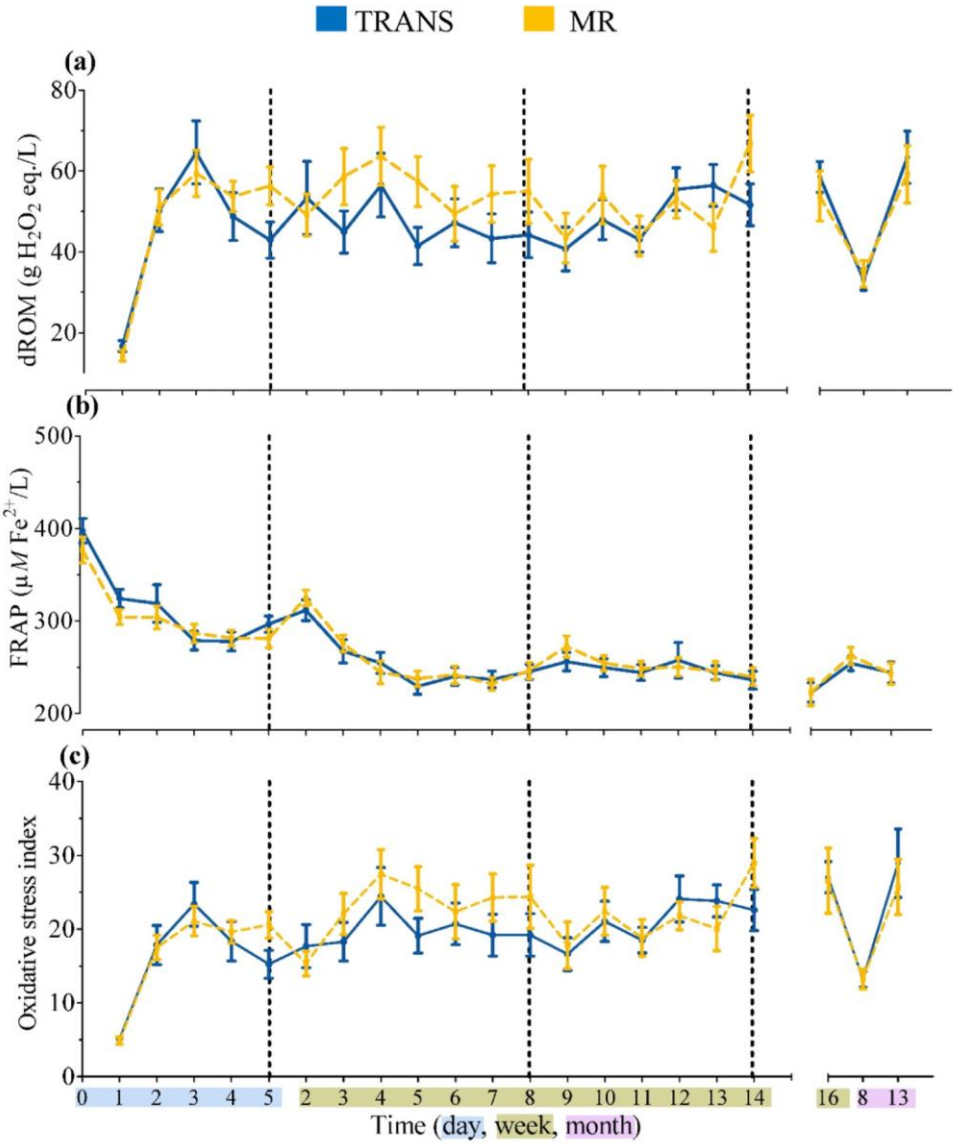

Figure 5. Serum measurements of dROM (a), FRAP (b), and oxidative stress index (c) in all calves during the rearing period and in female calves after weaning. Time points d 0 until wk 14 before weaning include all 50 male and female calves. Time points wk 16, mo 8, and mo 13 after weaning include only 30 female calves. The first vertical line marks the end of differential feeding, the second the beginning of weaning, and the third the end of weaning. Serum measurements of GSHPx (d) and TBARS (e), plasma measurement of AOPP per gram of protein (f), and serum total protein (g) in all calves during the rearing period and in female calves after weaning. Time points d 0 until wk 14 before weaning include all 50 male and female calves. Time points wk 16, mo 8, and mo 13 after weaning include only 30 female calves. The first vertical line marks the end of differential feeding, the second the beginning of weaning, and the third the end of weaning. Asterisks indicate differences between groups within time point ($P < 0.05$). Data are presented as mean ± SE.

ment and adaptation of the calves in the rearing phase. The lesser deviation after weaning could be a sign of having reached a mature status (Miller et al., 1993).

After colostrum intake, calves fed with TRANS had less GSHPx activity. The activity of this enzyme depends primarily on the supply of selenium in utero and via colostrum (Pilarczyk et al., 2012), and can also be increased in calves following a prolonged calving (Vannucchi et al., 2019). However, the effects of prolonged calving would become apparent immediately after birth

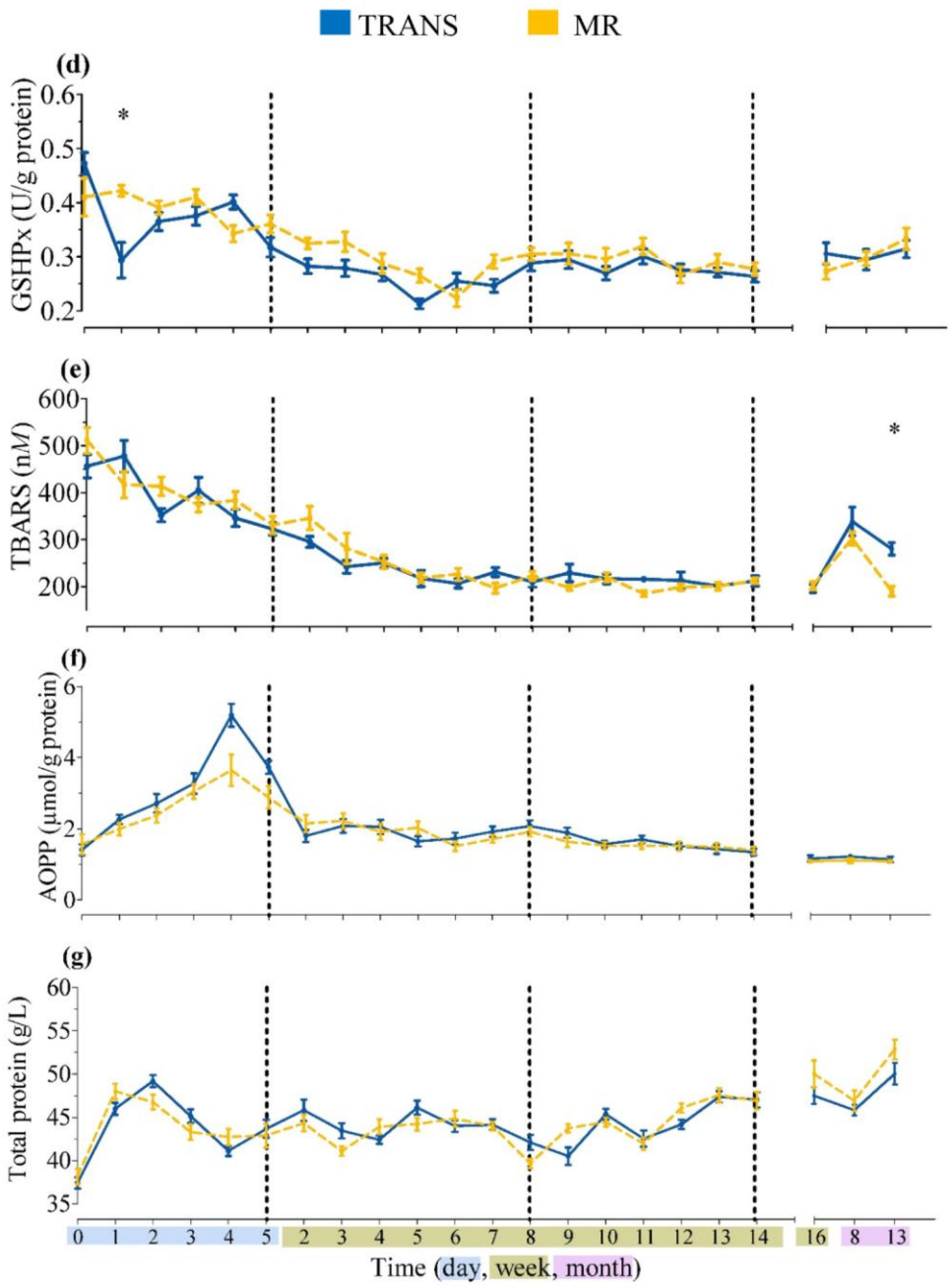

Figure 5 (continued). Serum measurements of dROM (a), FRAP (b), and oxidative stress index (c) in all calves during the rearing period and in female calves after weaning. Time points d 0 until wk 14 before weaning include all 50 male and female calves. Time points wk 16, mo 8, and mo 13 after weaning include only 30 female calves. The first vertical line marks the end of differential feeding, the second the beginning of weaning, and the third the end of weaning. Serum measurements of GSHPx (d) and TBARS (e), plasma measurement of AOPP per gram of protein (f), and serum total protein (g) in all calves during the rearing period and in female calves after weaning. Time points d 0 until wk 14 before weaning include all 50 male and female calves. Time points wk 16, mo 8, and mo 13 after weaning include only 30 female calves. The first vertical line marks the end of differential feeding, the second the beginning of weaning, and the third the end of weaning. Asterisks indicate differences between groups within time point ($P < 0.05$). Data are presented as mean ± SE.

Table 8. Blood concentrations of haptoglobin and adipokines of calves fed milk replacer (MR) or transition milk (TRANS) over 5 d

	Treatment			*P*-value		
Item	TRANS	MR	SEM	Group (G)	Time (T)	G × T
Haptoglobin (µg/mL)						
Treatment phase (d 1–5)	178.3	136.8	19.6	0.69	0.00	0.12
Preweaning (wk 2–8)	109.4	143.9	18.9	0.92	0.05	0.97
During weaning (wk 8–14)	194.3	149.6	25.7	0.96	0.00	0.03
Birth to weaning (d 0–wk 16)	169.6	144.3	12.7	0.86	<0.01	0.25
Adiponectin (µg/mL)						
Treatment phase (d 1–5)	12.9	11.6	0.22	0.00	<0.01	0.21
Preweaning (wk 2–8)	11.5	10.6	0.21	0.24	0.01	0.46
During weaning (wk 8–14)	12.9	13.8	0.31	0.29	<0.01	0.00
Birth to weaning (d 0–wk 16)	11.9	11.7	0.18	0.78	<0.01	0.00
Leptin (ng/mL)						
Treatment phase (d 1–5)	3.93	3.49	0.11	0.13	0.00	0.93
Preweaning (wk 2–8)	2.34	2.30	0.05	0.29	<0.01	0.16
During weaning (wk 8–14)	2.54	2.43	0.05	0.11	0.00	0.76
Birth to weaning (d 0–wk 16)	2.84	2.67	0.05	0.03	<0.01	0.54

and not 12 h later, as was the case in our study. Because feed intake did not yet differ between treatments and oxidative markers were neither assessed in colostrum nor in serum of the dams, the reason for this initial difference remains unknown. Before weaning, MR-fed calves showed higher GSHPx activity, which could be related to the higher ROM concentrations in these calves. A key enzyme of the antioxidant system, GSHPx acts as a scavenger of ROM and lipid peroxides (Ottaviano et al., 2009). This could also explain the slight increase in GSHPx activity observed in both groups in the first week after weaning, which coincided with an increase in ROM concentrations. A similar mechanism is observed in lactating cows, where GSHPx activity is greatest after calving and during the peak of lactation and then decreases toward the dry period (Bernabucci et al., 2005; Pilarczyk et al., 2012).

The concentrations of TBARS were highest in our calves immediately after birth and decreased thereafter. This trend is consistent with the results of Seibt et al. (2021) and Inanami et al. (1999), which is hypothesized to be due to the induction of antioxidant systems in newborns (Gaál et al., 2006). For example, Nrf2 (nuclear factor erythroid 2-related factor 2), a transcription factor that counteracts proinflammatory pathways and activates GSHPx, is upregulated after birth and activated in the liver (Gessner et al., 2014). As liver metabolism in neonates is lower than in adults and develops later, this could explain the higher TBARS and ROM levels observed after birth (Baldwin et al., 2004).

Our results on AOPP are consistent with those of previous studies (Ranade et al., 2014; Morittu et al., 2021; Seibt et al., 2021), which also observed the highest AOPP values in the first week of life, followed by a decline. As shown by Abuelo et al. (2014), the redox balance of colostrum significantly influences the oxidative status of the newborn calf. However, very few studies have investigated the effects of feeding regimens on AOPP concentrations in calves. Therefore, further studies are needed to determine whether the greater AOPP values in TRANS calves are due to a poorer redox balance in the transition milk compared with MR.

In agreement with Gaál et al. (2006), we observed the highest values for FRAP in the calves before colostrum intake. This may be due to higher bilirubin levels in neonates compared with adults, as observed in humans (Wiedemann et al., 2003), as bilirubin is a physiological cytoprotectant (Sedlak and Snyder, 2004). The FRAP values measured in our calves after wk 3 were similar to those measured in adult cows throughout lactation in Konvičná et al. (2015) and similar in their progression, as reported by Urh et al. (2019) in cows during mid-lactation.

The levels of FRAP, GSHPx, TBARS, and AOPP remained stable throughout the experimental period, regardless of initial feeding regimen, and developed in parallel, suggesting that the antioxidant system of the calves adapted during the first week of life. This is similar to the phenomenon observed in lactating cows, where levels are high after calving, decrease after parturition, and remain stable once they have adapted to the demands of lactation (Castillo et al., 2005). The OSi showed no significant fluctuations throughout the experiment, confirming a well-adapted antioxidant system in our calves.

Adipokines

Adiponectin is an adipokine with insulin-sensitizing effects that regulates lipid and glucose metabolism (Sauerwein and Häußler, 2016). High molecular weight adiponectin is not transferred to the fetus in utero in

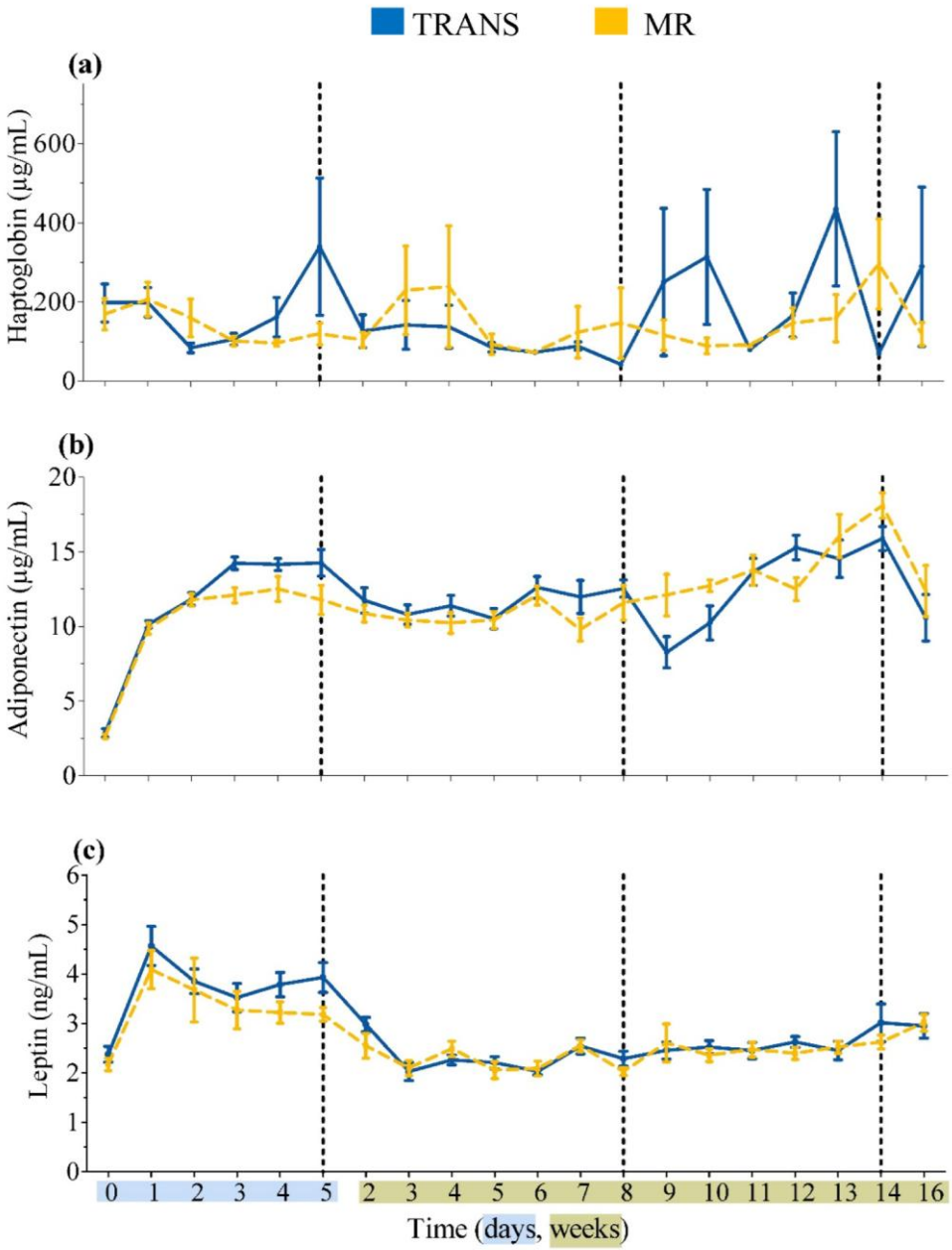

Figure 6. Serum measurements of haptoglobin (a), adiponectin (b), and leptin (c) in 30 female calves during the rearing period. The first vertical line marks the end of differential feeding, the second the beginning of weaning, and the third the end of weaning. Data are presented as mean ± SE.

cattle, and newborn calves have a low body fat content, so colostrum intake is the main source of adiponectin (Kesser et al., 2015). This was confirmed by the increase in adiponectin levels after colostrum intake in our calves. Kesser et al. (2015) showed that calves fed MR for 4 d after colostrum intake had lower plasma adiponectin levels than calves fed a transition milk volume of 6 L/d. This trend was also observed in our study, although not at a significant level. As adiponectin is upregulated at high nutrition levels in the diet, compared with low nutrition levels (English et al., 2018), the differences in our study may be less pronounced.

The presence of leptin in milk can be attributed to its local synthesis in the mammary gland and the transfer from blood to milk (Bonnet et al., 2002). The concentrations of leptin in colostrum are higher in colostrum than in mature milk (Blum et al., 2005; Kesser et al., 2017). It modulates feed intake and growth by acting on the hypothalamus and regulating metabolic and endocrine hormones (Langhans and Lutz, 2022). Leptin concentrations in calves appear to be influenced by both diet and adiposity, unlike in adult animals where diet is the most important factor (Ehrhardt et al., 2003). Leptin levels in our calves were similar to those reported by Block et al. (2003) and Seibt et al. (2021). We also saw the same increase in leptin concentrations as Kesser et al. (2017), which they attributed to the leptin transferred to the newborn via colostrum. Similar to their findings, we also saw no further differences when feeding TRANS compared with MR on the concentrations later on in life.

CONCLUSIONS

In summary, we found no long-term benefits of feeding transition milk on growth performance or oxidative markers. Calves initially fed TRANS had higher energy and feed intake and slightly improved health and antioxidant status. However, contrary to our hypothesis, these early benefits did not translate into significant long-term differences in growth, feeding behavior, performance or oxidative status (ROM, GSHPx, TBARS, AOPP). This lack of differences between feeding groups could be due to the high level of nutrition provided to the calves. Further studies are needed to determine whether different feeding regimens could lead to long-term effects. The observed patterns of different oxidative markers show the development of redox homeostasis in growing dairy calves and can serve as a basis for comparison in future studies. The results showed that the development of the antioxidant system in calves takes place in the first week of life and remains stable thereafter, even until puberty. Future studies should investigate different feeding strategies or longer duration of TRANS feeding to determine possible long-term benefits.

NOTES

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Nonstandard abbreviations used: AOPP = advanced oxidation protein products; dROM = detection of ROM; FRAP = ferric-reducing ability of plasma; GSHPx = glutathione peroxidase activity; Hp = haptoglobin; MR = milk replacer; OR = odds ratio; OSi = oxidative stress index; PA = percussion auscultation; ROM = reactive oxygen metabolites; ROS = reactive oxygen species; TBARS = thiobarbituric acid-reactive substances; TRANS = transition milk; WM = whole milk.

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IV. Transition milk– Effects on first lactation performance and oxidative status

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Long-term effects of early-life transition milk feeding on reproductive and lactation performance, and markers of oxidative status in Holstein heifers

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Summary

To determine the long-term effects of transition milk (TRANS), 30 female Holstein calves were allocated to two feeding groups (n = 15/group) after colostrum intake, receiving either 12 L of TRANS of their dam or 12 L of milk replacer (MR) per day. After 5 d of differential feeding, all calves received 12 L of MR/d. Until calving, heifers were weighed monthly. After calving, BW was recorded twice daily after milking. Body condition (BCS) and back fat thickness (BFT) were scored biweekly. Milk yield was recorded twice daily until d 200 in milk. Milk composition (protein, fat, and lactose), as well as somatic cell count (SCC) were analysed biweekly. Blood samples were taken 3 weeks before calving, at the day of calving and 3 weeks thereafter. Oxidative status was assessed as ferric reducing ability of plasma (FRAP) for antioxidative capacity, and as reactive oxygen metabolites via the dROM assay. Oxidative damage of lipids was measured via the thiobarbituric acid-reactive substances (TBARS) assay; peroxidized proteins were assessed using the advanced oxidation protein products (AOPP) assay. Performance until first insemination did not differ between the groups, as well as BW development until the first weeks of lactation. From week 7 of lactation onwards, TRANS had less BW than MR heifers but tended to have a higher BCS. Milk yield and composition did not differ between both treatments. Marker for oxidative stress showed typical patterns of increasing antioxidants before calving and increase in prooxidants after calving in both treatment groups. The results indicate that feeding TRANS in the first 5 days of life had no long-term effects on performance in the first lactation, except for lower postpartum BW in heifers fed TRANS than MR, under the current rearing and management conditions.

Early life nutrition is critical for the long-term development and performance of livestock. The concept of "lactocrine programming" states that bioactive, non-nutritive factors in colostrum transferred from dam to offspring can influence postnatal development with lasting effects into adulthood (Bagnell and Bartol, 2019). Colostrum is rich in immunoglobulins, cytokines, lactoferrin and hormones that mediate passive immunity and support the development of the gastrointestinal tract (Hammon *et al.,* 2000; Blum and Baumrucker, 2008). During the transition to mature milk, the concentration of bioactive compounds decreases, with transition milk (TRANS) containing lower levels of factors such as insulin-like growth factors, growth hormone, and nucleotides (Hammon *et al.,* 2000; McGrath *et al.,* 2016).

Feeding colostrum has been shown to improve the antioxidant-prooxidant balance in calves, increasing antioxidant levels and reducing reactive oxygen metabolites (ROM), which helps to regulate the redox balance and reduce oxidative stress (Kankofer and Lipko-Przybylska, 2008). In our companion study (Ostendorf *et al.,* 2025), we found that calves fed TRANS had greater energy and feed intake, along with moderate improvements in health and antioxidant status during the first 14 weeks of their life. However, these benefits did not translate into significant long-term differences in growth, feeding behavior or oxidative status during the pre-weaning and weaning period. TRANS feeding may reduce health risks (Conneely *et al.,* 2014; Kargar *et al.,* 2021), promote growth (Van Soest *et al.,* 2020) and improve the development of the gastrointestinal tract (Pyo *et al.,* 2020; Van Soest *et al.,* 2022) during the pre-weaning period. However, while the short-term benefits of TRANS feeding are well documented, its long-term effects on first lactation performance are still unclear and require further investigation.

Postnatal developmental programming is shaped by nutritional strategies in early life, including management of colostrum and pre-weaning nutrient intake (Faber *et al.,* 2005; Soberon *et al.,* 2012). Faber *et al.* (2005) demonstrated that calves fed 4 L of colostrum had greater milk production in both first and second lactations than calves fed 2 L of colostrum, suggesting that early feeding may influence future productivity. It is important to note that

feeding only 2 L of colostrum is insufficient for achieving optimal passive transfer of immunity in newborn calves. Current recommendations advise administering at least 10% to 12% of the calf's BW in high-quality colostrum within the first 2 hours after birth to ensure adequate passive transfer of immunoglobulins and immune protection (Godden et al., 2019). Thus, it is plausible that continued TRANS feeding could have lasting effects on long-term development, including performance in the first lactation.

To better understand how early nutrition affects developmental trajectories, it is important to conduct longitudinal studies that follow calves through all life stages, including the first lactation. However, such studies face challenges due to the limited infrastructure for long-term studies (van Niekerk *et al.,* 2021). This study is the first to examine the long-term effects of TRANS feeding on performance during first lactation, filling a significant gap in lactocrine programming. We hypothesized that feeding TRANS would improve reproductive performance, lactation outcomes and oxidative status, ultimately increasing overall health and productivity compared to feeding MR. The objective of this study was to investigate the long-term effects of TRANS feeding during the first five days of life on growth, reproductive and lactation performance, and oxidative and inflammatory status, as compared to MR.

MATERIALS AND METHODS

The experiment was conducted at the research facility Hofgut Neumühle with the approvement of the relevant authority (Landesuntersuchungsamt Rheinland-Pfalz, Germany, G23-20-082).

Animals, Diets, Feeding, and Management

Thirty female German Holstein heifers were monitored from birth until d 200 in their first lactation. The rearing practices of these heifers and results of their performance and oxidative status were described previously (Ostendorf et al., 2025). A total of 30 neonatal calves were enrolled in the study, which was conducted between July 2021 and March 2024. Calves recruited in this trial were born between September 2021 and April 2022. Within 2 hours of birth, each calf received 3.5 to 4.0 L of colostrum from its dam, administered via an

esophageal tube if necessary (Fig. 1). Afterwards they were moved to straw bedded single hutches and received a second feeding of 1 to 1.5 L of colostrum 11.5 h after birth. Immediately after the second colostrum feeding, calves were randomly allocated to one of two experimental groups: TRANS (12 L/d transition milk from their dam for 5 days) or MR (12 L/d milk replacer for 5 days), with n = 15 calves per group (Fig. 1). Calves were alternately assigned to either the TRANS or MR group, considering their sex and the parity of the dam. Continuous calving during the recruitment period ensured an even distribution of the animals between the two groups and in the group housing. After 10 differential feedings, all calves were fed with 12 L of MR. At day 14 calves were moved from the single housing into the group housing system in an open straw pen (Fig. 1). They received their 12 L of MR and pelleted starter via an automated feeding system (Vario Kombi, Förster Technik, Engen, Germany) until week 8 of life and were gradually weaned until week 14 (Fig. 1). After weaning, all heifers were fed TMR for growing heifers until late gestation, when they were switched to the ration fed to lactating cows (the composition of the TRANS, MR and TMR diets is shown in Ostendorf et al. (2025).

First insemination was carried out at a minimum of 400 kg BW and 13 months of age, when standing estrus was observed (Fig. 1). Three weeks before estimated calving, the heifers were moved to the milking herd to get used to the milking parlor (GEA Farm Technologies GmbH, Bönen, Germany). Before imminent calving, the heifers were moved to straw-bedded calving pens and re-introduced to the herd approximately 10 d after calving (Fig. 1). Daily milk production was recorded using the herd management system Dairy Plan C21 (GEA Farm Technologies GmbH, Bönen, Germany) and milk samples were collected biweekly as pooled aliquots from morning and evening milking (Fig. 1). The samples were analyzed for milk fat, protein, lactose and somatic cell content using an infrared analyzer (MilkoScan FT+ 600, Foss Analytical A/S, Hillerød, Denmark) in the regional laboratory of the milk recording organization, (Landeskontrollverband Rheinland-Pfalz-Saar e.V. Bad Kreuznach, Germany). Body weight was recorded monthly with a mobile electronic scale until calving. After calving, BW was recorded twice daily after milking using an automatic scale (GEA Farm Technologies

GmbH, Bönen, Germany). Backfat thickness was determined every second week using ultrasound and body condition was scored accordingly (BCS, 5-point scale, Edmonson et al., 1989).

Blood samples

Blood samples were taken 3 weeks before estimated calving, 12 to 24 h after calving and 3 weeks after calving. Nine mL of blood were collected from the coccygeal vain using EDTA vacutainer tubes (Beckton Dickinsons AG, Allschwil, Germany). Samples were centrifuged for 20 min at 1850 *g* at 4°C. Plasma was collected and the aliquots were stored at -80°C until further analysis.

The Ferric Reducing Ability of Plasma (FRAP) assay was used to determine the antioxidant capacity of plasma (Chen et al., 2003). Reactive Oxygen Metabolites (ROM) were measured using the detection of ROM (dROM) test (Alberti *et al.*, 2000). The Oxidative stress index (Osi) was calculated as the ratio of dROM/FRAP × 100. Oxidative damage was measured as the intensity of lipid peroxidation as Thiobarbituric Acid Reactive Substances (TBARS, Gutteridge and Quinlan, 1983) and as advanced oxidation protein products (AOPP, Witko-Sarsat et al., 1996). Total protein concentration as reference for AOPP was measured using the Bradford assay (Bradford, 1976). Haptoglobin and adiponectin in the plasma of the heifers were measured using in-house-developed ELISA (Hiss et al., 2004, Mielenz et al., 2013). The inter and intra assay CV of the oxidative markers were < 6% and of ELISA for haptoglobin and adiponectin measurements < 14%, indicating good assay performance.

One heifer from the MR group was euthanized at 21 months of age, due to a leg fracture. After completion of all blood samples, 6 heifers were sold during the first lactation for managerial reasons: 4 from the TRANS group and 2 of MR.

Statistical analysis

The sample size for this study was based on our companion study (Ostendorf et al., 2025) which assessed growth performance parameters in calves. The power analysis,

conducted with a significance level of 0.05 and a power of 0.8, indicated that a cohort of 30 calves (15 per treatment group) would be sufficient to detect meaningful differences.

Statistical analysis was conducted using PROC MIXED in SAS, with time (age) as a repeated measures factor. The model included fixed effects of treatment (feeding), time (week or month), treatment-time interaction, with individual heifers as random effects. Before analysis, the normality of residuals was assessed using the Shapiro-Wilk test, and homogeneity of variance was checked with the Levene test, both performed using the UNIVARIATE procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Data not meeting the normal distribution assumption were log10-transformed. Various variance-covariance structures (autoregressive type 1, compound symmetry, and Toeplitz) were tested to identify the best-fitting model. Based on the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), autoregressive type 1 was selected for all parameters. Tukey-Kramer adjustment was applied for post-hoc tests to evaluate the significance of the group × time interaction. Significance was declared at $P \leq 0.05$, trends at $0.05 < P \leq 0.10$. All heifers of the TRANS group and 14 heifers of the MR group were included in the analysis of the blood parameters, while post calving performance data included 11 TRANS heifers and 12 of the MR group.

Results

Performance during the Rearing Period

Detailed information about performance and feed intake data is presented in the companion paper (Ostendorf *et al.,* 2025). In brief, calves had similar BW, ADG and liquid feed intake during the first 14 weeks of life. Starter intake differed numerically, energy intake significantly between the groups. TRANS calves had higher energy intake during the differential feeding period, as well as during the weaning phase. Both groups showed high BW gains with ADG of over 1 kg/d up to 14 weeks of age, even during weaning. Health data showed a trend for fewer health incidences in TRANS calves, as well as numerical differences

in other health parameters observed. However, the samples size of this study was too small to make further assertions on the health effects of the treatments used.

Reproductive and Growth Performance

Our results showed no differences between heifers fed TRANS and MR for age at 400 kg BW, age at first insemination, BW at first insemination, number of inseminations or age at calving (Table 1). Both treatment groups had easy calving, needing no assistance ($P = 0.24$), with birth weights of calves at 41.0 ± 0.67 kg in TRANS heifers and 42.1 ± 0.69 kg in MR heifers ($P = 0.61$). Colostrum quality, measured as brix values, was consistent across both groups: 24.9% in TRANS and 24.5% in MR heifers ($P = 0.72$). Both treatment groups had similar BW development during the rearing period and reached BW of around 700 kg before calving (712 ± 10.7 kg in TRANS, 688 ± 9.5 kg in MR).

However, postpartum BW was greater in heifers fed MR compared to those fed TRANS (Table 2; Fig. 2). Body condition score tended (P = 0.06) to be higher in TRANS-fed heifers during the first 200 d of lactation, while overall BW was lower in TRANS-fed heifers (P = 0.03; Table 2). Milk yield, ECM yield and milk composition (fat, protein and lactose) were neither affected by treatment (TRANS vs. MR) nor the time x treatment interaction (Table 2). Time had a significant effect on all variables except BCS and BFT, without interaction between time and treatment for any variable (Table 2).

Blood parameter

No significant treatment effects were observed for FRAP (Fig. 3a), dROM (Fig. 3b), and Osi (Fig. 3c), although time and a significant treatment × time interaction affected these variables ($P < 0.01$), with larger changes in these values in the MR group. A trend in treatment effects was noted for TBARS ($P = 0.07$), but without time or treatment × time interaction (Fig. 3d). Similarly, no significant treatment effects were observed for AOPP (Fig. 3e) or total protein (Fig. 3f); however, treatment × time interactions were observed for both AOPP ($P = 0.01$) and total protein ($P = 0.02$), with values varying in the weeks relative to calving. No

treatment effects were observed for haptoglobin (Fig. 3g) and adiponectin (Fig. 3h). Haptoglobin was affected by time ($P < 0.01$) and peaked around calving, while adiponectin levels increased from pre-calving to post-calving. No significant interactions between treatment and time were observed for haptoglobin and adiponectin.

Discussion

Our results suggest that while TRANS may influence early developmental stages (Ostendorf *et al.,* 2025), its long-term effects on performance during first breeding and lactation were limited. Both treatment groups reached the target BW for insemination at a comparable age and had similar reproductive performance indices.

No differences were found between the TRANS and MR groups in terms of milk yield, ECM yield, or milk composition (fat, protein, lactose). TRANS heifers showed a numerical lower SCC than the MR group, especially during the transition period. However, differences were not significant and remained on levels below the threshold for a healthy udder of 100,00 cells/mL (Tho Seeth and Krömker, 2021).

In a meta-analysis Gelsinger *et al.* (2016) found that other aspects, like management, are more important for long-term benefits than early life nutrition. However, a positive correlation can be seen between the provision of adequate nutrients and ADG over 0.5 kg/d with first lactation performance (Gelsinger *et al.,* 2016, Jiang *et al.,* 2025). Both treatment groups of the present study were well above an ADG of 0.5 kg (1.02 kg/d in MR and 1.06 kg/d in TRANS, Ostendorf *et al.,* 2025) and had low health incidences. In terms of lactation performance, both groups achieved milk yields that were above the regional Holstein average, with heifers producing approximately 2,000 kg more than the current local standard (Annual Report of the Rhineland-Palatinate-Saar State Control Association, 2023).

The high milk yield of dams in our study herd likely resulted in some dilution of transition milk during the first 5 days postpartum. Nevertheless, the transition milk provided likely contained higher concentrations of bioactive compounds compared to the milk replacer.

Therefore, any potential dilution probably had minimal impact on observed differences between groups. This interpretation aligns with previous findings, although direct comparisons are challenging due to differences in experimental design and duration of transition milk feeding (Van Soest et al., 2022; Pyo et al., 2020). Notably, both cited studies focused only on short-term effects and did not assess long-term outcomes. While variations in early energy and nutrient intake may explain some findings, they do not account for the BW differences observed during lactation. Notably, the heifers in this study were exceptionally well managed, resulting in above-average daily weight gains and superior lactation performance.

This result challenges our original hypothesis and suggests that the early developmental benefits associated with TRANS for only 5 d after birth do not necessarily translate into sustained improvements in fertility or production outcomes under the conditions of this study.

The finding that BW was about 18 kg lower in the TRANS group than in the MR group only during lactation was surprising in view of the short-term TRANS feeding in the neonatal stage, and the lack of long-term effects on growth, reproduction or lactation performance. The BW loss around calving was not different between both groups; the differences became only evident when the animals started re-gaining weight around week 8 of lactation. Unfortunately, we have no feed intake data from this time and also did not assess body size to offer potential explanations for the BW difference. Further investigation into energy balance and metabolic parameters is needed to identify the underlying causes of these differences.

Markers of the Oxidative Status

When examining markers of oxidative stress, we observed typical patterns around calving (Ghaffari *et al.,* 2024), but no significant differences between treatment groups. Time and treatment × time interactions were significant for some markers, but the lack of clear treatment effects suggests that TRANS feeding had no sustained effect on oxidative stress. Although trends in certain blood factors such as TBARS and significant interactions in AOPP and total protein indicate areas for further investigation, their biological relevance in this context remains unclear. Haptoglobin, an acute phase protein indicative of inflammation,

increased around calving as expected. Variability between animals was high for Hp, consistent with previous findings (Nightingale *et al.*, 2015). Adiponectin, an important regulator of lipid and glucose metabolism that was also suggested as a negative acute phase protein, showed increasing concentrations in both groups at and after calving; the increase after calving is in line with the literature whereas mostly decreasing values from late pregnancy to calving were reported (Sauerwein and Häußler, 2016). For adiponectin, also no group differences were observed. In general, it should be noted that both groups were on a relatively high level of feed allowance, i.e., 12 L/d, from birth to the onset of weaning. At that level, the animals are likely better equipped for their start in life and thus the TRANS feeding might be less important.

In summary, under the conditions of this study, TRANS feeding did not provide any significant long-term advantage over MR feeding for heifer development or performance except for postpartum BW. Future studies should address the extent to which TRANS feeding affects re-gaining of BW during the first lactation and what the consequences are for energy homeostasis.

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Table 1. Reproductive and growth performance of heifers fed milk replacer (MR) or transition milk (TRANS) during the first 5 days after birth.

Item [1]	Treatment		SEM	*P*-value
	TRANS	MR		Group (G)
Age at 400 kg of BW (d)	404	403	9.15	0.94
Age at first insemination (d)	452	444	10.2	0.6
BW at first insemination (kg)	446	435	8.57	0.36
Number of inseminations	2.15	2.15	0.28	0.99
Age at calving (d)	774	755	10.8	0.25

Table 2. Milk production, composition, body weight, body condition scores, and back fat thickness of heifers fed milk replacer (MR) or transition milk (TRANS)

Item [1]		Treatment		SEM	*P*-value		
		TRANS	MR		Group (G)	Time (T)	G×T
Milk yield, kg/d	Early lactation	33.74	33.74	0.73			
	Peak lactation	37.5	37	0.55			
	Mid lactation	36.7	36.33	0.29			
	Overall	36.4	36	0.25	0.22	< 0.01	0.99
Milk fat (%)	Early lactation	4.27	4.5	0.10			
	Peak lactation	3.79	3.99	0.07			
	Mid lactation	4.05	4.11	0.04			
	Overall	4.06	4.15	0.03	0.81	< 0.01	0.84
Milk protein (%)	Early lactation	3.34	3.39	0.04			
	Peak lactation	3.22	3.24	0.03			
	Mid lactation	3.58	3.58	0.02			
	Overall	3.47	3.49	0.02	0.89	< 0.01	0.91
Milk lactose (%)	Early lactation	4.92	4.98	0.02			
	Peak lactation	4.97	5.03	0.02			
	Mid lactation	4.92	4.86	0.01			
	Overall	4.87	4.95	0.01	0.81	< 0.01	0.93
SCC (x 1000 per mL)	Early lactation	45	92.03	15.55			
	Peak lactation	35.9	44	0.51			
	Mid lactation	32.1	28.08	1.96			
	Overall	35.5	39.7	3.38	0.12	0.001	0.99
BCS	Early lactation	3.09	2.95	0.09			
	Peak lactation	3.06	2.98	0.07			
	Mid lactation	3.04	2.93	0.04			
	Overall	3.13	2.98	0.03	0.06	0.85	0.63
Back fat thickness (mm)	Early lactation	10.75	10.86	2.68			
	Peak lactation	9.58	9.93	0.33			
	Mid lactation	9.11	9.27	0.32			
	Overall	9.94	9.79	0.17	0.82	0.10	0.44
BW (kg)	Early lactation	615	618	16.7			
	Peak lactation	619	627	1.49			
	Mid lactation	635	651	5.12			
	Overall	627	645	0.63	0.03	< 0.01	0.1

[1] Early lactation period (week 1–5), Peak lactation (week 6–8), Mid lactation (week 9–28), Overall (week 1–28).

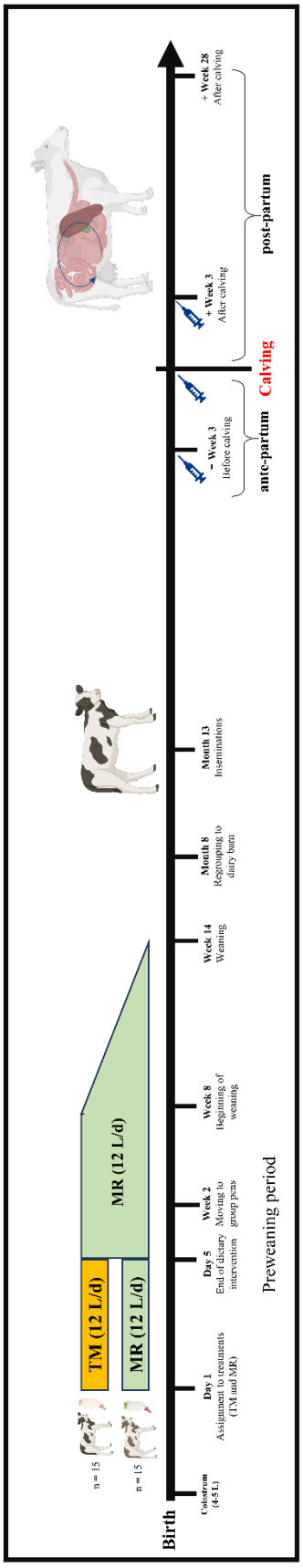

Fig. 1. Schematic representation of the study design and blood sampling including immediately after birth, pre-weaning, at weaning (week 14), post-weaning (months 8), first insemination (months 13) and calving (mean age 26 ± 2.3 months) and postpartum (until week 28).

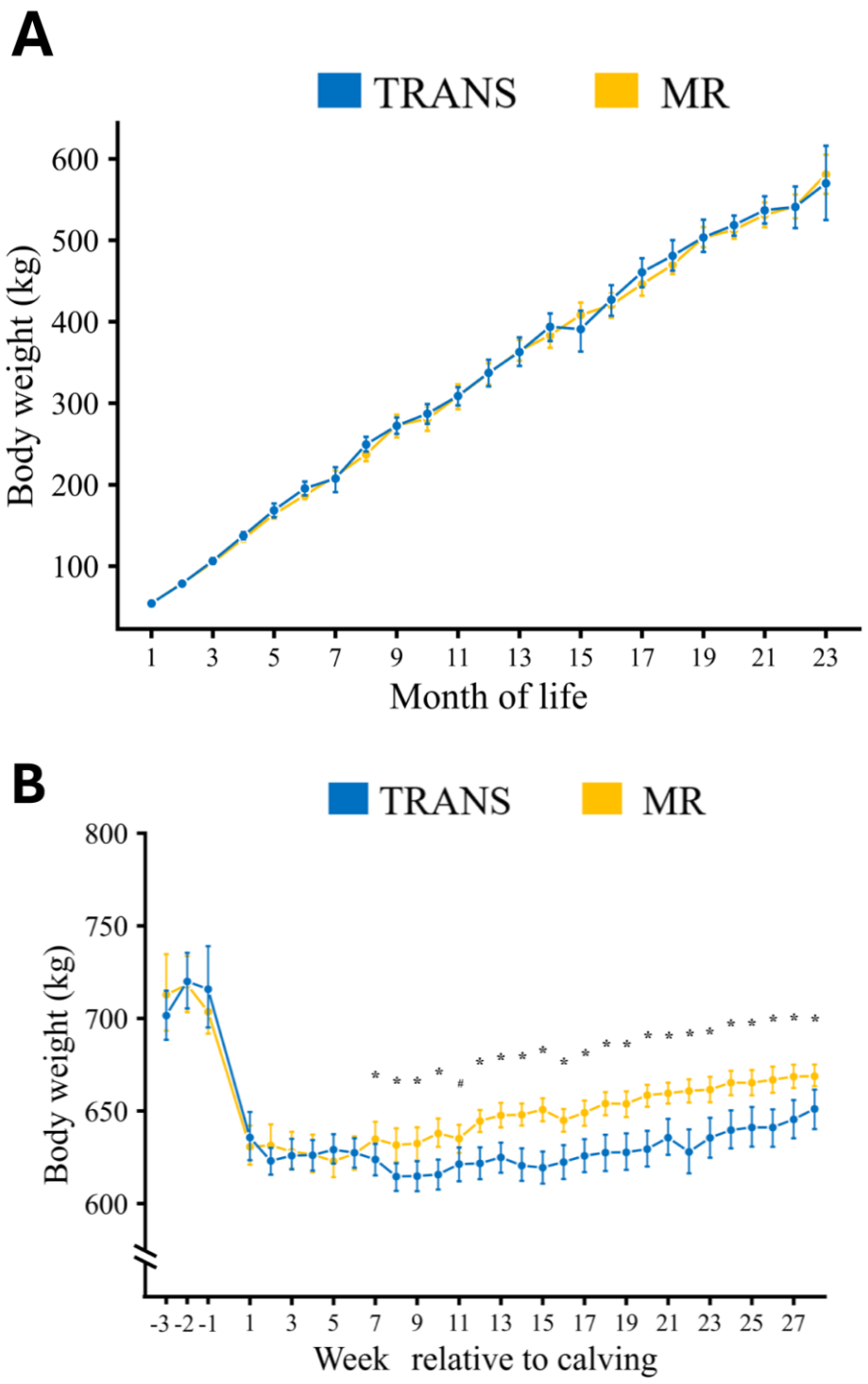

Fig. 2. Body weight of heifers fed milk replacer (MR) or transition milk (TRANS; during the first 5 days after birth) from birth to first calving (A) and during first lactation (B). Data is given as mean ± SEM.

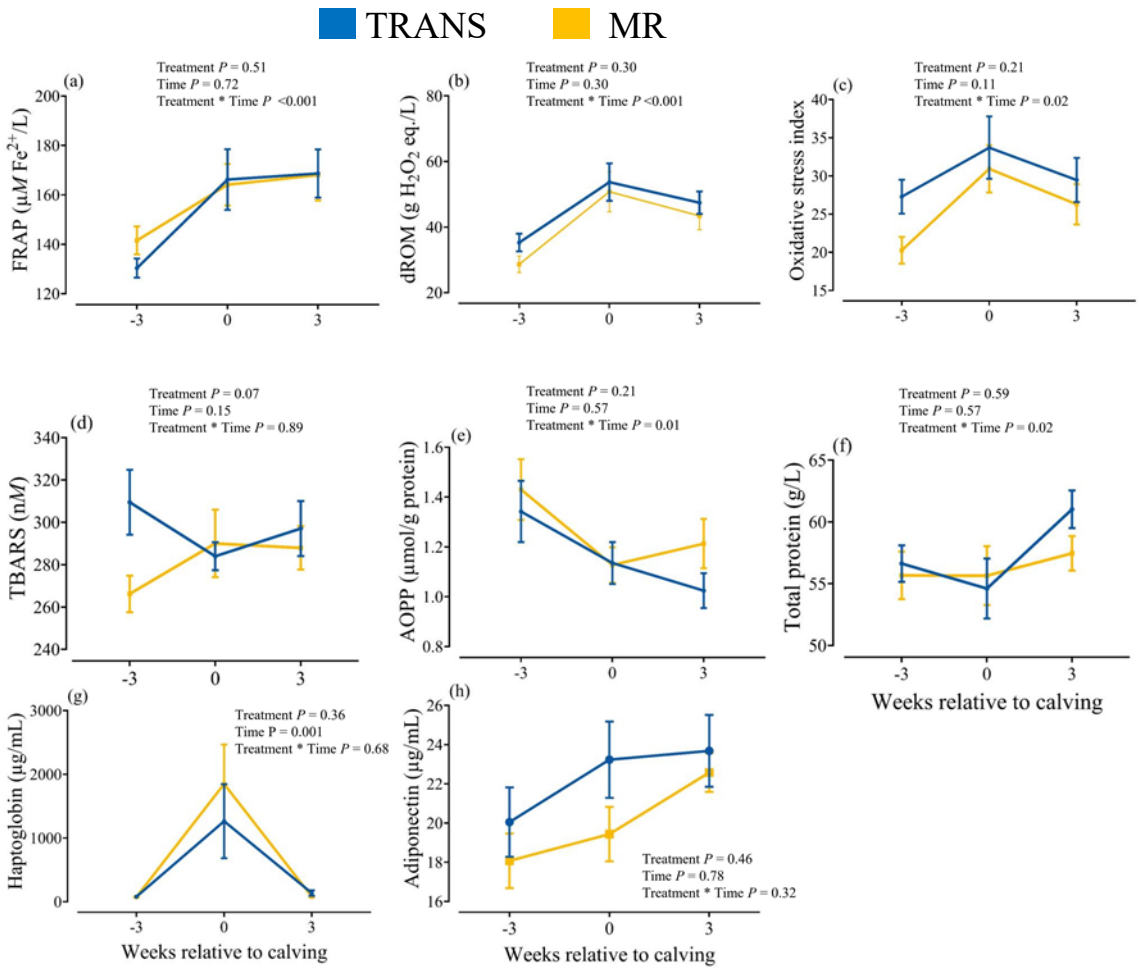

Fig. 3. Plasma measurements of Ferric Reducing Ability of Plasma (FRAP) (a), reactive Oxygen Metabolites (dROM) (b), oxidative stress index (c), Thiobarbituric Acid Reactive Substances (TBARS) (d), Advanced Oxidation Protein Products (AOPP) (e), total protein (f), haptoglobin (g), and adiponectin (h) in heifers, taken 3 weeks before parturition, 12-24 h after parturition, and 3 weeks after parturition. Data is given as mean ± SEM

V. DISCUSSION

1. Specifications for Transition Milk Feeding

The main objective in research into calf rearing is the optimization of the practices to enhance the calves' performance and development while balancing with economic and ecologic efficiency. Thus, recommendations for the best TRANS feeding protocol to maximize the calf's potential should be established. At present, the most consistent result in TRANS feeding research is that there are no negative effects in feeding TRANS to calves (CONNEELY et al., 2014; PYO et al., 2020; VAN SOEST et al., 2020; KARGAR et al., 2021; VAN SOEST et al., 2022). There is still much unknown about the full impact and the optimal feeding strategy. The objective of this thesis was to assess the effects seen by TRANS feeding in an optimized rearing system in which high feeding allowance is combined with a late and slow gradual weaning. The benefits of TRANS feeding are seen in the additional nutritional intake and increased supply of bioactive components compared to MR or WM (VAN SOEST, 2020). By providing both groups with a high feeding allowance in our study, the aim was to determine the influence of these higher levels of both TRANS feeding for the first 5 d of life and the subsequent MR feeding on the calf's development and performance.

Feeding volume

Previous studies showed improved development and health during the milk feeding period, when calves where fed TRANS (CONNEELY et al., 2014; VAN SOEST et al., 2020; KARGAR et al., 2021; VAN SOEST et al., 2022; ZOLOVA et al., 2022). VAN SOEST (2020) based the improvements in TRANS calves on the differences in energy intake between the feeding groups and questioned whether these differences could be maintained at higher nutritional levels. Considering that previous TRANS studies fed a maximum of 6 L per day to the calves (VAN SOEST et al., 2020; KARGAR et al., 2021; DA SILVA et al., 2023), this study was the first to test the effects of TRANS with a higher allowance. With 12 L of TRANS or MR for five days, TRANS calves gained 1.06 kg/d until the end of weaning and MR calves 1.02 kg/d, without significant difference between the groups. VAN SOEST et al. (2020) reported a significantly 10% higher ADG until weaning in their TRANS calves compared to MR calves, when they were fed 6 L of their treatment for 9 feedings. We achieved two times higher ADG in our TRANS calves during this time and 1.6 times higher ADG

in MR calves with our higher feeding protocol. Comparing the results of both trials on ADG is difficult, as other factors, like different feeding protocols and feed sources, and different rearing environments, also influence the development of the calves. Weight gain in our TRANS calves was still numerical higher and resulted in 5 kg more weight gain from birth to weaning compared to MR calves. However, the difference in weight gain between the treatment groups was substantially smaller than in VAN SOEST et al. (2020) This would indicate that VAN SOEST (2020) was correct in his theory that nutrient intake is more important for weight gain differences seen previously and that these differences would be smaller at higher feeding regimes. In contrast, DA SILVA et al. (2023) fed calves 4 L of TRANS or WM per day for 3 days and found no differences in weight gain between calves fed WM and calves fed TRANS. They based the differing results to VAN SOEST et al. (2020) on the lower amount fed to their calves. On the other hand, their results suggest that non-nutritive factors in milk, that are not found in MR, can improve the calves' performance (BÖSZE, 2008). However, PYO et al. (2020) showed a decrease in insulin-like growth-factor-1(IGF-1) and glucagon-like peptide-2 (GLP-2) in the first days after birth in calves fed 10 % of their BW TRANS or WM. This suggests that both of their feeding groups may not have consumed sufficient nutrients to meet their requirements. Concentrations of IGF-1 and GLP-2 are strongly correlated with adequate nutrient intake and are thought to be elevated with increased intestinal development (ELFSTRAND et al., 2002). The reduced *post natal* levels found by PYO et al. (2020) suggest that, regardless of the feeding group, the amount fed was insufficient and negatively affected the somatotropic growth axis. With similar amounts of feed allowance, the findings of PYO et al. (2020) further confirm the notion of VAN SOEST (2020), that the effects on growth performance of TRANS were due to energy differences between the diets. As already described previously, similar weight gains in our calves indicate that both groups were equally well supplied with energy for growth. The energy intake was significantly higher in TRANS calves, mainly due to the first five days of differential feeding. TRANS contained 1 Mcal/L ME, whereas the MR was at 0.68 Mcal/L ME, which led to around 30% more ME intake in TRANS calves during differential feeding. Afterwards, ME intake was similar in both groups, with slight differences during weaning, due to higher starter intake in TRANS calves. The recommendation for energy intake for maintenance at 50 kg of BW in Germany is 2.39 Mcal/d ME for dairy calves, for growth rates of over 1 kg it would be 5.25 Mcal/d ME intake (BUSKO, 2017). Both treatment groups were always above the

requirements for 1 kg of growth, apparently reaching their growth potential as BCS and BFT were similar in both groups. It would have been necessary to measure further body dimensions, like withers and hip height to get further knowledge about possible improvements on body development due to feeding.

Another factor that should be considered in relation to feeding amounts and nutrient intake is solid feed intake. It has been described that a higher milk allowance leads to a lower solid feed intake in suckling calves (KHAN et al., 2011). The higher allowance reduces the need for energy intake from solid feed, but solid feed intake was considered as prerequisite for full rumen development. With restrictive MR feeding, a higher solid feed intake can be achieved earlier and therefore an earlier, greater development of the rumen (SCHÄFF et al., 2018). Although many studies have since refuted this notion and emphasize the importance of an adequate milk supply (ROSENBERGER et al., 2017), the practice has been to feed a lower amount of milk or MR to accelerate the development of the forestomach to wean calves earlier (DIAO et al., 2019). However, these restrictive weaning strategies raise animal welfare concerns. Not only do restrictively fed calves visit the automatic feeder more often, a sign for hunger (KORST et al., 2017; SEIBT et al., 2021), they also show more and higher vocalizations (KHAN et al., 2011). In addition, high-starch and low-fiber diets, focusing on starter intake rather than forage, can lead to a delay in the development of the gastrointestinal tract and reduced rumen pH (KHAN et al., 2016). Feeding TRANS may help this problem by improving gut development compared to other liquid feed sources (PYO et al., 2020; VAN SOEST et al., 2022). The bioactive components in TRANS have been shown to enhance the development of the gastrointestinal tract (PYO et al., 2020) resulting in greater absorption of nutrients in the small intestine (STEINHOFF-WAGNER et al., 2014; INABU et al., 2019). We did not assess parameters to monitor these factors, but we have seen numerically earlier and increased starter intake in our TRANS calves during weaning. During the preweaning period from day 14 to day 56 TRANS calves consumed 120 g/d of starter, whereas MR calves consumed 90 g/d, while liquid intake was similar in both groups, resulting in higher energy uptake in the TRANS group. At day 45, around two weeks before the start of the weaning process, TRANS calves already consumed about 500 g/d and MR calves only about 250 g/d. During the weaning period, energy intake in TRANS was 6% higher compared to the MR calves. Relating this to improved gut development would be speculative, but grants for further research, to explore whether our results on higher starter intake in these animals were based on increased gut development. In

conventional weaning protocols, calves are usually weaned at an earlier age (SWEENEY et al., 2010) or over a shorter period (ECKERT et al., 2015). In these studies, lower solid feed intake and decreased ADG during weaning is reported (SWEENEY et al., 2010; ECKERT et al., 2015). Our results showed that both treatment groups were already consuming starter with the onset of weaning. With the slow weaning they could increase their solid intake accordingly and were able to sustain their energy intake and even increase their ADG: TRANS calves had a tendency for a higher ADG in week 9 and a significantly higher ADG in week 11 compared to MR calves, which resulted in 4 kg higher weight gain from the onset to the end of weaning. TRANS may be a way to improve weaning performance in environments with lower allowance or a shorter or earlier weaning protocol. However, the weaning strategy in our case seemed to be well suited to also allow MR calves to wean successfully and perform well afterwards as we saw continued weight gain during weaning in both groups.

Feeding duration

The duration of TRANS feeding is another way to influence the development of calves. There is no consensus in the literature on a period for labelling postpartum milk as 'transition milk'. Therefore, studies and practices differ in the duration of TRANS fed to calves. KARGAR et al. (2021) supplemented waste milk with different amounts of TRANS for two weeks. They saw the reason for the lower incidence of diarrhea in calves receiving 2 L of TRANS in the prolonged local protection of the gut in these calves, compared to calves receiving only waste milk (KARGAR et al., 2021). The theory behind this is that bioactive substances cannot be absorbed after the gut closure, but continue to act locally to protect the gut (KARGAR et al., 2021). This phenomenon was also observed by ZOLOVA et al. (2022). When feeding TRANS for 3 weeks, they found a lower risk of infection with *Cryptosporidium* spp., compared to calves only receiving WM. In a shorter feeding regimen of 3 days, DA SILVA et al. (2023) and CONNEELY et al. (2014) did not observe improved gut health. However, CONNEELY et al. (2014) found better eye/ear and nasal scores in their TRANS fed calves, again indicating the immune-enhancing effects of TRANS. We fed TRANS for a longer period than DA SILVA et al. (2023) or CONNEELY et al. (2014), albeit only by 2 days. Nevertheless, we observed a trend towards fewer cases of health disturbances in TRANS calves, i.e., numerically less incidences of diarrhea and respiratory illnesses, as compared to MR calves. This would further support the notion

that TRANS has immune-modulatory functions and that longer feeding periods may enhance this effect.

So far, TRANS appears to be a good option for improving lower allowance and shorter rearing programs, in particular when fed over a longer period to maximize performance and support gut health. In our high controlled environment with the optimized rearing strategy, effects of TRANS are smaller. An additional control group with a lower allowance would have been necessary to be able to compare the differences to a lower nutrient intake more clearly.

2. Development of the Oxidative Status in Calves

The oxidative balance is characterized by the concentrations of prooxidant properties and antioxidants to counteract them. If prooxidants exceed antioxidant concentrations, oxidative stress occurs, which may impede health.

The oxidative system contains many different parameters and mechanism that affect it, therefore only an estimate of the status can be made and the measurement of several parameters is needed to get a better picture (BRIVIBA et al., 2008). As part of the antioxidant system, we measured the ferric reducing ability of plasma (FRAP) and the activity of the glutathione peroxidase (GSHPx). We also determined the concentration of reactive oxygen metabolites (dROM) to represent prooxidants and measured the oxidative damage on lipids via the TBARS assay, and protein oxidation as advanced oxidation products of proteins (AOPP).

Oxidative status in neonates

It is known that infant formula and breast milk for human babies differ in terms of the content of antioxidants: AYCICEK et al. (2006) found a higher total antioxidant capacity in breastmilk than in formula milk, allowing the infant to cope better with oxidative stress at 3 to 6 months of age. Moreover, the OSi was higher in formula-fed infants. On the contrary, KORCHAZHKINA et al. (2006) found no effect in pre-term infants, as both sources of food were sufficient to prevent oxidative stress, but they only measured the TBARS concentrations in their infants. In the bovine species, it is also known that WM and TRANS have more antioxidative capacity compared to most MR (SOBERON et al., 2012b). Thus, as seen in the infants monitored by AYCICEK et al. (2006), TRANS feeding could also help calves to cope better with oxidative stress than MR. However, we did not see an effect of TRANS feeding on the oxidative status in the present study. The most, solely numerical, differences in measured parameters

were during the first five days of their life, during the differential feeding. Previously, RANADE et al. (2014) showed that the antioxidative defense in calves increases from birth onwards, which was also reported by ABUELO et al. (2014) and SEIBT et al. (2021). We were also able to verify these previous findings, with higher prooxidant and lower antioxidant concentrations immediately after birth and an inverse development of them in the following days. Interestingly, the working theory is that calves produce a large amount of ROM due to the short hypoxic state during and after birth (GAÁL et al., 2006). In contrast, our results showed no detectable amounts of ROM in the serum of the calves immediately after birth, but increased concentrations 12 h later. This could mean that this stress response takes time to manifest in the calf, as RANADE et al. (2014) showed that the oxidative status undergoes rapid changes in the first hours after birth. The concentration of ROM decreased afterwards in TRANS calves, whereas in MR a slight increase was observed until day two after birth. This could indicate that higher antioxidant concentrations in TRANS have beneficial effects in restoring the redox balance, but also confirms the theory of GAÁL et al. (2006), that healthy calves are usually able to cope with the oxidative disbalance after birth. The highest measurements of ROM were taken on day three in both treatment groups, with a decrease in FRAP at the same time. The changes were only minor but indicate a possible depletion of the still underdeveloped antioxidative system. The concentrations of ROM and therefor of OSi decreased or at least did not increase any further in the following days. This shows that our calves were able to cope with this situation (GAÁL et al., 2006) but also emphasizes the risk for an imbalance in the oxidative status of neonatal calves (RANADE et al., 2014).

Weaning

After week 3, all parameters assessed remained relatively stable and showed similar patterns in both treatment groups. This further supports the assumption that the antioxidative system of calves adapts during the first weeks of life (ALBERA & KANKOFER, 2011). Similar investigations were made in piglets. They are also exposed to massive oxidative stress after birth, which resolves in the first 7 days of life with the development of the antioxidative system (YIN et al., 2013). Once the redox balance is established, the system remains stable (BUCHET et al., 2017). A possible exception to this balance is weaning, if the weaning process is not well prepared (BUCHET et al., 2017). In calves, weaning is also stressful and can lead to oxidative burst responses (HULBERT et al., 2011). By reducing the WM or MR allowance,

calves are forced to consume their energy from solid feed and change their metabolism accordingly (SCHWARZKOPF et al., 2019). This leads to an increased ROM production and can overstrain the antioxidative system (MATTIOLI et al., 2020). In our study we did not see an increase in oxidative markers associated with weaning. Neither at the start of weaning at week 8, nor at the end at week 14, nor during the weaning period, nor during the two weeks thereafter. It is known that a late weaning allows calves to a smooth transition from liquid to solid feed intake and that this practice can reduce the stress response at weaning (SCHWARZKOPF et al., 2019). Starter intake and body weight development of our calves at weaning confirmed this finding. The starter intake was already high at the beginning of weaning and therefore the calves in our study were able to gain weight during this period (further discussed in chapter V.1). Combined with the stable oxidative balance throughout this period, we can confirm that the calves were able to adapt successfully to the slow changes during our weaning protocol.

After weaning

After weaning, sampling was performed at fewer time points to monitor critical milestones during maturation: two weeks after weaning, 24 hours after regrouping in the dairy barn, before first artificial insemination, 3 weeks before calving, after calving and 3 weeks after calving. The most interesting patterns were observed after regrouping. Surprisingly, ROM concentrations were low and TBARS concentration were high in both groups at this time. Cows are very social animals and therefore regrouping is a stressful situation (DENHAM & ADAMS PROGAR, 2023). It would be expected that due to the stress of being introduced to a new social group, ROM levels would increase and challenge the antioxidative system. RUBIO et al. (2021) found increased levels of antioxidant and prooxidant markers in the saliva 4 days after regrouping in weaned calves. In our heifers, ROM concentrations and GSH-Px activity were lower at this time compared to values seen before and after. TBARS measurements were highest at the regrouping sample and a slight increase was seen in FRAP. ROM may have already been neutralized, as social interactions happen especially within the first 6 hours (NOGUES et al., 2020) and heifers may have settled by the time of sampling. Further samples before and after regrouping would be needed as reference points to definitively assess these changes. In any case, OSi was low in both treatment groups and the heifers appeared to be well adapting to regrouping. The moving was already designed to minimize the stress in the heifers, as they were only

moved in the groups they lived in at the automatic feeder and received the same TMR in the dairy barn as they had previously in the calf barn.
Patterns at calving followed previously described measurements at this time (BERNABUCCI et al., 2005). An increase in antioxidants was observed 3 weeks before calving and prooxidants increased with calving. All parameters remained elevated during the *post partum* period, as the metabolism adapts to the demands of lactation (ABUELO et al., 2016). It would have been interesting to sample heifers more intensively during this period and further into lactation, to get further insight into the adaptation mechanisms happening.

Overall, the oxidative markers measured in our study were very similar in both treatment groups and in particular FRAP, dROM and TBARS were in a comparable range during calfhood and maturity.
At no time later in life were the parameters assessed as high as in the neonate. The first days after birth are a vulnerable time for the calf and optimizing rearing practices during this period is important. Irrespective of the diet, both of our treatment groups were able to cope with this challenge. The balance of the oxidative status seemed to happen slightly quicker in TRANS calves, but not on a significant level. The system appears to have adapted by week 3, with only minor changes thereafter and similar levels throughout.

Our results show that the effects of TRANS on the oxidative status need further research. As discussed in the previous chapter, our rearing protocol resulted in very good performance, regardless of the feeding group. As we are the first study to assess the effects of TRANS on the oxidative status of calves, we do not know whether the numerical differences seen during the first days in ROM and OSi throughout the rearing period were due to TRANS feeding or are part of the natural adaption of the oxidative system. Further studies would be required to test whether these numerical differences are caused by the different diets or whether they are simply a reflection of the developing and adapting metabolism in calves and to determine whether patterns seen in our trial would be more prominent in a more challenging environment.

3. Long-term Effects of Transition Milk Feeding

The requirements for first breeding in our study were mainly based on BW and age, as heifers needed to be at least 13 months old and to have a BW of at least 400 kg. Our heifers had a very similar weight development throughout their calfhood and therefore it is not surprising that the age at which they reached the required weight of 400 kg for first insemination was also not different between the two feeding groups. As puberty is mainly controlled by body weight (SCHILLO et al., 1992), it is not surprising that the same conception rates were found in our heifers. Long-term studies of different feeding regimes from calfhood to adulthood in calves are scarce, but the studies that did report different ages at first breeding also showed differences in body weight development during calfhood (MOALLEM et al., 2010; DAVIS RINCKER et al., 2011). Ultimately, TRANS feeding did not show any effects on first breeding performance in our heifers, but both treatment groups were 25 months at first calving and there was not much room for improvement to be achieved with the feeding.

There are even fewer reports on first lactation performance. We know that an initially higher colostrum supply can lead to numerically, but not significantly, higher milk production in the first lactation (FABER et al., 2005), but to significantly higher production in the second lactation. KORST et al. (2017) also found only numerical differences in first lactation milk yield in heifers fed WM *ad libitum* or MR *ad libitum*. MOALLEM et al. (2010) found trends towards increased milk production in WM vs. MR heifers and higher fat and protein yield. The protein intake of their heifers was very similar during calfhood and therefore the explanation is thought to be the lack of bioactive components in MR that are found in milk (MOALLEM et al., 2010). In contrast to these results, we did not observe any differences in milk yield or milk constituents in our heifers during the first 200 days of lactation. GELSINGER et al. (2016) concluded in a meta-analysis that other aspects, such as management, are more important than preweaning intake and growth rate for long-term performance benefits. However, they found a positive relation between providing adequate nutrients with a preweaning ADG greater than 0.5 kg/d and first lactation performance, particularly milk, fat and protein production, when these are combined with good postweaning practices (GELSINGER et al., 2016). Both of our treatment groups were well above a preweaning ADG of 0.5 kg/d (1.02 kg/d in MR and 1.06 kg/d in TRANS) and had low incidences of health issues over the examination period. During the first 14 weeks 3.9% of weekly veterinary checks and 0.96% of daily checks by staff required further

assistance. This shows that both groups had good pre-weaning management and thus a good basis for their later performance. That is further confirmed with the similar, above average, performance during the first 200 days in milk we saw in our heifers. Heifers from both treatments produced on average 36 ± 0.25 kg/d of milk. The average Holstein heifer in the region where the trial was done (Rhineland Palatine) produced 27 kg/d in 2023 during 300 days (LKV, 2023).

The somatic cell count (SCC) is a parameter used to assess the udder health, as it is usually an accumulation of cells of the immune defense in times of an inflammation (HARMON, 1994). The SCC in MR calves was numerically higher throughout the observation period. Especially during the *post partum* phase of the transition period, the cell count was almost double that of TRANS heifers (92,830 cells in MR, 54,630 in TRANS). The threshold for a healthy udder in Germany is considered to be < 100,000 cells (DLG, 2023). On average, all our heifers were below this threshold. However, combined with the trend towards fewer health events in TRANS calves that we saw during the rearing period and the numerical differences in the other established health parameters, this may indicate that TRANS can have long-term effects on health. Calf studies in which TRANS was fed more restrictively found more significant results regarding health effects of TRANS during calfhood, but did not monitor these calves further on in their life. As this is the first study to assess the long-term effects of TRANS feeding on the development of heifers even beyond weaning and the sample size is too low to definitively assess health data, definitive conclusions are not possible. Maintaining a large enough sample size and taking possible influencing factors, e.g., genetics or environmental factors, into account makes these long-term studies difficult. In order to be able to assess the effects of TRANS feeding with greater certainty, a larger sample size of heifers, as well as further consideration of genetic potential while allocating to the treatment groups, would be needed. Additionally, reevaluating the long-term effects with a lower feeding allowance performed in previous studies could also lead to more apparent results. With effects of TRANS already recorded during calfhood, differences during the first lactation seem more plausible.

Interestingly, TRANS heifers had a significantly lower BW during the first 200 days of lactation compared to the MR group. Since week 7 in milk, TRANS heifers were on average 30 kg lighter than MR heifers with similar BCS and RFT to the MR group. Further measurements of body dimensions, e.g., wither height or hip height, would be necessary to correlate this difference to altering body development. Additionally, milk

yield and components did not differ between the groups, resulting in the same energy requirements for milk production. To assess these BW differences further, feed intake data would be needed to compare feed efficiency of the two treatments.

4. Conclusion und Perspectives

Overall, it can be concluded that we still know very little about the effects and mechanisms of TRANS on calf development and performance and thus further research is needed. In our optimized rearing program, TRANS feeding showed no long-term effects on the measured parameters. Initial effects were seen in numerical differences in oxidative markers during the first days of life, in starter intake and in health parameters, as well as differences in BW during first lactation. The limits of TRANS seem to be reached in well-fed calves, as we could not reproduce the differences seen in studies with lower allowance. However, given our results of an increased starter intake at weaning and numerical differences in health parameters, TRANS has the potential to improve development and performance in less controlled and optimized environments. Our results also showed that the development of the oxidative status appears to be independent of the feeding regime in the early days of life. The mechanisms seen are similar to those described in adult cows and a functional balance is established in the first weeks of life with few changes afterwards. The values measured were never as high as in the days after birth, emphasizing the high stress situation to which calves are exposed.

The limitation of our study seems to be the optimized rearing regimen. Given previous results, it is inconclusive whether missing differences are based on true missing treatment effects or on the good rearing and high allowance in both groups. A control group with a lower feeding allowance would have been needed to accommodate for this. Considering our previous limited effects, most likely due to the high feeding plan, the effect of TRANS on the oxidative status should be re-tested with a different approach, possibly in a more challenging environment.

VI. SUMMARY

Early life nutrition lays the foundation for the development of the calf and can influence its performance in later life. Colostrum, with its nutritious and non-nutritious factors, plays an important role in this. However, these factors are not only found in colostrum, but also in decreased concentrations in the milk of the following days, the so-called 'transition milk'. The sale of this milk as bulk milk is prohibited in the EU; therefore, it could be a cheap and easy way to improve calf performance right from the neonatal stage.

One factor to consider is the oxidative status. The oxidative status is made up of pro- and antioxidants. Prooxidants are mostly reactive oxygen metabolites, a normal byproduct of the metabolism, that can cause oxidative damage to other substrates. Antioxidants are either endogenous systems, such as enzymes, or exogenous components, such as vitamins and minerals, that delay or prevent damage caused by reactive oxygen metabolites. The oxidative status plays a role in several physiological functions, as well as several diseases. A balance between pro- and antioxidants is therefore very important, especially in growing and still developing animals.

So far, we do not know how transition milk affects the calf after weaning. In addition, we do not have complete knowledge of the oxidative status in growing heifers. Therefore, the aim of this thesis was to examine the effects of five days transition milk feeding on the development and health of calves, as well as into maturity and first lactation. Furthermore, we aimed at assessing the oxidative status to determine whether transition milk feeding influences the redox balance and to describe the development of the oxidative status in growing heifers.

We studied the effect of five days of transition milk feeding in 50 Holstein calves (male and female) up to weaning and followed the 30 female Holstein calves further up to 200 days into their first lactation (Chapter III and IV). Twenty-five calves received 12 L transition milk of their dam for 5 days, while 25 calves received 12 L of milk replacer per day. After 5 days, all calves received 12 L of milk replacer per day until week 8, when the milk replacer allowance was gradually reduced until week 14. We assessed feed intake and feeding behavior throughout the rearing period, body weight and oxidative markers throughout the whole period, and lactation performance up to day 200 in the heifers.

There was no treatment effect on liquid intake and feeding behavior, but the transition milk-fed calves had higher energy intakes and numerically higher starter intake during the rearing period. Both treatment groups had high body weight gains, with average daily gains of over 1 kg/d up to 14 weeks of age, even during weaning. These observations confirm the benefit of the general feeding plan used, but the high feeding level and the late weaning time was obviously covering the animals' needs to such an extent that no further advantage could be achieved by feeding of TRANS. Furthermore, during this period, a trend towards fewer health incidents in the transition milk fed calves as well as numerical differences in the need for medication or antibiotic treatment could be observed, but the sample size of the study was too small to make further assertions on the health effects of the treatments.

The reproductive performance of the heifers was not different. First lactation milk yield and composition (fat, protein, and lactose content) during the first 200 days in milk were also not different between treatments. Numerical differences were observed in somatic cell count, particularly during the first three weeks of lactation with a lower cell count in heifers fed transition milk. This further suggests a potential effect of transition milk on the health of heifers. The body weight of the transition milk fed heifers was lower than that of the milk replacer fed animals during in these 200 days, but the former tended to have a higher body condition score. As the score was always within the desired optimal range and the backfat thickness of the animals did also not deviate, the differences in body weight observed seem not to have impaired health and performance.

The oxidative markers measured in the calves were the ferric reducing of plasma (FRAP) and detection of reactive oxygen metabolites (dROM) to calculate the oxidative stress index. In addition, glutathione-peroxidase (GSH-Px) activity was measured as an antioxidative enzyme during rearing and thiobarbiturate acid reactive substances (TBARS) and advanced oxidative protein products (AOPP) as oxidative damage on lipids and proteins, respectively throughout the period. Our results confirmed an undeveloped antioxidative status after birth and demonstrated the commencement of its regulatory mechanisms during the first days of life, with numerical differences between the treatments. An equilibrium was observed in both groups from week 3 onwards, without the stress response induced with weaning described in other studies. This further confirms the good rearing practices of our calves. The values around calving were very similar to those measured during rearing and maturation. All parameters were highest around birth and even at their own calving

changes were not as drastic. This confirms the high stress risk neonatal calves undergo, and the importance of supporting them as well as possible during this time. However, transition milk feeding had no effect on oxidative markers at any time. As with the other performance data evaluated, this could either be due to the lack of effects of transition milk on the oxidative status or due to the high feeding allowance and above average performance of our animals. Further research with different feeding regimes is needed to confirm or refute our findings.

Overall transition milk did not show major effects on the development and performance of calves and heifers at high feeding allowances. Knowledge of the effects of transition milk is still too limited to make definitive recommendations, and further research is needed to fill this knowledge gap. Until then we can confirm that transition milk feeding does seem to have positive effects on health parameters and solid feed intake.

VII. ZUSAMMENFASSUNG

Langfristige Entwicklung des oxidativen Status bei wachsenden Holstein Kälbern in Abhängigkeit einer anfänglichen postnatalen Fütterung mit Transitmilch oder Milchaustauscher

Constanze Sophie Ostendorf

Die Ernährung in den frühen Lebensstadien hat großen Einfluss auf die Entwicklung und Leistung von Kälbern. Besonders die nutritiven und nicht- nutritiven Faktoren von Kolostrum sind bekannt dafür, Einfluss auf die weitere Entwicklung des Neugeborenen zu nehmen. Doch nicht nur in Kolostrum sind diese Stoffe enthalten, auch die Milch der nächsten Tage, Transitmilch genannt, enthält diese in sinkenden Konzentrationen. Laut EU-Verordnung Nr. 853/2004 ist eine Abgabe dieser Milch für den Konsum untersagt und eine Fütterung an Kälber könnte daher eine einfache und günstige Möglichkeit bieten, bereits frühzeitig die spätere Leistung des Tieres zu beeinflussen.

Ein Faktor, der dabei von Bedeutung ist, ist der oxidative Status. Dieser setzt sich zusammen aus den Pro- und Antioxidantien. Pro-Oxidantien sind vor allem reaktive Sauerstoffmetaboliten, die ein normales Nebenprodukt in vielen Stoffwechselprozessen sind, und in zu hohen Konzentrationen zu oxidativen Schäden in anderen Substraten führen können. Antioxidantien sind entweder endogene Systeme oder exogen aufgenommene Stoffe, wie Vitamine und Mineralien, die die durch Pro-Oxidantien verursachte Schäden verzögern oder verhindern. Der oxidative Status ist eine Balance zwischen diesen Pro- und Antioxidantien und ist bedeutend für verschiedene physiologische Vorgänge, aber auch involviert in die Pathogenese verschiedenster Erkrankungen. Eine Balance zwischen den Komponenten ist daher für die Gesundheit und Entwicklung, besonders in noch heranwachsenden Tieren, wichtig.

Aktuell gibt es noch keine Informationen darüber, wie sich Transitmilchfütterung auf Rinder nach dem Absetzen auswirkt. Außerdem ist noch sehr wenig über die Entwicklung des oxidativen Status in wachsenden Rindern bekannt. Daher war das Ziel dieser Arbeit, die Effekte einer fünftägigen Transitmilchfütterung sowohl auf die

Entwicklung und Gesundheit von Kälbern als auch bis in deren Ausreifung und Leistung in der ersten Laktation, zu testen. Zusätzlich sollte durch die Messung von Parametern des oxidativen Status überprüft werden, ob Transitmilch einen Einfluss auf diesen Status hat und mehr Wissen über dessen Entwicklung in heranwachsenden Rindern bringen.

Wir haben dafür die fünftägige Transitmilch-Fütterung in 50 Holstein Kälbern (männlich und weiblich) bis zum Absetzen und in den 30 weiblichen Tieren bis Tag 200 in deren erster Laktation untersucht. 25 Kälber erhielten fünf Tage 12 L Transitmilch ihrer Mutter, während weitere 25 Kälber 12 L Milchaustauscher bekamen. Nach diesen fünf Tagen erhielten alle Kälber bis zur achten Lebenswoche 12 L Milchaustauscher am Tag und wurden dann bis zur 14. Lebenswoche graduell entwöhnt. Es wurden in der Tränkephase Futteraufnahme und Futteraufnahmeverhalten gemessen, sowie über die gesamte Periode bis in die Laktation hinein Körpergewicht und oxidative Marker. Bis zu Tag 200 in Milch wurden außerdem Milchleistung und -zusammensetzung erhoben.

Es konnten keine Effekte der Fütterungsgruppen auf die Aufnahme der flüssigen Futterkomponenten festgestellt werden, aber höhere Kraftfutter- und Energieaufnahme in den Transitmilch-gefütterten Kälbern. Die Körpergewichtsentwicklung in Kälbern beider Gruppen war in dieser Zeit sehr hoch, mit durchschnittlichen Tageszunahmen von über 1 kg. Diese Ergebnisse zeigen die sehr guten Erfolge, die ein hoher Fütterungsplan mit einem späten Absetzen erzielen kann, können aber auch der Grund für fehlende Gruppeneffekte sein, die von vorherigen Autoren beschrieben wurden. Außerdem konnte in dieser Zeit ein Trend zu weniger Krankheitsfällen in Transitmilch-Kälbern gefunden werden. Die Stichprobengröße war jedoch zu klein, um weitere Schlussfolgerungen über die Gesundheitseffekte der Behandlungen zu schließen.

Die Parameter der reproduktiven Performance der Färsen waren ohne Unterschiede. Die Milchleistung und die Konzentrationen von Fett, Protein und Laktose in den ersten 200 Laktationstagen unterschied sich außerdem nicht zwischen beiden Gruppen. Numerische Unterschiede konnten in der somatischen Zellzahl, besonders während der Transitperiode, festgestellt werden, bei der Transitmilch-gefütterte Färsen eine niedrigere Zellzahl aufwiesen. Dies ist ein weiterer Indikator für potenzielle Effekte, die Transitmilch auf den Gesundheitsstatus von Färsen haben kann. Das

Körpergewicht von Transitmilch-Färsen war in den ersten 200 Laktationstagen niedriger als in den Milchaustauscher-Tieren, sie tendierten aber dazu einen höheren Body-Condition-Score zu haben. Da der Score sich jederzeit im erstrebten Optimalbereich befand und die Rückenfettdicke der Tiere auch nicht unterschiedlich war, sind die Körpergewichtsunterschiede ohne offensichtliche Konsequenzen. Messungen der Futteraufnahme wären nötig, um Aussagen über die Energieeffizienz der Tiere treffen und die gemessenen Unterschiede im Körpergewicht besser einordnen zu können.

Die oxidativen Marker, die erhoben wurden, waren die eisenreduzierende Kapazität von Plasma (FRAP-Assay) und der Nachweis der reaktiven oxygenen Metaboliten (dROM) zur Kalkulation des oxidativen Stress-Indexes, als Verhältnis zwischen Pro- und Antioxidantien. Außerdem wurde die Aktivität der Glutathion-Peroxidase (GSH-Px) als antioxidativem Enzym und die Thiobarbiturat-reagierenden Substanzen (TBARS) und die fortgeschrittenen oxidierten Proteinprodukte (AOPP) als oxidative Schäden an Lipiden bzw. Proteinen gemessen. Unsere Ergebnisse zeigen, dass das oxidative System von Kälbern nach der Geburt unterentwickelt ist, sich aber in den ersten Lebenstagen etabliert, wobei numerische Unterschiede zwischen den Fütterungsgruppen zu beobachten waren. Eine gefestigte Balance kann ab Lebenswoche 3 in beiden Gruppen beobachtet werden, ohne den in vorherigen Studien beschriebenen oxidativen Stress durch das Absetzten. Das bestätigt zusätzlich die gute Aufzuchtspraxis, die in unserem Versuch durchgeführt wurde. Die Messungen rund um die Kalbung der Färsen sind sehr ähnlich zu denen während der Aufzuchtsphase. Alle gemessenen Werte hatten ihren Höhepunkt in der Neonatal-Phase und selbst die Veränderungen später bei der eigenen Kalbung waren demgegenüber nicht so deutlich. Das weist darauf hin, dass die Geburt für das Kalb eine Stressbelastung darstellt und wie wichtig damit eine bestmögliche Unterstützung in dieser Zeit ist.

Transitmilch hatte jedoch keinen Effekt auf die Entwicklung des oxidativen Status. Wie bereits bei den anderen Leistungsparametern festgestellt, hat das gute Fütterungsmanagement zu einer überdurchschnittlichen Leistung in den untersuchten Tieren geführt. Es sind also weitere Studien mit anderen Versuchsbedingungen und anderer Transitmilchfütterung notwendig, um tatsächlich zu bestätigen oder zu entkräften, dass Transitmilch einen Einfluss auf die Entwicklung des oxidativen Status hat.

Alles in allem hat Transitmilch bei dem hier getesteten Fütterungsniveau von 12 L/Tag keine signifikanten Effekte auf die Entwicklung und Leistung der Kälber bzw. Färsen gezeigt. Das Wissen über die Effekte von Transitmilch ist aber noch zu limitiert, um klare Empfehlungen auszusprechen. Weitere Forschung ist nötig, um diese Wissenslücke zu füllen. Bis dahin sind für die Gabe von Transitmilch von eher positiven Effekten auf die Tiergesundheit und die Aufnahme von Kraftfutter auszugehen.

VIII. REFERENCES

In the following the references for chapter I, II, and V are given. The references for the manuscripts in chapter III and IV are listed directly in the respective chapter.

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IX. PUBLICATIONS AND PROCEEDINGS DERIVED FROM THIS DOCTORATE THESIS

1. **C.S. Ostendorf**, M.H. Ghaffari, K.J. Hemmert, C. Koch, H. Sauerwein (2025*): Long-term effects of transition milk feeding on feed intake, feeding behavior, development, and oxidative status of Holstein calves,* Journal of Dairy Science, DOI: 10.3168/jds.2024-25435.
2. *Accepted:* **C.S. Ostendorf**, M.H. Ghaffari, I. Cohrs, C. Koch, H. Sauerwein (2025*): Effects of transition milk feeding after birth on performance and oxidative status during first lactation in Holstein heifers,* Journal of Dairy Research.
3. **C.S. Ostendorf**, M.H. Ghaffari, C. Koch, H. Sauerwein (2023): *Effects of transition milk on growth performance and health of Holstein calves*. Annual meeting of the American association of dairy science, Journal of Dairy Science Vol. 106, Suppl. 1, page 342.
4. **C.S. Ostendorf**, M.H. Ghaffari, B. Heitkönig, C. Koch, H. Sauerwein (2023): *Oxidative status in female Holstein calves fed with or without transition milk*. EAAP – 74th Annual Meeting, Lyon, Book of Abstracts, page 997.
5. **C.S. Ostendorf,** M. H. Ghaffari, T. Scheu, C. Koch, H. Sauerwein (2024): *Der Effekt von Transitmilchfütterung in den ersten fünf Lebenstagen auf die Entwicklung von Holstein Kälbern.* DVG-Vet-Kongress 2024, Berlin.
6. **C.S. Ostendorf,** M. H. Ghaffari, T. Scheu, C. Koch, H. Sauerwein (2024): *Lohnt sich die verlängerte Gabe von Transitmilch an Kälber?,* 124. Tagung des Arbeitskreises der Futterberater für die Länder Hessen, Rheinland-Pfalz und Saarland.
7. **C.S. Ostendorf** (2025): *Transitmilch an Kälber füttern,* Milchpraxis, Ausgabe 1/2025 (59. Jg.).
8. M. H. Ghaffari, **C. S. Ostendorf**, K. J. Hemmert, S. Schuchardt, C. Koch, and H. Sauerwein (2025): *Longitudinal characterization of plasma and fecal bile acids in dairy heifers from birth to first calving in response to transition milk feeding,* Journal of dairy science, DOI: 10.3168/jds.2025-26307
9. M. H. Ghaffari, **C. S. Ostendorf**, K. J. Hemmert, S. Schuchardt, C. Koch, and H. Sauerwein (2025): *Longitudinal characterization of plasma amino acids, biogenic amines, and amino acid-related metabolites in heifers from birth to first calving in response to colostrum and transition milk feeding,* Journal of dairy science, DOI: 10.3168/jds.2025-26814

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beruhigt eine Stunde länger schlafen, da ich wusste, dass du noch im Stall sein würdest, um mir bei meinen Proben zu helfen, was mir mein Schlafdefizit doch ein wenig verringert hat.

Danke, Anna, dass du auch immer wieder versucht hast, mir auch ein Leben außerhalb der Neumühle zu zeigen. Viele Dinge sind nur durch dich (und den Tee…) erträglich geworden. Du hattest für alle meine Probleme einen Lösungsvorschlag und ich konnte immer auf dich zählen. Auch wenn du nicht davon begeistert warst, ist es dir zu verdanken, dass am Ende alle weiblichen Kälber tragend geworden sind und die Möglichkeit bekamen, den Versuch zu beenden. War ja klar, dass selbst Simba es nicht schafft, die 2155 tragend zu bekommen, du dann aber im allerletzten Versuch!

Anke, deine Ruhe hat mir besonders in den Phasen geholfen, in denen alles zu viel wurde. Und danke auch dafür, dass du immer den Fahrservice gemacht hast, du hast definitiv noch einiges gut bei mir!

Mit niemandem hat das Probenziehen bei neugeborenen Kälbern so gut funktioniert, wie mit Elli. Auch wenn man dich sonst schon durch den halben Stall hören konnte (auch wenn das tatsächliche Verstehen teilweise an der Sprachbarriere scheiterte, an manche Dialekte gewöhnt man sich nicht so schnell…), bei den Kälbern hattest du immer die nötige Ruhe, um die Probennahme möglichst schnell über die Bühne zu bringen.

Ewald, dein Riecher für eine bevorstehende Kalbung hat mir noch die ein- oder andere Probe vor der Kalbung gerettet. Auch wenn ich bei deinen Wochenenddiensten immer besonders früh in den Stall kommen musste, bin ich dir für deine Schnelligkeit bei den frühmorgendlichen Kalbungen sehr dankbar!

Danke Daniel, für deine Bereitschaft, bei allem zu helfen, wo Hilfe benötigt wurde. Ob das Probleme am Wochenende am Tränkeautomaten waren, Proben, die gezogen werden mussten oder Kalbungen, die Geburtshelfer benötigt haben, ich kann mich nicht daran erinnern, dass du irgendwann mal eine Anfrage abgelehnt hättest.

Vielen Dank auch an Franzi! Auch wenn du rückblickend nur kurzzeitig Teil dieses Projektes warst, war das doch zu einer sehr entscheidenden Zeit. Danke, dass du mit mir nachts in den Stall gekommen bist, um Proben zu ziehen. Danke für deinen Beistand. Dank dir haben am Ende doch alle Kälber schon von Anfang an einen Namen bekommen, obwohl ich das eigentlich nicht vorgehabt habe. Die Plätzchenbackaktionen oder Filmabende mit Peter beim Eppi kleben haben den ersten Winter mit vielen schlaflosen Nächten erträglich gemacht.

Meine Familie ist natürlich auch nicht zu vergessen. Meine Eltern, die es mir überhaupt erst ermöglicht haben, Tiermedizin in München zu studieren und mich in diesem Entschluss immer unterstützt haben. Danke an meine Geschwister, die regelmäßig ihre Meinung und Rat zu diversen Graphiken, Präsentationen und allen möglichen anderen Kram abgeben mussten, obwohl sie nichts mit Tieren und Landwirtschaft am Hut haben.

Danke an meine Münchner Mädels, Kathrin, Maria, Anna, Verena und Anna. Allein das Studium zu beenden hätte ohne euch niemals funktioniert. Danke auch, dass ihr euch die ganze Zeit mein Gejammer angehört habt, obwohl ihr selbst in ähnlichen, wenn nicht schlimmeren, Situationen gesteckt habt. Eure Unterstützung hat mir wirklich den Rücken gestärkt.

Den wichtigsten Einfluss darauf, dass diese Arbeit in dieser Form jemals entstehen konnte, hatte aber vermutlich mein Großvater. Durch ihn gab es bei mir seit meiner Kindergartenzeit keinen anderen Wunsch, als irgendwann als Tierärztin im Stall zu stehen. Auch wenn es ihm vermutlich nicht bewusst war, war er am Ende die treibende Kraft, die mich dazu gebracht hat, die Zähne zusammenzubeißen und endlich meine Doktorarbeit zu Ende zu bringen. Er hat mir zwar immer davon abgeraten zu promovieren, aber auf alles muss man dann ja auch nicht hören… Ich hätte ihm diese Arbeit auch sehr gerne persönlich gezeigt, aber da hat er dann wiederum nicht auf mich hören wollen.