Effects of parity, farm, and dietary energy levels on circulating adiponectin concentrations and on oxidative status in high yielding dairy cows in late pregnancy and early lactation

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ABBREVIATIONS

Abbreviation	Meaning
BCS	Body condition score
BHB	Beta-hydroxybutyrate
dROM	Derivatives of reactive oxygen metabolites
FRAP	Ferric reducing ability of plasma
NEFA	Non-esterified fatty acids
OSi	Oxidative status index
ROM	Reactive oxygen metabolites
RQUICKI	Revised quantitative insulin sensitivity check index

I. INTRODUCTION

The transition period of dairy cows is commonly defined as the time from three weeks prepartum until three weeks postpartum and is a very important and challenging phase in the productive cycle of dairy cows (GRUMMER 1995). The development of the offspring and milk synthesis, i.e. energy and nutrient supply toward the placenta and mammary gland, have high priority in the transition period (BELL 1995; BELL and BAUMAN 1997). Due to decreasing dry matter intake in late pregnancy and a relative slow increase of dry matter intake in the first weeks of lactation, the rapidly increasing energy demands cannot be covered sufficiently (BERTICS et al. 1992; GRANT and ALBRIGHT 1995; BELL 1995). Cows' metabolism is adapting to this negative energy balance with hormonal changes, which lead, amongst other, to substantial mobilization of adipose tissue and protein (GRUMMER 1995; TAMMINGA et al. 1997). Glucose is the major nutrient for the conceptus and is the precursor for lactose, which is the main osmotic regulator of milk yield (BELL and BAUMAN 1997; HOLT 1983). To ensure sufficient glucose supply to the placenta and mammary gland, insulin sensitivity in peripheral tissues other than placenta and mammary gland is decreased. The placenta and mammary gland itself are not reliant on insulin due to the presence of insulin independent GLUT1 and GLUT3 glucose transporters (BELL and BAUMAN 1997). Hence, the glucose supply to peripheral tissues like muscle and adipose tissue is reduced in contrast to an increased glucose supply to the placenta and mammary gland. These metabolic changes due to the negative energy balance are considered as a major reason for the development of production diseases like ketosis, fatty liver, mastitis and metritis (BLOCK 2010). To improve the management of transition cows and enable breeding programs to work towards metabolically more stable and robust dairy cows it is necessary to identify and characterize cows with successful metabolic adaptations during the transition period. For this purpose the mechanisms behind these adaptations need to be understood as good as possible and indicators for a successful adaption need to be identified. Adiponectin, as insulin sensitizing hormone, and oxidative stress as one decreasing factor on insulin sensitivity, form the focus of this work to elucidate their role in the transition period and find out more about their interrelationship and influencing factors. In the end it will be discussed if one of

these factors or a combination of both could be suitable as indicator for metabolically successful transition cows or could at least be used as starting point for further research in this field.

II. LITERATURE REVIEW

1. Adiponectin

1.1. Adiponectin – general background

Adiponectin was discovered independently from four research groups 20 years ago (SCHERER et al. 1995; HU et al. 1996; MAEDA et al. 1996; NAKANO et al. 1996) and is also named ACRP30, apM1, adipoQ, or GBP28. It is a hormone exclusively produced in adipocytes and therefore belongs to the group of adipokines. Adiponectin contains 247 amino acids resulting in a molecular weight of around 30 kDa (SCHERER et al. 1995) and circulates in three different molecular weight forms: low molecular weight forms with three monomers, middle molecular weight forms with six monomers, and high molecular weight forms with 18 or more monomers (TSAO et al. 2003). The high molecular weight forms are considered the biologically most active form (FISHER et al. 2005; PAJVANI et al. 2004; WAKI et al. 2003), but TRUJILLO and SCHERER (2005) also stated in their review that measuring the total adiponectin concentrations remains legitimate as the levels of the high molecular weight form are mostly proportional to the total adiponectin concentration. The total adiponectin concentrations in the circulation make up around 0.01 % of total serum proteins and unlike other adipokines, i.e. leptin or resistin, circulating concentrations of adiponectin are decreased with increasing obesity (ARITA et al. 1999). As reviewed by KADOWAKI et al. (2006) adiponectin concentrations in humans are associated with several physiological and pathophysiological conditions, as sex, age, body condition, diet, and diseases like Type 2 diabetes, metabolic syndrome, hypertension and several more.

The insulin sensitizing effect of adiponectin is considered to be the main function, but it is still not clear in which direction the causal association between adiponectin and insulin sensitivity points. Insulin had a decreasing effect on adiponectin levels in humans and rodents (COMBS et al. 2001; YU et al. 2002), and hyperinsulinemia is associated with insulin resistance (SHANIK et al. 2008). Studies on Rhesus monkeys detected a drop of adiponectin already before the development of hyperinsulinemia (HOTTA et al. 1998; HOTTA et al. 2001). Hence, there is still need for research on the effect on and of adiponectin and especially

the underlying mechanisms, not only in terms of insulin sensitivity, but also on the several other effects reported in humans and rodents so far (Table 1).

Table 1. Effects of adiponectin detected in humans and rodents:

	Human (H)/Rodent (R)	Reference
Muscle	Increased glucose uptake (R) Increased lipid oxidation (R)	(YAMAUCHI et al. 2002) (YAMAUCHI et aL. 2001; YAMAUCHI et al. 2002)
Adipose tissue	Increased lipoprotein lipase activity (H, R)	(TRUJILLO and SCHERER 2006); (VRIES et al. 2005; EYNATTEN et al. 2004)
Liver	Decreased gluconeogenesis (R) Enhanced hepatic insulin sensitivity (H, R)	(COMBS et al. 2001) (TRUJILLO and SCHERER 2005)
Heart	Decreased myocardial infarct size in a cardiac ischemia/reperfusion model (R) Decreased risk for cardiomyopathy (H) Potent cardioprotective effects (H, R)	(OHASHI et al. 2006; OUCHI et al. 2006; SHIBATA et al. 2004; SHIBATA et al. 2005) (HOTTA et al. 2000; PISCHON et al. 2004; KUMADA et al. 2003) (YAMAMOTO et al. 2002; OHASHI et al. 2006; OUCHI et al. 2006; SHIBATA et al. 2004; SHIBATA et al. 2005)
Blood vessels	Decreased risk for atherosclerosis and thrombosis (H, R)	(KUBOTA et al. 2002; ARITA et al. 2002)

1.2. Adiponectin in dairy cows

There are already several studies on adiponectin in bovine animals revealing effects of adiponectin during different metabolic situation, mainly focusing on the dry period and lactation. Circulating adiponectin concentrations in dairy cows range between 20 and 40 µg/mL and are affected by several endogenous and exogenous factors, e.g. age, estrous, and feed (SAUERWEIN and HÄUßLER 2016).

The major changes in its concentrations occur in the peripartal period (SINGH et al. 2014a). Greatest concentrations can be found in late pregnancy, which then decline and reach a nadir around parturition. In the first weeks of lactation, the adiponectin concentrations increase again and reach basal levels again around the third week of lactation (GIESY et al. 2012; MIELENZ et al. 2013; SINGH et al. 2014b). It was supposed that the reduced adiponectin concentrations during the peripartal period belong to the subset of hormonal adaptations, which are necessary to deal with the physiological challenges and the negative energy balance (GIESY et al. 2012). The placenta and lactating mammary gland can take up glucose insulin-independently via the GLUT1 and GLUT3 receptor (BELL and BAUMAN 1997; ZHAO and KEATING 2007). Therefore, lower adiponectin concentrations, assumed to coincide with decreased insulin sensitivity in peripheral tissues like skeletal muscle and adipose tissue, would enhance the glucose uptake by the placenta and mammary gland in the peripartal period (GIESY et al. 2012; KOSTER and OPSOMER 2013).

In cooperation with a group from the University of Ghent, we could confirm with hyperinsulinemic euglycemic clamp tests during the dry period, that adiponectin was associated with the insulin responsiveness, i.e. the maximal effect of insulin on the glucose and lipid metabolism, but there was no correlation between adiponectin and the insulin sensitivity, i.e. the insulin concentration needed to achieve the half maximal effect, during the dry period (KOSTER et al. 2017).

KASIMANICKAM et al. (2013) demonstrated that the inverse relation between body condition and circulating adiponectin concentrations is also present in cows: Cows with a low body condition score (BCS; 2 and 2.5) had greater adiponectin concentrations compared with high BCS cows (3 to 4). SINGH et al. (2014b) further showed that the association is strongest with the mass of visceral adipose tissue compared to the subcutaneous or total adipose tissue mass. In addition, MELLOUK et al. (2017) reported a negative correlation between adiponectin and back fat thickness in dairy cows over two lactations. Around parturition the association between adiponectin and body condition seems to be decoupled, as the increase of adiponectin after parturition occurs independently from the loss of adiposity and there was no relation between change of body condition and change in plasma adiponectin during the peripartal study period in the study of KRUMM et al. (2017). During the dry period we showed a negative correlation between BCS

and adiponectin concentrations in the study with KOSTER et al. (2017) but in another study together with MANN et al. (2018) during the dry period we could not detect differences in the adiponectin concentrations between cows with a BCS ≤ 3.25 compared with cows with a BCS > 3.25. The latter could be explained by the smaller range of BCS and lower overall level of BCS compared with other studies (KOSTER et al. 2017; MANN et al. 2018).

Given the fact that adiponectin is associated with body condition, i.e. energy storage, and that the greatest changes occur around parturition when cows are dealing with a negative energy balance, the level of energy supply is likely to influence adiponectin.

SINGH et al. (2014a) tested the effect of a negative energy balance induced at around 100 days in milk by feed restriction to a similar extent as occurring physiologically in the first weeks of lactation, but this did not affect the circulating adiponectin concentrations. KAFI et al. (2015) reported a negative association between adiponectin and milk yield, again indicating an association of adiponectin with energy metabolism. Studies on the effect of feeding different energy levels on circulating adiponectin concentrations revealed different results. MELLOUK et al. (2017) fed cows either a diet calculated for 35 or 25 kg milk per day from one month before calving over two lactations. Adiponectin concentrations in the animals of the low energy group were lower and the drop of adiponectin towards calving was similar in both groups, whereas the increase after calving was greater in the high energy group. In contrast to that, there was no effect of feeding either 100 % or 150 % of the calculated energy demands during the dry period on the adiponectin concentrations (MANN et al. 2018). KRUMM et al. (2017) investigated the effect of energy balance on adiponectin by either milking fresh calved cows thrice daily or not milk them at all during the first four weeks after calving. In this approach adiponectin concentrations were 21 % greater in the non-milked cows compared with the milked cows. Hence, there seems to be a relation between energy status and adiponectin in early lactation, but it remains unclear which additional influencing factors are present at this time, but not in mid lactation or the dry period.

Age is also influencing the adiponectin concentrations in dairy cows. The concentrations are very low in newborn calves and increase rapidly after

colostrum intake. Until 52 days of life the adiponectin concentration remains more or less constant and then increases again until day 108 of life reaching around 20 µg/mL and therefore concentrations as seen in adult cows (KESSER et al. 2015). Later in life age dependent differences can be seen when comparing primiparous and pluriparous cows. SINGH et al. (2014c) reported that primiparous cows tended to have lower adiponectin concentrations than pluriparous cows from 21 days before until 252 days after calving. In contrast to that MELLOUK et al. (2017) found greater adiponectin concentrations in cows in the first compared to the second lactation. Hence, the results on the effect of age and parity are inconsistent so far and need further investigation.

There are some reports on associations between adiponectin and diseases. KASIMANICKAM et al. (2013) found that adiponectin serum concentrations in early lactation were greater in cows with metritis or clinical endometritis compared to healthy cows. Furthermore, cows that developed hyperketonemia postpartum had lower adiponectin concentrations during the dry period (MANN et al. 2018). Adiponectin serum concentrations change during the normal estrous cycle. HEINZ et al. (2015) reported 1.2-fold lower concentrations on day 10 than on day 0 and 3 of the estrous cycle. KAFI et al. (2015) reported a gradual decrease after ovulation with a following increase before the next ovulation. Furthermore, they found that cows with normal luteal activity and an earlier commencement of luteal activity had greater adiponectin concentrations postpartum compared with cows with a delayed commencement of luteal activity or cows with prolonged luteal phase, delayed first ovulation, or anovulation.

As the influencing factors on adiponectin in dairy cows are still not fully elucidated, this thesis focuses on possible factors affecting adiponectin during late gestation and early lactation. In the first study (Chapter III) we investigated the effects of parity, farm, and feeding different amounts of concentrate on the circulating adiponectin concentration. These results might help to answer the question whether adiponectin could be used as an indicator for dairy cows' metabolic situation.

2. Oxidative Status

2.1. Reactive oxygen metabolites

Reactive oxygen metabolites (ROM), also called reactive oxygen species, are considered the most abundant free radical systems in biological systems (MILLER et al. 1993) and describe oxygen-centered free radicals and their metabolites (POWELL 2011). Reactive nitrogen species also are part of the prooxidative site, but make up a smaller part of the prooxidants and will not be discussed further in this thesis.

Reactive oxygen metabolites occur as normal byproducts of cellular metabolism (REILLY et al. 1991) and can be derived from enzymatic actions of nicotinamide-adenine-dinucleotide phosphate oxidase (BABIOR 1999) and xanthine oxidase (MCCORD 1985) or non-enzymatic actions in the mitochondrial electron transport chain (KSENZENKO et al. 1983). They, or rather their derivatives (dROM), are involved in physiological processes and have regulatory effects (DRÖGE 2002; AGARWAL et al. 2005). These regulatory effects include the control of ventilation (ACKER et al. 1989), smooth muscle relaxation (SUZUKI and FORD 1999), and the enhancement of signal transduction from various membrane receptors including the antigen receptor of lymphocytes (HEHNER et al. 2000). Furthermore, ROM are antimicrobial and tumoricidal. The production of ROM and its derivatives by activated macrophages and neutrophils for the defense against environmental pathogens is termed oxidative burst and is part of the unspecific immune response (KEISARI et al. 1983). However, when produced in excess and not effectively and safely removed, ROM can cause direct and indirect damage, e.g. peroxidative damage of lipids and alterations in cell membranes and components (MILLER et al. 1993).

2.2. Antioxidants

The antioxidant defense mechanisms against ROM can be divided into enzymatic and non-enzymatic components. According to HALLIWELL AND GUTTERIDGE (2015) antioxidants are substances that are able to significantly delay or inhibit the oxidation of oxidizable substrates at a very low concentration. On the enzymatic site the superoxiddismutase, gluthatione peroxidase, and catalase should be mentioned (SIES 1985; HALLIWELL 1987) and on the non-enzymatic site: α-tocopherol, β-carotene, ascorbate, and gluthatione play important roles in the

defense against oxidative damage (SIES 1985). Amino acids, peptides and proteins also have scavenger functions, but to support the antioxidant mechanisms effectively they have to be present in high concentrations (DRÖGE 2002).

2.3. Oxidative stress in dairy cows

Oxidative stress is defined as the imbalance between prooxidants and antioxidants in an organism towards the prooxidative site (SIES 1991).

An increased abundance of ROM can cause a negative feedback mechanism to reduce the further production of ROM and can induce the expression of genes of antioxidant products. Moreover, an oxidative enhancement of proteolysis supports the antioxidative defense, as the scavenging activity of free amino acids is bigger than of proteins (DRÖGE 2002). Hence, moderate increases of ROM can be counteracted and the redox homeostasis can be maintained. Although, a shift towards the prooxidative site, even if sufficiently defended, can lead to pathological conditions via changed signal transduction and gene expression (DRÖGE 2002).

Oxidative stress is associated with several disease conditions in humans, such as diabetes, atherosclerosis, chronic inflammation, human immunodeficiency virus infection, ischemia-reperfusion injury (DRÖGE 2002).

To detect changes in the oxidative status accurately both sites of the oxidative status should be assessed. The ratio of reactive oxygen species and total antioxidant capacity was a superior discriminator for example between fertile and infertile men compared to the separate evaluation of these parameters (SHARMA et al. 1999). Similar to that ABUELO et al. (2013) suggested the ratio between oxidative damage to antioxidant capacity, i.e. oxidative status index (OSi), to assess the redox status in dairy cattle during the transition period.

Caloric restriction can ameliorate manifestations of oxidative stress, as the availability of mitochondrial energy substrates influences the mitochondrial ROM production (SOHAL and WEINDRUCH 1996). Therefore, it was objective of several (animal) studies to investigate the effect of caloric restriction on oxidative stress (DRÖGE 2002). For dairy cows ABUELO et al. (2013) assessed the OSi (dROM and OXY-Adsorbent assay) in four different production stages: late lactation, prepartum, postpartum, and peak of lactation and showed that cows undergo

oxidative stress after parturition. The increase of oxidative stress results from an increase of metabolic activity and thus enhanced accumulation of ROM at the onset of lactation, and at the same time the antioxidant defenses are depleted around calving (SORDILLO and AITKEN 2009). Furthermore, BERNABUCCI et al. (2005) showed that transition cows with greater beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) showed greater ROM and lower antioxidant levels and cows with higher BCS had greater ROM levels precalving.

Oxidative stress is considered to be involved in insulin signaling. Its associations with insulin resistance were reviewed in connection to diabetes mellitus in humans (CERIELLO and MOTZ 2004; RAINS and JAIN 2011). Oxidative stress can have a negative effect on insulin signaling by activating stress pathways, e.g. involving serine and threonine kinases (TALIOR et al. 2003; BLOCH-DAMTI and BASHAN 2005). In terms of diabetes mellitus, the hyperglycemia is considered as important factor increasing the oxidative stress at the cellular level (RAINS and JAIN 2011; MADDUX et al. 2001). At the same time, the increased production of ROM might cause a negative feedback mechanism resulting in decreased nutrient uptake by the cell to alleviate the further production of ROM. Therefore, the insulin resistance could be seen as compensatory mechanism to oxidative stress (CERIELLO and MOTZ 2004). Furthermore, elevated levels of ketone bodies can increase oxidative stress at the cellular level (PELLETIER and CODERRE 2007). On the other site, antioxidant supplementation has been shown to improve insulin sensitivity (CERIELLO 2000; PAOLISSO and GIUGLIANO 1996). Hence, there is strong evidence for an association between oxidative stress and insulin signaling in humans.

Ketosis in early lactation of dairy cows does not equal to diabetes mellitus, although it is also associated with insulin resistance; but in contrast it does not go in line with hyperglycemia, but hypoglycemia (DAVID BAIRD 1982). Nonetheless, also studies on dairy cows provided first evidence for an association of oxidative stress and insulin resistance. ABUELO et al. (2016b) have reported that the oxidative status was related to insulin sensitivity. They studied cows from two months before until two months after calving; animals with greater ROM or lower antioxidant potential, or both showed lower peripheral tissue insulin sensitivity as assessed by surrogate markers (ABUELO et al. 2016b). Moreover, the increased mobilization of NEFA in ketotic cows was associated with an increased

production of malondialdehyde, as marker for oxidative stress, and increased superoxide dismutase, as enzymatic antioxidant (XU SHI SHU 2014). Therefore, an association between oxidative stress and insulin sensitivity seems also to be evident in dairy cows. Further investigations are needed to elucidate the interrelationship of insulin resistance and problems in the transition period of dairy cows and possible treatments targeting oxidative stress. In the second study (Chapter IV) we investigated if the oxidative status in late gestation and early lactation is influenced by parity and energy supply provided by different amounts of concentrate.

III. ADIPONECTIN - EFFECTS OF PARITY, DIETARY ENERGY LEVEL, AND FARM

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Circulating adiponectin concentrations during the transition from pregnancy to lactation in high-yielding dairy cows: testing the effects of farm, parity, and dietary energy level in large animal numbers

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Highlights

• The examination of adiponectin in large animal numbers confirmed the typical and already reported time course of circulating adiponectin in late pregnancy and early lactation only in pluriparous cows.
• Primiparous cows showed an inverse pattern of adiponectin in the transition from late pregnancy to early lactation compared with pluriparous cows.
• Different fiber/straw content diets affected the adiponectin concentrations early in lactation, but not different concentrate levels.

Abstract

Dairy cows experience a negative energy balance due to increasing energy demands and insufficient voluntary feed intake in the transition from late pregnancy to early lactation. For supplying sufficient energy toward the conceptus and the mammary gland, the insulin sensitivity in peripheral tissues is reduced leading to adipose tissue mobilization. Adiponectin, an insulin-sensitizing adipokine, is presumably related to energy metabolism and could play an important role in these metabolic adaptations. We hypothesize (1) that primiparous cows would differ from pluriparous cows in their circulating adiponectin concentrations during the transition from late pregnancy to early lactation and (2) that feeding different energy levels would affect the adiponectin concentrations during early lactation in dairy cows. For the first hypothesis we examined 201 primiparous and 456 pluriparous Holstein dairy cows on three experimental farms. Ante partum, primiparous cows had lower adiponectin and greater NEFA concentrations than pluriparous cows, but vice versa post partum. Hence, adiponectin might be involved in the energy partitioning in primiparous cows (conceptus and lactation vs other still growing body tissues) with changing priorities from pregnancy to lactation. For the second hypothesis, 110 primiparous and 558 pluriparous Holstein and Simmental dairy cows in six experimental farms received either roughage with 6.1 or 6.5 MJ NEl/kg dry matter (adjusted with different amounts of wheat straw) *ad libitum*, combined with either 150 or 250 g concentrates/kg energy corrected milk. Greater amounts of concentrate lead to greater milk yield, but did not affect the blood variables. The higher energy level in the roughage led to greater glucose and IGF-1 but lower adiponectin in pluriparous cows. Further studies are needed to elucidate the mechanisms behind the roughage effect and its metabolic consequences.

Keywords: Adiponectin, Dairy cows, Parity, Feeding, optiKuh

1 Introduction

In the transition from late pregnancy to early lactation, during which voluntary feed intake is often insufficiently high, dairy cows are metabolically challenged due to the rapid increase of nutrient requirements for fetal growth and for milk secretion. In the phylogeny of most mammals, partitioning of nutrients toward the fetus and the mammary gland even at the cost of other tissues has evolved. Selective breeding for milk yield has intensified the teleologic drive to lactation, whereas feed intake was not considered directly in breeding programs. Consequently, the gap between energy output toward fetus and milk versus the input with feed, commonly termed negative energy balance, is particularly pronounced in high-yielding cows. The adaptive key mechanism activated in this situation concerns insulin sensitivity and insulin responsiveness: most peripheral tissues such as skeletal muscle and adipose tissue reduce their insulin sensitivity resulting in decreased glucose uptake and less anabolic reactions, for example, lipogenesis. By contrast, the uptake of glucose is mostly independent of insulin in the placenta and the mammary gland in late pregnancy and early lactation [1]. Adiponectin is considered as an adipokine with insulin-sensitizing effects [2,3] and thereby associated with glucose and lipid metabolism [4]. However, the question as to whether adiponectin is “driver or passenger on the road to insulin sensitivity” [5] is still not definitely answered. In dairy cows, positive correlations between adiponectin in blood and insulin responsiveness, the maximal effect of insulin on the glucose and lipid metabolism, were demonstrated during the dry period [6]. In addition, using surrogate markers for insulin sensitivity, parallel changes of insulin sensitivity, and adiponectin during the transition from late pregnancy to early lactation with a nadir in the first week of lactation were observed [7,8]. The peripheral insulin resistance is supposed to play an important role in the development of typical production diseases in dairy cows, for example, ketosis and fatty liver, although the mechanisms and exact relationships still need to be further investigated [9–11]. In view of the limitations for assessing insulin sensitivity via laborious and more invasive clamp studies in experimental conditions resembling the on-farm situation, measuring adiponectin in few blood samples might yield information about the adaptive capability of individual cows to cope with the metabolic challenge at that time. However, before being able to test whether circulating adiponectin during the transition period might be indicative for health risks, we aimed to investigate the effect of potentially

confounding factors on adiponectin in the present study. For doing so, we took advantage of a big national study in which 12 experimental farms throughout Germany participated (project optiKuh). In total, 1,710 cows were investigated in the project comprising three different breeds and different parities; the feeding regimens were harmonized across farms and individual feed intake could be recorded together with other variables on body condition, performance, and other variables assessed in blood (NEFA, beta-hydroxybutyrate [BHB], glucose, insulin, IGF-1) thus allowing to estimate, for example, energy balance (EB) and lipomobilization. We hypothesized that (1) primiparous Holstein cows would differ in their adiponectin concentrations compared with pluriparous cows over time and that (2) feeding different energy levels in Holstein and Simmental dairy cows would influence the adiponectin concentration. Both hypotheses were tested in combination with the results from other variables such as performance and the concentrations of metabolites and hormones in blood.

2 Materials and methods

All animal experiments used for this article were conducted between 2014 and 2017 at nine different experimental dairy farms in Germany as part of the national project optiKuh. They were approved by the responsible local authorities for animal welfare affairs[1] and therefore, have been carried out in accordance with EU Directive 2010/63/EU and the German animal protection law. All cows were housed in free stall barns and had permanent free access to feed and water. All cows were milked twice daily, with the exception of one farm, where cows were milked in a milking robot with a minimum break of 7 h between two milkings.

2.1 Experimental design for investigating the parity effects (Trial 1)

For comparing primiparous versus pluriparous cows, samples were considered only from Holstein cows kept in farm A, B, and C. Cows were fed the same specific diets during the dry period and during lactation, according to the recommendations of the Society of Nutrition Physiology in Germany

[1] **1**Regional council Tuebingen; Government Upper Bavaria; Food and veterinary institute (LAVES) Oldenburg; State office for agriculture food safety and fishing Mecklenburg-Western Pomerania (LALLF); Ministry for energy system transformation, agriculture, environment and digitization (MELUR); State office for nature, environment and consumer protection (LANUV); State office for chemical investigations Rhineland Palatinate (LUA); Government Middle Franconia.

(Gesellschaft für Ernährungsphysiologie, GfE) [12] and as agreed between the different partners of the optiKuh project. The chemical composition of the diets, which were fed as total mixed ration (TMR) in farm A and C and as partial mixed ration (PMR) in farm B, are summarized in Table 1. In total, samples from 201 primiparous and 456 pluriparous cows were included into the analyses. Cows were considered as primiparous from their first pregnancy until the end of their first lactation; cows, when pregnant, were considered as pluriparous with beginning of the dry period after the first lactation. The number of animals and their mean age and lactation number per farm are displayed in Table 2 and the time points and the procedure of blood sample collection are provided in section 2.3.

Table 1 Chemical composition of rations in Trial 1

Trial 1	Dry period	Lactation (1-100 DIM)
Dry matter (DM; g/kg)	430	438
Nutrient (/kg DM)		
Crude ash (g)	84	63
Crude protein (g)	135	164
Ether extract (g)	33	38
Crude fibre (g)	231	163
aNeutral detergent fiber om[a] (g)	435	338
Acid detergent fiber om (g)	257	205
Energy (NEl)	6.1	7
Energy (ME)	10.2	12

Values are presented as means over all farms in the dry-period and the first 100 days of lactation, respectively. [a] organic matter

Table 2 Number, age, and number of lactation of cows per parity and farm in Trial 1

Trial 1	Farm A	Farm B	Farm C	Total
Primiparous (n)	86	92	23	201
Mean age (mo) ± SEM	24 ± 2	24 ± 2	24 ± 2	24 ± 2
Pluriparous (n)	171	203	82	456
Mean age (mo) ± SEM	59 ± 23	54 ± 19	52 ± 22	56 ± 21
Mean lnr[a] ± SEM	3.7 ± 0.13	3.29 ± 0.09	3.29 ± 0.18	3.4 ± 0.07
Min lnr	2	2	2	2
Max lnr	10	9	10	10

[a] lnr: number of lactation

2.2 Experimental design for investigating dietary energy supply via roughage and concentrate (Trial 2)

Samples and data were considered from six experimental farms in which the different feeding variants were performed. Two different breeds were investigated, that is, Holstein cows on farms D, E, and F, and Simmental cows on farms G, H, and I. During lactation, feeding differed either in the energy content in the roughage portion or the amount of concentrates or in both factors. The energy content in the roughage portion was adjusted by adding different amounts of wheat straw resulting in either a moderate energy concentration (6.1 MJ NEl/kg dry matter [DM], groups MR) or a high energy concentration (6.5 MJ NEl/kg DM, groups HR). The concentrates were fed according to the expected milk yield and supplied for either 150 g/kg energy-corrected milk yield (ECM; MC groups) or 250 g/kg ECM (HC groups). Thus, four feeding groups were compared: MR-MC, MR-HC, HR-MC, and HR-HC. Farms D, E, and G conducted the feeding trial with all four groups; Farms H and I only varied the amount of concentrates (HR-MC vs HR-HC) and farm F only varied the energy content in the roughage portion (MR-HC vs HR-HC). The number of animals per groups and farms is shown in Table 3. During the dry period, all animals were fed the farm-specific ration according to the recommendations of the GfE [12]. The feeding was offered either as TMR (farm F and H) or PMR (farm D, E, G, and I). The components and chemical composition of the diets are displayed in Table 4.

Table 3 Number, age, and number of lactation of primiparous and pluriparous cows per feeding group and farm in Trial 2

	Farm Breed	Farm D Holstein	Farm E Holstein	Farm F Holstein	Farm G Simmental	Farm H Simmental	Farm I Simmental	Total
MR[a]-MC[c]	Primiparous: n	10			6			16
	Mean age (mo)	25			29			27
	Pluriparous: n	14	16		29			59
	Mean age (mo)	54	43		65			56
	Mean lnr[e] ± SEM	3.3 ± 0.3	2.7 ± 0.1		3.9 ± 0.3			3.4 ± 0.2
	Min lnr	2	2		2			2
	Max lnr	6	3		8			8
MR-HC[d]	Primiparous: n	4			8			12
	Mean age (mo)	26			27			27
	Pluriparous: n	19	16	25	28			88
	Mean age (mo)	54	55	46	56			53
	Mean lnr ± SEM	3.4 ± 0.3	3.5 ± 0.4	2.4 ± 0.1	3.3 ± 0.3			3.1 ± 0.1
	Min lnr	2	2	2	2			2
	Max lnr	7	9	3	6			9
HR[b]-MC	Primiparous: n	5			8	6	11	30
	Mean age (mo)	24			27	28	26	26
	Pluriparous: n	20	16		28	50	44	158
	Mean age (mo)	53	45		64	62	57	58
	Mean lnr ± SEM	3.3 ± 0.3	2.9 ± 0.2		3.9 ± 0.4	3.98 ± 0.2	3.7 ± 0.2	3.6 ± 0.1
	Min lnr	2	2		2	2	2	2
	Max lnr	6	4		10	9	8	10
HR-HC	Primiparous: n	8			13	7	8	36
	Mean age (mo)	26			28	28	27	27
	Pluriparous: n	13	16	26	28	57	44	184
	Mean age (mo)	55	46	44	55	62	58	56
	Mean lnr ± SEM	3.4 ± 0.4	2.8 ± 0.3	2.5 ± 0.1	3.3 ± 0.3	4 ± 0.2	3.6 ± 0.2	3.4 ± 0.1
	Min lnr	2	2	2	2	2	2	2
	Max lnr	6	5	3	7	9	7	9
Total	Primiparous: n	27			35	13	19	94
	Mean age (mo)	25			28	28	26	27
	Pluriparous: n	66	64	51	113	107	88	489
	Mean age (mo)	54	47	45	60	62	57	56
	Mean lnr ± SEM	3.3 ± 0.2	3 ± 0.1	2.5 ± 0.1	3.6 ± 0.1	3.9 ± 0.2	3.6 ± 0.2	3.4 ± 0.1
	Min lnr	2	2	2	2	2	2	2
	Max lnr	7	9	3	10	9	8	10

[a] MR: moderate energy level in the roughage portion (6.1 MJ NEL/kg DM); [b] HR: high energy level in the roughage portion (6.5 MJ NEL/kg DM); [c] MC: moderate amount of concentrate (150 g/kg energy corrected milk); [d] HC: high amount of concentrate (250 g/kg energy corrected milk); [e] lnr: number of lactations.

Table 4 Chemical composition and proportions of straw and concentrates in the rations fed in Trial 2

	Farm D (HF) (PMR)				Farm E (HF) (PMR)				Farm F (HF) (TMR)		Farm G (ST) (PMR)				Farm H (ST) (TMR)		Farm I (ST) (PMR)	
Feeding group	MR[a]-MC[c]	MR-HC[d]	HR[b]-MC	HR-HC	MR-MC	MR-HC	HR-MC	HR-HC	MR-HC	HR-HC	MR-MC	MR-HC	HR-MC	HR-HC	HR-MC	HR-HC	HR-MC	HR-HC
Concentrate in ration	33%	42%	34%	43%	32%	31%	43%	42%	18%	16%	29 %	40%	28 %	35%	22%	35%	31%	38%
Straw in ration	12%		3%		18%		6%		6%	4%	11%		3%		10%		20%	
DM (g/kg)	533	580	517	569	547	601	523	580	428	412	476	514	450	476	429	468	466	514
XA (g)	74	72	71	70	66	65	67	65	55	52	64	65	64	65	80	79	71	70
XP (g)	153	160	158	164	129	138	135	142	195	195	148	159	153	158	152	157	158	159
XL (g)	35	35	37	36	37	39	38	40	43	39	36	36	37	37	30	28	45	44
XF (g)	189	173	172	157	205	184	188	173	160	161	208	190	183	177	195	172	177	167
aNDF om [e] (g)	384	366	355	340	404	366	372	345	354	352	417	387	377	368	346	310	377	362
ADF om (g)	224	210	204	192	241	221	221	207	204	200	251	234	223	217	223	197	222	212
Energy (NEl)	6.7	6.9	6.9	7.1	6.7	7.1	6.9	7.2	7.0	7.0	6.6	6,8	6.8	6,9	6.7	7.0	6.76	6.82
Energy (ME)	11.0	11.2	11.3	11.6	11.0	11.5	11.3	11.7	11.5	11.5	10.8	11.1	11.2	11.3	11.0	11.3	11.10	11.15

Values are presented as means over the study period (1-100 DIM) in the respective farms with either Holstein Friesian (HF) or Simmental (ST) cows. The portions of concentrates and straw are provided on a dry matter (DM) basis. Rations were either provided as total mixed ration (TMR) or partial mixed ration (PMR).

[a] MR: moderate energy level in the roughage portion (6.1 MJ NEL/kg DM); [b] HR: high energy level in the roughage portion (6.5 MJ NEL/kg DM); [c] MC: moderate amount of concentrate (150 g/kg energy corrected milk); [d] HC: high amount of concentrate (250 g/kg energy corrected milk); [e] organic matter

2.3 Sample collection and performance records (Trial 1 and 2)

Blood samples were collected after the morning milking (between 08:00 and 11:00 h) from a jugular vein on the following days (d) relative to calving [desired d (±accepted range of deviation)]: −50 (±10 d), −14 (±4 d), +8 (±2 d), +28 (±2 d), and +100 (±4 d). Samples were allowed to clot at 20 to 25°C for 60 to 90 min and were then centrifuged at 1,800 × g for 10 min. Serum samples were stored at −20°C until analyzed. Blood samples which were not taken in the accepted range of days were excluded from the statistical analyses. Body weight (BW) was recorded at least every 2 wk during lactation in all farms. Individual feed intake was assessed via weighing troughs for the roughage portion and with automatic feeders for concentrates (in case of PMR feeding). Milk yield was recorded daily and milk composition (fat, protein, lactose, urea, and somatic cell count) was assessed at least once a week.

2.4 Calculations

For the calculation of weekly EB, weekly means of BW, DM intake (DMI), milk yield, and milk composition were used. The ECM was calculated according to the recommendations of the GfE [12] using the following equation:

$$ECM\ \left[\frac{kg}{d}\right] =$$

$$Milk\ yield\ [kg/d] \cdot \left(\frac{1.05 + 0.38 \cdot Milk\ fat\ [\%] + 0.21 \cdot Milk\ protein\ [\%]}{3.28}\right)$$

The net energy requirements for maintenance, lactation (NEl), and pregnancy were calculated using the following equations given by GfE [12]:

$$NEm\ [MJ\ NEl/d] = 0.293 \cdot BW^{0.75}\ [kg]$$

$$Milk\ energy\ [MJ\ NEl/kg] = 0.38 \cdot Milk\ fat\ [\%] + 0.21\ \cdot Milk\ protein\ [\%] + 0.95$$

$$NEl\ [MJ\ NEl/d] = (Milk\ energy\ [MJ\ NEl/kg] + 0.086) \cdot Milk\ yield\ [kg/d]$$

$$NEp\ [MJ\ NEl/d] = \frac{(\ 0.044\ \cdot e^{0.0162 \cdot t} + Development\ of\ the\ udder)}{0.29}$$

During the peripartal period, 1.5 MJ NEl/d were added to the requirements as additional energy demand for udder development [12]. For primiparous cows, 10% of bodyweight at calving were assumed as body gain and were included into the calculation of energy requirements [12].

Daily energy intake was calculated by multiplying the DMI by the energy content of the ration. Feed efficiency (MJ NEl/kg milk) was calculated by dividing the energy intake (MJ NEl) by the milk yield (kg). For the calculation of EB, the calculated net energy demands for maintenance, lactation, gestation, udder development (only peripartal period), and growth (NEg; only primiparous cows) were subtracted from the energy intake.

$$EB\ \left[MJ\frac{NEl}{d}\right] = Energy\ intake\ [MJ\ NEl/d] - (NEm\ [MJ\ NEl/d] + NEl\ [MJ\ NEl/d] + NEp\ [MJ\ NEl/d] + NEg[MJNEg/d])$$

2.5 Blood sample analyses

Serum adiponectin concentrations (µg/mL) were assessed in duplicate by an in-house developed indirect competitive bovine-specific ELISA [8]. The interassay CV was 10.2% and the mean intra-assay CV was 5.2%. Serum concentrations of glucose (mmol/L), BHB (mmol/L), and NEFA (µmol/L) were analyzed photometrically with a Cobas Mira analyzer (Hoffmann-La Roche, Basel, Switzerland). The serum concentrations of insulin and IGF-1 were measured with commercial radioimmunoassay kits (Insulin: IRMA IM3210 and IGF-1: IRMA A15729, Beckman Coulter, Brea, CA), which were executed according to the manufacturer's instructions. Insulin concentrations are given in µU/mL: the intra-assay CV was 7.6% and the inter-assay CV was 10.7%. The lower limit of the measurement range for insulin was 3 µU/mL and samples with concentrations below this limit (448 of 4,129 samples) were randomly assigned to a concentration between 0 and 3 µU/mL from a uniform distribution in this range. Concentrations of IGF-1 are given in ng/mL: the intra-assay CV was 5.1% and the inter-assay CV was 9.3%. The lower limit of the measurement range for IGF-1 was 33 ng/mL and samples with concentrations below this limit (380 of 4,129 samples) were randomly assigned to a concentration between 0 and 33 ng/mL from a uniform distribution in this range.

2.6 Statistical analyses

Data were analyzed using SPSS (IBM® SPSS® Statistics 25). The assumptions for the linear mixed model were tested in terms of normal distribution and homoscedasticity of the residuals and, if necessary, data were transformed by the power function of Box and Cox [13]. Transformation was conducted for all blood variables and the transformed data were then used for the statistical analysis, now fulfilling the required model assumptions. The original data were used for the graphical display in the figures shown herein and are reported as means ± SEM. Cows that participated in the study over more than one lactation (Trial 1: n = 78; Trial 2: n = 205) were statistically regarded as different cows for their different lactations. Cow was set as random effect in all models. For the post-hoc analyses the Bonferroni adjustment was used to account for multiple comparisons. In a first step for the analysis of parity effects a linear mixed model with the fixed effects number of lactation, time, farm, and all interactions between these effects was performed. For adiponectin the number of lactation was not significant in the model, but the interaction time by number of lactation was. The graphical display showed that the interaction was due to an inverse relationship between primi- and pluriparous cows before and after calving, therefore, further analyses were performed separately for the pre- and postpartum period. In addition, parity was dichotomized into primiparous and pluriparous cows, since differences in adiponectin concentrations between different parities were limited to lactation number 1 versus all other numbers, whereas lactation numbers > 1 were not different amongst each other in the post-hoc analyses. Thus the final linear mixed model was:

$$y_{ijkl} = \mu + T_i + P_j + F_k + TP_{ij} + TF_{ik} + TPF_{ijk} + i_l + e_{ijkl}$$

y_{ijkl} = response variable, μ = overall mean, T_i = fixed time effect (for prepartum analyses: i = d −50, −14; for postpartum analyses: i = d +8, +28, +100), P_j = fixed parity effect (j = primiparous, pluriparous), F_k = fixed farm effect (k = Farm A, B, C), TP_{ij}, TF_{ik}, and TPF_{ijk} = fixed interactions, i_l = random effect of the individual cow (l = 1, …, 657), and e_{ijkl} = residual error.

The analyses of feeding effects in Trial 2 were performed separately for primi- and pluriparous cows. In a first linear mixed model the effect of the four feeding groups (MR-MC, MR-HC, HR-MC, and HR-HC) was investigated with the fixed

effects time, feeding group, farm, and all interactions therefrom. Feeding group was significant for pluriparous cows (p = 0.006) and the MR-HC group had greater adiponectin concentrations (26.9 ± 0.4 µg/mL) than the HR-MC (25.8 ± 0.2 µg/mL; P = 0.012). Therefore, the two factors in the feeding group, i.e. energy level in the roughage portion (MR vs. HR) and amount of concentrate (MC vs. HC) were tested as separate effects in the final linear mixed model:

$$y_{ijklm} = \mu + T_i + R_j + C_k + F_l + TR_{ij} + TC_{ik} + TF_{il} + RC_{jk} + RF_{jl} + CF_{kl} + TRC_{ijk} + TRF_{ijl} + TCF_{ikl} + RCF_{jkl} + TRCF_{ijkl} + i_m + e_{ijklm}$$

y_{ijklm} = response variable, μ = overall mean, T_i = time effect (i = d +8, +28, +100), R_j = roughage effect (j = MR, HR), C_k = concentrate effect (k = MC, HC), F_l = farm effect (l = Farm D, E, F, G, H, I), TR_{ij}, TC_{ik},, TF_{il}, RC_{jk}, RF_{jl}. CF_{kl}, TRC_{ijk}, TRF_{ijl}, TCF_{ikl}, RCF_{jkl}, and $TRCF_{ijkl}$ = fixed interactions, i_m = random effect of the individual cow (primiparous: m = 1, …, 94; pluriparous: m = 1,…, 489), and e_{ijklm} = residual error.

Data was tested for normal distribution using the Kolmogorov-Smirnov test to choose between parametric and non-parametric tests for the pairwise comparison and correlation analyses. To ensure that the groups in Trial 2 did not already differ in their adiponectin concentrations before the feeding treatment started, Mann-Whitney-U tests were performed for the adiponectin concentrations on d −14 comparing the MR and the HR groups as well as the MC and the HC groups. Spearman correlation coefficients were calculated to check for correlations between adiponectin concentrations and the other variables. Results are presented as means ± SEM for the prepartum period (mean of each, d −50 and −14) and the postpartum period (mean of each, d +8, +28, and +100). Significance was declared for $P < 0.05$ and trends were declared at $0.05 < P < 0.10$. For the analyses only data from the five sampling time points was used.

3 Results and discussion

3.1 Differences between primiparous and pluriparous cows (Trial 1)

The concentrations of the measured hormones and metabolites varied differently between primiparous and pluriparous cows during the study period and are shown in Figure 1 and the statistical results are reported in Table 5. The time course of

adiponectin with a nadir directly after parturition as reported earlier [14] was confirmed for the pluriparous cows in our study. In the prepartum period, primiparous cows had lower adiponectin concentrations than the pluriparous cows and there were no interactions or differences between time points or farms. Postpartum the primiparous cows had greater adiponectin concentrations than pluriparous cows and there were time by farm and time by parity by farm interactions. These interactions were mainly driven by the different pattern of the adiponectin concentrations over time for primiparous cows in farm C and the parity effect could be interpreted despite these interactions, at least for farms A and B (Fig. 2). The different time course of adiponectin concentrations in primiparous cows in farm C compared with farm A and B may have resulted from the lower number of animals compared with the other farms, resulting in greater SEM or from other unknown factors, for example, in management. At first examination, our postpartum results partly disagree with the report of Singh et al who reported that primiparous compared with pluriparous cows tended to have lower adiponectin concentrations from d −21 to d +250 relative to parturition [14], but they did not analyze the data separately for the prepartum and postpartum period. Their graphs show a steeper increase in primiparous cows (approx. 2-fold) from the nadir around parturition to the peak concentration 3 wk after parturition compared with pluriparous cows (approx. 1.6-fold), which corresponds with our results. Hence the comparison of primiparous and pluriparous cows should always take into account the stage of gestation and lactation. In addition, when comparing across studies the possibility of individual farm variation should be considered. The differences in circulating adiponectin between primiparous and pluriparous cows could reflect the differences in energy partitioning in primiparous cows. Primiparous cows are not yet physically mature at the time of their first calving [15] and still need energy for their own body growth [16]. Therefore, Wathes et al [16] suggested differences in the control of tissue mobilization between primiparous and pluriparous cows, which may promote nutrient partitioning toward growth as well as milk production during the first lactation. Greater adiponectin concentrations could alleviate the insulin resistance in peripheral tissues other than the mammary gland and thus allow for an increased glucose uptake in body tissues [6]. The lesser adiponectin concentrations in primiparous than pluriparous cows prepartum suggests that the energy demands for the conceptus are prioritized over the own body growth, which is not yet completed at

this time. The earlier increase of circulating adiponectin and greater concentrations after calving in primiparous cows might reflect a compensation for this prepartum prioritization and suggests that milk production in primiparous cows is not as prioritized (over the demands for the own body growth) as pregnancy, and as milk production in pluriparous cows.

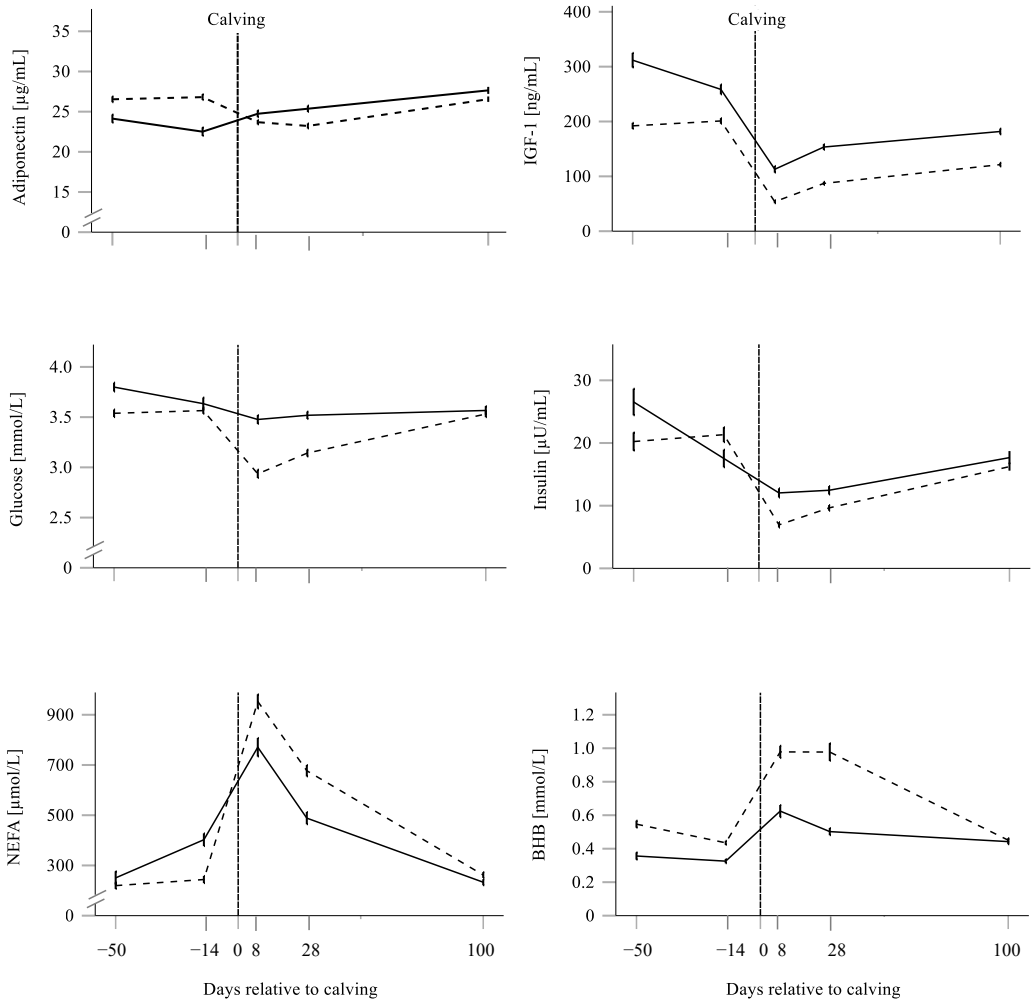

Fig. 1. Circulating concentrations (means ± SEM) of adiponectin, IGF-1, glucose, insulin, NEFA, and β-hydroxybutyrate (BHB) in primiparous (solid lines) and pluriparous (dashed lines) cows in Trial 1 (mean over all farms). The results from the statistical evaluation are presented in Table 5

Table 5 Circulating concentrations of various metabolites and hormones in primiparous and pluriparous cows in Trial 1

	Means ± SEM				P – values												
	primiparous		pluriparous		Time (T)		Parity (P)		Farm (F)		T by P		T by F		P by F		T by P by F
	ap[a]	pp[b]	ap	pp	ap	pp	ap	pp	ap	pp	ap	pp	ap	pp	ap	pp	pp
Adiponectin	23.3	26	26.7	24.4		0.001	<0.001	0.002						0.001			0.001
µg/mL	± 0.4	± 0.2	± 0.3	± 0.2													
IGF-1	286	150	197	86.4		0.001	0.001	0.001	0.001	0.001	0.015	0.003	0.034				0.001
ng/mL	± 8	± 3	± 4	± 2													
Glucose	3.7	3.4	3.6	3.2		0.001	0.001	0.001		0.001		0.001		0.001	0.036	0.008	
mmol/L	± 0.03	± 0.02	± 0.02	± 0.02													
Insulin	22.1	14.1	20.8	10.8		0.001		0.002	0.001	0.001	0.05	0.018		0.001	0.002		
µU/mL	± 1.3	± 0.5	± 0.9	± 0.3													
NEFA	326	491	232	640	0.03	<0.001	0.005	<0.001	0.001	<0.001		0.016		<0.001	0.026		
µmol/L	± 19	± 18	± 10	± 16													
BHB[a]	0.34	0.52	0.49	0.81	0.01	<0.001	<0.001	<0.001	0.005	<0.001		<0.001		0.003	<0.001	<0.001	0.001
mmol/L	± 0.01	± 0.02	± 0.01	± 0.02													

P-values given are limited to $P \leq 0.05$ for the fixed factors time (T), parity (P), farm (F), and interactions between these.

[a] ante partum (d −50 to d −14; ap)

[b] post partum (d + 8 to d 100; pp)

[c] beta-hydroxybutyrate

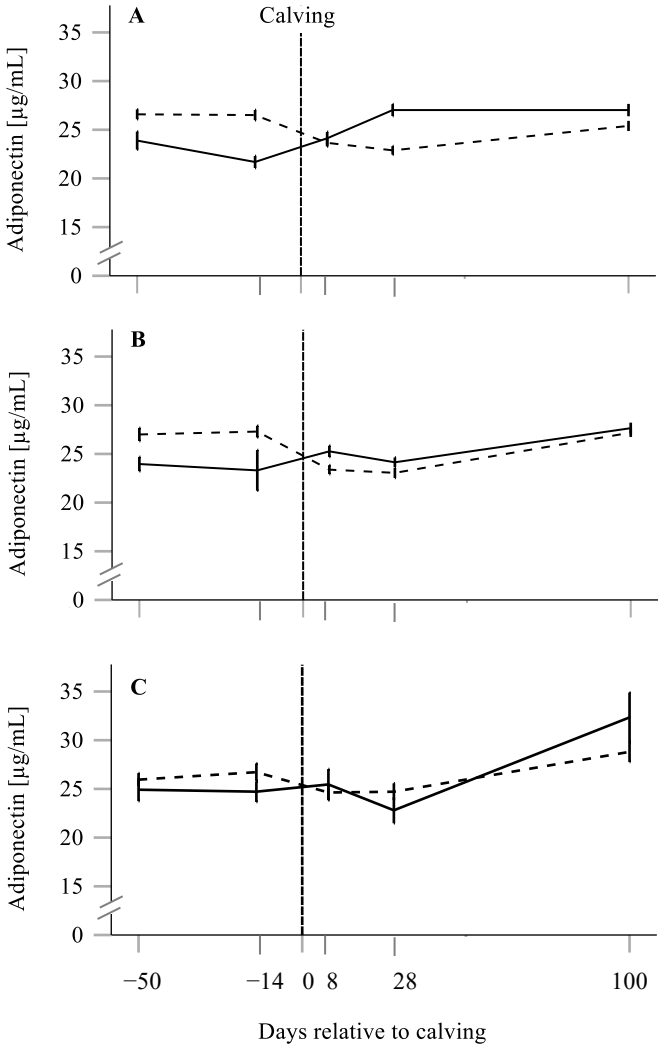

Fig. 2. Circulating concentrations (means ± SEM) of adiponectin in primiparous (solid lines) and pluriparous cows (dashed lines) from three experimental farms (A: Farm A, B: Farm B, C: Farm C) in Trial 1.

Our results on blood glucose, IGF-1, BHB, and insulin are in accordance with the literature [16–20]. Primiparous cows had greater blood glucose and IGF-1 concentrations, and lower BHB concentrations than pluriparous cows prepartum and postpartum. Insulin concentrations did not show any parity effect prepartum, but postpartum primiparous cows had greater insulin concentrations than pluriparous cows. Insulin and IGF-1 were positively correlated with each other, both in primiparous ($r = 0.32$) and in pluriparous cows ($r = 0.38$, both $P < 0.001$). Furthermore, adiponectin was positively correlated with insulin ($r = 0.17$; $P < 0.001$) and IGF-1 ($r = 0.2$, $P < 0.001$) for pluriparous but not for primiparous cows in our study (insulin: $P > 0.1$; IGF-1: $r = -0.1$, $P = 0.011$). Insulin as well as IGF-1 have growth promoting effects [21,22]. Thus, the greater concentrations of these

hormones in primiparous cows support the notion that body growth or storage of body reserves is more important in primiparous than in pluriparous cows. The differences in insulin concentrations were apparent only postpartum but not prepartum, this and the positive, albeit weak correlations between insulin and IGF-1 with adiponectin support the notion that body growth in primiparous cows is prioritized over lactation but not over pregnancy and that adiponectin is involved in this regulation of energy partitioning. In our study, NEFA concentrations showed an inverse pattern for primiparous and pluriparous cows: primiparous cows had greater concentrations prepartum, but lower concentrations postpartum compared with pluriparous cows. Adiponectin and NEFA were weakly negatively correlated with each other in both primiparous ($r = -0.12$, $P = 0.002$) and pluriparous cows ($r = -0.26$, $P < 0.001$). Wathes et al [16] showed that the NEFA peak in primiparous cows occurs earlier (1 wk before calving) than in pluriparous cows, (around 3 wk after calving) suggesting that tissue mobilization in primiparous cows starts earlier than in pluriparous cows. Owing to the low frequency of blood sampling in our study, we could not detect the shifted peaks, but the inverse ratio of NEFA between primiparous and pluriparous before and after calving, is in support of the hypothesis of time-dependent differences in energy partitioning between primiparous and pluriparous cows. Besides the hormonal and metabolic results, the different energy partitioning is also visible in the performance data. Primiparous cows produced less milk (29.2 ± 0.29 kg) than pluriparous cows (39.1 ± 0.28 kg, $P < 0.001$) as also reported in several studies before [16,23]. Furthermore, primiparous cows had lower energy intakes during the first 100 d of lactation (114 ± 1.95 MJ NEl/d) than pluriparous cows (141 ± 1.55 MJ NEl/d, $P < 0.001$) resulting in a lower feed efficiency for primiparous cows (4.1 ± 0.08 MJ NEl/kg milk) than for pluriparous cows (3.73 ± 0.05 MJ NEl/kg milk, $P = 0.015$). This confirms additional energy requirements in primiparous cows. The calculated EB postpartum did not differ between primiparous and pluriparous cows ($P = 0.2$), what can be explained by the inclusion of the additional energy requirements for primiparous cows in terms of 10% of their body weight into the calculation of EB in our study. By producing less milk, primiparous cows may save energy for their own body growth. Wathes et al [16] suggested that the differences in nutrient partitioning between primiparous and pluriparous cows may be caused by a differing endocrine background. Our results imply that adiponectin is likely involved in these

mechanisms and possibly indicates energy partitioning. A better energy supply for the own body (during the period of negative EB) and thus less mobilization of body fat to meet the requirements for milk production could reduce the risk for metabolic diseases during early lactation. Lee et al [24] found a lower incidence of metabolic disorders in the periparturient period in primiparous compared with pluriparous cows. Therefore, including adiponectin in further studies on energy partitioning could be useful to improve the understanding of the metabolic differences and may provide explanations for the varying risks for periparturient metabolic diseases.

3.2 Investigation of dietary energy supply (Trial 2)

The adiponectin concentrations of primiparous and pluriparous cows on d −14, that is, before the differential feeding started, did not differ between the feeding groups ($P > 0.1$); in the following sections, only results from the first 100 days in milk (DIM) are presented.

3.2.1 Primiparous cows

Varying the energy density of the diet affected the performance and the measured blood variables in primiparous cows. Greater concentrate allowances resulted in greater energy intake (118 ± 2.4 MJ NEl/d) and less negative EB (−6.5 ± 2 MJ NEl/d) in the HC groups compared with the MC groups (energy intake: 106 ± 2.6 MJ NEl/d, $P = 0.003$; EB: −11.3 ± 2.2 MJ NEl/d, $P = 0.024$). The higher energy content in the roughage portion did not affect energy intake ($P = 0.468$) and the effect on energy balance could not be interpreted due to a time by roughage by concentrate by farm interaction ($P = 0.036$). The milk yield, glucose, NEFA, insulin, BHB, and IGF-1 concentrations did not differ between the feeding groups ($P > 0.1$). A time by concentrates by farm interaction ($P = 0.048$) impeded a reliable interpretation of the feeding effects for adiponectin. These results are in accordance with the findings from Mellouk et al [25], despite that they reported greater adiponectin and a tendency toward greater insulin for their higher energy group. The concentrations of glucose, NEFA, and BHB differed between the farms ($P < 0.05$) in our trial, but we could not detect any reasons for this.

3.2.2 Pluriparous cows

Varying the amounts of concentrates in the diet in pluriparous cows mainly affected the performance but not the measured blood variables. Pluriparous cows in HC groups had greater energy intake (136 ± 1.4 MJ NEl/d), EB (−15.3 ± 1.2 MJ NEl/d), and milk yield (35.1 ± 0.3 kg) than in the MC groups (energy intake: 125 ± 1.6 MJ NEl/d; EB: −19.4 ± 1.3 MJ NEl/d; milk yield: 32.7 ± 0.4 kg; all $P \leq 0.001$). The concentrations of the blood variables in the MC and HC groups during the study period did not differ and are shown in Figure 3; the statistical results are reported in Table 6. These results are in accordance with Schmitz et al [26], who already reported the results from one farm of the optiKuh project. In the study of Reist et al, higher energy content in the diet lead to greater insulin, glucose, and IGF-1 but lesser NEFA concentrations [27]. A tendency for greater insulin concentrations, but not the difference in NEFA in cows fed a high energy diet compared with a lower energy diet was also reported by Mellouk et al [25]. In addition, they reported greater concentrations of adiponectin for cows receiving the higher energy diet [25]. Krumm et al [28] also found greater adiponectin in cows with a higher EB, but this was not achieved by differential feeding but by an immediate dry-off after calving. In our study, the difference in the energy content between the MC and HC groups may not have been great enough to affect the concentrations of the blood variables as in the cited studies. Furthermore the variation in literature concerning the energy effect on blood variables suggests that energy is not the only important factor to look at.

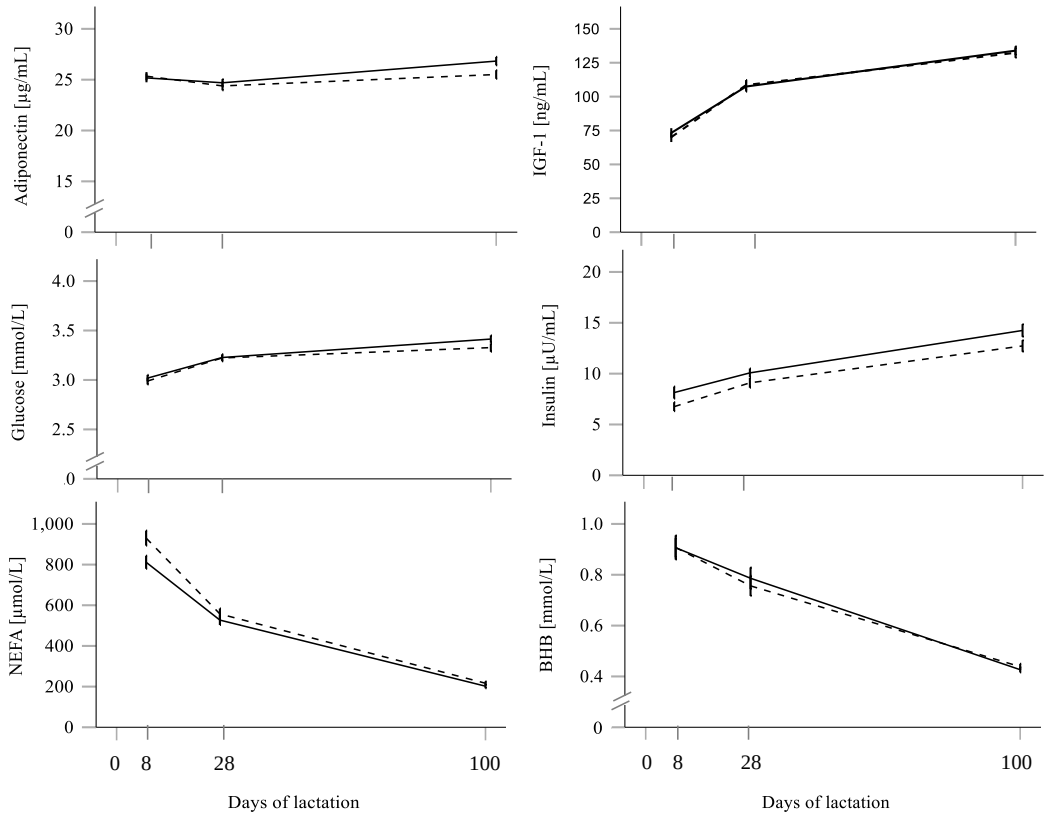

Fig. 3. Circulating concentrations (means ± SEM) of adiponectin, IGF-1, glucose, insulin, NEFA, and β-hydroxybutyrate (BHB) in pluriparous cows fed either 150 g concentrate/kg Energy corrected milk (MC, dashed lines) or 250 g concentrate/kg Energy corrected milk (HC, solid lines) in Trial 2 (means over all farms). The results from the statistical evaluation are presented in Table 6.

Table 6 Mean (± SEM) concentrations of various blood variables in pluriparous cows from Trial 2

	Mean ± SEM				P – values							
	MC[a]	HC[b]	MR[c]	HR[d]	T	C	R	F	T by C	T by R	T by F	T by R by F
Adiponectin	25.7	26.2	26.5	25.7	<0.001		0.001	<0.001			<0.001	
µg/mL	± 0.2	± 0.2	± 0.3	± 0.2								
IGF-1	132	135	127	137	<0.001		0.036	<0.001			<0.001	
ng/mL	± 2.6	± 2.5	± 3.4	± 2.2								
Glucose	3.3	3.3	3.3	3.3	<0.001		0.001	<0.001			<0.001	
mmol/L	± 0.02	± 0.02	± 0.02	± 0.01								
Insulin	12.7	13.6	12	13.7	<0.001			<0.001	0.018		<0.001	
µU/mL	± 0.4	± 0.4	± 0.5	± 0.4								
NEFA	474	434	480	440	<0.001			0.015			<0.001	
µmol/L	± 15	± 12.7	± 20.5	± 10.9								
BHB[e] mmol/L	0.62	0.62	0.67	0.6	<0.001			<0.001		0.046	0.001	0.044
	± 0.02	± 0.02	± 0.03	± 0.01								

Results are presented for pluriparous cows during Trial 2 (d 8 to 100 of lactation). The P-values listed are limited to those $P \leq 0.05$ for the fixed factors time (T), concentrate (C), roughage (R), farm (F), and interactions between those, respectively.

[a] MR: moderate energy level in the roughage portion (6.1 MJ NEL/kg DM); [b] HR: high energy level in the roughage portion (6.5 MJ NEL/kg DM); [c] MC: moderate amount of concentrate (150 g/kg energy corrected milk); [d] HC: high amount of concentrate (250 g/kg energy corrected milk); [e] beta-hydroxybutyrate.

The different energy contents in the roughage portion affected the performance in pluriparous cows to a lesser extent than the amounts of concentrates, but the measured metabolites and hormones were affected. Cows in the HR groups had greater energy intake (134 ± 1.3 MJ NEl/d) and EB (−15.7 ± 1.1 MJ NEl/d) than in the MR groups (energy intake: 127 ± 1.8 MJ NEl/d, P = 0.02; EB: −19.4 ± 1.5 MJ NEl/d, P = 0.007), but milk yield did not differ between HR and MR groups (P = 0.16). These results, except for the difference in EB, were also observed by Schmitz et al [26], who concluded that greater energy content in roughage was beneficial for energy efficiency and would allow a reduction in concentrate feeding. The concentrations of the blood variables during the study period for HR and MR groups are shown in Figure 4 and the statistical results are reported in Table 6. All measured blood variables in the pluriparous cows showed a farm effect, but as for the primiparous cows we could not detect any reasons for that. There was no evidence that the farm effect was driven by the two different breeds, as for example, the greatest as well as the lowest adiponectin concentrations were found in farms with Simmental cows, farm H and G, respectively, and the third farm with Simmental cows and the farms with Holstein cows were ranked in between. The concentrations of NEFA and BHB were not affected by the energy content in the roughage portion. Glucose and IGF-1 were greater and insulin tended to be greater in the HR groups than in the MR groups. By contrast, adiponectin was lower in the HR groups than in the MR groups. The latter was surprising as we would have rather expected greater adiponectin concentrations in the groups with greater energy content, that is, HC and HR due to the reported positive association of adiponectin and EB in dairy cows [25,28]. In our study, adiponectin and EB were only very weakly positively correlated (r = 0.1, P < 0.002). The correlation seems to depend on the stage of production, as there was no correlation between EB and circulating adiponectin in nonpregnant, nonlactating cows [29], or during the dry period [30]. The inverse correlation between body fat and adiponectin in humans [31] was confirmed in nonlactating, nonpregnant dairy cows [29], but this relationship seems to be disrupted in early lactation. The mobilization of body reserves, mainly fat, during the transition period in dairy cows is not accompanied by increased concentrations of adiponectin; instead concentrations decline when the portion of body fat decreases. This goes in line with the inverse relation between circulating adiponectin and NEFA, the latter is regarded as marker for lipolysis [32]. Weight

loss due to low caloric diets was accompanied by increased concentrations of adiponectin in human patients and rats [33,34], but as reviewed by Klempel and Varady, there are also studies in which no such adiponectin increases were observed [35]. Reducing the energy supply for cows around d 100 of lactation did not affect the circulating adiponectin concentrations either [36]. Therefore, it is still unclear which (dietary) factors are mainly affecting the adiponectin concentrations in dairy cows and how these effects change dependent on the stage of production.

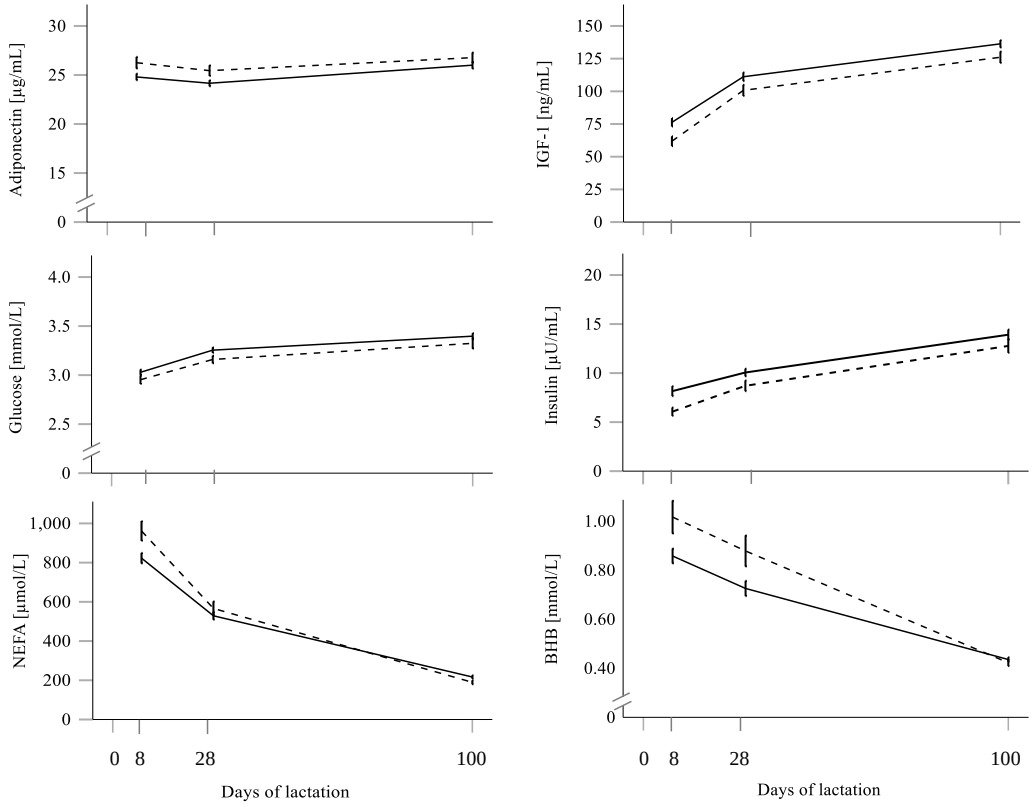

Fig. 4. Circulating concentrations (means ± SEM) of adiponectin, IGF-1, glucose, insulin, NEFA, and β-hydroxybutyrate (BHB) in pluriparous cows fed either a roughage portion containing 6.1 MJ NEl/kg dry matter (MR, dashed lines) or 6.5 MJ NEl/kg dry matter (HR, solid lines) in Trial 2 (mean over all farms). The results from the statistical evaluation are presented in Table 6.

One possible explanation for the roughage effect in our study might be the greater content of wheat straw in the MR groups' roughage portion (eg, for farm E 18% straw in the roughage portion in the MR groups and 6% in the HR groups) resulting in a higher fiber to starch ratio than in the HR groups. To date, there is no information on how adiponectin in ruminants is affected by different diet

composition, especially concerning the fiber content. Studies on humans revealed a positive relation between adiponectin and the fiber content in the diet [33,37]. Although results from humans as monogastrics, and cows as ruminants cannot directly be compared, these reports indicate a possible explanation for our results. In ruminants, a greater roughage portion in the diet leads to a greater production of acetate and butyrate in the rumen [38,39]. Butyrate and propionate stimulated the adiponectin secretion of porcine adipocytes in vitro [40]. However, in another study, butyrate tended to decrease the mRNA expression of adiponectin in bovine adipocytes and the adiponectin system seemed to be more sensitive to propionate than to butyrate [41]. Hence, the roughage effect on adiponectin could result from the greater fiber content and subsequent greater butyrate and acetate production in the rumen; but this hypothesis is only based on in vitro studies, and further in vivo studies are needed to investigate the effect of dietary fiber on circulating adiponectin in ruminants and the metabolic consequences.

4 Conclusion

We could confirm the reported time course of circulating adiponectin in the transition from late pregnancy to early lactation in pluriparous but not in primiparous cows. The direction and extent of differences between the metabolic and hormonal profiles of primiparous and pluriparous cows were time-dependent, showing that parity comparisons can vary according to the stage of production. These data support the notion of changes in energy partitioning in primiparous cows from late pregnancy to early lactation and the involvement of adiponectin in the regulation of energy partitioning. In our feeding trial, the adiponectin concentrations in pluriparous cows were greater in the groups with a bigger portion of straw and therefore, lower energy content in the roughage, but were not affected by higher or lower amounts of concentrates in the diet. Further studies are needed to elucidate the mechanisms behind the roughage effect and its metabolic consequences.

Declaration of interest

None.

Author Contribution

Christiane Urh: Investigation, Formal analysis, Writing – original draft,
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Eckhard Stamer: Data curation, Validation, Writing – review and editing,
Hubert Spiekers: Conceptualization, Resources, Writing – review and editing, Supervision, Project administration, Funding acquisition,
Helga Sauerwein: Writing – review and editing, Supervision, Resources.

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IV. OXIDATIVE STATUS - EFFECTS OF PARITY, DIFFERENT DIETARY ENERGY LEVELS, AND FARM

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Short communication: Pro- and antioxidative indicators in serum of dairy cows during late pregnancy and early lactation: Testing the effects of parity, different dietary energy levels and farm

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Interpretive summary:

Dysbalances between antioxidants and pro-oxidants may occur as concomitants of the metabolic load and the inflammatory status during the transition period. Assessing the oxidative status may help to estimate the level of distress in transition dairy cows. For sorting out factors potentially influencing oxidative status, we investigated the effects of time, parity, diet, and farm on two variables in serum reflecting the pro- and antioxidative side, respectively. Oxidative status was elevated in the first and second lactation compared with that of later lactations. Diet had no detectable effect, but one farm stuck out with greater oxidative status for unknown reasons.

ABSTRACT

Dairy cows face metabolic challenges in the transition from late pregnancy to early lactation. The energy demands for the growing fetus and the onset of milk production are increasing but voluntary feed intake often decreases around parturition and cannot meet these demands. This energy balance, amongst others, can change the oxidative status. Oxidative stress occurs when the antioxidant defense mechanisms are not sufficient to cope with the increasing generation of reactive oxygen species. Our objectives were to investigate (1) the effect of parity on the oxidative status of dairy cows (n = 247) in late pregnancy and early lactation and (2) the effect of different inclusion rates of concentrate feeding (150 vs. 250 g/kg energy-corrected milk) during early lactation on 2 farms including 87 cows in total. In addition, we aimed to compare the oxidative status across the 2 farms using equal portions of concentrate feeding. For these purposes, we measured the concentrations of the derivatives of reactive oxygen metabolites (dROM) and the ferric reducing ability (FRAP) in serum on d −50, −14, +8, +28, and +100 relative to calving. Furthermore, we calculated the oxidative status index (OSi) as dROM / FRAP × 100. Data were analyzed using a linear mixed model. Cows in the first and second lactation had greater dROM, FRAP, and OSi than cows in their third and greater lactations. Hence, supporting the antioxidative side of the balance might be of particular importance in the first and second lactation. Feeding different amounts of concentrates did not affect dROM, FRAP, or OSi under our experimental conditions, suggesting that the relatively small differences in energy intake were not affecting the oxidative status. Comparing farms, cows from one farm were notable for having greater dROM and lower FRAP, resulting in a greater OSi as compared with cows in the other farm. Milk yield showed a time by farm interaction with 7% less milk on d 100 in the farm with the greater OSi. Moreover, cows on that farm had 1.4-fold greater β-hydroxybutyrate concentrations.

Our results emphasize the value of assessing the oxidative status with regard to both, the pro- and antioxidative sides, and support the association between oxidative and metabolic status. Further investigations are needed to determine the applicability of OSi as a prognostic tool during early lactation and to determine which factors have the greatest influence on oxidative status.

Key words: dairy cow, oxidative status, parity, feeding concentrates, optiKuh

SHORT COMMUNICATION

Dairy cows face metabolic challenges in the transition from late pregnancy to early lactation; for example, increasing energy demands for the growing fetus and the onset of milk production. In addition, systemic inflammation is an epiphenomenon of early lactation (Bradford et al., 2015). Oxidative stress is defined as the imbalance between pro-oxidants and antioxidants in an organism towards the pro-oxidative side (Sies, 1991). Reactive oxygen species are assumed to be produced proportionally to metabolic rate resulting from mitochondrial function and the electron transport chain (Monaghan et al., 2009; Speakman and Garratt, 2014). Oxidative stress is common in transition cows (Sordillo and Aitken, 2009). To characterize the pro-oxidative side, measuring the derivatives of reactive oxygen metabolites (**dROM**) is a common option; dROM comprise oxygen-centered free radicals and nonradical derivatives that are normal byproducts of cellular metabolism (Reilly et al., 1991). They are important in several physiological processes such as protein phosphorylation, apoptosis, cell immunity and others (Agarwal et al., 2005). Apart from that, they can impair cell functions by affecting cellular lipids, proteins, and DNA if they are produced in excess and the antioxidative defense is insufficient (Miller et al., 1993; Sugino, 2006). The antioxidative defense comprises enzymatic and nonenzymatic components. Enzymatic antioxidants such as superoxide dismutase and glutathione peroxidase represent the main intracellular defense, and nonenzymatic antioxidants such as sulfhydryl groups of albumin, α-tocopherol, and uric acid represent the main extracellular defense (Miller et al., 1993; Urban-Chmiel, 2006; Halliwell and Gutteridge, 2015). Assessing individual antioxidative compounds is very laborious; therefore, methods to assess the antioxidant capacity of serum as a function of their overall activity has been suggested (Cao and Prior, 1998), for example, the ferric reducing ability of plasma (**FRAP**; Benzie and Strain, 1996). To detect changes in the oxidative status, both sides of the oxidative status should be assessed (Sharma et al., 1999). Abuelo et al. (2013) suggested the oxidative status index (**OSi**) as a tool to assess the redox status in dairy cattle during the transition period. The OSi is calculated as the ratio of pro-oxidant to antioxidant capacity. Oxidative status increases postpartum because of an increase in metabolic activity and systemic inflammation and thus enhanced accumulation of dROM and, at the same time, depletion of the antioxidant compounds (Sordillo and Aitken, 2009; Abuelo et al., 2013).

When considering assessments of oxidative status beyond strictly controlled experiments, potentially confounding factors need to be evaluated. We hypothesized that cows in their first lactation would have a greater OSi related to their greater metabolic activity for their own body growth. Moreover, we hypothesized that increasing the portion of concentrate in the diet would elevate the OSi, and that cows on different farms fed similar rations would not differ in OSi.

Therefore, our objectives were to investigate the effect of parity on the oxidative status of dairy cows in late pregnancy and early lactation. Primiparous cows are still growing at the time of their first calving and therefore, face different metabolic challenges around parturition than pluriparous cows (Coffey et al., 2006; Wathes et al., 2007). We also aimed to test the effect of different energy levels in the lactation diet by feeding different amounts of concentrate during early lactation in 2 different farms. Increasing the dietary level of starch was reported to increase oxidative status (Gabai et al., 2004); thus, the amount and portion of concentrates in the diet might need to be considered when aiming to assess the oxidative status. Finally, we aimed to test the effect of farm under similar feeding conditions.

The animal experiments were conducted between 2014 and 2017 at 3 experimental dairy farms in Germany as part of the national project “optiKuh”. The experiments were approved by the responsible local authorities for animal welfare affairs and therefore, were carried out in accordance with EU Directive 2010/63/EU and the *German Animal Protection Law*. All cows were housed in free stall barns, had permanent free access to feed and water and were milked twice daily (farm A: 0500 and 1630 h; farm B: 0545 and 1630 h; farm C: 0530 and 1515 h). Milk yield was recorded daily and milk components were analyzed weekly (Denissen et al., 2018).

For the first objective, investigating the effect of parity, we analyzed data from 257 Holstein dairy cows from one experimental farm (farm A). Cows were fed according to the recommendations of the Society of Nutrition Physiology in Germany (GfE, 2001). For the second and third objective, investigating the effect of feeding different amounts of concentrates and the differences between farms, samples and data from Simmental cows (lactation number ≥ 3) on 2 experimental farms (farms B and C) were considered. During the dry periods all, animals were fed the farm-specific ration according to the recommendations of the GfE (2001).

During lactation, cows were fed roughage containing 6.5 MJ NE_L / kg of DM and either 150 g (farm B: n = 24; farm C: n = 19) or 250 g (farm B: n = 25; farm C: n = 19) of concentrate / kg ECM yield. The feed was offered as a TMR in farm B and as partial mixed ration (**PMR**) in farm C. The components and chemical composition of the diets are shown in Table 1.

Table 1. Chemical composition and proportions of concentrates in rations in Farm B and C[1]

Item	Farm B (TMR)		Farm C (PMR)	
g concentrate / kg ECM	150	250	150	250
concentrate portion (% of DM)	22	35	31	38
DM, g/kg	429	468	466	514
Chemical composition (g/kg DM)				
Crude ash	80	79	71	70
Crude protein	152	157	158	159
Crude fat	30	28	45	44
Crude fibre	195	172	177	167
$aNDF_{OM}$[2]	346	310	377	362
ADF_{OM}[3]	223	197	222	212
Energy, MJ NE_L / kg DM	6.72	6.96	6.76	6.82
Energy, MJ ME / kg DM	11.0	11.3	11.1	11.1

[1]Values are presented as means over the study period (1-100 DIM) in the respective farms. Rations were either provided as TMR or partial mixed ration (PMR).

[2]NDF in OM: that is, without residual ash and pretreated with amylase.

[3]ADF without residual ash.

The ECM was calculated using the following equation by the GfE (2001):

$$ECM\ \left[\frac{kg}{d}\right] =$$

$$Milk\ yield\ [kg/d] \cdot \left(\frac{1.05 + 0.38 \cdot Milk\ fat\ [\%] + 0.21 \cdot Milk\ protein\ [\%]}{3.28}\right)$$

Individual feed intake was measured daily with weighing troughs and BCS was assessed monthly using a 5-point scale with 0.25 increments (Edmonson et al., 1989). Blood samples were collected after the morning milking (between 0800 and 1100 h) from a jugular vein on the following days relative to calving [desired day (± accepted range of deviation)]: −50 (± 10) d, −14 (± 4) d, +8 (± 2) d, +28 (± 2) d, and +100 (± 4) d. Samples were allowed to clot at 20 to 25 °C for 60 to 90 min and were then centrifuged at 1800 x g for 10 min. Serum samples were stored at −20 °C until analyzed.

Serum concentrations of dROM were measured with N, N-diethyl-*para*-phenylendiamine as chromogenic substrate (Alberti et al., 2000) with modifications according to Regenhard et al. (2014). The results are expressed as H_2O_2 equivalents. The mean intra-assay coefficient of variation (**CV**) was 2.9 % and the inter-assay CV was 11.8 %. The FRAP was measured according to Benzie and Strain (1996). The standard curve included 7 points (0.05, 0.1, 0.2, 0.3, 0.4, 0.6, and 0.8 m*M* $FeSO_4$ $7H_2O$; concentrations were given as m*M* Fe^{2+}). The mean intra-assay CV was 1.1 % and the inter-assay CV was 0.5 %. The OSi was calculated as dROM / FRAP * 100 (µg H_2O_2 / mL / m*M* Fe^{2+}) * 100. Serum concentrations of nonesterified fatty acids (NEFA) and BHB were analyzed photometrically with a Cobas Mira analyzer (Hoffmann-La Roche, Basel, Switzerland). Data were analyzed using SPSS (IBM SPSS Statistics 25, Ehningen, Germany). Cow was set as random effect in all models. A first analysis with lactation number as fixed effect revealed that cows in the first and second lactations did not differ from each other in their oxidative status but that they did differ compared with cows in greater lactations. Therefore, parity was dichotomized into cows in their first and second lactation (n = 133; mean lactation number: 1.4 ± 0.04; mean age: 28 ± 0.6 mo) and cows in their third and greater lactation (n = 114; mean lactation number: 4.5 ± 0.15; mean age: 70 ± 2 mo). Bonferroni adjustment was used for post hoc analyses. The linear mixed model for investigating the parity effect was:

$$y_{ijk} = \mu + T_i + P_j + TP_{ij} + i_k + e_{ijk}$$

where y_{ijk} = response variable, μ = overall mean, T_i = fixed time effect (i = d −50, −14, +8, +28, +100), P_j = fixed parity effect (j = 1st and 2nd lactation, ≥3 lactations), TP_{ij} = fixed interaction, i_k = random effect of the individual cow (k = 1, …, 247), and e_{ijk} = residual error.

The linear mixed model for investigating the feeding and farm effect was:

$$y_{ijkl} = \mu + T_i + Cj + F_k + TC_{ij} + TF_{ik} + CF_{jk} + i_l + e_{ijkl}$$

where y_{ijkl} = response variable, μ = overall mean, T_i = time effect (i = d +8, +28, +100), C_j = concentrate effect (j = 150 g/kg ECM, 250 g/kg ECM), F_k = farm effect (k = Farm B, Farm C), TC_{ij}, TF_{ik}, and CF_{jk} = fixed interactions, i_l = random effect of the individual cow (l = 1,…, 87), and e_{ijkl} = residual error. The 3-way interaction between time, treatment, and farm was not significant for any of the tested response variables and was, therefore, excluded from the model. The assumptions for the linear mixed model, in terms of normal distribution and

homoscedasticity of the residuals were met. Results are presented as means ± standard error of the means. Significance was declared for $P < 0.05$. The results for dROM, FRAP, and OSi in farm A are shown in Figure 1.

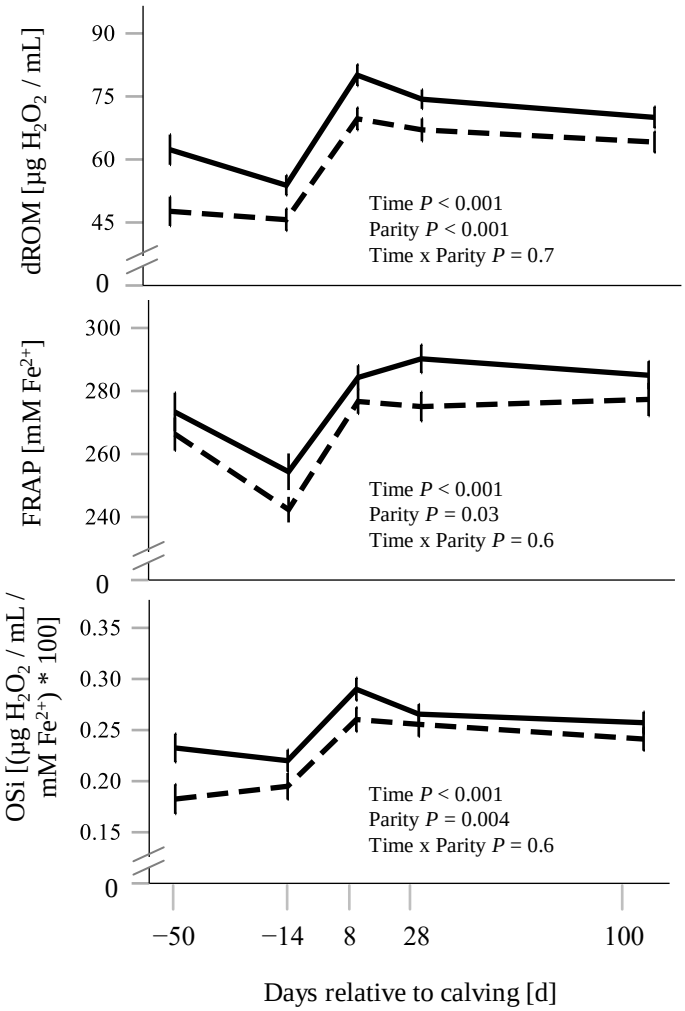

Figure 1. Mean (± SEM) serum concentrations of derivatives of reactive oxygen metabolites (dROM) and ferric reducing ability (FRAP) and their ratio (OSi) on farm A for Holstein cows in their first and second lactations (solid lines, n = 133) and in their third or higher lactations (dashed lines, n = 114). *P*-values from the linear mixed model for the fixed effects time, parity, and the interaction between time and parity are presented.

The dROM, FRAP and OSi showed similar trends over time for cows in all lactations and were lowest during prepartum and increased until d 8, remaining at this level for the subsequent sampling time points. The increased OSi postpartum observed is in accordance with the study of Abuelo et al. (2013), although these authors did not detect differences when looking at the pro- and antioxidative variables separately. The rapid increase of milk yield and insufficient feed intake early postpartum might enhance the production of reactive oxygen species, for example, because of increased metabolic activity and lipid peroxidation (Castillo

et al., 2005; Sordillo and Aitken, 2009). Additionally, antioxidants are likely depleted prepartum to some extent because of the production of colostrum (Goff and Stabel, 1990) and to balance the increased oxidants (Abuelo et al., 2013). Nevertheless, the effect of this oxidative status on milk yield and health might be not critical (Wullepit et al., 2009) and needs to be evaluated together with clinical data. In our study, cows in the first and second lactations had greater dROM, FRAP, and OSi than cows in their third or higher lactations. Only a few studies are available on the oxidative status in cows of different parities. Abuelo et al. (2016) reported greater pro-oxidant production in primiparous cows than in pluriparous cows, whereas Elischer et al. (2015) demonstrated that primiparous cows had a greater antioxidant potential. In contrast, Omidi et al. (2017) reported a lower total antioxidant capacity for primiparous cows than pluriparous cows.

The individual variation (Castillo et al., 2005) and the variation between studies in oxidative status emphasizes the importance of assessing both the pro-oxidant and antioxidant side, to obtain a more precise picture of oxidative status in dairy cows. Greater levels of pro-oxidants might not always equal greater oxidative stress but could be controlled by greater antioxidant capacity. Our results suggest that not only cows in their first lactation but those in second lactation should be evaluated with regard to potential burdens arising from increased oxidative status.

Results of the feeding trial are shown in Figure 2.

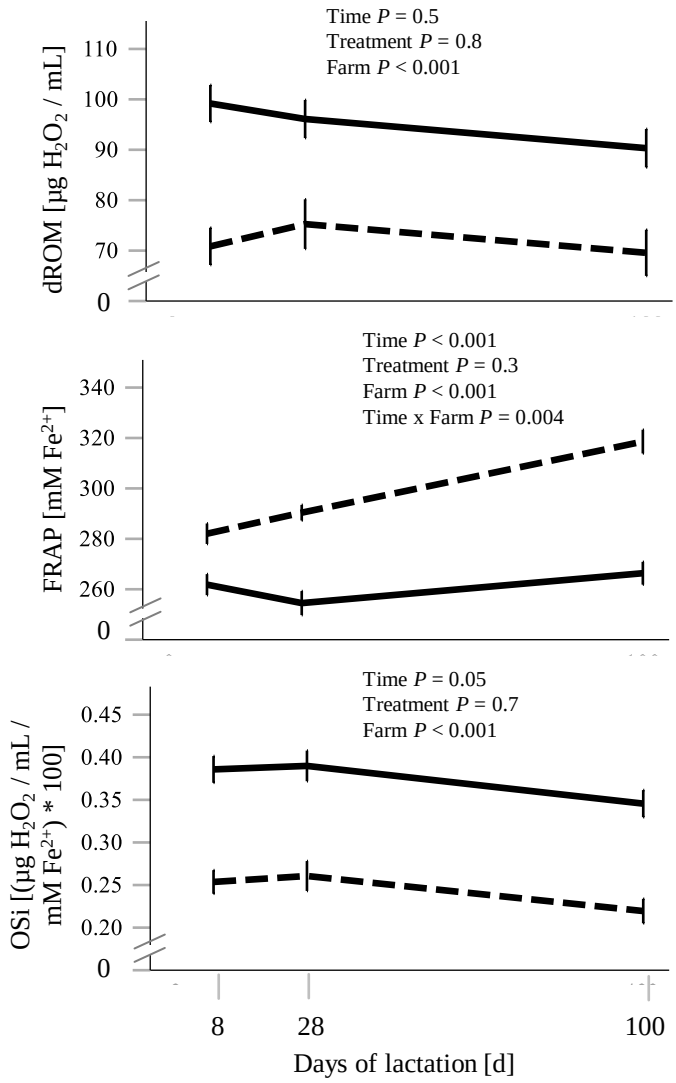

Figure 2. Mean (± SEM) serum concentrations of derivatives of reactive oxygen metabolites (dROM) and ferric reducing ability (FRAP) and their ratio (OSi) of all cows, irrespective of feeding group, in farm B (solid lines, n = 49) and C (dashed lines, n = 38) with Simmental cows. *P*-values from the linear mixed model for the fixed effects time, treatment, farm and significant interactions between these effects are presented.

Feeding different amounts of concentrates did not affect dROM, FRAP, or OSi. Previous studies have shown an increase in oxidative status related to high starch contents in the diet of dairy cows (Gabai et al., 2004) and ewes (Sgorlon et al., 2008). Pederna et al. (2010) reported an indirect effect of diet on oxidative status in a study with pasture-based feeding. When they compared cows with greater mobilization of body reserves, cows fed more concentrate had lower antioxidant capacity than cows fed less concentrate. Cows with medium or low mobilization of body reserves showed no differences in oxidative status related to the diet (Pedernera et al., 2010). The difference between the amounts of concentrates in

our study might not have been great enough to affect oxidative status; the recommendations of GfE (2001) were still met in both groups. The BHB concentrations (Table 2) were not affected by the different inclusion rates of concentrate (mean from d 8 to d 100: 150 g/kg ECM group: 0.67 ± 0.05 mmol/L; 250 g/kg ECM group: 0.66 ± 0.05 mmol/L; P = 0.9). Greater NEFA concentrations for cows in the 150 g/kg ECM group (566 ± 27 µmol/L) indicated a greater mobilization of body reserves compared with cows in the 250 g/kg of ECM group (NEFA: 468 ± 0.01 µmol/L; P = 0.01).

In our study, all 3 variables for oxidative status differed between the 2 farms compared directly, with greater dROM, lower FRAP, and a greater OSi on farm B than on farm C. To avoid intermingling of breed and feeding with the farm effect, we only compared farms B and C to investigate the farm effect. There was an interaction between time and farm for FRAP, resulting from a 12 % increase from d 8 to d 100 on farm C, whereas FRAP in farm B remained lower during the study period.

The differences between the farms might be explained, to some extent, by individual variation, as noted by Castillo et al. (2005). In comparing oxidative status between different farms, Abuelo et al. (2015) reported a greater OSi on organically managed farms than on conventionally managed farms but no difference between 2 organically managed farms. They suggested that the different external supply of antioxidants explained the lower antioxidant capacity on the organically managed farms (Abuelo et al., 2015). The amount of antioxidants (e.g. vitamin E and selenium) in the roughage of the 2 farms in our study might have varied and could have affected the cows' antioxidant capacity. However, because both farms provided mineral and vitamin mix to their herds to cover dietary needs, it is unlikely that divergent supply would account for the differences in the oxidative status between the farms. When comparing milk yields (at 100 d) between the 2 farms with the divergent oxidative status, we found an interaction between time and farm, which resulted from greater yields in farm B on d 8 (33.9 ± 0.8 kg/d) but lower milk yield on d 100 (33.8 ± 0.8 kg/d) compared with farm C (d 8: 32.2 ± 0.8 kg/d; d 100: 36.4 ± 0.8 kg/d). The lower milk yield on 100 d might be related to the greater oxidative status that the cows in farm B experienced. In agreement, Pederna et al. (2010) reported a greater OSi related to lower milk yield. For the cows in our study, Spiekers et al. (2018) reported a higher energy expenditure per kilogram of ECM on farm B than on

farm C. Although the cows on farm B had greater intake of DM and energy, their BCS loss from d 28 to d 100 tended to be greater (− 0.2 BCS points) than on farm C (− 0.1 BCS points, time x farm: $P = 0.079$). Bernabucci et al. (2005) stated that cows with greater BCS loss are more sensitive to oxidative stress and reported a positive association between oxidative status with NEFA and BHB as indicators for lipomobilization and ketogenesis. In our study, cows on farm B had 1.3-fold greater BHB concentrations (0.76 ± 0.04 mmol/L) compared with those on farm C (0.57 ± 0.05 mmol/L; $P = 0.003$) but the mean BHB concentrations were below the critical value of 1.2 mmol/L (Dirksen et al., 2012) in both farms at all time points tested. Concentrations of NEFA did not differ between the farms (farm B: 516 ± 26 µmol/L; farm C: 518 ± 29 µmol/L, $P = 0.97$). Hence, the greater oxidative status on farm B might indicate a metabolic or health problem, on which the cows had to spent additional energy, resulting in lower milk yields and greater loss of BCS towards d 100. However, the relatively small number of animals and farms is a limitation of our study and the question on the causal connection and its direction between the different variables remain unclear. Further investigations including a greater number of farms and animals are needed to narrow down the reasons for the individual farm-level differences. For example, management factors, such as rearing of heifers, likely plays an important role in the oxidative status of animals (Celi, 2011). In a further step the suitability of OSi in early lactation as a prognostic tool for metabolic health or milk yield in the ongoing lactation should be investigated.

In conclusion, our results emphasize the value of investigating both sides of the pro- and antioxidant balance because greater concentrations of pro-oxidants might not always result in greater oxidative status but might be controlled by a greater antioxidant capacity. Moreover, not only the special management of primiparous cows is important, but also cows in their second lactation also need to be regarded with special care related to their greater oxidative status. Further investigations are needed to find out which factors, in management for example, have the greatest influence on oxidative status and result in farm individual variation. Furthermore, the suitability of the OSi as prognostic marker in early lactation should be investigated.

Table 2. Mean (± SEM) concentrations of various blood variables in cows (lactation number ≥3) from the feeding trial[1]

Blood variable[2]	MC	HC	*p*-value	Farm B	Farm C	*p*-value
dROM	84.4 ± 3.8	83.1 ± 3.8	0.8	96.4 ± 3.6	71.1 ± 4	<0.001
FRAP	280 ± 4	274 ±4	0.3	258 ± 4	296 ± 4	<0.001
OSi	0.31 ± 0.02	0.32 ± 0.02	0.7	0.38 ± 0.01	0.24 ± 0.02	<0.001
NEFA µmol/L	566 ± 27	468 ± 27	0.01	516 ± 26	518 ± 29	0.97
BHB mmol/L	0.67 ± 0.05	0.66 ± 0.05	0.9	0.76 ± 0.04	0.57 ± 0.05	0.003
Insulin µU/mL	10 ± 0.9	12.4 ± 1	0.065	11 ± 0.9	11.4 ± 1	0.7
Glucose mmol/L	3.15 ± 0.04	3.22 ± 0.04	0.3	3.38 ± 0.04	2.98 ± 0.04	<0.001
IGF-1 ng/mL	98 ± 6	106 ± 6	0.3	89 ± 5	115 ± 6	0.002
Adiponectin µg/mL	25.4 ± 0.7	24.3 ± 0.7	0.3	26.3 ± 0.7	23.4 ± 0.7	0.004

[1]Values are presented as means over the study period (1-100 DIM) in the respective feeding group (MC or HC) or farm (b or C). MC = medium concentrate group receiving 150 g of concentrate/kg of ECM yield; HC = high concentrate group receiving 250 g of concentrate/kg of ECM yield. Additional measurements were performed as described in Urh et al. (2019).

[2]dROM = derivatives of oxygen metabolites; FRAP = ferric reducing ability of plasma; OSi = oxidative status index; NEFA = nonesterified

fatty acids.

P-values are from the linear mixed model with the fixed effects time, feeding group, farm, and all 2-way interactions, and the random effect of cow.

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V. DISCUSSION

1. Associations between adiponectin and oxidative stress

A relation between adiponectin and oxidative stress was already proposed in human studies and on the cellular level. Especially within the scope of obesity, diabetes mellitus, artherosclerosis, and the metabolic syndrome several studies in human medicine and mice models were performed to gain knowledge on the mechanisms acting between adiponectin and oxidative stress. OUEDRAOGO et al. (2006) showed that adiponectin reverses the hyperglycemia-associated endothelial ROM production via cAMP/PKA-linked pathways and therefore could be possibly used for the prevention or treatment of vascular complications of type 1 diabetes. Furthermore, adiponectin was shown to suppress the development of artherosclerosis in vascular walls by its different anti-inflammatory effects (MATSUDA et al. 2002; OUCHI et al. 2000; OKAMOTO et al. 2002). In contrast, oxidative stress is supposed to play an important role in the development of artherosclerosis and diabetes (MATSUDA and SHIMOMURA 2014). FURUKAWA et al. (2004) stated that adipose tissue might be a major source of ROM in patients with metabolic syndrome. Furthermore, ROM suppressed the adiponectin production in adipocytes and treatment with antioxidants restored adiponectin production and improved insulin sensitivity. Adiponectin was negatively correlated with markers of oxidative stress and systemic inflammation (FURUKAWA et al. 2004; FRÜHBECK et al. 2017). Hence, there is a close connection between oxidative stress and adiponectin in humans, at least during obesity linked disease complexes like the metabolic syndrome, and both should be involved in the further research (MATSUDA and SHIMOMURA 2014).

For dairy cows there are fewer studies on the relation of adiponectin and oxidative stress so far. HÄUSSLER et al. (2013) reported that an increase of oxidative status due to excessive adipose tissue accumulation coincided with decreased circulating adiponectin concentrations in non-pregnant, non-lactating dairy cows and adiponectin concentrations tended to be negatively correlated with dROM ($r = -0.37$, $p = 0.07$). They concluded that the increased dROM concentrations might indicate mitochondrial dysfunction, which results in decreased insulin sensitivity via a reduced adiponectin secretion (HÄUSSLER et al. 2013). Looking at the changes in concentrations of dROM and adiponectin from two weeks before until

one to four weeks after calving, an inverse pattern can be observed. In pluriparous cows, adiponectin is decreasing in this time, whereas dROM is increasing (URH et al. 2019). In conclusion, there are hints (HÄUSSLER et al. 2013) that circulating adiponectin and oxidative status are linked to each other also in dairy cows, but further investigations are needed to investigate the mechanisms and specific relationships. Especially in the transition period, the interaction might differ due to the several metabolic and hormonal adaptations. Based on the insulin resistance in this period, the metabolic situation of transition dairy cows is often compared with diabetes type 2. It has to be mentioned that different from diabetes the insulin resistance does not coincide with high blood glucose levels in the transition dairy cow. Hence, some of the mechanisms and pathways mentioned above for humans might not fit to the situation in dairy cows and alternative explanations need to be addressed.

2. Suitability of adiponectin as metabolic indicator

Adiponectin is supposed to play a role in regulating energy metabolism. Especially during the transition period it might be involved in the energy partitioning between the mammary gland and the rest of the body as we concluded from the differences between primiparous and pluriparous cows (URH et al. 2019, 2019). Consequently, it might be involved in the development of metabolic diseases like ketosis, as we observed lower adiponectin concentrations in dairy cows which developed hyperketonemia postpartum (MANN et al. 2018). The relatively high variability of the adiponectin concentrations during the transition period and the individual differences can be seen as positive for the use as a metabolic indicator but also imply difficulties in the interpretation. The variation might help to distinct between cows being successful or not in their adaptive reactions. At the same time, the individual and sampling time dependent variation complicates the definition of reliable general thresholds. Furthermore, the adiponectin concentrations change in a relatively small range even in the transition period, e.g. approximately 0.9-fold from d −14 to d +28 for pluriparous cows (URH et al. 2019), thus further impeding the establishment of reliable thresholds. For a clinical use of adiponectin, reference values would be necessary. Reference values are often established as the 95% confidence interval based on a clinically healthy population (LUMSDEN and MULLEN 1978). Clinical diagnoses like ketosis, mastitis, and hypocalcaemia were recorded in the present study, but

the quality of the records differed very much between the different farms, as some farms distinguished in their records between clinical and subclinical diagnoses, others only recorded the general diagnoses, and others have not recorded any diagnoses at all. Therefore, we could not determine animals as clinically healthy or unhealthy reliably. To do so regular clinical examinations would have been necessary. The other measured blood metabolites, e.g. glucose and BHB, give an additional orientation on the health status but could not replace the regular clinical examination completely due to the low sampling frequency, especially in the precarious days after calving. Hence, we did not calculate reference values for adiponectin in the present study, as we would not have been able to determine a healthy population and could not check the sensitivity and specificity of possible reference values.

It is not yet clear which factors are affecting the adiponectin concentrations besides the time relative to calving and the parity. For example, we could not explain the observed farm effect satisfactorily and the individual variation is not yet assured to be linked to successful metabolic adaptation. Therefore, adiponectin as a separate measurement is not a very promising candidate for a new indicator of metabolic health in the transition period.

Adiponectin is known from human medicine to indicate the risk for diseases in the complex of metabolic syndrome, which is linked to low insulin sensitivity (TRUJILLO and SCHERER 2005). In dairy cows, the relation between adiponectin and insulin sensitivity was shown in terms of moderate correlations between adiponectin and the revised quantitative insulin sensitivity check index (RQUICKI) (SINGH et al. 2014a; SINGH et al. 2014b; SINGH et al. 2014c) but only few studies assessed the insulin sensitivity and responsiveness directly. In a study with hyperinsulinemic euglycemic clamp tests, the gold standard for the assessment of insulin sensitivity, a positive correlation between adiponectin and insulin responsiveness in the dry period was shown, but not with insulin sensitivity (KOSTER et al. 2017). In the present study we did calculate the RQUICKI, but could not find any significant or strong correlations with adiponectin (data not shown). According to KOSTER et al. (2016) surrogate markers like the RQUICKI are not reliable at the end of the dry-period and further research is needed to test their reliability in early and later stages of lactation. As hyperinsulinemic euglycemic clamp tests are very laborious, an improved

surrogate marker, which could also be used around parturition could be a goal for further studies. Maybe a combination of surrogate markers like the RQUICKI with adiponectin could be an option to improve the informatory power and reliability.

3. Suitability of dROM and FRAP as metabolic indicators

Oxidative stress affects and is affected by metabolic changes, e.g. increased oxidative stress after parturition in dairy cows (CASTILLO et al. 2005; ABUELO et al. 2013). Hence, assessing the oxidative status in the transition period could be an option to evaluate the chance of successfully passing the transition period. Furthermore, SORDILLO and AITKEN (2009) reviewed that oxidative stress is related to impaired immune and inflammatory responses especially in transition dairy cows. LI et al. (2016) reported that ketotic cows experience oxidative stress, as prooxidants were increased and the antioxidant capacity was decreased in ketotic cows in their study. According to ABUELO et al. (2016a), oxidative stress is rather a consequence than a predictor of inflammatory responses related to lameness.

In our study (Chapter IV), the oxidative status was affected by lactation number and time relative to parturition. Hence, when establishing a reference value, these factors need to be considered. Furthermore, differences could be observed between the two investigated farms, and we suspect a relation between the greater oxidative status, already prepartum, and greater BHB concentrations and lower milk yield in the following lactation. Consequently, there are reasons to put dROM, FRAP, and OSi on the candidate list for metabolic indicators in the transition period. Although, as already discussed for adiponectin, the fold change seen in the transition period, e.g. 1.4-fold for dROM and 1.1-fold for FRAP from d −14 to d +8, is not optimal to set reliable reference values. Moreover, the details of the association between the oxidative status and milk yield or potential production diseases needs to be investigated further, before dROM, FRAP or both can be established as metabolic indicators. Similar as discussed for, we did not calculate reference values for dROM and FRAP in this study, but it might make sense to include these oxidative variables in indices describing the metabolic status of dairy cows.

4. Conclusion und perspectives

To sum it up, the tested factors parity, farm, and feed did all affect the adiponectin serum concentrations. Interestingly the effect of feed was different than expected, as not the energy supply as such affected the adiponectin concentration, but rather the content of straw in the roughage showed an effect. The time dependent differences in the comparison between primi- and pluriparous cows emphasize the importance of taking into account multiple factors at the same time. The oxidative status was affected by farm and parity as well. Other than for adiponectin, cows in their second lactation showed a similar pattern like those in their first lactation, and differed compared with cows in higher lactations. Furthermore, the study on the oxidative status emphasized the importance of assessing both, pro- and antioxidative parameters.

As discussed above, the parameters investigated herein are not perfectly suitable as indicators for a successfully passed transition period. Nevertheless, we could expand the knowledge on influencing factors on adiponectin and the oxidative status of transition dairy cows. Furthermore, there is evidence that also in dairy cows insulin sensitivity, and therefore adiponectin, and oxidative status interact, as it was already reported for humans. Further studies should focus on the possibilities to include adiponectin in a surrogate marker for insulin sensitivity or to establish new indices for metabolic health in the transition period.

VI. SUMMARY

During late pregnancy and early lactation the energy demands of high yielding dairy cows exceed their nutrient intake. The resulting negative energy balance leads to hormonal changes, substantial mobilization of body reserves, and an increased risk for production diseases like ketosis, fatty liver, mastitis and metritis. Glucose and insulin sensitivity play a pivotal role in this period, as the reduced insulin sensitivity in tissues like liver, muscle and adipose tissue in the transition period is supposed to be one reason for the development of the production diseases and glucose is the main energy supply for the conceptus and the precursor for lactose. On the one hand, insulin sensitivity can be increased by adiponectin, an insulin sensitizing hormone produced by adipose tissue and circulating in relatively high concentrations. On the other hand, insulin sensitivity can be decreased by oxidative stress, the imbalance between pro- and antioxidants. In this thesis influencing factors on adiponectin and the oxidative status are examined, leading to the discussion, if they could possibly serve as indicators for metabolically successful cows in the transition period.

In our studies (chapter III and IV) we examined the effects of parity, farm, and feeding different energy levels on adiponectin and the oxidative status in a large number of dairy cows.

Primiparous cows had lower adiponectin prepartum and greater adiponectin postpartum compared with pluriparous cows. Our results suggest that adiponectin is likely involved in the mechanisms of energy partitioning between the the offspring on the one hand and the own body, which is still growing in primiparous cows, on the other hand. This energy partitioning may change from pregnancy to lactation. Unexpectedly, not a greater energy supply, but a greater amount of straw in the roughage portion lead to greater adiponectin concentrations in early lactation. Further studies are needed to elucidate the mechanisms behind the roughage effect and its metabolic consequences.

To examine the oxidative status we measured the concentrations of the derivatives of reactive oxygen metabolites (dROM) and the ferric reducing ability (FRAP) in serum and calculated the oxidative status index (OSi) as dROM / FRAP * 100. Cows in their first and second lactation had greater dROM, FRAP, and OSi than cows in their third and higher lactation. Hence, not only cows in their first

lactation, but also cows in their second lactation should be regarded with special care, like antioxidant supplements. Furthermore, these results emphasize the value of assessing the oxidative status with regard to both, the pro- and antioxidative side. Feeding different amounts of concentrates did not affect dROM, FRAP, and OSi, but cows from one farm were sticking out by greater dROM and lower FRAP, resulting in a greater OSi as compared with cows in the other farm. A lower milk yield on day 100 and greater BHB concentrations in this farm with higher Osi support the association of oxidative status and metabolic status. Further investigations are needed to determine the applicability of OSi as a prognostic tool during early lactation and to sort out which factors, e.g. in management, are influencing the oxidative status the most.

To sum it all up, circulating adiponectin was affected by parity, farm, and feeding and the oxidative status was affected by farm and parity. The time dependent differences of the effects emphasize the importance of taking multiple factors at the same time into account.

By now there are no reliable thresholds for adiponectin concentrations or the oxidative parameters investigated in our studies. The various effects, the individual and sampling time dependent variation, and the fact that the concentrations change in a relatively small range complicate the definition of thresholds. Therefore, these parameters are not perfectly suitable as indicators for a successfully passed transition period. Nevertheless, we could expand the knowledge on influencing factors on adiponectin and the oxidative status of transition dairy cows. Furthermore, there is evidence that also in dairy cows insulin sensitivity, and therefore adiponectin, and oxidative status interact, as it was already reported for humans. Further studies should focus on the possibilities to include adiponectin in a surrogate marker for insulin sensitivity or to establish new indices for metabolic health in the transition period.

VII. ZUSAMMENFASSUNG

In der Transitphase, also den letzten Wochen der Trächtigkeit und den ersten Wochen der Laktation, übersteigt der Energiebedarf von hochleistenden Milchkühen die Energieaufnahme mit dem Futter. Daraus folgt eine negative Energiebilanz, die zu Änderungen im Hormonhaushalt, Mobilisation von Körperreserven und einem erhöhten Risiko für Produktionskrankheiten wie Ketose, Fettleber, Mastitis und Metritis führt. In dieser Phase spielen Glukose und Insulinsensitivität eine wichtige Rolle. Die Insulinsensitivität in Geweben wie Leber, Muskel und Fett wird reduziert, was als ein Grund für die Entwicklung der Produktionskrankheiten angesehen wird. Adiponectin als insulinsensitivierendes Hormon, welches im Fettgewebe produziert wird und in relativ hohen Konzentrationen im Blut vorkommt, kann die Insulinsensitivität steigern. Oxidativer Stress, also das Ungleichgewicht zwischen Pro- und Antioxidantien, können die Insulinsensitivität hingegen reduzieren. In dieser Arbeit werden die Einflussfaktoren auf Adiponectin und den oxidativen Status untersucht. Weiterhin wird diskutiert ob sie als Indikatoren dienen können, um Kühe mit einem stabilen Stoffwechsel zu erkennen.

In unseren Studien (Kapitel III und IV) haben wir die Effekte der Lakatationsnummer, verschiedener Betriebe und der Fütterung verschiedener Energielevels auf Adiponectin und den oxidativen Status untersucht.

Primipare Kühe hatten geringere Adiponectinkonzentrationen vor der Kalbung und größere Adiponectinkonzentrationen nach der Kalbung als pluripare Kühe. Unsere Ergebnisse lassen vermuten, dass Adiponectin wahrscheinlich in die Vorgänge der Energieverteilung zwischen dem Nachwuchs auf der einen und dem eigenen, bei primiparen Kühen noch im Wachstum befindlichen, Körper auf der anderen Seite involviert ist. Des Weiteren scheint sich diese Energieverteilung Verlauf von Hochträchtigkeit zur Laktation zu verändern. Anders als erwartet, hat nicht eine erhöhte Energiezufuhr, sondern ein erhöhter Strohgehalt im Grundfutter zu erhöhten Adiponectinkonzentrationen geführt. Um die Gründe für diesen Grundfuttereffekt und die Folgen daraus zu erörtern, sind weitere Studien notwendig.

Zur Erfassung des oxidativen Status haben wir in den Serumproben die Derivate der Sauerstoffmetaboliten (dROM), und die eisenreduzierende Kapazität (FRAP)

gemessen und daraus den oxidativen Status Index (OSi) (dROM/FRAP*100) gemessen. Kühe in ihrer ersten und zweiten Laktation zeigten höhere dROM, FRAP und OSi Werte als Kühe in der dritten oder höheren Laktation. Diese Ergebnisse sprechen dafür, dass nicht nur Kühe in ihrer ersten Laktation besondere Behandlung, wie z.B. die Zugabe von Antioxidantien erfahren sollten, sondern auch Kühe in der zweiten Laktation. Außerdem zeigen sie, dass beide Seiten des oxidativen Status, also sowohl die pro- als auch die antioxidativen Parameter, erfasst werden sollten. Die Fütterung verschiedener Kraftfuttermengen hatte keinen Einfluss auf den oxidativen Status. Allerdings hatten Kühe in einem Betrieb deutlich höhere dROM und niedrigere FRAP-Werte als in dem anderen betrachteten Betrieb, was sich ebenfalls in einem höheren OSi zeigte. Eine niedrigere Milchleistung an Tag 100 der Laktation und höhere BHB-Konzentrationen in diesem Betrieb unterstützen die Idee, dass der oxidative Status mit der Stoffwechselsituation in Verbindung steht. Die Verwendbarkeit des OSi als Prognosewert in der frühen Laktation und weitere Einflussfaktoren auf den oxidativen Status müssen in weiteren Studien geklärt werden.

Bis jetzt gibt es noch keine zuverlässigen Grenzwerte für Adiponectin oder die oxidativen Parameter aus unserer Studie. Die verschiedenen Effekte und die individuellen und Zeitpunkt abhängigen Streuungen sowie die Tatsache, das sich die Konzentrationsänderungen nur in einem relativ kleinen Bereich bewegen, erschweren die Festlegung solcher Grenzwerte. Dadurch sind diese Parameter bisher nicht gut als Indikatoren für eine erfolgreiche Transitphase zu gebrauchen. Nichtsdestotrotz zeigt diese Dissertation neue Erkenntnisse über die Einflussfaktoren auf Adiponectin und den oxidativen Status bei Milchkühen. Außerdem geben die Ergebnisse Hinweise darauf, dass auch bei Milchkühen die Insulinsensitivität und somit auch Adiponectin mit dem oxidativen Status interagieren, wie es bereits für den Menschen berichtet wurde. In zukünftigen Studien sollte der Fokus darauf liegen Adiponectin zum Beispiel in Schätzgleichungen für die Insulinsensitivität zu integrieren oder Indices für Stoffwechselgesundheit in der Transitphase zu entwickeln.

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IX. PUBLICATIONS AND PROCEEDINGS DERIVED FROM THIS DOCTORATE THESIS

1. submitted **: C. Urh**, J. Denißen, E. Gerster, E. Stamer, B. Heitkönig, H. Spiekers, H. Sauerwein (2019): *Short Communication: Pro- and anti-oxidative indicators in serum of dairy cows during late pregnancy and early lactation: Testing effects of parity, different dietary energy levels and farms.* Journal of Dairy Science.

2. **C. Urh**, J. Denißen, I. Harder, C. Koch, E. Gerster, T. Ettle, N. Kraus, R. Schmitz, B. Kuhla, E. Stamer, H. Spiekers, H. Sauerwein (2019): *Circulating adiponectin concentrations during the transition from pregnancy to lactation in high yielding dairy cows: testing the effects of farm, parity, and dietary energy level in large animal numbers.* Domestic Animal Endocrinology, DOI: 10.1016/j.domaniend.2019.01.001.

3. **C. Urh**, J. Denißen, I. Harder, C. Koch, E. Stamer, H. Spiekers, H. Sauerwein (2018): PSI-18 Adiponectin serum concentrations in late pregnancy and early lactation in primiparous and multiparous Holstein dairy cows., *Journal of Animal Science*, Volume 96, Issue suppl_3, 7 December 2018, Pages 65, https://doi.org/10.1093/jas/sky404.144

4. **C. Urh**, K. Schuh, G. Dusel, C. Koch, H. Sauerwein (2018): *Effect of body condition on the oxidative status in dairy cows*. 5th DairyCare Conference Thessaloniki.

5. **C. Urh**, J. Denißen, T. Ettle, E. Meyer, R. Schmitz, E. Stamer, H. Spiekers, H. Sauerwein (2018): *Varying the energy density of the diets by roughage composition and the amount of concentrates: effects on the circulating concentrations of adiponectin in Holstein and in Simmental cows*. Proceedings of the Society of Nutrition Physiology: 72nd Conference 13th - 15th March 2018 in Göttingen, S. 84

6. **C. Urh**, J. Denißen, I. Harder, C. Koch, E. Gerster, T. Ettle, N. Kraus, R. Schmitz, B. Kuhla, E. Stamer, H. Sauerwein (2018): *Adiponectin – Neue Erkenntnisse aus optiKuh?!* Tagungsband der Abschlussveranstaltung Verbundprojekt optiKuh, 29.-30.01.2018, Braunschweig, Bayerische Landesanstalt für Landwirtschaft (LfL), Freising-Weihenstephan.

7. S. Mann; **C. Urh**; H. Sauerwein; J. J. Wakshlag; F. A. L Yepes; T. R. Overton; D. V. Nydam. (2018): Short communication: The association of adiponectin and leptin concentrations with prepartum dietary energy supply, parity, body condition, and postpartum hyperketonemia in transition dairy cows. Journal of dairy science 101 (1), S. 806–811. DOI: 10.3168/jds.2017-13752.

8. **C. Urh**, T. Ettle, E. Gerster, U. Mohr, H. Sauerwein (2017): *Evaluation of the suitability of a marker for oxidative stress in dairy cows of a dual purpose breed.* EAAP - 68th Annual Meeting, Tallinn, Book of Abstracts no. 23.

9. J. De Koster, **C. Urh**, M. Hostens, W. Van den Broeck, H. Sauerwein, G. Opsomer (2017): *Relationship between serum adiponectin concentration, body condition score, and peripheral tissue insulin response of dairy cows during the dry period*. Domestic Animal Endocrinology. 59, S. 100–104.
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