Aus dem Institut für Tierernährung
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

und

dem Institut für Ernährungsphysiologie "Oskar Kellner"
am Leibniz-Institut für Nutztierbiologie Dummerstorf

Effects of the Flavonoids Quercetin and Rutin in Dairy Cows

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der Freien Universität Berlin

vorgelegt von
Ann-Kathrin Stoldt
Tierärztin aus Halle (Saale)

Berlin 2017
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Abbreviations

ap	ante partum
ATP	adenosine triphosphate
AST	aspartate aminotransferase
BHBA	beta-hydroxybutyrate
BW	body weight
GLDH	glutamate dehydrogenase
mRNA	messenger ribonucleic acid
NEFA	non-esterified fatty acids
pp	post partum
RNS	reactive nitrogen species
ROS	reactive oxygen species
TCA	tricarboxylic acid

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1 General Introduction

1.1 Properties of flavonoids

Belonging to the highly diverse group of polyphenols (Scalbert and Williamson, 2000), flavonoids are secondary plant metabolites with a variable phenolic structure first discovered in 1930 (Lakhanpal and Rai, 2007). In plants, flavonoids contribute to the defense system against radiation and microbial invasion (Harborne and Williams, 2000) and have antioxidative, antifungal and antibacterial effects (Materska, 2008). Additionally, flavonoids provide color, texture and taste to flowers, fruits and leaves (Formica and Regelson, 1995). High flavonoid concentrations are found in sun-exposed parts of leaves and fruit peel (Lesser and Wolffram, 2006). Under more than 6000 different forms of flavonoids (Harborne and Williams, 2000), quercetin is one of the most abundant in plants and food, found in fruits, vegetables, grain, flowers, tea, and wine (Formica and Regelson, 1995; Lesser and Wolffram, 2006; Lakhanpal and Rai, 2007). Over the last 30 years (Heim et al., 2002), many studies revealed that quercetin exhibits antiviral, antibacterial, anticarcinogenic, antihepatotoxic and antiinflammatory properties in vitro and in rodents and humans (Miles et al., 2014; Kawabata et al., 2015). Furthermore, quercetin is one of the most potent antioxidants among polyphenols (Formica and Regelson, 1995; Materska, 2008). Only little references for toxic effects of quercetin where found in in vivo studies (Boots et al., 2008; Lesser and Wolffram, 2006; Russo et al., 2012).

1.1.1 Biochemical structure

Flavonoids are benzopyran derivates consisting of a benzodihydropyran and a phenyl group (Narayana et al., 2001; Heim et al., 2002). Several subclasses differ in their molecular structure, functional groups and substitutions (Heim et al., 2002; Nijveldt et al., 2001; **Figure 1**). In plants, flavonoids are usually bound to sugars forming glycosides. Glycosidic bonds occur on 3rd or 7th position with mono-, di- and oligosaccharides, often rhamnose, glucose, glucorhamnose or galactose (Manach et al., 2004). Free flavonoids, which are not bound to sugars are called aglyca (Lesser and Wolffram, 2006). Methylated derivates do also exist (Lesser and Wolffram, 2006).

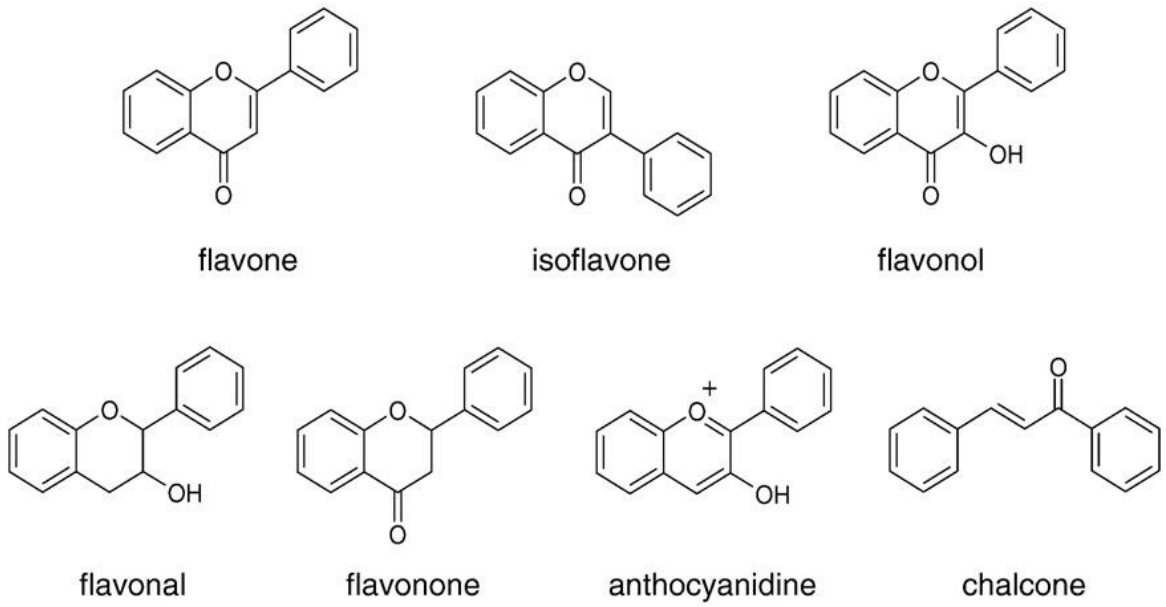

Figure 1. Main subclasses of flavonoids (from: Boots et al., 2008)

Quercetin (**Figure 2)**, belongs to the flavonoid subclass of flavonols and can be found in onions, kale, tomatoes, apples, tea and grapes. Flavonols contain a hydroxyl group in 3rd position of the B-ring heterocycle (Nijveldt et al., 2001; Lakhanpal and Rai, 2007). Consisting of three rings and five hydroxyl groups, quercetin itself is an aglycone without carbohydrate moiety. Main derivates of quercetin are glycosides mostly glycosylated at the hydroxyl group at 3rd position (Materska, 2008). Well known glycosides are rutin, a quercetin-3-rutinoside found in tea and buckwheat, and quericitrin, a quercetin-3-L-rhamnoside (Lakhanpal and Rai, 2007). The aglycone form is lipophilic, whereas glycosylation enhances hydrophilicity (Materska, 2008).

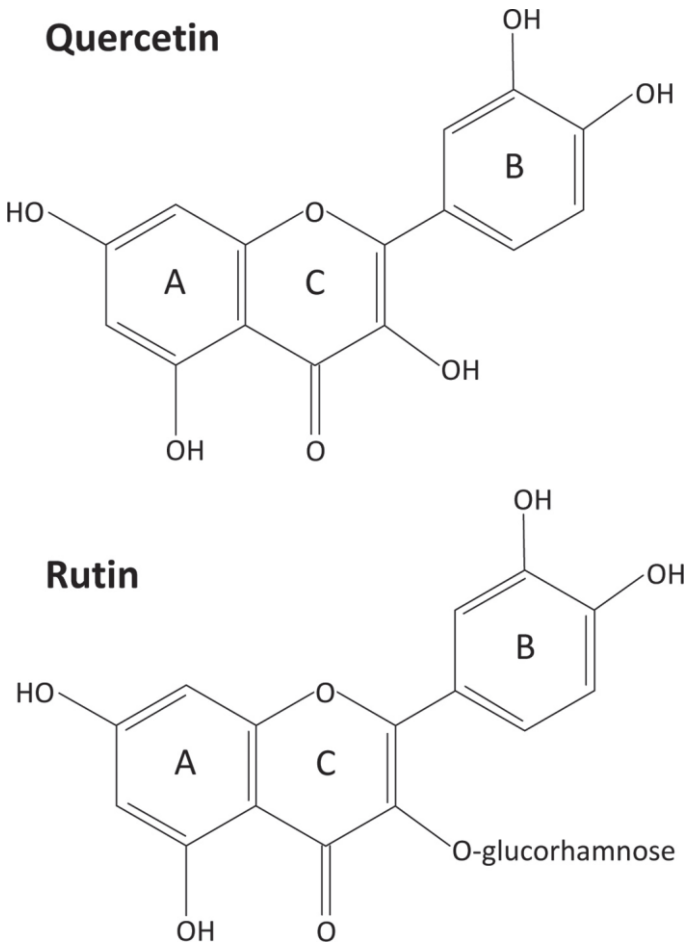

Figure 2. Molecular structure of quercetin and rutin (from: Berger et al., 2015)

1.1.2 Anti-oxidative and anti-inflammatory properties

One of the best described properties of flavonoids, and particularly of quercetin, is the antioxidant activity (Nijveldt et al., 2001). In addition, various health-promoting, especially anti-inflammatory and hepatoprotective effects are postulated for flavonoids (Rathee et al., 2009; Aydin, 2011).

Free radicals are reactive molecules because they possess one or more unpaired electrons (Boots et al., 2008). They are continuously produced in physiological oxygen metabolism or induced by exogenous noxious substances (Nijveldt et al., 2001). Important radicals are reactive oxygen species (ROS), e.g. the superoxide ($O_2^{\cdot-}$) and hydroxyl radical ($^{\cdot}OH$), and reactive nitrogen species (RNS), e.g. nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^-$; Boots et al., 2008). These radicals are physiologically involved in smooth muscle relaxation,

metabolism of xenobiotics and in respiratory burst against microorganisms (Boots et al., 2008). They also induce and attract inflammatory mediators and cytokines by activation of transcription factors, leading to a general inflammatory response (Nijveldt et al., 2001). Nevertheless, radicals react with all molecules via oxidation by subtraction of a hydrogen atom or an electron from the attacked molecule. This can lead to an uncontrolled radical chain reaction (Halliwell and Chirico, 1993; Boots et al., 2008). These reactions are a threat to cells and tissues, possibly leading to DNA lesions, loss of function of enzymes, increased cell permeability, disturbed signaling, mutagenesis and carcinogenesis, and necrotic cell death, referred to as oxidative damage (Nijveldt et al., 2001; Heim et al., 2002; Boots et al., 2008).

For protection, the body possesses antioxidative mechanisms. Antioxidants are defined as "a substances that, when present in low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate" (Halliwell, 1991), reacting directly with the radical (Boots et al., 2008). Antioxidants are enzymes like superoxide dismutase, catalase and glutathione peroxidase, and nonenzymatic substances (Nijveldt et al., 2001). In lipoproteins and membranes, hydrophobic nonenzymatic antioxidants like tocopherol and carotenoide A are present, whereas in mitochondrial and aqueous compartments, hydrophilic substances like glutathione and ascorbic acid dominate (Nijveldt et al., 2001; Boots et al., 2008; Sordillo and Aitken, 2009). Oxidative stress is "an imbalance between production of and protection against reactive species" (Boots et al., 2008), leading to oxidative damage by "overproduction of free radicals or by an impairment of the endogenous antioxidative defense system" (Boots et al., 2008). Because oxidative stress is associated with various diseases, there is a growing and recent interest in antioxidants as treatments. For dietary antioxidants like flavonoids, many health benefits are claimed, but definitive proof of efficacy in vivo lacks in many cases.

Flavonoids are effective scavengers of ROS and RNS because they donate hydrogen atoms or electrons to radicals (Heim et al., 2002). For this reason, they are sacrificial agents and get oxidized instead of other molecules (Nijveldt et al., 2001; Heim et al., 2002). The antioxidative activity of flavonoids is based on the number and form of the hydroxyl groups, and the B-ring hydroxyl groups are the most important determinants of scavenging properties (Heim et al., 2002). The antioxidative efficiency decreases with the presence of sugar moieties because they block hydroxyl groups (Bravo, 1998; Materska et al., 2008). Consequentially, quercetin aglycone is one of the most potent natural antioxidants because of

its structure (Bravo, 1998; Boots et al., 2008). Indeed, quercetin is not only a radical scavenger, but also a chelator of iron, thus removing a causal factor for the generation of radicals (Bravo, 1998; Materska, 2008). Further, quercetin interacts with enzyme systems that produce free radicals, inhibits lipid peroxidation directly and modulates gene expression via interaction with intracellular signaling pathways (Nijveldt et al., 2001; Lakhanpal and Rai, 2007; Kobori et al., 2015). In addition, quercetin accumulates in mitochondria, a critical source of ROS, so quercetin may be functional for protection of mitochondria function and integrity (Fiorani et al., 2010).

The hepatoprotective potential of flavonoids, especially of quercetin, has been shown in various recent in vivo studies using rodents with experimentally induced hepatic injury (Chen, 2010; De David et al., 2011; Sikder et al., 2014), indicated by lowered plasma levels of injury marker enzymes like aspartate aminotransferase (AST) and alanine amino transferase (Abo-Salem et al., 2011; Aydin, 2011; Cui et al., 2014; Zou et al., 2015). Authors ascribed these effects to the antioxidative properties of quercetin. Furthermore, quercetin influences the expression of hepatic genes related to lipid metabolism (Hoek-van den Hil e al., 2013; Jung et al., 2013; Kim et al., 2015) and prevents liver lipid accumulation under adverse dietary or health conditions, i.e. high fat diets or hepatic steatosis (Marcolin et al., 2013; Hoek-van den Hil et al., 2014; Surapaneni and Jainu, 2014; Arias et al., 2015). A steatotic liver may overproduce ROS because of electron leakage in mitochondrial β-oxidation (Begriche et al., 2006; Hassan et al., 2014) and accumulation of fatty acids that are a prone to lipid peroxidation. The resulting oxidative stress is involved in hepatic lipid dysregulation and primes the liver for further lipid storage (Hassan et al., 2014), and also cytokines produced because of ROS are involved in liver steatosis development (Hassan et al., 2014; Surapaneni and Jainu, 2014). In turn, liver lipid lowering effects of quercetin may be, at least in part, attributed to its antioxidative properties.

Quercetin is also known for its antiinflammatory properties (Heeba et al., 2014; Kawabata et al., 2015) These are based on the inhibition of the enzymes cyclooxygenase, phospholipase A2 and lipoxygenase, thus reducing inflammatory metabolites prostanoids and leukotrienes and inhibiting the arachidonic acid metabolism (Nijveldt et al., 2001; Rathee et al., 2009). Boots et al. (2008) suggested a connection to the ROS scavenging properties of quercetin. Since ROS promote inflammation via activation of transcription factors like NF-Kappa-B,

which induce the production of inflammatory cytokines, scavenging of ROS may also alleviate inflammation (Boots et al., 2008).

1.1.3 Bioavailability and metabolism

1.1.3.1 Monogastric species

Because bioavailability of a substance is fundamental for its biological effects, the bioavailability of quercetin has been intensively studied, especially in rodents and humans. Bioavailability "is determined by absorption, dispositions, metabolism and excretion" (Lesser and Wolffram, 2006) of the substance of interest. In plants and foods, quercetin occurs predominately in glycosylated forms, and the type of glycosylation is important for site of absorption and bioavailability (Nijveldt et al., 2001; Materska, 2008; Russo et al., 2012). As aglycone, quercetin may passively pass through cell membranes in stomach and small intestine by diffusion because of its lipophilic character (Lesser and Wolffram, 2006; Materska, 2008). Glycosides are too polar to be absorbed directly (Lin et al., 2003). Quercetin monoglycosides are absorbed from small intestine after hydrolysis by β-glucosidases or lactose phlorizin hydrolase in the brush-border membrane of enterozytes or by carrier-mediated transport via the sodium-dependent glucose transporter SGLT-1(Lesser and Wolffram, 2006). After uptake of intact monoglycosides, these are hydrolyzed by intracellular β-glucosidases (Boots et al., 2008; Materska, 2008; Russo et al., 2012). More complex glycosides like rutin are not cleaved by endogenous enzymes and are absorbed in the distal small and large intestine, where microbial α-rhamnosidases and β-glucosidases release the aglycone from the sugar moiety (Kim et al., 1998; Lin et al., 2003; Materska et al., 2008). After uptake, quercetin is intensively conjugated during the first pass in intestinal mucosa and liver by phase II metabolism, including O-methylation, glucuronidation and sulfation (Nijveldt et al., 2001; Boots et al., 2008; Lesser and Wolffram, 2006). In plasma, quercetin and its metabolites like isorhamnetin and tamarixetin are bound to albumin (Manach et al., 2004; Lakhanpal and Rai, 2007). A major part, mostly large and conjugated metabolites, is excreted into bile during first pass in liver, only a minor part via urine (Manach et al., 2004). Excreted flavonoids may be reabsorbed in small intestine or hydrolyzed by microbes in the large intestine and subsequently reabsorbed, thus enterohepatic cycling is possible (Manach et al, 2004; Lesser and Wolffram, 2006). In general, quercetin has a relatively high bioavailability compared to other phytochemicals (Russo et al., 2012), but absorption,

metabolism, tissue distribution and excretion depended on species (Lin et al., 2003; De Boer et al., 2005). In pigs, quercetin and its metabolites tamarixetin and isorhamnetin are mainly present as conjugated derivates in plasma and nearly the complete metabolism occurs in intestinal mucosa (Ader et al., 2000; Cermak et al., 2003; Wiczkowski et al., 2014). In dogs, intensive enterohepatic circulation has been shown and the bioavailability of rutin differs from other species, possible due to different composition of large intestine bacteria (Reinboth et al., 2010). For horses, a large part of quercetin was found unconjugated in plasma and one derivate was kaempferol, which is not found in other species (Wein and Wolffram, 2013).

1.1.3.2 Polygastric species

For dairy cows, there are only a few studies investigating the bioavailability of quercetin. Interestingly, the flavonoid concentration in plasma is considerably higher after intraruminal administration of rutin, the glucorhamnoside of quercetin, compared to quercetin (Berger et al., 2012), which is in contrast to monogastric species, where complex glycosides have a lower bioavailability than the aglycone. The aglycone is believed to be rapidly degraded by rumen microbes, whereas rutin seems to be partly protected from microbial degradation (Berger et al., 2012). If the flavonoids are given directly into the small intestine, the aglycone is absorbed to an extent comparable to pigs, but rutin is not absorbed (Gohlke et al., 2013a). Gohlke et al. (2013a) assumed a low hydrolysis of rutin in the large intestine due to a short retention time combined with a rapid degradation of the released quercetin aglycone. In plasma, the main metabolite is conjugated quercetin with low levels of isorhamnetin and tamarixetin. Metabolism and elimination are much more rapid in cows compared to monogastric animals (Berger et al., 2012; Gohlke et al., 2013a).

1.2 The peripartal period in dairy cattle

The peripartal period comprises the transition from late gestation to early lactation, usually defined as “the last 3 weeks before parturition to 3 weeks after parturition” (Drackley, 1999) and determines the health, productivity and profitability of a high yielding dairy cow. In the last weeks of pregnancy, nutrient demands of the calf and placenta are at maximum (Drackley et al., 2005), whereas the feed intake decreases up to 30 % (Drackley et al., 2005). The initiation of milk synthesis and the increasing milk production create a sudden increase in energy and nutrient demands (Drackley et al., 2005), that far exceed the demands prepartum (Goff and Horst, 1997) and cannot be met by feed intake (Drackley et al., 2005; Esposito et

al., 2014). Under control of catecholamines and growth hormone (Vernon et al., 2005; Van der Drift et al., 2012), amino acids from muscles and long chain fatty acids from adipose tissue are mobilized to fuel hepatic gluconeogenesis (Van der Drift et al., 2012). Still, a negative energy balance is unavoidable, especially in the first weeks post partum (Drackley et al., 2005; Gross et al., 2013) and lasting for ten to twelve weeks (Butler, 2003). The major energy source in this period are mobilized long chain fatty acids (Drackley et al., 2005), circulating as non-esterified fatty acids (NEFA) and taken up by the liver, that plays a key role in coordinating the modifications to adapt to the negative energy balance (Drackley et al., 2005; Wathes et al., 2007). NEFA are either oxidized completely to CO_2 or partially to ketone bodies or reconverted to triglycerides (Drackley et al., 2005).

1.2.1 Health status in peripartal period

Most metabolic diseases of dairy cows occur in the first two weeks of lactation (Goff and Horst, 1997) and every second to third cow (Drackley et al., 2005) experiences health problems in this period, especially milk fever, ketosis, retained placenta and displaced abomasums (Goff and Horst, 1997; Drackley et al., 2005). Regarding the high priority to maintain milk secretion (Bauman and Currie, 1980), the selection for high milk production led to a dairy cow with high susceptibility to digestive and metabolic disorders (Drackley et al., 2005). Furthermore, immunosuppression occurs in the transition period (Ingvartsen and Moyes, 2015) and a large proportion of cows are affected by inflammation (Bertoni et al., 2008; Esposito et al., 2014), leading to high incidence of infectious diseases like mastitis and metritis (Drackley et al., 2005). Many authors observed a significant relationship between negative energy balance and appearing health problems (Collard et al., 2000; Butler, 2003; Ingvartsen and Moyes; 2015). Since glucose is the preferred nutrient of immune cells (Ingvartsen and Moyes, 2015), the low plasma glucose level may, at least in part, explain the impaired immune function (Ingvartsen and Moyes, 2015). In turn, inflammation negatively affects liver function and exacerbates the negative energy balance (Bertoni et al., 2008; Esposito et al., 2014). Other contributing factors to peripartal health problems are ketosis, fatty liver and oxidative stress (Drackley et al., 2005; Ma et al., 2015).

1.2.2 Metabolic diseases

The oxidation of circulating NEFA, mostly in the liver, generates acetyl-CoA to be further metabolized in the tricarboxylic acid (TCA) cycle and subsequently to reform ATP (Goff and Horst, 1997). However, in times of negative energy balance, gluconeogenesis is usually favored which creates a lack of oxalacetate to maintain the TCA cycle. Thus, acetyl-CoA that cannot be used in TCA cycle is converted to the ketone bodies acetoacetic acid, acetone and beta-hydroxybutyrate (BHBA) in mitochondria of hepatocytes to maintain fatty acid oxidation (Goff and Horst, 1997; Esposito et al., 2014). Ketosis is a typical peripartal disease characterized by hypoglycemia and hyperketonemia (Esposito et al., 2014). Plasma BHBA values over 1.2 mmol/L are defined as subclinical ketosis, values over 3 mmol/L as clinical ketosis (McArt et al., 2015). The importance of ketosis in dairy farming is reflected, on the one hand, by the high incidence of the disease. For subclinical ketosis, between 20 % (Suther et al., 2013) and 50 % (McArt et al., 2012; Esposito et al., 2014) of peripartal dairy cows are affected, whereas clinical ketosis concerns up to 20 % of dairy cows (McArt et al., 2015). On the other hand, ketosis is associated with immunosuppression (Esposito et al., 2014), impaired liver function and fatty liver disease (Li et al., 2012; Djokovic et al., 2013; Simonov and Vlizlo, 2015), poor performance (Walsh et al., 2007) and milk production, and subsequently with high medical costs (McArt et al., 2015).

Fatty liver is a further major disease (Bobe et al., 2004) in periparturient dairy cows and a symptom of a dysregulated metabolism (Grummer, 1993; Bobe et al., 2004). It is associated with reduced feed intake, severe negative energy balance, and high NEFA and BHBA values in plasma (Duffield et al., 2009; Weber et al., 2013). If NEFA mobilization becomes excessive, the lipid uptake by the liver can exceed the oxidation capacity and triglycerides are formed (Bobe et al., 2004; Vernon, 2005). Because ruminants have a low capacity for the synthesis and secretion of very low density lipoprotein to export triglycerides from liver (Drackley et al., 2005), the triglyceride concentration increases from one day after calving and peaks between the first and second week after calving (Vernon, 2005; Gross et al., 2013). Fatty liver is categorized from mild, physiologically occurring in healthy peripartal cows (Djokovic et al., 2013), to severe fatty liver, that can lead to hepatic encephalopathy and death (Bobe et al., 2004). Affected livers are enlarged and swollen, with fatty cysts in hepatocytes, cell organelles damage, necrosis and cellular leakage (Bobe et al, 2004). In consequence, fatty infiltration alters metabolism in hepatocytes with decreased gluconeogenesis (Bobe et al.,

2004), further deteriorating the energy provision, decreased synthesis of immune system compounds, and impaired detoxification ability (Grummer 1993; Drackley et al., 2005; Merrel and Cherrington, 2011). Furthermore, fatty liver disease is accompanied by inflammation and increased oxidative stress (Drackley et al., 2005). Up to 50 % of dairy cows experience fatty liver in the first 4 weeks after calving, followed by decreased well-being, productivity and fertility and increased length and severity of infectious diseases (Bobe et al., 2004).

1.2.3 Oxidative stress in dairy cows

Ma et al. (2015) considered oxidative stress as a major problem in dairy farming. High milk yield seems to be associated with oxidative stress (Löhrke et al., 2005; Vernunft et al., 2014). The metabolic demands of parturition and milk production increase the oxidative metabolism and thereby the production of ROS (Sharma et al., 2011; Abuelo et al., 2013; Esposito et al., 2014) in a period of an impaired antioxidative defense (Turk et al., 2013; Konvicna et al., 2015). It is noteworthy, that cows with high lipid mobilization are more sensitive to oxidative stress (Bernabucci et al., 2005). Therefore, oxidative stress may contribute to a higher susceptibility to peripartal disorders (Sordillo and Aitken, 2009; Celi, 2011), and, given that oxidative stress is a relatively young field of research in veterinary medicine (Celi, 2011), antioxidant requirements may be higher than recognized and supplementation with antioxidative nutrients can potentially be a conceivable component of a treatment for diseases related to oxidative stress (Miller et al., 1993; Lykkesfeldt and Svendsen, 2007).

1.2.4 Indications of flavonoids in dairy cows

Regarding an increasing public concern about the use of pharmaceuticals in animal farming (Rochfort et al., 2008), increasing research on plants and their non-nutrient ingredients as alternative treatment and performance enhancing factors has occurred, but little information is available on ruminant health benefits (Greathead, 2003; Rochfort et al., 2008), especially about the use of flavonoids (Gohlke et al., 2013a).

It has been shown that supplementation of antioxidants, e.g. vitamin E and selenium, has significant positive effects on health and fertility of dairy cows (Allison and Laven, 2000; Spears and Weiss, 2008; Jovanovic et al., 2015). Quercetin, owning not only antioxidative,

but also hepatoprotective, lipid lowering and antiinflammatory properties, is an interesting approach for the use in dairy cows.

First studies using flavonoids in dairy cows have shown an influence on glucose metabolism as well as improved rumen fermentation and production performance (Guo et al., 2010; Balcells et al., 2012; Gohlke et al., 2013b; Cui et al., 2015). Furthermore, flavonoids may reduce ruminal methane production (Broudiscou et al., 2000; Santra et al., 2012), a greenhouse gas contributing to climate warming (Etim et al., 2013) by influencing rumen microbes population (Oskoueian et al., 2013; Seradj et al., 2014). In vivo, quercetin and rutin had no negative effects on rumen fermentation processes (Berger et al., 2015; Cui et al., 2015).

1.3 Aims of the studies

The flavonoids quercetin and rutin possess various constitutional effects and have been intensively studied in rodents and humans, but still, knowledge on flavonoid effects in ruminants is scarce. Consequentially, effects of the flavonoids rutin and quercetin in dairy cows were examined. In the first study, the hypothesis that quercetin has beneficial effects on energy metabolism and performance, antioxidative response as well as liver health in periparturient dairy cows was tested. The objectives were to investigate effects of quercetin on plasma metabolites, indirect calorimetry measurements, liver lipid content, plasma antioxidative status, and hepatic messenger ribonucleic acid (mRNA) abundance of genes related to lipid metabolism and oxidative defense. Because quercetin as aglycone is not bioavailable if given orally, quercetin was administered intraduodenally (Gohlke et al., 2013a).

To investigate whether flavonoids affect health, energy metabolism and methane emission in dairy cows in established lactation, a further feeding trial was conducted with rutin, a naturally rumen-protected source of quercetin (Berger et al., 2012), including indirect calorimetry and gas exchange measurements (Derno et al., 2009) as well as examination of plasma metabolites. Given that buckwheat has a high rutin content (Fabjan et al., 2003) and is suitable as feed for ruminants (Amelchanka et al., 2010; Kälber et al., 2012), it was also tested whether supplementation of tartary buckwheat (*Fagopyrum tartaricum*) seeds have properties comparable to pure rutin.

2 Publication I

Effects of a 6 wk intraduodenal supplementation with quercetin on energy metabolism and indicators of liver damage in periparturient dairy cows

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3 Publication II

Effects of rutin and buckwheat seeds on energy metabolism and methane production in dairy cows

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4 Publication III

Effects of a six-week intraduodenal supplementation with quercetin on liver lipid metabolism and oxidative stress in peripartal dairy cows[1]

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ABSTRACT

The periparturient period is associated with enormous metabolic changes for dairy cows. Energy needs for incipient lactation are too high to be balanced by feed intake leading to negative energy balance and body fat mobilization. It has been estimated that this leads to the development of fatty liver in about 50% of cows which are at high risk for disease. Furthermore, the antioxidative status of these cows may be impaired. Quercetin (Q) is a plant flavonoid having hepatoprotective and antioxidative potential and the ability to reduce liver lipid accumulation in monogastric animals. Little information is available in regard to these effects in ruminants. Thus, we evaluated effects of Q on liver lipid metabolism and antioxidative status in periparturient dairy cows. To prevent microbial Q degradation in the rumen, Q was administered via a duodenal fistula to improve systemic availability. Five cows of the Q-treated group received daily 100 mg of Q dihydrate per kilogram of body weight in a 0.9% sodium chloride solution from d -20 until d 20 relative to calving whereas 5 control cows (CTR) received sodium chloride solution only. Blood samples were taken weekly and liver biopsies were performed in wk -4 and -2 and 3 wk relative to calving. Cows postpartum treated with Q showed a tendency for lower liver fat content than CTR cows. Liver glycogen, glutathione concentrations, and relative mRNA abundance of genes related to hepatic lipid metabolism and antioxidative status as well as parameters of antioxidative status in plasma were not affected by Q supplementation. In conclusion, liver fat content in dairy cows tended to be reduced by Q supplementation but potential underlying mechanisms remain unclear because parameters related to hepatic lipid metabolism and antioxidative defense were not altered by Q supplementation. These findings as well as longer-term implications on animal performance need to be clarified in a larger number of cows.

Key Words:
Transition dairy cows, metabolism, quercetin, antioxidative status, liver fat

INTRODUCTION

The periparturient period is critical for high-yielding dairy cows (Drackley, 1999). Actual feed intake is insufficient, leading to negative energy balance and lipolysis (Bell, 1995; Coffey et al., 2002). If subsequently released NEFA exceed the capacity for complete hepatic oxidation, ketone bodies are formed, and NEFA are re-esterified to triacylglycerols (TG; Bobe et al., 2004). These may accumulate in hepatocytes leading to fatty liver, a typical periparturient metabolic disorder (Goff and Horst, 1997; Gross et al., 2013) often associated with hepatocellular damage, impaired hepatocyte function and health problems (Wensing et al., 1997; Bobe et al., 2004; Esposito et al., 2014). Additionally, in early lactating cows both high milk output and high energy intake increase oxidative metabolism and production of reactive oxygen species (Löhrke et al., 2005; Sordillo et al., 2007; Celi, 2011) which may contribute to impaired immune response (Spears and Weiss, 2008), retained placenta and mastitis (Miller et al., 1993). Fatty hepatocytes are especially vulnerable to oxidative damage (Yang et al., 2000). Thus, an antioxidant supplement may provide alleviation of peripartum diseases of dairy cows (Miller et al., 1993; Lykkesfeldt and Svendsen, 2007).

Flavonoids are phenolic substances found in higher plants (Nijveldt et al., 2001). Quercetin (**Q**) is one of the most potent flavonoids (Ader et al., 2000) and has been shown, mainly in rodents, to reduce liver lipid accumulation and incidence of fatty liver (Aguirre et al., 2011; Beltrán-Debón et al., 2011; Joven et al., 2012). In addition, Q has proven antioxidant (Nijveldt et al., 2001; Cui et al., 2014) and anti-inflammatory (Rathee et al., 2009) properties in several species. Recently, we have reported that Q decreases markers of liver damage in peripartal dairy cows (Stoldt et al., 2015). Contents of Q and its derivatives in forages are relatively low, somewhat higher in buckwheat (Fabjan et al., 2003; Besle et al., 2010) but still low as compared to commercial supplements containing mixtures of Q and other flavonoids (Balcells et al., 2012). The efficacy of these products in cows is not known. However, in view of the increasing public concern with the use of pharmaceuticals and efforts to restrict antibiotics, flavonoids may be an interesting approach as alternative to alleviate metabolic stress in animal production (Rochfort et al., 2008). Still, knowledge on flavonoid effects in ruminants is scarce, so we tested the hypothesis that Q has beneficial effects on liver lipid metabolism and antioxidative response in periparturient dairy cows. Our objectives were to investigate effects of Q on liver lipid content, plasma antioxidative status, and hepatic mRNA abundance of genes related to lipid metabolism and oxidative defense. To analyze principal

effects of Q on dairy cow liver metabolism, Q was administered intraduodenally (Gohlke et al., 2013a).

MATERIALS AND METHODS

Animals and Diet

The procedures performed in this study were in accordance to the guidelines of the German animal protection regulations and were approved by the relevant authorities (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischereiwesen Mecklenburg-Vorpommern, Germany; LALLF M-V/TSD/7221.3-2.1-019/12).

A group of 10 duodenally fistulated German Holstein cows were monitored over a period of 7 wk, starting 4 wk prior to the estimated date of their third calving. Housing, feeding, milking procedures and disease-related treatments, measurements of BW, DMI and milk performance data were previously reported (Stoldt et al., 2015). Since the fistula itself and the housing of fistulated animals is considered as wearing, we decided to keep the number of animals as small as possible. The Q-treated cows (n = 5) were dosed daily with 100 mg Q dihydrate (Carl Roth GmbH, Karlsruhe, Germany) per kg of BW via the duodenal fistula starting 3 wk prior to 3 wk after estimated calving (d -20 ± 2.6 to d +20 ± 1.5 relative to true calving date). The dose was calculated based on the results of our previous study (Gohlke et al., 2013a) to generate an elevated plasma flavonoid level throughout the day. The amount was partitioned into three equal portions and dissolved in 0.9% NaCl (Serumwerk Bernburg AG, Bernburg, Germany), which were administered at 0630, 1200 and 1800 h to 5 cows. Another 5 cows received a corresponding volume of NaCl solution only and served as control (CTR).

Blood Sampling and Analysis

Blood samples for isolation of plasma and leukocytes were drawn from the left jugular vein by venipuncture using the vacutainer system (Becton, Dickinson and Company, Plymouth, UK). Every experimental wk, on Mondays at 0800 h, blood samples for leukocyte glutathione (GSH) measurement were taken (d -26 ± 0.9, -19 ± 0.7, -12 ± 0.8, -5 ± 0.8, 3 ± 0.8, 8 ± 1.1 and 17 ± 0.7 relative to true calving date). Blood samples for ferric ion reducing antioxidant power (Frap), Trolox equivalent antioxidative capacity (TEAC), thiobarbituric acid reactive substances (TBARS) and F2-isoprostanes were withdrawn on Wednesdays at 1200 h (d -26 ± 0.4, -18 ± 0.5, -11 ± 0.6, -4 ± 0.6, 4 ± 0.4, 11 ± 0.6 and 18 ± 0.6 relative to true calving date). One wk before the start of the Q supplementation (d -23 ± 2.5), one wk before estimated

calving (d -2 ± 0.3) and every wk postpartum (pp) (d 8 ± 0.3, 14 ± 0.6 and 21 ± 0.5 relative to true calving date), blood samples for haptoglobin (Hp) measurement were taken also on Wednesday at 1200 h. Lithium-heparin vacutainers (10 mL) were used to prepare plasma for the analysis of anti-/oxidative parameters and GSH, and potassium-EDTA vacutainers (10 mL) were used for analysis of Hp.

Blood samples (10 mL) for parameters of oxidative status and Hp concentrations were immediately centrifuged at 1,500 × *g* and 4°C for 20 min and plasma aliquots were stored at -80°C. Leukocytes for GSH measurements were immediately isolated from blood samples (30 mL) by centrifugation at 1,200 × *g* and 4°C for 20 min using Biocoll (Biochrom GmbH, Berlin, Germany) as separation medium, than thrice resuspended in phosphate-buffered saline (PAA Lab GmbH, Pasching, Austria), centrifuged at 300 × *g* and 4°C for 10 min and stored at -80°C. Plasma NEFA and BHBA were analyzed using an automatic analyzer and commercial kits as described by Stoldt et al. (2015).

Plasma concentrations of Frap, TEAC, TBARS and F2-isoprostanes were analyzed as described (Re et al., 1999; Luehring et al., 2011). Intra- and inter-assay CV were 4.3 % and 1.9 % for Frap, 0.9 % and 3.1 % for TEAC, 11.1 % and 2.5 % for TBARS, and 35.7 % and 33.5 % for F2-isoprostanes, respectively.

To measure leukocyte GSH levels the leukocyte pellet was re-suspended in 150 µL ice-cold PBS, mixed and centrifuged at 2,000 × *g* (4 °C) for 5 min. Afterwards, 150 µL cold 5% meta-phosphoric acid were added to the supernatant, mixed and centrifuged at 13,000 × *g* (4 °C) for 10 min. Supernatants were transferred to HPLC vials and injected immediately or stored at -80 °C until analysis. HPLC analysis was done according to Bayram et al. (2014) .Intra- and inter-assay CV were 3.7 % and 5.3%, respectively.

Plasma Hp levels were analyzed using an ELISA as described by Hiss et al. (2004). The serum used as standard has been calibrated against a standard obtained from a European Union Concerted Action on the standardization of animal acute phase protein (QLK5-CT-1999-0153; Skinner, 2001). Intra- and inter-assay CVs were 3.9 % and 12.2 %, respectively. Plasma flavonoid concentrations were analyzed as previously described (Gohlke et al., 2013a). Plasma total flavonoid concentrations were reported previously (Stoldt et al., 2015). All analyses were performed in duplicate and merged as means.

Liver Sampling and Analysis

Before, in the first and in the last wk of Q supplementation, corresponding to 4 and 2 wk ante partum (ap) and 3 wk pp (d -26 ± 1.9, -12 ± 2.0 and 21 ± 0.7 relative to true calving date), liver biopsies (1 – 2 g tissue on average) were aseptically taken at the 11th intercostal space after local anesthesia (procaine hydrochloride 20 mg/mL and epinephrine 0.025 mg/mL, Selectavet, Weyarn/Holzolling, Germany) using a custom-made biopsy needle with a 6 mm outside diameter. Biopsied liver tissue samples were immediately frozen in liquid nitrogen and stored at -80°C.

Using a commercial photometric test kit (kit no. 10207748035, Boehringer Mannheim, Mannheim, Germany), liver glycogen content was determined in 25 mg of fresh liver tissue based on amyloglucosidase catalyzed glucose release. Carbon and nitrogen content of liver samples, at least 5 mg DM, were determined at the Institute of Chemistry, University of Rostock, Germany. Based on this analysis, total liver fat content was calculated as described (Kuhla et al., 2004) using the following equation:

Liver fat (% of DM) = 1.3038 × C (% of DM) – 4.237 × N (% of DM) – 0.58 × glycogen (% of DM) – 0.5215 × glucose (% of DM).

To measure GSH in liver tissue, preparation of liver samples was done according to Rebrin and Sohal (2004). Liver samples (50 mg) were homogenized in 1 mL isolation buffer (0.25 M sucrose, 3 mM EDTA, 10 mM Tris buffer, pH 7.4) at 25 Hz using TissueLyser II (Qiagen, Hilden, Germany) for 2.5 min. Immediately after homogenization, 200 µL aliquots were mixed with 200 µL of ice-cold 10% meta-phosphoric acid. Samples were incubated on ice for 30 min. After centrifugation for 10 min at 14,000 × *g* (4°C), the supernatant was transferred to HPLC vials and injected immediately or stored at -80 °C until analysis. HPLC analysis was done in duplicate according to Bayram et al. (2014).

Quantitative Real-time PCR in Liver samples

For determination of transcript abundance of selected genes related to lipid metabolism and antioxidative status (fatty acid synthase (*FASN*), glycerol-3-phosphate acyltransferase 1 (*GPAM*), acetyl-CoA carboxylase (*ACACA*), peroxisome proliferator-activated receptor *alpha* (*PPARA*), peroxisome proliferator-activated receptor *gamma* (*PPARG*), aryl hydrocarbon receptor (*AHR*), paraoxonase (*PON1*), glutathione peroxidase (*GPX1*), superoxide dismutase (*SOD1*), catalase (*CAT*), diglyceride acyltransferase (*DGAT1*), microsomal triglyceride

transfer protein (*MTTP*) and apolipoprotein B100 (*APOB*)), RNA extraction, cDNA synthesis and relative quantification of mRNA was performed as described by Saremi et al. (2012) with some adaptations. Primer sequences and PCR conditions are shown in Table 1.

Liver samples were pulverized in liquid nitrogen and homogenized using FastPrep 120 (Thermo Fischer Scientific, Karlsruhe, Germany) in TriFast (PeqLab Biotechnology, Erlangen, Germany). Total RNA was immediately extracted and stored at -80°C. DNase digestion was done in solution (Qiagen, Hilden, Germany) and RNA was purified using spin column (RNeasy mini Kit, Quiagen, Hilden, Germany). Purity and concentration of RNA were controlled using NanoPhotometer (Implen, München, Germany) by absorbance reading at 260 and 280 nm. Corresponding RNA integrity numbers were assessed by microcapillary electrophoresis using the Bioanalyzer 2100 (Agilent, Waldbronn, Germany) and RNA 6000 Nano Kit system (Agilent, Waldbronn, Germany). The mean RNA integrity factor was 6.1.

Independent reverse transcription of 2 × 750 ng total RNA was performed with random hexamer primers (Metabion, Planegg, Germany) and RevertAid reverse transcriptase (Thermo Fischer Scientific, Karlsruhe, Germany) with dNTP Mix (Thermo Fischer Scientific, Karlsruhe, Germany) in a total volume of 40 µL using the Thermocycler (Biometra, Göttingen, Germany) and afterwards combined. Each run included a cDNA negative template control and no reverse transcriptase control. For inter-run calibrator preparation, samples were pooled before cDNA synthesis and transcribed. Inter-run calibrator was diluted 1:4 and 1:8 in water.

Before real-time PCR, cDNA was diluted 1:4 in water. Each real-time PCR run consisted of an inter-run calibrator, negative template control for real-time PCR, cDNA negative template control and no reverse transcriptase control of cDNA and all samples as duplicates. Two µL cDNA as template and Luminaris Color HiGreen qPCR Master Mix (Thermo Fischer Scientific Karlsruhe, Germany) in a total volume of 10 µl were run in a Light Cycler 2.0 (Roche, Mannheim, Germany).

After each PCR cycle, a melting curve analysis was performed. The PCR products were verified by sequencing (ABI 3130 Genetic Analyzer Life Technologies GmbH, Darmstadt, Germany). Amplification efficiencies (Table 1) were established using LinRegPCR version 2013.0 (Ruijter, Heart Failure Research Center, Amsterdam, Netherlands; Ruijter et al., 2013).

Quantification cycle values were imported in qbase+ version 2.6.1 (Biogazelle, Ghent, Belgium) and all subsequent calculations and data quality controls were done with this software (Hellemans et al., 2007). For normalization of the efficiency corrected data, emerin (*EMD*), hippocalcin-like 1 (*HPCAL1*) and low density lipoprotein receptor-related protein 10 (*LRP10*) were selected and used as reference genes (Saremi et al., 2012). Data are presented as ratios of genes of interest and geometric mean of the selected reference genes' abundances.

Statistical Analysis

Data were analyzed with SAS/STAT 9.3 (SAS Institute Inc. 2011, Cary, NC) using repeated-measurement ANOVA (PROC MIXED). Factors were Q (yes or no) and wk relative to calving (wk -3, -2, -1 ap and wk 1, 2, 3 pp for blood parameters; wk -1 ap and wk 1, 2, 3 pp for Hp; wk -2 ap and 3 pp for liver parameters) as well as Q × wk interaction. The covariance structure of the repeated factor wk was modelled by autoregressive first-order (AR (1); smallest AIC). Effects were considered significant at $P \leq 0.05$ and LSM-differences were tested using the Tukey-Kramer test. Significance levels of $P \leq 0.10$ were considered a statistical trend. Data are presented as LSM ± SE.

RESULTS

Health Status

Plasma NEFA and BHBA in cows were not affected by Q but by time (Stoldt et al., 2015; Figure 1). As mentioned previously (Stoldt et al., 2015), one of the control cows became clinical ketotic after calving, was appropriately treated with propylene glycol, Amynin (Merial GmbH, Halbergmoos, Germany), Catosal (Bayer Vital GmbH, Leverkusen, Germany), and was provided with additional hay. Furthermore, 3 cows of each group were considered subclinical ketotic. These cows were not treated because of the absence of clinical signs (i.e. feed intake and milk yield decline). One cow of the Q treated group and 2 of the CTR had retained placenta and were treated with antibiotics in case of fever.

Plasma and Leukocyte Oxidative Status

Plasma oxidative and antioxidative parameters (Figure 2) as well as leukocyte GSH were not affected by Q treatment. Plasma Frap increased ($P = 0.034$) from ap (91.1 ± 5.2 μmol/L) to pp (105.2 ± 5.4 μmol/L), whereas TBARS decreased ($P = 0.043$; 0.4 ± 0.1 μmol/L to 0.3 ± 0.1

µmol/L). Plasma TEAC tended to decrease (P = 0.081) from ap (2.2 ± 0.1 mmol/L) to pp (2.1 ± 0.1 mmol/L). Plasma Hp (Figure 3) was affected by time (P = 0.006) and increased from ap (229.5 ± 402.1 µg/mL) to the first wk pp (2,182.6 ± 402.1 µg/mL), then decreased again until wk 3 pp (386.5 ± 427.5 µg/mL).

Fat, Glycogen and Glutathione in Liver

Liver fat content (Table 2) was affected by Q treatment and time. The time × treatment interaction tended to be significant (P = 0.087). Liver fat content (per DM) ranged pp from 9.5 to 43.8 % in the Q group, and from 38.8 to 62.1 % in CTR. Liver glycogen content (Table 2) was not affected by Q but by time (P = 0.002). After calving, liver glycogen ranged from 0.5 to 1.4 % in the Q group and from 0.4 to 3.7 % in CTR. Liver GSH (Table 2) was neither affected by Q treatment nor by time.

Transcript Abundances in Liver

Abundances of all transcripts measured (Table 3) were not affected by Q treatment. Relative mRNA expression of *MTTP* increased (P = 0.004) from ap (0.985 ± 0.1) to pp (1.197 ± 0.2). Relative mRNA expression of *SOD1* increased (P = 0.036) from ap (0.956 ± 0.1) to pp (1.166 ± 0.1).

DISCUSSION

Effects of Q on Lipid Metabolism

The results of the present study suggest that Q supplementation reduces liver fat content in metabolically burdened dairy cows after calving. We have previously reported that plasma concentrations of Q and its metabolites were significantly increased in these cows whereas plasma NEFA, glucose and BHBA concentrations as well as DMI and milk yield were not affected by Q (Stoldt et al., 2015). As indicated by plasma BHBA values, some of our cows suffered from clinical (1 of 10) or subclinical ketosis (6 of 10) (McArt et al., 2012). Since it is known that Q exerts its effects especially under adverse health conditions (Shao et al., 2013, Xu et al., 2014), we deliberately choose the periparturient period to examine Q effects in cows.

In rodents, Q administration resulted in hepatic Q accumulation and regulated hepatic gene expression, also of genes involved in lipid metabolism (Kobayashi et al., 2010; Jung et al.,

2013) and particularly in Ω oxidation (Hoek–van den Hil et al., 2013) via regulation of nuclear receptors or transcription factors. In mice fed a high fat diet (Jung et al., 2013) or with induced steatohepatitis (Marcolin et al., 2013), Q supplementation reduced liver fat content. On d -26 prior to calving, all our cows had a low liver fat content, comparable to levels reported elsewhere (Djokovic et al., 2013; Weber et al., 2013), but on d 21 after calving, Q-treated cows showed a moderately increased liver fat content whereas CTR cows reached considerably higher values. This is in line with our finding, that the liver enzymes glutamate dehydrogenase and aspartate aminotransferase (AST), which are markers of liver damage, where higher after calving in CTR than in Q-treated cows (Stoldt et al., 2015). This is of particular interest given that fatty liver is a relevant health problem in the periparturient period (Bobe et al., 2004) In accordance with former studies, liver glycogen decreased after calving (Veenhuizen et al., 1991; Vazquez-Anon et al., 1994; Duske et al., 2009), known to indicate an increased risk for metabolic disorders (Bobe et al., 2004).

Hepatic mRNA abundances of genes involved in fatty acid oxidation, synthesis and transport, glycerolipid and VLDL synthesis as well as peroxisomal fatty acid β-oxidation were not influenced by Q administration. Others reported changes of hepatic mRNA abundance of related genes throughout the periparturient period (Graber et al., 2010; Loor et al., 2005 and 2006), but in our cows, these transcripts were not influenced by time relative to calving. Indeed, the literature is inconsistent here and there are other studies (Loor et al., 2007; Van Dorland et al., 2012) that did not report changes in gene expression from ap to pp. It is also possible that differences between ap and pp depend on the sample time relative to calving (Schäff et al., 2012), and 21 d after calving was perhaps too late to detect changes in transcript expression. Only for *MTTP*, a protein assisting the assembly of apolipoprotein B-containing lipoproteins, a higher relative expression could be found after calving, but Schlegel et al. (2012) and Bernabucci et al. (2004) considered *MTTP* as not relevant for etiology of fatty liver in ruminants.

Plasma Hp, a major acute phase protein in ruminants (Eckersall, 2000) peaked at wk 1 pp. This is in agreement with other studies (Ametaj et al., 2005; Bertoni et al., 2008; Hiss et al., 2009). In particular, Hp is related to the development of fatty liver (Eckersall, 2000; Katoh, 2002), because Hp is mainly produced in the liver (Ametaj et al., 2005) and fatty infiltration is a stimulus for hepatocytes leading to production of Hp (Katoh, 2002), but the mechanistic link is not fully understood. Although cows of the Q-treated group experienced fatty liver to a somewhat lower extent, plasma haptoglobin in our study did not differ among groups.

Effects of Q on Oxidative Metabolism

Flavonoids act as antioxidants through their capacity to transfer electrons to free radicals, chelate metal catalyst and by activation of antioxidative enzymes (Middleton et al., 2000; Nijveldt et al., 2001; Heim et al., 2002). By reducing the generation of peroxides, Q lowers the activation of redox-sensitive transcription factors (Crespo et al., 2008). Given that oxidative stress plays a central role in liver disease pathology and progression, antioxidants such as Q may be interesting as preventive agents (Crespo et al., 2008). This is particularly relevant for early lactating cows which are sensitive to oxidative stress and depleted antioxidative status (Bernabucci et al., 2005; Sordillo et al., 2007; Celi, 2011). Interestingly, hepatic steatosis in rats is associated with lower antioxidant capacity and higher susceptibility to oxidative damage (Grattagliano et al., 2000).

To obtain a comprehensive picture of possible Q effects on antioxidative status, we investigated antioxidative defense at different levels and measured antioxidant enzymes, main constituent of intracellular defense (Bernabucci et al., 2005), at the mRNA level in liver tissue, and GSH in leukocytes and liver tissue as low-molecular-weight antioxidant (Szczubial, 2010). Additionally, we investigated lipid peroxidation by measurements of TBARS and F2-isoprostanes, as well as plasma Frap and TEAC as a measure of total antioxidant capacity (Janssen, 2001; Aoki et al., 2008; Celi, 2011). Although mRNA abundance of antioxidative enzymes was not influenced by Q treatment, we found increased mRNA concentration of *SOD1* after calving in both groups, an enzyme that converts superoxides to less harmful compounds (Halliwell and Chirico, 1993). Likewise, in a former study in mid-lactation cows, we found no effects of Q on mRNA abundance levels related to antioxidative status albeit at a lower supplementation dose level of Q (Gohlke et al., 2013b). Plasma TBARS decreased from ap to pp. This is surprising given that other studies found an increase of TBARS around or after calving (Bernabucci et al., 2005) and when fatty liver was present (Vendemiale et al., 2001). However, TBARS measurements may be inadequate to assess lipid peroxidation in vivo. In addition to malondialdehyde, an end product of lipid peroxidation, TBARS assays also measure other aldehydes with large variability, so there are also studies that failed to find changes in TBARS (Aoki et al., 2008; Celi, 2011).

A possible reason for the lack of Q effects on hepatic mRNA abundance might be that the dose of Q applied was too low to produce changes or the time plasma flavonoids were at effective levels was too short. Berger et al. (2012) showed that decline in Q plasma concentration is much more rapid in cows compared with monogastric species. On the other

hand, it cannot be excluded that Q induced changes on the protein level (De David et al., 2011; Pan et al., 2014, Schäff et al., 2012), which was not analyzed in this study. Additionally, it is also possible that the time of liver biopsy pp was too late to detect changes, because this might be a period where liver fat is already at its maximum or even already declining (Graugnard et al., 2013).

Given that Q tended to reduce liver fat, it is possible that there are other underlying mechanisms than assessed herein. In addition, if we consider parameters related to oxidative stress, it is not clear if our cows are as stressed as expected (Bernabucci et al., 2005), because markers of lipid peroxidation were not increased and antioxidative capacity showed contradictory values around calving. Nevertheless, our cows were clearly challenged by lipid mobilization, indicated by clinical relevant plasma BHBA values. In this situation, Q seems to have a certain constitutional effect on liver metabolism, shown by the less severe fatty liver in Q-treated cows pp. This is of particular interest because fatty liver is a major metabolic disorder in peripartal cows and there is a need for a suitable treatment (Bobe et al., 2004). Furthermore, the liver fat lowering effect is in line with results of our previous study (Stoldt et al., 2015) were we found that Q lowered AST plasma values, which is a marker of liver health. Further research is needed to clarify the mode of Q action in dairy cows.

CONCLUSION

The aim of this study was to examine Q effects on liver lipid metabolism and antioxidative status in periparturient dairy cows intraduodenally supplemented with Q for a 6-wk-period starting 3 wk before calving. In conclusion, we found no effect of Q on plasma antioxidative status and relative mRNA abundances of hepatic genes related to lipid metabolism and antioxidative defence, but liver fat content tended to be lower in Q-treated cows pp, suggesting a hepatoprotective effect. Further studies in a larger number of cows are needed to substantiate the effects of Q in periparturient dairy cows and clarify underlying mechanisms.

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REFERENCES

Ader, P., A. Wessmann, and S. Wolffram. 2000. Bioavailability and metabolism of the flavonol quercetin in the pig. Free Radic. Biol. Med. 28:1056-1067. http://dx.doi.org/10.1016/S0891-5849(00)00195-7.

Aguirre, L., N. Arias, M. T. Macarulla, A. Gracia, and M. P. Portillo. 2011. Beneficial effects of quercetin on obesity and diabetes. Open Nutraceuticals J. 4:189-198.

Ametaj, B. N., B. J. Bradford, G. Bobe, R. A. Nafikov, Y. Lu, J. W. Young, and D. C. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. Can. J. Anim. Sci. 85:165-175.

Aoki, M., T. Oshita, and M. Sakaguchi. 2008. The comparison of thiobarbituric acid reactive substances (TBARS) concentrations in plasma and serum from dairy cattle. J. Vet. Med. Sci. 70:107-110. http://dx.doi.org/10.1292/jvms.70.107.

Balcells, J., A. Aris, A. Serrano, A. R. Seradj, J. Crespo, and M. Devant. 2012. Effects of an extract of plant flavonoids (Bioflavex) on rumen fermentation and performance in heifers fed high-concentrate diets. J. Anim. Sci. 90:4975-4984. http://dx.doi.org/10.2527/jas.2011-4955.

Bayram, B., G. Rimbach, J. Frank, and T. Esatbeyoglu. 2014. Rapid method for glutathione quantitation using high-performance liquid chromatography with coulometric electrochemical detection. J. Agric. Food Chem. 62:402-408. http://dx.doi.org/10.1021/jf403857h.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Beltrán-Debón, R., A. Rull, F. Rodríguez-Sanabria, I. Iswaldi, M. Herranz-López. G. Aragonès, J. Camps, C. Alonso-Villaverde, J. A. Menéndez, V. Micol, A. Segura-Carretero, and J. Joven. 2011. Continuous administration of polyphenols from aqueous rooibos (*Aspalathus linearis*) extract ameliorates dietary-induced metabolic disturbances in hyperlipidemic mice. Phytomedicine 18:414-424. http://dx.doi.org/10.1016/j.phymed.2010.11.008.

Berger L. M., S. Wein, R. Blank, C. C. Metges, and S. Wolffram. 2012. Bioavailability of the flavonol quercetin in cows after intraruminal application of quercetin aglycone and rutin. J. Dairy Sci. 95: 5047–5055. http://dx.doi.org/10.3168/jds.2012-5439.

Bernabucci, U., B. Ronchi, L. Basirico, D. Pirazzi, F. Rueca, N. Lacetera, and A. Nardone. 2004. Abundance of mRNA of apolipoprotein B_{100}, apolipoprotein E, and microsomal triglyceride transfer protein in liver from periparturient dairy cows. J. Dairy Sci. 87:2881-2888.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy. Sci. 88:2017-2026.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91:3300-3310. http://dx.doi.org/10.3168/jds.2008-0995.

Besle, J. M., D. Viala, B. Martin, P. Pradel, B. Meunier, J. L. Berdagué, D. Fraisse, J. L. Lamaison, and J. B. Coulon. 2010. Ultraviolet-absorbing compounds in milk are related to forage polyphenols. J. Dairy Sci. 93:2846-2856. http://dx.doi.org/10.3168/jds.2009-2939.

Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited review: Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J. Dairy Sci. 87:3105-3124.

Celi, P. 2011. Biomarkers of oxidative stress in ruminant medicine. Immunopharm. Immunot. 33:233-240. http://dx.doi.org/10.3109/08923973.2010.514917.

Coffey, M. P., G. Simm, and S. Bortherstone. 2002. Energy balance profiles for the first three lactations of dairy cows estimated using random regression. J. Dairy Sci. 85:2669-2678. http://dx.doi.org/10.3168/jds.S0022-0302(02)74352-X.

Crespo, I., M. V. Garcia-Medlavilla, M.Almar, P. Gonzalez, M. J. Tunon, S. Sanchez-Campos, and J. Gonzalez-Gallego. 2008. Differential effects of dietary flavonoids on reactive oxygen and nitrogen species generation and changes in antioxidant enzyme

expression induced by proinflammatory cytokines in Chang Liver cells. Food. Chem. Toxicol. 46:1555-1569. http://dx.doi.org/10.1016/j.fct.2007.12.014.

Cui, Y. M., Y. Han, X. B. Yang, Y. F. Sun, and Y. Zhao. 2014. Protective effects of quercetin and quercetin-5',8-disulfonate against carbon tetrachloride-caused oxidative liver injury in mice. Molecules 19:291-305. http://dx.doi.org/10.3390/molecules19010291.

De David, C., G. Rodrigues, S. Bona, L. Meurer, J. Gonzalez-Gallego, M. J. Tunon, and N. P. Marroni. 2011. Role of quercetin in preventing thioacetamide-induced liver injury in rats. Toxicol. Pathol. 39:949-957. http://dx.doi.org/ 10.1177/0192623311418680.

Djokovic, R., H. Samanc, M. Jovanovic, N. Fratric, V. Doskovic, and Z. Stanimirovic. 2013. Relationship among blood indicators of hepatic function and lipid content in the liver during transitional period in high-yielding dairy cows. Acta Sci. Vet. 41:1128.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 82:2259-2273.

Duske, K., H. M. Hammon, A.-K. Langhof, O. Bellmann, B. Losand, K. Nürnberg , G. Nürnberg , H. Sauerwein, H. M. Seyfert, and C. C. Metges. 2009. Metabolism and lactation performance in dairy cows fed a diet containing rumen-protected fat during the last twelve weeks of gestation. J. Dairy Sci. 92:1670–1684.

Eckersall, P. D. 2000. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. Revue Méd. Vét. 151:577-584.

Esposito, G., P. C. Irons, E.C. Webb, and A. Chapwanya. 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim. Reprod. Sci. 144:60-71. http://dx.doi.org/10.1016/j.anireprosci.2013.11.007.

Fabjan, N., J. Rode, I. J. Kosir, Z. H. Wang, Z. Zhang, and I. Kreft. 2003. Tartary buckwheat (*Fagopyrum tartaricum Gaertn*.) as a source of dietary rutin and quercitrin. J. Agric. Food. Chem. 51:6452-6455. http://dx.doi.org/10.1021/jf034543e.

Goff, J. P., and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80:1260:1268.

Gohlke A., C. J. Ingelmann, G. Nürnberg, A. Starke, S. Wolffram, and C. C. Metges. 2013a. Bioavailability of quercetin from its aglycone and its glucorhamnoside rutin in lactating dairy cows after intraduodenal administration. J. Diary Sci. 96:1-11. http://dx.doi.org/10.3168/jds.2012-6234.

Gohlke A., C. J. Ingelmann, G. Nürnberg, J. M. Weitzel, H. M. Hammon, S. Görs, A. Starke, S. Wolffram, and C. C. Metges. 2013b. Influence of 4-week intraduodenal

supplementation of quercetin on performance, glucose metabolism, and mRNA abundance of genes related to glucose metabolism and antioxidative status in dairy cows. J. Dairy Sci. 96:6986-7000. http://dx.doi.org/10.3168/jds.2013-6852.

Graber, M., S. Kohler, T. Kaufmann, M. G. Doherr, R. M. Bruckmaier, and H. A. van Dorland. 2010. A field study on characteristics and diversity of gene expression in the liver of dairy cows during the transition period. J. Dairy Sci. 93:5200-5215. http://dx.doi.org/10.3168/jds.2010-3265.

Grattagliano, I., G. Vendemiale, P. Caraceni, M. Domenicali, B. Nardo, A. Cavallari, F. Trevisani, M. Bernardi, and E. Altomare. 2000. Starvation impairs antioxidant defense in fatty livers of rats fed a choline-deficient diet. J. Nutr. 130: 2131-2136.

Graugnard, D. E., K. M. Moyes, E. Trevisi, M. J. Khan, D. Keisler, J. K. Drackley, G. Bertoni, and J. J. Loor. 2013. Liver lipid content and inflammometabolic indices in peripartal dairy cows are altered in response to prepartal energy intake and postpartal intramammary inflammatory challenge. J. Dairy Sci. 96:1-18. http://dx.doi.org/10.3168/jds.2012-5676.

Gross, J. J., F. J. Schwarz, K. Eder, H. A. van Dorland, and R. M. Bruckmaier. 2013. Liver fat content and lipid metabolism in dairy cows during early lactation and during a mid-lactation feed restriction. J. Dairy Sci. 96:5008-5017. http://dx.doi.org/10.3168/jds.2012-6245.

Halliwell, B., and S. Chirico. 1993. Lipid peroxidation: Its mechanism, measurement, and significance. Am. J. Clin. Nutr. 57:715S-25S.

Heim, K. E., A. R. Tagliaferro, and D. J. Bobilya. 2002. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. 13:572-584. http://dx.doi.org/10.1016/S0955-2863(02)00208-5.

Hellemans, J., G. Mortier, A. De Paepe, F. Speleman, and J. Vandesompele. 2007. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 8:R19. http://dx.doi.org/10.1186/gb-2007-8-2-r19.

Hiss, S., M. Mielenz, R. M. Bruckmaier, and H. Sauerwein. 2004. Haptoglobin concentrations in blood and milk after endotoxin challenge and quantification of mammary Hp mRNA expression. J. Dairy Sci. 87:3778-3784.

Hiss, S., C. Weinkauf, S. Hachenberg, and H. Sauerwein. 2009. Relationship between metabolic status and the milk concentrations of haptoglobin and lactoferrin in dairy cows

during early lactation. J. Dairy Sci. 92 :4439–4443. http://dx.doi.org/10.3168/jds.2008-1632.

Hoek-van den Hil E. F., J. Keijer, A. Bunschoten, J. J. M. Vervoort, B. Stankova, M. Bekkenkamp, L. Herreman, D. Venema, P. C. H. Hollman, E. Tvrzicka, I. M. C. M. Rietjens, and E. M. van Schothorst. 2013. Quercetin induces hepatic lipid omega-oxidation and lowers serum lipid levels in mice. PLoS ONE 8:e51588. http://dx.doi.org/10.1371/journal.pone.0051588.

Hosseini, A., C. Behrendt, P. Regenhard, H. Sauerwein, and M. Mielenz. 2012. Differential effects of propionate or β-hydroxybutyrate on genes related to energy balance and insulin sensitivity in bovine white adipose tissue explants from a subcutaneous and a visceral depot. J. Anim. Physiol. Anim. Nutr. (Berl.). 2012; 96: 570–580. http://dx.doi.org/10.1111/j.1439-0396.2011.01180.x.

Janssen, L. J. 2001. Isoprostanes: An overview and putative roles in pulmonary pathophysiology. Am. J. Physiol. Lung Cell Mol. Physiol. 280:L1067-L1082.

Joven J., E. Espinel, A. Rull, G. Aragonès, E. Rodríguez-Gallego, J. Camps, V. Micol, M. Herranz-López, J. A. Menéndez, I. Borrás, A. Segura-Carretero, C. Alonso-Villaverde, and R. Beltrán-Debón. 2012. Plant-derived polyphenols regulate expression of miRNA paralogs miR-103/107 and miR-122 and prevent diet-induced fatty liver disease in hyperlipidemic mice. Biochim. Biophys. Acta 1820:894-899. http://dx.doi.org/10.1016/j.bbagen.2012.03.020.

Jung, C. H., I. Cho, J. Ahn, T. Jeon, and T. Ha. 2013. Quercetin reduces high-fat diet-induced fat accumulation in the liver by regulating lipid metabolism genes. Phytother. Res. 27:139-142. http://dx.doi.org/10.1002/ptr.4687.

Katoh, N. 2002. Relevance of apolipoproteins in the development of fatty liver and fatty liver-related peripartum diseases in dairy cows. J. Vet. Med. Sci. 64:293-307. http://dx.doi.org/10.1292/jvms.64.293.

Kobayashi, Y., M. Miyazawa, A. Kamei, K. Abe, and T. Kojima. 2010. Ameliorative effects of mulberry (*Morus alba L.*) leaves on hyperlipidemia in rats fed a high-fat diet: Induction of fatty acid oxidation, inhibition of lipogenesis, and suppression of oxidative stress. Biosci. Biotechnol. Biochem. 74:2385-2395. http://dx.doi.org/10.1271/bbb.100392.

Kuhla, S., M. Klein, U. Renne, W. Jentsch, P. E. Rudolph, and W. B. Souffrant. 2004. Carbon and nitrogen content based estimation of the fat content of animal carcasses in various species. Arch. Anim.Nutr. 58:37–46.

Löhrke, B., T. Viergutz, W. Kanitz, B. Losand, D. G. Weiss, and M. Simko. 2005. Short communication: Hydroperoxides in circulating lipids from dairy cows: Implications for bioactivity of endogenous-oxidized lipids. J. Dairy Sci. 88:1708-1710.

Loor, J. J., H. M. Dann, R. E. Everts, R. Oliveira, C. A. Green, N. A. J. Guretzky, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. Physiol. Genomics 23:217-226. http://dx.doi.org/10.1152/physiolgenomics.00132.2005.

Loor, J. J., H. M. Dann, N. A. Janovick Guretzky, R. E. Everts. R. Oliveira, C. A. Green, N. B. Litherland, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2006. Plane of nutrition prepartum alters hepatic gene expression and function in dairy cows as assessed by longitudinal transcript and metabolic profiling. Physiol. Genomics 27:29-41. http://dx.doi.org/10.1152/physiolgenomics.00036.2006.

Loor, J. J., R. E. Everts, M. Bionaz, H. M. Dann, D. E. Morin, R. Oliveira, S. L. Rodriguez-Zas, J. K. Drackley, and H. A. Lewin. 2007. Nutrition-induced ketosis alters metabolic and signaling gene networks in liver of periparturient dairy cows. Physiol. Genomics. 32:105-116. http://dx.doi.org/10.1152/physiolgenomics.00188.2007.

Luehring, M., R. Blank, and S. Wolffram. 2011. Vitamin E-sparing and vitamin E-independent antioxidative effects of the flavonol quercetin in growing pigs. Anim. Feed Sci. Technol. 168:199-207. http://dx.doi.org/10.1016/j.anifeedsci.2011.06.006.

Lykkesfeldt, J., and O. Svendsen. 2007. Oxidants and antioxidants in disease: Oxidative stress in farm animals. Vet. J. 173:502-511. http://dx.doi.org/ 10.1016/j.tvjl.2006.06.005.

Marcolin, E., L. F. Forgiarini, G. Rodrigues, J. Tieppo, G. S. Borghetti, V. L. Bassani, J. N. Picada, and N. P. Marroni. 2013. Quercetin decreases liver damage in mice with non-alcoholic steatohepatitis. Basic Clin. Pharmacol. Toxicol. 112:385-391. http://dx.doi.org/10.1111/bcpt.12049.

McArt, J. A. A., D. V. Nydam, and G. R. Oetzel. 2012. Epidemiology of subclinical ketosis in early lactation dairy cattle. J. Dairy Sci. 95:5056-5060. http://dx.doi.org/10.3168/jds.2012-5443.

Middleton, E. J. R., C. Kandaswami, and T. C. Theoharides. 2000. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 52:673-751.

Miller, J. K., E. Brzezinska-Slebodzinska, and F. C. Madsen. 1993. Oxidative Stress, antioxidants, and animal Function. J. Dairy Sci. 76:2812-2823.

Nijveldt, R. J., E. van Nood, D. E. C. van Hoorn, P. G. Boelens, K. van Norren, and P. M. van Leeuwen. 2001. Flavonoids: A review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. 74:418-425.

Pan, M. H., C. S. Lai, M. L. Tsai, and C. T. Ho. 2014. Chemoprevention of nonalcoholic fatty liver disease by dietary natural compounds. Mol. Nutr. Res. 58:147-171. http://dx.doi.org/10.1002/mnfr.201300522.

Rathee, P., H. Chaudhary, S. Rathee, D. Rathee, V. Kumar, and K. Kohli. 2009. Mechanism of action of flavonoids as anti-inflammatory agents: A review. Inflamm. Allergy Drug Targets. 8:229-235.

Re, R. N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio. Med. 26:1231-1237. http://dx.doi.org/10.1016/S0891-5849(98)00315-3.

Rebrin, I., and R. S. Sohal. 2004. Comparison of thiol redox state of mitochondria and homogenates of various tissues between two strains of mice with different longevities. Exp. Gerontol. 39:1513-1519. http://dx.doi.org/10.1016/j.exger.2004.08.014.

Rochfort, S., A. J. Parker, and F. R. Dunshea. 2008. Plant bioactives for ruminant health and productivity. Phytochem. 69:299-322. http://dx.doi.org/10.1016/j.phytochem.2007.08.017

Ruijter, J. M., M. W. Pfaffl , S. Zhao, A. N. Spiess, G. Boggy, J. Blom, R. G. Rutledge, D. Sisti, A. Lievens, K. De Preter , S. Derveaux , J. Hellemans, and J. Vandesompele. 2013. Evaluation of qPCR curve analysis methods for reliable biomarker discovery: Bias, resolution, precision, and implications. Methods 59:32-46. http://dx.doi.org/10.1016/j.ymeth.2012.08.011.

Saremi, B, H. Sauerwein, S. Dänicke, and M. Mielenz. 2012. Identification of reference genes for gene expression studies in different bovine tissues focusing on different fat depots. J. Dairy Sci. 95:3131-3138. http://dx.doi.org/10.3168/jds.2011-4803.

Schäff, C., S. Börner, S. Hacke, U. Kautzsch, D. Albrecht, H. M. Hammon, M. Röntgen, and B. Kuhla. 2012. Increased anaplerosis, TCA cycling, and oxidative phosphorylation in the liver of dairy cows with intensive body fat mobilization during early lactation. J. Proteome Res. 11:5503−5514.

Schlegel, G., R. Ringseis, J. Keller, F. J. Schwarz, and K. Eder. 2012. Changes in the expression of hepatic genes involved in cholesterol homeostasis in dairy cows in the transition period and at different stages of lactation. J. Dairy Sci. 95:3826-3836. http://dx.doi.org/10.3168/jds.2011-5221.

Shao, L., K. Liu, F. Huang, X. D. Guo, M. Wang, and B. L. Liu. 2013. Opposite Effects of Quercetin, Luteolin, and Epigallocatechin Gallate on Insulin Sensitivity Under Normal and Inflammatory Conditions in Mice. Inflammation 36:1-14. http://dx.doi.org/10.1007/s10753-012-9514-x.

Skinner, J. G. 2001. International standardization of acute phase proteins. Vet. Clin. Pathol. 30:2–7.

Sordillo, L. M., N. O'Boyle, J. C. Gandy, C. M. Corl, and E. Hamilton. 2007. Shifts in thioredoxin reductase activity and oxidant status in mononuclear cells obtained from transition dairy cattle. J. Dairy Sci. 90:1186-1192.

Spears, J. W., and W. P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 176:70-76. http://dx.doi.org/10.1016/j.tvj1.2007.12.015.

Stoldt, A., M. Derno, G. Nürnberg, J. M. Weitzel, W. Otten, A. Starke, S. Wolffram, and C. C. Metges. 2015. A 6-wk intraduodenal supplementation of Quercetin reduces indicators of liver damage in periparturient dairy cows. J. Dairy Sci. 98:4509–4520. http://dx.doi.org/10.3168/jds.2014-9053.

Szczubial, M. 2010. Non-enzymatic antioxidative defense mechanism in plasma of pigs during periparturient period: Vitamin C and Glutathione. Bull. Vet. Inst. Pulawy 54:373-376.

Van Dorland, H. A., H. Sadri, I. Morel, and R. M. Bruckmaier. 2012. Coordinated gene expression in adipose tissue and liver differs between cows with high or low NEFA concentrations in early lactation. J. Anim. Physiol. An. N. 96:137-147. http://dx.doi.org/10.1111/j.1439-0396.2011.01130.x.

Vazquez-Anon, M., S. Bertics, M. Luck, and R. R. Grummer. 1994. Peripartum liver triglyceride and plasma metabolites in dairy cows. J. Dairy Sci. 77.1521-1528.

Veenhuizen, J. J., J. K. Drackley, M. J. Richard, T. P. Sanderson, L. D. Miller, and J. W. Young. 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. J. Dairy Sci. 74:4238-4253.

Vendemiale, G., I. Grattagliano, P. Caraceni, G. Caraccio, M. Domenicali, M. Dall'Agata, F. Trevisani, F. Guerrieri, M. Bernardi, and E. Altomare. 2001. Mitochondrial oxidative injury and energy metabolism alteration in rat fatty liver: Effect of the nutritional status. Hepatology 33:808-815. http://dx.doi.org/10.1053/jhep.2001.23060.

Weber C., C. Hametner, A. Tuchscherer, B. Losand, E. Kanitz, W. Otten, S.P. Singh, R.M. Bruckmaier, F. Becker, W. Kanitz, and H.M. Hammon. 2013. Variation in fat

mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. J. Dairy Sci. 96:165–180. http://dx.doi.org/10.3168/jds.2012-5574

Wensing, T., T. Kruip, M. J. H. Geelen, G. H. Wentink, and A. M. van den Top. 1997. Postpartum fatty liver in high-producing dairy cows in practice and in animal studies. The connection with health, production and reproduction problems. Comp. Haematol. Int. 7:167-171. http://dx.doi.org/10.1007/BF02652596.

Yang, S. Q., H. Zhu, Y. B Li, H. Z. Lin, K. Gabrielson, M. A. Trush, and A. M. Diehl. 2000. Mitochondrial adaptations to obesity-related oxidant stress. Arch. Biochem. Biophys. 378:259-268. http://dx.doi.org/10.1006/abbi.2000.1829.

Xu, M. L., J. J. Hu, W. W. Zhao, X. J. Gao, C. H. Jiang, K. Liu, B. L. Liu, and F. Huang. 2014. Quercetin differently regulates insulin-mediated glucose transporter 4 translocation under basal and inflammatory conditions in adipocytes. Mol. Nutr. Food Res. 58:931-941. http://dx.doi.org/10.1002/mnfr.201300510.

Table 1. Characteristics of primers and real-time PCR conditions.

Primer	Sequence (5′-3′)	NIH Genbank accession number	Bp	Mean Conc. (nM)	Cq[14]	Annea-ling (s/°C)	Effici-ency
FASN[1]		NM_001	204	600	27.1	10/60	1.850
Forward	AACACCTCGGTGCAGTTCAT	012669					
Reverse	GGTGACGCCTTTCTCTTTGC						
GPAM[2]		NM_001	133	600	24.5	10/60	1.865
Forward	CAGATGAATCCCGCCGAAGA	012282					
Reverse	CCAATTCCCTGCCTGTGTCT						
ACACA[3]		NM_174	104	600	26.8	10/57	1.870
Forward	CGTTTGGGGTTATTTCAGTG	224					
Reverse	TTGCTTCCTCTCGGTTTTC						
PPARA[4]		NM_001	171	400	22.3	10/59	1.776
Forward	CGGAAGTCCGCATTTTCCAC	034036					
Reverse	TCACAGAAGACAGCATCGCA						
PPARG[5]		Y12420	121	600	28.5	10/59	1.892
Forward	AGGATGGGGTCCTCATATCC						
Reverse	GCGTTGAACTTCACAGCAAA						
AHR[6]		NM_001	164	600	22.9	10/55	1.877
Forward	CCAGGACAACTGTCGAACCA	206026					
Reverse	CATCAGACTGCTGAAACCCCA						
PON1[7]		NM_001	208	600	20.6	10/57	1.813
Forward	CTGGATCAGAGGTGCTCCAAA	046269					
Reverse	AGCTCCCACTTGTCAGGGTA						
GPX1[8]		X13684	62	600	21.3	10/55	1.840
Forward	CTTCCCCTGCAACCAGTTTG						
Reverse	GGCAATTCAGGATCTCCTCGTT						
SOD1[9]		M81129	246	600	22.1	10/57	1.795
Forward	AAGGCCGTGTGCGTGCTGAA						
Reverse	CAGGTCTCCAACATGCCTCT						
CAT[10]		NM_001	162	600	17.2	10/59	1.780
Forward	TCACTCAGGTGCGGACTTTC	035386					
Reverse	GGATGCGGGAGCCATATTCA						
DGAT1[11]		NM_174693	121	600	24.1	10/58	1.775

Forward	ACATCCCTGTTCACAAGTG						
Reverse	AGGTACTCGTGGAAGAAGG						
APOB[12]		XM_0108175	149	600	18.2	10/57	1.799
Forward	GCCAGAGATGAAGTGCTGGA	52					
Reverse	ATCTTGGTGGCGCTTCTTGA						
MTTP[13]		NM_0011018	84	600	21.5	10/58	1.808
Forward	GCCACCACACTCCAGAGATATG	34					
Reverse	ACGATTCTGGTGGTATATCCTGTTC						

[1]Fatty acid synthase
[2]Glycerol-3-phosphate acyltransferase 1
[3]Acetyl-CoA carboxylase
[4]Peroxisome proliferator-activated receptor *alpha*
[5]Peroxisome proliferator-activated receptor *gamma*; according to Hosseini et al. (2012)
[6]Aryl hydrocarbon receptor
[7]Paraoxonase
[8]Glutathione peroxidase; according to Gohlke et al. (2013b)
[9]Superoxide dismutase; according to Gohlke et al. (2013b)
[10]Catalase
[11]Diglyceride acyltransferase
[12]Apolipoprotein B100
[13]Microsomal triglyceride transfer protein, according to Loor et al. (2006)
[14]Cq: Quantification cycle

Table 2. Liver fat, glycogen and glutathione (GSH) status (LSM ± SE) before (wk -4) and during (wk -2, wk 3) quercetin supplementation from wk 3 before to wk 3 after parturition in cows (n = 5 per group).

Item	Content		*P*-Value			
			Period	ap[1] vs. pp[2]		
	Quercetin	Control	Treatment	Treatment	Time	Time × Treatment
Liver fat[4]						
basal[3]						
DM	16.0 ± 4.6	15.3 ± 3.9	0.484			
WM[5]	5.8 ± 0.3	4.9 ± 0.2				
ap						
DM	15.7 ± 3.4	15.9 ± 3.4	0.935			
WM	5.5 ± 0.6	5.8 ± 0.4				
pp				0.082	0.001	0.087
DM	29.4 ± 4.6	48.3 ± 4.6	0.204			
WM	12.3 ± 4.0	20.1 ± 1.5				
Liver glycogen[4]						
basal						
DM	8.0 ± 1.4	9.2 ± 1.2	0.427			
WM	2.8 ± 0.5	3.0 ± 0.5				
ap						
DM	6.7 ± 1.1	9.5 ± 1.1	0.149			
WM	2.2 ± 0.5	3.5 ± 0.3				
pp				0.204	0.002	0.483
DM	1.5 ± 1.4	2.0 ± 1.4	0.388			
WM	0.5 ± 0.1	1.0 ± 0.3				

Liver GSH[6]										
basal	3.4	±	0.9	1.2	±	0.9	0.124			
ap	0.9	±	0.9	1.2	±	0.8	0.533	0.749	0.338	0.564
pp	2.5	±	1.3	1.6	±	1.1	0.738			

[1]Ante partum; values of wk -2

[2]Postpartum; values of wk 3

[3]Values of wk -4

[4]%

[5]Wet matter

[6]µmol/L

Table 3. Relative mRNA abundance (LSM ± SE) of genes related to lipid metabolism and oxidative stress in the liver of cows (n = 5 per group) before, ante and post partum during quercetin supplementation from wk 3 before to wk 3 after parturition

	Relative mRNA expression						*P*-Value		
							Period	ap[1] vs. pp[2]	
Item	Quercetin			Control			Treatment	Treatment	Time[4]
FASN[5]									
basal[3]	0.688	±	0.13	1.019	±	0.11	0.106		
ap	1.369	±	0.51	1.011	±	0.51	0.631	0.768	0.513
pp	0.864	±	0.76	1.983	±	0.76	0.355		
GPAM[6]									
basal	0.712	±	0.16	1.153	±	0.14	0.087		
ap	0.906	±	0.11	1.161	±	0.11	0.127	0.170	0.537
pp	0.986	±	0.22	1.243	±	0.22	0.461		
ACACA[7]									
basal	1.062	±	1.10	2.303	±	0.95	0.434		
ap	1.828	±	0.64	1.124	±	0.64	0.461	0.928	0.962
pp	1.180	±	1.00	1.838	±	1.00	0.666		
PPARA[8]									
basal	0.928	±	0.15	1.063	±	0.13	0.529		
ap	1.048	±	0.11	0.968	±	0.11	0.628	0.663	0.565
pp	1.074	±	0.21	1.075	±	0.21	0.999		
PPARG[9]									
basal	1.027	±	0.11	0.969	±	0.10	0.711		
ap	0.891	±	0.09	1.023	±	0.09	0.338	0.879	0.295
pp	1.155	±	0.27	1.127	±	0.27	0.946		
DGAT[10]									

basal	0.857	±	0.13	0.977	±	0.12	0.529		
ap	1.088	±	0.12	0.936	±	0.12	0.392	0.209	0.336
pp	1.228	±	0.08	1.037	±	0.08	0.191		
APOB[11]									
basal	1.019	±	0.06	1.011	±	0.05	0.929		
ap	0.913	±	0.07	1.069	±	0.07	0.141	0.126	0.255
pp	0.995	±	0.09	1.150	±	0.09	0.305		
MTTP[12]									
basal	0.900	±	0.08	0.916	±	0.07	0.892		
ap	0.936	±	0.08	1.043	±	0.08	0.377	0.764	0.004
pp	1.203	±	0.19	1.192	±	0.19	0.967		
PON1[13]									
basal	1.055	±	0.09	1.156	±	0.07	0.425		
ap	1.081	±	0.15	1.194	±	0.15	0.605	0.843	0.134
pp	0.863	±	0.24	0.861	±	0.24	0.996		
GPX1[14]									
basal	1.634	±	0.09	1.040	±	0.08	0.368		
ap	0.934	±	0.10	1.061	±	0.10	0.4155	0.651	0.971
pp	1.116	±	0.07	0.898	±	0.07	0.100		
SOD1[15]									
basal	0.999	±	0.09	0.962	±	0.08	0.775		
ap	0.954	±	0.07	0.959	±	0.07	0.960	0.495	0.036
pp	1.082	±	0.13	1.250	±	0.13	0.406		
CAT[16]									
basal	1.090	±	0.14	0.963	±	0.13	0.542		
ap	0.903	±	0.07	1.067	±	0.07	0.138	0.373	0.242
pp	1.081	±	0.14	1.107	±	0.14	0.904		

AHR[17]									
basal	0.943	±	0.21	1.394	±	0.18	0.165		
ap	1.087	±	0.27	1.280	±	0.27	0.633	0.666	0.268
pp	0.781	±	0.13	0.883	±	0.13	0.599		

[1]Ante partum; values of wk -2

[2]Postpartum; values of wk 3

[3]Values of wk -4

[4]There were no significant interactions of treatment × time.

[5]Fatty acid synthase

[6]Glycerol-3-phosphate acyltransferase 1

[7]Acetyl-CoA carboxylase

[8]Peroxisome proliferator-activated receptor *alpha*

[9]Peroxisome proliferator-activated receptor *gamma*

[10]Diglyceride acyltransferase

[11]Apolipoprotein B100

[12]Microsomal triglyceride transfer protein

[13]Paraoxonase

[14]Glutathione peroxidase

[15]Superoxide dismutase

[16]Catalase

[17]Aryl hydrocarbon receptor

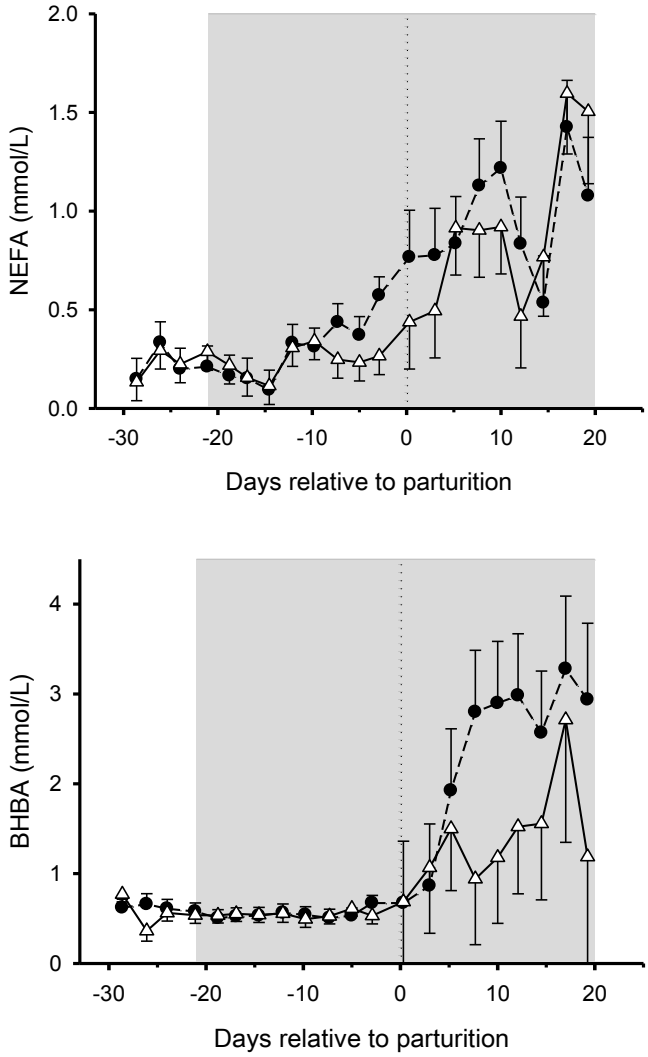

Figure 1. Plasma NEFA and BHBA content in cows with (Δ; solid line) or without (●; dashed line) intraduodenal quercetin supplementation in the periparturient period. Values are LSM ± SE of n = 5 cows per group. The shaded area indicates the period of quercetin supplementation from wk – 3 to wk + 3 relative to calving. The day of calving is represented by a dotted vertical line. NEFA and BHBA where influenced by time ($P < 0.05$), but not by quercetin supplementation ($P > 0.1$).

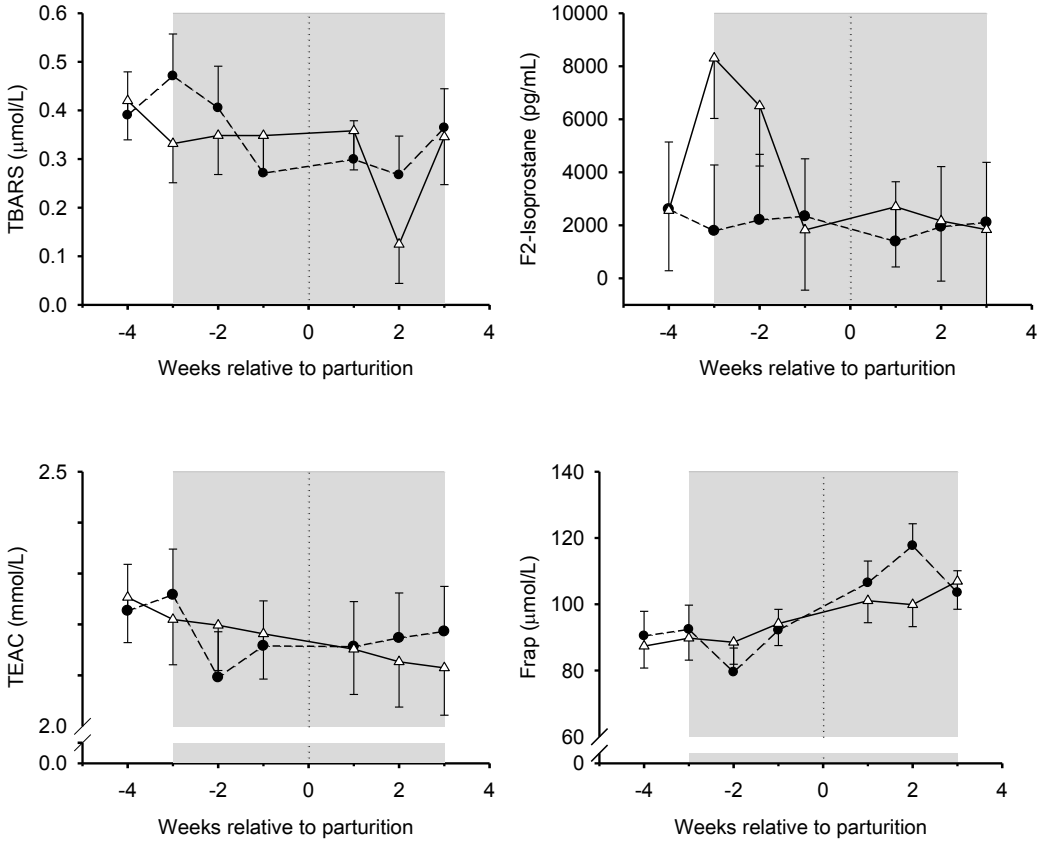

Figure 2. Plasma ferric ion reducing antioxidant power (Frap), Trolox equivalent antioxidative capacity (TEAC), thiobarbituric acid reactive substances (TBARS) and F2-isoprostanes content in cows with (Δ; solid line) or without (●; dashed line) intraduodenal quercetin supplementation in the periparturient period. Values are LSM ± SE of n = 5 cows per group. The shaded area indicates the period of quercetin supplementation from wk – 3 to wk + 3 relative to calving. The day of calving is represented by a dotted vertical line. *P*-values for time were $P = 0.034$ for Frap, $P = 0.081$ for TEAC, $P = 0.043$ for TBARS, and $P > 0.1$ for F2-isoprostanes. *P*-values for treatment were $P > 0.1$ for all shown parameters.

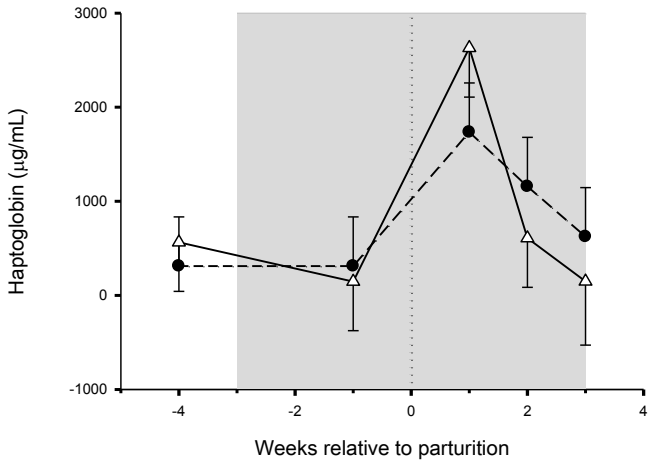

Figure 3. Plasma haptoglobin content in cows with (Δ; solid line) or without (●; dashed line) intraduodenal quercetin supplementation in the periparturient period. Values are LSM ± SE of n = 5 cows per group. The shaded area indicates the period of quercetin supplementation from wk – 3 to wk + 3 relative to calving. The day of calving is represented by a dotted vertical line. *P*-value for time was $P < 0.006$, for treatment and time × treatment interaction *P*-values were $P > 0.1$.

5 Summarizing discussion

The first study was conducted to reveal effects of the flavonoid quercetin, administered intraduodenally, in peripartal dairy cows, whereas the second study examined effects of rutin fed orally in cows in established lactation.

5.1 Plasma flavonoid concentrations

In the first study, 100 mg quercetin aglycone per kg body weight (BW) per day were administered intraduodenally in three daily aliquots to generate a constantly elevated plasma flavonoid level with quercetin as major flavonoid in plasma and minor levels of kaempferol, tamarixetin and isorhamnetin. The dose given was derived from preliminary studies using lower doses (Berger at al., 2012; Gohlke et al., 2013a,b), and adjusted to reach preferably high plasma flavonoid levels.

Plasma flavonoid concentrations at midday were higher post partum (pp) than ante partum (ap), suggesting an effect of lactation stage, liver status or both on plasma total flavonoids concentration. The liver is of special importance for elimination of quercetin and its metabolites (Lesser and Wolffram, 2006). Drug metabolism and elimination in the liver can be impaired in early lactating cows (Lake et al., 2011; Merrel and Cherrington, 2011), maybe an explanation for the higher plasma quercetin pp. On the other hand, in the morning samples, we observed lower plasma quercetin levels pp than ap. This seems to be due to a lower absorption of quercetin because of faster passage through the digestive tract after calving (Aikman et al., 2008).

Because the intraduodenal application of quercetin is not suitable for practical use, also effects of oral rutin, a form of quercetin which is resistant to rumen microbes (Berger et al., 2012) were investigated, yet in cows in established lactation. In the second study, rutin trihydrate supplementation of 100 mg per kg BW per day elevated total flavonoid levels in plasma with quercetin as the main metabolite. Indeed, total plasma flavonoid levels were comparably lower than in former studies with 50 mg per kg BW quercetin as rutin given intraruminally (Berger et al., 2012) or in the first study. The different levels of plasma flavonoids are most likely due to the different ways of flavonoid application and different stages of lactation of the cows examined.

Although the plasma flavonoid concentrations differed between quercetin and rutin treatment, it remains unclear, if the higher plasma flavonoid concentration generates the more definite effects. Cui et al. (2014) examined mice with experimentally induced oxidative stress, treated them with differed dosages of quercetin and found the more distinct effects the higher the dose given was. Interestingly, in a recent study using two dosages of rutin in dairy cows (Cui et al., 2015), the lower dose showed better effects on the milk yield. In any case, the dosage and the plasma concentration of flavonoids seem to have an influence on their biological effects.

Oral supplementation of ground buckwheat seeds was not sufficient to increase total plasma flavonoids above detection limit. A possible reason is a low digestibility of the buckwheat seeds, because a recognizable amount of the seeds was found unmodified in cows' feces despite milling. Others (Kälber et al., 2012, 2013) reported feeding of buckwheat as silage, which seems to be more digestible and suitable as dietary component.

5.2 Health status and performance

As indicated by plasma BHBA values, some of the peripartal cows suffered from clinical or subclinical ketosis (McArt et al., 2012), and one cow of the quercetin treated group and two of the control cows had retained placenta. Since it is known that quercetin exerts its effects especially under adverse health conditions (Shao et al., 2013, Xu et al., 2014), we deliberately choose the periparturient period to examine quercetin effects in cows. Cows supplemented with rutin were healthy throughout the experiment.

Quercetin administration in peripartal cows had no effects on dry matter intake, milk yield and BW. As expected, energy balance decreased after calving and reached its nadir on day nine pp, mostly caused by increasing milk yield. Production performance of cows in established lactation was not altered by rutin supplementation. Improved milk yield or milk protein content, as reported for rutin (Guo et al., 2010; Cui et al., 2015), intraduodenally administered quercetin (Gohlke et al., 2013b) or a grape extract rich in polyphenols (Gessner et al., 2015) could not be confirmed.

5.3 Energy metabolism and methane production

Effects of quercetin and other flavonoids on energy expenditure in humans (Dulloo et al., 1999; Egert et al., 2011; Hursel et al., 2011) and mice (Klaus et al., 2005; Stewart et al., 2008) are contradictory. In the present studies, several components of energy expenditure where measured via indirect calorimetry but no quercetin or rutin effects were found. Nevertheless, with the onset of lactation, energy expenditure in peripartal cows increased with milk production (Jentsch et al., 2001). The respiratory quotient and the carbohydrate oxidation suggest an increase of fatty acid oxidation reflecting the higher supply of fatty acids as energy source from lipomobilization after calving (Dulloo et al., 1999; Börner et al., 2013). Likewise, changes in energy metabolism found in cows in established lactation indicate the metabolic shift towards fat oxidation as triggered by the feed restriction period, consistent with former studies (Derno et al., 2013).

It has been previously shown that quercetin can lower plasma lipids (Hoek-van den Hil et al., 2013; Jung et al., 2013; Wein and Wolffram, 2014) which was explained by up regulation of genes involved in ß and Ω fatty acid oxidation and down regulation of ketogenic genes in the liver (Kobayashi et al., 2010). However, quercetin did not improve metabolic status of peripartal cows. Changes in plasma metabolites reflect the typical peripartal changes in dairy cows with indication of lipid mobilization and affected liver metabolism indicated by high plasma values of NEFA and BHBA and low values of glucose. Particularly, plasma NEFA and BHBA concentrations are indicators for energy status and lipomobilization (Cozzi et al., 2011), thus it is interesting to note that pp plasma BHBA levels were numerically lower in quercetin than in control cows. This observation suggests that quercetin may have a beneficial effect on liver metabolism because lower BHBA levels in periparturient cows have been associated with a lower risk of ketosis and hepatic lipidosis (Gonzalez et al, 2011; Mostafavi et al., 2013).

In cows in established lactation, plasma glucose values were higher under rutin supplementation. This is in contrast to findings in cows in established lactation supplemented with quercetin intraduodenally (Gohlke et al., 2013b), where quercetin decreased plasma glucose and with the first study, where plasma glucose was not altered by quercetin. It is known that quercetin may affect glucose tolerance and insulin effects (Shao et al., 2013), but plasma insulin was not changed by flavonoid treatments in both studies. In contrast to the first

study and similar to mid-lactation cows under quercetin supplementation (Gohlke et al., 2013b), plasma BHBA values tended to be higher under rutin supplementation. It is established for the carbohydrate metabolism that flavonoids exert their effects differently under different health conditions (Shao et al., 2013; Xu et al., 2014). Furthermore, Hoek-van den Hil et al. (2014) described that quercetin effects in mice depend on the composition of the diet. Consequentially, the influence of cows health and state of lactation with the corresponding diet on the effects of flavonoid supplementation should not be underestimated and may be an explanation for the contradictory findings.

Flavonoids including rutin and quercetin were found to reduce ruminal production of methane, a greenhouse gas with global warming potential (Etim et al., 2013) by reducing the number of methanogenic microbiota in vitro (Leiber et al., 2012; Oskoueian et al., 2013; Wang et al., 2013). However, quercetin and rutin supplementation in the present studies did not result in a reduced methane emission in vivo. Possibly, the residence time of rutin in rumen was too short to suppress rumen methanogens. Additionally, in vitro studies may achieve considerably higher flavonoid concentrations than in vivo experiments. Thus, the rutin dose given may be to low to induce changes in rumen gas production similar to the mentioned studies. Interestingly, Berger et al. (2015) found no effects of quercetin and rutin on in vitro methane production using the Hohenheimer gas test. Due to the intraduodenal application, an effect of quercetin of rumen metabolism was unlikely.

5.4 Liver health and metabolism

To investigate liver damage in the transition period, the activities of AST and glutamate dehydrogenase (GLDH) in plasma were measured in the first study. Severe fatty infiltration causes hepatocyte destruction with release of the cytosolic enzyme AST (Liu et al., 2012) and increased activity levels of GLDH in plasma are suggestive of hepatocyte injury and death (Ok et al., 2013). Both quercetin and control group showed an increase in plasma AST levels from ap to pp, but the increase in AST values pp was considerably less in the quercetin treated than in the control group. This observation is similar to findings in rodents with non-alcoholic steatohepatitis and cirrhosis, where quercetin supplementation inhibited the development of steatosis and reduced AST (Peres et al., 2000; Marcolin et al., 2013). Because plasma AST values may also be increased by muscle injury, plasma creatine kinase was measured to identify the origin of the elevated plasma AST values. In respect of plasma creatine kinase

values, our observation is solely due to differences in liver damage among the groups. Plasma GLDH concentrations were not significantly affected by quercetin treatment. However, in control cows the numerical increase of plasma GLDH values from ap to pp was about 300 % while in quercetin cows it was only 50 %, suggesting that quercetin supplementation potentially acts hepatoprotective also in respect to GLDH values. A possible explanation for the more distinct effect on plasma AST is that GDLH is a mitochondrial enzyme which is only elevated in cases of complete cell destruction, whereas AST is a hepatocellular leakage enzyme released from cytosol through cell damage (Ok et al., 2013).

Further, the results suggest that quercetin supplementation may reduce liver fat content after calving. In rodents, quercetin administration resulted in hepatic quercetin accumulation and regulated hepatic gene expression, also of genes involved in lipid metabolism (Kobayashi et al., 2010; Hoek–van den Hil et al., 2013; Jung et al., 2013). In mice fed a high fat diet (Jung et al., 2013) or with induced steatohepatitis (Marcolin et al., 2013), quercetin supplementation reduced liver fat content. Four weeks prior to calving, all our cows had a low liver fat content, but on day 21 after calving, quercetin treated cows showed a moderately increased liver fat content whereas control cows reached considerably higher values. This is of particular interest given that fatty liver is a relevant health problem in the periparturient period (Bobe et al., 2004).

Hepatic mRNA abundances of genes involved in fatty acid oxidation metabolism were not influenced by quercetin administration. It cannot be excluded that quercetin induced changes on the protein level (De David et al., 2011; Schäff et al., 2012; Pan et al., 2014), which was not analyzed in this study.

A possible explanation for the rather slight quercetin effects on the liver metabolism might be that the time of liver biopsy pp was too late to detect changes, because this is a period where liver fat is already at its maximum or even already declining (Drackley et al., 2005; Graugnard et al., 2013). Furthermore, the tissue flavonoid concentration should also be considered. Although there are no data on flavonoid tissue distribution in dairy cows, it is likely that there is a certain accumulation of quercetin in the liver as found for pigs (De Boer et al., 2005; Bieger et al., 2008) and rats (De Boer et al., 2005). It is unclear, if the tissue concentrations reached an effective level in the present study, and the tissue concentrations are often below the plasma concentration in other species (Lesser and Wolffram, 2006).

5.5 Oxidative stress

To obtain a comprehensive picture of possible quercetin effects on antioxidative status, it was investigated at different levels in the first study. Antioxidant enzymes, the main constituent of intracellular defense (Bernabucci et al., 2005), were measured at the mRNA level in liver tissue, and glutathione was measured in leukocytes and liver tissue as low-molecular-weight antioxidant (Szczubial, 2010). Additionally, lipid peroxidation was analyzed by thiobarbituric acid reactive substances and F2-isoprostanes, and plasma ferric ion reducing antioxidant power and Trolox equivalent antioxidative capacity were used as a measure of total antioxidant capacity (Janssen, 2001; Aoki et al., 2008; Celi, 2011). In periparturient cows, parameters of oxidative metabolism and mRNA abundance levels of antioxidative enzymes were not influenced by quercetin treatment. Likewise, in a former study in mid-lactation cows, no effects of quercetin on mRNA abundances related to antioxidative status were found (Gohlke et al., 2013b). In general, it is not clear if the cows were as stressed as expected (Bernabucci et al., 2005), because markers of lipid peroxidation were not increased and antioxidative capacity showed contradictory values around calving.

5.6 Conclusion

The main novel finding of this study is that quercetin supplementation can possibly be beneficial in periparturient cows challenged by lipid mobilization, because liver damage seems to be reduced, as indicated by plasma AST and GLDH values as well as by liver fat values. Although the mechanistic link remains unclear, these findings are of particular interest, because fatty liver is a major metabolic disorder in peripartal cows and there is a need for a suitable treatment (Bobe et al., 2004).

In summary, the results indicate that the absolute concentrations of quercetin in cows' plasma and tissue as well as health conditions and the stage of lactation of the cow influence the specificity of quercetin effects on energy metabolism and production performance. Effects of quercetin are contradictory under different settings and health promoting properties of quercetin seem to be more pronounced in cows under challenged metabolic health conditions.

6 Summary - Effects of the Flavonoids Quercetin and Rutin in Dairy Cows

Periparturient high yielding dairy cows experience metabolic challenges that result in a negative energy balance and a range of postpartum health problems. To compensate for the negative energy balance, cows mobilize fatty acids from adipose tissues, which can lead to ketosis and fatty liver disease, typical periparturient metabolic disorders. Furthermore, the antioxidative status of these cows may be impaired.

Flavonoids, such as quercetin and rutin, are polyphenolic substances found in all higher plants and have antioxidative and hepatoprotective potential and the ability to prevent or reduce lipid accumulation in the liver of rodents and humans. In addition, flavonoids can reduce the ruminal production of the greenhouse gas methane in vitro through their antibacterial properties. In ruminants, only few studies on the metabolic effects of flavonoids are available, thus the present studies were conducted to determine possible effects of flavonoids in dairy cows. The first study examined quercetin effects on energy expenditure, lipid and oxidative metabolism, as well as liver health in periparturient dairy cows. Quercetin was supplemented intraduodenally to circumvent microbial degradation in the rumen. Ten transition cows with duodenal fistulas were monitored for seven weeks. Beginning three weeks before expected calving, five cows were treated with 100 mg of quercetin dihydrate per kilogram of BW daily for 6 weeks, whereas the control cows received only the physiological saline solution. The total plasma flavonoid levels were more than 30 times higher in the quercetin-treated cows than in the control cows. The pp increases in plasma AST and GLDH activities as well as liver fat were less in the quercetin-treated cows than in the control cows. Quercetin had no effect on energy expenditure, lipid metabolism and oxidative status.

A second study was conducted to investigate whether rutin, a relatively rumen stable form of quercetin, has effects on energy metabolism, methane production and production performance in dairy cows. Rutin trihydrate at a dose of 100 mg per kg BW was fed to a group of seven lactating dairy cows for two weeks in a cross-over-design. In a second step, two cows were fed the same ration but were supplemented with buckwheat seeds (*Fagopyrum tartaricum*) providing rutin at a comparable dose. Two other cows receiving barley supplements were used as controls in a change-over mode. Supplementation of pure rutin but not of rutin contained in buckwheat seeds increased the plasma quercetin content. Methane production and performance were not affected by rutin treatment in either form, but plasma glucose and

BHBA were increased by pure rutin treatment, indicating a possible metabolic effect in dairy cows.

In summary, the results indicate that the absolute concentrations of flavonoids in cows' plasma and tissue as well as health conditions and the stage of lactation of the cow influence the specificity of quercetin effects. Effects of quercetin are contradictory under different settings and health promoting properties of quercetin seem to be more pronounced in cows under challenging metabolic health conditions.

7 Zusammenfassung – Effekte der Flavonoide Quercetin und Rutin bei Milchkühen

Hochleistende Milchkühe in der peripartalen Phase durchleben aufgrund der einsetzenden Laktation vielfältige metabolische Herausforderungen, die in einer negativen Energiebilanz und einer Reihe postpartaler Gesundheitsprobleme resultieren können. Um die negative Energiebilanz zu kompensieren, mobilisieren die Kühe Fettgewebe, was zu Ketose und Fettlebersyndrom, den typischen metabolischen Problemen der peripartalen Phase, führen kann. Weiterhin kann der antioxidative Stoffwechsel dieser Kühe beeinträchtigt sein.

Flavonoide wie Quercetin und Rutin sind pflanzliche polyphenolische Substanzen die antioxidatives sowie hepatoprotektives Potential besitzen. Außerdem können sie die Akkumulation von Lipiden in der Leber reduzieren oder verhindern. Darüber hinaus können Flavonoide in vitro, aufgrund ihrer antibakteriellen Wirkung, die Produktion des klimaschädlichen Gases Methan im Pansen reduzieren. Zu weiteren Wirkungen von Flavonoiden bei Wiederkäuern gibt es nur sehr wenige Studien, weshalb die vorliegenden Studien durchgeführt wurden, um mögliche Effekte der Flavonoide bei Milchkühen zu erforschen.

Die erste Studie untersucht die Effekte des Flavonoids Quercetin auf den Energieumsatz, den Lipid- und den oxidativen Stoffwechsel sowie die Lebergesundheit bei Milchkühen in der peripartalen Phase. Das verwendete Quercetin wurde intraduodenal verabreicht, um einen mikrobiellen Abbau im Pansen zu vermeiden. Zehn duodenal fistulierte Milchkühe wurden über einen Zeitraum von sieben Wochen untersucht. Beginnend in der dritten Woche vor der erwarteten Kalbung erhielten fünf Tiere täglich 100 mg Quercetindihydrat pro kg Körpergewicht für eine Dauer von sechs Wochen, während die fünf Kontrolltiere lediglich physiologische Kochsalzlösung über die Fistel erhielten. Die mit Quercetin behandelten Kühe zeigten erhöhte Plasmaflavonoidwerte. Der Anstieg der Plasmaaktiviäten der leberspezifischen Enzyme Aspartataminotransferase und Glutamatdehydrogenase sowie des Leberfettgehalts nach der Kalbung waren in der Behandlungsgruppe geringer als in der Kontrollgruppe. Die Quercetingabe hatte keinen Effekt auf den Energieumsatz, den Lipidstoffwechsel und den oxidativen Status.

Die zweite Studie wurde durchgeführt um die Wirkungen des pansenstabilen Flavonoids Rutin auf den Energiestoffwechsel, die Methanproduktion und die Leistung von Milchkühen zu untersuchen. Über zwei Wochen wurden sieben laktierenden Milchkühen in einem cross-over-Design täglich 100 mg Rutintrihydrat pro kg Körpergewicht mit dem Futter verabreicht.

In einem weiteren Schritt bekamen zwei der Tiere rutinhaltige Buchweizensaat (*Fagopyrum tartaricum*) zugefüttert, wobei die gewählte Menge dem Rutingehalt der ersten Phase entsprach. Die Zufütterung von Rutin, aber nicht von rutinhaltigem Buchweizen, führte zu einem erhöhten Flavonoidspiegel im Plasma der Kühe. Die Methanproduktion sowie die Leistung der Tiere wurden durch keine der beiden Applikationsformen beeinflusst, allerdings stiegen die Plasmakonzentrationen von Glucose und BHBA unter Behandlung mit purem Rutin an. Dies weißt möglicherweise auf einen metabolischen Effekt von Rutin bei Milchkühen hin.

Zusammenfassend weisen die Resultate beider Studien darauf hin, dass die absolute Flavonoidkonzentration im Plasma und im Gewebe sowie der Gesundheitszustand und das Laktationsstadium der Tiere die Art und Ausprägung der Flavonoideffekte beeinflusst. Die gesundheitsfördernden Effekte von Quercetin erscheinen ausgeprägter bei Milchkühen mit beeinträchtigtem Gesundheitsstatus.

8 References cited in Introduction and Discussion

Abo-Salem, O. M., M. F.Abd-Ellah, and M. M. Ghonaim. 2011. Hepatoprotective activity of quercetin against acrylonitrite-induced hepatotoxycity in rats. J. Biochem. Mol. Toxicol. 25:6.

Abuelo, A., J. Hernández, J. L. Benedito, and C. Castillo. 2013. Oxidative stress index (OSi) as a new tool to assess redox status in dairy cattle during the transition period. Animal 7:1374-1378. http://dx.doi.org/10.1017/S1751731113000396.

Ader, P., A. Wessmann, and S. Wolffram. 2000. Bioavailability and metabolism of the flavonol quercetin in the pig. Free Radic. Biol. Med. 28:1056-1067. http://dx.doi.org/10.1016/S0891-5849(00)00195-7.

Aikman, P. C., C. K. Reynolds, and D. E. Beever. 2008. Diet digestibility, rate of passage, and eating and rumination behavior of Jersey and Holstein cows. J. Dairy. Sci. 91:1103-1114.

Allison, R. D., and R. A. Laven. 2000. Effect of vitamin E supplementation on the health and fertility of dairy cows: A review. Vet. Rec. 147:703-708.

Amelchanka, S. L., M. Kreuzer, and F. Leiber. 2010. Utility of buckwheat (*Fagopyrum esculentum Moench*) as feed: Effects of forage and grain on in vitro ruminal fermentation and performance of dairy cows. Anim. Feed Sci. Tech. 155:111-121. http://dx.doi.org/10.1016/j.anifeedsci.2009.10.007.

Ametaj, B. N., B. J. Bradford, G. Bobe, R. A. Nafikov, Y. Lu, J. W. Young, and D. C. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. Can. J. Anim. Sci. 85:165-175.

Aoki, M., T. Oshita, and M. Sakaguchi. 2008. The comparison of thiobarbituric acid reactive substances (TBARS) concentrations in plasma and serum from dairy cattle. J. Vet. Med. Sci. 70:107-110. http://dx.doi.org/10.1292/jvms.70.107.

Arias, N., M. T. Macarulla, L. Aguirre, J. Miranda, and M. P. Portillo. 2015. Liver delipidating effect of a combination of resveratol and quercetin in rats fed a obesogenic diet. J. Physiol. Biochem. 71:569-576.

Aydin, B. 2011. Quercetin prevents methotrexate-induced hepatotoxicity without interfering methotrexate metabolizing enzymes in liver of mice. JABS 5:75-80.

Balcells, J., A. Aris, A. Serrano, A. R. Seradj, J. Crespo, and M. Devant. 2012. Effects of an extract of plant flavonoids (Bioflavex) on rumen fermentation and performance in heifers fed high-concentrate diets. J. Anim. Sci. 90:4975-4984. http://dx.doi.org/10.2527/jas.2011-4955.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Begriche, K., A. Igoudjil, D. Pessayre, and B. Fromenty. 2006. Mitochondrial dysfunction in NASH: Causes, consequences and possible means to prevent it. Mitochondrion 6:1-28.

Berger L. M., S. Wein, R. Blank, C. C. Metges, and S. Wolffram. 2012. Bioavailability of the flavonol quercetin in cows after intraruminal application of quercetin aglycone and rutin. J. Dairy Sci. 95: 5047–5055. http://dx.doi.org/10.3168/jds.2012-5439.

Berger, L. M., R. Blank, F. Zorn, S. Wein, C. C. Metges, and S. Wolffram. 2015. Ruminal degradation of quercetin and its influence on fermentation in ruminants. J. Dairy Sci. . doi: 10.3168/jds.2015-9633.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy. Sci. 88:2017-2026.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91:3300-3310. http://dx.doi.org/10.3168/jds.2008-0995.

Bieger, J., R. Cermak, R. Blank, V. C. J. De Boer, P. C. H. Hollman, J. Kamhues, and S. Wolffram. 2008. Tissue Distribution of Quercetin in Pigs after Long-Term Dietary Supplementation. J. Nutr. 138:1417-1420.

Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited review: Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J. Dairy Sci. 87:3105-3124.

Boots, A. W., G. R. M. M. Haenen, and A. Bast. 2008. Health effects of quercetin: From antioxidant to nutraceutical. Eur. J. Pharmacol. 585:325-337. http://dx.doi.org/10.1016./j.ephar.2008.03.008.

Börner, S., E. Albrecht, C. Schäff, S. Hacke, U. Kautzsch, M. Derno, H. M. Hammon, M. Röntgen, H. Sauerwein and B. Kuhla. 2013. Reduced AgRP activation in the hypothalamus of cows with high extent of fat mobilization after parturition. Gen. Comp. Endocr. 193:167-177. http://dx.doi.org/ 10.1016/j.ygcen.2013.08.002.

Bravo, L. 1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56:11.

Broudiscou, L.-P., y. Papon, and A. F. Broudiscou. 2000. Effects of dry plant extracts on fermentation and methanogenesis in continuous culture of rumen microbes. Anim. Feed Sci. Technol. 87:263-277.

Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest. Prod. Sci. 83:211-218. http://dx.doi.org/10.1016/S0301-6226(03)00112-X.

Celi, P. 2011. Biomarkers of oxidative stress in ruminant medicine. Immunopharm. Immunot. 33:233-240. http://dx.doi.org/10.3109/08923973.2010.514917.

Cermak, R., S. Landgraf, and S. Wolffram. 2003. The bioavailability of quercetin in pigs depends on the glycoside moiety and on dietary factors. J. Nutr. 133:2802-2807.

Chen, X. 2010. Protective effects of quercetin on liver injury induced by ethanol. Phcog. Mag. 6:135-141. http://dx.doi.org/10.4103/0973-1296.62900.

Collard, B. L., P. J. Boettcher, J. C. M. Dekkers, D. Petitclerc. and L. R. Schaeffer. 2000. Relationships between energy balance and health traits of dairy cattle in early lactation. J. Dairy Sci. 83:2683-2690. http://dx.doi.org/10.3168/jds.S0022-0302(00)75162-9.

Cozzi, G., L. Ravarotto, F. Gottardo, A. L. Stefani, B. Contiero, L. Moro, M. Brscic, and P. Dalvit. 2011. Short communication: Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. J. Dairy Sci. 94:3895-3901. http://dx.doi.org/10.3168/jds.2010-3687.

Cui, Y. M., Y. Han, X. B. Yang, Y. F. Sun, and Y. Zhao. 2014. Protective effects of quercetin and quercetin-5',8-disulfonate against carbon tetrachloride-caused oxidative liver injury in mice. Molecules 19:291-305. http://dx.doi.org/10.3390/molecules19010291.

Cui, K., X. D. Guo, Y. Tu, N. F. Zhang, T. Ma and Q. Y. Diao. 2015. Effect of dietary supplementation of rutin on lactation performance, ruminal fermentation and metabolism in dairy cows. J. Anim. Physiol. Anim. Nutr. 99:1065-1073. http://dx.doi.org/ 10.1111/jpn.12334.

De Boer, V. C. J., A. A. Dihal, H. van der Woude, I. C. W. Arts, S. Wolffram, G. M. Alink, I. M. C. M. Rietjens, J. Keijer, and P. C. H. Hollman. 2005. Tissue distribution of quercetin in rats and pigs. J. Nutr. 135:1617-1618.

De David, C., G. Rodrigues, S. Bona, L. Meurer, J. Gonzalez-Gallego, M. J. Tunon, and N. P. Marroni. 2011. Role of quercetin in preventing thioacetamide-induced liver injury in rats. Toxicol. Pathol. 39:949-957. http://dx.doi.org/ 10.1177/0192623311418680.

Derno M., H. G. Elsner, E.-A. Paetow, H. Scholze, and M. Schweigel. 2009. Technical note: A new facility for continuous respiration measurements in lactating cows. J. Diary Sci. 92:2804-2808. http://dx.doi.org/10.3168/jds.2008-1839.

Derno M., G. Nürnberg, P. Schön, A. Schwarm, M. Röntgen, H.M. Hammon, C.C. Metges, R.M. Bruckmaier, and B. Kuhla. 2013. Short-term feed intake is regulated by

macronutrient oxidation in lactating Holstein cows. J. Dairy Sci. 96:971-980. http://dx.doi.org/10.3168/jds.2012-5727.

Djokovic, R., H. Samanc, M. Jovanovic, N. Fratric, V. Doskovic, and Z. Stanimirovic. 2013. Relationship among blood indicators of hepatic function and lipid content in the liver during transitional period in high-yielding dairy cows. Acta Sci. Vet. 41:1128.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 82:2259:2273.

Drackley, J. K., H. M. Dann. N. Douglas. N. A. Janovick Guretzky, N. B. Litherland, J. P. Underwood, and J. J. Loor. 2005. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Ital. J. Anim. Sci. 4:323-344.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92:571-580.

Dulloo, A. G., C. Duret, D. Rohrer, L. Girardier, N. Mensi, M. Fathi, P. Chantre, and J. Vandermander. 1999. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. Am. J. Clin. Nutr. 70:1040-1045.

Eckersall, P. D. 2000. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. Revue Méd. Vét. 151:577-584.

Egert, S., G. Rimbach, and M. J. Müller. 2011. No evidence for a thermic effect of the dietary flavonol quercetin: a pilot study in healthy normal-weight women. Eur. J. Appl. Physiol. 111:869-873. http://dx.doi.org/10.1007/s00421-010-1674-5.

Esposito, G., P. C. Irons, E.C. Webb, and A. Chapwanya. 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim. Reprod. Sci. 144:60-71. http://dx.doi.org/10.1016/j.anireprosci.2013.11.007.

Etim, N. N., E. E. A. Offiong, and U. A. Ukpanah. 2013. Farm animals: Culprits and victims of global warming and climate change. AJAAR 1:10-24.

Fabjan, N., J. Rode, I. J. Kosir, Z. H. Wang, Z. Zhang, and I. Kreft. 2003. Tartary buckwheat (*Fagopyrum tartaricum Gaertn*.) as a source of dietary rutin and quercitrin. J. Agric. Food. Chem. 51:6452-6455. http://dx.doi.org/10.1021/jf034543e.

Fiorani, M., A. Guidarelli, M. Blasa, C. Azzolini, M. Candiracci, W. Piatti, and O. Cantoni. 2010. Mitochondria accumulate large amounts of quercetin: Prevention of mitochondrial

damage and release upon oxidation of the extramitochondrial fraction of the flavonoid. J. Nutr. Biochem. 21:397-404. http://dx.doi.org/10.1016/j.jnutbio.2009.01.014.

Formica, J. V., and W. Regelson. 1995. Review of the biology of quercetin and related bioflavonoids. Fd. Chem. Toxic. 33:1061-1080. http://dx.doi.org/10.1016/0278-6915(95)00077-1.

Gessner, D. K., C. Koch, F.-J. Romberg, A. Winkler, G. Dusel, E. Herzog, E. Most, and K. Eder. 2015. The effect of grape seed and grape marc meal extract on milk performance and the expression of genes of endoplasmic reticulum stress and inflammation in the liver of dairy cows in early lactation. J. Dairy Sci. 98:8856-8868. http://dx.doi.org/10.3168/jds.2015-9478.

Goff, J. P., and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80:1260:1268.

Gohlke A., C. J. Ingelmann, G. Nürnberg, A. Starke, S. Wolffram, and C. C. Metges. 2013a. Bioavailability of quercetin from its aglycone and its glucorhamnoside rutin in lactating dairy cows after intraduodenal administration. J. Diary Sci. 96:1-11. http://dx.doi.org/10.3168/jds.2012-6234.

Gohlke A., C. J. Ingelmann, G. Nürnberg, J. M. Weitzel, H. M. Hammon, S. Görs, A. Starke, S. Wolffram, and C. C. Metges. 2013b. Influence of 4-week intraduodenal supplementation of quercetin on performance, glucose metabolism, and mRNA abundance of genes related to glucose metabolism and antioxidative status in dairy cows. J. Dairy Sci. 96:6986-7000. http://dx.doi.org/10.3168/jds.2013-6852.

Gonzalez, F. D., R. Muino, V. Pereira, R. Campos, and J. L. Benedito. 2011. Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. J. Vet. Sci. 12:251.255. http://dx.doi.org/10.4142/jvs.2011.12.3.251.

Graber, M., S. Kohler, T. Kaufmann, M. G. Doherr, R. M. Bruckmaier, and H. A. van Dorland. 2010. A field study on characteristics and diversity of gene expression in the liver of dairy cows during the transition period. J. Dairy Sci. 93:5200-5215. http://dx.doi.org/10.3168/jds.2010-3265.

Graugnard, D. E., K. M. Moyes, E. Trevisi, M. J. Khan, D. Keisler, J. K. Drackley, G. Bertoni, and J. J. Loor. 2013. Liver lipid content and inflammometabolic indices in peripartal dairy cows are altered in response to prepartal energy intake and postpartal intramammary inflammatory challenge. J. Dairy Sci. 96:1-18. http://dx.doi.org/10.3168/jds.2012-5676.

Greathead, H. 2003. Plants and plant extracts for improving animal productivity. Proc. Nutr. Soc. 62:279-290. http://dx.doi.org/10.1079/PNS2002197.

Gross, J. J., F. J. Schwarz, K. Eder, H. A. van Dorland, and R. M. Bruckmaier. 2013. Liver fat content and lipid metabolism in dairy cows during early lactation and during a mid-lactation feed restriction. J. Dairy Sci. 96:5008-5017. http://dx.doi.org/10.3168/jds.2012-6245.

Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882-3896. http://dx.doi.org/10.3168/jds.S0022-0302(93)77729-2.

Guo, X. D., Q.Y. Diao; Y. Tu, G. L. Yan N. F. Zhang, K. D. Deng, C. F. Ju, G. Q. Zhao Y. Y. Wang, X. W. Gu, Z. Y. Lu, and X. J. Wang. 2010. Effect of rutin supplementation on lactation performance and serum parameters in lactating Chinese Holstein cows. Sci. Agric. Sin. 43: 5137-5146.

Halliwell, B. 1991. Reactive oxygen species in living systems: Source, biochemistry, and role in human disease. Am. J. Med. 91:S14-S22.

Halliwell, B., and S. Chirico. 1993. Lipid peroxidation: Its mechanism, measurement, and significance. Am. J. Clin. Nutr. 57:715S-25S.

Hassan, W., G. Rongyin, A. Daoud, L. Ding, L. Wang, J. Liu, and J. Shang. 2014. Reduced oxidative stress contributes to the lipid lowering effects of isoquericitrin in free fatty acids induced hepatocytes. Oxid. Med. Cell. Longev. 313602. http://dx.doi.org/10.1155/2014.313602.

Harborne, J. B., and C. A. Williams. 2000. Advances in flavonoid research since 1992. Phytochem. 55:481-504. http://dx.doi.org/10.1016/S0031-9422(00)00235-1.

Heeba, G. H., M. E. Mahnoud, and A. A. El Hanafy. 2014. Anti-inflammatory potential of curcumin and quercetin in rats: Role of oxidative stress, heme oxygenase-I and TNF-α. Toxicol. Ind. Health 30:551-560. http://dx.doi.org/1/0.1177/0748233712462444.

Heim, K. E., A. R. Tagliaferro, and D. J. Bobilya. 20nb 02. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. 13:572-584. http://dx.doi.org/10.1016/S0955-2863(02)00208-5.

Hiss, S., C. Weinkauf, S. Hachenberg, and H. Sauerwein. 2009. Relationship between metabolic status and the milk concentrations of haptoglobin and lactoferrin in dairy cows during early lactation. J. Dairy Sci. 92 :4439–4443. http://dx.doi.org/10.3168/jds.2008-1632.

Hoek-van den Hil, E. F., J. Keijer, A. Bunschoten, J. J. M. Vervoort, B. Stankova, M. Bekkenkamp, L. Herreman, D. Venema, P. C. H. Hollman, E. Tvrzicka, I. M. C. M.

Rietjens, and E. M. van Schothorst. 2013. Quercetin induces hepatic lipid omega-oxidation and lowers serum lipid levels in mice. PLoS ONE 8:e51588. http://dx.doi.org/10.1371/journal.pone.0051588.

Hoek-van den Hil, E. F., E. M. van Schothorst, I. van der Stelt, H. J. M. Swarts, D. Venema, M. Sailer, J. J. M. Vervoort, P. C. H. Hollman, I. M. C. M. Rietjens, and J. Keijer. 2014. Quercetin decreases high-fat diet induced body weight gein and accumulation of hepatic and circulating lipids in mice. Genes Nutr. 9:418.

Hursel, R., W. Viechtbauer, A. G. Dulloo, A. Tremblay, L. Tappy, W. Rumpler, and M. S. Westerterp-Plantenga. 2011. The effects of catechin rich teas and caffeine on energy expenditure and fat oxidation: a meta-analysis. Obes. Rev.12:e573-e581. http://dx.doi.org/10.1111/j.1467-789X.2011.00862.x.

Ingvartsen, K. L., and K. M. Moyes. 2015. Factors contributing to immunosuppression in the dairy cow during the periparturient period. Jpn. J. Vet. Res. 63:S15-S24. http://dx.doi.org/10.14943/jjvr.63.suppl.s15.

Janssen, L. J. 2001. Isoprostanes: An overview and putative roles in pulmonary pathophysiology. Am. J. Physiol. Lung Cell Mol. Physiol. 280:L1067-L1082.

Jentsch, W., M. Derno, and O. Weiher. 2001. Heat production of dairy cows in dependence on milk yield – A study. Arch. Tierz. 44:599-610.

Jovanovic, B. I., M. Velickovic, S. Milanovic, O. Valcic, D. Gvozdic, and S. Vranjes-Duric. 2015. Supplemental selenium reduces the levels of biomarkers of oxidative and general stress in peripartum dairy cows. Acta Vet. (Beogr.). 65:191-201. http://dx.doi.org/10.1515/acve-2015-0016.

Jung, C. H., I. Cho, J. Ahn, T. Jeon, and T. Ha. 2013. Quercetin reduces high-fat diet-induced fat accumulation in the liver by regulating lipid metabolism genes. Phytother. Res. 27:139-142. http://dx.doi.org/10.1002/ptr.4687.

Kälber, T., M. Kreuzer, and F. Leiber. 2012. Silages containing buckwheat and chicory: Quality, digestibility and nitrogen utilization by lactating cows. Arch. Anim. Nutr. 66:50-65. http://dx.doi.org/10.1080/1745039X.2011.630213.

Kälber, T., M. Kreuzer, and F. Leiber. 2013. Effect of feeding buckwheat and chicory silages on fatty acid profile and cheese-making properties of milk from dairy cows. J. Dairy Res. 0:1-8.

Katoh, N. 2002. Relevance of apolipoproteins in the development of fatty liver and fatty liver-related peripartum diseases in dairy cows. J. Vet. Med. Sci. 64:293-307. http://dx.doi.org/10.1292/jvms.64.293.

Kawabata, K., R. Mukai, and A. Ishisaka. 2015. Quercetin and related polyphenols: New insights and implications for their bioactivity and bioavailability. Food Funct. 6:1399-1417. http://dx.doi.org/10.1039/C4FO01178C.

Kim, D., E. Jung, I. Sohng, J. Han, T. Kim, and M. J. Han. 1998. Intestinal bacterial metabolism of flavonoids and its relation to some biological activities. Arch. Pharm. Res. 21:17-23.

Kim, C., Y. Kwon, S. Choe, S. Hong, H. Yoo, T. Goto, T. Kawada, H. Choi, Y. Joe, H. T. Chung, and R. Yu. 2015. Quercetin reduses obesity-induced hepatosteatosis by enhancing mitochondrial oxidative metabolism via heme oxygenase-I. Nutr. Metab. 12:33. http://dx.doi.org/10.1186/s12986-015-0030-5.

Klaus, S., S. Pultz, C. Thone-Reineke, and S. Wolffram. 2005. Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. Int. J. Obesity 29:615-623. http://dx.doi.org/10.1038/sj.ijo.0802926.

Kobayashi, Y., M. Miyazawa, A. Kamei, K. Abe, and T. Kojima. 2010. Ameliorative Effects of Mulberry (Morus alba L.) Leaves on hyperlipidemia in rats fed a high-fat diet: Induction of fatty acid oxidation, inhibition of lipogenesis, and suppression of oxidative stress. Biosci. Biotechnol. Biochem. 74:2385-2395. http://dx.doi.org/10.1271/bbb.100392.

Kobori, M., Y. Takahashi, Y. Akimoto, M. Sakurai, I. Matsunaga, H. Nishimuro, K. Ippoushi, H. Oike, and M. Ohnishi-Kameyama. 2015. Chronic high intake of quercetin reduces oxidative stress and induces expression of the antioxidant enzymes in the liver and visceral adipose tissues in mice. J. Funct. Foods 15:551-560. http://dx.doi.org/10.1016/j.jff.2015.04.006.

Konvicná, J., M. Vargová, I. Pauliková, G. Kovác, and Z. Kostecká. 2015. Oxidaitve stress and antioxidant status in dairy cows during prepartal and postpartal periods. Acta Vet. Brno 84:133-140. http://dx.doi.org/10.2754/avb201584020133.

Lake, A. D., P. Novak, C. D. Fisher, J. P. Jackson, R. N. Hardwick, D. D. Billheimer, W. T. Klimecki, and N. J. Cherrington. 2011. Analysis of global and absorption, distribution, metabolism, and elimination gene expression in the progressive stages of human nonalcoholic fatty liver disease. Drug Metab. Dispos. 39:1954-1960.

Lakhanpal, P., and D. K. Rai. 2007. Quercetin: A versatile flavonoid. IJMU 2: No.2.

Leiber, F., C. Kunz, and M. Kreuzer. 2012. Influence of different morphological parts of buckwheat (*Fagopyrum esculentum*) and its major secondary metabolite rutin on rumen fermentation in vitro. Czech J. Anim. Sci. 57: 10–18.

Lesser, S., and S. Wolffram. 2006. Oral Bioavailability of the Flavonol Quercetin. Curr. Top. Nutraceut. R. 4:239-256.

Li, P., X. B. Li, S. X. Fu, C. C. Wu, X.X. Wang, G. J. Yu, M. Long, Z. Wang, and G. W. Liu. 2012. Alterations of fatty acid β-oxidation capability in the liver of ketotic cows. J. Dairy Sci. 95:1759-1766. http://dx.doi.org/10.3168/jds.2011-4580.

Lin,Y., S. Hsiu, Y. Hou, H. Chen, and P. L. Chao. 2003. Degradation of flavonoid aglycones by rabbit, rat and human fecal flora. Biol. Pharm. Bull. 26:747-751.

Liu, P., B. X. He, X. L. Yang, X. L. Hou, H. Y. Zhao, Y. H. Han, P. Nie, H. F. Deng, and L. Cheng. 2012. Activities of aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, alkaline phosphatase in plasma of postpartum Holstein cows. J. Anim. Vet Adv. 11:1270-1274.

Löhrke, B., T. Viergutz, W. Kanitz, B. Losand, D. G. Weiss, and M. Simko. 2005. Short communication: Hydroperoxides in circulating lipids from dairy cows: Implications for bioactivity of endogenous-oxidized lipids. J. Dairy Sci. 88:1708-1710.

Loor, J. J., H. M. Dann, R. E. Everts, R. Oliveira, C. A. Green, N. A. J. Guretzky, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. Physiol. Genomics 23:217-226. http://dx.doi.org/10.1152/physiolgenomics.00132.2005.

Loor, J. J., H. M. Dann, N. A. Janovick Guretzky, R. E. Everts. R. Oliveira, C. A. Green, N. B. Litherland, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2006. Plane of nutrition prepartum alters hepatic gene expression and function in dairy cows as assessed by longitudinal transcript and metabolic profiling. Physiol. Genomics 27:29-41. http://dx.doi.org/10.1152/physiolgenomics.00036.2006.

Loor, J. J., R. E. Everts, M. Bionaz, H. M. Dann, D. E. Morin, R. Oliveira, S. L. Rodriguez-Zas, J. K. Drackley, and H. A. Lewin. 2007. Nutrition-induced ketosis alters metabolic and signaling gene networks in liver of periparturient dairy cows. Physiol. Genomics. 32:105-116. http://dx.doi.org/10.1152/physiolgenomics.00188.2007.

Lykkesfeldt, J., and O. Svendsen. 2007. Oxidants and antioxidants in disease: Oxidative stress in farm animals. Vet. J. 173:502-511. http://dx.doi.org/ 10.1016/j.tvjl.2006.06.005.

Ma, Y., L. Song, M. Gao, and L. Wang. 2015. Effect of oxidative stress on transition dairy cows mastitis and its regulation mechanisms. Chin. J. Anim. Nutr. 27:671-676.

Manach, C., A. Scalbert, C. Morand, C. Rémésy, and L. Jiménez. 2004. Polyphenols: Food sources and bioavailability. Am. J. Clin. Nutr. 79:727-747.

Marcolin, E., L. F. Forgiarini, G. Rodrigues, J. Tieppo, G. S. Borghetti, V. L. Bassani, J. N. Picada, and N. P. Marroni. 2013. Quercetin decreases liver damage in mice with non-alcoholic steatohepatitis. Basic Clin. Pharmacol. Toxicol. 112:385-391. http://dx.doi.org/10.1111/bcpt.12049.

Materska, M. 2008. Quercetin and its derivatives: Chemical structure and bioactivity – A review. Pol. J. Food Nutr. Sci. 58:407-413.

McArt, J. A. A., D. V. Nydam, and G. R. Oetzel. 2012. Epidemiology of subclinical ketosis in early lactation dairy cattle. J. Dairy Sci. 95:5056-5060. http://dx.doi.org/10.3168/jds.2012-5443.

McArt, J. A. A., D. V. Nydam, and M. W. Overton. 2015. Hyperketonemia in early lactation dairy cattle: A deterministic estimate of component and total cost per case. J. Dairy Sci. 98:2043-2054. http://dx.doi.org/10.3168/jds.2014-8740.

Merrel, M. D., and N. J. Cherrington. 2011. Drug metabolism alterations in nonalcoholic fatty liver disease. Drug Metab. Rev. 43:317-334. http://dx.doi.org/10.3109/03602532.2011.577781.

Miles, S. L., M. McFarland, and R. M. Niles. 2014. Molecular and physiological actions of quercetin: Need for clinical trials to assess its benefits in human disease. Nutr. Rev. 72:720-734. http://dx.doi.org/10.111/nure.12152.

Miller, J. K., E. Brzezinska-Slebodzinska, and F. C. Madsen. 1993. Oxidative Stress, antioxidants, and animal Function. J. Dairy Sci. 76:2812-2823.

Mostafavi, M., H. A. Seifi, M. Mohri, and A. Jamshidi. 2013. Optimal thresholds of metabolic indicators of hepatic lipidosis in dairy cows. Revua Méd. Vét. 164:564-571.

Narayana, K. R., M. S. Reddy, M. R. Chaluvadi, and D. R. Krishna. 2001. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J. Pharmacol. 33:2-16.

Nijveldt, R. J., E. van Nood, D. E. C. van Hoorn, P. G. Boelens, K. van Norren, and P. M. van Leeuwen. 2001. Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. 74:418-425.

Ok, M., I. Sen, H. Guzelbektes, M. Boydak, C. Er, U. Aydogdu, and R. Yildiz. 2013. The importance of concentrations of sorbitol dehydrogenase and glutamate dehydrogenase and B-mode ultrasonographic examination in the diagnosis of hepatic lipidosis in dairy cows. Kafkas Univ. Vet. Fak. Derg. 19:A117-A123. http://dx.doi.org/10.9775/kvfd.2012.8146.

Oskoueian, E., N. Abdullah, and A. Oskoueian. 2013. Effects of flavonoids on rumen fermentation activity, methane production, and microbial population. Biomed. Res. Int. 2013: 349129. http://dx.doi.org/10.1016/10.1155/2013/349129.

Pan, M. H., C. S. Lai, M. L. Tsai, and C. T. Ho. 2014. Chemoprevention of nonalcoholic fatty liver disease by dietary natural compounds. Mol. Nutr. Res. 58:147-171. http://dx.doi.org/10.1002/mnfr.201300522.

Peres, W., M. J. Tunon, P. S. Collado, S. Herrmann, N. Marroni, and J. Gonzalez-Gallego. 2000. The flavonoid quercetin ameliorates liver damage in rats with biliary obstruction. J. Hepatol. 33:742-750. http://dx.doi.org/10.1016/S0168-8278(00)80305-0.

Rathee, P., H. Chaudhary, S. Rathee, D. Rathee, V. Kumar, and K. Kohli. 2009. Mechanism of action of flavonoids as anti-inflammatory agents: A review. Inflamm. Allergy Drug Targets. 8:229-235.

Reinboth, M., S. Wolffram, G. Abraham, F. R. Ungemach, and R. Cermak. 2010. Oral bioavailability of quercetin from different quercetin glycosides in dogs. Br. J. Nutr. 104:198-203. http://dx.doi.org/10.1017/S000711451000053X.

Rochfort, S., A. J. Parker, and F. R. Dunshea. 2008. Plant bioactives for ruminant health and productivity. Phytochem. 69:299-322. http://dx.doi.org/10.1016/j.phytochem.2007.08.017.

Russo, M., C. Spagnuolo, I. Tedesco, S. Bilotto, and G. L. Russo. 2012. The flavonoid quercetin in disease prevention and therapy: Facts and fancies. Biochem. Pharmacol. 83:6-15. http://dx.doi.org/10.1016/j.bcp.2011.08.010.

Santra, A., A. Saikia, and K. K. Baruah. 2012. Scope of rumen manipulation using medicinal plants to mitigate methane production. J. Pharmacogn. 3:115-120.

Scalbert, A., and G. Williamson. 2000. Dietary intake and bioavailability of polyphenols. J. Nutr. 130:2073S-2085S.

Schäff, C., S. Börner, S. Hacke, U. Kautzsch, D. Albrecht, H. M. Hammon, M. Röntgen, and B. Kuhla. 2012. Increased anaplerosis, TCA cycling, and oxidative phosphorylation in the liver of dairy cows with intensive body fat mobilization during early lactation. J. Proteome Res. 11:5503−5514.

Seradj, A. R., L. Abecia, J. Crespo, D. Villalba, M. Fondevila, and J. Balcells. 2014. The effect of Bioflavex and its pure flavonoid components on in vitro fermentation parameters and methane production in rumen fluid from steers given high concentrate diets. Anim. Feed Sci. Tech. 197:85-91. http://dx.doi.org/10.1016/j.anifeedsci.2014.08.013.

Shao, L., K. Liu, F. Huang, X. D. Guo, M. Wang, and B. L. Liu. 2013. Opposite effects of quercetin, luteolin, and epigallocatechin gallate on insulin sensitivity under normal and inflammatory conditions in mice. Inflammation 36:1-14. http://dx.doi.org/10.1007/s10753-012-9514-x.

Sharma, N., N. K. Singh, O. P. Singh, V. Pandey, and P. K. Verma. 2011. Oxidative stress and antioxidant status during transistion period in dairy cows. Asian-Aust. J. Anim. Sci. 24:479-484.

Sikder, K. S. B. Kesh, N. Das, K. Manna, and S. Dey. 2014. The high antioxidative power of quercetin (aglycone flavonoid) and its glycone (rutin) avert high cholesterol diet induced hepatotoxicity and inflammation in Swiss albino mice. Food Funct. 5:1294-1303. http://dx.doi.org/10.1039/c3fo60526d.

Simonov, M., and V. Vlizlo. 2015. Some blood markers of the functional state of liver in dairy cows with clinical ketosis. Bulg. J. Vet. Med. 18:74-82. http://dx.doi.org/10.15547/bjvm.814.

Sordillo, L. M., and S. L. Aitken. 2009. Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol. 128:104-109. http://dx.doi.org/10.1016/j.vetimm.2008.10.305.

Spears, J. W., and W. P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 176:70-76. http://dx.doi.org/10.1016/j.tvj1.2007.12.015.

Stewart, L. K., J. L. Soileau, D. Ribnicky, Z. Q. Wang, I. Raskin, A. Poulev, M. Majewski, W. T. Cefalu, and T. W. Gettys. 2008. Quercetin transiently increases energy expenditure but persistently decreases circulating markers of inflammation in C57BL/6J mice fed a high-fat diet. Metabolism 57:S39-S46. http://dx.doi.org/10.1016/j.metabol.2008.03.003.

Surapaneni, K. M., and M. Jainu. 2014. Comparative effect of pioglitazone, quercetin and hydroxyl citric acid on the status of lipid peroxidation and antioxidants in experimental non-alcoholic steatohepatitis. J. Physiol. Pharmacol. 65:67-74.

Suthar, V. S., J. Canelas-Raposo, A. Deniz, and W. Heuwieser. 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in european dairy cows. J. Dairy Sci. 96:2925-2938. http://dx.doi.org/10.3168/jds.2012-6035.

Szczubial, M. 2010. Non-enzymatic antioxidative defense mechanism in plasma of pigs during periparturient period: Vitamin C and glutathione. Bull. Vet. Inst. Pulawy 54:373-376.

Turk, R., O. Podpecan, J. Mrkun, M. Kosec, Z. Flegar-Mestric, S. Perkov, J. Staric, M. Robic, M. Belic, and P. Zrimsek. 2013. Lipid mobilization and oxidative stress as metabolic adaptation processes in dairy heifers during transition period. Anim. Reprod. Sci. 141:109-115. http://dx.doi.org/10.1016/j.anireprosci.2013.07.014.

Van der Drift, S. G. A., M. Houweling, J. T. Schonewille, A. G. M. Tielens, and R. Jorritsma. 2012. Protein and fat mobilization and assosciations with serum β-hydroxybutyrate concentrations in dairy cows. J. Dairy Sci. 95:4911-4920. http://dx.doi.org/10.3168/jds.2011-4771.

Van Dorland, H. A., H. Sadri, I. Morel, and R. M. Bruckmaier. 2012. Coordinated gene expression in adipose tissue and liver differs between cows with high or low NEFA concentrations in early lactation. J. Anim. Physiol. An. N. 96:137-147. http://dx.doi.org/10.1111/j.1439-0396.2011.01130.x.

Vendemiale, G., I. Grattagliano, P. Caraceni, G. Caraccio, M. Domenicali, M. Dall'Agata, F. Trevisani, F. Guerrieri, M. Bernardi, and E. Altomare. 2001. Mitochondrial oxidative injury and energy metabolism alteration in rat fatty liver: Effect of the nutritional status. Hepatology 33:808-815. http://dx.doi.org/10.1053/jhep.2001.23060.

Vernon, R. G. 2005. Lipid metabolism during lactation: A review of adipose tissue-liver interactions and development of fatty liver. J. Dairy Res. 72: 460-469. http://dx.doi.org/10.1017/S0022029905001299.

Vernuft, A., T. Viergutz, C: Plinski, and J. M. Weitzel. 2014. Postpartum levels of 8-iso-prostaglandin $F_{2\alpha}$ in plasma and milk phospholipid fractions as biomarker of oxidative stress in first-lactating dairy cows. Prostaglandins Other Lipid Mediat. 112:34-38. http://dx.doi.org/10.1016/j.prostaglandines.2014.07.004.

Walsh, R. B., J. S. Walton, D. F. Kelton, S. J. LeBlanc, K. E. Leslie, and T. F. Duffield. 2007. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. J. Dairy Sci. 90:2788-2796. http://dx.doi.org/10.3168/jds.2006-560.

Wang, D. S., J. L. Huang, Z. H. Zhang, X. J. Tian, H. Huang, Y. Z. Yu, G. H. Zhang, J. N. Ding, and R. L. Huang. 2013. Influences of *Portulaca oleracea* extracts on in vitro methane emissions and rumen fermentation of forage. J. Food Agric. Environ. 11:483-488.

Wathes, D. C., M. Fenwick, Z. Cheng, N. Bourne, S. Llewellyn, D. G. Morris, D. Kenny, J. Murphy, and R. Fitzpatrick. 2007. Influence of negative energy balance on cyclicity and

fertility in the high producing dairy cow. Theriogenology 68S:S232-S241. http://dx.doi.org/10.1016/j.theriogenology.2007.04.006.

Weber C., C. Hametner, A. Tuchscherer, B. Losand, E. Kanitz, W. Otten, S.P. Singh, R.M. Bruckmaier, F. Becker, W. Kanitz, and H.M. Hammon. 2013. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. J. Dairy Sci. 96:165–180. http://dx.doi.org/10.3168/jds.2012-5574.

Wein, S., and S. Wolffram. 2013. Concomitant Intake of Quercetin with a Grain-Based Diet Acutely Lowers Postprandial Plasma Glucose and Lipid Concentrations in Pigs. Biomed. Res. Int. Article ID 748742. http://dx.doi.org/10.1155/2014/748742.

Wiczkowski, W., D. Szawara-Nowak, J. Topolska, K. Olejarz, H. Zielinski, and M. K. Piskula. 2014. Metabolites of dietary quercetin: Profile, isolation, identification, and antioxidant capacity. J. Funct. Foods 11:121-129. http://dx.doi.org/10.1016/j.jff.2014.09.013.

Xu, M. L., J. J. Hu, W. W. Zhao, X. J. Gao, C. H. Jiang, K. Liu, B. L. Liu, and F. Huang. 2014. Quercetin differently regulates insulin-mediated glucose transporter 4 translocation under basal and inflammatory conditions in adipocytes. Mol. Nutr. Food Res. 58:931-941. http://dx.doi.org/10.1002/mnfr.201300510.

Zou, W., W. Liu, B. Yang, L. Wu, J. Yang, T. Zou, F. Liu, L. Xia, and D. Zhang. 2015. Quercetin protects against perfluorooctanoic acid-induced liver injury by attenuating oxidative stress and inflammatory response in mice. Int. Immunopharmacol. 28:129-135. http://dx.doi.org/10.1016/j.intimp.2015.05.043.

9 Publication list

Abstracts

Stoldt, A., A. Starke, S. Wolffram, and C. C. Metges. Effects of the flavonoid Quercetin on energy metabolism and oxidative stress in periparturient dairy cows. 2014. 15th Day of the Doctoral Student, Leibniz Institute for Farm Animal Biology.

Stoldt A., M. Derno, G. Nürnberg, A. Starke, S. Wolffram, and C. C. Metges. Effects of 6-wk intraduodenal supplementation of quercetin on blood parameters and energy metabolism in periparturient dairy cows. 2015. Proceedings of the Society of Nutrition Physiology 24, 2015.

Stoldt, A., M. Mielenz, A. Starke, S. Wolffram, and C. C. Metges. Effects of a 6-week duodenal supplementation of quercetin on metabolic stress and liver health in peripartal dairy cows. 2015. ADSA-ASAS Joint Annual Meeting 2015. 318. J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2.

Stoldt A, M. Derno, G. Nürnberg, A. Starke, S. Wolffram, C. C. Metges. Effects of quercetin on liver health in dairy cows during periparturient period. 2015. 66th Annual Meeting of the European Federation of Animal Science (EAAP).

Research articles

Stoldt, A., M. Derno, G. Nürnberg, J. M. Weitzel, W. Otten, A. Starke, S. Wolffram, and C. C. Metges. 2015. Effects of a 6-wk intraduodenal supplementation with quercetin on energy metabolism and indicators of liver damage in periparturient dairy cows. J. Dairy Sci. 98(7):4509-20.

Stoldt, A., M. Derno, G. Das, J. M. Weitzel, S. Wolffram, and C. C. Metges. 2015. Effects of rutin and buckwheat seeds on energy metabolism and methane production in dairy cows. J. Dairy Sci. J. Dairy Sci. TBC:1–8.

Stoldt, A., M. Mielenz, G. Nürnberg, H. Sauerwein, T. Esatbeyoglu, A. E. Wagner, G. Rimbach, A. Starke, S. Wolffram, and C. C. Metges. Effects of a 6-wk intraduodenal supplementation with quercetin on liver lipid metabolism and oxidative stress in peripartal dairy cows. J Anim Sci. 2016 May;94(5):1913-23.

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11 Declaration of independence

I hereby confirm that the present work was solely composed by my own. I certify that I have used only the specified sources and aids.

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen Anspruch genommen habe.

Rostock, 23.2.2016

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