Aus dem Institut für Veterinär-Physiologie
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Effects of the Flavonol Quercetin on Glucose Metabolism and Health Status in Neonatal Calves

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

vorgelegt von
Jeannine Gruse
Tierärztin
aus Räckelwitz

Berlin 2016

Journal-Nr.: 3914

Gedruckt mit Genehmigung

des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Univ.-Prof. Dr. Jörg Aschenbach
Zweiter Gutachter:	PD Dr. Harald M. Hammon
Dritter Gutachter:	Univ.-Prof. Dr. Jürgen Zentek

Deskriptoren (nach CAB-Thesaurus):
calves, Holstein-Friesian, newborn animals, cow colostrum, feeding habits, feed additives, phytochemicals, quercetin, glucose, xylose, digestive absorption, blood glucose (MeSH), metabolism, nutrient nutrient interactions, nutrition physiology, biopsy, polymerase chain reaction, clinical trials

Tag der Promotion: 24.10.2016

Coverbild: © greenpapillion, fotolia.com

CONTENT

LIST OF TABLES

Appendix

LIST OF FIGURES

ABBREVIATIONS

8-iso-$PGF_{2\alpha}$	8-epimer of Prostaglandin $F_{2\alpha}$
ANOVA	Analysis of variance
ASCE	ascorbic acid equivalents
AUC	area under the curve
BW	body weight
C/Col	colostrum
CAT	catalase (gene)
cDNA	complementary DNA
CRP	C-reactive protein (gene)
CV	coefficient of variation
DM	dry matter
ELISA	enzyme-linked immunosorbent assay
Emax	maximal enrichment
F/For	formula
FBPase	frucose-1,6-bisphosphatase (protein)
FGA	fibrinogen alpha chain (gene)
FPU	first pass uptake
FRAP	ferric reducing ability of plasma
G6Pase	glucose-6-phosphatase (protein)
G6PC	glucose-6-phosphatase (gene)
GCK/GCK	glucokinase
GIP	glucose-dependent insulinotropic polypeptide
GIT	gastrointestinal tract
GLUT	faciliated glucose transporter
GNG	gluconeogenesis
GPX1	glutathione peroxidase 1 (gene)
H_2O_2	hydrogen peroxide
HP	haptoglobin (gene)
HPCAL1	hippocalcin-like 1 (gene)
HPLC	high-performance liquid chromatography
i.m.	intramuscular
i.v.	intravenous
Ig	immunoglobulin
IGF	insulin-like growth factor
IL1A	interleukin-1α (gene)

IL1B	interleukin-1β (gene)
LRP10	low-density lipoprotein receptor-related protein 10 (gene)
LSM	least squares mean
MDAE	malondialdehyde equivalents
NEFA	non-esterified fatty acids
PC	pyruvate carboxylase (protein)
PCK1	phosphoenolpyruvate carboxykinase, cytosolic (gene)
PCK2	phosphoenolpyruvate carboxykinase, mitochondrial (gene)
PCR	polymerase chain reaction
PEPCK	phosphoenolpyruvate carboxykinase (protein)
PGF	prostaglandin F
POLR2A	RNA polymerase II (gene)
PYG	glycogen phosphorylase (protein)
PYGL	glycogen phosphorylase (gene)
Q-	not supplemented with quercetin
Q+	supplemented with quercetin
Ra	rate of appearance
RIA	radioimmunoassay
ROS	reactive oxygen species
s.c.	subcutaneous
SAA	serum-amyloid A (protein)
SAA2	serum-amyloid A2 (gene)
SE(M)	standard error (of the mean)
SGLT	sodium-dependent glucose transporter
SOD	superoxide dismutase (gene)
TBARS	thiobarbituric acid reactive species
TE	trolox equivalents
TEAC	trolox equivalent antioxidative capacity
Tmax	time at maximal enrichment
TNF	tumor necrosis factor (gene)
TNFα	tumor necrosis factor α (protein)

1. GENERAL INTRODUCTION

Calf losses are still a major problem in the dairy industry, seeing that the overall preweaning calf mortality on U.S. dairy operations is approximately 15% [1]. Newborn calves are particularly prone to sickness, with gastrointestinal imbalances, especially neonatal diarrhea, being responsible for more than 50% of the mortality rate in neonates [2]. The reasons for this are manifold, ranging from infectious to noninfectious causes. After birth, the calf loses the relative protection by the uterus, which entails three fundamental consequences: first, its energy supply shifts from a continuous flow of mainly glucose and amino acids to a discontinuous provision with fat and lactose, the latter being insufficient to meet the neonate's glucose demands [3]. Thus, endogenous glucose production needs to be established fast. Second, the newborn is for the first time exposed to a germ-rich environment, and the acquisition of passive immunity is essential to support the calf's naïve immune system during the first weeks of life [4]. Third, the process of birth itself causes the excessive production of reactive oxygen species, which promote the susceptibility to disease if they are not properly counterbalanced by antioxidative defenses [5]. With the ingestion of first colostrum, the calf is not only provided with macronutrients but also with bioactive factors, e.g. hormones and growth factors supporting the maturation of its gastrointestinal tract and the adaptation of its glucose metabolism [6]. Furthermore, colostrum contains antioxidants and immunoglobulins to support neonatal health; unfortunately, the colostrum supply is often insufficient [7, 8].

The prophylactic application of antibiotic performance enhancers was banned by the European Union in 2006; since then, research has been intensified to identify natural alternatives for enhancing the productivity in farm animal rearing. Special focus has been directed on flavonoids for their attributed health-promoting properties [9]. Flavonoids are polyphenolic plant metabolites with strong antioxidative potential and are involved in the actions of plant enzymes, growth hormones and antimicrobial defense [10]. Because of their antioxidative, antiinflammatory, immunomodulatory and antimicrobial activities, flavonoids have long been used by traditional medicine [11]. In humans, the incidence of some chronic diseases, e.g. obesity, mental and neurological disorders, seems to be lower with increasing dietary flavonoid intake [12, 13]. Furthermore, flavonoids are known to interact with glucose metabolism and to normalize the blood glucose level in diabetic individuals, e.g. by suppressing hepatic glucose release and reducing intestinal carbohydrate digestion, thus they ameliorate metabolic conditions in case of diabetes type II [14]. In farm animals, the usage of flavonoid-rich extracts decreases the number of deaths in calves [15] and exerts immunostimulatory effects in sows and their offspring [16]. Other feed additives with antioxidative potential, e.g. tocopherol, have been shown to improve the performance of farm animals [9]. However, to date, there are no studies on the impact of quercetin on the glucose metabolism and immune

system of newborn farm animals.

The aim of the present work was to characterize the health promoting properties of an oral quercetin supplementation in neonatal calves with regard to its impact on the neonate's glucose metabolism, because any disadvantageous effects of quercetin supplementation on the same would forbid the usage of flavonoids in the upbringing of newborn calves. Another aspect was to determine whether quercetin could be useful in stabilizing the calves' health status by compensating for an insufficient initial colostrum supply.

In the present thesis, chapter 2 introduces major aspects of the glucose metabolism in neonatal calves as well as their immune and antioxidative status, and outlines the importance of colostrum feeding for the maturation of aforesaid aspects. Furthermore, an overview is given on the current state of knowledge concerning flavonoids and their impact on carbohydrate metabolism as well as their health promoting properties. Chapter 3 deals with the effects of an oral quercetin supplementation on the glucose metabolism of neonatal calves according to the initial colostrum supply. Chapter 4 focuses on selected metabolic, antioxidative and inflammatory parameters in newborn calves and how these are affected by initial colostrum deprivation and a seven-day quercetin supplementation. In a final general discussion, the main findings of chapters 3 and 4 are critically reviewed and put into context to the present literature.

REFERENCES

1. USDA (United States Department of Agriculture) 2010. Dairy 2007, Heifer Calf Health and Management Practices on U.S. Dairy Operations, 2007. USDA:APHIS:VS, CEAH. Fort Collins, CO. #550.0110.
2. Uetake K. Newborn calf welfare: A review focusing on mortality rates. Animal Science Journal. 2013; 84(2):101-5.
3. Girard J, Ferre P, Pegorier J, Duee P. Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. Physiological Reviews. 1992; 72(2):507-62.
4. Chase CC, Hurley DJ, Reber AJ. Neonatal immune development in the calf and its impact on vaccine response. Veterinary Clinics of North America: Food Animal Practice. 2008; 24(1):87-104.
5. Mutinati M, Pantaleo M, Roncetti M, Piccinno M, Rizzo A, Sciorsci R. Oxidative stress in neonatology. A review. Reproduction in Domestic Animals. 2014; 49(1):7-16.
6. Hammon HM, Steinhoff-Wagner J, Flor J, Schonhusen U, Metges CC. Lactation Biology Symposium: role of colostrum and colostrum components on glucose metabolism in neonatal calves. Journal of Animal Science. 2013; 91(2):685-95.
7. Albera E, Kankofer M. Antioxidants in colostrum and milk of sows and cows. Reproduction in Domestic Animals. 2009; 44(4):606-11.
8. Godden S. Colostrum management for dairy calves. Veterinary Clinics of North America: Food Animal Practice. 2008; 24(1):19-39.
9. Rochfort S, Parker AJ, Dunshea FR. Plant bioactives for ruminant health and productivity. Phytochemistry. 2008; 69(2):299-322.
10. Middleton E, Jr., Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews. 2000; 52(4):673-751.
11. Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry. 2000; 55(6):481-504.
12. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, et al. Flavonoid intake and risk of chronic diseases. The American Journal of Clinical Nutrition. 2002; 76(3):560-8.
13. Passamonti S, Terdoslavich M, Franca R, Vanzo A, Tramer F, Braidot E, et al. Bioavailability of flavonoids: a review of their membrane transport and the function of bilitranslocase in animal and plant organisms. Current Drug Metabolism. 2009; 10(4):369-94.
14. Hanhineva K, Torronen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkanan H, et al. Impact of Dietary Polyphenols on Carbohydrate Metabolism. International Journal of Molecular Sciences. 2010; 11(4):1365-402.
15. Ishihara N, Chu DC, Akachi S, Juneja LR. Improvement of intestinal microflora balance and prevention of digestive and respiratory organ diseases in calves by green tea extracts. Livestock Production Science. 2001; 68(2–3):217-29.
16. Kuhn G, Ender K, Thomann R, Tuchscherer M, Tuchscherer A, Stabenow B, et al. Einsatz von Echinacea-Extrakt bei tragenden und säugenden Sauen. Archiv für Tierzucht. 2005; 48:270-82.

2. LITERATURE OVERVIEW

2.1 GENERAL ASPECTS ON NUTRITIONAL AND IMMUNE STATUS IN NEONATAL CALVES

2.1.1 Postnatal adaptation of glucose metabolism in calves

With birth, energy supply of the calf switches from continuous transplacental provision of mainly carbohydrates (glucose) and amino acids to discontinuous oral intake of colostrum and milk [1, 2]. Colostrum is rich in fat but relatively low in carbohydrates when compared to the fetal diet, thus in most mammalian species, the glucose intake with food is insufficient to meet the glucose requirements of the newborn. Figure 2.1 illustrates the altering percentage composition of the bovine perinatal diet.

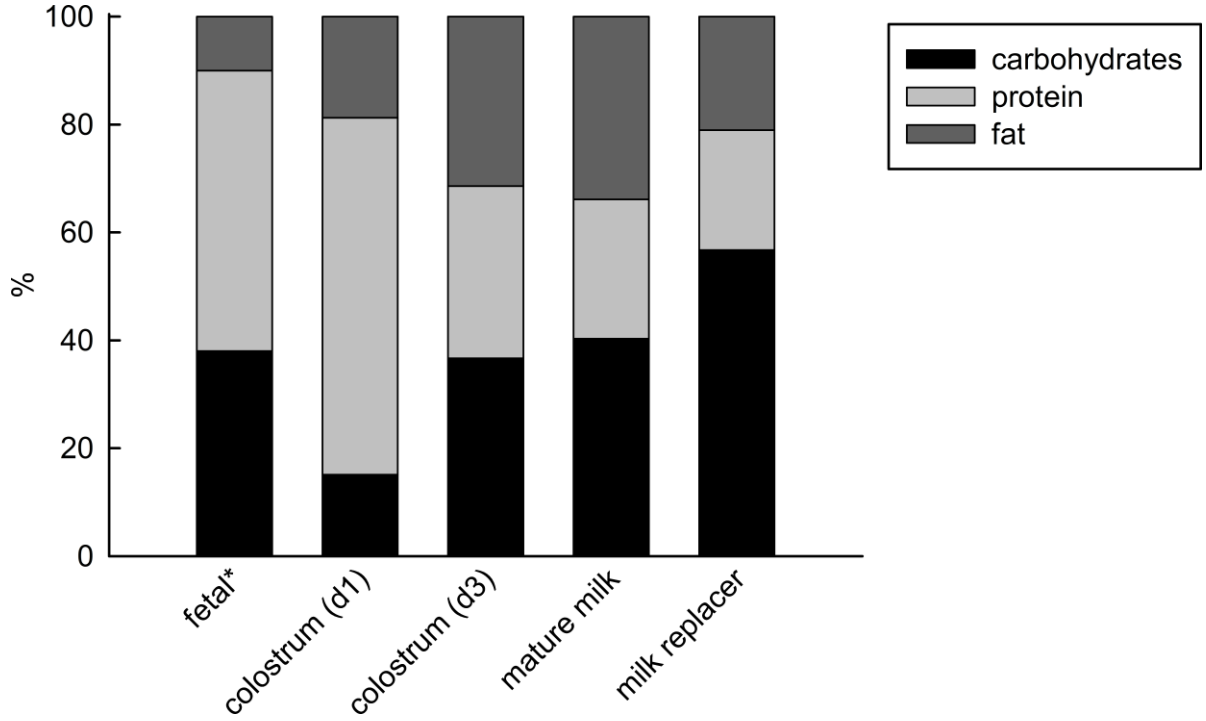

Figure 2.1: Percentage composition of macronutrient intake in calves (minor nutrients are disregarded) [3-6]. Composition of colostrum and mature milk was obtained from pluriparous Holstein cows. *The fat fraction in the fetal diet refers to the short-chain fatty acid acetate.

In neonatal ruminants, milk lactose accounts for only 25% of total glucose demand [7]. To ensure glucose homeostasis, it is crucial for newborn calves to produce glucose endogenously, that is by glycogenolysis and by gluconeogenesis. Towards term, the rise in the

fetal glucocorticoid concentration causes the functional and morphological maturation of several tissues and promotes hepatic glycogen deposition as well as the induction of gluconeogenic enzymes [8-10].

Immediately after birth, glycogenolysis, i.e. the degradation of (hepatic) glycogen stores, is the first action to overcome postnatal starvation and to maintain euglycemia for at least one day of food deprivation [10, 11]. However, within the first days of life, these glycogen stores are depleted [12-14], thus calves have to establish gluconeogenesis (GNG), i.e. the *de-novo* synthesis of glucose from different precursors, as the main glucose-producing metabolic pathway to further keep glucose homeostasis in circulation [15].

Both glucose-producing pathways are controlled by rate-limiting enzymes such as glycogen phosphorylase (PYG), pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase), whose activities are regulated by different hormonal and metabolic triggers. Figure 2.2 gives an overview of the hepatic glucose production including key enzymes and different precursors.

In neonates, the gluconeogenic precursors are mainly lactate, amino acids (alanine, glycine, glutamine) and, to a small extent, glycerol [2, 7]. Lactate and glucogenic amino acids are converted to pyruvate, the first substrate of the gluconeogenic pathway. Inside mitochondria, PC catalyzes the reaction of pyruvate to oxaloacetate, which is then converted to phosphoenolpyruvate by PEPCK [7], both being rate-limiting steps of GNG. Depending on whether lactate or other substrates serve as gluconeogenic precursor, the latter conversion occurs either inside mitochondria or in the cytoplasm. Phosphoenolpyruvate is then transformed by five different enzymes to fructose-1,6-bisphosphate. Another rate-limiting step is the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate by fructose-1,6-bisphosphatase (FBPase). The isomerization of fructose-6-phosphate results in glucose-6-phosphate. Glucose-6-phosphate is also generated during glycogenolysis from glucose-1-phosphate, which is sequentially removed from glycogen by PYG. Finally, G6Pase controls the rate of endogenous glucose production as it catalyzes the final step to free glucose, which can then be released into the circulation.

According to Steinhoff-Wagner et al. [15], the fraction of gluconeogenesis on total endogenous glucose production increases during the first week of life, while Haga et al. [12] showed that the activities of PEPCK and PC are lower in weaned than in 1-week-old calves. Furthermore, gluconeogenesis from lactate is eightfold greater in hepatocytes from 1-week-old than from ruminating calves [16]. In neonatal lambs, lactate accounts for 17-31% of total glucose synthesis [17], whereas propionate becomes the preferred gluconeogenic substrate with development of a functional rumen [7].

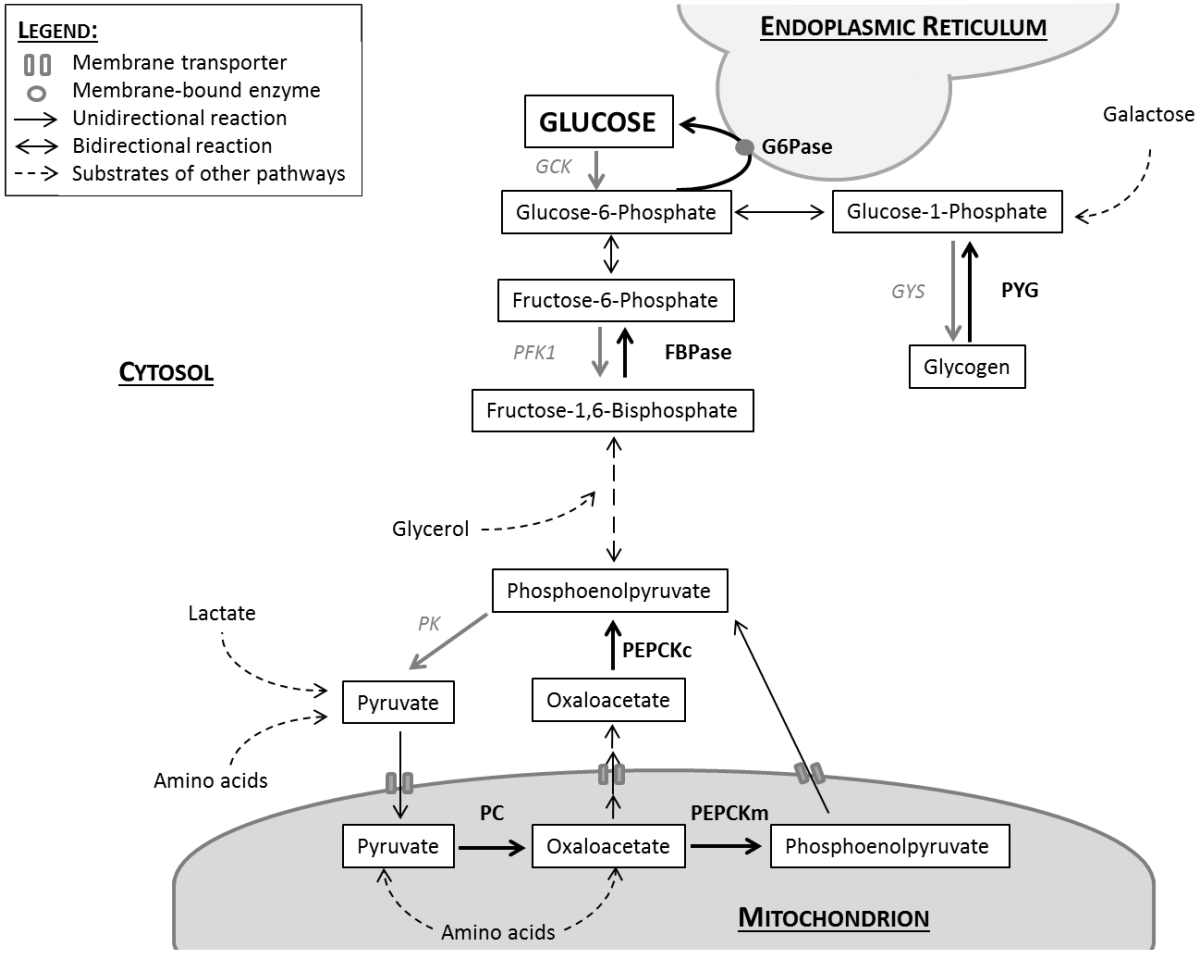

Figure 2.2: Simplified scheme of the hepatic glucose metabolism. Rate-limiting enzymes of endogenous glucose production (gluconeogenesis, glycogenolysis) are bold black, enzymes of glucose consuming pathways (glycolysis, glycogen synthesis) are italic grey. Abbreviations: FBPase, fructose-1,6-bisphosphatase (EC 3.1.3.11); G6Pase, glucose 6-phosphatase (EC 3.1.3.9); GCK, glucokinase (EC 2.7.1.2); GYS, glycogen synthase (EC 2.4.1.11); PC, pyruvate carboxylase (EC 6.4.1.1); PEPCK, phosphoenolpyruvate carboxykinase, (c, soluble; m, mitochondrial; EC 4.1.1.32); PFK, phosphofructokinase (EC 2.7.1.11); PK, pyruvate kinase (EC 2.7.1.40); PYG, glycogen phosphorylase (EC 2.4.1.1).

2.1.2 Maturation of the digestive system

Though calves are born relatively mature, their gastrointestinal tract (GIT) is not fully developed at birth. Therefore, the timing and rate of gastrointestinal maturation are considered as intermediate, meaning that major developmental changes occur both pre- and postnatally [18, 19]. Neonatal calves are regarded as pseudomonogastrics because they are preruminants without considerable forestomach activity [20] making them more dependent on abomasal and intestinal digestive processes than adults to cover their energy needs.

To ensure energy provision *ex utero*, maturation of the GIT is, next to the establishment of hepatic glucose metabolism, a key element of adaptation to postnatal life. Indeed, the most severe changes in the gastrointestinal development of ruminants happen during the first days

of life: Gut regulatory peptides (cholecystokinin, gastrin) rise radically in concentration and promote the development of digestive organs [20, 21]. Functional maturation of the digestive system is evident in elevated activities of brush border membrane enzymes [22] as well as enhanced synthesis and secretion of pancreatic juice within the first week of life [23]. In neonatal calves and lambs, pancreas mass and weight of abomasal mucosa increase by 20-30% within the first week [21]. Morphologic changes of the intestine include accelerated growth rate as well as increase of crypt depth and villus height in calves and piglets, respectively, all contributing to the enlargement of absorptive surface area [22, 24, 25]. Vacuolated fetal-type enterocytes that are able to transport macromolecules disappear resulting in intestinal barrier closure [26, 27].

Microbial colonization of the GIT begins with birth and physiological flora needs to establish during the first week of life by ingestion of first food [28]. Finally, food intake triggers the production of endogenous substances that further enhance maturational processes inside and outside the GIT [18, 24, 29].

2.1.3 Immune system and antioxidative status

Neonatal calves are prone to diseases because they are considered immunologically naïve at birth [30, 31]. On the one hand, the bovine placenta epitheliochorialis prohibits intrauterine transfer of immunoglobulins hence the calf is born hypogammaglobulinemic [32]. Although endogenous production of immunoglobulins could be demonstrated in colostrum-deprived calves during the first days of life, IgA and IgG do not reach appreciable levels until 16-32 days of life [33, 34]. On the other hand, the immune system is immature at birth and maturation continues until the 6th month of age [31]. Complement activity within the first month of life reaches only 50% of the activity in adults [35] and functional capacity of phagocytes (neutrophils, macrophages) as well as numbers of circulating B- and T-cells are reduced at birth [30, 36]. Due to high concentrations of corticosteroids and cytokines (IL-4, IL-10), the cell-mediated immune response around birth is suppressed [37]. Although present in neonates, many immune components are not (fully) functional until 2-4 weeks of age [31] so the health status of calves mainly depends on the acquisition of passive immunity with colostrum.

Irrespective of age, immune cells are particularly susceptible to oxidative damage because of their phospholipid-rich membrane structure [38-40]. After birth, neonates are for the first time exposed to an oxygen-rich environment. The initiation of aerobic metabolism and reperfusion after hypoxic ischemia during birth imply an increased generation of reactive oxygen species (ROS) [41, 42]. ROS are highly reactive molecules that originate from several physiologic/endogenous processes and are liberated either as mediators or as byproducts [43]. Those processes include immune response, inflammatory reactions, oxidative metabolism or detoxification as well as ischemic processes [44]. During immune response, immune cells,

e.g. neutrophils or macrophages, produce superoxide during respiratory burst to degrade internalized pathogens [45]. However, ROS can also arise from exogenous sources, e.g. radiation, toxins, chemicals or drugs [42, 46, 47]. In a balanced system, ROS are inactivated rapidly by either antioxidative enzymes or antioxidants, which, already at low concentrations, significantly delay or inhibit the oxidation of susceptible substrates and therefore prevent free radical injury. Figure 2.3 opposes the endogenous production of ROS and their inactivation by antioxidants. An imbalance between production of and protection against ROS results in oxidative stress, thus increased ROS are able to react with biomolecules including DNA, RNA, proteins, carbohydrates and lipids, impairing their physiological functions [43, 47].

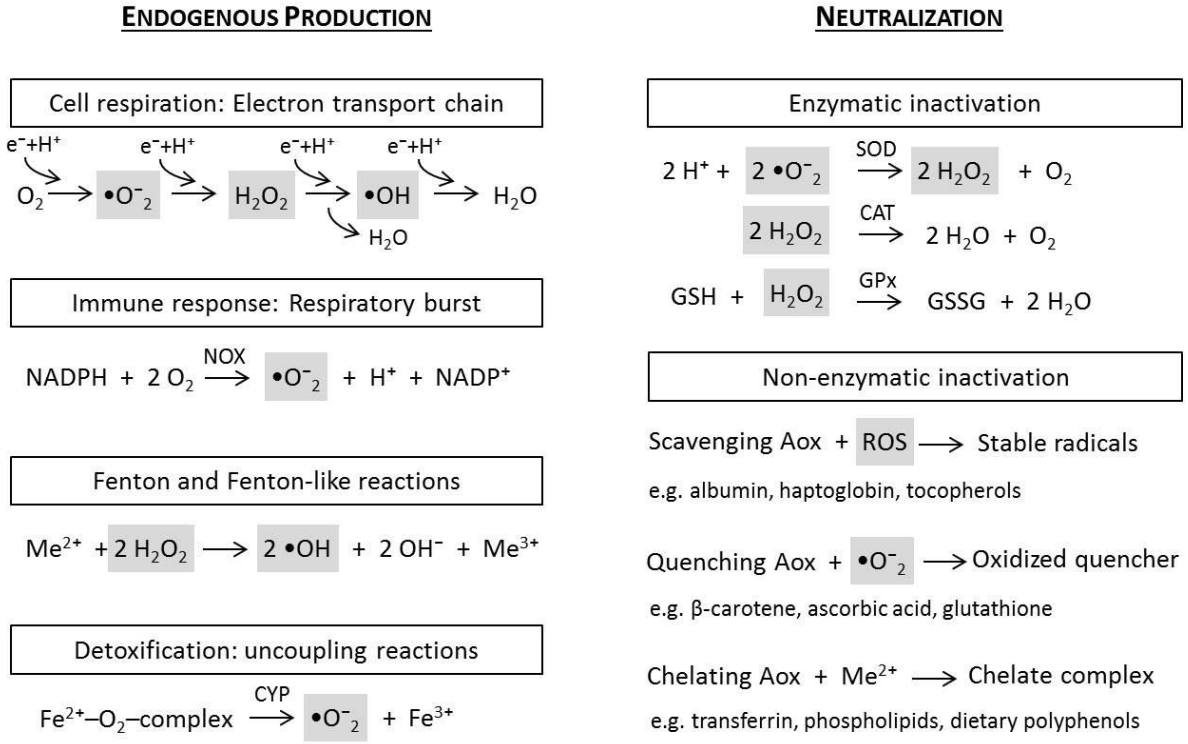

Figure 2.3: Endogenous formation and neutralization of ROS [44, 48, 49]. Abbreviations: Aox, antioxidant; CAT, catalase (EC 1.11.1.6); CYP, cytochrome P450 (EC 1.14); GPx, glutathione peroxidase (EC 1.11.1.9); GSH, glutathione; GSSG, glutathione disulfide; Me, transition metal ion (e.g. Fe^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}); $NADP^+$/NADPH, nicotinamide adenine dinucleotide phosphate; NOX, NADPH oxidase (EC 1.6.3.1); SOD, superoxide dismutase (EC 1.15.1.1).

ROS are involved in the pathogenesis of inflammatory and other diseases [46, 50, 51]. Furthermore, oxidative stress is also considered as a catalyzer for neonatal disease [52, 53]. Gastrointestinal anatomical and functional alterations, reduced intestinal absorption as well as failure of nutrient and immunoglobulin transfer can be consequences of ROS-induced damages in neonates of different species [52]. Conversely, growing calves, puppies and piglets with enteric disease have been shown to have significantly elevated markers for oxidative stress and decreased antioxidant capacity in plasma [54-56]. Supplementing calves and lambs with antioxidants, e.g. vitamin E, reduces morbidity and mortality after exposure to

stress, enhances the immune response and protects against tissue damage [57, 58]. In comparison to adult cattle, concentrations of free radicals in the blood of newborn calves are high while concentrations of antioxidative vitamins are low, leading to an impaired antioxidative status [41, 59-61]. At the same time, the activities of antioxidative enzymes increase. However, data on the antioxidative status are inconsistent and strongly dependent on the regarded parameter and the method of determination [62], thus, comparison of different studies is intricate [63].

2.2 IMPORTANCE OF COLOSTRUM

2.2.1 General aspects

According to the European Commission, colostrum is defined as "the fluid secreted by the mammary glands of milk-producing animals up to three to five days post parturition that is rich in antibodies and minerals, […]" [European Commission Regulation (EC) No. 1662/2006, Annex II, point 3]. A compositional analysis of 55 Holstein dairy herds revealed macronutrient contents of 6.7 ± 4.2% fat, 14.9 ± 3.3% protein and 2.5 ± 0.7% lactose in colostrum 4 h after calving [64]. While the macronutrients' major purpose is to provide the initial substrate supply for the neonate's energy metabolism, colostrum also contains large amounts of non-nutrient bioactive factors such as immunoglobulins, hormones, enzymes, growth factors and antioxidants [65-67].

The main site of action for milk-borne nonnutritive compounds is the GIT whose growth and development in farm animals is dramatic during the neonatal period [68, 69]. However, some bioactive factors also exert systemic effects: for instance, the absorption of essential fatty acids and vitamins (β-carotene, vitamins A and E) affects the status of plasma of respective compounds [24]. Although some proteins and peptides, e.g. immunoglobulins and lactoferrin, are also intestinally absorbed and appear in the circulation, this does not apply for insulin and IGF-I. However, colostral insulin and IGF-I indirectly modulate the somatotropic axis by enhancing the intestinal nutrient uptake and stimulating endogenous IGF-I production [24, 70]. Thus, the indirect anabolic effect of human IGF-I added to milk replacer becomes apparent by an enhanced lipid status in calves with increased concentrations of essential fatty acids and fat-soluble vitamins in their plasma [65].

Delayed intake of first colostrum alters plasma concentrations of several metabolites and hormones, but is rapidly compensated and does not indicate permanent imprinting effects on hematological, metabolic or endocrine traits [71, 72]. However, absorption of IgG is reduced thus performance is indirectly impaired [11, 73, 74]. Up to now, there is no clear evidence

whether colostral bioactive factors may program the animal and its later productivity [75].

2.2.2 Effects on GIT and glucose metabolism

The development and maturation of the GIT are essential for an efficient uptake of ingested nutrients, which in turn is necessary for proper growth and development of the young.

On the one hand, colostrum modulates the intestinal microbial population as it contains not only antimicrobial substances (e.g. lactoferrin, lactoperoxidase, lysozyme, oligosaccharides) to prevent growth of pathogens, but also pre- and probiotics to establish the physiological intestinal microflora [24, 76, 77].

On the other hand, colostrum is proven to modulate the GIT morphology [10, 69]. Feeding colostrum or colostrum-extracts resulted in greater villus circumferences, areas and heights [29, 78, 79] and increased crypt cell proliferation rates in the small intestine [78] when compared to colostrum-free alimentation. According to this, the intestinal absorption of monosaccharides depends on initial colostrum supply and is reduced when calves are colostrum-deprived (Figure 2.4) [5, 29, 80, 81]. However, the gene expression and protein concentrations of glucose transporters GLUT2 and sodium-dependent glucose transporter-1 (SGLT1) seem not to be affected by colostrum deprivation [25, 79].

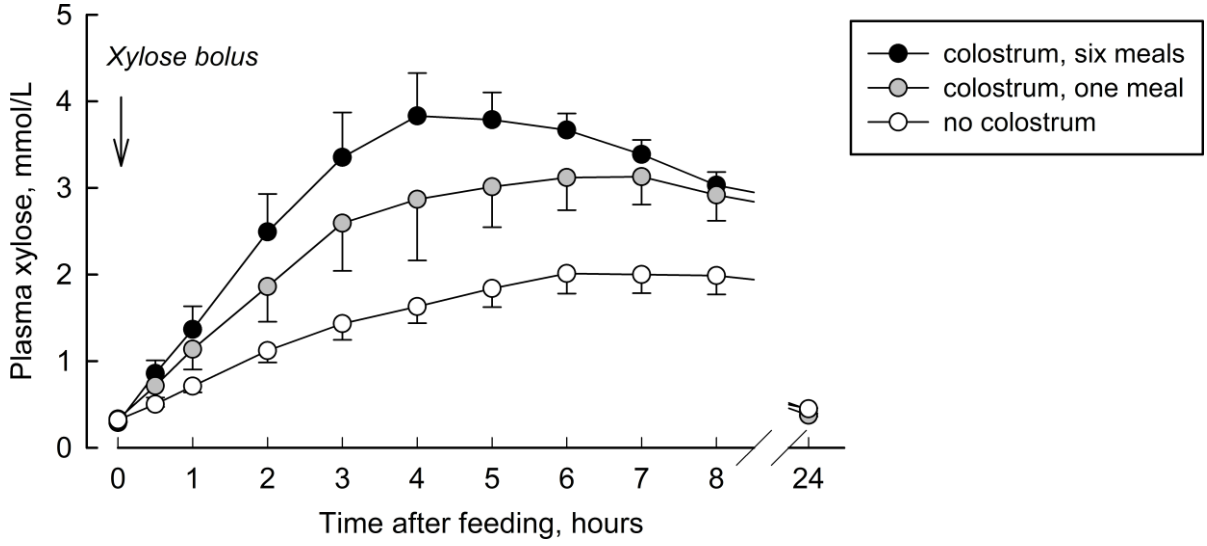

Figure 2.4: Plasma xylose concentrations following intestinal absorption in 5d old calves after different initial colostrum supply, modified from Hammon and Blum [81].

Moreover, the digestive capacity of the GIT is also affected by colostrum intake. Plasma concentrations of gut regulatory peptides, e.g. gastrin, cholecystokinin, and secretin increase after colostrum feeding thus exert favorable effects on gastrointestinal growth and digestive functions by enhancing gastric and pancreatic secretions [20]. In neonatal calves and piglets, activities of intestinal lactase as well as other brush border membrane enzymes seem to be only marginally affected by the initial feeding of formula versus colostrum [25, 78, 79], but

they are positively correlated with the intestinal villus size [29]. However, prolonged colostrum-feeding enhances activities of pancreatic lipase, elastase II and trypsin [78].
Even under conditions of similar lactose intake, colostrum-deprived calves have lower pre- and postprandial plasma glucose concentrations [5, 82, 83]. In newborn piglets, capacity for GNG from lactate is reduced when colostrum intake was restricted [84]. In contrast to this, GNG and total endogenous glucose production do not differ between colostrum-fed and colostrum-deprived calves [5]. However, dietary effects on mRNA abundance or activities of rate-limiting enzymes of GNG in newborn calves are inconsistent [5, 82, 85].

2.2.3 Effects on immune system and antioxidative status

Colostrum is rich in immunoglobulins, thus it is essential for neonatal calves to acquire passive immunity. As IgG accounts for 85-90% of total immunoglobulins in colostrum [86], its concentration in newborns' plasma is used to assess the success of passive transfer. According to this, a serum IgG concentration of 10 g/L (equivalent to serum protein concentration of 52 g/L) at the age of 24-48 h is considered as threshold for an adequate passive transfer [30, 87, 88]. Closure of the intestinal barrier coincides with the disappearance of vacuolated enterocytes and marks the cessation of macromolecule absorption [89]. Although delayed colostrum feeding can slow down this process, the efficiency of absorption impairs with increasing age at first colostrum ingestion hence incidence for failure of passive transfer rises [90, 91]. Absorbed colostral antibodies activate and regulate innate immune responses in calves [31] therefore health status and productivity are enhanced [92-94]. In addition to immunoglobulins, maternal leukocytes ingested with colostrum also enhance the neonatal immune system by increasing phagocytosis, stimulating cellular immune response and increasing the development of antigen-presenting cells [31, 95, 96].
Despite of immunity-promoting properties, colostrum is also a source of ROS. Those are produced by viable maternal leukocytes as well as enzymes like xanthine oxidase and lactoperoxidase in colostrum [67]. Opposing to this, there is also a variety of antioxidants present in colostrum. While catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) inactivate ROS through enzymatic reactions, vitamins (A, C, E), minerals (Se, Cu, Zn) as well as lactoferrin, caseins and whey proteins are non-enzymatic colostral compounds exerting antioxidative properties [67, 97]. In porcine and bovine colostrum, activities of antioxidative enzymes and lactoferrin increase with time after parturition [98-100] and an assessment of the antioxidative status revealed that the total antioxidant capacity in colostrum and milk of cows rises during the first week after parturition [99, 101]. Abuelo and collegues [61] could show that the levels of lipoperoxides in colostrum are positively correlated with ROS in the serum of newborn calves fed respective colostrum, while colostral antioxidative capacity and serum ROS were negatively correlated. Figure 2.5 opposes the intensity of protein peroxidation and the total antioxidant capacity of colostrum and newborn

calves' plasma of Holstein-Friesian primiparous cows.

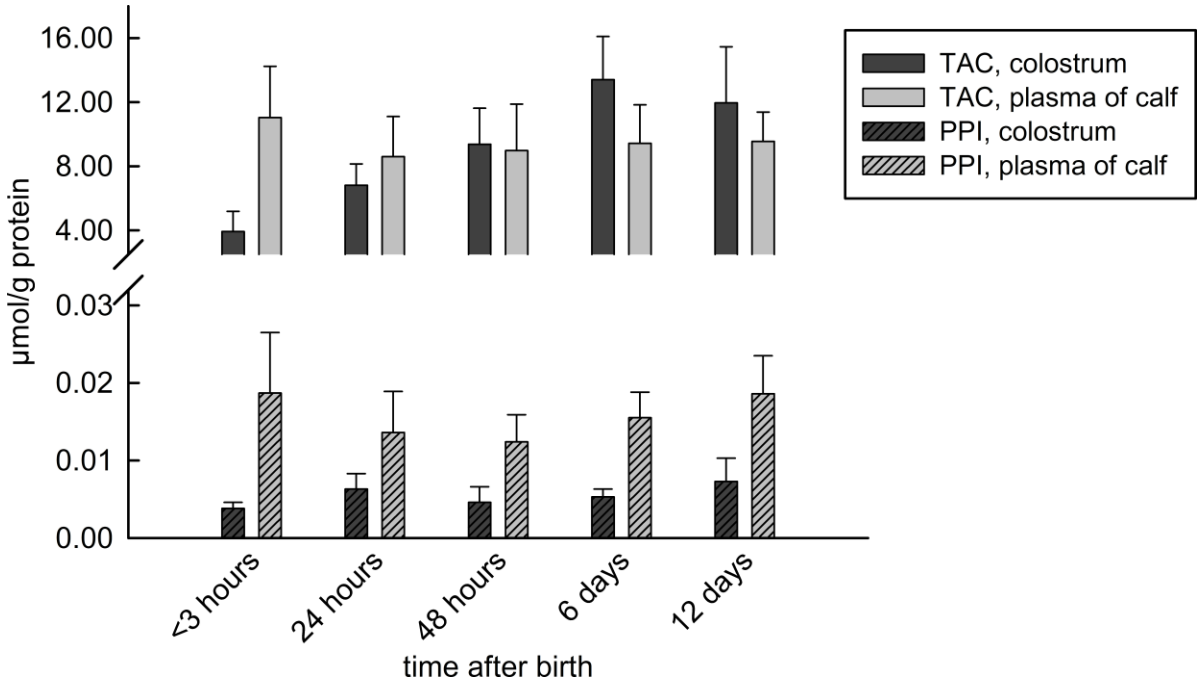

Figure 2.5: Antioxidative status of colostrum and plasma of calves fed with respective colostrum, modified from Albera and Kankofer [99]. Abbreviations: PPI, protein peroxidation intensity; TAC, total antioxidant capacity.

According to Retskii et al. [102], correcting the calf's antioxidant status before the first feeding improves the acquisition of passive immunity. Indeed, serum IgG concentrations have been shown to be positively correlated with the colostral antioxidative capacity [61]. Nonetheless, research on the connection of antioxidative status with IgG acquisition in calves is scarce as studies mostly focus on the effect of single colostral compounds rather than antioxidative capacity in general. Another obstacle is that middle- or long-term effects have been investigated in species whose offspring usually suckle the dam for a longer period of time, therefore the explicit influence of colostral antioxidative capacity on acquisition of passive immunity and health status is ambiguous and results can hardly be transferred to dairy calves.

Finally, the early change from colostrum to artificial milk replacers in calf rearing might attribute to a prolonged imbalance in antioxidant status as milk replacers often contain high levels of pro-oxidative unsaturated fatty acids while their antioxidative capacity is low [103].

2.3 CHARACTERISTICS OF FLAVONOIDS

2.3.1 General aspects

Flavonoids are secondary plant metabolites ubiquitously present in higher plants, where they are involved in photosynthesis, growth, development and pigmentation as well as defense against pathogens and other biotic and abiotic stresses [45, 104, 105]. They are polyphenols with a three-ring structure and are divided into subclasses according to the degree of saturation and oxidation as well as the position of the B-ring (Figure 2.6). Up to now, more than 8,000 different flavonoid compounds have been identified [105]. As animals are not able to synthesize flavonoids, they can only ingest them with vegetarian food components. However, the flavonoid content of green fodder is not fixed, but it depends on many factors, e.g. ripeness, harvesting time, degree of dryness, processing of foodstuff, storage etc. [104, 106]. Flavonols are the most ubiquitous flavonoids with quercetin being the most abundant dietary flavonol [104, 107]. As with other flavonoids except for flavanols, quercetin naturally rarely occurs in its free form (aglycone) but is bound to a sugar moiety by beta-glycosidic linkage, which can be glucose (glucoside) or other mono-, as well as di-, tri- or tetrasaccharides (glycoside) [106].

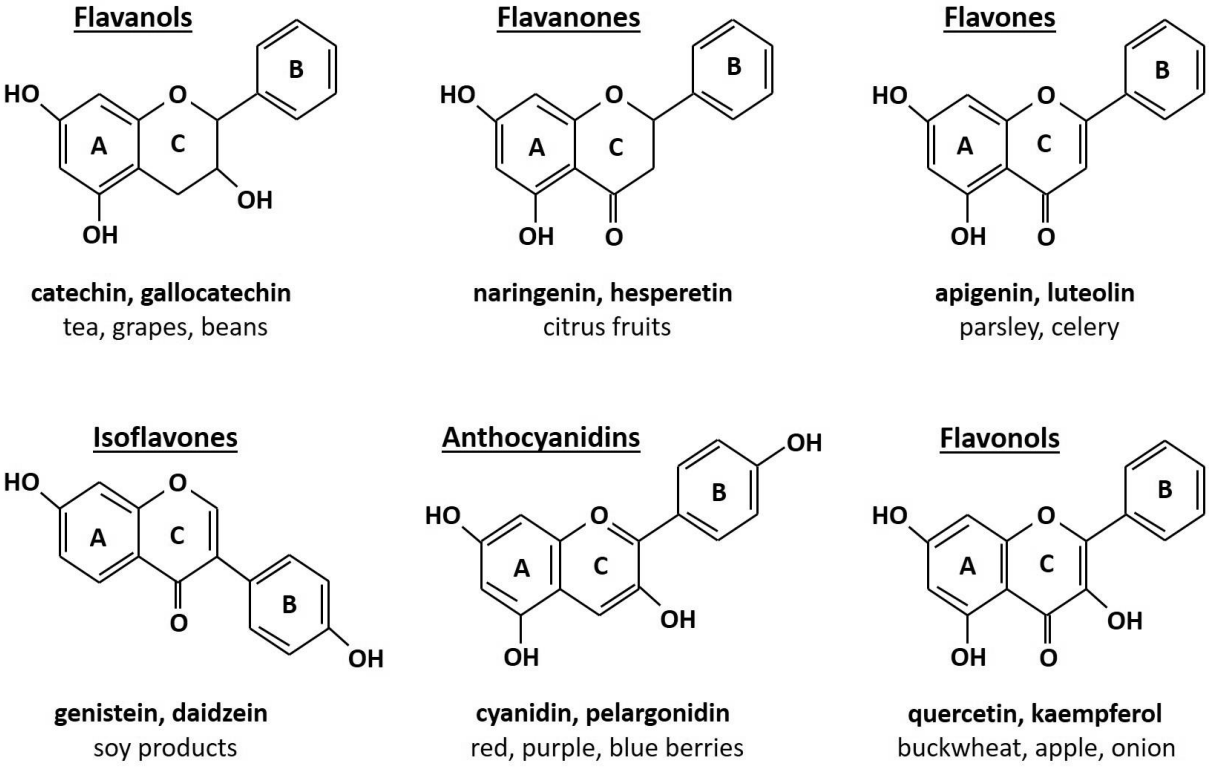

Figure 2.6: Chemical structure of the most common flavonoid subclasses and names of some representative compounds as well as their dietary sources [45, 104, 105].

Flavonoid-rich plants or plant extracts have been used for centuries in traditional medicine [108, 109], taking advantage of their antioxidative, anti-inflammatory, antiproliferative, and antimicrobial properties [110]: In humans, flavonoid-rich diets are related to reduced incidence of diseases associated with oxidative stress, e.g. cancer, cardiovascular, neurodegenerative and inflammatory diseases as well as metabolic disorders [105, 107, 109, 111, 112]. However, the excessive intake of flavonoids may not promote health, but exert adverse effects [49, 113]; thus supplementing flavonoids to the natural diet should be diligently supervised.

2.3.2 Absorption and metabolism of flavonols

Although the oral bioavailability of quercetin is relatively high [107], it depends on many factors: 1) the nature of the compound (hydrophilic glucoside resp. glycoside versus lipophilic aglycone) [114-116], 2) the dietary matrix with which it is ingested [111, 117, 118], 3) availability and activity of intestinal enzymes [107, 119, 120], 4) the gut microflora [107, 121], and 5) the duration of the flavonol administration (single dose vs. long-term intake/application) [111, 122]. For these reasons, interindividual variability of flavonol metabolites in plasma after intestinal absorption is high [123-125].

While quercetin aglycones are passively absorbed from the small intestine by glucose transporter 2 (GLUT2), its glucosides can be taken up actively by sodium-dependent glucose transporter-1 (SGLT1) [126, 127]. More complex glycosides are mainly microbially degraded before absorbed in distal parts of the intestine [104]. If not immediately excreted back into the intestinal lumen by multidrug-resistance-associated proteins [110, 115], absorbed flavonols are metabolized by various organs, e.g. the small intestine, liver and kidneys, where they are mainly glucuronidated, sulphated (intestine, liver, kidney) and methylated (liver, kidney) via phase II metabolism [43, 107, 118]. Thus, after oral administration, there are several glucuronide and sulfate conjugates but almost no aglycones detectable in the circulation of humans [125, 128], rats and pigs [110, 122, 129, 130]. Metabolic detoxification increases the hydrophilicity thus facilitates biliary and urinary excretion [104]. However, the slow elimination from blood, their presence in the bile as well as re-entry peaks in plasma indicate an enterohepatic cycling of flavonols [45, 114, 115, 125].

In adult cattle, intraruminal application of quercetin glycoside (rutin) but not its aglycone increases plasma flavonol concentrations, indicating that glycosylation protects from ruminal degradation [131]. In newborn calves, oral bioavailability changes with increasing age: on d 2 and d 29 of life, feeding of quercetin aglycone results in higher plasma flavonol concentrations than rutin feeding (quercetin-3-O-rutinoside), which is comparable to data obtained in monogastrics or in cows after intraduodenal application [4, 114, 124]. However, the plasma flavonol concentration on d 29 is significantly reduced when compared to d 2 and the proportion of single flavonol metabolites corresponds to patterns found in adult cattle,

indicating maturation of detoxification and elimination processes.

2.3.3 Interaction with glucose metabolism

Quercetin is well-known for its antihyperglycemic properties, which is why it is used as a natural anti-diabetic agent. After oral administration, the first side of action for quercetin and other flavonols is the GIT, where they interact with digestive enzymes and nutrient transporters. Quercetin and kaempferol were shown to inhibit α-glucosidase as well as α-amylase *in vitro* [132, 133] thus reducing polysaccharide digestion. Similar effects on activities of intestinal glucosidases maltase and sucrase were also observed *in vivo* in diabetic quercetin-fed rats and mice; however, lactase activity was unaltered [134, 135]. Several flavonols are transported across the intestinal barrier via similar transporters as monosaccharides; hence intestinal transport of the latter is impaired. Quercetin glucosides competitively inhibit intestinal glucose uptake via SGLT1 [114, 129, 136, 137], whereas quercetin aglycone is a non-competitive inhibitor of GLUT2 [126, 137, 138]. GLUT2 is not only located at the basolateral side of enterocytes but it can also be translocated to the apical side if the luminal glucose concentration is high [139]. Furthermore, GLUT2 is also the major glucose transporter in pancreatic β-cells and hepatocytes [137].

Hepatic glucose metabolism is also influenced by flavonols. Kato et al. [140] demonstrated in isolated rat hepatocytes that quercetin inhibits PYG and therefore concluded the inhibition of hepatic glucose production by quercetin. In contrast to this, Gasparin et al. [141] could show in isolated perfused rat liver that quercetin aglycone stimulates glycogenolysis and glucose release in a concentration-dependent manner, which indicates that effects in situ might differ from effects observed in cell culture. Same authors also demonstrated that quercetin in doses above 50 µM inhibited glycolysis from endogenous as well as exogenous glucose [141, 142] by decreasing the activity of glucokinase (GCK), thus glucose could not be phosphorylated for subsequent reaction steps. In diabetic mice, the hepatic expression of *GCK* was unaltered by quercetin-feeding [143]. Yet another enzyme, namely G6Pase, which catalyzes the dephosphorylation of glucose during gluconeogenesis, has been shown to be inhibited by quercetin (50 µM) and its dehydroxylated metabolite kaempferol [141, 144].

Quercetin interacts not only with hepatocytes but also with pancreatic β-cells, hence glucose metabolism might also be indirectly affected by alteration of its hormonal regulation. Anti-inflammatory properties account for the protection against oxidative damage as well as cytokine-induced cell death in the pancreas [145, 146], and it was shown that insulin release increases in diabetic mice and rats treated with quercetin [143, 147]. Insulin is a key regulator of glucose homeostasis as it affects not only hepatic metabolism but is also crucial for glucose uptake in muscle and adipose tissue by GLUT4. However, direct effects of quercetin on insulin-dependent glucose uptake are controversial: while some authors identified quercetin as competitive inhibitor of GLUT4 [148], others postulate that reduction of

insulin-dependent glucose uptake occurs via inhibition of GLUT4 translocation [149]. In contrast to this, Fang et al. [150] postulated that kaempferol and quercetin improve glucose-uptake in the presence of insulin. However, basal, insulin-independent glucose uptake in adipocytes seems to be unaffected by quercetin [148, 150].
In animal models of diabetes mellitus, quercetin treatment was shown to reduce postprandial hyperglycemia and thus ameliorate metabolic conditions [126, 147, 151, 152]. Anyhow, to what extent quercetin might influence overall glucose production *in vivo* depends on its metabolism as well as on biological activity and concentration of flavonols and respective derivatives at the side of action after application of the compound. Until now, the biological relevance of quercetin in healthy, normoglycemic individuals is not clear [153].

2.3.4 Effects on performance and intestinal health

Several flavonoids and flavonoid extracts that have been used by traditional medicine for centuries have attracted special notice during the last decades and thus became subject to intensive scientific research for their health promoting properties.
Diarrhea is a major problem in calves during the first weeks of life and causes substantial economic losses. In 1991, Capasso et al. [154] showed that quercetin inhibited prostaglandin- and leukotriene-induced contractions in isolated guinea pig ileum and concluded that the flavonol might be useful in relieving gastrointestinal colic, diarrhea and other gastrointestinal disorders. The same group also demonstrated that an intraperitoneal injection of quercetin reduced castor oil induced diarrhea in rats and mice [155, 156]. In humans, the spasmolytic effect of a quercetin-rich guava extract reduced abdominal pain, however, the consistency or frequency of diarrhea were not affected [157]. A comparable extract was shown by Chinese scientists to have a good curative effect on infantile rotaviral enteritis, and rotavirus antigen in stool was significantly reduced after oral application of the extract [158]. The antiviral property of quercetin was also evaluated *in vitro*: although several flavonols showed a favorable anti-rotavirus activity, quercetin was ineffective to inhibit the virus' cytopathic effect [159, 160]. However, quercetin and its 7-rhamnoside are able to reduce the infectivity of several strains of cornonaviruses, which are relevant enteropathogens in neonatal farm animals [161]. Intestinal bacteria have also been investigated for their interference with quercetin: Pakar et al. [162] demonstrated the inhibitory action on growth and cell-adhesion of selected enteropathogenic bacteria *in vitro*, while at the same time, probiotics were relatively unaffected, concluding that quercetin may help to stabilize the intestinal flora. In laying hens, dietary quercetin improved the performance by beneficially modulating the intestinal microflora populations [163]. In contrast to this, the intraruminal application of quercetin aglycone or its glycorhamnosid rutin did not alter ruminal fermentation processes in dairy cows, thus the ruminal microflora seemed not to be substantially influenced [131].

2.3.5 Effects on antioxidative and anti-inflammatory status

Quercetin possesses strong antioxidant and anti-inflammatory properties. The catechol structure in the B-ring accounts for the metal-chelating properties of quercetin, hence cations are unavailable for the generation of ROS by Fenton-type reactions [118, 164]. As quercetin inhibits myeloperoxidase and NADPH oxidase, the superoxide production during respiratory burst is reduced [45].

The phenolic hydroxyl groups are able to act as electron donors, thus quercetin scavenges ROS [118, 164]: Due to its hydroxyl groups, quercetin also exerts chain-breaking antioxidant action during lipid-peroxidation and is able to inhibit the autoxidation of fatty acids [165, 166].

As mentioned earlier, biological effects always depend on the chemical structure of circulating metabolites: in accordance to this, quercetin was found to act more as an antioxidant than its monoglucosides [165]. Furthermore, Shirai and coworkers [167] could show that although co-incubation with H_2O_2 and quercetin aglycone inhibited H_2O_2-induced ROS production in mouse fibroblasts, the pre-incubation with quercetin aglycone prevented its antioxidative activity, while quercetin-3-glucuronide retained its effectiveness.

Flavonols do not only exert antioxidative activity by directly scavenging ROS or inhibiting their production but they can also protect the antioxidant ascorbic acid from oxidative degradation [45], conserve tocopherols in biological membranes [168] and reduce α-tocopherol radicals [164]. Further indirect impact on the antioxidative system occurs by the interaction with antioxidative enzymes, e.g. SOD, CAT and GPx [164] but data on potential effects are inconsistent as both stimulating and inhibiting effects have been described *in vivo* and *in vitro* [169-172].

The antioxidative activity of flavonols also affects the immune system: ROS are produced by phagocytes during respiratory burst as part of the innate immune response to fight invading pathogens. Flavonoids are not only able to scavenge ROS, but they can also inhibit their production. In case of immune cells, e.g. macrophages and neutrophils, ROS release is impaired by quercetin as it inhibits relevant enzymes, namely myeloperoxidase and NADPH oxidase [45]. Quercetin was also shown to inhibit the gene expression and enzyme activity of cyclooxygenase-2 and inducible nitric oxide synthetase, which results in a reduced release of pro-inflammatory mediators (nitric oxide, prostaglandin E_2) and thus contributes to the anti-inflammatory properties of quercetin [173]. In isolated macrophages, incubation with quercetin was shown to reduce mRNA levels of proinflammatory cytokines including TNFα and IL-1β [174, 175]. In non-immune cells, e.g. pancreatic β-cells or adipocytes, quercetin treatment attenuated the TNFα-mediated inflammation by interfering with signal transduction [145, 175, 176]. According to these findings, quercetin has also been shown to ameliorate the metabolic alterations evoking from chronic inflammation in animal models of diabetes mellitus type 2, obesity or atherosclerosis [147, 175, 177].

In rat and mouse models for inflammatory bowel disease, oral treatment with quercetin

aglycone or its O-methylated metabolite isorhamnetin attenuated chemically-induced symptoms of inflammation [178, 179]. Similar intestinal anti-inflammatory effects could be shown in mice fed the quercetin-glucorhamnosid rutin [180]. On a whole body level, however, only the acute administration of quercetin aglycone was useful to prevent endotoxemia and death in mice intraperitoneally injected with lipopolysaccharide, while the anti-inflammatory effect was missing after chronic administration [181]. When administered 12 h after lipopolysaccharide treatment, only quercetin aglycone but not its glucuronide affected the cytokine secretions by peritoneal macrophages [182].

Although numerous experiments have been undertaken *in vitro* or in laboratory rodents to emphasize the anti-inflammatory properties of quercetin, studies in farm animals are scarce and mostly focus on the effects of plant extracts. For example, in piglets of Echinacea-supplemented sows, immunostimulatory effects were evident from increased Ig concentrations when compared to unsupplemented controls [183]. Similar effects could be shown in growing pigs, whose specific immune response to vaccination was higher when animals received Echinacea [184]. Elevated immunoglobulin titers were also observed in buffalo calves fed propolis, a flavonol-rich natural compound produced by honey bees [185]. Furthermore, addition of propolis to an inactivated vaccine against bovine herpesvirus-5 also resulted in significantly higher antibody titers in vaccinated Hereford cattle when compared to animals receiving a propolis-free vaccine [186].

However, as extracts of natural compounds contain mixtures of multiple components, it is not possible to distinguish whether observed immune-stimulatory effects are caused by single substances or by their synergism.

2.4 REFERENCES

1. Girard J. Metabolic adaptations to change of nutrition at birth. Biology of the Neonate. 1990; 58(Suppl. 1):3-15.
2. Girard J, Ferre P, Pegorier J, Duee P. Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. Physiological Reviews. 1992; 72(2):507-62.
3. Bell A, Greenwood P, Ehrhardt R. Regulation of metabolism and growth during prenatal life. Biology of Metabolism in Growing Animals. 2005; 3:3-34. D.G. Bunin and H. Meismann (Eds.); Elsevier Limited.
4. Maciej J, Schäff CT, Kanitz E, Tuchscherer A, Bruckmaier RM, Wolffram S, et al. Bioavailability of the flavonol quercetin in neonatal calves after oral administration of quercetin aglycone or rutin. Journal of Dairy Science. 2015; 98(6): 3906-3917.
5. Steinhoff-Wagner J, Gors S, Junghans P, Bruckmaier RM, Kanitz E, Metges CC, et al. Intestinal Glucose Absorption but Not Endogenous Glucose Production Differs between Colostrum- and Formula-Fed Neonatal Calves. Journal of Nutrition. 2011; 141(1):48-55.
6. Weber C, Hametner C, Tuchscherer A, Losand B, Kanitz E, Otten W, et al. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. Journal of Dairy Science. 2013; 96(1):165-80.
7. Donkin S, Hammon H. Hepatic gluconeogenesis in developing ruminants. Biology of Growing Animals. 2005; 3:375-90. D.G. Bunin and H. Meismann (Eds.). Elsevier Limited.
8. Fowden AL, Li J, Forhead AJ. Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? Proceedings of the Nutrition Society. 1998; 57(01):113-22.
9. Fowden AL, Mundy L, Silver M. Developmental regulation of glucogenesis in the sheep fetus during late gestation. The Journal of Physiology. 1998; 508(3):937-47.
10. Hammon HM, Steinhoff-Wagner J, Schonhusen U, Metges CC, Blum JW. Energy metabolism in the newborn farm animal with emphasis on the calf: endocrine changes and responses to milk-born and systemic hormones. Domestic Animal Endocrinology. 2012; 43(2):171-85.
11. Hadorn U, Hammon H, Bruckmaier RM, Blum JW. Delaying colostrum intake by one day has important effects on metabolic traits and on gastrointestinal and metabolic hormones in neonatal calves. The Journal of Nutrition. 1997; 127(10):2011-23.
12. Haga S, Fujimoto S, Yonezawa T, Yoshioka K, Shingu H, Kobayashi Y, et al. Changes in hepatic key enzymes of dairy calves in early weaning production systems. Journal of Dairy Science. 2008; 91(8):3156-64.
13. Hammon H, Philipona C, Zbinden Y, Blum J, Donkin S. Effects of dexamethasone and growth hormone treatment on hepatic gluconeogenic enzymes in calves. Journal of Dairy Science. 2005; 88(6):2107-16.
14. Ontsouka E, Zbinden Y, Hammon H, Blum J. Ontogenesis of mRNA levels and binding sites of hepatic α-adrenoceptors in young cattle. Domestic Animal Endocrinology. 2006; 30(3):170-84.
15. Steinhoff-Wagner J, Gors S, Junghans P, Bruckmaier RM, Kanitz E, Metges CC, et al.

Maturation of endogenous glucose production in preterm and term calves. Journal of Dairy Science. 2011; 94(10):5111-23.

16. Donkin S, Armentano L. Insulin and glucagon regulation of gluconeogenesis in preruminating and ruminating bovine. Journal of Animal Science. 1995; 73(2):546-51.

17. Townsend S, Rudolph C, Wood C, Rudolph A. Perinatal onset of hepatic gluconeogenesis in the lamb. Journal of Developmental Physiology. 1989; 12(6):329-35.

18. Georgiev IP. Effect of colostrum insulin-like growth factors on growth and development of neonatal calves. Bulgarian Journal of Veterinary Medicine. 2008; 11(2):75-88.

19. Sangild PT. Gut responses to enteral nutrition in preterm infants and animals. Experimental Biology and Medicine. 2006; 231(11):1695-711.

20. Guilloteau P, Huërou-Luron IL, Chayvialle J, Toullec R, Zabielski R, Blum J. Gut regulatory peptides in young cattle and sheep. Zentralblatt für Veterinärmedizin. Reihe A. 1997; 44(1-10):1-23.

21. Guilloteau P, Zabielski R, Blum JW. Gastrointestinal tract and digestion in the young ruminant: ontogenesis, adaptations, consequences and manipulations. Journal of Physiology and Pharmacology. 2009; 60 Suppl 3:37-46.

22. Bittrich S, Philipona C, Hammon H, Romé V, Guilloteau P, Blum J. Preterm as compared with full-term neonatal calves are characterized by morphological and functional immaturity of the small intestine. Journal of Dairy Science. 2004; 87(6):1786-95.

23. Zabielski R, Morisset J, Podgurniak P, Romé V, Biernat M, Bernard C, et al. Bovine pancreatic secretion in the first week of life: potential involvement of intestinal CCK receptors. Regulatory Peptides. 2002; 103(2):93-104.

24. Blum JW. Nutritional physiology of neonatal calves. Journal of Animal Physiology and Animal Nutrition. 2006; 90(1-2):1-11.

25. Thymann T, Burrin DG, Tappenden KA, Bjornvad CR, Jensen SK, Sangild PT. Formula-feeding reduces lactose digestive capacity in neonatal pigs. British Journal of Nutrition. 2006; 95(6):1075-81.

26. Baintner K. Transmission of antibodies from mother to young: Evolutionary strategies in a proteolytic environment. Veterinary immunology and immunopathology. 2007; 117(3):153-61.

27. Skrzypek T, Valverde PJ, Skrzypek H, Kazimierczak W, Biernat M, Zabielski R. Gradual disappearance of vacuolated enterocytes in the small intestine of neonatal piglets. Journal of Physiology and Pharmacology. 2007; 58:87-95.

28. Taschuk R, Griebel PJ. Commensal microbiome effects on mucosal immune system development in the ruminant gastrointestinal tract. Animal Health Research Reviews. 2012; 13(01):129-41.

29. Sauter SN, Roffler B, Philipona C, Morel C, Rome V, Guilloteau P, et al. Intestinal development in neonatal calves: Effects of glucocorticoids and dependence on colostrum feeding. Biology of the Neonate. 2004; 85(2):94-104.

30. Barrington GM, Parish SM. Bovine neonatal immunology. Veterinary Clinics of North America Food Animal Practice. 2001; 17(3):463-76.

31. Chase CC, Hurley DJ, Reber AJ. Neonatal immune development in the calf and its impact on vaccine response. Veterinary Clinics of North America: Food Animal Practice. 2008; 24(1):87-104.

32. Weaver DM, Tyler JW, VanMetre DC, Hostetler DE, Barrington GM. Passive transfer of colostral immunoglobulins in calves. Journal of Veterinary Internal Medicine. 2000; 14(6):569-77.

33. Devery J, Davis C, Larson B. Endogenous production of immunoglobulin lgG1 in newborn calves. Journal of Dairy Science. 1979; 62(11):1814-8.

34. Husband A, Brandon M, Lascelles A. Absorption and endogenous production of immunoglobulins in calves. The Australian Journal of Experimental Biology and Medical Science. 1972; 50(4):491-8.

35. Firth MA, Shewen PE, Hodgins DC. Passive and active components of neonatal innate immune defenses. Animal Health Research Reviews. 2005; 6(02):143-58.

36. Kampen AH, Olsen I, Tollersrud T, Storset AK, Lund A. Lymphocyte subpopulations and neutrophil function in calves during the first 6 months of life. Vet Immunol Immunopathol. 2006; 113(1):53-63.

37. Jacob S, Ramnath V, Philomina P, Rahunandhanan K, Kannan A. Assessment of physiological stress in periparturient cows and neonatal calves. Indian journal of physiology and pharmacology. 2001; 45(2):233-8.

38. Carroll JA, Forsberg NE. Influence of stress and nutrition on cattle immunity. Veterinary Clinics of North America: Food Animal Practice. 2007; 23(1):105-49.

39. Meydani SN, Wu D, Santos MS, Hayek MG. Antioxidants and immune response in aged persons: overview of present evidence. The American Journal of Clinical Nutrition. 1995; 62(6):1462S-76S.

40. Sordillo LM, Aitken SL. Impact of oxidative stress on the health and immune function of dairy cattle. Veterinary Immunology and Immunopathology. 2009; 128(1):104-9.

41. Gaál T, Ribiczeyne-Szabo P, Stadler K, Jakus J, Reiczigel J, Kövér P, et al. Free radicals, lipid peroxidation and the antioxidant system in the blood of cows and newborn calves around calving. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 2006; 143(4):391-6.

42. Sies H. Reactive oxygen species: Prooxidants and antioxidants in biology and medicine. Rheinisch-Westfaelische Akademie der Wissenschaften Natur- Ingenieur- und Wirtschaftswissenschaften Vortraege. 1993; 0(399):27-50.

43. Boots AW, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. European Journal of Pharmacology. 2008; 585(2):325-37.

44. Abd Ellah RM. The role of liver biopsy in detection of hepatic oxidative stress. Veterinary Medicine International. 2011; 2011.

45. Middleton E, Jr., Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews. 2000; 52(4):673-751.

46. Lykkesfeldt J, Svendsen O. Oxidants and antioxidants in disease: oxidative stress in farm animals. Veterinary Journal. 2007; 173(3):502-11.

47. Montuschi P, Barnes P, Jackson Roberts L. Insights into oxidative stress: the isoprostanes. Current Medicinal Chemistry. 2007; 14(6):703-17.

48. Choe E, Min DB. Mechanisms of antioxidants in the oxidation of foods. Comprehensive Reviews in Food Science and Food Safety. 2009; 8(4):345-58.

49. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics. 2002; 96(2-3):67-202.

50. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods of their quantification. 2002; 30 (6): 620-50. Toxicologic Pathology. 2002; 30(6):620-50.

51. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Sciences. 1993; 90(17):7915-22.

52. Mutinati M, Pantaleo M, Roncetti M, Piccinno M, Rizzo A, Sciorsci R. Oxidative stress in neonatology. A review. Reproduction in Domestic Animals. 2014; 49(1):7-16.

53. Saugstad OD. Oxidative stress in the newborn–a 30-year perspective. Biology of the Neonate. 2005; 88(3):228-36.

54. Ahmed W, Hassan SE. Applied studies on coccidiosis in growing Buffalo-calves with special reference to oxidant/antioxidant status. World Journal of Zoology. 2007; 2:40-8.

55. De UK, Mukherjee R, Nandi S, Patel BHM, Dimri U, Ravishankar C, et al. Alterations in oxidant/antioxidant balance, high-mobility group box 1 protein and acute phase response in cross-bred suckling piglets suffering from rotaviral enteritis. Tropical animal health and production. 2014; 46(7):1127-33.

56. Kizil O, Yuce A. Oxidative stress in dogs with coccidiosis. Revue de Médecine Vétérinaire. 2009; 160(11):495-9.

57. Morán L, Andrés S, Bodas R, Benavides J, Prieto N, Pérez V, et al. Antioxidants included in the diet of fattening lambs: Effects on immune response, stress, welfare and distal gut microbiota. Animal Feed Science and Technology. 2012; 173(3):177-85.

58. Nockels CF. Antioxidants improve cattle immunity following stress. Animal Feed Science and Technology. 1996; 62(1):59-68.

59. Inanami O, Shiga A, Okada K, Sato R, Miyake Y, Kuwabara M. Lipid peroxides and antioxidants in serum of neonatal calves. American Journal of Veterinary Research. 1999; 60(4):452-7.

60. Porzig S. Untersuchung zum antioxidativen Status von Kühen und deren neugeborenen Kälbern [Doctoral dissertation]. München: Ludwig-Maximilians-Universität 2004.

61. Abuelo A, Perez-Santos M, Hernandez J, Castillo C. Effect of colostrum redox balance on the oxidative status of calves during the first 3 months of life and the relationship with passive immune acquisition. Veterinary Journal. 2014; 199(2):295-9.

62. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. The American Journal of Clinical Nutrition. 1993; 57(5):715S-24S.

63. Celi P. Biomarkers of oxidative stress in ruminant medicine. Immunopharmacology and Immunotoxicology. 2011; 33(2):233-40.

64. Kehoe S, Jayarao B, Heinrichs A. A survey of bovine colostrum composition and colostrum management practices on Pennsylvania dairy farms. Journal of Dairy Science. 2007; 90(9):4108-16.

65. Blum JW, Hammon H. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Production Science. 2000; 66(2):151-9.

66. Gauthier SF, Pouliot Y, Maubois J-L. Growth factors from bovine milk and colostrum: composition, extraction and biological activities. Le Lait. 2006; 86(2):99-125.

67. Przybylska J, Albera E, Kankofer M. Antioxidants in bovine colostrum. Reproduction in

Domestic Animals. 2007; 42(4):402-9.

68. Blum JW, Baumrucker CR. Insulin-like growth factors (IGFs), IGF binding proteins, and other endocrine factors in milk: role in the newborn. Bioactive Components of Milk. 2008; 397-422. Bosze (Ed.); Springer Science & Business Media.

69. Odle J, Zijlstra RT, Donovan SM. Intestinal Effects of Milkborne Growth Factors in Neonates of Agricultural Importance1, 2. Journal of Animal Science. 1996; 74:2509-22.

70. Hammon H, Blum J. The somatotropic axis in neonatal calves can be modulated by nutrition, growth hormone, and Long-R3-IGF-I. American Journal of Physiology. Endocrinology and Metabolism. 1997; 273(1):E130-E8.

71. Blum J, Baumrucker C. Colostral and milk insulin-like growth factors and related substances: mammary gland and neonatal (intestinal and systemic) targets. Domestic Animal Endocrinology. 2002; 23(1):101-10.

72. Zanker I, Hammon H, Blum J. Delayed feeding of first colostrum: are there prolonged effects on haematological, metabolic and endocrine parameters and on growth performance in calves? Journal of Animal Physiology and Animal Nutrition. 2001; 85(3-4):53-66.

73. DeNise S, Robison J, Stott G, Armstrong D. Effects of passive immunity on subsequent production in dairy heifers. Journal of Dairy Science. 1989; 72(2):552-4.

74. Wittum TE, Perino L. Passive immune status at postpartum hour 24 and long-term health and performance of calves. American Journal of Veterinary Research. 1995; 56(9):1149-54.

75. Bach A. Ruminant Nutrition Symposium: Optimizing Performance of the Offspring: Nourishing and managing the dam and postnatal calf for optimal lactation, reproduction, and immunity. Journal of Animal Science. 2012; 90(6):1835-45.

76. Gopal PK, Gill H. Oligosaccharides and glycoconjugates in bovine milk and colostrum. British Journal of Nutrition. 2000; 84(S1):69-74.

77. Lindner JDD, Santarelli M, Yamaguishi CT, Soccol CR, Neviani E. Recovery and identification of bovine colostrum microflora using traditional and molecular approaches. Food Technology and Biotechnology. 2011; 49(3):364.

78. Blattler U, Hammon HM, Morel C, Philipona C, Rauprich A, Rome V, et al. Feeding colostrum, its composition and feeding duration variably modify proliferation and morphology of the intestine and digestive enzyme activities of neonatal calves. Journal of Nutrition. 2001; 131(4):1256-63.

79. Steinhoff-Wagner J, Zitnan R, Schönhusen U, Pfannkuche H, Hudakova M, Metges CC, et al. Diet effects on glucose absorption in the small intestine of neonatal calves: Importance of intestinal mucosal growth, lactase activity, and glucose transporters. Journal of Dairy Science. 2014; 97(10):6358-69.

80. Kuhne S, Hammon HM, Bruckmaier RM, Morel C, Zbinden Y, Blum JW. Growth performance, metabolic and endocrine traits, and absorptive capacity in neonatal calves fed either colostrum or milk replacer at two levels. Journal of Animal Science. 2000; 78(3):609-20.

81. Hammon H, Blum JW. Prolonged colostrum feeding enhances xylose absorption in neonatal calves. Journal of Animal Science. 1997; 75(11):2915-9.

82. Hammon HM, Sauter SN, Reist M, Zbinden Y, Philipona C, Morel C, et al. Dexamethasone and colostrum feeding affect hepatic gluconeogenic enzymes differently in neonatal calves. Journal of Animal Science. 2003; 81(12):3095-106.

83. Rauprich ABE, Hammon HM, Blum JW. Effects of feeding colostrum and a formula with nutrient contents as colostrum on metabolic and endocrine traits in neonatal calves. Biology of the Neonate. 2000; 78(1):53-64.

84. Lepine AJ, Boyd RD, Whitehead DM. Effect of colostrum intake on hepatic gluconeogenesis and fatty acid oxidation in the neonatal pig. Journal of Animal Science. 1991; 69(5):1966-74.

85. Scheuer BH, Zbinden Y, Schneiter P, Tappy L, Blum J, Hammon H. Effects of colostrum feeding and glucocorticoid administration on insulin-dependent glucose metabolism in neonatal calves. Domestic Animal Endocrinology. 2006; 31(3):227-45.

86. Larson B, Heary H, Devery J. Immunoglobulin production and transport by the mammary gland. Journal of Dairy Science. 1980; 63(4):665-71.

87. Tyler JW, Hancock DD, Parish SM, Rea DE, Besser TE, Sanders SG, et al. Evaluation of 3 assays for failure of passive transfer in calves. Journal of Veterinary Internal Medicine. 1996; 10(5):304-7.

88. Godden S. Colostrum management for dairy calves. Veterinary Clinics of North America: Food Animal Practice. 2008; 24(1):19-39.

89. Sangild P, Harrison A, Nielsen T, Bjørnvad C. Neonatal maturation of intestinal motility and structure is diet-dependent. International Symposium on Digestive Physiology in Pigs, Proceedings of the 9th International Symposium on Digestive Physiology in Pigs; Banff, BC, Canada; 2003.

90. Hadorn U, Blum J. Effects of Feeding Colostrum, Glucose or Water on the First Day of Life on Plasma Immunoglobulin G Concentrations and γ-Glutamyltransferase Activities in Calves. Zentralblatt für Veterinärmedizin. Reihe A. 1997; 44(1-10):531-7.

91. Stott G, Marx D, Menefee B, Nightengale G. Colostral immunoglobulin transfer in calves I. Period of absorption. Journal of Dairy Science. 1979; 62(10):1632-8.

92. Besser TE, McGuire T, Gay C, Pritchett L. Transfer of functional immunoglobulin G (IgG) antibody into the gastrointestinal tract accounts for IgG clearance in calves. Journal of Virology. 1988; 62(7):2234-7.

93. Furman-Fratczak K, Rzasa A, Stefaniak T. The influence of colostral immunoglobulin concentration in heifer calves' serum on their health and growth. Journal of Dairy Science. 2011; 94(11):5536-43.

94. Wells S, Dargatz D, Ott S. Factors associated with mortality to 21 days of life in dairy heifers in the United States. Preventive Veterinary Medicine. 1996; 29(1):9-19.

95. Le Jan C. Cellular components of mammary secretions and neonatal immunity: a review. Veterinary Research. 1996; 27(4-5):403-17.

96. Tuboly S, Bernath S, Glavits R, Kovacs A, Megyeri Z. Intestinal absorption of colostral lymphocytes in newborn lambs and their role in the development of immune status. Acta Veterinaria Hungarica. 1994; 43(1):105-15.

97. Chiang S-H, Chang C-Y. Antioxidant properties of caseins and whey proteins from colostrums. Journal of Food and Drug Analysis. 2005; 13(1).

98. Albera E, Kankofer M. Antioxidants in colostrum and milk of sows and cows. Reproduction in Domestic Animals. 2009; 44(4):606-11.

99. Albera E, Kankofer M. The Comparison of Antioxidative/Oxidative Profile in Blood, Colostrum and Milk of Early Post-partum Cows and Their Newborns. Reproduction in Domestic Animals. 2011; 46(5):763-9.

100. Lipko-Przybylska J, Kankofer M. Antioxidant defence of colostrum and milk in consecutive lactations in sows. Irish Veterinary Journal. 2012; 65(4):2-8.

101. Kankofer M, Lipko-Przybylska J. Physiological antioxidative/oxidative status in bovine colostrum and mature milk. Acta Veterinaria. 2008; 58(2-3):231-9.

102. Retskii M, Shakhov A, Chusov D, Zolotarev A, Lebedev M, Ermolova T, et al. Correcting the antioxidant status of newborn calves for forming higher colostral immunity. Russian Agricultural Sciences. 2010; 36(2):127-9.

103. Soberon M, Liu R, Cherney D. Short communication: Antioxidant activity of calf milk replacers. Journal of Dairy Science. 2012; 95(5):2703-6.

104. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition. 2004; 79(5):727-47.

105. Passamonti S, Terdoslavich M, Franca R, Vanzo A, Tramer F, Braidot E, et al. Bioavailability of flavonoids: a review of their membrane transport and the function of bilitranslocase in animal and plant organisms. Current Drug Metabolism. 2009; 10(4):369-94.

106. Hollman PCH, Arts ICW. Flavonols, flavones and flavanols–nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture. 2000; 80(7):1081-93.

107. Russo M, Spagnuolo C, Tedesco I, Bilotto S, Russo GL. The flavonoid quercetin in disease prevention and therapy: facts and fancies. Biochemical Pharmacology. 2012; 83(1):6-15.

108. Eid HM, Martineau LC, Saleem A, Muhammad A, Vallerand D, Benhaddou-Andaloussi A, et al. Stimulation of AMP-activated protein kinase and enhancement of basal glucose uptake in muscle cells by quercetin and quercetin glycosides, active principles of the antidiabetic medicinal plant Vaccinium vitis-idaea. Molecular Nutrition & Food Research. 2010; 54(7):991-1003.

109. Kobayashi Y, Miyazawa M, Kamei A, Abe K, Kojima T. 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. Bioscience, Biotechnology, and Biochemistry. 2010; 74(12):2385-95.

110. Crespy V, Morand C, Manach C, Besson C, Demigne C, Remesy C. Part of quercetin absorbed in the small intestine is conjugated and further secreted in the intestinal lumen. American Journal of Physiology-Gastrointestinal and Liver Physiology. 1999; 277(1):G120-G6.

111. Martel F, Monteiro R, Calhau C. Effect of polyphenols on the intestinal and placental transport of some bioactive compounds. Nutrition Research Reviews. 2010; 23(01):47-64.

112. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. Critical Reviews in Food Science and Nutrition. 2005; 45(4):287-306.

113. Zung A, Reifen R, Kerem Z, Zadik Z. Phytoestrogens: the pediatric perspective. Journal of Pediatric Gastroenterology and Nutrition. 2001; 33(2):112-8.

114. Cermak R, Landgraf S, Wolffram S. The bioavailability of quercetin in pigs depends on the glycoside moiety and on dietary factors. The Journal of Nutrition. 2003; 133(9):2802-7.

115. Crespy V, Morand C, Besson C, Cotelle N, Vézin H, Demigné C, et al. The splanchnic metabolism of flavonoids highly differed according to the nature of the compound. American Journal of Physiology. Gastrointestinal and Liver Physiology. 2003;

284(6):G980-G8.

116. Hollman P, De Vries J, van Leeuwen SD, Mengelers M, Katan MB. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. The American Journal of Clinical Nutrition. 1995; 62(6):1276-82.

117. Lesser S, Cermak R, Wolffram S. The fatty acid pattern of dietary fat influences the oral bioavailability of the flavonol quercetin in pigs. British Journal of Nutrition. 2006; 96(06):1047-52.

118. Murota K, Terao J. Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. Archives of Biochemistry and Biophysics. 2003; 417(1):12-7.

119. Day AJ, Canada FJ, Diaz JC, Kroon PA, McLauchlan R, Faulds CB, et al. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. FEBS Letters. 2000; 468(2-3):166-70.

120. Walle T, Otake Y, Walle UK, Wilson FA. Quercetin glucosides are completely hydrolyzed in ileostomy patients before absorption. The Journal of Nutrition. 2000; 130(11):2658-61.

121. Simmering R, Pforte H, Jacobasch G, Blaut M. The growth of the flavonoid-degrading intestinal bacterium, Eubacterium ramulus, is stimulated by dietary flavonoids in vivo. FEMS Microbiology Ecology. 2002; 40(3):243-8.

122. Manach C, Texier O, Morand C, Crespy V, Régérat F, Demigné C, et al. Comparison of the bioavailability of quercetin and catechin in rats. Free Radical Biology and Medicine. 1999; 27(11):1259-66.

123. Egert S, Wolffram S, Bosy-Westphal A, Boesch-Saadatmandi C, Wagner AE, Frank J, et al. Daily quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans. The Journal of Nutrition. 2008; 138(9):1615-21.

124. Gohlke A, Ingelmann CJ, Nurnberg G, Starke A, Wolffram S, Metges CC. Bioavailability of quercetin from its aglycone and its glucorhamnoside rutin in lactating dairy cows after intraduodenal administration. Journal of Dairy Science. 2013; 96(4):2303-13.

125. Moon YJ, Wang L, DiCenzo R, Morris ME. Quercetin pharmacokinetics in humans. Biopharmaceutics & Drug Disposition. 2008; 29(4):205-17.

126. Song J, Kwon O, Chen SL, Daruwala R, Eck P, Park JB, et al. Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), intestinal transporters for vitamin C and glucose. The Journal of Biological Chemistry. 2002; 277(18):15252-60.

127. Wolffram S, Blöck M, Ader P. Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine. The Journal of Nutrition. 2002; 132(4):630-5.

128. Walle T. Absorption and metabolism of flavonoids. Free Radical Biology and Medicine. 2004; 36(7):829-37.

129. Ader P, Wessmann A, Wolffram S. Bioavailability and metabolism of the flavonol quercetin in the pig. Free Radical Biology and Medicine. 2000; 28(7):1056-67.

130. de Boer VC, Dihal AA, van der Woude H, Arts IC, Wolffram S, Alink GM, et al. Tissue distribution of quercetin in rats and pigs. The Journal of Nutrition. 2005; 135(7):1718-25.

131. Berger LM, Wein S, Blank R, Metges CC, Wolffram S. Bioavailability of the flavonol quercetin in cows after intraruminal application of quercetin aglycone and rutin. Journal

of Dairy Science. 2012; 95(9):5047-55.

132. Li YQ, Zhou FC, Gao F, Bian JS, Shan F. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of α-glucosidase. Journal of Agricultural and Food Chemistry. 2009; 57(24):11463-8.

133. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. Journal of Nutritional Science and Vitaminology. 2006; 52(2):149-53.

134. Barrenetxe J, Aranguren P, Grijalba A, Martinez-Penuela JM, Marzo F, Urdaneta E. Effect of dietary quercetin and sphingomyelin on intestinal nutrient absorption and animal growth. The British Journal of Nutrition. 2006; 95(3):455-61.

135. Ramachandra R, Shetty AK, Salimath PV. Quercetin alleviates activities of intestinal and renal disaccharidases in streptozotocin-induced diabetic rats. Molecular Nutrition & Food Research. 2005; 49(4):355-60.

136. Walgren RA, Lin JT, Kinne RKH, Walle T. Cellular uptake of dietary flavonoid quercetin 4 '-beta-glucoside by sodium-dependent glucose transporter SGLT1. Journal of Pharmacology and Experimental Therapeutics. 2000; 294(3):837-43.

137. Chen C-H, Hsu H-J, Huang Y-J, Lin C-J. Interaction of flavonoids and intestinal facilitated glucose transporters. Planta Medica. 2007; 73(4):348-54.

138. Kwon O, Eck P, Chen S, Corpe CP, Lee J-H, Kruhlak M, et al. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. The FASEB Journal. 2007; 21(2):366-77.

139. Kellett G, Helliwell P. The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane. The Biochemical Journal. 2000; 350:155-62.

140. Kato A, Nasu N, Takebayashi K, Adachi I, Minami Y, Sanae F, et al. Structure− activity relationships of flavonoids as potential inhibitors of glycogen phosphorylase. Journal of Agricultural and Food Chemistry. 2008; 56(12):4469-73.

141. Gasparin FR, Salgueiro-Pagadigorria CL, Bracht L, Ishii-Iwamoto EL, Bracht A, Constantin J. Action of quercetin on glycogen catabolism in the rat liver. Xenobiotica; the fate of foreign compounds in biological systems. 2003; 33(6):587-602.

142. Gasparin FR, Spitzner FL, Ishii-Iwamoto EL, Bracht A, Constantin J. Actions of quercetin on gluconeogenesis and glycolysis in rat liver. Xenobiotica; the fate of foreign compounds in biological systems. 2003; 33(9):903-11.

143. Kobori M, Masumoto S, Akimoto Y, Takahashi Y. Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. Molecular Nutrition & Food Research. 2009; 53(7):859-68.

144. Estrada O, Hasegawa M, Gonzalez-Mujíca F, Motta N, Perdomo E, Solorzano A, et al. Evaluation of flavonoids from Bauhinia megalandra leaves as inhibitors of glucose-6-phosphatase system. Phytotherapy Research. 2005; 19(10):859-63.

145. Kim EK, Kwon KB, Song MY, Han MJ, Lee JH, Lee YR, et al. Flavonoids protect against cytokine-induced pancreatic beta-cell damage through suppression of nuclear factor kappaB activation. Pancreas. 2007; 35(4):e1-9.

146. Youl E, Bardy G, Magous R, Cros G, Sejalon F, Virsolvy A, et al. Quercetin potentiates insulin secretion and protects INS-1 pancreatic β-cells against oxidative damage via the ERK1/2 pathway. British Journal of Pharmacology. 2010; 161(4):799-814.

147. Vessal M, Hemmati M, Vasei M. Antidiabetic effects of quercetin in streptozocin-

induced diabetic rats. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2003; 135(3):357-64.

148. Strobel P, Allard C, Perez-Acle T, Calderon R, Aldunate R, Leighton F. Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. The Biochemical Journal. 2005; 386:471-8.

149. Nomura M, Takahashi T, Nagata N, Tsutsumi K, Kobayashi S, Akiba T, et al. Inhibitory mechanisms of flavonoids on insulin-stimulated glucose uptake in MC3T3-G2/PA6 adipose cells. Biological and Pharmaceutical Bulletin. 2008; 31(7):1403-9.

150. Fang X-K, Gao J, Zhu D-N. Kaempferol and quercetin isolated from Euonymus alatus improve glucose uptake of 3T3-L1 cells without adipogenesis activity. Life Sciences. 2008; 82(11):615-22.

151. Kim J-H, Kang M-J, Choi H-N, Jeong S-M, Lee Y-M, Kim J-I. Quercetin attenuates fasting and postprandial hyperglycemia in animal models of diabetes mellitus. Nutrition Research and Practice. 2011; 5(2):107-11.

152. Shetty A, Rashmi R, Rajan M, Sambaiah K, Salimath P. Antidiabetic influence of quercetin in streptozotocin-induced diabetic rats. Nutrition Research. 2004; 24(5):373-81.

153. Babu PVA, Liu D, Gilbert ER. Recent advances in understanding the anti-diabetic actions of dietary flavonoids. The Journal of Nutritional Biochemistry. 2013; 24(11):1777-89.

154. Capasso A, Pinto A, Mascolo N, Capasso F. Reduction of agonist-induced contractions of guinea-pig isolated ileum by flavonoids. Phytotherapy Research. 1991; 5(2):85-7.

155. Di Carlo G, Mascolo N, Izzo AA, Capasso F. Effects of quercetin on the gastrointestinal tract in rats and mice. Phytotherapy Research. 1994; 8(1):42-5.

156. Meli R, Di Carlo G, Capasso F. Inhibitory action of quercetin on intestinal transit in mice. Phytotherapy Research. 1990; 4(5):201-2.

157. Lozoya X, Reyes-Morales H, Chávez-Soto MA, del Carmen Martínez-García Ma, Soto-González Y, Doubova SV. Intestinal anti-spasmodic effect of a phytodrug of Psidium guajava folia in the treatment of acute diarrheic disease. Journal of Ethnopharmacology. 2002; 83(1):19-24.

158. Wei L-b, Li Z-J, Chen B-T, Sun S-Y, Luan T, Gao Y-F, et al. Clinical study on treatment of infantile rotaviral enteritis with guava leaf. Chinese Journal of Integrative Medicine. 2001; 7(2):86-9.

159. Bae E-A, Han MJ, Lee M, KIM D-H. In vitro inhibitory effect of some flavonoids on rotavirus infectivity. Biological and Pharmaceutical Bulletin. 2000; 23(9):1122-4.

160. Savi LA, Caon T, de Oliveira AP, Sobottka AM, Werner W, Reginatto FH, et al. Evaluation of antirotavirus activity of flavonoids. Fitoterapia. 2010; 81(8):1142-6.

161. Choi H-J, Kim J-H, Lee C-H, Ahn Y-J, Song J-H, Baek S-H, et al. Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. Antiviral Research. 2009; 81(1):77-81.

162. Parkar SG, Stevenson DE, Skinner MA. The potential influence of fruit polyphenols on colonic microflora and human gut health. International Journal of Food Microbiology. 2008; 124(3):295-8.

163. Liu H, Liu Y, Hu L, Suo Y, Zhang L, Jin F, et al. Effects of dietary supplementation of quercetin on performance, egg quality, cecal microflora populations, and antioxidant status in laying hens. Poultry Science. 2014; 93(2):347-53.

164. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry. 2002; 13(10):572-84.

165. Ioku K, Tsushida T, Takei Y, Nakatani N, Terao J. Antioxidative activity of quercetin and quercetin monoglucosides in solution and phospholipid bilayers. Biochimica et Biophysica Acta-Biomembranes. 1995; 1234(1):99-104.

166. Peng I-W, Kuo S-M. Flavonoid structure affects the inhibition of lipid peroxidation in Caco-2 intestinal cells at physiological concentrations. The Journal of Nutrition. 2003; 133(7):2184-7.

167. Shirai M, Yamanishi R, Moon J-H, MUROTA K, TERAO J. Effect of quercetin and its conjugated metabolite on the hydrogen peroxide-induced intracellular production of reactive oxygen species in mouse fibroblasts. Bioscience, Biotechnology, and Biochemistry. 2002; 66(5):1015-21.

168. Terao J, Piskula M, Yao Q. Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. Archives of Biochemistry and Biophysics. 1994; 308(1):278-84.

169. Doronicheva N, Yasui H, Sakurai H. Chemical structure-dependent differential effects of flavonoids on the catalase activity as evaluated by a chemiluminescent method. Biological and Pharmaceutical Bulletin. 2007; 30(2):213-7.

170. Jeong SM, Kang MJ, Choi HN, Kim JH, Kim JI. Quercetin ameliorates hyperglycemia and dyslipidemia and improves antioxidant status in type 2 diabetic db/db mice. Nutrition Research and Practice. 2012; 6(3):201-7.

171. Krych J, Gebicka L. Catalase is inhibited by flavonoids. International Journal of Biological Macromolecules. 2013; 58:148-53.

172. Wiegand H, Boesch-Saadatmandi C, Regos I, Treutter D, Wolffram S, Rimbach G. Effects of Quercetin and Catechin on Hepatic Glutathione-S Transferase (GST), NAD(P)H Quinone Oxidoreductase 1 (NQO1), and Antioxidant Enzyme Activity Levels in Rats. Nutrition and Cancer. 2009; 61(5):717-22.

173. Raso GM, Meli R, Di Carlo G, Pacilio M, Di Carlo R. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A. 1. Life Sciences. 2001; 68(8):921-31.

174. Nair MP, Mahajan S, Reynolds JL, Aalinkeel R, Nair H, Schwartz SA, et al. The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-κβ system. Clinical and Vaccine Immunology. 2006; 13(3):319-28.

175. Overman A, Chuang C, McIntosh M. Quercetin attenuates inflammation in human macrophages and adipocytes exposed to macrophage-conditioned media. International Journal of Obesity. 2011; 35(9):1165-72.

176. Chuang CC, Martinez K, Xie GX, Kennedy A, Bumrungpert A, Overman A, et al. Quercetin is equally or more effective than resveratrol in attenuating tumor necrosis factor-alpha-mediated inflammation and insulin resistance in primary human adipocytes. American Journal of Clinical Nutrition. 2010; 92(6):1511-21.

177. Bhaskar S, Kumar KS, Krishnan K, Antony H. Quercetin alleviates hypercholesterolemic diet induced inflammation during progression and regression of atherosclerosis in rabbits. Nutrition. 2013; 29(1):219-29.

178. Dodda D, Chhajed R, Mishra J. Protective effect of quercetin against acetic acid induced inflammatory bowel disease (IBD) like symptoms in rats: Possible

morphological and biochemical alterations. Pharmacological Reports. 2014; 66(1):169-73.

179. Dou W, Zhang J, Li H, Kortagere S, Sun K, Ding L, et al. Plant flavonol isorhamnetin attenuates chemically induced inflammatory bowel disease via a PXR-dependent pathway. The Journal of Nutritional Biochemistry. 2014; 25(9):923-33.

180. Mascaraque C, Aranda C, Ocón B, Monte MJ, Suárez MD, Zarzuelo A, et al. Rutin has intestinal antiinflammatory effects in the CD4+ CD62L+ T cell transfer model of colitis. Pharmacological Research. 2014; 90:48-57.

181. Chang Y-C, Tsai M-H, Sheu WH-H, Hsieh S-C, Chiang A-N. The Therapeutic Potential and Mechanisms of Action of Quercetin in Relation to Lipopolysaccharide-Induced Sepsis In Vitro and In Vivo. Plos One. 2013; 8(11).

182. Liao Y-R, Lin J-Y. Quercetin intraperitoneal administration ameliorates lipopoly-saccharide-induced systemic inflammation in mice. Life Sciences. 2015; 137:89-97.

183. Kuhn G, Ender K, Thomann R, Tuchscherer M, Tuchscherer A, Stabenow B, et al. Einsatz von Echinacea-Extrakt bei tragenden und säugenden Sauen. Archiv für Tierzucht. 2005; 48:270-82.

184. Maass N, Bauer J, Paulicks B, Böhmer B, Roth-Maier D. Efficiency of Echinacea purpurea on performance and immune status in pigs. Journal of Animal Physiology and Animal Nutrition. 2005; 89(7-8):244-52.

185. Metwally M, Khafagy A, Eid H, ALgammal A. Studies on the Immunostimulatory Effect of Propolis in Diarrheic Buffalo Calves. Journal of Advanced Veterinary Research. 2013; 3(3):114-7.

186. Fischer G, Cleff MB, Dummer LA, Paulino N, Paulino AS, de Oliveira Vilela C, et al. Adjuvant effect of green propolis on humoral immune response of bovines immunized with bovine herpesvirus type 5. Veterinary Immunology and Immunopathology. 2007; 116(1):79-84.

3. EFFECTS OF ORAL QUERCETIN SUPPLEMENTATION ON SPLANCHNIC GLUCOSE METABOLISM IN 1-WEEK-OLD CALVES DEPEND ON DIET AFTER BIRTH

Jeannine Gruse[1], Solvig Görs[1], Armin Tuchscherer[2], Winfried Otten[3], Joachim M Weitzel[4], Cornelia C Metges[1], Siegfried Wolffram[5], and Harald M Hammon[1]*

[1] Institute of Nutritional Physiology ‘‘Oskar Kellner’’,
[2] Institute of Genetics and Biometry,
[3] Institute of Behavioural Physiology, and
[4] Institute of Reproductive Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany; and
[5] Institute of Animal Nutrition and Physiology, Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University of Kiel, Kiel, Germany

Received June 3, 2015
Accepted August 27, 2015
*Corresponding author: hammon@fbn-dummerstorf.de

Published in *The Journal of Nutrition* (2015; 145:2486–95), American Society for Nutrition. doi:10.3945/jn.115.218271.

Please read this part online.
https://dx.doi.org/10.3945/jn.115.218271

4. QUERCETIN FEEDING IN NEWBORN DAIRY CALVES CANNOT COMPENSATE COLOSTRUM DEPRIVATION: STUDY ON METABOLIC, ANTIOXIDATIVE AND INFLAMMATORY TRAITS

Jeannine Gruse[1], Ellen Kanitz[2], Joachim M. Weitzel[3], Armin Tuchscherer[4], Tadeusz Stefaniak[5], Paulina Jawor[5], Siegfried Wolffram[6] and Harald M. Hammon[1]*

[1] Institute of Nutritional Physiology "Oskar Kellner",

[2] Institute of Behavioural Physiology,

[3] Institute of Reproductive Biology, and

[4] Institute of Genetics and Biometry, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany

[5] Department of Immunology, Pathophysiology and Veterinary Preventive Medicine, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland

[6] Institute of Animal Nutrition and Physiology, Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University of Kiel, Kiel, Germany

Received November 6, 2015

Accepted December 26, 2015

*Corresponding author: hammon@fbn-dummerstorf.de

Published in *PLoS ONE* 2016; 11(1): e0146932. doi:10.1371/journal.pone.0146932

4.1 ABSTRACT

Immaturity of the neonatal immune system is causative for high morbidity in calves and colostrum intake is crucial for acquiring passive immunity. Pathogenesis is promoted by reactive oxygen species accumulating at birth if counter-regulation is inadequate. The flavonol quercetin exerts antioxidative and anti-inflammatory effects that may enhance neonatal health. The aim of this work was to study effects of quercetin feeding on metabolic, antioxidative and inflammatory parameters in neonatal calves to investigate whether quercetin could compensate for insufficient colostrum supply. Twenty-eight newborn calves were assigned to two dietary groups fed colostrum or milk-based formula on day 1 and 2 and milk replacer thereafter. From day 2 onward, 7 calves per dietary group were additionally fed quercetin aglycone (50 mg/(kg body weight × day)). Blood samples were taken repeatedly to measure plasma concentrations of flavonols, glucose, lactate, total protein, albumin, urea, non-esterified fatty acids, triglycerides, cholesterol, insulin, glucagon, cortisol, immunoglobulins, fibrinogen, haptoglobin and serum amyloid A. Trolox equivalent antioxidative capacity, ferric reducing ability of plasma, thiobarbituric acid reactive species and F2-isoprostanes were analyzed to evaluate plasma antioxidative status. Expression of tumor necrosis factor, interleukin-1α, interleukin-1β, serum amyloid A, haptoglobin, fibrinogen, C-reactive protein, catalase, glutathione peroxidase and superoxide dismutase mRNA were measured in liver tissue on day 8. Plasma flavonol concentrations were detectable only after quercetin-feeding without differences between colostrum and formula feeding. Plasma glucose, lactate, total protein, immunoglobulins, triglycerides, cholesterol, trolox equivalent antioxidative capacity and thiobarbituric acid reactive species were higher after colostrum feeding. Body temperature, fecal fluidity and plasma concentrations of cortisol and haptoglobin were higher in formula- than in colostrum-fed groups. Hepatic mRNA expression of tumor necrosis factor was higher after quercetin feeding and expression of C-reactive protein was higher after formula feeding. Data confirm that colostrum improves neonatal health and indicate that quercetin feeding cannot compensate for insufficient colostrum supply.

4.2 INTRODUCTION

Calfhood diseases play a key role in the economy of dairy farms because they increase operating costs and reduce long-term productivity of the animal. Incidence of disease is associated with increased mortality rates [1], and enteritis is the most common diagnosis in young calves [2], which, according to Svensson, Linder and Olsson [3], contributes to 23% of calf losses during the first 14 days of life. Neonatal calves are prone to sickness because their immune system is immature. Furthermore, the process of birth itself causes an elevated stress level for the newborn and exposure to an oxygen-rich environment leads to an increased generation of reactive oxygen species [4, 5]. Reactive oxygen species induce peroxidation of lipids and other macromolecules, leading to alteration of cellular components, interaction with signaling cascades and modification of physiological cell functions [6]. If not properly counterbalanced by antioxidative defenses, excessive production of reactive oxygen species results in oxidative stress, which is a cofactor of disease in humans and farm animals [5, 7, 8]. Adequate colostrum supply is vital to calves because colostrum ensures ingestion of nutrients and contains immunoglobulins (Ig), peptides, antioxidants and other bioactive factors supporting maturation, antioxidative and immune defense as well as local intestinal immunity [9].

The ban on antibiotic performance promoters by the European Union in 2006 increased efforts to establish natural alternatives to enhance health and productivity in breeding. Special focus has been directed to phytochemicals because their use can be manifold according to the respective compound [10]. Flavonoids are secondary plant metabolites that are widely distributed in the plant kingdom and are able to modulate inflammation and immune function and exert antioxidative activity [11-13].

Quercetin, which belongs to the subclass of flavonols, is ubiquitous in most plants and is of interest for scientists for its beneficial use in humans and farm animals. Its antioxidative capacity can ameliorate the acquisition of passive immunity in neonates, based on the finding that feeding antioxidant-enriched colostrum enhanced IgG absorption and antioxidative status in newborn calves and piglets [14, 15]. Similarly, Retskii et al. [16] showed that correcting the antioxidative balance in newborn calves prior to first colostrum ingestion increases the acquisition of colostral immunity and reduces the incidence of enteric colibacillosis. Another beneficial effect of quercetin is its local action in the gastrointestinal tract. *In vitro* studies on intestinal epithelium demonstrated that quercetin down-regulates the expression of genes related to inflammation in inflamed epithelium [17], and Lozoya et al. [18] showed in a clinical study that oral quercetin administration reduced abdominal pain in acute diarrheic disease in humans. In guinea pigs, mice and rats, the inhibitory action of quercetin on prostaglandin E_2-induced ileal contractions and on castor-oil-induced diarrhea has been demonstrated [19, 20]. Furthermore, quercetin acts as a prebiotic, thus inhibiting

adhesion of enteropathogens to Caco-2 cells without affecting the viability of probiotics [21], and improves performance in hens by modulating cecal microflora populations [22].

Although a multitude of research on quercetin has been performed *in vitro* or in animal models for medical conditions, studies of the effects in neonatal farm animals are scarce. The aim of the present work was to investigate the potential health-promoting effects of feeding quercetin to newborn calves during the first week of life and to evaluate whether the health-promoting effects of quercetin compensate for initial colostrum deprivation in calves. We hypothesized that quercetin improves antioxidative balance and immune function and that local antibacterial and anti-inflammatory effects reduce the incidence of diarrhea and gastrointestinal dysfunctions.

4.3 MATERIALS AND METHODS

4.3.1 Animals, Husbandry and Feeding

Experimental procedures were conducted in compliance with the German Animal Protection regulations with approval of the authorities of the state Mecklenburg-Western Pomerania, Germany (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern; LALLF M-V/TSD/7221.3-1.1-044/12). Liver biopsies were performed under local lidocaine anesthesia and all calves received metamizole post-operatively for pain relief.

Twenty-eight male Holstein Friesian calves were separated from their dams immediately after birth and were housed in single boxes during their first 8 days of life. Before the trial started, separate colostrum pools were prepared from the first and third milkings after parturition. According to the colostrums' macronutrient compositions, milk-based formulas with comparable amounts of macronutrients [23] but without bioactive factors were provided (Bergin MAT-Formula; Bergophor Futtermittelfabrik, Kulmbach, Germany). Calves were randomly assigned to two dietary groups and were bucket-fed twice daily, receiving either colostrum (Col, n = 14) or corresponding formula (For, n = 14) on days 1 and 2 of life (10% and 12% of body weight/day, respectively). If appetite was reduced, calves were tube fed to ensure complete ingestion of colostrum or formula. From day 3 until day 8, all calves were fed commercial milk replacer (12% of body weight/day; 150 g/L; SALVAlac MIRApro 45; Salvana Tiernahrung, Klein-Offenseth Sparrieshoop, Germany).

On day 2, the dietary groups were subdivided into control (ColQ- and ForQ-; n = 7 per group) and treatment groups (ColQ+ and ForQ+; n = 7 per group), the latter receiving quercetin aglycone twice daily with feeding (50 mg/(kg body weight × day); quercetin aglycone dihydrate ≥98%, Carl Roth, Karlsruhe, Germany). The control groups received no quercetin aglycone.

4.3.2 Treatment

Navels were disinfected with 10% povidone iodine solution (Vet-Sept; aniMedica, Senden-Bösensell, Germany). Neonatal calves received an oral dose of 1 g iron dextran with their first meal on day 1 (Ursoferran; Serumwerk Bernburg, Bernburg, Germany). To support immunological defense during the first 5 days of life, all calves received chicken egg-derived immunoglobulins with the morning feeding (0.25 g/kg body weight; Globigen Life Start 25%, EW Nutrition, Visbek, Germany) [24]. To prevent cryptosporidiosis, calves were treated with halofuginone (0.1 mg/kg body weight per *os*; Halocur, Intervet, Igoville, France) after the evening feeding from day 1 to day 7.

Colostrum-deprived calves (ForQ+, ForQ-) additionally received B-vitamins (100 mg nicotinamide/calf, 40 mg thiamin chlor*i*de hydrochloride/calf, s.c.; Vitamin-B-Komplex, Serumwerk Bernburg, Germany) and bovine colostral immunoglobulins on days 1 (s.c.), 3 and 5 (per *os)* (2 g gammaglobulins/calf with antibodies against *Escherichia coli*, rotavirus, coronavirus; Aniserin orinject; aniMedica, Seden-Börsensell, Germany). Furthermore, formula-fed calves were treated metaphylactically with colistin sulfate from day 2 to day 8 (3 mg/kg body weight, i.m.; Belacol; BelaPharm, Vechta, Germany).

All calves were weighed immediately after birth and before evening meals on days 2 and 6. Every morning, health status was examined and appetite, general condition, heart rate, respiratory rate, rectal temperature and gut motility were assessed. Fecal fluidity was scored according to Larson et al. [25]. Calves with reduced vitality after the first 2 days of life were allowed one recovery day before further sample taking. In these cases, the times referred to as days 3, 4, 7 and 8 in the results section are days 4, 5, 8 and 9 after birth, respectively. Due to gastrointestinal imbalances, two calves (one calf of group ColQ+ and one calf of group ForQ+) had to be excluded from the study.

4.3.3 Blood Analyses

4.3.3.1 Sample Taking

Basal blood samples were taken before the morning feeding on days 1, 2, 4 and 7 from the jugular vein using evacuated tubes containing either potassium-EDTA (1.2-2 mg/mL EDTA) for analyses of plasma metabolites, insulin, glucagon, immunoglobulins and acute-phase proteins or Li-heparin (12-30 IU heparin) for the determination of the cortisol and flavonol concentrations and the antioxidative status in the plasma. For flavonol analysis, additional blood samples were taken before the morning feeding on days 3 and 8. After centrifugation (1,500 × *g*, 4°C, 20 min), plasma aliquots were stored at -20°C until analyses (-80°C for analyses of flavonol concentrations and antioxidative status, respectively).

4.3.3.2 Plasma Flavonols, Metabolites and Hormones

Plasma concentrations of flavonols (quercetin, isorhamnetin, tamarixetin and kaempferol) were measured via HPLC as previously described [26] with a detection limit of 2 nmol/L and a recovery rate of 92 ± 2%. The intra- and inter-assay coefficients were 0.5 and 7.2%, respectively.

Plasma metabolites were analyzed using an automatic spectrophotometer (ABX Pentra 400; Horiba ABX, Montpellier, France) and respective kits: glucose (#A11A01667), lactate (#A11A01721), albumin (#A11A01664) and triacylglycerides (#A11A01640) from HORIBA ABX, Montpellier, France; total protein (#553-412) and cholesterol (#553-127) from mti-diagnostics, Idstein, Germany; urea (#LT-UR 0010) from Labor+Technik, E. Lehmann, Berlin, Germany; and non-esterified fatty acids (#434-91795, #436-91995) from WAKO Chemicals, Neuss, Germany.

Plasma concentrations of insulin (#RIA-1257) and glucagon (#RIA-1258) were determined by RIA using kits from DRG Instruments, Marburg, Germany, which were adapted to bovines [27]. Intra- and inter-assay coefficients of variation were 3.7% and 5.5% for insulin, and 3.4% and 22.5% for glucagon, respectively. Plasma cortisol concentrations were analyzed in duplicate after extraction with diethylether using a commercially available ELISA kit (#EIA1887; DRG Instruments GmbH, Marburg, Germany) according to the instructions of the manufacturer. Cross reactivities of the antibody to corticosterone and progesterone were 45% and 9%, respectively, and <2% to any further competing plasma steroids. The assay was validated for use with bovine plasma. The test sensitivity was 3.4 µg/L, and intra- and inter-assay coefficients of variation were 5.3% and 12.1%.

4.3.3.3 Immunoglobulins (Ig) and Acute-Phase Proteins

The concentrations of IgG1, IgG2 and IgM, as well as acute-phase proteins (haptoglobin, serum amyloid A and fibrinogen), were measured in the EDTA plasma samples taken on days 1, 2, 4 and 7. IgG1 was analyzed by radial immunodiffusion [28] (modified by Gasowska and Stefaniak [29]) using bovine reference serum (RS10-103; Bethyl Laboratories Inc., Montgomery, USA) as standard. IgG2 (#E10-117) and IgM (#E10-101) were determined by ELISA using kits from Bethyl Laboratories Inc., Montgomery, USA. Intra-assay coefficients of variation were 10.9% and 4.0% for IgG2 and IgM, respectively. The detection limit of IgG2 was 7.8 µg/L and that of IgM was 15.6 µg/L. For detection of serum amyloid A (SAA; #TP-802), we used a multispecies ELISA kit from Tridelta Development, Maynooth, Ireland. The detection limit of SAA was 9.4 mg/L. The intra-assay coefficient of variation was 12.0%. The haptoglobin concentration was analyzed using the guaiacol method developed by Jones and Mould [30] with human haptoglobin Hp 2-2 (Sigma #H9762) as a standard. The detection limit of haptoglobin was 0.01 g/L. Plasma fibrinogen was determined by rapid heat

precipitation according to Millar, Simpson and Stalker [31].

4.3.3.4 Antioxidative Status

Li-heparinized plasma samples taken on days 1, 4 and 7 were used to analyze the Trolox-Equivalent Antioxidative Capacity (TEAC, as trolox equivalents (TE) in mmol/L) and Ferric Reducing Ability of Plasma (FRAP, as Ascorbic Acid Equivalents, (ASCE) in µmol/L) as parameters of antioxidative capacity as well as Thio-Barbituric Acid Reactive Species (TBARS, as Malondialdehyde Equivalents (MDAE) in µmol/L) and 8-iso-$PGF_{2\alpha}$ (F2-isoprostanes, in ng/L) as markers for oxidative stress.

TEAC was analyzed as described by Miller et al. [32] and modified by Re et al. [33]. FRAP and TBARS were determined according to Luehring et al. [34]. For determination of F2-isoprostanes, a commercial ELISA kit (#ADI-900-091; Enzo Life Sciences, Lause, Switzerland) was used. Cross-reactivities of the assay to $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were 4.6% and 1.85%, respectively, and <1% to any further eicosanoids.

4.3.4 Liver Tissue Analyses

On day 8, a liver biopsy was conducted 2 h after the morning meal using a custom-made biopsy trocar [23]. Biopsy tissue was immediately frozen in liquid nitrogen and stored at -80°C until further analysis. Hippocalcin-like 1 (*HPCAL1*; NM_001098964), low-density lipoprotein receptor-related protein 10 (*LRP10*; BC149232) and RNA polymerase II (*POLR2A*; NM_001206313.1) were used as reference genes (given accession numbers related to NIH GenBank). Primer sequences, accession numbers and PCR conditions for target genes related to antioxidative status (catalase (*CAT*); glutathione peroxidase 1 (*GPX1*); superoxide dismutase (*SOD*)) and inflammation (tumor necrosis factor (*TNF*); interleukin-1α and -1β (*IL1A*, *IL1B*); haptoglobin (*HP*); fibrinogen (*FGA*); serum amyloid A2 (*SAA2*); and C-reactive protein (*CRP*)) are listed in **Table 4.1**. As recently described [23], primer products were verified by sequencing using the BigDye Terminator version 1.1 Cycle Sequencing kit and an ABI 3130 Genetic Analyzer (Life Technologies, Carlsbad, USA). Real-time PCR was performed using a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany); SYBR Green I was used as the fluorescent dye. Melting curve analysis and agarose gel electrophoresis were used to confirm the specificity of the PCR products. Quantification cycle values and amplification efficiencies obtained using LinRegPCR version 2013.0 [35] were imported into qBASE+ version 2.6.1 (Biogazelle, Gent, Belgium) for all subsequent calculations and quality controls. The geometric mean of the reference gene abundances was used for normalization. Data are presented as the ratio of the copy numbers of genes of interest and the geometric mean of the reference genes' abundances.

Table 4.1: Characteristics of Primers and Real-Time RT-PCR Conditions[1]

Gene	Forward (5'→ 3')	Reverse (5'→ 3')	NIH GenBank accession number	Amplicon size (bp)	Mean Cq[2]	Efficiency
Proinflammatory Cytokines						
TNF	AGAGGGAAGAGCAGTCCCCAG	TTCACACCGTTGGCCATGAG	NM_173966.3	181	27.30	1.90
IL1A	TGAACGACGCCCTCAATCAA	GGTGTCTCAGGCATCTCCTTT	NM_174092.1	226	27.98	1.90
IL1B	AACGTCCTCCGACGAGTTTC	GCTCATGCAGAACACCACTTC	NM_174093.1	163	25.70	1.89
Acute-Phase Proteins						
HP	GGCCCCCGAGATTGCTAATA	CTCTGGGCAGCTGTCATCTT	NM_001040470.2	172	15.53	1.88
SAA2	CCACTGGGGATCAGCACAAT	CCTCTTTGGGCAGCGTCATA	NM_001075260.2	212	16.25	1.82
FGA	CGCGATTGAAAGCAAGCACT	GAAGTGTGGATACCTCTGGCA	NM_001033626.1	129	14.71	1.87
CRP	CAGGCCAGACAGACTTGCATA	TGCTGCTTGGTGGCATAA	NM_001144097.1	181	18.52	1.90
Antioxidative Enzymes						
CAT	TCACTCAGGTGCGGACTTTC	GGATGCGGGAGCCATATTCA	NM_001035386.2	162	18.40	1.87
GPX1	CTTCCCCTGCAACCAGTT	GGCAATTCAGGATCTCCTCGTT	X13684.1	62	20.97	1.87
SOD	AAGGCCGTGTGCGTGCTGAA	CAGGTCTCCAACATGCCTCT	M81129.1	246	20.44	1.87

[1] Initial denaturation = 10 min at 95°C; denaturation =15 s at 95°C; annealing = 10 s at 60°C; extension = 30 s at 72°C.
[2] Quantification cycle.

4.3.5 Statistical Analyses

Statistical analyses were conducted using SAS software, version 9.3 for Windows, SAS Institute Inc., Cary, USA. Descriptive statistics and tests for normality were calculated using the UNIVARIATE procedure of Base SAS software. Body weight, average daily weight gain and data for hepatic gene expression were analyzed by ANOVA with the MIXED procedure of SAS/STAT taking a model with the fixed factors diet (levels: Col vs. For), quercetin (levels: Q+ vs. Q-) and the interaction diet×quercetin. Feed intake, body temperature, heart and respiratory rate and plasma concentrations of metabolites, hormones, flavonols and markers of antioxidative status were analyzed by repeated measurement ANOVA using the MIXED procedure of SAS/STAT software and a model with the fixed factors diet, quercetin and day of life (repeated variable) and all interactions between the fixed factors. Repeated measures on the same calf were taken into account using the REPEATED statement of the MIXED procedure and a type for the block diagonal residual covariance matrix chosen in dependence on the levels of day of life. For concentrations of plasma metabolites, hormones and data on antioxidative status in plasma, an unstructured type was used. For the plasma concentrations of flavonols, acute-phase proteins and Ig, a compound symmetry type was used. For data on heart and respiratory rate, as well as body temperature and feed intake, an autoregressive (1) type was applied. Least-squares means (LSM) and their standard errors (SE) were computed for each fixed effect in the models and all pairwise differences of LSM were tested by the Tukey-Kramer procedure. The SLICE statement of the MIXED procedure was used to conduct partitioned analyses of the LSM for interactions. Fecal score was analyzed by a generalized linear mixed model using the GLIMMIX procedure of SAS/STAT and a Poisson model with the fixed factors diet, quercetin and day of life (repeated variable) and all interactions between these fixed factors. Repeated measurements on the same animal were taken into account by the RESIDUAL option of the RANDOM statement of the GLIMMIX procedure using a compound symmetry structure for the block diagonal residual covariance matrix. Sick frequencies of calves with respect to diet and quercetin were analyzed with the FREQ procedure of SAS/STAT software using two-way tables of diet by sick and quercetin by sick and the exact Pearson chi-square test. Effects and differences were considered significant at $P < 0.05$.

4.4 RESULTS

4.4.1 Feeding, Growth Performance and Health Status

Mean body weight at birth was 45.5 ± 1.9 kg and increased with age ($P < 0.01$) by 368 ± 140 g/d, without group differences, respectively. All calves received their first meal 2.2 ± 0.1 h after birth. Milk intake related to body weight did not differ among groups on

days 1 and 2 but was lower on days 3 and 4 in the colostrum-deprived calves ($P < 0.01$; **Figure 4.1 A**). On day 2, appetite was reduced in the formula-fed calves and the amount of tube-fed milk was higher than in colostrum-fed calves ($P < 0.01$; **Figure 4.1 B**). Heart rate decreased with age ($P < 0.01$) and respiratory rate tended to decrease ($P = 0.08$; **Figures 4.1 C and D**). Rectal temperature was highest on days 3 and 4 and subsequently decreased ($P < 0.01$). Rectal temperature and fecal score were higher in formula-fed than in colostrum-fed calves ($P < 0.01$; **Figures 4.1 E and F**). The number of calves with an allowed recovery day was similar among groups. With the exception of ColQ+, we treated one calf per group medically for navel infection. Four calves in group ForQ+ needed antispasmodic/analgesic treatment during a recovery day because of abdominal pain. Thus, Col-fed calves tended to be less susceptible to illness ($P = 0.08$), whereas quercetin treatment did not affect well-being ($P = 0.38$). Due to severe disease, we had to remove two calves from the study on day 4 (ForQ+) and day 7 (ColQ+).

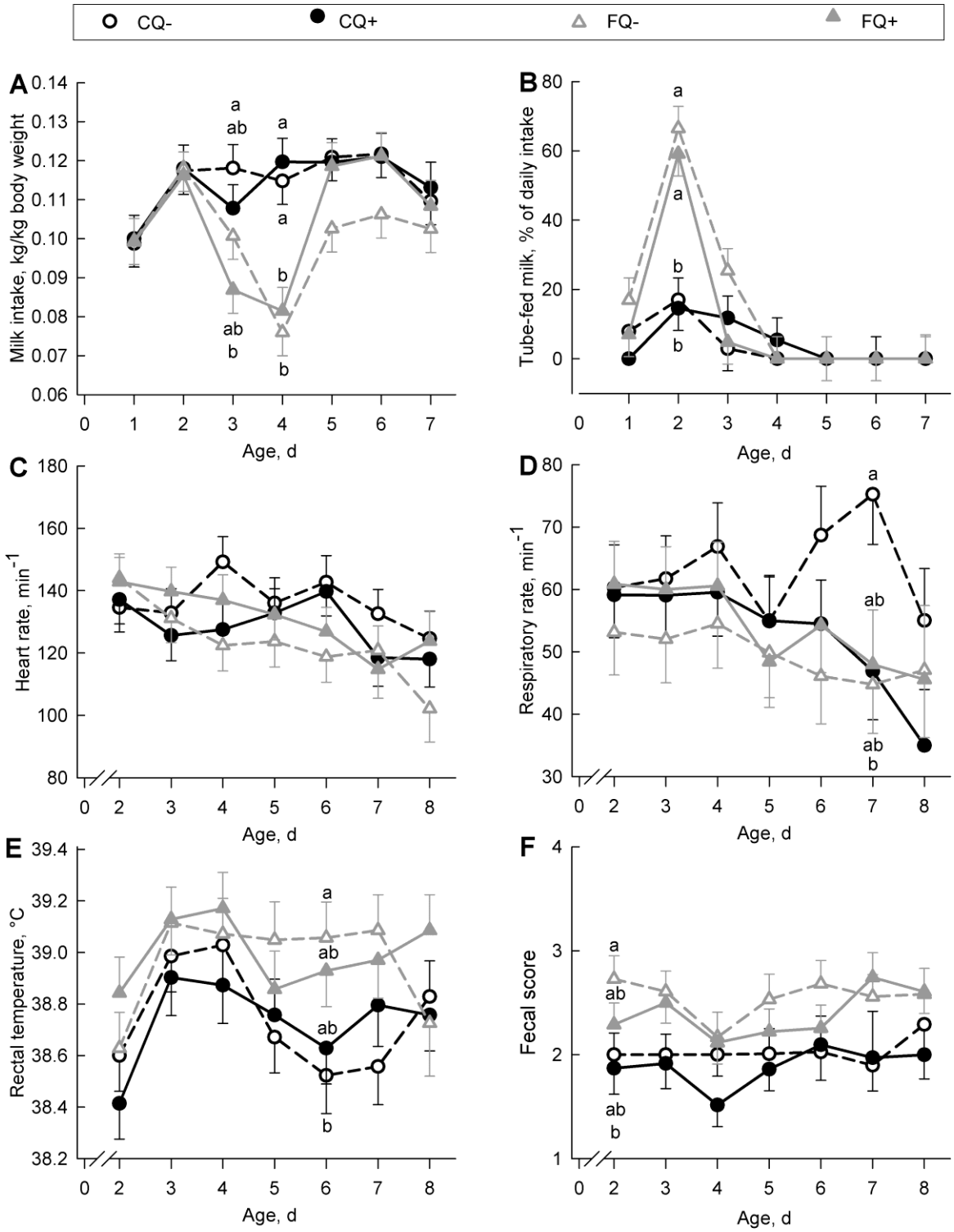

Figure 4.1: Feed Intake and Health Parameters. Neonatal calves were fed either colostrum (black circles) or formula (gray triangles) on days 1 and 2 and were supplemented with (filled symbols, solid lines) or without (open symbols, dashed lines) quercetin aglycone from day 2 to day 8 (50 mg/(kg body weight × day)). (A) Milk intake, (B) percent milk intake by tube feeding, (C) heart rate, (D) respiratory rate, (E) body temperature and (F) fecal score (according to Larson et al. [25]; 1 = normal, 2 = soft, 3 = runny, 4 = watery) were observed daily. Data are presented as the least squares means ± standard errors. Least squares means with different lowercase letters (a, b) differ among groups within the same day ($P < 0.05$).

4.4.2 Flavonoid Content

Preprandial concentrations of total flavonols in plasma on days 1 and 2 (before first quercetin supplementation) did not reveal significant differences among groups, but very low concentrations were detectable in five colostrum-fed calves. In the control groups ForQ- and ColQ-, the plasma concentrations did not change with age. In ColQ+ and ForQ+, the plasma flavonol concentrations changed with age ($P < 0.01$); they reached the maximum on day 3 and decreased subsequently but tended to differ among groups only on day 4 ($P = 0.08$; **Figure 4.2 A**). Comparing the flavonol fractions in the plasma, the quercetin fraction (60% of total flavonoids) was highest in both dietary groups. The concentrations of isorhamnetin and tamarixetin on day 3 were higher in ColQ+ than in ForQ+ (**Figure 4.2 B**).

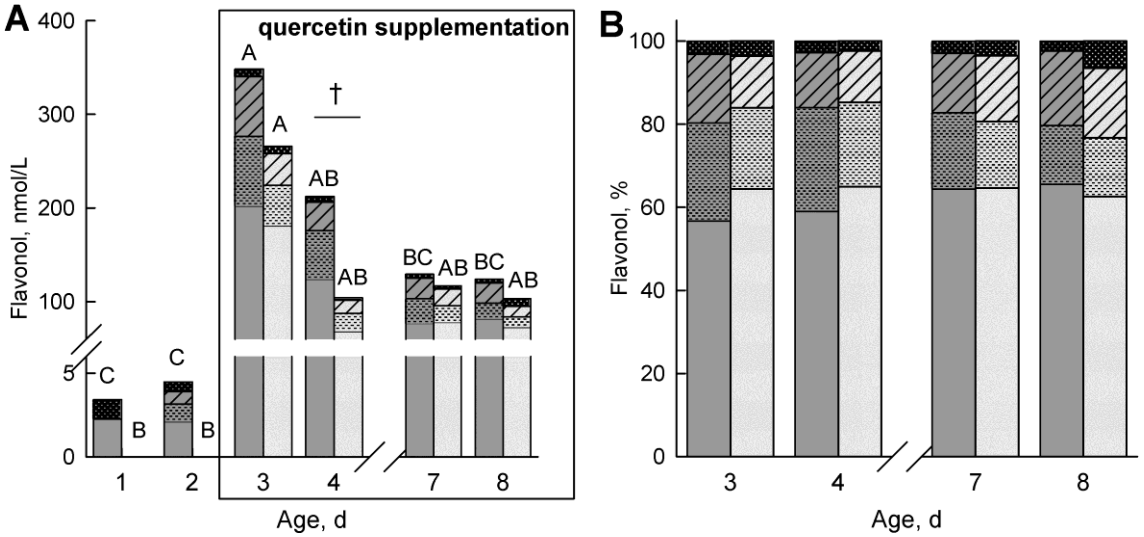

Figure 4.2: Flavonol Concentrations in Plasma. Stacked concentrations (A) and percentage composition (B) of flavonols in basal plasma samples of calves fed either colostrum (dark grey) or formula (light grey) on days 1 and 2 and supplemented with quercetin aglycone from day 2 to day 8 (50 mg/(kg body weight × day)). Flavonol metabolites: quercetin (without fill pattern), isorhamnetin (horizontal lines), tamarixetin (crossline pattern) and kaempferol (horizontal bold lines). Data are presented as the least squares means. Different uppercase letters (A, B, C) symbolize differences in the total flavonol concentration within the same group on different days ($P < 0.05$). † tend to differ among groups on the same day ($P < 0.10$)

4.4.3 Plasma Metabolites and Hormones

Plasma glucose concentrations were lowest on day 1, increased on day 2 in ColQ-, ForQ+ and ForQ- ($P < 0.05$) and decreased on day 4 only in ForQ+ and ForQ- ($P < 0.05$). The mean plasma concentration of glucose was higher in the colostrum-fed than in the formula-fed calves ($P < 0.03$; **Table 4.2**). The plasma lactate and non-esterified fatty acid concentrations were highest on day 1 and decreased with age ($P < 0.01$) without any group differences (Table 4.2). The concentrations of total protein in the plasma increased only in the colostrum-fed calves ($P < 0.01$) and were higher in the colostrum-fed than in the formula-fed calves on day 2, day 4 and day 7 ($P < 0.01$; **Figure 4.3 A**). Plasma albumin decreased with age ($P < 0.01$) in all groups (**Figure 4.3 B**). For both total protein and albumin, the diet×age interaction was significant ($P < 0.01$). The plasma urea concentrations increased on day 2 in the formula-fed and on day 7 in the colostrum-fed calves ($P < 0.01$; Table 4.2). The plasma triglyceride concentrations increased until day 4 only in the colostrum-fed calves ($P < 0.05$) and continuously decreased from day 2 to day 7 in the formula-fed calves ($P < 0.01$). The plasma cholesterol concentration increased with age ($P < 0.01$). The plasma triglyceride (day 7) and cholesterol concentrations (day 4 and day 7) were higher in the colostrum-fed than in the formula-fed calves ($P < 0.01$; Table 4.2).

The plasma insulin concentrations decreased with age ($P < 0.01$; Table 4.2). Glucagon increased to a maximum plasma concentration on day 2 and subsequently decreased in all calves ($P < 0.01$). The glucagon concentration on day 4 and day 7 was higher in the colostrum-fed than in the formula-fed calves ($P < 0.01$; Table 4.2). The cortisol concentrations decreased with age in all groups ($P < 0.01$) but decreased earlier in the colostrum-fed calves. The mean cortisol concentrations in plasma during the first week of life were lower in the colostrum-fed than in the formula-fed calves ($P = 0.02$; Table 4.2). Quercetin treatment did not affect the concentrations of metabolites nor hormones in the plasma.

Table 4.2: Metabolites and Hormones of Calves during the First Week of Life.

	Age	Group (Diet, Quercetin)				ANOVA *P*-values[1]			
		Colostrum		Formula					
Item	(d)	Q-	Q+	Q-	Q+	Diet	Quercetin	Age	Diet × Age
Glucose (mmol/L)	1	4.3 ± 0.6	4.7 ± 0.6	4.1 ± 0.6	4.1 ± 0.6	0.03	0.89	<0.01	0.06
	2	6.0 ± 0.4	5.5 ± 0.4	5.8 ± 0.4	5.8 ± 0.4				
	4	5.5 ± 0.2^{a}	5.6 ± 0.2^{a}	4.4 ± 0.2^{b}	4.4 ± 0.2^{b}				
	7	5.2 ± 0.2^{a}	5.2 ± 0.2^{a}	4.4 ± 0.2^{b}	4.6 ± 0.2^{ab}				
Lactate (mmol/L)	1	4.0 ± 0.7	2.8 ± 0.7	3.7 ± 0.7	3.1 ± 0.7	0.88	0.78	<0.01	0.32
	2	2.4 ± 0.3	3.0 ± 0.3	2.7 ± 0.3	2.9 ± 0.3				
	4	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.4 ± 0.2				
	7	0.5 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.4 ± 0.1				
Urea (mmol/L)	1	3.3 ± 0.5	3.6 ± 0.5	3.1 ± 0.5	3.3 ± 0.5	0.99	0.55	<0.01	0.02
	2	3.5 ± 0.6	4.3 ± 0.6	5.0 ± 0.6	4.4 ± 0.6				
	4	3.7 ± 0.5	3.9 ± 0.5	3.8 ± 0.5	4.6 ± 0.6				
	7	5.0 ± 0.4	5.4 ± 0.4	4.5 ± 0.4	4.0 ± 0.4				
NEFA (µmol/L)	1	830 ± 156	709 ± 156	767 ± 156	733 ± 156	0.99	0.70	<0.01	0.95
	2	359 ± 43	368 ± 43	387 ± 43	372 ± 43				
	4	290 ± 71	269 ± 71	226 ± 71	285 ± 75				
	7	152 ± 38	157 ± 40	198 ± 38	164 ± 40				
Triglycerides (mmol/L)	1	0.24 ± 0.04	0.25 ± 0.04	0.26 ± 0.04	0.23 ± 0.04	<0.01	0.63	<0.01	<0.01
	2	0.31 ± 0.04	0.26 ± 0.04	0.30 ± 0.04	0.26 ± 0.04				
	4	0.40 ± 0.06	0.43 ± 0.06	0.21 ± 0.06	0.19 ± 0.07				
	7	0.21 ± 0.02^{a}	0.23 ± 0.02^{a}	0.10 ± 0.02^{b}	0.09 ± 0.02^{b}				

Table 4.2 Continuation.

	Age	Group (Diet, Quercetin)				ANOVA *P*-values[1]			
		Colostrum		Formula					
Item	(d)	Q-	Q+	Q-	Q+	Diet	Quercetin	Age	Diet × Age
Cholesterol (mmol/L)	1	0.62 ± 0.08	0.59 ± 0.08	0.63 ± 0.08	0.60 ± 0.08	0.01	0.90	<0.01	<0.01
	2	0.78 ± 0.07	0.76 ± 0.07	0.92 ± 0.07	0.76 ± 0.07				
	4	1.34 ± 0.09^{a}	1.51 ± 0.09^{a}	1.00 ± 0.09^{b}	0.85 ± 0.09^{b}				
	7	1.49 ± 0.12^{a}	1.71 ± 0.12^{a}	1.03 ± 0.12^{b}	0.96 ± 0.12^{b}				
Insulin (µg/L)	1	0.59 ± 0.43	0.56 ± 0.45	1.59 ± 0.43	0.96 ± 0.43	0.10	0.21	<0.01	0.43
	2	0.77 ± 0.19	0.89 ± 0.19	1.14 ± 0.19	0.85 ± 0.19				
	4	0.34 ± 0.06	0.29 ± 0.06	0.35 ± 0.06	0.26 ± 0.07				
	7	0.36 ± 0.15	0.39 ± 0.16	0.51 ± 0.15	0.18 ± 0.16				
Glucagon (ng/L)	1	82 ± 17	93 ± 17	120 ± 17	118 ± 17	0.01	1.00	<0.01	<0.01
	2	331 ± 27	324 ± 27	288 ± 27	303 ± 27				
	4	194 ± 15^{a}	181 ± 15^{ab}	130 ± 15^{b}	138 ± 15^{ab}				
	7	163 ± 10^{a}	159 ± 10^{a}	82 ± 10^{b}	73 ± 10^{b}				
Cortisol (µg/L)	1	36.8 ± 3.7	39.9 ± 3.7	34.3 ± 3.7	42.8 ± 3.7	0.02	0.30	<0.01	0.01
	2	15.6 ± 4.1^{ab}	14.3 ± 4.1^{b}	28.5 ± 4.1^{ab}	31.2 ± 4.1^{a}				
	4	13.2 ± 2.7	9.6 ± 2.7	11.5 ± 2.7	15.7 ± 2.8				
	7	9.9 ± 1.3	8.3 ± 1.4	8.1 ± 1.3	11.9 ± 1.4				

Data are given as the least squares means ± standard errors. Least squares means within a row with different lowercase letters (a, b) differ ($P < 0.05$).
Q+, quercetin-supplemented; Q-, control (no quercetin); $n = 7$ per group. NEFA, non-esterified fatty acids.
[1]Effects: For interactions that are not listed, the probability was $P > 0.10$.

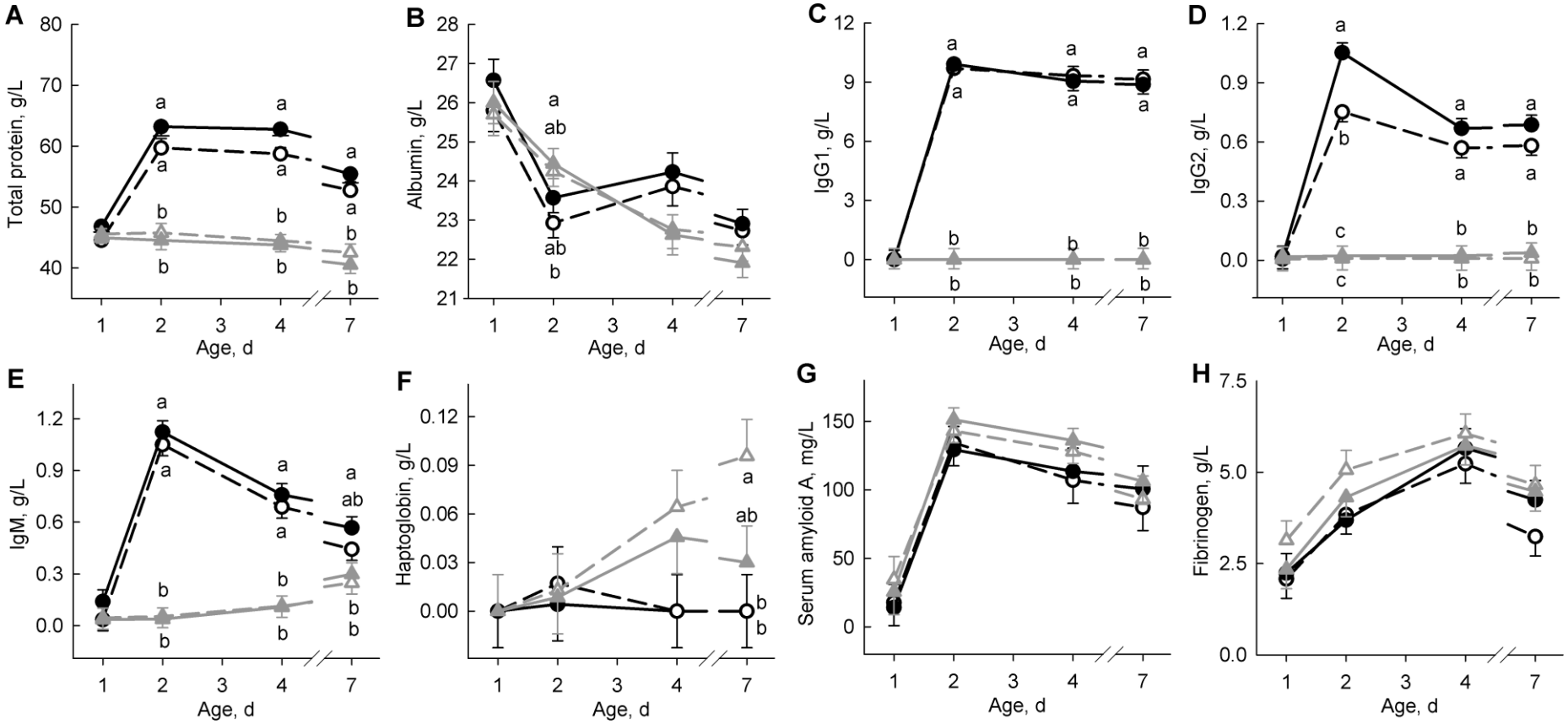

Figure 4.3: Total Protein, Albumin and Immune and Inflammatory Status in Blood Plasma. Basal concentrations of total protein (A) and albumin (B), immunoglobulins G1, G2 and M (C, D, E) and acute-phase proteins haptoglobin, serum amyloid A and fibrinogen (F, G, H) in the plasma of calves fed either colostrum (black circles) or formula (gray triangles) on days 1 and 2 and supplemented with (filled symbols, solid lines) or without (open symbols, dashed lines) quercetin aglycone from day 2 to day 8 (50 mg/(kg body weight × day)). Data are presented as the least squares means ± standard errors. Least squares means with different lowercase letters (a, b, c) differ among groups within the same day ($P < 0.05$).

4.4.4 Immunoglobulins and Acute-Phase Proteins

The plasma concentrations of IgG1 and IgG2 increased on day 2 only in colostrum-fed calves ($P < 0.01$; **Figures 4.3 C and D**). The plasma concentrations of IgM increased sharply until day 2 in colostrum-fed calves and then slowly decreased ($P < 0.01$). In the formula-fed groups, the IgM concentration increased until day 7 ($P < 0.01$) but was still lower than in the colostrum-fed groups ($P < 0.01$) at the end of the trial (**Figure 4.3 E**). The concentrations of haptoglobin in blood plasma were below the detection limit on day 1 in all calves and increased only in the formula-fed calves ($P = 0.01$) and were highest in ForQ- on day 7 (**Figure 4.3 F**). The mean haptoglobin concentration was higher in formula-fed than in the colostrum-fed calves ($P = 0.03$). The concentrations of SAA increased until day 2 and slowly decreased afterwards in all calves ($P < 0.01$; **Figure 4.3 G**). The concentrations of fibrinogen increased until day 4 and then decreased ($P < 0.01$) without differences among groups (**Figure 4.3 H**). However, the mean fibrinogen concentration tended to be higher after formula feeding ($P = 0.10$).

4.4.5 Antioxidative Status

Plasma TEAC increased in all groups until day 4 of life ($P < 0.01$) but was higher in the colostrum-fed than in the formula-fed calves ($P < 0.01$; **Figure 4.4 A**). FRAP decreased from day 1 to day 7 only in the formula-fed groups ($P \leq 0.01$) and was higher ($P < 0.05$) on day 7 in the colostrum-fed groups (**Figure 4.4 B**). Although not statistically significant, the FRAP decrease was delayed in the quercetin-fed calves compared with the control groups. The mean concentrations of TBARS in plasma were higher in the colostrum-fed than in the formula-fed calves ($P = 0.01$) and revealed a diet×age interaction ($P = 0.01$) with higher concentrations in the colostrum-fed than in the formula-fed calves on day 4 (**Figure 4.4 C)**. The mean concentrations of F2-isoprostanes decreased from day 1 to day 4 ($P < 0.01$) in all calves and tended to increase on day 7 only in ForQ- ($P = 0.08$; **Figure 4.4 D**).

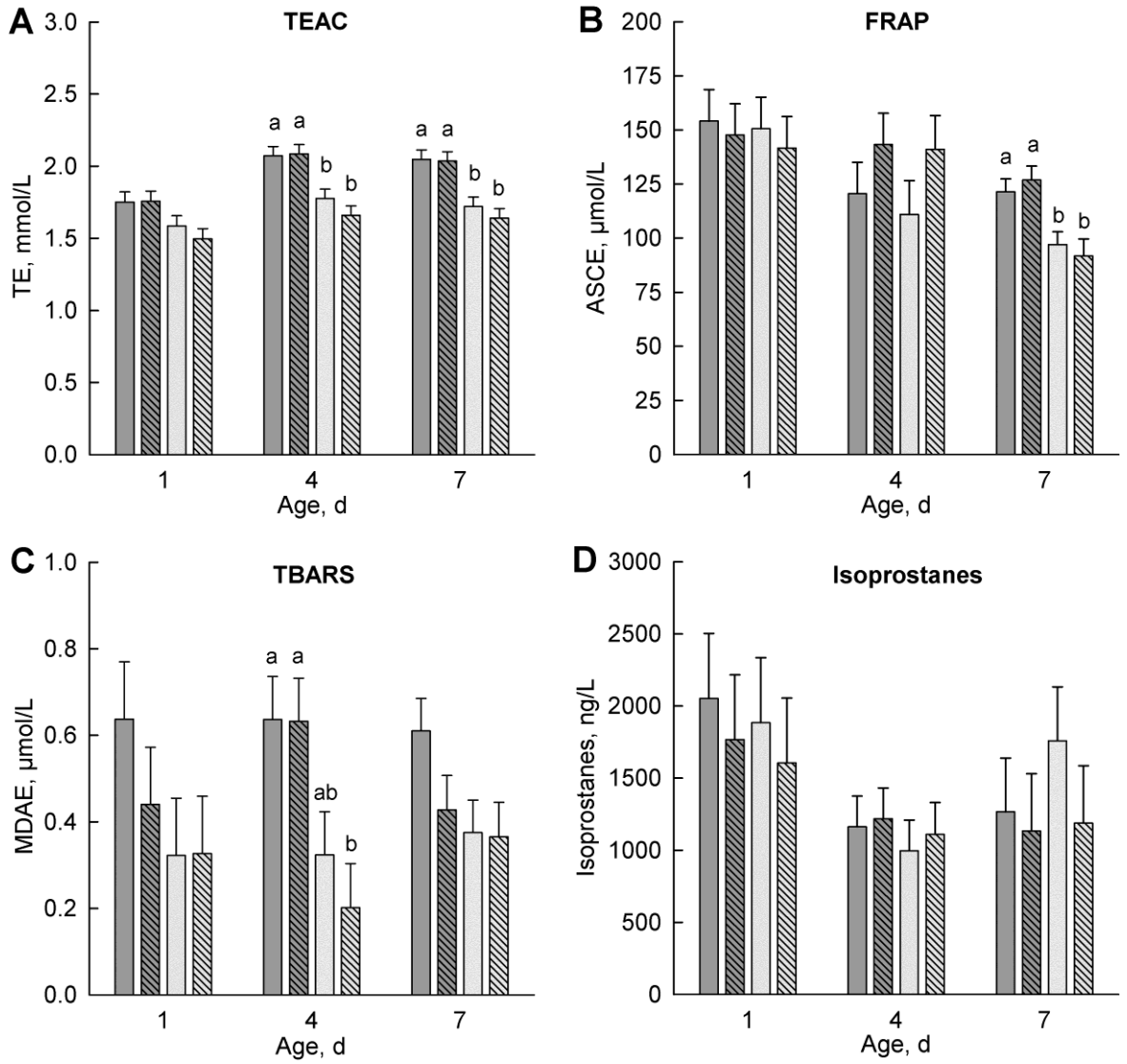

Figure 4.4: Antioxidative Status in Plasma. Plasma parameters for antioxidative capacity (A, B) and oxidative stress (C, D) in calves fed either colostrum (dark grey) or formula (light grey) on days 1 and 2 and supplemented with (crossline pattern) or without (without fill pattern) quercetin on days 2-8 (50 mg/(kg body weight × day)). Data are presented as the least squares means ± standard errors. Bars with different lowercase letters (a, b) differ among groups within the same day ($P < 0.05$). TEAC, trolox equivalent antioxidative capacity (in trolox equivalents, TE); FRAP, ferric reducing ability of plasma (in ascorbic acid equivalents, ASCE); TBARS, thiobarbituric acid reactive species (in malondialdehyde equivalents, MDAE)

4.4.6 Hepatic Gene Expression

Regarding inflammation markers, the relative mRNA abundance of *TNF* was higher after quercetin feeding (P = 0.04; **Table 4.3**). The relative mRNA abundance of *CRP* was higher (P = 0.03) and that of *SAA2* tended to be higher (P = 0.09) in the formula-fed than in the colostrum-fed groups (Table 4.3). For the antioxidative enzymes, the mRNA abundances of *GPX1* and *SOD* were not different among groups, whereas the relative mRNA abundance of *CAT* tended to be higher in ColQ- than in ColQ+ (P = 0.06) but did not differ between ForQ- and ForQ+, revealing a diet×quercetin interaction (P = 0.04; Table 4.3).

Table 4.3: Hepatic mRNA Expression of Inflammatory and Antioxidative Traits in Newborn Calves.

	Group (Diet, Quercetin)					ANOVA *P*-values		
	Colostrum		Formula					
	Q-	Q+	Q-	Q+	SE	Diet	Quercetin	Interaction
Proinflammatory Cytokines								
TNF	0.98	1.15	0.89	1.48	0.10	0.41	0.04	0.39
IL1A	1.17	0.90	1.05	1.18	0.08	0.62	0.66	0.22
IL1B	3.00	3.68	3.11	2.69	0.30	0.49	0.83	0.38
Acute-Phase Proteins								
HP	14.74	29.14	39.78	23.09	4.61	0.30	0.90	0.10
FGA	2.73	3.28	3.73	2.18	0.35	0.95	0.48	0.15
SAA2	0.91	0.88	1.20	1.12	0.07	0.09	0.72	0.87
CRP	0.90	0.96	1.11	1.06	0.04	0.03	0.99	0.42
Antioxidative Enzymes								
CAT	1.22	0.77	1.01	1.08	0.06	0.68	0.11	0.04
GPX1	1.08	0.95	0.98	1.05	0.03	0.99	0.65	0.13
SOD	1.03	1.08	1.09	0.90	0.04	0.42	0.36	0.11

Relative mRNA expression of genes related to inflammation and antioxidative status in the liver of 8-d-old calves; data are given in arbitrary units and are presented as the means ± standard errors (SE). Q+, quercetin-supplemented; Q-, control (no quercetin); n = 6 per group.
CAT, catalase; *GPX1*, glutathione peroxidase; *SOD*, superoxide dismutase; *TNF*, tumor necrosis factor; *IL1A*, interleukin-1α; *IL1B*, interleukin-1β; *HP*, haptoglobin; *FGA*, fibrinogen; *SAA2*, serum amyloid A2; *CRP*, C-reactive protein.

4.5 DISCUSSION

In this study, we were able to confirm the oral bioavailability of quercetin in colostrum-fed calves and further showed that after colostrum deprivation, quercetin is absorbed in equal amounts. As indicated earlier, oral bioavailability decreases with age due to intestinal maturation and therefore reduced permeability or more effective elimination processes [24]. The pattern of flavonol fractions in the plasma in this study was different from the patterns in 29-day-old calves or adult cattle but comparable to observations in 2-day-old calves [24, 36]. This result can be explained by the maturation of intestinal metabolism because it has been shown for rats and pigs [37, 38] that orally administered flavonols undergo complete metabolism inside the intestinal mucosa, where glucuronidation is of major importance [39].

Although flavonols were present in the plasma of quercetin-fed calves, we failed to detect any effect on the metabolic parameters or hormone concentrations, which was also the case in previous studies in cattle [24, 36, 40]. However, we could confirm a variety of effects caused by colostrum-deprivation during the first 2 days of life. Cortisol is crucial for initiation of parturition and catabolic activity, especially during hypoxia at birth. However, a decrease of the plasma cortisol concentration is delayed in formula-fed calves, as seen in previous studies [41, 42]. We assume that the delay was possibly caused by either abdominal pain or by reduced utilization of nutrients, which is proven to increase stress parameters in sheep [43, 44].

Higher plasma glucose in colostrum-fed groups was most likely caused by enhanced intestinal maturation and therefore enlarged absorptive surface [45, 46]. Additionally, colostrum feeding seems to accelerate maturation of the pancreas [47] because glucagon concentrations were also higher in colostrum-fed calves from day 4 onwards. Although the basal insulin concentrations were similar among dietary groups, we showed in a companion paper that the postprandial insulin response is more pronounced in colostrum-fed groups [23].

Higher triglyceride and cholesterol concentrations in colostrum-fed calves are in accordance with observations in other studies [41, 42] and are due to the enhanced stimulation of intestinal differentiation by colostral growth factors and hormones. However, it must be considered that diarrhea in formula-fed calves accelerated intestinal transit and therefore reduced absorption time, which might also account for differences in the plasma triglyceride and cholesterol concentrations between dietary groups. Similar concentrations of non-esterified fatty acids indicate that the energy balance in the dietary groups is equal and that lipid mobilization seems to be of minor importance to maintain energy balance; previous studies also failed to demonstrate consistent effects in neonatal calves [41, 42, 48].

As we expected, the concentration of total protein in the plasma increased only in the colostrum-fed groups, although the protein content of the formula and colostrum was similar.

The decrease of the albumin concentration from day 1 to day 2 was probably caused by hemodilution after first feed intake and coincides with previous findings [49, 50]. Because only Ig, but not the albumin concentrations in plasma were affected by colostrum feeding, differences in total protein are most likely caused by absorption of colostral immunoglobulins after colostrum intake [42]. Colostrum intake is important not only for maturational processes but also to acquire passive immunity because the bovine placenta is impermeable to antibodies. As reviewed by Weaver et al. [51], calves with serum IgG concentrations below 10 g/L (total protein < 52 g/L) 24 h after birth suffer from failure of passive transfer, which is associated with increased morbidity and mortality as well as reduced performance. In our study, colostrum-fed calves exceeded this threshold; however, failure of passive transfer was a relevant problem in formula-fed calves, although they were parenterally supplemented with bovine immunoglobulins on day 1. Only formula-fed calves showed slight hyperthermia on the first days of life and a higher incidence of diarrhea, probably caused by gastrointestinal inflammation due to missing local immunity [52], accompanied by abdominal pain and diminished appetite. Obviously, parenteral and oral treatments with immunoglobulins could not prevent local or systemic infections in formula-fed calves, probably because of missing herd-specific immunoglobulins in the formula and the administered drugs. However, we did not perform microbiological analyses in the feces of the calves to determine pathogens that might have caused loose feces. The time course of plasma IgM concentration in formula-fed calves indicates that the indigenous production of IgM is evident as early as day 4 of life, but the concentrations are too low to effectively protect against infections [9].

We assume that inflammatory processes also activated the synthesis of acute phase proteins. Although the increase of the plasma acute phase proteins in all calves emphasized the immunological burden of the new environment, higher haptoglobin concentrations in formula-fed calves suggest more severe inflammatory processes than in colostrum-fed calves [53, 54], which was underlined by the greater incidence of gastrointestinal infections. Because serum amyloid A is more susceptible to stress [53, 55], including physical stress, we suppose that the plasma concentrations were equally high in all groups due to the experimental procedures (e.g., continued venipuncture, restricted feeding, single penning, temporal fixation and dietary changes).

On the mRNA level, we did not find differences in the acute phase proteins between dietary groups because hepatic transcription precedes translation and protein release into circulation. Thus, we obviously missed the time point of elevated mRNA abundances of *HP*. However, mRNA abundance of *CRP*, a moderate acute phase protein, was elevated in the formula-fed groups on day 8, which could indicate the importance of C-reactive protein as a major component of the bovine innate immune system [56]. Hence, increased C-reactive protein production is compensative for the absence of immunoglobulins in colostrum-deprived calves.

Regarding pro-inflammatory cytokines, we found elevated hepatic mRNA abundance of *TNF* in quercetin-supplemented calves but no differences between dietary groups. Under immunocompetent conditions, the impact of various noxae leads to local production of pro-inflammatory cytokines, e.g., TNF or interleukin-1, which in turn induce the systemic acute phase response, including synthesis of acute phase proteins [57]. Within signal transduction, reactive oxygen species act as second messengers, thus enhancing TNF-induced gene expression, and oxidative conditions potentiate the activation of respective pathways [57, 58]. Although high doses of quercetin were repeatedly shown to reduce mRNA expression of *TNF in vitro* [59, 60] application of prophylactic doses increased the ratio of pro- to anti-inflammatory cytokines in murine macrophages [60]. We assume that the hepatic quercetin concentration in our experiment did not exceed the prophylactic dose; thus, quercetin might have increased the expression of *TNF*. Unfortunately, we did not measure TNF protein concentration, but we suppose that posttranscriptional processes anticipated TNF signal transduction [17, 61], because quercetin scavenged reactive oxygen species necessary for signal transduction. Therefore, the observed quercetin effects on *TNF* gene expression could not have been forwarded on the expression of target genes, e.g., *IL1B* or acute phase proteins, as would have been expected otherwise.

Concerning the antioxidative status in the plasma, previous findings in neonatal calves are inconsistent. Inanami et al. [62] and Stohrer, Lutz and Stangassinger [63] concluded from comparisons between calves and dams that the former are highly susceptible to oxidative stress due to immature defense systems, whereas Gaál et al. [64] deduced from high FRAP values, despite the high reactive oxygen species, that calves are well-prepared to address oxidative stress. Although the increase of TEAC in this and a previous study of our group [65] indicates rising antioxidative capacity during the first week of life, this result was not supported by determination of FRAP, another marker of antioxidative capacity. Furthermore, the values of TBARS, which serve as a proxy to measure the products of lipid peroxidation, were unaffected by age in this study, which contradicts earlier studies [62, 64, 66]. However, the reliability of TBARS is criticized for low sensitivity and specificity, and the use of F2-isoprostanes is recommended as the most reliable approach to assess oxidative stress *in vivo* [67, 68]. The time courses of F2-isoprostanes were significantly decreased in all calves; thus, we support the theory that exposure to an oxygen-rich environment following hypoxia during birth results in severe oxidative stress in the newborn [5]. Although quercetin is known to exert antioxidative effects, we did not find improved antioxidative status in the plasma of calves nor increased hepatic expression of antioxidative enzymes, which is in line with previous findings of our group in research conducted on neonatal calves and lactating dairy cows [65, 69]. Orally administered quercetin is completely metabolized inside the intestinal mucosa; thus, the gastrointestinal tract is the first site of action for quercetin [39], as it is for pathogens and gastrointestinal disorders commonly occurring during the first weeks of life.

Because we did not examine the intestinal tissue, we cannot exclude local effects of quercetin on antioxidative status. Except for F2-isoprostanes, the parameters of the antioxidative system in the plasma were higher after colostrum feeding. Colostrum is a source of reactive oxygen species, but it also contains a variety of antioxidative factors [66, 70], the latter increasing with time after parturition [71]. The absorption of pro- and antioxidants present in colostrum [72] might have contributed to the rise of the respective parameters in the plasma of colostrum-fed calves and triggered immunological processes, which in turn caused elevated plasma levels. However, the hepatic mRNA abundances of antioxidative enzymes seemed not to be affected by diet, but all groups were fed milk replacer from day 3 onwards and potential dietary effects on hepatic antioxidative status might not be long-lasting.

In conclusion, oral administration of quercetin aglycone at a daily dose of 50 mg/kg body weight to newborn calves during the first week of life is unable to compensate for inadequate colostrum supply. Quercetin did not show any positive effects on neonatal antioxidative or anti-inflammatory status, whereas colostrum feeding improves neonatal health status by supporting passive immunity and by promoting antioxidative/oxidative status.

4.6 ACKNOWLEDGMENTS

We gratefully thank C. Fiedler for assistance in conducting the experimental procedures, O. Bellmann for veterinary assistance and K.D. Witt and B. Stabenow for providing experimental facilities. We also thank W. Kühl, P. Schulz, C. Reiko, P. Müntzel and U. Wiedemuth for excellent laboratory assistance.

4.7 AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: HMH JG SW. Performed the experiments: JG HMH. Analyzed the data: EK JW AT TS SW. Contributed reagents/materials/analysis tools: EK JW TS PJ SW. Wrote the paper: JG HMH.

4.8 REFERENCES

1. Gulliksen SM, Lie KI, Loken T, Osteras O. Calf mortality in Norwegian dairy herds. Journal of Dairy Science. 2009; 92(6):2782-95.
2. Lorenz I, Fagan J, More SJ. Calf health from birth to weaning. II. Management of diarrhoea in pre-weaned calves. Irish Veterinary Journal. 2011; 64(9):10.1186.
3. Svensson C, Linder A, Olsson S-O. Mortality in Swedish dairy calves and replacement heifers. Journal of Dairy Science. 2006; 89(12):4769-77.
4. Kane AD, Hansell JA, Herrera EA, Allison BJ, Niu Y, Brain KL, et al. Xanthine oxidase and the fetal cardiovascular defence to hypoxia in late gestation ovine pregnancy. The Journal of Physiology. 2014; 592(3):475-89.
5. Mutinati M, Pantaleo M, Roncetti M, Piccinno M, Rizzo A, Sciorsci R. Oxidative stress in neonatology. A review. Reproduction in Domestic Animals. 2014; 49(1):7-16.
6. Miller J, Brzezinska-Slebodzinska E, Madsen F. Oxidative stress, antioxidants, and animal function. Journal of Dairy Science. 1993; 76(9):2812-23.
7. Lykkesfeldt J, Svendsen O. Oxidants and antioxidants in disease: oxidative stress in farm animals. Veterinary Journal. 2007; 173(3):502-11.
8. Sordillo LM, Aitken SL. Impact of oxidative stress on the health and immune function of dairy cattle. Veterinary Immunology and Immunopathology. 2009; 128(1):104-9.
9. Chase CC, Hurley DJ, Reber AJ. Neonatal immune development in the calf and its impact on vaccine response. Veterinary Clinics of North America: Food Animal Practice. 2008; 24(1):87-104.
10. Rochfort S, Parker AJ, Dunshea FR. Plant bioactives for ruminant health and productivity. Phytochemistry. 2008; 69(2):299-322.
11. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics. 2002; 96(2-3):67-202.
12. Middleton E, Jr., Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews. 2000; 52(4):673-751.
13. Provenza FD, Villalba JJ. The role of natural plant products in modulating the immune system: an adaptable approach for combating disease in grazing animals. Small Ruminant Research. 2010; 89(2):131-9.
14. Kamada H, Nonaka I, Ueda Y, Murai M. Selenium addition to colostrum increases immunoglobulin G absorption by newborn calves. Journal of Dairy Science. 2007; 90(12):5665-70.
15. Lee SD, Kim JH, Jung HJ, Kim YH, Kim IC, Kim SB, et al. The effect of ginger extracts on the antioxidant capacity and IgG concentrations in the colostrum and plasma of neo-born piglets and sows. Livestock Science. 2013; 154(1):117-22.
16. Retskii M, Shakhov A, Chusov D, Zolotarev A, Lebedev M, Ermolova T, et al. Correcting the antioxidant status of newborn calves for forming higher colostral immunity. Russian Agricultural Sciences. 2010; 36(2):127-9.
17. Sergent T, Piront N, Meurice J, Toussaint O, Schneider Y-J. Anti-inflammatory effects of dietary phenolic compounds in an in vitro model of inflamed human intestinal epithelium. Chemico-Biological Interactions. 2010; 188(3):659-67.

18. Lozoya X, Reyes-Morales H, Chávez-Soto MA, del Carmen Martínez-García Ma, Soto-González Y, Doubova SV. Intestinal anti-spasmodic effect of a phytodrug of Psidium guajava folia in the treatment of acute diarrheic disease. Journal of Ethnopharmacology. 2002; 83(1):19-24.

19. Capasso A, Pinto A, Mascolo N, Capasso F. Reduction of agonist-induced contractions of guinea-pig isolated ileum by flavonoids. Phytotherapy Research. 1991; 5(2):85-7.

20. Di Carlo G, Mascolo N, Izzo AA, Capasso F. Effects of quercetin on the gastrointestinal tract in rats and mice Phytotherapy Research. 1994; 8(1):42-5.

21. Parkar SG, Stevenson DE, Skinner MA. The potential influence of fruit polyphenols on colonic microflora and human gut health. International Journal of Food Microbiology. 2008; 124(3):295-8.

22. Liu H, Liu Y, Hu L, Suo Y, Zhang L, Jin F, et al. Effects of dietary supplementation of quercetin on performance, egg quality, cecal microflora populations, and antioxidant status in laying hens. Poultry Science. 2014; 93(2):347-53.

23. Gruse J, Görs S, Tuchscherer A, Otten W, Weitzel JM, Metges CC, et al. The Effects of Oral Quercetin Supplementation on Splanchnic Glucose Metabolism in 1-Week-Old Calves Depend on Diet after Birth. The Journal of Nutrition. 2015; 145(11):2486-95

24. Maciej J, Schäff CT, Kanitz E, Tuchscherer A, Bruckmaier RM, Wolffram S, et al. Bioavailability of the flavonol quercetin in neonatal calves after oral administration of quercetin aglycone or rutin. Journal of Dairy Science. 2015; 98(6):3906-17.

25. Larson LL, Owen FG, Albright JL, Appleman RD, Lamb RC, Muller LD. Guidelines toward More Uniformity in Measuring and Reporting Calf Experimental-Data. Journal of Dairy Science. 1977; 60(6):989-91.

26. Berger LM, Wein S, Blank R, Metges CC, Wolffram S. Bioavailability of the flavonol quercetin in cows after intraruminal application of quercetin aglycone and rutin. Journal of Dairy Science. 2012; 95(9):5047-55.

27. Hammon HM, Sturmer G, Schneider F, Tuchscherer A, Blum H, Engelhard T, et al. Performance and metabolic and endocrine changes with emphasis on glucose metabolism in high-yielding dairy cows with high and low fat content in liver after calving. Journal of Dairy Science. 2009; 92(4):1554-66.

28. Mancini G, Vaerman JP, Carbonara AO, Heremans JF. A single-radial-diffusion method for the immunological quantitation of proteins. Proceedings of the Biological Fluids; Amsterdam, Netherlands; 1964; 370-379. Elsevier.

29. Gasowska A, Stefaniak T. Ocena efektow doustnego podania immunoglobuliny zoltka jaja [IgY] cieletom w okresie wchlaniania makromolekul z jelita. [Polish] Folia Universitatis Agriculturae Stetinensis Zootechnica. 2003; (45):87-92.

30. Jones G, Mould D. Adaptation of the guaiacol (peroxidase) test for haptoglobins to a microtitration plate system. Research in Veterinary Science. 1984; 37(1):87-92.

31. Millar H, Simpson J, Stalker A. An evaluation of the heat precipitation method for plasma fibrinogen estimation. Journal of Clinical Pathology. 1971; 24(9):827-30.

32. Miller NJ, Sampson J, Candeias LP, Bramley PM, RiceEvans CA. Antioxidant activities of carotenes and xanthophylls. FEBS Letters. 1996; 384(3):240-2.

33. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1999; 26(9-10):1231-7.

34. Luehring M, Blank R, Wolffram S. Vitamin E-sparing and vitamin E-independent anti-

oxidative effects of the flavonol quercetin in growing pigs. Animal Feed Science and Technology. 2011; 169(3-4):199-207.

35. Ruijter JM, Pfaffl MW, Zhao S, Spiess AN, Boggy G, Blom J, et al. Evaluation of qPCR curve analysis methods for reliable biomarker discovery: Bias, resolution, precision, and implications. Methods. 2013; 59(1):32-46.

36. Gohlke A, Ingelmann CJ, Nurnberg G, Starke A, Wolffram S, Metges CC. Bioavailability of quercetin from its aglycone and its glucorhamnoside rutin in lactating dairy cows after intraduodenal administration. Journal of Dairy Science. 2013; 96(4):2303-13.

37. Cermak R, Landgraf S, Wolffram S. The bioavailability of quercetin in pigs depends on the glycoside moiety and on dietary factors. The Journal of Nutrition. 2003; 133(9):2802-7.

38. Spencer JP, Chowrimootoo G, Choudhury R, Debnam ES, Srai SK, Rice-Evans C. The small intestine can both absorb and glucuronidate luminal flavonoids. FEBS Letters. 1999; 458(2):224-30.

39. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition. 2004; 79(5):727-47.

40. Stoldt A-K, Derno M, Nürnberg G, Weitzel JM, Otten W, Starke A, et al. Effects of a 6-wk intraduodenal supplementation with quercetin on energy metabolism and indicators of liver damage in periparturient dairy cows. Journal of Dairy Science. 2015; 98(7):4509-20.

41. Kuhne S, Hammon HM, Bruckmaier RM, Morel C, Zbinden Y, Blum JW. Growth performance, metabolic and endocrine traits, and absorptive capacity in neonatal calves fed either colostrum or milk replacer at two levels. Journal of Animal Science. 2000; 78(3):609-20.

42. Rauprich ABE, Hammon HM, Blum JW. Effects of feeding colostrum and a formula with nutrient contents as colostrum on metabolic and endocrine traits in neonatal calves. Biology of the Neonate. 2000; 78(1):53-64.

43. Hyder I, Ramesh K, Sharma S, Uniyal S, Yadav V, Panda R, et al. Effect of different dietary energy levels on physio-biochemical, endocrine changes and mRNA expression profile of leptin in goat (Capra hircus). Livestock Science. 2013; 152(1):63-73.

44. Kania B, Zaremba-Rutkowska M, Romanowicz K. Experimental intestinal stress induced by duodenal distention in sheep. Journal of Animal and Feed Sciences. 1999; 8:233-45.

45. Blattler U, Hammon HM, Morel C, Philipona C, Rauprich A, Rome V, et al. Feeding colostrum, its composition and feeding duration variably modify proliferation and morphology of the intestine and digestive enzyme activities of neonatal calves. The Journal of Nutrition. 2001; 131(4):1256-63.

46. Steinhoff-Wagner J, Gors S, Junghans P, Bruckmaier RM, Kanitz E, Metges CC, et al. Intestinal Glucose Absorption but Not Endogenous Glucose Production Differs between Colostrum- and Formula-Fed Neonatal Calves. The Journal of Nutrition. 2011; 141(1):48-55.

47. Guilloteau P, Zabielski R, Blum JW. Gastrointestinal tract and digestion in the young ruminant: ontogenesis, adaptations, consequences and manipulations. Journal of Physiology and Pharmacology. 2009; 60 Suppl 3:37-46.

48. Hammon HM, Blum JW. Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum for different durations or only milk replacer. The Journal of Nutrition. 1998; 128(3):624-32.

49. Rauprich AB, Hammon HM, Blum JW. Influence of feeding different amounts of first colostrum on metabolic, endocrine, and health status and on growth performance in

neonatal calves. Journal of Animal Science. 2000; 78(4):896-908.

50. Steinhoff-Wagner J, Gors S, Junghans P, Bruckmaier RM, Kanitz E, Metges CC, et al. Maturation of endogenous glucose production in preterm and term calves. Journal of Dairy Science. 2011; 94(10):5111-23.

51. Weaver DM, Tyler JW, VanMetre DC, Hostetler DE, Barrington GM. Passive transfer of colostral immunoglobulins in calves. Journal of Veterinary Internal Medicine. 2000; 14(6):569-77.

52. Besser TE, McGuire T, Gay C, Pritchett L. Transfer of functional immunoglobulin G (IgG) antibody into the gastrointestinal tract accounts for IgG clearance in calves. Journal of Virology. 1988; 62(7):2234-7.

53. Gånheim C, Alenius S, Waller KP. Acute phase proteins as indicators of calf herd health. Veterinary Journal. 2007; 173(3):645-51.

54. Pourjafar M, Badiei K, Nazifi S, Naghib S. Acute phase response in Holstein dairy calves affected with diarrhoea. Bulgarian Journal of Veterinary Medicine. 2011; 14(3):142-9.

55. Alsemgeest S, Lambooy I, Wierenga H, Dieleman S, Meerkerk B, Van Ederen A, et al. Influence of physical stress on the plasma concentration of serum amyloid-a (SAA) and haptoglobin (HP) in calves. Veterinary Quarterly. 1995; 17(1):9-12.

56. Schroedl W, Jaekel L, Krueger M. C-reactive protein and antibacterial activity in blood plasma of colostrum-fed calves and the effect of lactulose. Journal of Dairy Science. 2003; 86(10):3313-20.

57. Koj A. Initiation of acute phase response and synthesis of cytokines. Biochimica et Biophysica Acta-Molecular Basis of Disease. 1996; 1317(2):84-94.

58. Gloire G, Legrand-Poels S, Piette J. NF-κB activation by reactive oxygen species: Fifteen years later. Biochemistry & Pharmacology. 2006; 72(11):1493-505.

59. Boesch-Saadatmandi C, Loboda A, Wagner AE, Stachurska A, Jozkowicz A, Dulak J, et al. Effect of quercetin and its metabolites isorhamnetin and quercetin-3-glucuronide on inflammatory gene expression: role of miR-155. The Journal of Nutritional Biochemistry. 2011; 22(3):293-9.

60. Liao Y-R, Lin J-Y. Quercetin, but not its metabolite quercetin-3-glucuronide, exerts prophylactic immunostimulatory activity and therapeutic antiinflammatory effects on lipopolysaccharide-treated mouse peritoneal macrophages ex vivo. Journal of Agricultural and Food Chemistry. 2014; 62(13):2872-80.

61. Granado-Serrano AB, Martín MÁ, Bravo L, Goya L, Ramos S. Quercetin attenuates TNF-induced inflammation in hepatic cells by inhibiting the NF-κB pathway. Nutrition and Cancer. 2012; 64(4):588-98.

62. Inanami O, Shiga A, Okada KJ, Sato R, Miyake Y, Kuwabara M. Lipid peroxides and antioxidants in serum of neonatal calves. American Journal of Veterinary Research. 1999; 60(4):452-7.

63. Stohrer M, Lutz S, Stangassinger M, Schubert R, Flachowsky G, Jahreis G, et al. Antioxidant status of newborn and young animals. Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier 8 Symposium; Jena/Thüringen, Germany; 2001.

64. Gaál T, Ribiczeyne-Szabo P, Stadler K, Jakus J, Reiczigel J, Kövér P, et al. Free radicals, lipid peroxidation and the antioxidant system in the blood of cows and newborn calves around calving. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 2006; 143(4):391-6.

65. J. Maciej, C. T. Schäff, E. Kanitz, A. Tuchscherer, R. M. Bruckmaier, S. Wolffram, et al.

Short communication: Effects of oral flavonoid supplementation on the metabolic and antioxidative status of newborn dairy calves. Journal of Dairy Science. 2016; 99(1):805-11.

66. Albera E, Kankofer M. The Comparison of Antioxidative/Oxidative Profile in Blood, Colostrum and Milk of Early Post-partum Cows and Their Newborns. Reproduction in Domestic Animals. 2011; 46(5):763-9.

67. Montuschi P, Barnes P, Jackson Roberts L. Insights into oxidative stress: the isoprostanes. Current Medicinal Chemistry. 2007; 14(6):703-17.

68. Roberts LJ, Morrow JD. Measurement of F 2-isoprostanes as an index of oxidative stress in vivo. Free Radical Biology and Medicine. 2000; 28(4):505-13.

69. Gohlke A, Ingelmann CJ, Nurnberg G, Weitzel JM, Hammon HM, Gors S, et al. 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. Journal of Dairy Science. 2013; 96(11):6986-7000.

70. Przybylska J, Albera E, Kankofer M. Antioxidants in bovine colostrum. Reproduction in Domestic Animals. 2007; 42(4):402-9.

71. Kankofer M, Lipko-Przybylska J. Physiological antioxidative/oxidative status in bovine colostrum and mature milk. Acta Veterinaria. 2008; 58(2-3):231-9.

72. Abuelo A, Perez-Santos M, Hernandez J, Castillo C. Effect of colostrum redox balance on the oxidative status of calves during the first 3 months of life and the relationship with passive immune acquisition. Veterinary Journal. 2014; 199(2):295-9.

4.9 SUPPORTING INFORMATION

Supplemental Table 4.1: Complete data set of parameters regarding daily milk intake and health status as shown in Figure 4.1.

Calf	Group	Feeding	Quercetin	Age, d	BW, kg	Milk intake, kg/kg BW	Force-fed milk, % of intake/day	Fecal score	Rectal temp, °C	Heart rate, per min	Resp. rate, per min
1	ColQ-	COL	Q-	1	38.5	0.0961	27.027	.	.	.	.
4	ColQ-	COL	Q-	1	46	0.09565	0	.	.	.	.
5	ColQ-	COL	Q-	1	54.5	0.09725	28.302	.	.	.	.
10	ColQ-	COL	Q-	1	46	0.1	0	.	.	.	.
12	ColQ-	COL	Q-	1	44	0.1	0	.	.	.	.
23	ColQ-	COL	Q-	1	48.5	0.10103	0	.	.	.	.
28	ColQ-	COL	Q-	1	39.5	0.10127	0	.	.	.	.
2	ColQ+	COL	Q+	1	44	0.1	0	.	.	.	.
3	ColQ+	COL	Q+	1	52.5	0.09333	0	.	.	.	.
7	ColQ+	COL	Q+	1	42.5	0.09647	0	.	.	.	.
8	ColQ+	COL	Q+	1	50	0.1	0	.	.	.	.
11	ColQ+	COL	Q+	1	43	0.10465	0	.	.	.	.
19	ColQ+	COL	Q+	1	51	0.10588	0	.	.	.	.
26	ColQ+	COL	Q+	1	46	0.1	0	.	.	.	.
9	ForQ-	FOR	Q-	1	51	0.09804	48	.	.	.	.
15	ForQ-	FOR	Q-	1	43	0.1	0	.	.	.	.
18	ForQ-	FOR	Q-	1	44.5	0.09888	13.636	.	.	.	.
21	ForQ-	FOR	Q-	1	45	0.1	37.778	.	.	.	.
22	ForQ-	FOR	Q-	1	37	0.1	18.919	.	.	.	.
24	ForQ-	FOR	Q-	1	48	0.1	0	.	.	.	.
29	ForQ-	FOR	Q-	1	47.5	0.09895	0	.	.	.	.
13	ForQ+	FOR	Q+	1	44.5	0.09888	31.818	.	.	.	.
14	ForQ+	FOR	Q+	1	52	0.1	0	.	.	.	.
17	ForQ+	FOR	Q+	1	43	0.09302	0	.	.	.	.
20	ForQ+	FOR	Q+	1	33	0.1	0	.	.	.	.
25	ForQ+	FOR	Q+	1	46.5	0.10108	0	.	.	.	.
27	ForQ+	FOR	Q+	1	46.5	0.10108	17.021	.	.	.	.
30	ForQ+	FOR	Q+	1	47	0.1	0	.	.	.	.
1	ColQ-	COL	Q-	2	38.5	0.1039	82.5	.	38.7	120	78
4	ColQ-	COL	Q-	2	46	0.12174	0	2	38.4	150	66
5	ColQ-	COL	Q-	2	54.5	0.1156	0	2	38.2	138	56
10	ColQ-	COL	Q-	2	46	0.12174	0	3	38.9	138	66
12	ColQ-	COL	Q-	2	44	0.12045	0	2	38.4	126	48
23	ColQ-	COL	Q-	2	48.5	0.11959	36.207	2	38.9	132	60
28	ColQ-	COL	Q-	2	39.5	0.11899	0	1	38.7	138	48
2	ColQ+	COL	Q+	2	44	0.12273	0	.	38.3	126	48
3	ColQ+	COL	Q+	2	52.5	0.1181	85.484	1	38.5	138	96
7	ColQ+	COL	Q+	2	42.5	0.11765	0	2	38.3	180	48
8	ColQ+	COL	Q+	2	50	0.12	0	2	38.2	150	54
11	ColQ+	COL	Q+	2	43	0.11395	16.327	.	38.6	132	54
19	ColQ+	COL	Q+	2	51	0.11176	0	2	38.5	126	60
26	ColQ+	COL	Q+	2	46	0.12174	0	.	38.5	108	54
9	ForQ-	FOR	Q-	2	51	0.11765	95	.	38.9	162	48
15	ForQ-	FOR	Q-	2	43	0.11628	42	.	38.2	168	60
18	ForQ-	FOR	Q-	2	44.5	0.11685	80.769	3	38.6	144	60
21	ForQ-	FOR	Q-	2	45	0.11778	100	2	38.9	132	66
22	ForQ-	FOR	Q-	2	37	0.12162	100	2.75	38.6	144	54
24	ForQ-	FOR	Q-	2	48	0.12083	29.31	3	38.9	138	48
29	ForQ-	FOR	Q-	2	47.5	0.11579	18.182	3	38.3	120	36
13	ForQ+	FOR	Q+	2	44.5	0.11011	0	.	39.5	166	48
14	ForQ+	FOR	Q+	2	52	0.10962	26.316	2	39.4	126	66
17	ForQ+	FOR	Q+	2	43	0.11395	0	2.5	38.9	138	48
20	ForQ+	FOR	Q+	2	33	0.12424	95.122	2	38.1	156	72
25	ForQ+	FOR	Q+	2	46.5	0.11613	88.889	2.5	38.7	132	72
27	ForQ+	FOR	Q+	2	46.5	0.12043	100	3	38.9	168	60
30	ForQ+	FOR	Q+	2	47	0.11915	103.571	2	38.4	114	60
1	ColQ-	COL	Q-	3	40	0.115	0	2	38.8	108	66
4	ColQ-	COL	Q-	3	46	0.12174	0	2	38.7	132	60
5	ColQ-	COL	Q-	3	56	0.11786	0	1	38.8	126	54
10	ColQ-	COL	Q-	3	48.5	0.11753	0	3	39.4	150	90
12	ColQ-	COL	Q-	3	46	0.12391	0	2	39.1	126	66
23	ColQ-	COL	Q-	3	50.5	0.10693	20.37	2	39	108	48
28	ColQ-	COL	Q-	3	39.5	0.12405	0	2	39.1	180	48
2	ColQ+	COL	Q+	3	46.5	0.11613	0	2	38.8	72	.
3	ColQ+	COL	Q+	3	55	0.09273	19.608	1	38.6	126	90
7	ColQ+	COL	Q+	3	44	0.11818	0	.	.	.	.
8	ColQ+	COL	Q+	3	52	0.11923	0	.	38.7	132	54
11	ColQ+	COL	Q+	3	45	0.10889	38.776	3	39.2	120	42
19	ColQ+	COL	Q+	3	52.5	0.08	23.81	2	38.6	138	42
26	ColQ+	COL	Q+	3	47	0.11915	0	.	39.6	132	66
9	ForQ-	FOR	Q-	3	51	0.11765	91.667	3	39.4	144	48
15	ForQ-	FOR	Q-	3	45.5	0.11868	0	2	38.8	132	36
18	ForQ-	FOR	Q-	3	45.5	0.1011	0	3	38.7	174	72
21	ForQ-	FOR	Q-	3	48	0.12083	0	2	39.5	120	54

Supplemental Table 4.1 Continuation.

Calf	Group	Feeding	Quercetin	Age, d	BW, kg	Milk intake, kg/kg BW	Force-fed milk, % of intake/day	Fecal score	Rectal temp, °C	Heart rate, per min	Resp. rate, per min
22	ForQ-	FOR	Q-	3	38	0.12105	0	2.75	38.8	114	66
24	ForQ-	FOR	Q-	3	48	0.06042	86.207	2.5	39.4	138	.
29	ForQ-	FOR	Q-	3	50.5	0.06535	0	3	39.2	96	39
13	ForQ+	FOR	Q+	3	47.5	0.08421	17.5	2	39.3	168	48
14	ForQ+	FOR	Q+	3	53	0.11698	0	2	39.2	126	54
17	ForQ+	FOR	Q+	3	44.5	0.11685	0	2	39.7	138	42
20	ForQ+	FOR	Q+	3	34.5	0.08986	16.129	2	38.6	162	90
25	ForQ+	FOR	Q+	3	48	0.12083	0	2.5	39.4	126	72
27	ForQ+	FOR	Q+	3	44	0.01364	0	4	38.6	144	48
30	ForQ+	FOR	Q+	3	45.5	0.06593	0	3	39.1	114	66
1	ColQ-	COL	Q-	4	39.25	0.11849	0	2	39.2	120	102
4	ColQ-	COL	Q-	4	46	0.12174	0	.	38.4	138	54
5	ColQ-	COL	Q-	4	56	0.11786	0	2	39.2	.	.
10	ColQ-	COL	Q-	4	48.5	0.11959	0	2	39.4	168	102
12	ColQ-	COL	Q-	4	46	0.11957	0	2	39.4	162	72
23	ColQ-	COL	Q-	4	49.5	0.08233	0	2	38.5	132	48
28	ColQ-	COL	Q-	4	39.5	0.12405	0	2	39.1	180	33
2	ColQ+	COL	Q+	4	46.5	0.12043	0	2	39.3	102	66
3	ColQ+	COL	Q+	4	53.75	0.11996	37.903	1	39	156	84
7	ColQ+	COL	Q+	4	44	0.11818	0	1	38.7	162	78
8	ColQ+	COL	Q+	4	51	0.12162	0	.	.	.	.
11	ColQ+	COL	Q+	4	45	0.12	0	2	38.9	114	42
19	ColQ+	COL	Q+	4	52.5	0.1181	0	2	38.5	108	30
26	ColQ+	COL	Q+	4	46.5	0.11936	0	1	39.2	126	66
9	ForQ-	FOR	Q-	4	51	0.04706	0	3	39.4	120	48
15	ForQ-	FOR	Q-	4	44.25	0.06837	0	.	38.9	150	42
18	ForQ-	FOR	Q-	4	45	0.04948	0	2	38.9	.	.
21	ForQ-	FOR	Q-	4	46.5	0.08153	0	2	39	114	60
22	ForQ-	FOR	Q-	4	37.5	0.07945	0	2	39.5	108	60
24	ForQ-	FOR	Q-	4	48	0.12083	0	.	39.2	114	54
29	ForQ-	FOR	Q-	4	50.5	0.08515	0	.	38.6	108	48
13	ForQ+	FOR	Q+	4	47.5	0.12	0	2	39.4	126	30
14	ForQ+	FOR	Q+	4	52.5	0.09022	0	.	39.1	138	66
17	ForQ+	FOR	Q+	4	44.5	0.11685	0	2	39.4	150	54
20	ForQ+	FOR	Q+	4	34.5	0.13333	0	2	39.4	168	84
25	ForQ+	FOR	Q+	4	47.25	0.02718	0	2	39	132	78
27	ForQ+	FOR	Q+	4	44	0.02045	0	3	38.7	.	.
30	ForQ+	FOR	Q+	4	46.25	0.06218	0	2	39.2	114	66
1	ColQ-	COL	Q-	5	40	0.12	0	.	38.9	116	72
4	ColQ-	COL	Q-	5	46	0.12174	0	.	37.6	138	42
5	ColQ-	COL	Q-	5	56	0.11786	0	2	39.1	132	42
10	ColQ-	COL	Q-	5	48.5	0.11959	0	2	39.1	156	54
12	ColQ-	COL	Q-	5	46	0.12174	0	.	38.2	.	.
23	ColQ-	COL	Q-	5	50.5	0.11881	0	2	38.9	120	60
28	ColQ-	COL	Q-	5	39.5	0.12658	0	2	38.9	156	.
2	ColQ+	COL	Q+	5	46.5	0.12043	0	2	39.1	102	54
3	ColQ+	COL	Q+	5	55	0.12	0	2	38.9	138	84
7	ColQ+	COL	Q+	5	44	0.12045	0	2	38.8	162	.
8	ColQ+	COL	Q+	5	52	0.11923	0	2	37.7	120	42
11	ColQ+	COL	Q+	5	45	0.12	0	.	39	138	54
19	ColQ+	COL	Q+	5	52.5	0.1181	0	2	38.8	132	30
26	ColQ+	COL	Q+	5	47	0.11915	0	1	39	138	66
9	ForQ-	FOR	Q-	5	51	0.04706	0	4	39	114	42
15	ForQ-	FOR	Q-	5	45.5	0.12088	0	2	39.1	162	42
18	ForQ-	FOR	Q-	5	45.5	0.07253	0	.	.	.	.
21	ForQ-	FOR	Q-	5	48	0.12083	0	2	39.4	120	48
22	ForQ-	FOR	Q-	5	38	0.12105	0	2	38.8	132	60
24	ForQ-	FOR	Q-	5	48	0.12083	0	.	39.3	108	60
29	ForQ-	FOR	Q-	5	50.5	0.11485	0	.	38.7	102	36
13	ForQ+	FOR	Q+	5	47.5	0.12	0	.	38.8	126	30
14	ForQ+	FOR	Q+	5	53	0.12075	0	2	39.7	132	54
17	ForQ+	FOR	Q+	5	44.5	0.12135	0	2	38.7	144	.
20	ForQ+	FOR	Q+	5	34.5	0.14493	0	2	38.6	148	76
25	ForQ+	FOR	Q+	5	48	0.12083	0	2	38.8	138	60
27	ForQ+	FOR	Q+	5	44	0.14091	0	3	38.4	114	30
30	ForQ+	FOR	Q+	5	45.5	0.06154	0	.	.	.	.
1	ColQ-	COL	Q-	6	40	0.12	0	.	38.1	126	84
4	ColQ-	COL	Q-	6	46	0.12174	0	.	38.1	.	.
5	ColQ-	COL	Q-	6	56	0.11964	0	.	38.3	168	54
10	ColQ-	COL	Q-	6	48.5	0.12371	0	2	.	.	.
12	ColQ-	COL	Q-	6	46	0.12174	0	2	39.1	126	126
23	ColQ-	COL	Q-	6	50.5	0.11881	0	.	38.7	108	60
28	ColQ-	COL	Q-	6	39.5	0.12658	0	2	38.7	186	.
2	ColQ+	COL	Q+	6	46.5	0.12043	0	.	38.9	126	.
3	ColQ+	COL	Q+	6	55	0.12364	0	2	38.5	126	78
7	ColQ+	COL	Q+	6	44	0.12045	0	.	38.3	180	36
8	ColQ+	COL	Q+	6	52	0.12115	0	.	38.3	150	42
11	ColQ+	COL	Q+	6	45	0.12222	0	2	38.1	144	48
19	ColQ+	COL	Q+	6	52.5	0.12	0	.	38.9	120	48
26	ColQ+	COL	Q+	6	47	0.11915	0	2	39.4	132	72

Supplemental Table 4.1 Continuation.

Calf	Group	Feeding	Quercetin	Age, d	BW, kg	Milk intake, kg/kg BW	Force-fed milk, % of daily intake	Fecal score	Rectal temp, °C	Heart rate, per min	Resp. rate, per min
9	ForQ-	FOR	Q-	6	51	0.06078	0	4	38.7	90	30
15	ForQ-	FOR	Q-	6	45.5	0.11868	0	.	38.8	.	.
18	ForQ-	FOR	Q-	6	45.5	0.1033	0	3	39.2	108	.
21	ForQ-	FOR	Q-	6	48	0.12083	0	2	38.9	132	54
22	ForQ-	FOR	Q-	6	38	0.1	0	2	39.1	126	48
24	ForQ-	FOR	Q-	6	48	0.12083	0	.	39.1	126	54
29	ForQ-	FOR	Q-	6	50.5	0.11881	0	3	39.6	96	42
13	ForQ+	FOR	Q+	6	47.5	0.12	0	3	38.9	108	.
14	ForQ+	FOR	Q+	6	53	0.12075	0	.	39.2	132	60
17	ForQ+	FOR	Q+	6	44.5	0.12135	0	2	38.7	132	36
20	ForQ+	FOR	Q+	6	34.5	0.12464	0	2	39.1	126	84
25	ForQ+	FOR	Q+	6	48	0.12083	0	2	38.5	156	72
27	ForQ+	FOR	Q+	6	45	0.12	0	.	38.7	102	30
30	ForQ+	FOR	Q+	6	45.5	0.12088	0	2	39.4	132	.
1	ColQ-	COL	Q-	7	40	0.1125	0	2	38.9	116	78
4	ColQ-	COL	Q-	7	46.5	0.11613	0	2	38.7	116	.
5	ColQ-	COL	Q-	7	57.5	0.10087	0	2	37.6	114	.
10	ColQ-	COL	Q-	7	51	0.10784	0	.	38.6	126	42
12	ColQ-	COL	Q-	7	48	0.11042	0	.	38.3	138	138
23	ColQ-	COL	Q-	7	52	0.10962	0	.	39.2	114	84
28	ColQ-	COL	Q-	7	41	0.10976	0	2	.	204	.
2	ColQ+	COL	Q+	7	47.5	0.12	0	.	38.3	96	54
3	ColQ+	COL	Q+	7	56.5	0.12035	0	.	38.9	126	66
7	ColQ+	COL	Q+	7	45.5	0.10989	0	.	.	.	.
8	ColQ+	COL	Q+	7	52.5	.	.	.	.	.	.
11	ColQ+	COL	Q+	7	46.5	0.11183	0	.	38.7	126	36
19	ColQ+	COL	Q+	7	53.5	0.10654	0	2	39.1	.	.
26	ColQ+	COL	Q+	7	49	0.1102	0	.	39.2	120	60
9	ForQ-	FOR	Q-	7	50	0.1	0	4	39.1	96	36
15	ForQ-	FOR	Q-	7	46.5	0.10968	0	.	38.5	162	.
18	ForQ-	FOR	Q-	7	43.5	0.11264	0	3	39.2	108	.
21	ForQ-	FOR	Q-	7	51	0.10588	0	2	38.6	138	54
22	ForQ-	FOR	Q-	7	38	0.11316	0	2.5	38.9	126	42
24	ForQ-	FOR	Q-	7	48	0.11042	0	2	39.3	120	48
29	ForQ-	FOR	Q-	7	52	0.06538	0	2	40	96	42
13	ForQ+	FOR	Q+	7	51	0.10784	0	.	39.1	.	.
14	ForQ+	FOR	Q+	7	55	0.10545	0	3	39.5	132	60
17	ForQ+	FOR	Q+	7	47	0.10851	0	3	38.6	.	.
20	ForQ+	FOR	Q+	7	36	0.10833	0	3	38.9	114	60
25	ForQ+	FOR	Q+	7	47.5	0.10947	0	.	.	.	.
27	ForQ+	FOR	Q+	7	45	0.11111	0	.	38.6	108	36
30	ForQ+	FOR	Q+	7	42.5	.	.	2	39.3	102	.
1	ColQ-	COL	Q-	8	.	.	.	.	38.6	104	60
4	ColQ-	COL	Q-	8	.	.	.	.	38.9	120	36
5	ColQ-	COL	Q-	8	.	.	.	3	38.8	.	.
10	ColQ-	COL	Q-	8	.	.	.	2	38.9	.	.
12	ColQ-	COL	Q-	8	.	.	.	2	39	126	126
23	ColQ-	COL	Q-	8	.	.	.	2	38.8	126	42
28	ColQ-	COL	Q-	8	.	.	.	.	38.8	162	.
2	ColQ+	COL	Q+	8	.	.	.	2	38.7	.	.
3	ColQ+	COL	Q+	8	.	.	.	2	38.8	114	.
7	ColQ+	COL	Q+	8	.	.	.	2	38.5	96	24
8	ColQ+	COL	Q+	8	.	.	.	.	38.9	.	.
11	ColQ+	COL	Q+	8	.	.	.	.	38.6	120	30
19	ColQ+	COL	Q+	8	.	.	.	2	39.1	120	.
26	ColQ+	COL	Q+	8	.	.	.	2	38.7	150	42
9	ForQ-	FOR	Q-	8	.	.	.	3	.	.	.
15	ForQ-	FOR	Q-	8	.	.	.	.	.	.	.
18	ForQ-	FOR	Q-	8	.	.	.	3	39	102	.
21	ForQ-	FOR	Q-	8	.	.	.	2	38.7	102	51
22	ForQ-	FOR	Q-	8	.	.	.	3	38.3	108	48
24	ForQ-	FOR	Q-	8	.	.	.	.	.	.	.
29	ForQ-	FOR	Q-	8	.	.	.	.	.	.	.
13	ForQ+	FOR	Q+	8	.	.	.	3	39.1	.	.
14	ForQ+	FOR	Q+	8	.	.	.	2	39.2	132	54
17	ForQ+	FOR	Q+	8	.	.	.	.	38.8	132	.
20	ForQ+	FOR	Q+	8	.	.	.	3	38.9	.	.
25	ForQ+	FOR	Q+	8	.	.	.	2	38.9	114	48
27	ForQ+	FOR	Q+	8	.	.	.	2	39.1	132	42
30	ForQ+	FOR	Q+	8	.	.	.	3.5	39.6	.	.

doi:10.1371/journal.pone.0146932.s001

Supplemental Table 4.2: Complete data set of flavonoid measurements in blood plasma as shown in Figure 4.2.

Calf	Group	Feeding	Quercetin	Age, d	Quercetin, nmol/L	Isorhamnetin, nmol/L	Tamarixetin, nmol/L	Kaempferol, nmol/L	Total flavonols, nmol/L	Quercetin, % of total flav.	Isorhamnetin, % of total flav.	Tamarixetin, % of total flav.	Kaempferol, % of total flav.	Ratio KIT:Q
2	CQ+	COL	Q+	1	7.85	0	0	3.9759	11.826	66.381	0	0	33.6187	0.50645
3	CQ+	COL	Q+	1	7.969	0	0	4.0806	12.05	66.136	0	0	33.8638	0.51203
7	CQ+	COL	Q+	1	0	0	0	0	0	0	0	0	0	0
8	CQ+	COL	Q+	1	0	0	0	0	0	0	0	0	0	0
11	CQ+	COL	Q+	1	0	0	0	0	0	0	0	0	0	0
13	FQ+	FOR	Q+	1	0	0	0	0	0	0	0	0	0	0
14	FQ+	FOR	Q+	1	0	0	0	0	0	0	0	0	0	0
17	FQ+	FOR	Q+	1	0	0	0	0	0	0	0	0	0	0
19	CQ+	COL	Q+	1	0	0	0	0	0	0	0	0	0	0
20	FQ+	FOR	Q+	1	0	0	0	0	0	0	0	0	0	0
25	FQ+	FOR	Q+	1	0	0	0	0	0	0	0	0	0	0
26	CQ+	COL	Q+	1	0	0	0	0	0	0	0	0	0	0
27	FQ+	FOR	Q+	1	0	0	0	0	0	0	0	0	0	0
30	FQ+	FOR	Q+	1	0	0	0	0	0	0	0	0	0	0
2	CQ+	COL	Q+	2	6.606	2.098	0	4.0518	12.756	51.789	16.4465	0	31.7649	0.93092
3	CQ+	COL	Q+	2	8.006	5.358	5.213	0	18.577	43.098	28.8405	28.0613	0	1.32028
7	CQ+	COL	Q+	2	0	0	0	0	0	0	0	0	0	0
8	CQ+	COL	Q+	2	0	0	0	0	0	0	0	0	0	0
11	CQ+	COL	Q+	2	0	0	0	0	0	0	0	0	0	0
13	FQ+	FOR	Q+	2	0	0	0	0	0	0	0	0	0	0
14	FQ+	FOR	Q+	2	0	0	0	0	0	0	0	0	0	0
17	FQ+	FOR	Q+	2	0	0	0	0	0	0	0	0	0	0
19	CQ+	COL	Q+	2	0	0	0	0	0	0	0	0	0	0
20	FQ+	FOR	Q+	2	0	0	0	0	0	0	0	0	0	0
25	FQ+	FOR	Q+	2	0	0	0	0	0	0	0	0	0	0
26	CQ+	COL	Q+	2	0	0	0	0	0	0	0	0	0	0
27	FQ+	FOR	Q+	2	0	0	0	0	0	0	0	0	0	0
30	FQ+	FOR	Q+	2	0	0	0	0	0	0	0	0	0	0
2	CQ+	COL	Q+	3	.	.	.	.	.	.	.	.	.	.
3	CQ+	COL	Q+	3	62.32	59.12	30	0	151.44	41.152	39.0386	19.8098	0	1.43004
7	CQ+	COL	Q+	3	442.07	164.64	173.79	15.75	796.25	55.519	20.6769	21.8261	1.978	0.80119
8	CQ+	COL	Q+	3	146.25	56.897	54.945	8.7674	266.859	54.804	21.321	20.5896	3.2854	0.82469
11	CQ+	COL	Q+	3	251.482	50.674	43.272	12.1299	357.559	70.333	14.1723	12.1022	3.3924	0.42181
13	FQ+	FOR	Q+	3	338.903	85.44	88.522	12.8381	525.703	64.467	16.2525	16.8389	2.4421	0.55119
14	FQ+	FOR	Q+	3	287.612	57.044	33.413	14.0687	392.138	73.345	14.547	8.5207	3.5877	0.36343
17	FQ+	FOR	Q+	3	.	.	.	.	.	.	.	.	.	.
19	CQ+	COL	Q+	3	.	.	.	.	.	.	.	.	.	.
20	FQ+	FOR	Q+	3	.	.	.	.	.	.	.	.	.	.
25	FQ+	FOR	Q+	3	24.192	19.666	8.65	2.7849	55.293	43.753	35.5671	15.6434	5.0367	1.28557
26	CQ+	COL	Q+	3	96.844	34.327	14.712	2.9816	148.865	65.055	23.0594	9.8831	2.0029	0.53717
27	FQ+	FOR	Q+	3	167.896	32.723	18.219	5.8081	224.646	74.738	14.5666	8.11	2.5854	0.33801
30	FQ+	FOR	Q+	3	96.733	23.013	21.926	3.5314	145.204	66.619	15.849	15.1	2.4321	0.50108
2	CQ+	COL	Q+	4	248.16	94.744	56.899	13.4129	413.215	60.056	22.9284	13.7697	3.246	0.66512
3	CQ+	COL	Q+	4	58.998	30.973	24.604	7.8548	122.43	48.189	25.2984	20.0967	6.4158	1.07516
7	CQ+	COL	Q+	4	142.42	69.31	29.24	0	240.97	59.103	28.7629	12.1343	0	0.69197
8	CQ+	COL	Q+	4	143.141	64.269	51.01	7.3421	265.762	53.861	24.183	19.1938	2.7627	0.85665
11	CQ+	COL	Q+	4	130.985	38.251	20.963	7.3405	197.539	66.308	19.3636	10.6121	3.716	0.50811
13	FQ+	FOR	Q+	4	.	.	.	.	.	.	.	.	.	.
14	FQ+	FOR	Q+	4	87.991	21.646	13.398	4.8179	127.853	68.822	16.9306	10.4794	3.7683	0.45303
17	FQ+	FOR	Q+	4	35.987	12.32	8.637	3.2762	60.221	59.759	20.4581	14.3427	5.4404	0.67339

Supplemental Table 4.2 Continuation.

Calf	Group	Feeding	Quercetin	Age, d	Quercetin, nmol/L	Isorhamnetin, nmol/L	Tamarixetin, nmol/L	Kaempferol, nmol/L	Total flavonols, nmol/L	Quercetin, % of total flav.	Isorhamnetin, % of total flav.	Tamarixetin, % of total flav.	Kaempferol, % of total flav.	Ratio KIT:Q
19	CQ+	COL	Q+	4	93.096	47.86	30.393	5.6078	176.956	52.609	27.0463	17.1753	3.169	0.9008
20	FQ+	FOR	Q+	4	37.224	15.659	2.205	0	55.088	67.572	28.4259	4.002	0	0.4799
25	FQ+	FOR	Q+	4	69.207	22.767	9.919	2.0801	103.973	66.563	21.8965	9.5401	2.0007	0.50234
26	CQ+	COL	Q+	4	50.863	18.959	0	0	69.822	72.846	27.1538	0	0	0.37276
30	FQ+	FOR	Q+	4	91.096	20.46	31.805	3.105	146.466	62.196	13.9689	21.7152	2.1199	0.60782
2	CQ+	COL	Q+	7	49.054	20.021	9.63	6.1586	84.864	57.803	23.5922	11.3476	7.2571	0.73001
3	CQ+	COL	Q+	7	37.108	21.098	14.833	2.1688	75.209	49.341	28.0527	19.7229	2.8837	1.02672
7	CQ+	COL	Q+	7	114.75	30.36	22.76	3.45	171.32	66.98	17.7212	13.2851	2.0138	0.49298
11	CQ+	COL	Q+	7	82.213	31.798	30.46	6.4607	150.931	54.47	21.0676	20.1815	4.2806	0.83586
13	FQ+	FOR	Q+	7	133.499	29.247	33.533	4.4738	200.752	66.499	14.5685	16.7037	2.2285	0.50377
14	FQ+	FOR	Q+	7	103.231	14.519	12.442	5.0035	135.196	76.357	10.7396	9.2027	3.7009	0.30964
17	FQ+	FOR	Q+	7	16.76	5.331	4.908	2.072	29.07	57.651	18.3365	16.8846	7.1274	0.73456
19	CQ+	COL	Q+	7	151.937	54.495	52.604	7.8448	266.88	56.931	20.4192	19.7106	2.9395	0.75652
20	FQ+	FOR	Q+	7	107.243	22.783	15.851	2.6877	148.564	72.186	15.3352	10.6695	1.8091	0.38531
25	FQ+	FOR	Q+	7	50.308	23.449	20.763	3.2455	97.765	51.458	23.9847	21.238	3.3197	0.94335
26	CQ+	COL	Q+	7	30.657	0	0	0	30.657	100	0	0	0	0
27	FQ+	FOR	Q+	7	62.657	14.244	18.096	3.1707	98.167	63.826	14.5099	18.4337	3.2299	0.56675
2	CQ+	COL	Q+	8	63.343	19.154	9.372	4.6322	96.501	65.639	19.8488	9.7117	4.8001	0.52348
3	CQ+	COL	Q+	8	.	.	.	.	.	.	.	.	.	.
7	CQ+	COL	Q+	8	.	.	.	.	.	.	.	.	.	.
11	CQ+	COL	Q+	8	.	.	.	.	.	.	.	.	.	.
13	FQ+	FOR	Q+	8	161.704	19.12	16.33	5.0544	202.208	79.969	9.4555	8.0757	2.4996	0.25048
14	FQ+	FOR	Q+	8	89.841	10.458	6.72	3.9153	110.935	80.986	9.4272	6.0578	3.5294	0.23479
17	FQ+	FOR	Q+	8	.	.	.	.	.	.	.	.	.	.
19	CQ+	COL	Q+	8	74.96	16.3	35.961	5.1305	132.351	56.637	12.316	27.1706	3.8764	0.76563
20	FQ+	FOR	Q+	8	76.688	14.205	11.083	29.195	131.17	58.464	10.8292	8.4493	22.2573	0.71045
25	FQ+	FOR	Q+	8	43.227	9.308	13.496	2.47	68.5	63.105	13.5877	19.7017	3.6058	0.58466
26	CQ+	COL	Q+	8	100.191	11.644	15.68	2.0032	129.518	77.357	8.99	12.1067	1.5467	0.29271
27	FQ+	FOR	Q+	8	7.801	6.937	9.782	.	24.52	31.816	28.2917	39.8922	.	.

doi:10.1371/journal.pone.0146932.s002

Supplemental Table 4.3: Complete data set of plasma concentrations of metabolites and hormones as shown in Figure 4.3 and Table 4.2.

Calf	Group	Feeding	Quercetin	Age, d	NEFA, µmol/L	Urea, mmol/L	Triglycerides, mmol/L	Lactate, mmol/L	Glucose, mmol/L	Cholesterol, mmol/L	Albumin, g/L	Protein, g/L	Cortisol, µg/L	Insulin, µg/L	Glucagon, ng/L
1	ColQ-	COL	Q-	1	895	4.27	0.23	2.57	2.62	0.39	26.1	45.3	29.9	0.42164	74.45
4	ColQ-	COL	Q-	1	1130	3.24	0.23	1.78	3.62	0.41	24.7	42.4	28.8	0.27239	89.945
5	ColQ-	COL	Q-	1	1156	3	0.24	1.92	4.27	0.82	24	41.6	39.6	0.52239	71.655
10	ColQ-	COL	Q-	1	223	4.25	0.25	7.68	6.02	0.96	24.9	44	32	1.47948	83.6
12	ColQ-	COL	Q-	1	1393	3.14	0.36	2.96	6.07	0.59	28.4	47.3	38.4	0.92351	78.35
23	ColQ-	COL	Q-	1	368	2.47	0.16	6.06	3.39	0.69	26.4	45.4	57.6	0.24254	97.3
28	ColQ-	COL	Q-	1	646	2.79	0.22	5.29	3.81	0.45	26.1	45.8	31.6	0.23507	80
2	ColQ+	COL	Q+	1	566	3.39	0.18	2.28	4.35	0.73	25.9	46.7	39.4	0.48694	57.25
3	ColQ+	COL	Q+	1	1478	2.89	0.33	2.2	2.97	0.53	25.8	44.5	44.1	0.375	66.65
7	ColQ+	COL	Q+	1	626	3.28	0.14	4.27	6.89	0.52	26.1	46.9	46.9	0.21269	104.3
8	ColQ+	COL	Q+	1	46	6.99	0.49	.	7.67	0.42	27.5	47.9	23.8	.	187
11	ColQ+	COL	Q+	1	500	2.6	0.16	3.02	5.08	0.36	27.8	48.8	40.2	1.73507	62.35
19	ColQ+	COL	Q+	1	1070	3.1	0.32	2.91	2.72	0.64	26.7	47.9	43.2	0.54664	95
26	ColQ+	COL	Q+	1	679	3.13	0.15	2.28	3.48	0.94	26.2	45	41.8	0.13993	80.85
9	ForQ-	FOR	Q-	1	321	5.96	0.45	8.11	2.63	0.59	23.7	49.3	44.7	0.52425	260.9
15	ForQ-	FOR	Q-	1	1597	3.21	0.42	1.67	5.13	0.85	27.2	50	19.8	0.41978	57.9
18	ForQ-	FOR	Q-	1	308	2.65	0.22	6.31	5	0.48	24.2	42.3	34.9	3.35261	97.45
21	ForQ-	FOR	Q-	1	382	2.84	0.14	2.9	2.72	0.84	27.3	45.9	38.9	0.32649	93.95
22	ForQ-	FOR	Q-	1	950	1.34	0.19	2.555	2.885	0.66	24.4	41.35	32.2	0.54851	127.4
24	ForQ-	FOR	Q-	1	966	2.72	0.19	2.9	6.28	0.68	27.8	46	29.8	5.45709	127.75
29	ForQ-	FOR	Q-	1	844	3.27	0.21	1.75	3.83	0.29	25.3	44.2	39.5	0.49627	74.25
13	ForQ+	FOR	Q+	1	565	2.93	0.27	2.72	5.16	0.39	26.3	43.5	27.6	0.31343	63.3
14	ForQ+	FOR	Q+	1	448	3.85	0.18	3.25	3.54	0.58	24.2	42.3	44.6	1.13806	85.25
17	ForQ+	FOR	Q+	1	659	2.65	0.29	3.38	3.57	0.54	26.9	47.3	38.5	1.13806	121.2
20	ForQ+	FOR	Q+	1	1160	3.31	0.32	4.01	6.51	0.78	26.5	47.3	46.9	1.59328	146.65
25	ForQ+	FOR	Q+	1	514	2.57	0.11	3.35	3.85	0.85	24.6	43.4	67.6	0.20149	136.95
27	ForQ+	FOR	Q+	1	833	5.29	0.18	2.84	3.63	0.4	24.8	43.5	38.9	0.66045	88.55
30	ForQ+	FOR	Q+	1	952	2.57	0.23	2.45	2.52	0.67	28.7	47.4	35.7	1.65485	181.6
1	ColQ-	COL	Q-	2	233	4.11	0.2	1.43	5.8	0.84	22.7	65.7	11.2	0.94963	400
4	ColQ-	COL	Q-	2	389	3.64	0.26	2.33	6.13	0.52	23.6	56.3	10	0.4403	398.8
5	ColQ-	COL	Q-	2	199	3.41	0.27	2.8	5.35	0.96	21.2	58.8	15.6	0.93843	241.35
10	ColQ-	COL	Q-	2	399	4.4	0.36	1.94	6.31	1.11	22.2	63.2	8.1	0.62127	227.6
12	ColQ-	COL	Q-	2	402	3.3	0.24	2.85	7	0.61	23.9	55	23.4	1.47575	308.5
23	ColQ-	COL	Q-	2	609	3.51	0.4	2.69	5.61	0.82	23.5	63	27.3	0.63806	341.5
28	ColQ-	COL	Q-	2	279	2.21	0.47	2.9	5.46	0.57	23.4	55.9	13.7	0.33955	400
2	ColQ+	COL	Q+	2	348	5.08	0.24	4.13	5.63	0.87	23.3	70.4	10.1	0.375	313.4
3	ColQ+	COL	Q+	2	410	3.82	0.47	2.85	5.08	0.65	22.1	71.2	9.6	1.38246	399.85
7	ColQ+	COL	Q+	2	390	3.71	0.17	3.35	5.87	0.63	23.2	62.8	30.9	0.54291	333.15
8	ColQ+	COL	Q+	2	554	6.49	0.32	2.47	5.27	0.87	24.6	60.7	6.5	1.33396	400
11	ColQ+	COL	Q+	2	293	3.83	0.24	3.75	6.57	0.68	24.7	62.3	16.6	1.5	346.15
19	ColQ+	COL	Q+	2	174	4.05	0.23	2.37	5.43	0.73	23.7	58	14.2	0.93843	265
26	ColQ+	COL	Q+	2	410	3.09	0.16	1.96	4.82	0.87	23.4	57.1	12	0.18097	208.95
9	ForQ-	FOR	Q-	2	314	10.71	0.57	4	7.01	1.21	23	51.4	46.5	1.54104	363.65
15	ForQ-	FOR	Q-	2	535	4.37	0.2	2.43	6.85	0.92	25.2	48.2	19	1.79851	317.2
18	ForQ-	FOR	Q-	2	366	3.57	0.29	2.89	3.59	1.05	23.7	44.3	18	0.86567	323
21	ForQ-	FOR	Q-	2	370	6	0.4	3.22	4.96	1.08	26.3	47.4	32.2	0.59888	251.6
22	ForQ-	FOR	Q-	2	566	2.43	0.21	1.43	4.93	0.86	23.5	40.8	45.4	0.26679	266.25

Supplemental Table 4.3 Continuation.

Calf	Group	Feeding	Quercetin	Age, d	NEFA, µmol/L	Urea, mmol/L	Triglycerides, mmol/L	Lactate, mmol/L	Glucose, mmol/L	Cholesterol, mmol/L	Albumin, g/L	Protein, g/L	Cortisol, µg/L	Insulin, µg/L	Glucagon, ng/L
24	ForQ-	FOR	Q-	2	295	3.49	0.14	3.09	7.45	0.74	25	45.9	16.5	1.07463	304.95
29	ForQ-	FOR	Q-	2	262	4.32	0.28	1.67	5.94	0.55	23	42.4	21.6	1.84515	187.25
13	ForQ+	FOR	Q+	2	368	6.26	0.24	1.49	4.86	0.53	25.3	45.6	29.6	0.58582	249.75
14	ForQ+	FOR	Q+	2	309	4.77	0.25	2.06	7.12	0.76	23.1	41.8	27.6	1.42537	175.9
17	ForQ+	FOR	Q+	2	345	5.29	0.41	2.89	5.7	0.89	25.6	47.1	14.7	1.03545	302.2
20	ForQ+	FOR	Q+	2	387	3.02	0.2	5.23	6.17	0.87	23.8	43.2	33.9	1.13246	400
25	ForQ+	FOR	Q+	2	403	4.64	0.34	2.02	7.23	0.95	23.9	42.8	25.7	1.13433	248.95
27	ForQ+	FOR	Q+	2	307	3.4	0.24	3.91	5.31	0.67	24.6	44.9	28	0.40299	370.7
30	ForQ+	FOR	Q+	2	484	3.7	0.13	2.66	4.12	0.66	24.8	46.4	58.6	0.26306	374.3
1	ColQ-	COL	Q-	4	179	5.72	0.24	0.97	4.89	1.58	23	59.3	5.7	0.50933	220.8
4	ColQ-	COL	Q-	4	179	3.89	0.31	0.88	6.23	0.95	24.3	56.9	18.1	0.16604	231.05
5	ColQ-	COL	Q-	4	371	3.66	0.43	1.58	5.34	1.8	23.4	60.9	7.7	0.39925	167.55
10	ColQ-	COL	Q-	4	345	3.37	0.52	0.68	5.5	1.47	23.1	61.4	12.9	0.41045	191.55
12	ColQ-	COL	Q-	4	710	2.8	0.77	0.9	5.74	1.15	25.5	59.7	14.6	0.19216	145.95
23	ColQ-	COL	Q-	4	139	3.89	0.23	0.98	5.96	1.16	23.2	60.2	20	0.41978	227.3
28	ColQ-	COL	Q-	4	110	2.74	0.29	1.07	5.14	1.28	24.5	52.7	13.2	0.25933	172.7
2	ColQ+	COL	Q+	4	147	4.39	0.62	0.78	5.45	1.69	23.1	65.6	2.9	0.31903	187.35
3	ColQ+	COL	Q+	4	346	7.45	0.42	0.72	5.86	1.34	21.7	64.1	10.1	0.40299	239.55
7	ColQ+	COL	Q+	4	408	2.84	0.53	1.07	6.26	1.65	24	64.6	10.4	0.32276	189.8
8	ColQ+	COL	Q+	4	179	4.09	0.12	1.22	5.82	1.54	25.3	62.3	5.6	0.38246	160.5
11	ColQ+	COL	Q+	4	211	2.36	0.74	1.27	5.32	1.09	24.6	62.7	14.6	0.28358	164.45
19	ColQ+	COL	Q+	4	417	3.47	0.34	1.08	4.88	1.76	26.3	63.3	14.7	0.16418	157.15
26	ColQ+	COL	Q+	4	177	2.62	0.23	0.81	5.42	1.51	24.6	56.8	8.8	0.18097	165.3
9	ForQ-	FOR	Q-	4	227	4.18	0.36	0.48	3.55	1.04	20.6	49.5	3.3	0.26306	156.9
15	ForQ-	FOR	Q-	4	130	3.41	0.3	0.33	4.91	1.3	24.9	47.6	14.4	0.78731	101.85
18	ForQ-	FOR	Q-	4	95	4.5	0.16	1.63	4.98	0.85	22	42.5	11.1	0.20709	112.7
21	ForQ-	FOR	Q-	4	113	3.47	0.07	0.72	4.74	1.07	22.7	41.9	14.1	0.6903	106.55
22	ForQ-	FOR	Q-	4	91	3.15	0.1	0.66	4.96	1.02	22.8	40.7	14	0.1903	112
24	ForQ-	FOR	Q-	4	569.5	3.02	0.26	1.075	3.63	0.985	23.85	44.9	3.5	0.09328	164
29	ForQ-	FOR	Q-	4	356	4.9	0.23	1.76	3.87	0.77	22.5	44.1	20.1	0.24813	152.6
13	ForQ+	FOR	Q+	4	315	7.76	0.18	2.47	3.81	0.58	23.5	44.3	9	0.28358	85.05
14	ForQ+	FOR	Q+	4	73	5.9	0.09	1.23	4.66	0.67	20.8	42.3	6.6	0.41791	55.25
17	ForQ+	FOR	Q+	4	211	2.7	0.19	0.91	3.86	0.95	23.3	45.8	8	0.21828	170.9
20	ForQ+	FOR	Q+	4	692	2.6	0.27	1.57	4	0.83	24.6	45.6	31.1	0.09328	227.25
25	ForQ+	FOR	Q+	4	55	4.26	0.25	1.32	5.86	1.15	22.4	41.3	11.6	0.36567	136
27	ForQ+	FOR	Q+	4	.	.	.	.	.	.	.	.	.	.	.
30	ForQ+	FOR	Q+	4	325	5.77	0.17	0.72	3.64	0.93	21.8	44	30	0.1847	159.6
1	ColQ-	COL	Q-	7	236	6.18	0.26	0.4	5.07	2.1	22.6	56.2	12.6	0.34515	194.45
4	ColQ-	COL	Q-	7	105	5.65	0.18	0.44	5.73	1.05	23.3	51.2	4.9	0.46642	206.65
5	ColQ-	COL	Q-	7	145	4.58	0.11	0.55	5.37	2.07	21.4	49.9	14.7	0.42724	141.55
10	ColQ-	COL	Q-	7	133	4.69	0.26	0.47	4.88	1.52	22.3	55.5	10.8	0.28545	177.9
12	ColQ-	COL	Q-	7	233	4.15	0.24	0.47	5.26	1.07	23.2	49	8.1	0.20522	124.3
23	ColQ-	COL	Q-	7	93	4.73	0.21	0.48	5.44	1.15	23.1	57.2	8	0.45149	143.1
28	ColQ-	COL	Q-	7	118	5.06	0.2	0.55	4.93	1.47	23.2	50.2	10.2	0.33022	150.45
2	ColQ+	COL	Q+	7	117	6.89	0.23	1.62	5.32	2.26	22.4	59.9	7.7	0.28545	183.9
3	ColQ+	COL	Q+	7	162	6.62	0.24	0.32	5.17	1.56	20.6	57.3	5.2	0.47201	160
7	ColQ+	COL	Q+	7	134	5.9	0.1	0.51	6.08	1.71	23.5	58	6.1	0.375	169.2
8	ColQ+	COL	Q+	7	.	.	.	.	.	.	.	.	.	.	.
11	ColQ+	COL	Q+	7	141	4.31	0.34	1.17	4.99	1.48	23	53.8	7.5	0.32836	124.55
19	ColQ+	COL	Q+	7	153	4.04	0.26	0.8	5.08	1.71	23.5	52	10.9	0.52985	182.3

Supplemental Table 4.3 Continuation.

Calf	Group	Feeding	Quercetin	Age, d	NEFA, µmol/L	Urea, mmol/L	Triglycerides, mmol/L	Lactate, mmol/L	Glucose, mmol/L	Cholesterol, mmol/L	Albumin, g/L	Protein, g/L	Cortisol, µg/L	Insulin, µg/L	Glucagon, ng/L
26	ColQ+	COL	Q+	7	234	4.38	0.16	0.53	4.61	1.43	23.6	52.8	11	0.125	154.9
9	ForQ-	FOR	Q-	7	316	5.15	0.18	0.5	4.52	1	21	53.2	1.3	0.18284	77.95
15	ForQ-	FOR	Q-	7	179	3.32	0.15	0.53	4.75	1.45	23.1	44.4	9.6	2.21455	55.6
18	ForQ-	FOR	Q-	7	511	6.34	0.07	0.4	3.47	0.5	22.7	40.2	9.1	0.09328	89.45
21	ForQ-	FOR	Q-	7	100	2.98	0.07	0.55	4.46	0.92	23.6	40	9.3	0.15299	75.15
22	ForQ-	FOR	Q-	7	87	5.28	0.08	0.04	5.01	1.07	21.5	38.4	7.2	0.24254	81.05
24	ForQ-	FOR	Q-	7	100	3.54	0.11	0.74	4.92	1.2	23.6	42.8	10	0.33209	80.45
29	ForQ-	FOR	Q-	7	95	4.58	0.06	1.35	3.86	1.06	20.7	38.7	10.4	0.36381	111.65
13	ForQ+	FOR	Q+	7	239	3.8	0.11	0.39	4.06	0.69	21.8	41.4	11.7	0.13993	46.4
14	ForQ+	FOR	Q+	7	153	5.2	0.11	0.28	5.05	0.82	21.8	42.3	8.7	0.35634	47.6
17	ForQ+	FOR	Q+	7	290	3.3	0.1	0.45	3.95	0.98	23	43.7	12	0.09328	95.5
20	ForQ+	FOR	Q+	7	90	3.36	0.13	0.46	5.28	0.89	22	38.4	20.5	0.28731	120.4
25	ForQ+	FOR	Q+	7	80	4.14	0.03	0.5	4.82	1.2	21.6	37.3	5.4	0.22575	69.05
27	ForQ+	FOR	Q+	7	159	3.65	0.07	0.63	5.25	1.08	21.5	38.4	11.2	0.20336	54.05
30	ForQ+	FOR	Q+	7	.	.	.	.	.	.	.	.	.	.	.

doi:10.1371/journal.pone.0146932.s003

Supplemental Table 4.4: Complete data set of immune and inflammatory status in blood plasma as shown in Figure 4.3.

Calf	Group	Feeding	Quercetin	Age, d	IgG1, g/L	IgG2, g/L	IgM, g/L	Fibrinogen, g/L	Haptoglobin, g/L	Serum amyloid A, mg/L
1	ColQ-	COL	Q-	1	0	0	37.29	3.81	0	14.888
4	ColQ-	COL	Q-	1	0	0	30.82	0	0	13.438
5	ColQ-	COL	Q-	1	0	8.65	27.15	0	0	1.208
10	ColQ-	COL	Q-	1	0	11.15	25.41	2.17	0	26.579
12	ColQ-	COL	Q-	1	0	3.04	20.08	3.5	0	33.281
23	ColQ-	COL	Q-	1	0	9.81	48.12	2.86	0	14.13
28	ColQ-	COL	Q-	1	0	6.53	52.32	2.22	0	20.482
2	ColQ+	COL	Q+	1	0	4.53	13.46	2.71	0	1.499
3	ColQ+	COL	Q+	1	0	.	.	0.81	0	16.725
7	ColQ+	COL	Q+	1	0	8.1	72.09	0	0	0
8	ColQ+	COL	Q+	1	0	23.71	57.07	4.52	0	29.727
11	ColQ+	COL	Q+	1	0	0	20.25	3.75	0	9.764
19	ColQ+	COL	Q+	1	0	19.59	342.08	2.22	0	27.851
26	ColQ+	COL	Q+	1	0	5.35	20.82	1.67	0	13.159
9	ForQ-	FOR	Q-	1	.	.	129.64	5.24	0	107.84
15	ForQ-	FOR	Q-	1	.	.	27.75	3.49	0	18.943
18	ForQ-	FOR	Q-	1	0	5.32	10.54	1.95	0	36.547
21	ForQ-	FOR	Q-	1	0	4.19	44.36	4.35	0	22.804
22	ForQ-	FOR	Q-	1	0	5.01	18.69	1.46	0	17.747
24	ForQ-	FOR	Q-	1	0	5.41	39.22	2.86	0	14.659
29	ForQ-	FOR	Q-	1	0	5.07	42.59	2.6	0	23.306
13	ForQ+	FOR	Q+	1	0	4.37	16.99	2.86	0	26.197
14	ForQ+	FOR	Q+	1	0	2.97	26.66	3.25	0	40.492
17	ForQ+	FOR	Q+	1	0	91.95	48.33	2.1	0	32.56
20	ForQ+	FOR	Q+	1	0	5.32	22.17	1.96	0	23.143
25	ForQ+	FOR	Q+	1	0	3.2	15.97	2.04	0	17.394
27	ForQ+	FOR	Q+	1	0	7	15.31	2.34	0	21.011
30	ForQ+	FOR	Q+	1	0.01	7.81	105.56	1.84	0	18.453
1	ColQ-	COL	Q-	2	10.46	986.05	1386.49	4.6	0.07	139.162
4	ColQ-	COL	Q-	2	7.58	1021.1	726.9	3.42	0	126.353
5	ColQ-	COL	Q-	2	10.7	605.89	1151.22	2.86	0.05	200.51
10	ColQ-	COL	Q-	2	10.17	762.41	1234.9	4.88	0	168.81
12	ColQ-	COL	Q-	2	8.9	652.35	852.23	3.9	0	94.11
23	ColQ-	COL	Q-	2	10.46	573.12	1246.63	4.17	0	121.418
28	ColQ-	COL	Q-	2	9.6	665.97	752.27	3.04	0	90.493
2	ColQ+	COL	Q+	2	12.54	1381.67	1512.83	4.21	0	116.879
3	ColQ+	COL	Q+	2	13.43	1270.24	1661.41	2.86	0	119.036
7	ColQ+	COL	Q+	2	10.13	1316.29	1065.97	2.63	0	95.949
8	ColQ+	COL	Q+	2	9.47	1368.68	773.73	5.53	0.03	130.268
11	ColQ+	COL	Q+	2	10.13	906.28	1087.25	4.69	0	183.342
19	ColQ+	COL	Q+	2	6.4	589.25	865.14	3.02	0	128.851
26	ColQ+	COL	Q+	2	7.28	533.12	893.9	2.88	0	131.3
9	ForQ-	FOR	Q-	2	.	.	110.07	7.67	0	239.091
15	ForQ-	FOR	Q-	2	.	.	39.03	4.65	0	136.486
18	ForQ-	FOR	Q-	2	0	7.59	27.21	5.12	0	146.964
21	ForQ-	FOR	Q-	2	0	8.36	59.27	6.52	0	170.803
22	ForQ-	FOR	Q-	2	0	9.52	31.99	3.04	0	93.978
24	ForQ-	FOR	Q-	2	0	8.97	49.69	4.22	0	99.581
29	ForQ-	FOR	Q-	2	0	11.88	53.25	4.22	0.09	113.654
13	ForQ+	FOR	Q+	2	0	3.9	12.46	5.53	0.01	203.424
14	ForQ+	FOR	Q+	2	0	3.41	25.95	5.5	0.03	113.708
17	ForQ+	FOR	Q+	2	0	103.22	53.13	4.05	0.02	158.502
20	ForQ+	FOR	Q+	2	0	8.95	31.21	2.32	0	152.436
25	ForQ+	FOR	Q+	2	0	5.96	26.23	4.08	0	132.006
27	ForQ+	FOR	Q+	2	0	19.38	27	5.32	0	124.418
30	ForQ+	FOR	Q+	2	0.01	9.05	89.02	3.41	0	173.474
1	ColQ-	COL	Q-	4	8.51	638.67	922.58	5	0	91.405
4	ColQ-	COL	Q-	4	6.98	532.44	432.24	5.13	0	56.168
5	ColQ-	COL	Q-	4	11.86	620.97	881.6	7.32	0	222.568
10	ColQ-	COL	Q-	4	9.25	540.47	789.27	5.13	0	153.284
12	ColQ-	COL	Q-	4	8.6	424.71	549.25	5.23	0	98.394
23	ColQ-	COL	Q-	4	10.8	554.4	801.91	5.12	0	44.04
28	ColQ-	COL	Q-	4	9.25	674.06	440.74	3.69	0	83.346
2	ColQ+	COL	Q+	4	9.47	758.35	1108.26	7.43	0	125.193
3	ColQ+	COL	Q+	4	11.14	673	1032.83	4.42	0	51.286
7	ColQ+	COL	Q+	4	9.47	659.7	805.85	6.67	0	113.302
8	ColQ+	COL	Q+	4	9.8	916.82	478.51	5.58	0	61.533
11	ColQ+	COL	Q+	4	9.47	664.11	645.61	7.07	0	192.768
19	ColQ+	COL	Q+	4	6.98	511.52	765.53	4.65	0	112.308
26	ColQ+	COL	Q+	4	6.98	497.49	476.63	3.78	0	138.403
9	ForQ-	FOR	Q-	4	.	.	90.94	7.5	0.12	251.532
15	ForQ-	FOR	Q-	4	.	.	103.3	7.14	0	153.666
18	ForQ-	FOR	Q-	4	0	6.66	72.26	6.82	0.04	102.339
21	ForQ-	FOR	Q-	4	0	7.45	166.01	6.52	0	96.74
22	ForQ-	FOR	Q-	4	0	7.93	169.98	0.53	0	65.347
24	ForQ-	FOR	Q-	4	0	7.7	85.58	7.02	0	102.713
29	ForQ-	FOR	Q-	4	0.01	11.22	102.12	6.87	0.29	123.58
13	ForQ+	FOR	Q+	4	0	3.31	57.21	7.27	0.08	249.745

Supplemental Table 4.4 Continuation.

Calf	Group	Feeding	Quercetin	Age, d	IgG1, g/L	IgG2, g/L	IgM, g/L	Fibrinogen, g/L	Haptoglobin, g/L	Serum amyloid A, mg/L
14	ForQ+	FOR	Q+	4	0	2.19	112.34	6.67	0.07	64.798
17	ForQ+	FOR	Q+	4	0	128.55	64.52	6.34	0.03	162.574
20	ForQ+	FOR	Q+	4	0	9.02	72.88	4.54	0	68.362
25	ForQ+	FOR	Q+	4	0	5.87	96.97	5.1	0	111.713
27	ForQ+	FOR	Q+	4	0	8.09	85.65	3.12	0	134.697
30	ForQ+	FOR	Q+	4	0.01	7.87	263.18	7.08	0.14	160.24
1	ColQ-	COL	Q-	7	7.89	630.33	555.9	3.9	0	106.728
4	ColQ-	COL	Q-	7	7.28	550.14	297.2	3.95	0	56.409
5	ColQ-	COL	Q-	7	9.8	841.64	426.18	0	0	104.311
10	ColQ-	COL	Q-	7	12.93	503.05	475.9	4.28	0	85.881
12	ColQ-	COL	Q-	7	8.8	534.17	424.38	3.02	0	105.054
23	ColQ-	COL	Q-	7	9.47	516.66	532.58	3.96	0	82.552
28	ColQ-	COL	Q-	7	7.84	491.83	388.82	3.57	0	69.185
2	ColQ+	COL	Q+	7	9.47	894.82	759.6	4.62	0	130.413
3	ColQ+	COL	Q+	7	13.98	694.06	955.99	3.25	0	17.208
7	ColQ+	COL	Q+	7	9.15	726.69	586.46	4.05	0	40.651
8	ColQ+	COL	Q+	7	9.47	849.34	335.79	4.5	0	166.134
11	ColQ+	COL	Q+	7	6.69	615.4	419.04	7.5	0	98.994
19	ColQ+	COL	Q+	7	6.4	501.25	518.34	2.13	0	115.404
26	ColQ+	COL	Q+	7	6.98	524.29	395.64	3.62	0	135.623
9	ForQ-	FOR	Q-	7	.	.	595.76	7.32	0.51	234.857
15	ForQ-	FOR	Q-	7	.	.	129.49	4.77	0	95.298
18	ForQ-	FOR	Q-	7	0	6.7	150.86	4.39	0.15	74.682
21	ForQ-	FOR	Q-	7	0	9.24	208.17	4.13	0	84.651
22	ForQ-	FOR	Q-	7	0	7.31	137.5	3.62	0	38.79
24	ForQ-	FOR	Q-	7	0	7.38	171.53	4.03	0	40.025
29	ForQ-	FOR	Q-	7	0.01	11.33	340.5	4.31	0.01	84.758
13	ForQ+	FOR	Q+	7	0	3.07	333.9	5.36	0.05	198.715
14	ForQ+	FOR	Q+	7	0	5.13	314.56	4.39	0	89.401
17	ForQ+	FOR	Q+	7	0	219.75	150.65	4.88	0	102.721
20	ForQ+	FOR	Q+	7	0	8.63	170.05	2.55	0	115.235
25	ForQ+	FOR	Q+	7	0	7.73	186.71	3.67	0	56.304
27	ForQ+	FOR	Q+	7	0	10.59	165.77	4.89	0	33.937
30	ForQ+	FOR	Q+	7	0.01	6.89	774.94	5.53	0.16	147.578

doi:10.1371/journal.pone.0146932.s004

Supplemental Table 4.5: Complete data set of antioxidative status in blood plasma as shown in Figure 4.4.

Calf	Group	Feeding	Quercetin	Age, d	FRAP, ASCE (µmol/L)	TEAC, TE (mmol/L)	TBARS, MDAE (µmol/L)	Isoprostanes, ng/L
1	ColQ-	COL	Q-	1	134.6153846	1.681916039	0.233250176	872.8109473
4	ColQ-	COL	Q-	1	165.3846154	1.766782194	0.421821589	3323.513168
5	ColQ-	COL	Q-	1	113.0769231	1.761031366	0.202773465	1845.892853
10	ColQ-	COL	Q-	1	191.547619	1.995746654	1.693583504	3432.077597
12	ColQ-	COL	Q-	1	155.952381	2.003353121	1.096887807	1497.934204
23	ColQ-	COL	Q-	1	153.7777778	1.567096855	0.314685315	1316.667206
28	ColQ-	COL	Q-	1	164.6666667	1.482471879	0.498251748	2079.781082
2	ColQ+	COL	Q+	1	106.9230769	1.870161257	0.299751167	2065.667459
3	ColQ+	COL	Q+	1	133.8461538	1.787868564	0.393335698	2070.592066
7	ColQ+	COL	Q+	1	151.1904762	1.658526638	0.165721649	1115.406874
8	ColQ+	COL	Q+	1	221.9047619	1.855027023	0.453178694	1855.912236
11	ColQ+	COL	Q+	1	126.4285714	2.074663744	0.890731728	3667.339625
19	ColQ+	COL	Q+	1	144.4444444	1.508613992	0.613879004	769.8431956
26	ColQ+	COL	Q+	1	148.6666667	1.547190497	0.262237762	818.9533998
9	ForQ-	FOR	Q-	1	267.1428571	2.102237185	0.07109375	2352.334682
15	ForQ-	FOR	Q-	1	143.3333333	1.55579468	0.613879004	1097.712239
18	ForQ-	FOR	Q-	1	102.6666667	1.469952654	0.720640569	917.4523105
21	ForQ-	FOR	Q-	1	149.5555556	1.498516495	0.5625	2462.551238
22	ForQ-	FOR	Q-	1	136.8888889	1.387163857	0.104895105	391.0270488
24	ForQ-	FOR	Q-	1	121.7777778	1.499549741	0.078671329	704.2283563
29	ForQ-	FOR	Q-	1	132.8888889	1.586838851	0.104895105	5267.394514
13	ForQ+	FOR	Q+	1	166.4444444	1.549688221	0.640569395	573.8641745
14	ForQ+	FOR	Q+	1	136.4444444	1.465232911	0.427046263	812.8168926
17	ForQ+	FOR	Q+	1	181.7777778	1.572874309	0.266903915	1320.77096
20	ForQ+	FOR	Q+	1	168.5	1.465563684	0.427046263	1118.56926
25	ForQ+	FOR	Q+	1	127.1111111	1.424822272	0.236013986	1937.449516
27	ForQ+	FOR	Q+	1	125.1111111	1.468917919	0.20979021	3083.518788
30	ForQ+	FOR	Q+	1	86.22222222	1.529527086	0.078671329	2385.765122

Supplemental Table 4.5 Continuation.

Calf	Group	Feeding	Quercetin	Age, d	FRAP, ASCE (μmol/L)	TEAC, TE (mmol/L)	TBARS, MDAE (μmol/L)	Isoprostanes, ng/L
1	ColQ-	COL	Q-	4	154.6153846	1.992423615	0.420020275	681.7809869
4	ColQ-	COL	Q-	4	108.7179487	2.165506277	0.391534384	1110.758263
5	ColQ-	COL	Q-	4	102.0512821	2.196177361	0.426345819	866.6391585
10	ColQ-	COL	Q-	4	101.9047619	2.248661666	1.384797643	2145.207851
12	ColQ-	COL	Q-	4	132.6190476	2.217601928	0.756937329	1343.370792
23	ColQ-	COL	Q-	4	118.2222222	1.89554019	0.576923077	637.0641473
28	ColQ-	COL	Q-	4	126.2222222	1.790207411	0.498251748	1350.548847
2	ColQ+	COL	Q+	4	137.3076923	2.324278587	0.61104331	1351.585625
3	ColQ+	COL	Q+	4	278.4615385	2.198094303	0.493036317	808.6143913
7	ColQ+	COL	Q+	4	102.1428571	2.043920942	0.595317869	607.5221159
8	ColQ+	COL	Q+	4	133.8095238	2.094630719	0.306271478	1079.303575
11	ColQ+	COL	Q+	4	89.28571429	2.273382682	1.36020748	1860.142827
19	ColQ+	COL	Q+	4	135.5555556	1.851817733	0.774021352	1287.525435
26	ColQ+	COL	Q+	4	126.2222222	1.815953359	0.288461538	1532.568858
9	ForQ-	FOR	Q-	4	107.6190476	2.203022867	0.354947917	1702.926659
15	ForQ-	FOR	Q-	4	96	1.862789135	0.507117438	706.0255733
18	ForQ-	FOR	Q-	4	124.2222222	1.681541604	0.346975089	717.9125686
21	ForQ-	FOR	Q-	4	114	1.67239252	0.348214286	533.4365606
22	ForQ-	FOR	Q-	4	115.3333333	1.685813929	0.183566434	529.8769064
24	ForQ-	FOR	Q-	4	106	1.626439486	0.20979021	482.1558393
29	ForQ-	FOR	Q-	4	.	1.702090346	0.314685315	2301.740353
13	ForQ+	FOR	Q+	4	144.4444444	1.605298125	0.226868327	1248.038414
14	ForQ+	FOR	Q+	4	140.8888889	1.64281134	0.18683274	1011.200921
17	ForQ+	FOR	Q+	4	127.5555556	1.710609697	0.18683274	730.7388027
20	ForQ+	FOR	Q+	4	204	1.614742913	0.320284698	942.8627723
25	ForQ+	FOR	Q+	4	97.33333333	1.723530667	0.20979021	321.7424991
27	ForQ+	FOR	Q+	4	.	.	.	.
30	ForQ+	FOR	Q+	4	133.5555556	1.684214853	0.157342657	1802.367305
1	ColQ-	COL	Q-	7	131.5384615	1.998245632	0.621432282	742.713916
4	ColQ-	COL	Q-	7	136.9230769	2.144419908	0.597931422	1135.866266
5	ColQ-	COL	Q-	7	98.46153846	2.144419908	0.462749112	703.2490998
10	ColQ-	COL	Q-	7	123.8095238	2.221088225	0.854614925	1904.189486
12	ColQ-	COL	Q-	7	124.5238095	2.173230873	0.815552425	2309.539948
23	ColQ-	COL	Q-	7	102.8888889	1.838914714	0.340909091	598.8128756
28	ColQ-	COL	Q-	7	131.7777778	1.814688312	0.576923077	1463.093822
2	ColQ+	COL	Q+	7	103.4615385	2.242770349	0.406468531	1155.626195
3	ColQ+	COL	Q+	7	130	2.14633685	0.616258741	703.7163919
7	ColQ+	COL	Q+	7	162.8571429	2.090827486	0.311039519	985.0262514
8	ColQ+	COL	Q+	7				
11	ColQ+	COL	Q+	7	102.6190476	2.167842959	0.811197917	1920.390602
19	ColQ+	COL	Q+	7	119.7777778	1.800379136	0.346975089	565.4206329
26	ColQ+	COL	Q+	7	141.5555556	1.73951696	0.236013986	1562.882356
9	ForQ-	FOR	Q-	7	76.66666667	2.176400234	0.662239583	2730.653029
15	ForQ-	FOR	Q-	7	99.11111111	1.790522278	0.453736655	872.2010944
18	ForQ-	FOR	Q-	7	94.22222222	1.55631307	0.346975089	4620.767468
21	ForQ-	FOR	Q-	7	93.55555556	1.580970984	0.348214286	556.8655332
22	ForQ-	FOR	Q-	7	99.77777778	1.571634283	0.262237762	607.706398
24	ForQ-	FOR	Q-	7	102.8888889	1.720128713	0.20979021	508.053522
29	ForQ-	FOR	Q-	7	112.6666667	1.642747715	0.340909091	2413.288349
13	ForQ+	FOR	Q+	7	.	.	0.18683274	420.791816
14	ForQ+	FOR	Q+	7	91.55555556	1.740558764	0.160142349	527.6681207
17	ForQ+	FOR	Q+	7	98.88888889	1.707662508	0.320284698	713.2756069
20	ForQ+	FOR	Q+	7	.	1.539562447	0.37366548	900.2003889
25	ForQ+	FOR	Q+	7	94.44444444	1.604049263	0.839160839	1540.91929
27	ForQ+	FOR	Q+	7	80.88888889	1.612179518	0.314685315	2460.959659
30	ForQ+	FOR	Q+	7	.	.	.	.

doi:10.1371/journal.pone.0146932.s005

Supplemental Table 4.6: Complete data set of hepatic mRNA expression of inflammatory and antioxidative traits as shown in Table 4.3.

				Targets of interest, AU										Reference genes, AU			
Calf	Group	Feeding	Quercetin	CAT	GPX	SOD	CRP	IL1A	SAA2	TNF	FGA	Hp	IL1B	LRP10	POL2A	beta_actin	HPCAL1
1	ColQ-	COL	Q-	1.17143	1.06366	1.15192	0.7976	1.23514	0.62615	0.39087	3.82877	14.275	3.3734	1.11062	1.32371	1.78423	2.98448
4	ColQ-	COL	Q-	1.09433	1.00378	0.90348	0.718	1.1661	0.53025	1.14482	1.44347	6.0121	4.12951	1.30542	1.56374	1.28497	1.50192
5	ColQ-	COL	Q-	1.26334	1.38293	1.19406	0.89045	1.3085	0.73128	0.53663	4.34262	22.7932	4.76277	1.19393	1.85821	1.18233	3.27089
10	ColQ-	COL	Q-	1.68193	1.07982	1.16457	0.87412	0.84428	1.06089	0.99429	1.47065	4.1538	1.53206	1.62734	1.50812	1.0688	1.56687
12	ColQ-	COL	Q-	0.98027	0.93044	0.8111	1.04122	1.27395	1.4042	1.35465	1.71678	21.3998	2.12321	2.44541	1.07265	1.00	1.62079
23	ColQ-	COL	Q-	1.13387	1.0099	0.93922	1.09176	1.18664	1.12075	0.94523	3.57125	19.7832	2.06005	1.20449	1.28786	1.69099	3.03024
28	ColQ-	COL	Q-	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	ColQ+	COL	Q+	0.73922	0.64333	1.02115	0.63041	1.29465	0.86595	1.69928	1.25948	19.6812	2.20152	1.80704	1.36213	1.06567	1.01059
3	ColQ+	COL	Q+	0.44354	1.25023	1.08499	1.02144	0.45686	0.8613	0.74644	3.56168	48.5291	2.98425	1.01548	1.62021	1.59429	1.49852
7	ColQ+	COL	Q+	0.94772	0.81753	0.92369	0.95628	0.99084	0.43953	0.74649	5.33002	24.5246	2.57569	1.60655	1.48464	1.09975	2.40735
11	ColQ+	COL	Q+	0.76525	0.92833	1.04868	1.09882	0.80116	1.29573	1.34743	2.70274	39.7927	3.44802	1.14434	1.45122	1.57952	1.99151
19	ColQ+	COL	Q+	0.54456	0.95776	1.12808	1.13751	0.56584	1.03359	1.47307	3.84848	36.3452	7.37029	1.04446	1.66645	1.50704	2.24306
26	ColQ+	COL	Q+	1.20786	1.0994	1.27642	0.89764	1.27159	0.80903	0.87578	2.99053	5.9909	3.52465	1	1.33272	1.9682	2.53918
9	ForQ-	FOR	Q-	0.88953	0.93322	1.30995	1.39457	0.93068	1.51382	0.76048	6.07127	97.2782	3.19267	1.03933	1.27779	1.97514	2.13348
15	ForQ-	FOR	Q-	1.27149	0.97648	1.3739	0.90225	1.33036	1.01482	0.58024	7.42837	57.9337	3.63976	1.27616	1.2705	1.61782	3.82103
18	ForQ-	FOR	Q-	1.53709	0.97762	1.26895	1.02068	0.347	1.67499	1.43352	1.43329	13.5158	2.81554	1.33933	1.07143	1.82794	1.50244
21	ForQ-	FOR	Q-	1.152	0.97053	0.83668	1.1456	1.2084	1.00005	0.79529	4.03869	22.7161	5.04311	1.30082	1.38654	1.45432	2.3124
22	ForQ-	FOR	Q-	0.7968	0.91963	0.80882	1.1498	1.00232	0.7198	1.22524	1.72817	9.4138	2.239	1.17379	1.41489	1.57941	1.52159
24	ForQ-	FOR	Q-	.	.	.	.	.	.	.	.	.	.	.	.	.	.
29	ForQ-	FOR	Q-	0.44006	1.0961	0.93117	1.05969	1.47852	1.28236	0.52395	1.70676	37.8081	1.72565	1.19509	1.27504	1.72141	1.14704
13	ForQ+	FOR	Q+	0.92814	1.11748	0.90468	0.94202	0.96915	1.82901	1.39742	4.00911	67.3544	5.47918	1.06309	1.67006	1.47743	2.43242
14	ForQ+	FOR	Q+	1.07636	1.2076	0.71457	0.92361	0.73109	1.15721	1.5481	1.34891	14.6176	2.11813	1.38901	1.11465	1.69421	1.40983
17	ForQ+	FOR	Q+	1.11213	0.8946	1.09038	0.93096	1.16911	1.03578	0.60566	3.4702	26.4486	2.42419	1.08941	1.4429	1.66873	2.19312
20	ForQ+	FOR	Q+	0.99311	0.98436	1.02362	0.9965	2.08857	1.37232	1.62735	1.80067	17.9123	3.68457	1.2915	1.27819	1.58899	1.68524
25	ForQ+	FOR	Q+	0.92992	0.88046	0.90038	1.3269	1.49731	0.6904	1.06856	1.46064	11.2364	1.4396	1.42924	1.27971	1.43415	1.75185
27	ForQ+	FOR	Q+	1.42541	1.21503	0.75697	1.21643	0.61317	0.65656	2.66255	1.00	1.00	1.00	1.43461	1.59914	1.14338	1.98326

doi:10.1371/journal.pone.0146932.s006

5. GENERAL DISCUSSION

For newborn farm animals, the energy supply during the first stage of life significantly determines developmental and maturational processes and thus, affects health and productivity of the animal. In dairy calves, the external energy supply with colostrum and milk needs to be complemented by endogenous glucose production to fully meet the demands of the organism [1], and colostrum feeding is essential to promote gastrointestinal development and advance nutrient uptake [2]. By adding flavonoids to the neonate's diet, productivity may be improved as flavonoids possess antioxidative, anti-inflammatory, and antimicrobial properties that may promote health [3, 4]. However, as flavonoids also interact with glucose metabolism [5], it is of major importance to exclude potential inhibitory effects on the development of the neonate's glucose metabolism before considering the use of flavonoids as health-promoting feed additives in calf rearing.

During the last decade, various studies have been conducted to evaluate the health-promoting properties of quercetin, one of the main flavonoids [6]. Most of these studies focused on quercetin effects either *in vitro* or in experiments with laboratory rodents, where the great antioxidative potential of the compound was highlighted [7, 8]. This antioxidative capacity indeed was identified to be causative for the health-promoting properties in model animals of metabolic disorders, e.g. diabetic mice or obese rats, where quercetin treatment lowers elevated plasma glucose concentrations to normal levels, thus enhancing the metabolic status of the animal [9, 10]. While this effect is intended to ameliorate chronic or degenerative disorders, it would be highly undesirable in newborn calves: here, a quercetin-induced decrease of carbohydrate digestion and inhibition of hepatic glucose production would cause a detrimental energy deficiency, as glucose is a main energy source during the first days of life. As a consequence, not only growth in general, but also immune function and overall health would be diminished [11].

For the above-mentioned reasons, the main objective of this thesis was to investigate the effects of an oral quercetin supplementation on the glucose metabolism in newborn calves during the first week of life, according to the initial colostrum supply (**Chapter 3**). Calves were fed quercetin aglycone from d 2 until d 8 of life, the daily dose of 50 mg/kg BW resulting from data obtained in companion studies in calves [12, 13]. After an initial phase of colostrum or non-colostrum feeding, intestinal monosaccharide absorption and splanchnic glucose extraction, as well as the hepatic expression of genes related with glucose metabolism were investigated. As, based on the data obtained in this study, quercetin-feeding does not affect the neonates' glucose metabolism, the health-promoting potential of quercetin in newborn calves was further explored. For this purpose, blood plasma and liver biopsy samples obtained during the trial were analyzed for parameters of oxidative stress and immune

function, and performance and health parameters of the calves were evaluated in the second study of this thesis (**Chapter 4**).

In both studies, although the impact of an initial colostrum deprivation was significant and persistent during the first week of life, the little effects caused by quercetin treatment on some of the parameters regarding glucose metabolism and calf health seemed to be random and inconsistent. As the quercetin supplementation was increased five-fold compared to previous studies [12, 13] and quercetin-supplemented calves showed increased plasma flavonol concentrations, the absence of quercetin effects was unlikely to be caused by inaccurate application or insufficient absorption of the compound. In humans, about 99% of plasma quercetin is bound to proteins, mainly albumin [14], and *in vitro* experiments revealed that the covalent attachment of quercetin to bovine serum albumin reduces its total antioxidant activity [15]. Even after proteolysis of the quercetin-albumin complex, the free flavonol does not fully regain its antioxidative activity [15, 16]. If excessive binding between quercetin and albumin also occurred in the plasma of the calves in the present study, quercetin might have lost its biological activity, which would explain the lack of effects on plasma antioxidative status.

As mentioned in Chapter 2, flavonols rarely occur as aglycones, but mainly in conjugated form in plasma of pigs and humans [17, 18]. In the present studies, plasma flavonol concentrations were determined after enzymatic treatment of the plasma [19] hence the fraction of glucuronidated and sulfated conjugates to total flavonols in plasma is unknown. This is an important aspect, given that the biological effects of quercetin vary with its derivatives [20, 21]. *In vitro* experiments reveal that the antioxidative and anti-inflammatory properties of conjugated quercetin are reduced compared to the non-conjugated parent compound [18, 22]. In adult cattle, approximately 15% of total flavonols in plasma are unconjugated, whereas the predominant fraction appears as glucuronidated or sulfated derivatives [19]. If quercetin metabolism in the newborn calf is comparable to that in cows, this might be an explanation for the lack of quercetin effects on plasma metabolites and hormones analyzed herein.

Referring to the main objective of the present work, it was shown here that quercetin does not impair glucose metabolism when fed to newborn calves during the first week of life in a daily dosage of 50 mg/kg BW (Chapter 3). Contrariwise, data indicate that quercetin feeding might accelerate intestinal glucose absorption on d 7 of life, which contradicts previous studies where quercetin was shown to inhibit carbohydrate digestion and intestinal glucose transport [23, 24]. In milk-fed calves, the most important intestinal disaccharidase is lactase, whose activity seems to be unaffected by quercetin [25]. Additionally, the extensive flavonol metabolism inside the enterocytes and the subsequent luminal excretion of flavonol derivatives [26] is not considered in studies on quercetin's inhibitory potential on specific glucose transporters; thus, it is possible that intestinal glucose transport inhibition is of minor

importance after quercetin feeding *in vivo*. The results of the present study could be insofar beneficial to newborn calves as the improved intestinal monosaccharide absorption might enhance the overall energy supply. Thus, the glucose excess could be used as a fuel to cover the elevated energy demand in terms of infection and accruing immune defense [27]. Unfortunately, it could not be shown in the second study (Chapter 4) that quercetin supplementation alone is effectual to improve the newborn's health status. Although a specific immune challenge was not conducted, it is assumed from general health and performance parameters that quercetin feeding did not favor supplemented calves to better resist or more effectively fight infections. This conclusion is further supported by plasma concentrations of immunoglobulins and acute phase proteins, which were similar between quercetin-treated and untreated animals. Rather, it was shown that an adequate colostrum provision is essential for the newborn to stand against the new environment and support the immune system. Data obtained here indicate that negligence concerning the initial colostrum supply cannot be compensated by adding plant bioactives such as quercetin to the ensuing diet.

In growing pigs, it has been shown that after feeding a single quercetin dose, the flavonol concentration in the liver exceeds the plasma flavonol concentration, although this difference diminishes when quercetin is fed for a long-term period [28]. Further, it has been pointed out that in contrast to flavonols in plasma, quercetin and its metabolites isorhamnetin and tamarixetin appear to approximately 90% as unconjugated aglycones in the liver. If this is transferable to newborn calves, it would be expected that, first, effects in the liver might emerge earlier than in plasma, and second, that findings gained in cell culture experiments with aglycones could be confirmed in liver tissue of calves fed quercetin. To follow up this theory, PCR analyses were performed to assess the expression of different genes involved in glucose metabolism, immune function or antioxidative defense in the course of the studies that underlie the present thesis. In Chapter 3, the focus was put on key enzymes of glucose metabolism. As it has been shown in rat liver, it was anticipated that quercetin may stimulate glycogenolysis on the one hand [29], but on the other hand, inhibition of GNG was apprehended [30]. Thus it was tested to what extent key enzymes of both pathways are influenced by quercetin treatment. Interpreting the mRNA abundances of *PYGL*, coding for a key enzyme of glycogen breakdown, combined with hepatic glycogen concentrations that were also measured in the first study of this work, quercetin feeding seems to not affect hepatic glycogen metabolism in newborn calves. It is assumed that, because the neonate's energy supply is already delicate, glycogenolysis is physiologically exceedingly active. Therefore, glycogen stores are depleted shortly after birth [1, 31] which was confirmed by very low hepatic glycogen concentrations in all calves involved in this study. At the same time, GNG is upregulated, which is crucial to cover the energy demand during the first stage of life [32]. Because of the high priority of endogenous glucose production, it would be

reasonable if hepatic glucose metabolism in the newborn is autonomous and insensible to foreign impacts such as quercetin feeding. This conclusion is supported by another study in newborn calves which shows that endogenous glucose production is even independent of colostrum feeding [33], which is yet crucial for other maturational processes.

As mentioned above, quercetin not only interacts with glucose metabolism but also possesses antiinflammatory and antioxidative properties. For that reason, gene expression of proinflammatory cytokines, acute phase proteins and antioxidative enzymes were analyzed in the second study (Chapter 4) but again, effects evoking from quercetin treatment were scarce. Solely the mRNA abundance of *TNF* was increased in quercetin-fed calves, which contradicts studies *in vitro* and *in vivo* demonstrating that quercetin downregulates this cytokine. However, those experiments were conducted after inducing a high *TNF* expression, and quercetin treatment approximated TNFα levels back to the range of unstimulated, non-inflamed cells [34-36]. Therefore, it is assumed that the calves investigated in this study did not fight a general infection, thus hepatic *TNF* was not elevated and quercetin was needless to antagonize inflammatory gene expression.

A general influence of quercetin on the hepatic targets investigated in both studies, however, cannot be excluded because due to the limited amount of liver tissue, translation products and enzyme activities were disregarded. Furthermore, the flavonol concentration and the pattern of conjugates and derivatives in the liver was not analyzed, thus it could not be proven whether flavonol distribution patterns and subsequent effects obtained in growing pigs are actually deducible to newborn calves.

Effects resulting from treatment with flavonoids are generally difficult to summarize, as they are very variable, depending on the bioavailability of the compound. In this context, different doses of the same substance can cause adverse effects, and flavonoids known for their antioxidative properties can also act as prooxidants when supplemented in doses that exceed the physiologically achievable range [37]. The duration of flavonoid treatment influences its biological properties, too: Reviewing results from different studies, it can be concluded that acute and chronic treatment with polyphenols do not produce parallel results and that different compounds belonging to the same subclass can have opposite effects [38]. Finally, the physiological condition of the biological system seems to play an important role, because the actions of polyphenols on intestinal epithelium largely differ between inflamed and non-inflamed cells [39]. Concerning the glucose metabolism, Shao and colleagues have shown that the oral treatment with quercetin impairs glucose tolerance and attenuates insulin-stimulated glucose uptake in healthy mice. In mice with inflammation-induced insulin resistance, however, quercetin reversed glucose intolerance and improved insulin sensitivity, hence beneficially modulated glucose homeostasis [40]. There are several studies demonstrating that although quercetin has ameliorative effects on metabolic conditions in animal models of chronic inflammation, e.g. diabetes type II, it is of little value in healthy

animals or humans [41-43]. A possible explanation for this is that the generation of the biologically active aglycone from its inactive glucuronidated/sulfated derivatives exceedingly happens at sites of inflammation [44]. Hence, it is assumed that the metabolic stress in newborn calves that occurs with the adaptation to extrauterine life is a necessary and physiological process incomparable to deranged metabolic conditions evoking from chronic inflammation; therefore, data obtained in both studies underlying this thesis indicate that the metabolism of newborn calves cannot be altered substantially by quercetin feeding.

In contrast to quercetin treatment, the systemic effects of colostrum deprivation were distinct and consistent throughout the first week of life and agree with the results described in the literature [31]. Whether quercetin might improve the local defense against pathogens in the gastrointestinal tract cannot be concluded from the data gained herein. Although quercetin did not affect the fecal score or general health status of the animals, it must be kept in mind that the calves were probably stressed because of intensive sample taking. Stress is known to accelerate gastrointestinal motility [45] and reduce immune function, which could mask local effects of quercetin. To make a reliable statement on quercetin effects inside the gastrointestinal tract, the characterization of its microbiota under field conditions could expose whether quercetin is able to stabilize the calf's microflora, which has already been demonstrated for flavonoids of other subclasses in calves [46] and for quercetin in laying hens [47]. Furthermore, it would be of value to analyze tissue samples with particular focus on the gut associated lymphoid tissue, and to conduct special tests to evaluate its immune function.

In conclusion, the studies underlying this thesis demonstrated that quercetin feeding does not impair the glucose metabolism of newborn calves, but that it enhances the gastrointestinal absorption of labelled glucose. However, this effect alone seems to be insufficient to improve the overall immune function. Whether adding quercetin to the diet would be more advantageous in weakish calves if at the same time the glucose (as lactose) content of the meal is increased, needs to be probed in further studies.

REFERENCES

1. Girard J. Metabolic adaptations to change of nutrition at birth. Biology of the Neonate. 1990; 58(Suppl. 1):3-15.
2. Blum JW, Hammon H. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Production Science. 2000; 66(2):151-9.
3. Boots AW, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. European Journal of Pharmacology. 2008; 585(2):325-37.
4. Rochfort S, Parker AJ, Dunshea FR. Plant bioactives for ruminant health and productivity. Phytochemistry. 2008; 69(2):299-322.
5. Hanhineva K, Torronen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkanan H, et al. Impact of Dietary Polyphenols on Carbohydrate Metabolism. International Journal of Molecular Sciences. 2010; 11(4):1365-402.
6. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics. 2002; 96(2-3):67-202.
7. Kobori M, Takahashi Y, Akimoto Y, Sakurai M, Matsunaga I, Nishimuro H, et al. Chronic high intake of quercetin reduces oxidative stress and induces expression of the antioxidant enzymes in the liver and visceral adipose tissues in mice. Journal of Functional Foods. 2015; 15:551-60.
8. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends in Plant Science. 1997; 2(4):152-9.
9. Jeong SM, Kang MJ, Choi HN, Kim JH, Kim JI. Quercetin ameliorates hyperglycemia and dyslipidemia and improves antioxidant status in type 2 diabetic db/db mice. Nutrition Research and Practice. 2012; 6(3):201-7.
10. Seiva FR, Chuffa LGA, Braga CP, Amorim JPA, Fernandes AAH. Quercetin ameliorates glucose and lipid metabolism and improves antioxidant status in postnatally monosodium glutamate-induced metabolic alterations. Food and Chemical Toxicology. 2012; 50(10):3556-61.
11. Wang H, Ye J. Regulation of energy balance by inflammation: Common theme in physiology and pathology. Reviews in Endocrine and Metabolic Disorders. 2015; 16(1):47-54.
12. Maciej J, Schäff C, Kanitz E, Tuchscherer A, Bruckmaier R, Wolffram S, et al. Short communication: Effects of oral flavonoid supplementation on the metabolic and antioxidative status of newborn dairy calves. Journal of Dairy Science. 2016; 99(1):805-11.
13. Maciej J, Schäff CT, Kanitz E, Tuchscherer A, Bruckmaier RM, Wolffram S, et al. Bioavailability of the flavonol quercetin in neonatal calves after oral administration of quercetin aglycone or rutin. Journal of Dairy Science. 2015; 98(6): 3906-3917.
14. Boulton DW, Walle UK, Walle T. Extensive binding of the bioflavonoid quercetin to human plasma proteins. Journal of Pharmacy and Pharmacology. 1998; 50(2):243-9.
15. Rohn S, Rawel HM, Kroll J. Antioxidant activity of protein-bound quercetin. Journal of Agricultural and Food Chemistry. 2004; 52(15):4725-9.
16. Kroll J, Rawel HM, Rohn S. Reactions of plant phenolics with food proteins and enzymes under special consideration of covalent bonds. Food Science and Technology Research. 2003; 9(3):205-18.

17. Cermak R, Landgraf S, Wolffram S. The bioavailability of quercetin in pigs depends on the glycoside moiety and on dietary factors. The Journal of Nutrition. 2003; 133(9):2802-7.

18. Manach C, Morand C, Crespy V, Demigné C, Texier O, Régérat F, et al. Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. FEBS Letters. 1998; 426(3):331-6.

19. Berger LM, Wein S, Blank R, Metges CC, Wolffram S. Bioavailability of the flavonol quercetin in cows after intraruminal application of quercetin aglycone and rutin. Journal of Dairy Science. 2012; 95(9):5047-55.

20. Lotito SB, Zhang W-J, Yang CS, Crozier A, Frei B. Metabolic conversion of dietary flavonoids alters their anti-inflammatory and antioxidant properties. Free Radical Biology and Medicine. 2011; 51(2):454-63.

21. Murota K, Terao J. Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. Archives of Biochemistry and Biophysics. 2003; 417(1):12-7.

22. Winterbone MS, Tribolo S, Needs PW, Kroon PA, Hughes DA. Physiologically relevant metabolites of quercetin have no effect on adhesion molecule or chemokine expression in human vascular smooth muscle cells. Atherosclerosis. 2009; 202(2):431-8.

23. Cermak R, Landgraf S, Wolffram S. Quercetin glucosides inhibit glucose uptake into brush-border-membrane vesicles of porcine jejunum. The British Journal of Nutrition. 2004; 91(6):849-55.

24. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. Journal of Nutritional Science and Vitaminology. 2006; 52(2):149-53.

25. Ramachandra R, Shetty AK, Salimath PV. Quercetin alleviates activities of intestinal and renal disaccharidases in streptozotocin-induced diabetic rats. Molecular Nutrition & Food Research. 2005; 49(4):355-60.

26. Crespy V, Morand C, Manach C, Besson C, Demigne C, Remesy C. Part of quercetin absorbed in the small intestine is conjugated and further secreted in the intestinal lumen. American Journal of Physiology. Gastrointestinal and Liver Physiology. 1999; 277(1):G120-G6.

27. Segerstrom SC. Stress, Energy, and Immunity An Ecological View. Current Directions in Psychological Science. 2007; 16(6):326-30.

28. Bieger J, Cermak R, Blank R, de Boer VC, Hollman PC, Kamphues J, et al. Tissue distribution of quercetin in pigs after long-term dietary supplementation. The Journal of Nutrition. 2008; 138(8):1417-20.

29. Gasparin FR, Salgueiro-Pagadigorria CL, Bracht L, Ishii-Iwamoto EL, Bracht A, Constantin J. Action of quercetin on glycogen catabolism in the rat liver. Xenobiotica; the fate of foreign compounds in biological systems. 2003; 33(6):587-602.

30. Gasparin FR, Spitzner FL, Ishii-Iwamoto EL, Bracht A, Constantin J. Actions of quercetin on gluconeogenesis and glycolysis in rat liver. Xenobiotica; the fate of foreign compounds in biological systems. 2003; 33(9):903-11.

31. Hammon HM, Steinhoff-Wagner J, Flor J, Schonhusen U, Metges CC. Lactation Biology Symposium: role of colostrum and colostrum components on glucose metabolism in neonatal calves. Journal of Animal Science. 2013; 91(2):685-95.

32. Steinhoff-Wagner J, Gors S, Junghans P, Bruckmaier RM, Kanitz E, Metges CC, et al. Maturation of endogenous glucose production in preterm and term calves. Journal of Dairy Science. 2011; 94(10):5111-23.

33. Steinhoff-Wagner J, Gors S, Junghans P, Bruckmaier RM, Kanitz E, Metges CC, et al. Intestinal Glucose Absorption but Not Endogenous Glucose Production Differs between Colostrum- and Formula-Fed Neonatal Calves. The Journal of Nutrition. 2011; 141(1):48-55.

34. Boesch-Saadatmandi C, Loboda A, Wagner AE, Stachurska A, Jozkowicz A, Dulak J, et al. Effect of quercetin and its metabolites isorhamnetin and quercetin-3-glucuronide on inflammatory gene expression: role of miR-155. The Journal of Nutritional Biochemistry. 2011; 22(3):293-9.

35. Kawada N, Seki S, Inoue M, Kuroki T. Effect of antioxidants, resveratrol, quercetin, and N-acetylcysteine, on the functions of cultured rat hepatic stellate cells and Kupffer cells. Hepatology. 1998; 27(5):1265-74.

36. Vidyashankar S, Varma RS, Patki PS. Quercetin ameliorate insulin resistance and up-regulates cellular antioxidants during oleic acid induced hepatic steatosis in HepG2 cells. Toxicology in Vitro. 2013; 27(2):945-53.

37. Skibola CF, Smith MT. Potential health impacts of excessive flavonoid intake. Free Radical Biology and Medicine. 2000; 29(3):375-83.

38. Martel F, Monteiro R, Calhau C. Effect of polyphenols on the intestinal and placental transport of some bioactive compounds. Nutrition Research Reviews. 2010; 23(01):47-64.

39. Sergent T, Piront N, Meurice J, Toussaint O, Schneider Y-J. Anti-inflammatory effects of dietary phenolic compounds in an in vitro model of inflamed human intestinal epithelium. Chemico-Biological Interactions. 2010; 188(3):659-67.

40. Shao L, Liu K, Huang F, Guo X, Wang M, Liu B. Opposite effects of quercetin, luteolin, and epigallocatechin gallate on insulin sensitivity under normal and inflammatory conditions in mice. Inflammation. 2013; 36(1):1-14.

41. Egert S, Wolffram S, Bosy-Westphal A, Boesch-Saadatmandi C, Wagner AE, Frank J, et al. Daily quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans. The Journal of Nutrition. 2008; 138(9):1615-21.

42. Kobori M, Masumoto S, Akimoto Y, Takahashi Y. Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. Molecular Nutrition & Food Research. 2009; 53(7):859-68.

43. Vessal M, Hemmati M, Vasei M. Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2003; 135(3):357-64.

44. Shimoi K, Saka N, Nozawa R, Sato M, Amano I, Nakayama T, et al. Deglucuronidation of a flavonoid, luteolin monoglucuronide, during inflammation. Drug Metabolism and Disposition. 2001; 29(12):1521-4.

45. Konturek PC, Brzozowski T, Konturek SJ. Stress and the gut: Pathophysiology, clinical consequences, diagnostic approach and treatment options. Journal of Physiology and Pharmacology. 2011; 62(6):591-9.

46. Ishihara N, Chu DC, Akachi S, Juneja LR. Improvement of intestinal microflora balance and prevention of digestive and respiratory organ diseases in calves by green tea extracts. Livestock Production Science. 2001; 68(2–3):217-29.

47. Liu H, Liu Y, Hu L, Suo Y, Zhang L, Jin F, et al. Effects of dietary supplementation of quercetin on performance, egg quality, cecal microflora populations, and antioxidant status in laying hens. Poultry Science. 2014; 93(2):347-53.

SUMMARY

Effects of the Flavonol Quercetin on Glucose Metabolism and Health Status in Neonatal Calves

With birth, calves are confronted with a new, extrauterine environment and have to meet their energy demand autonomously. For this reason, it is essential that not only overall energy intake, but also endogenous glucose production work effectively, because glucose is one of the main energy sources during the first stage of life. With the intake of first colostrum calves ingest not only nutrients, but also a plethora of bioactive factors which support the maturation and development of metabolic processes. Colostrum is further essential to support the naïve immune system of newborn calves during the first weeks of life and to mediate passive immunity. Unfortunately, colostrum supply is often inadequate. To support the health status, flavonoids could play an important role in the upbringing of newborn calves. These are secondary plant metabolites with numerous attributed health-promoting properties. One of the most important flavonoids is quercetin, which is proven to possess antioxidative, anti-inflammatory and antimicrobial capacity and to modulate the intestinal microflora. However, quercetin also interacts with glucose metabolism by inhibiting intestinal carbohydrate absorption and reducing plasma glucose concentrations. For newborn calves, such an effect would be crucial. Therefore, it was the main objective of the present work to investigate the effects of an oral quercetin supplementation on the glucose metabolism of newborn calves, because this knowledge would be the prerequisite to consider the usage of quercetin as a health promoting feed additive in calf rearing.

In the first part of the study, 28 newborn male Holstein Friesian calves were assigned to two dietary groups and fed colostrum or a milk-based formula with same macronutrient composition, but without bioactive factors, during the first two days of life. On d 2 of life, groups were subdivided into control and treatment groups, the latter receiving quercetin aglycone with meals during the first week of life. On d 3, intestinal xylose absorption was probed, on d 7, a tracer study was conducted to investigate the first pass uptake of glucose, and on d 8, a liver biopsy sample was taken and analyzed via PCR. The postabsorptive recovery rate of orally administered xylose and $^{13}C_6$-labelled glucose in plasma was higher in calves that initially received colostrum, indicating a better intestinal absorption capacity and a lower splanchnic glucose extraction when compared to colostrum-deprived calves. Irrespective of quercetin supplementation, the mRNA abundance of hepatic mitochondrial phosphoenolpyruvate carboxykinase and several plasma metabolites were also reduced in formula-fed calves, pointing to a delayed maturation of metabolic pathways after colostrum deprivation. Although quercetin-fed calves initially showed higher peak concentrations of $^{13}C_6$-glucose in plasma on d 7, this effect did not last for the following hours.

Based on the finding that an oral quercetin supplementation does not seem to disadvantage

the glucose metabolism in newborn calves, the health parameters recorded during the trial were evaluated in the second part of this work. Additionally, plasma samples of the calves were analyzed for concentrations of immunoglobulins, acute-phase proteins, as well as parameters of the antioxidative system. Furthermore, the expression of genes for some proinflammatory cytokines, antioxidative enzymes and acute-phase proteins was analyzed in cDNA samples generated from the liver biopsy tissue. Data indicate that an adequate initial colostrum supply supports neonatal health and prepares the calves very well to cope with the new environment. Colostrum-fed calves showed reduced signs of inflammation and were more vital than calves initially fed formula, further, the metabolic status was improved. In contrast to this, the quercetin supplementation did not induce any detectable health-promoting effects, a finding that underlines that results proven *in vitro* are not always transferable to the organism as a whole.

In summary, it can be concluded from the present work that an oral quercetin supplementation does not impair the glucose metabolism of newborn dairy calves. However, health-promoting properties cannot be reproduced *in vivo*, hence quercetin feeding cannot compensate for an inadequate initial colostrum supply.

ZUSAMMENFASSUNG

Einfluss des Flavonols Quercetin auf Glucosestoffwechsel und Gesundheitsstatus beim neugeborenen Kalb

Mit der Geburt werden Kälber mit einer neuen, extrauterinen Umgebung konfrontiert und müssen ihren Energiebedarf selbstständig decken. Dafür ist es essentiell, dass nicht nur ausreichend Nährstoffe aufgenommen werden, sondern auch die endogene Glucoseproduktion effizient funktioniert, da Glucose in der ersten Lebensphase als eine Hauptenergiequelle dient. Mit der Aufnahme von Kolostrum werden neben Nährstoffen auch zahlreiche bioaktive Substanzen zugeführt, die die Reifung und Entwicklung von Stoffwechselprozessen fördern. Kolostrum ist außerdem essentiell, um das naive Immunsystem des Kalbes während der ersten Lebenswochen zu unterstützen und passive Immunität zu vermitteln. Leider ist die Kolostrumversorgung oft suboptimal. Um die Gesundheit zu fördern, könnte dem Einsatz von Flavonoiden hier eine wichtige Rolle zukommen. Es handelt sich dabei um sekundäre Pflanzenstoffe, denen zahlreiche gesundheitsfördernde Eigenschaften zugeschrieben werden. Einer der Hauptvertreter ist Quercetin, welches nachweislich antioxidativ, antiinflammatorisch und antimikrobiell wirken und die Darmflora modulieren kann. Allerdings interagiert es auch mit dem Glucosestoffwechsel, wodurch die intestinale Aufnahme sowie der Blutglucosespiegel gesenkt werden. Für das Kalb wäre dies fatal. Somit war es das Hauptziel dieser Arbeit, die Wirkung einer oralen Quercetinsupplementierung auf den Glucosestoffwechsel neugeborener Kälber zu untersuchen, da die Kenntnis darüber die Voraussetzung ist, um den Einsatz von Quercetin als gesundheitsfördernden Futterzusatzstoff in der Kälberaufzucht überhaupt in Betracht ziehen zu können.

Zu diesem Zweck wurden 28 neugeborene männliche Kälber der Rasse Deutsche Holstein in zwei Fütterungsgruppen unterteilt und während der ersten beiden Lebenstage entweder mit Kolostrum oder einer Formula mit gleicher Makronährstoffzusammensetzung, jedoch ohne bioaktive Substanzen, gefüttert. Am 2. Lebenstag erfolgte eine Unterteilung in Kontroll- und Behandlungsgruppen, wobei letztere während der ersten Lebenswoche Quercetin mit der Fütterung erhielten. Am 3. Lebenstag wurde eine Xyloseabsorptionsstudie durchgeführt, am 7. Lebenstag erfolgte eine Tracerstudie zum first pass uptake von Glucose, und am 8. Tag wurde Lebergewebe mittels Biopsie entnommen und mit Hilfe von PCR analysiert. Die postabsorptive Wiederfindung von oral verabreichter Xylose und $^{13}C_6$-markierter Glucose im Plasma war höher in initial Kolostrum-gefütterten Kälbern, was im Vergleich zu Formula-gefütterten Tieren auf eine bessere Absorptionskapazität sowie eine geringere Glucoseextraktion im Splanchnikusgewebe schließen lässt. Ferner waren die mRNA Abundanz der hepatischen mitochondrialen Phosphoenolpyruvatcarboxykinase sowie verschiedene Plasmametaboliten in den Formulagruppen unabhängig von der Quercetin-

supplementierung reduziert, was auf eine verzögerte Reifung des Stoffwechsels nach Kolostrumentzug hindeutet. Obwohl die Quercetinsupplementierung am 7. Lebenstag den intestinalen Glucoseabsorptionspeak steigern konnte, verlor sich dieser Effekt im Verlauf der darauffolgenden Stunden.

Basierend darauf, dass eine orale Quercetinsupplementierung den Glucosestoffwechsel bei neugeborenen Kälbern nicht nachteilig beeinflusst, wurden im zweiten Teil der Arbeit die während der Studie aufgenommenen Gesundheitsparameter genauer ausgewertet. Weiterhin wurden Plasmaproben der Tiere auf Konzentrationen von Immunglobulinen, Akute-Phase-Proteinen sowie auf Parameter des antioxidativen Systems untersucht und die Genexpression verschiedener proinflammatorischer Zytokine, antioxidativer Enzyme und akuter-Phase-Proteine in den zuvor angelegten cDNA Umschreibungen analysiert. Die Ergebnisse zeigten, dass die adäquate Versorgung mit Kolostrum während der ersten Lebenstage die Kälber gut für die erste Lebensphase ausstattet und die Kälbergesundheit fördert. Die Tiere zeigten verminderte Anzeichen von Inflammation und waren in den ersten Lebenstagen vitaler als Kälber der Formulagruppe, ferner war der metabolische Status verbessert. Die Quercetinsupplementierung hingegen brachte keine gesundheitsfördernden Effekte, was beweist, dass *in vitro* nachgewiesene Effekte nicht auf den Gesamtorganismus übertragbar sind.

Zusammenfassend lässt sich aus dieser Arbeit ableiten, dass die Fütterung von Quercetin Aglykon beim neugeborenen Kalb zwar keine nachteiligen Effekte auf den Glucose-stoffwechsel hat. Dennoch sind die gesundheitsfördernden Eigenschaften nicht *in vivo* reproduzierbar, sodass der Einsatz von Quercetin als Futterzusatz keinesfalls eine unzureichende Kolostrumversorgung kompensieren kann.

APPENDIX

Table A1: Correlation (Pearson coefficients) between flavonol concentration and selected parameters in plasma of calves fed quercetin twice daily from d 2-8 of life.

		Colostrum (CQ+)		Formula (FQ+)		All calves (Q+)	
Parameter	**Day**	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**
Metabolites							
Glucose	4	0.11	0.83	0.12	0.87	0.44	0.16
	7	0.34	0.54	0.14	0.81	0.25	0.44
	∑ (4,7)	0.33	0.28	0.16	0.64	0.39	0.06
Lactate	4	0.10	0.84	-0.48	0.45	-0.25	0.54
	7	0.06	0.92	-0.36	0.51	0.04	0.90
	∑ (4,7)	0.16	0.62	-0.36	0.29	0.07	0.76
Albumin	4	-0.08	0.87	-0.88	**0.05**	-0.14	0.68
	7	0.39	0.48	-0.58	0.25	0.17	0.60
	∑ (4,7)	0.26	0.39	-0.64	**0.03**	0.23	0.29
Total protein	4	0.69	0.09	-0.63	0.29	0.64	**0.02**
	7	-0.33	0.56	-0.18	0.75	-0.01	0.99
	∑ (4,7)	0.47	0.11	-0.37	0.27	0.45	**0.02**
NEFA	4	-0.30	0.53	-0.49	0.45	-0.22	0.51
	7	-0.46	0.39	-0.24	0.68	-0.29	0.36
	∑ (4,7)	-0.01	0.97	-0.35	0.30	-0.11	0.61
Urea	4	-0.02	0.97	0.98	**<0.01**	0.06	0.85
	7	-0.41	0.45	0.25	0.66	-0.13	0.70
	∑ (4,7)	-0.30	0.32	0.51	0.11	-0.05	0.81
TAG	4	0.36	0.45	-0.61	0.32	0.52	0.08
	7	0.21	0.71	0.38	0.49	0.23	0.48
	∑ (4,7)	0.47	0.11	-0.23	0.50	0.50	**0.01**
Cholesterol	4	0.35	0.46	-0.10	0.89	0.60	**0.04**
	7	0.05	0.93	-0.68	0.16	-0.01	0.98
	∑ (4,7)	0.04	0.91	-0.43	0.19	0.28	0.18
Hormones							
Cortisol	4	-0.59	0.17	0.01	0.99	-0.42	0.18
	7	0.17	0.76	0.23	0.69	0.12	0.72
	∑ (4,7)	-0.26	0.41	0.01	0.98	-0.22	0.30
Insulin	4	0.35	0.46	0.48	0.46	0.37	0.35
	7	0.72	0.12	0.33	0.55	0.56	0.06
	∑ (4,7)	0.34	0.27	0.33	0.34	0.41	**0.05**
Glucagon	4	-0.08	0.88	-0.65	0.28	0.04	0.90
	7	0.23	0.69	-0.32	0.57	0.06	0.85
	∑ (4,7)	0.16	0.60	-0.45	0.17	0.15	0.48

Table A1 Continuation.

		Colostrum (CQ+)		Formula (FQ+)		All calves (Q+)	
Parameter	**Day**	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**
Antioxidative status							
FRAP	4	-0.28	0.57	-0.45	0.50	-0.23	0.49
	7	-0.06	0.92	-0.50	0.58	0.14	0.71
	∑ (4,7)	-0.13	0.68	-0.20	0.63	-0.04	0.88
TEAC	4	0.62	0.15	0.14	0.85	0.74	**<0.01**
	7	-0.15	0.80	-0.40	0.55	0.06	0.86
	∑ (4,7)	0.33	0.27	-0.22	0.56	0.49	**0.02**
TBARS	4	0.09	0.85	-0.70	0.22	0.39	0.21
	7	0.02	0.97	-0.32	0.57	-0.10	0.76
	∑ (4,7)	0.19	0.55	-0.22	0.52	0.25	0.23
Isoprostanes	4	-0.06	0.89	0.55	0.38	0.21	0.52
	7	-0.40	0.46	-0.33	0.56	-0.33	0.30
	∑ (4,7)	-0.15	0.62	-0.06	0.87	-0.04	0.85
Immunoglobulins							
IgG1	4	0.31	0.53	0.66	0.26	0.61	**0.04**
	7	-0.41	0.46	.	.	-0.05	0.89
	∑ (4,7)	-0.04	0.90	0.25	0.47	0.34	0.10
IgG2	4	0.63	0.13	-0.55	0.38	0.68	**0.01**
	7	-0.31	0.58	-0.77	0.08	-0.06	0.85
	∑ (4,7)	0.25	0.41	-0.68	**0.02**	0.38	0.07
IgM	4	0.44	0.34	0.81	0.11	0.68	**0.01**
	7	-0.24	0.67	0.74	0.10	0.05	0.89
	∑ (4,7)	0.31	0.31	0.73	**0.01**	0.50	**0.01**
Acute phase proteins							
SAA	4	0.02	0.98	0.12	0.87	0.03	0.94
	7	-0.04	0.95	0.63	0.20	0.24	0.47
	∑ (4,7)	0.10	0.75	0.42	0.21	0.16	0.47
Haptoglobin	4	.	.	0.79	0.13	-0.17	0.61
	7	.	.	0.70	0.14	0.35	0.47
	∑ (4,7)	.	.	0.43	0.19	-0.01	0.97
Fibrinogen	4	0.81	**0.02**	0.67	0.25	0.56	0.06
	7	-0.17	0.77	-0.05	0.93	-0.14	0.68
	∑ (4,7)	0.48	0.10	0.02	0.95	0.33	0.12

Table A2: Correlation (Pearson coefficients) between flavonol concentration and selected metabolites and hormones on d 7 in plasma in calves fed quercetin twice daily from d 2-8 of life.

		Colostrum (CQ+)		Formula (FQ+)		All calves (Q+)	
Parameter	**Time**	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**
Metabolites							
Glucose	0 h	0.34	0.54	0.14	0.81	0.25	0.44
	1 h	-0.37	**0.50**	0.72	0.11	0.06	0.85
	2 h	-0.68	0.24	-0.80	0.06	-0.42	0.21
	3 h	-0.15	0.79	-0.25	0.66	-0.11	0.74
	4 h	-0.94	**0.01**	-0.39	0.47	-0.71	**0.01**
	5 h	-0.31	0.65	-0.09	0.88	-0.53	0.09
	6 h	0.73	0.19	0.53	0.30	-0.14	0.68
	8 h	-0.01	0.98	0.35	0.53	-0.09	0.79
	10 h	0.90	**0.04**	0.56	0.27	-0.02	0.95
	All	-0.04	0.78	0.08	0.58	-0.07	0.46
Lactate	0 h	0.06	0.92	-0.36	0.51	0.04	0.90
	1 h	0.25	0.66	0.69	0.14	0.46	0.13
	2 h	-0.63	0.30	-0.68	0.16	-0.41	0.21
	3 h	0.33	0.55	-0.9	**0.01**	0.23	0.49
	4 h	0.30	0.66	-0.66	0.17	-0.15	0.67
	5 h	0.57	0.52	-0.34	0.54	-0.11	0.78
	6 h	-0.62	0.47	-0.37	0.50	-0.30	0.42
	8 h	-0.08	0.89	-0.33	0.56	-0.10	0.76
	10 h	0.94	**0.01**	-0.43	0.43	-0.01	0.98
	All	0.07	0.63	-0.13	0.34	-0.04	0.67
Urea	0 h	-0.41	0.45	0.25	0.66	-0.13	0.70
	1 h	-0.66	0.17	-0.14	0.81	-0.19	0.56
	2 h	-0.78	0.13	0.34	0.54	-0.07	0.85
	3 h	-0.69	0.14	0.84	**0.04**	-0.11	0.75
	4 h	-0.59	0.33	0.33	0.55	-0.43	0.19
	5 h	-0.59	0.34	0.15	0.79	-0.49	0.13
	6 h	0.51	0.43	0.35	0.52	-0.28	0.41
	8 h	-0.39	0.47	-0.44	0.41	-0.42	0.19
	10 h	0.50	0.43	0.19	0.74	-0.31	0.37
	All	-0.39	**0.01**	0.29	**0.03**	-0.20	**0.04**

Table A2 Continuation.

		Colostrum (CQ+)		Formula (FQ+)		All calves (Q+)	
Parameter	**Time**	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**
NEFA	0 h	-0.46	0.39	-0.24	0.68	-0.29	0.36
	1 h	-0.39	0.48	-0.73	0.11	-0.53	0.08
	2 h	0.21	0.77	0.71	0.13	0.47	0.15
	3 h	0.64	0.19	0.16	0.78	0.43	0.17
	4 h	0.30	0.66	0.14	0.81	0.17	0.63
	5 h	0.80	0.12	0.40	0.46	0.47	0.15
	6 h	0.45	0.49	0.14	0.81	0.11	0.76
	8 h	0.38	0.49	0.35	0.52	0.29	0.38
	10 h	-0.21	0.76	-0.68	0.15	-0.55	0.08
	All	-0.07	0.63	-0.28	**0.04**	-0.17	0.08
Hormones							
Insulin	0 h	0.72	0.12	0.33	0.55	0.56	0.06
	1 h	-0.29	0.61	0.77	0.08	0.03	0.92
	2 h	-0.18	0.79	0.34	0.54	0.04	0.92
	3 h	0.46	0.39	0.76	0.08	0.53	0.08
	4 h	<0.01	1.00	0.05	0.93	0.15	0.67
	5 h	-0.43	0.51	-0.45	0.41	-0.56	0.08
	6 h	0.11	0.88	0.74	0.10	-0.13	0.72
	8 h	-0.18	0.75	-0.21	0.72	-0.25	0.44
	10 h	0.84	0.09	0.18	0.76	0.18	0.60
	All	0.10	0.50	0.13	0.34	0.07	0.51
Glucagon	0 h	0.23	0.69	-0.32	0.57	0.06	0.85
	1 h	0.29	0.61	0.25	0.66	0.38	0.23
	2 h	-0.92	**0.02**	-0.15	0.79	-0.17	0.63
	3 h	0.89	**0.02**	-0.27	0.63	0.31	0.33
	4 h	-0.44	0.51	-0.16	0.78	-0.57	0.07
	5 h	-0.32	0.64	0.26	0.64	-0.40	0.23
	6 h	0.68	0.24	0.05	0.93	-0.41	0.21
	8 h	-0.64	0.18	0.59	0.25	-0.23	0.49
	10 h	0.11	0.88	0.84	**0.04**	-0.37	0.28
	All	0.05	0.71	0.07	0.63	-0.10	0.32

Table A2 Continuation.

		Colostrum (CQ+)		Formula (FQ+)		All calves (Q+)	
Parameter	**Time**	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**
Noradrenaline	0 h	-0.01	0.99	-0.12	0.83	<0.01	0.99
	1 h	-0.10	0.87	-0.63	0.19	0.08	0.81
	2 h	-0.21	0.77	-0.77	0.08	-0.20	0.57
	3 h	-0.17	0.76	-0.02	0.97	<0.01	0.99
	4 h	0.88	0.05	-0.12	0.84	0.13	0.71
	5 h	0.28	0.68	-0.32	0.56	-0.27	0.43
	6 h	0.44	0.51	-0.23	0.68	-0.10	0.78
	8 h	-0.12	0.83	-0.77	0.08	-0.25	0.44
	10 h	0.85	0.08	-0.39	0.47	0.04	0.91
	All	-0.11	0.47	-0.23	0.09	-0.19	**0.05**
Adrenaline	0 h	-0.64	0.19	-0.23	0.69	-0.30	0.35
	1 h	-0.25	0.65	-0.58	0.25	-0.15	0.65
	2 h	-0.03	0.97	-0.56	0.28	-0.26	0.45
	3 h	-0.63	0.20	-0.09	0.88	-0.31	0.33
	4 h	0.56	0.37	-0.26	0.65	-0.17	0.62
	5 h	0.66	0.26	-0.86	**0.02**	-0.50	0.12
	6 h	0.50	0.44	-0.47	0.37	-0.14	0.69
	8 h	-0.34	0.55	-0.68	0.15	-0.34	0.30
	10 h	0.42	0.53	-0.57	0.26	-0.29	0.40
	All	-0.28	0.05	-0.21	0.13	-0.26	**0.01**

Table A3: Correlations (Pearson correlation coefficients) between plasma flavonol concentrations (2 h after feeding on d 7) and mRNA abundances of hepatic genes involved in antioxidative status and inflammation (2 h after feeding on d 8) in calves orally supplemented with quercetin aglycone (50 mg/(kg BW × d)) for 7 days.

	Colostrum (CQ+)		Formula (FQ+)		All calves (Q+)	
Gene	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**	**Pearson r**	***P*-value**
Cytokines						
TNF	-0.50	0.44	-0.15	0.80	-0.22	0.52
IL1A	-0.35	0.60	-0.13	0.82	-0.18	0.60
IL1B	0.94	**0.02**	0.82	**0.05**	0.82	**<0.01**
Antioxidative status						
CAT	0.28	0.68	-0.59	0.24	-0.20	0.58
GPX	0.63	0.30	0.29	0.61	0.36	0.29
SOD	0.41	0.53	-0.14	0.81	0.03	0.93
Acute phase proteins						
SAA2	0.35	0.61	0.85	**0.03**	0.67	**0.03**
HP	0.16	0.82	0.86	**0.03**	0.68	**0.02**
CRP	0.87	0.06	-0.46	0.39	-0.04	0.91
FGA	0.39	0.57	0.56	0.28	0.45	0.18

CAT, catalase; *GPX1*, glutathione peroxidase; *SOD*, superoxide dismutase; *TNF*, tumor necrosis factor; *IL1A*, interleukin-1α; *IL1B*, interleukin-1β; *HP*, haptoglobin; *FGA*, fibrinogen; *SAA2*, serum amyloid A2; *CRP*, C-reactive protein.

PUBLICATION LIST

Publications (peer reviewed)

Gruse J, Kanitz E, Weitzel JM, Tuchscherer A, Stefaniak T, Jawor P, et al. **Quercetin Feeding in Newborn Dairy Calves Cannot Compensate Colostrum Deprivation: Study on Metabolic, Antioxidative and Inflammatory Traits.** Plos One. 2016; 11(1).

Gruse J, Görs S, Tuchscherer A, Otten W, Weitzel JM, Metges CC, et al. **The Effects of Oral Quercetin Supplementation on Splanchnic Glucose Metabolism in 1-Week-Old Calves Depend on Diet after Birth.** J Nutr. 2015; 145(11):2486-95.

Abstracts in proceedings and participation in conferences

Gruse J, Kanitz E, Tuchscherer A, Stefaniak T, Wolffram S, Hammon HM. **Effects of quercetin feeding on metabolic and inflammatory parameters in neonatal calves according to colostrum supply**. Tagung der DVG-Fachgruppe ,,Physiologie und Biochemie", 2016, Berlin, Germany. Sektion Immunologie, p.90-91.

Hammon HM, Gruse J, Mielenz M, Wolffram S. **Anti-oxidative status and inflammatory response in neonatal calves fed quercetin with or without colostrum.** ADSA-ASAS Joint Annual Meeting, 2015, Orlando, Florida; Section Growth and Development I, p.392.

Gruse J, Görs S, Otten W, Weitzel JM, Wolffram S, Metges CC, Hammon HM. **Effects of oral quercetin supplementation on glucose metabolism in newborn dairy calves fed colostrum or a milk-based formula.** 15th Day of the Doctoral Student, 2015, Dummerstorf, Germany; Schriftenreihe / Leibniz-Institut für Nutztierbiologie, 23:5-8.

Gruse J, Mielenz M, Wolffram S, Hammon HM. **Effects of different milk diets and quercetin feeding on parameters concerning antioxidative and health status in neonatal calves**.; 69th Conference of the Society of Nutrition Physiology, 2015, Göttingen, Germany; Section Feed additives, 24:94.

Gruse J, Görs S, , Otten W, Weitzel JM, Wolffram S, Metges CC, Hammon HM. **Milk diet but not quercetin intake affects postprandial glucose metabolism in neonatal calves.** ADSA-ASAS-CSAS Joint Annual Meeting, 2014, Kansas City, Kansas; Section Growth and Development I, p.580.

Gruse J, Görs S, Otten W, Weitzel JM, Wolffram S, Metges CC, Hammon HM, 2014. **Effects of different milk diets and oral quercetin supplementation on postprandial glucose metabolism in neonatal calves.** 68th Conference of the Society of Nutrition Physiology, 2014, Göttingen, Germany; Section Feed additives, 23:114.

DANKSAGUNG

An dieser Stelle möchte ich mich bei all denen bedanken, die an der Entstehung dieser Arbeit Anteil hatten. Insbesondere gilt mein Dank

PD Dr. Harald M. Hammon sowohl für die thematische Ausrichtung und wissenschaftliche Betreuung als auch für die Möglichkeit, meine eigenen Ideen und Vorschläge in diese Arbeit einbringen zu können. Ebenfalls möchte ich mich bei **Univ. Prof. Dr. Jörg R. Aschenbach** bedanken, der die universitäre Betreuung und Begutachtung meiner Arbeit bereitwillig übernommen hat und mir bei Fragen und Problemen stets beratend zur Seite stand. **Prof. Dr. Siegfried Wolffram** danke ich für die Projektidee und -leitung sowie konstruktive Kommentierung der verschiedenen Publikationen. Außerdem bedanke ich mich beim **Bundesministerium für Bildung und Forschung** für die finanzielle Unterstützung des Projektes und beim **Leibniz-Institut für Nutztierbiologie** (FBN) für die Bereitstellung der Ressourcen.

Den **Mitarbeitern der Tierexperimentellen Anlage Rind** (EAR) des FBN Dummerstorf unter der Leitung von Dr. Bernd Stabenow danke ich für die Unterstützung bei der Aufstallung und Versorgung der Tiere. Besonderer Dank gilt dem Herdenmanager **Klaus Dieter Witt**, der mit seinem Organisations- und Improvisationstalent beim Stallumbau all meine kleinen und großen Wünsche umgesetzt hat.

Bei der **Milchhof Scharstorf KG** sowie der **Gut Dummerstorf GmbH** bedanke ich mich für die Bereitstellung der Versuchskälber; dem Milchhof Scharstorf danke ich darüber hinaus auch für das aufwendige Sammeln und anschließende Überlassen des Kolostrums, welches ein integraler Bestandteil dieser Arbeit war.

Ein riesiges Dankeschön geht auch an die großartigen **Handwerker des FBN** für ihr tatkräftiges Zupacken beim Transport der Kälber sowie ihre vielen innovativen Ideen und deren Umsetzung während der tierexperimentellen Phase meiner Arbeit.

Besonderer Dank gilt meiner Vorreiterin Josi, mittlerweile Frau **Dr. Josefine Maciej**, die mich herzlichst aufgenommen und eingearbeitet hat, mir ihre Tipps und Tricks weitergegeben hat und mir vor allem in der Anfangsphase der Arbeit stets mit Rat und Tat zur Seite stand. Auch **Christa Fiedler** danke ich zutiefst für ihre aufopfernde Hilfe im Versuch, zu jeder Tages- und Nachtzeit, dafür, dass sie die Kälber immer zuerst als lebende Wesen und nie als reine Untersuchungsobjekte behandelt hat und mich bei allen Entscheidungen widerspruchslos unterstützt hat.

Dem Institutstierarzt **Dr. Olaf Bellmann** danke ich für die Zusammenarbeit und Beratung bei der tierärztlichen Versorgung der Tiere und für die Einarbeitung in die Methodik der Leberbiopsie.

Für die hervorragende Laborarbeit möchte ich mich ebenfalls bedanken. Bei **Ute Lüdtke** und **Kirsten Karpati** für die Analysen der Tracerstudie sowie **Dr. Solvig Görs** für die

mathematische Aufbereitung der Ergebnisse; **Claudia Reiko** für die PCR-Analyse der Leberproben sowie **PD Dr. Manfred Mielenz** für die Etablierung und Einarbeitung in die entsprechende Software; **Ulrike Wiedemuth**, **Dagmar Mähling** und **Petra Müntzel** für die Hormonanalysen; **Petra Schulz** und **Maike Gosse** für die Analysen von Plasmaflavonolkonzentrationen und Parametern des antioxidativen Status; und schließlich **Paulina Jawor** für die Messung von Immunglobulinen und Akute-Phase-Proteinen im Plasma.

Weiterhin danke ich meiner Weggefährtin **Ann-Kathrin Stoldt**, die mit ihrem unerschütterlichen Optimismus fast drei Jahre lang nicht nur das Büro, sondern auch Freud und Leid mit mir geteilt hat, während sie parallel ihre Kühe „quercetint“ hat; außerdem **Dr. Christine Schäff** für das Korrekturlesen, Ratschläge und die Offenbarung ihrer gesammelten PC-Geheimnisse. Beiden danke ich auch für ihre Freundschaft, offene Ohren und ihr bereitwilliges Ertragen gelegentlicher Jammertiraden, wenn ich das Gefühl hatte, nicht weiter zu kommen.

Dr. Armin Tuchscherer danke ich dafür, dass er mir mit seiner ruhigen und freundlichen Art und immerwährender Geduld die Statistik nahegebracht hat. Ferner danke ich **Lutz Hasselmann** und seinen Kollegen der IT-Abteilung des Instituts für die Bereitstellung der Software und die Schlichtung sämtlicher Uneinigkeiten zwischen mir und meinem Computer.

Mein größter Dank aber gilt **meinen Lieben**. Für eure Unterstützung, euren unbeirrbaren Glauben an mich und mein Tun, eure Wärme und dafür, dass ihr mir immer ein Zuhause seid – von ganzem Herzen danke.

Kweli, kweli hallelujah!

DECLARATION

med. vet. Jeannine Gruse

Statutory Declaration:

Herewith I declare on oath that the submitted dissertation under the title
“Effects of the Flavonol Quercetin on Glucose Metabolism and Health Status in Neonatal Calves”

has been authored independently and without illegitimate external help and that it has not been formerly submitted to another university department.

Jeannine Gruse
Berlin, 24th October 2016

Herewith I declare that I am not subject to any pending case of public prosecution.

Jeannine Gruse
Berlin, 24th October 2016