8TH INTERNATIONAL CONGRESS ON FARM ANIMAL ENDOCRINOLOGY 2015

27-29 AUGUST, HOTEL LEGOLAND BILLUND, DENMARK

MOGENS VESTERGAARD, RUPERT BRUCKMAIER, IAIN CLARKE, THEODORE ELSASSER, AKIO MIYAMOTO, JAMES L. SARTIN AND HELGA SAUERWEIN

DCA REPORT NO. 064 · AUGUST 2015

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Scientific report
The reports contain mainly the final reportings of research projects, scientific reviews, knowledge syntheses, commissioned work for authorities, technical assessments, guidelines, etc.

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Welcome and Acknowledgements

On behalf of the organizing committee, I welcome you to the 8th International Conference on Farm Animal Endocrinology (IcFAE). This conference has been running regularly every three to four years since it's re-vitalization in 1998 (3rd), which could largely be acknowledged to Robert Renaville. So, with meetings in 1998 (Bruxelles), 2001 (Salsomaggiore-Parma), 2004 (Budapest), 2008 (Roanoke-Blacksburg) and 2011 (Bern), it was decided to have the 8th IcFAE meeting in Denmark in 2015 and to have the meeting immediately before the annual EAAP meeting in Warsaw. The small-town venue Billund was chosen because it had easy access to an international airport and had a combined hotel-conference center that could house all participants and keep people together in a more intimate atmosphere allowing for good scientific discussions. Being close to LegoLand amusement park would allow participants to visit this nice garden Wednesday night or after the meeting. We hope you will have a nice stay, enjoy good food, make new friends, and have a memorable experience.

The scientific program has been put together by the help of the organizers and we are indebted to the 25 invited speakers who have agreed to contribute with talks of high international standard. The invited contributions will be published in Domestic Animal Endocrinology. Besides the invited talks, we have received 38 abstracts to be presented as posters. Thus, despite a slightly lower number of participants compared with the previous meeting, I am sure you will get a fantastic outcome of the two-days meeting.

I should like also to acknowledge our sponsors, both private companies and universities/organizations, who made it possible to sponsor most of the costs for the invited speakers, publications, poster session etc. Thank you.

Finally, I should like to specifically acknowledge our secretary Mette Graves Madsen, who has been responsible for all practicalities including the website, the registrations, the correspondence with authors, participants, sponsors etc., the technical handling of abstracts/the proceedings and much more. Thank you, Mette. It has been great working with you.

After this meeting, I will step down as chair of the organizing committee. The organizing committee suggests that at least 4 of the current 7 members are being replaced. Some of us have been around for decades. Please contact me during the meeting, if you are interested in joining the organizing committee of the 9th IcFAE.

I wish you all a fruitful meeting.

Mogens Vestergaard
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PROGRAM

8th International congress on Farm Animal Endocrinology August 27-29 2015, Hotel LEGOLAND, Billund/Denmark

Thursday August 27

Session 1: ENDOCRINE CONTROL OF METABOLISM
Chair: Helga Sauerwein

8.00-8.10	**Welcome and Acknowledgements** Mogens Vestergaard, Aarhus University
8.10-8.45	**Exogenous and endogenous factors influencing lipid and energy metabolism in dairy cows** Björn Kuhla, FBN Dummerstorf, Germany
8.45-9.20	**Regulation of nutrient metabolism and immune function in chicken** Mark E Cook, University of Wisconsin, USA
9.20-9.55	**Regulation of skeletal muscle protein synthesis and protein breakdown** Tracy G. Anthony, Rutgers University, NJ, USA
9.55-10.20	Coffee (Conference center)
10.20-10.55	**"Metabolic syndrome" in mammals and birds?** Korinna Huber, University of Veterinary Medicine Hannover, Foundation, Germany
10.55-11.30	**A survey of the endogenous and exogenous factors influencing the concentrations of adiponectin in body fluids and tissues through all phases of bovine life** Helga Sauerwein, Uni Bonn, Germany
11.30-11.40	Discussion session 1

Session 2: THE GUT: NEW CRITICAL CONTROL POINTS FOR ENDOCRINE-IMMUNE-METABOLIC TARGETING

Chair: Ted Elsasser

11.40-12.15 **The Gut Microbiome as a Virtual Endocrine Organ: Implications for Physiology, Brain and Behaviour**
Gerard Clarke, Biosciences Institute, University College Cork, Ireland

12.15-13.00 Lunch (Hotel restaurant)

13.00-13.35 **Glucagon-like peptide 2 and its beneficial effects on gut function and health in production animals**
Erin E Connor, USDA, Beltsville Agricultural Research Center, USA

13.35-14.10 **Adrenomedullin regulates microbiota and intestinal pathophysiology**
Alfredo Martinez, Center for Biomedical Research of La Rioja (CIBIR), Spain

14.10-14.45 **Natural bioactives to maintain gut homeostasis**
Torres Sweeney, University College Dublin, Ireland

14.45-15.10 Coffee (Conference center)

15.10-15.45 **Dietary influence on gut maturation in early postnatal life in pigs**
Thomas Thymann, University of Copenhagen, Denmark

15.45-16.00 Discussion – Session 2

Session 3: ANIMAL HEALTH AND STRESS. CONSEQUENCES AND STRATEGIES

Chair: Jim Sartin

16.00-16.35 **Adrenergic and noradrenergic regulation of poultry behavior and production**
Rachael Dennis, University of Maryland, USA

16.35-17.10 **A comparison of the equine and bovine pituitary-adrenocortical axis**
Han van der Kolk, Swiss Institute for Equine Medicine, University of Bern and Agroscope, Switzerland

17.10-17.45 **Behavioral and metabolic differences in ewes characterized as high or low cortisol responders**
Belinda Henry, Monash University, Australia

17.45-19.15 **Poster session** (Conference center)

20.00 Conference Dinner (Hotel restaurant)

Friday 28 August

Session 3 continued

8.00-8.35 **Glucocorticoid programming of intrauterine development**
Abigail Fowden, Cambridge University, UK

8.35-9.10 **The role of mitochondrial DNA copy number, variants and haplotypes in farm animal developmental outcome**
Justin St. John. Hudson Institute of Medical Research, Australia

9.10-9.20 Discussion Session 3

9.20-9.40 Coffee (Conference center)

Session 4: ENDOCRINE CONTROL OF LACTATION

Chair: Rupert Bruckmaier

9.40-10.15 **Serotonin regulates calcium transport during lactation**
Laura Hernandez, University of Wisconsin, USA

10.15-10.50 **Effect of trans-10, cis-12 CLA on mammary Lipid Synthesis**
Kevin Harvatine, Cornell Univ, NY, USA

10.50-11.05 **Supplementation of conjugated linoleic acid during the transition to lactation period improves milk production and reproductive efficiency**
Tawny Chandler et al. presented by Heather White, Univ of Wisconsin-Madison

11.05-11.40 **Altering prolactin concentrations in sows**
Chantal Farmer, AgCanada, Lennoxville, Canada

11.40-12.15 **Quantification of nutrients absorbed, metabolized in the liver, and secreted into milk of lactating sows**
Peter Theil, Aarhus Univ, Denmark

12.15-12.25 Discussion session 4

12.25-13.10 Lunch (Hotel restaurant)

Session 5: REPRODUCTION AND HEALTH

Chair: Akio Miyamoto

13.10-13.45 **New Concepts of the Central Control of Reproduction, integrating influence of Stress, Metabolic State and Season**
Iain Clarke, Monash University, Melbourne, Australia

13.45-14.20 **Impact of nutritional programming on growth, health and sexual development of bull calves**
Heinrich Bollwein, University of Zurich, Switzerland

14.20-14.55	**Intra-pituitary mechanisms underlying the control of fertility: key players in seasonal breeding** Domingo Tortonese, University of Bristol, UK
14.55-15.15	Coffee (Conference center)
15.15-15.50	**Local immune system in oviduct physiology and pathophysiology: Attack or tolerance?** Mohamed Marey, Damanhour University, Egypt
15.50-16.25	**Early pregnancy in the mare: old concepts re-visited** Claudia Klein, University of Calgary, Canada
16.25-17.00	**The deleterious effects and the interaction of mastitis and heat stress on ovarian function in lactating cow: basic and applied aspects** Zvi Roth, The Hebrew University, Israel
17.00-17.10	Discussion session 5
17.10-17.30	Closing and New Organizing Committee
20.00	Dinner (Hotel restaurant)

Abstracts

Oral presentations

Endogenous and dietary lipids influencing energy metabolism and feed intake of dairy cows

Kuhla, B., Metges C.C., Hammon, H.M.
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The high metabolic priority of the mammary gland for milk production accompanied by limited feed intake around parturition results in a high propensity to mobilize body fat reserves. Under these conditions, fuel selection of many peripheral organs switches e.g. from carbohydrate to fat utilization to ensure partitioning of tissue- and dietary-derived nutrients toward the mammary gland. For example, muscle tissue releases non-esterified fatty acids (NEFA), lactate and amino acids in a coordinated order thereby providing precursors for milk synthesis or hepatic gluconeogenesis (Kuhla et al., 2011, J Proteome Res.10:4252-62). Additionally, muscle fatty acid oxidation is highly activated around calving but declines within 4 weeks after calving, thus saving NEFA for milk fat production (Schäff et al., 2013, J Dairy Sci. 96:6449-60). Tissue metabolism and in concert nutrient partitioning are controlled by the endocrine system involving a reduction in insulin secretion and systemic insulin sensitivity (Kautzsch et al., 2012, J Dairy Sci 95 (Suppl 2): 78) and orchestrated changes in plasma insulin, IGF-I, growth hormone, glucagon, leptin, glucocorticoids catecholamines etc. However, the endocrine system is highly sensitive and responsive to an overload of fatty acids, no matter if excessive NEFA load originates from exogenous or endogenous sources. Feeding a diet containing rumen-protected fat from late lactation until calving (van Knegsel et al., 2007, J Dairy Sci. 90:3397-409; Duske et al., 2009, J Dairy Sci. 92:1670-84) and a high extent of body fat mobilization around parturition alike exert negative effects on energy intake, glucose and insulin concentrations, but support the development of ketosis and fatty liver (Hammon et al., J Dairy Sci. 2009, 92:1554-66). High plasma NEFA concentrations are thought not to act at the brain level (Laeger et al., 2013, J Dairy Sci. 96:2883-93), but increase the energy charge of the liver which is signaled to the brain to diminish feed intake (Schäff et al., 2012, J Proteome Res.11:5503-14). Cows with a different extent of fat mobilization during the transition phase differ in their hepatic energy charge, whole body fat oxidation, glucose metabolism, plasma ghrelin concentrations and feed intake already several weeks before parturition (Börner et al., 2013, J Endocrinol.216:217-29; Schäff et al., 2012, J Proteome Res.11:5503-14; Weber et al., 2013, J Dairy Sci.96:165-80). Hence, a high lipid load, no matter if stored, mobilized or fed, affects the endocrine, metabolism and feed intake, and increases the risk for metabolic disorders.

Keywords: dairy cow, fat feeding, fat mobilization

Regulation of nutrient metabolism and immune function in chickens

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Balancing nutrient partitioning between immune priorities and growth is the basis for key management decisions in farm animals production systems. Studies involving germ free chickens and pigs show growth improvement of 12% or greater than those mono-associated with a bacterial species or housed conventionally. Additional losses resulting from vaccination or limited sanitation, both directly linked to immune activation, result in animal performance at less than 75% of the genetic potential. Antibiotic use has demonstrated the value in limiting immune activation for improving efficiency in animal production systems and has served as a basic strategy for immune manipulation. Recently, discovery of immune mechanisms for recruiting nutrients away from growth into defense have provided novel tools to monitor and manage immune regulation of nutrient metabolism. For example, immune-induced muscle catabolism and amino acid release for fuel and synthesis of acute phase proteins can be real-time measured in animal breath by quantifying carbon fractionation. Released tumor necrosis factor and interleukin-1 following an acute inflammatory response induces skeletal muscle catabolism through an eicosanoid signal transduction pathway. Systemically released amino acids can be used for synthesis of acute phase proteins or as fuel for the ensuing defense. Enzymatic processes during the metabolism of amino acids to CO_2 discriminate against naturally abundant ^{13}carbon metabolites through the kinetic isotope effect, rendering breath enriched in $^{12}CO_2$. Also, arachidonate derived eicosanoid-signaling events that drive early catabolic and inflammatory cytokine pathways can be suppressed using linoleic acid derivatives such as conjugated linoleic acid. Conjugated linoleic acid has been shown to prevent immune-induced weight loss without an adverse affect on the adaptive immune system. Regulation of immune peptides released into the gastrointestinal lumen has also been shown to stimulate animal growth. For example, inhibiting secretory phospholipase A_2B1 function in the intestinal lumen has been shown to help maintaining gut barrier function and prevent the feed forward loop attributed to antigen translocation across the intestinal mucosa. Cost effective systems, which produce sIgA as blocking antibodies against antigen translocation, have been shown to markedly improve feed efficiency and growth of broilers. Recently, a feed additive that prevents pathogen mediated up-regulation of interleukin-10 can promote a swift adaptive immune defense against selected pathogens and eliminate the needs for anticoccidial antibiotics and chemicals in broiler production. Basic science continues to point toward new directions and new tools to improve the health and production efficiency of the domestic fowl.

Keywords: immunity, cytokines, nutrient partitioning, growth

Regulation of skeletal muscle protein synthesis and protein breakdown

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Anabolic growth is achieved when rates of new synthesis exceed turnover, producing a positive net protein balance. Conversely, deterioration or atrophy of lean mass is a consequence of a net negative protein balance. The role of nutrition and hormones in regulating these processes has received great attention over the past 20 years. These investigations have created a larger understanding of the signal transduction events instigated by meal feeding and the role of specific amino acids in guiding postprandial anabolism and overall proteostasis. Intracellular signaling via the mammalian target of rapamycin complexes and the amino acid stress response or unfolded protein response work together to respond and adapt to environmental changes in energy and nutrient supply. More recent research efforts have studied the impact of dose and timing of amino acid intake on muscle net balance, particularly as it relates to physical activity and growth throughout the lifespan. Finally, the influence of overweight and metabolic disease on protein synthesis and turnover in domestic farm animals can affect meat quality and milk production. An examination of these issues as well as an overview of methods to assess proteostasis will be discussed.

Keywords: mRNA translation, protein metabolism

„Metabolic Syndrome“ in mammals and birds?

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According to the International Diabetes Federation the metabolic syndrome (MS) in humans clusters the most dangerous risk factors for heart attacks: diabetes and prediabetes, abdominal obesity, high cholesterol and high blood pressure. Central obesity and insulin resistance are accepted causative factors in the pathogenesis of human MS (hMS). In cows, horses and broiler chicken, MS is also described; however it is questionable whether these “metabolic syndromes” match the pathophysiology of hMS. The aim of this paper is to compare “metabolic syndromes” of horses, cows and chicken to identify similarities and disparities of metabolic (dys) regulation with a focus on obesity, insulin resistance and inflammation. The coincidence of these metabolic conditions is imperative; however, the causal relationship is unclear. In humans two concepts are discussed for the development of obesity as initial process in MS; the *concept of energy imbalance* with high energy intake and low energy expenditure and the *concept of metabolic inflexibility* with inadequate utilization of energy in oxidative pathways, both leading to obesity. Obesity is causative for insulin resistance by lipotoxicity in tissues, resulting in defects of the insulin signaling pathway. Compensatory insulin secretion is enhanced, and hyperinsulinemia leads to a further down-regulation of insulin signaling in tissues. The reduced inhibition of hepatic gluconeogenesis increases plasma glucose, thereby provoking additive pancreatic hypersecretion, and later exhaustion. Massive lipolysis due to the lack of energy in insulin resistant cells leads to hyperlipidemia, ketosis and ectopic fat deposition. Reduced mitochondrial function and impaired oxidative pathways are linked to proinflammatory pathways which generate a chronic low-grade inflammation.

MS in horses is commonly observed in native breeds which are obese due to overfeeding and low exercise. Special adipose tissue depots are predominant, i.e. at the neck region and the tailhead. Hyperinsulinemia in response to postprandial glucose and inflammation is observed. MS in dairy cows is observed in ante partum overconditioned cows which are physiologically insulin insensitive postpartum due to nutrient partitioning at the onset of lactation. Plasma insulin and glucose concentrations are low, but hyperlipidemia, hepatic lipid deposition and inflammation are prevailing. MS in the modern broiler-type chicken - genetically selected for growth and coincidentally, for high food intake - is associated with obesity and inflammation. Due to these metabolic dysregulations, species-specific MS-associated pathologies develop which cause severe health problems in humans, mammals and birds.

Key words: metabolic syndrome, obesity, insulin resistance

A survey of the endogenous and exogenous factors influencing the concentrations of adiponectin in body fluids and tissues through all phases of bovine life

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Adiponectin, one of the messenger molecules secreted from adipose tissue that are collectively termed adipokines, has been demonstrated to play a central role in lipid and glucose metabolism in humans and laboratory rodents; it improves insulin sensitivity and exerts anti-diabetic, anti-atherosclerotic and anti-inflammatory actions. Adiponectin is synthesized as a 28 kDa monomer but is not secreted as such; instead it is glycosylated and undergoes multimerization to form different molecular weight (MW) multimers prior to secretion (Kadowaki et al. 2006; J Clin Invest 116:1784-92). Adiponectin is one of the most abundant adipokines that is present in the µg/mL range in the circulation. The concentrations are negatively correlated with adipose depot size, in particular with visceral fat mass in humans (Cnop et al. 2003; Diabetologia 46:459-69). Adiponectin exerts its effects by activating a range of different signaling molecules via binding to two transmembrane receptors, AdipoR1 and AdipoR2. AdipoR1 is expressed primarily in the skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver (Yamauchi et al. 2003; Nature 423:762-9). Many of the functions of adiponectin are relevant for growth, lactation and health and are thus of interest in both beef and dairy production. Studies on the role of the adiponectin protein in cattle have been impeded by the lack of reliable assays for bovine adiponectin. While there are species specific bovine adiponectin assays commercially available, they suffer from a lack of scientific peer-review of validity. Quantitative data about the adiponectin protein in cattle available in the literature emerged only during the last three years and were largely based on Western blotting (WB) using either antibodies against human adiponectin or partial peptides from the bovine sequence. Using native bovine high MW adiponectin purified from serum, we were able to generate a polyclonal antiserum that can be used for WB but also in an ELISA system recently validated (Mielenz et al. 2013; Domest Anim Endocrinol 44:121-30). The review presented will focus on the available literature about the adiponectin protein in cattle and will summarize the following aspects: (a) the course of the adiponectin serum concentrations during development in both sexes, during inflammation (mastitis), nutritional energy deficit and energy surplus, and lactation-induced changes including the response to supplementation with conjugated linoleic acids, (b) adiponectin concentrations in subcutaneous versus visceral fat depots of dairy cows, (c) adiponectin protein expression in tissues other than adipose and (d) concentrations in different body fluids including milk.

Keywords: adiponectin, adipose tissue, cattle, insulin sensitivity

The Gut Microbiome as a Virtual Endocrine Organ: Implications for Physiology, Brain and Behaviour

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The gut microbiome exerts a marked influence on host physiology and manipulating its composition has repeatedly been shown to influence host metabolism and body-weight gain while obese subjects appear to carry distinct microbial signatures. This virtual endocrine organ also regulates the plasma concentrations of tryptophan, an essential amino acid and precursor to serotonin, a key neurotransmitter within both the enteric and central nervous systems. Control over the hypothalamic-pituitary-adrenal axis also appears to be under the influence of our gut microbiota. This is clear from studies in microbiota-deficient germ-free animals with exaggerated responses to psychological stress that can be normalized by monocolonization with certain bacterial species including Bifidobacterium infantis. Therapeutic targeting of the gut microbiota may thus be useful in treating or preventing stress-related microbiome-gut-brain axis disorders and metabolic diseases. Moreover, the implications of these findings also need to be considered in the context of farm and domestic animal physiology and behaviour.

Keywords: microbiome; endocrine organ; stress; tryptophan; behavior

Glucagon-like peptide 2 and its beneficial effects on gut function and health in production animals

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Numerous endocrine cell subtypes exist within the intestinal mucosa and produce peptides contributing to the regulation of critical physiological processes including appetite, energy metabolism, gut function, and gut health. The mechanisms of action and the extent of the physiological effects of these enteric peptides are only beginning to be uncovered. One peptide in particular, glucagon-like peptide 2 (GLP-2) produced by enteroendocrine L cells, has been fairly well characterized in rodent and swine models in terms of its ability to improve nutrient absorption and healing of the gut after injury. In fact, a long-acting form of GLP-2 recently has been approved for the management and treatment of human conditions like inflammatory bowel disease and short bowel syndrome. However, novel functions of GLP-2 within the gut continue to be demonstrated, including its beneficial effects on intestinal barrier function and reducing intestinal inflammation. As knowledge continues to grow about GLP-2's effects on the gut and its mechanisms of release, the potential to use GLP-2 to improve gut function and health of food animals becomes increasingly more apparent. Thus the purpose of this review is to summarize: 1) the current understanding of GLP-2's functions and mechanisms of action within the gut; 2) novel applications of GLP-2 (or stimulators of its release) to improve general health and production performance of food animals; and 3) recent findings, using dairy calves as a model, that suggest the therapeutic potential of GLP-2 to reduce the pathogenesis of intestinal protozoan infections.

Keywords: glucagon-like peptide 2, gut health, intestinal barrier function, protozoan infection

Adrenomedullin regulates microbiota and intestinal pathophysiology

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Adrenomedullin (AM) and proadrenomedullin N-terminal 20 peptide (PAMP) are two biologically active peptides produced by the same gene, adm, with ubiquitous distribution and many physiological functions. AM is composed of 52 amino acids, has an internal molecular ring composed by 6 amino acids and a disulfide bond, and shares structural similarities with calcitonin gene-related peptide (CGRP), amylin, and intermedin. The AM receptor consists of a 7-transmembrane domain protein called calcitonin receptor-like receptor in combination with a single transmembrane domain protein known as receptor activity modifying protein. Using morphological techniques, it has been shown that AM and PAMP are expressed throughout the gastrointestinal tract, being specially abundant in the neuroendocrine cells of the gastrointestinal mucosa; in the enterochromaffin-like and chief cells of the gastric fundus; in the ephitelial microvilli; and in the submucosa of the duodenum, ileum and colon. This wide distribution in the gastrointestinal tract suggests that AM and PAMP may act as gut hormones regulating many physiological and pathological conditions. To date it has been proven that, acting through different molecular pathways, AM and PAMP act as autocrine/paracrine growth factors in the gastrointestinal epithelium, play key roles in the protection of gastric mucosa from various kinds of injury, accelerate healing in diseases such as gastric ulcer and inflammatory bowel diseases. In addition, both peptides are potent inhibitors of gastric acid secretion and gastric emptying, they regulate the active transport of sugars in the intestine, regulate water and ion transport in the colon, modulate colonic bowel movements and small-intestinal motility, improve endothelial barrier function, and stabilize circulatory function during gastrointestinal inflammation. Furthermore, AM and PAMP are antimicrobial peptides and they contribute to the mucosal host defence system by regulating gut microbiota. To get a formal demonstration of the effects that endogenous AM and PAMP may have in gut microbiota, we developed an inducible knockout (KO) of the adm gene. Using this model, we have shown, for first time, that lack of AM/PAMP leads to changes in gut microbiota composition in mice. Further studies are needed to investigate whether this lack of AM/PAMP may have an impact in the development and/or progression of intestinal diseases through their effect on microbiota composition.

Keywords: adrenomedullin, intestinal physiology, antimicrobial, microbiota

Natural bioactives to maintain gut homeostasis

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The mammalian gastrointestinal tract is a dynamic environment, where a symbiotic relationship exists between the resident microbiota and the digestive and immune systems of the host. The development of the immune system begins in-utero and is further developed following the colonization of the GIT with microbiota during birth and postnatal life. The early establishment of this relationship is fundamental to the development and long-term maintenance of gut homeostasis. Regulatory mechanisms ensure an appropriate level of immune reactivity in the gut to accommodate the presence of beneficial and dietary microorganisms, while allowing effective immune responses to clear pathogens. However, unfavorable alterations in the composition of the microbiota, known as dysbiosis, have been implicated in many conditions including post-weaning diarrhorea in pigs. Weaning is a major critical period in pig husbandry. It involves complex dietary, social and environmental stresses that interfere with gut development. Post-weaning complications in piglets are characterized by a reduction in feed intake, atrophy of small intestine architecture, up-regulation of intestinal inflammatory cytokines, alterations in GIT microflora, diarrhea, and heightened susceptibility to infection. In the past, these challenges were controlled with in-feed prophylactic antibiotics and dietary minerals. However, these strategies are now banned because of their role in promoting multi-drug resistant bacteria and the accumulation of minerals in the environment, respectively. Therefore, significant efforts are being made to identify natural alternatives to support homeostasis in the piglet GIT, in particular during the weaning period. Chemodiversity in nature, including microorganisms, terrestrial plants, seaweeds and marine organisms, offers a valuable source for novel bioactives. Diverse organisms have evolved diverse chemical and molecular mechanisms for a variety of homeostatic activities including cell-to-cell signaling, receptor sensitivity, inflammasome activity and gene activation. Hence they offer great potential as preventatives and prophylactics in mammals. Bioactives vary greatly in biochemical structure including complex oligosaccharides (eg. Inulin), glucose polymers (eg. β glucans), fatty acids, minerals, antioxidants and organic acids. In this review, we discuss the advances in our understanding of the immune mechanisms by which the dynamic interplay of the intestinal microbiota and its host normally favours a homeostatic, symbiotic relationship, and how feeding natural bioactives in both the maternal diet and the piglet diet, can be utilized to support this symbiotic relationship in times of challenge.

Keywords: bioactives, pigs, gastrointestinal tract, immune, microbiota

Dietary influence on gut maturation in early postnatal life

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Following birth, the newborn must meet the acute challenges of circulatory changes, active respiration, thermoregulation, microbial colonization, and enteral nutrition. Whereas these processes normally occur without clinical complications in full-born neonates, birth at a preterm state of pregnancy is associated with high morbidity and mortality. One major clinical complication is necrotizing enterocolitis (NEC), a condition mainly seen among preterm human neonates. This is a severe and life-threatening condition and today NEC is the most commonly observed gastroenteropathy in intensive care units.

Because of the great similarities between humans and swine across many physiological and anatomical systems, swine are considered a premier model for human biomedical investigation. Accordingly, piglets born preterm display clinical complications relevant to preterm human neonate stresses and as such have provided a valuable model that helps investigate etiology, pathogenesis and prevention of NEC. The model is based on preterm delivery and formula-feeding using feeding paradigms that are not only clinically relevant in the human setting. We have investigated a range of medical, dietary and microbial interventions including bovine colostrum, human milk, milk replacers and amniotic fluid to understand disease progression and alternative feeding strategies towards mitigation of NEC. Data derived from the model suggests that natural biological fluids are superior to artificial milk replacers in regard to protection against NEC.

On the intervention fluids tested, colostrum is unique. Colostrum provides support to the neonate by providing many regulatory peptides in the early days before its own endocrine system is fully functioning. The importance of regulatory compounds is likely highest in the first 1-2 days where the intestine absorbs intact proteins and peptides via endocytosis. After this the newborn animal gradually becomes endocrinologically independent as endogenous regulatory functions increase. The actions of regulatory peptides and hormones include regulation of gastric emptying, absorption, transit time, and gut trophicity. The regulatory peptides are produced in response to ingestion of a meal and provide coordinated regulation of the digestive process that follows. One of the peptides that have received some attention is glucagon-like peptide 2 (GLP-2) as it has potent trophic effects on the intestine. It is secreted mainly by enteroendocrine L-cells in the distal small intestine in response to presence of nutrients. The intestine grows rapidly during the last three weeks of gestation and this coincides with an increase in fetal plasma GLP-2. These observations suggest that GLP-2 is associated with intestinal growth from the last three weeks of gestation, and endogenous levels of GLP-2 seem to peak in the immediate postnatal phase which coincides with the onset of enteral nutrition and rapid intestinal growth. The dietary influences on gut maturation in early postnatal life are profound. In perspective, new diets may be designed that reduce morbidity and mortality in neonates. This has clinical implications not only for human neonates but also for neonatal piglets in the industry.

Key words: preterm birth, colostrum, GLP-2, gut development, necrotizing enterocolitis

Adrenergic and noradrenergic regulation of poultry behavior and production

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Norepinephrine and epinephrine (NE and EP; noradrenaline and adrenaline) are integral in maintaining behavioral and physiological homeostasis during both aversive and rewarding events. They regulate the response to stressful stimuli through hormonal activity, direct activation of adrenergic receptors in the central and the sympathetic nervous systems, as well as through the interaction of the brain, gut and microbiome. The multiple functions of these catecholamines work synergistically to prepare an individual for a "fight or flight" response. However, hyper-reactivity of this system can lead to increased fearfulness and aggression, decreased health and productivity and a reduction in overall well-being. Maladaptive behaviors, such as aggression and fear-related behaviors are a serious problem in the poultry industry, leading to injury and cannibalism. For decades, catecholamines have been used as a measure of stress in animals. However, few studies have specifically targeted the adrenergic systems as a means to reduce maladaptive behaviors and improve animal well-being.

Recent selective breeding and microarray studies show that genetic differences in the catecholamine pathway, including in genes regulating enzymes involved in biosynthesis and metabolism, can be predictors of bird aggression and fearfulness. While environmental influences that affect the individual's aggressiveness or dominance status have long-lived impacts on catecholamine levels that interact with genetic background. Recent advances in our understanding of the development of the noradrenergic and adrenergic systems and their role in the brain-gut-microbial axis present us with new opportunities for therapeutic intervention in this system. Studies from our lab show that pharmaceutical intervention and maternal diet can alter the neurodevelopmental environment, resulting in morphological and biochemical changes within the adrenergic neurons. Early life vagal and enteric stimulation from microbiome-produced catecholamines has recently been shown to have long-lasting implications on neural development and anxiety-related behaviors. Together these data suggest the need for more research into targeted intervention to the noradrenergic and adrenergic systems to reduce damaging behaviors and improve animal well-being and productivity.

Keywords: chicken, epinephrine, norepinephrine, catecholamines, behavior

A comparison between the equine and bovine hypothalamus-pituitary-adrenocortical axis

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Cortisol secretion is regulated by concerted action of hypothalamic corticotropin-releasing factor (CRF) and arginine vasopressin on pituitary adrenocorticotropic hormone release in addition to hypothalamic and pituitary cortisol feedback. Studies on the neuroregulatory mechanisms on the secretion of anterior pituitary hormones in animals require in vivo approaches. Of interest, a nonsurgical technique involving cannulation of a venous pathway unique to equids can be performed under local anesthesia and mild tranquilization thereby facilitating research on neuroregulatory mechanisms in horses. Although both horses and cows show diurnal variation in plasma cortisol concentration with high values in the morning and low values in the evening, total basal plasma cortisol concentration is much higher in horses than in cows with similar free cortisol fractions. In accord, plasma glucose concentrations are much lower in cows than in horses. Furthermore, CRF concentrations in equine pituitary venous blood are lower than compared with other species, whereas plasma ACTH concentrations in cows are higher than in horses. A CRF challenge test induced a more pronounced plasma cortisol response in horses compared to cattle, whereas regarding ACTH the opposite seems true. Based on data from literature, the bovine species is characterised by relative high basal CRF and ACTH and low cortisol concentrations associated with high sensitivity to ACTH. As a consequence, the cortisol sensitivity to ACTH increases gluconeogenesis and ketogenesis more rapidly in ruminants than in monogastric plant eaters.

Keywords: bovine, equine, hypothalamus-pituitary-adrenocortical axis, cortisol, ACTH

Behavioral and metabolic differences in ewes characterized as high or low cortisol responders.

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We have identified sub-populations of ewes that have either high (HR) or low (LR) cortisol responses to adrenocorticotropin (ACTH) (10% from each extreme). When placed on a high energy diet, HR have a greater increase in adiposity than LR. We have extensively characterised the phenotype of HR and LR and show that HR display a distinct suite of metabolic, neuroendocrine and behavioral physiology that aligns with altered predisposition to gain adipose tissue.

In the non-stressed state, HR animals display increased gene expression of corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) in the paraventricular nucleus (PVN) of the hypothalamus. In addition, pro-opiomelanocortin (POMC) mRNA levels are higher in the anterior pituitary of HR than LR. Despite, differences in the cortisol response to ACTH, adrenal gland expression of various steroidogenic enzymes was similar between groups. Adrenal weights and histology (ratio of cortex: medulla) were not different. We show that HR have an innate up-regulation of the HPA axis at the level of the hypothalamus and the pituitary.

In response to feeding, HR animals exhibit reduced skeletal muscle thermogenesis. HR animals also show a greater catabolic state in response to stress than LR. Psychosocial stress (barking dog) reduced food intake in LR only, whereas immune challenge (lipopolysaccharide) reduced food intake in both groups, albeit this effect was greater in HR. Similarly, the LPS-induced increase in skeletal muscle heat production was greater in HR than LR. In addition to differences in the effects of stress on feeding, HR are resistant to the satiety effects of α-melanocyte stimulating hormone (αMSH); this resistance is associated with lower expression the melanocortin 3 and 4 receptors in the PVN.

Finally, LR and HR animals display distinct behavioral and coping strategies in response to stress. LR have reduced fear, increased physical activity and a proactive coping style (HR are reactive). These behavioral differences are associated with increased physical activity and may contribute to differences in the propensity to gain weight. In conclusion, we show that high cortisol response identifies individuals with a complex series of traits that associate with reduced energy expenditure and increased food intake.

Keywords: cortisol, adiposity, thermogenesis, food intake and behavior

Glucocorticoid programming of intrauterine development

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In adults, glucocorticoids (GCs) are stress hormones that aid survival during challenges to homeostasis. In fetuses, they have an even wider range of roles. Towards term, GCs act as the maturational signal in preparing fetuses for birth. Earlier in gestation, they can act as environmental signals that alter fetal development in relation to resource availability for intrauterine growth. These functions improve viability before and at birth, particularly when conditions are sub-optimal for survival. However, by altering fetal growth, early exposure to excess GCs modifies the developing phenotype with life-long consequences. Consequently, GCs also act as programming signals that adapt intrauterine development to optimise offspring viability and fitness.

Both before and at term, GCs affect development of a wide range of fetal tissues by inducing changes in cellular expression of structural, transport and signalling proteins, which have widespread functional consequences at whole organ and system levels. Glucocorticoids, therefore, activate many of the physiological systems that have little or no function in utero but are vital at birth, such as pulmonary respiration, hepatic glucogenesis and themoregulation. At the tissue level, their developmental effects can be direct via glucocorticoid receptors or mediated indirectly via changes in the placenta or other endocrine systems. At the molecular level, GCs can act directly on gene expression via the promotors or indirectly by epigenetic modifications to the genome. However, in switching tissues to differentiation from accretion to improve immediate viability, GCs can lead to long term functional deficits, particularly if excess exposure occurs before full term. In sheep, early prenatal exposure to excess GCs has been shown to alter glucose tolerance, blood pressure, insulin secretion and hypothalamic-pituitary-adrenal (HPA) axis function of the adult offspring. In horses in which fetal HPA development occurs comparatively late in gestation, the main period of susceptibility to glucocorticoid programming may be immediately after rather than before birth. Certainly, raising cortisol concentrations in newborn foals for 5 days leads to altered insulin secretion in older foals and to abnormal insulin sensitivity and HPA function in yearlings. Thus, GCs are important regulatory signals during both fetal and early neonatal life in farm animals. They program a phenotype best suited to the prevailing environmental conditions, thereby maximising the chances of offspring survival to reproductive age. However, if postnatal conditions differ from those signalled in utero, the glucocorticoid-induced developmental adaptations can become maladaptive with adverse outcomes for offspring health in the long term.

Keywords: glucocorticoids, programming, development

The role of mitochondrial DNA copy number, variants and haplotypes in farm animal developmental outcome

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The vast majority of cellular energy is generated through the process of oxidative phosphorylation (OXPHOS), which takes place in the electron transfer chain (ETC) in the mitochondria. The ETC is encoded by two genomes, the chromosomal and the mitochondrial (mtDNA) genomes. MtDNA replication is strictly regulated during development through DNA methylation of the nuclear-encoded mtDNA-specific replication factor, Polymerase Gamma. During oogenesis mtDNA copy number increases exponentially so that mature, fertilisable, metaphase II oocytes possess >150,000 copies. Following fertilization, mtDNA copy number is significantly reduced with no mtDNA replication taking place during preimplantation development until the blastocyst stage when it is initiated but restricted to the trophectodermal cells.

The inner cell mass cells, which give rise to the embryo proper, do not replicate mtDNA and continue to reduce mtDNA copy number so that, prior to gastrulation, they possess fewer copies of mtDNA. This establishes the 'mtDNA-set point', which enables all differentiating cells to acquire the appropriate numbers of mtDNA copy to meet their specific demands for ATP generated through OXPHOS in order that they can perform their designated cellular functions. Consequently, cells with a high requirement for OXPHOS-derived ATP, such as neurons, possess thousands of copies of mtDNA per cell whilst cells with a low requirement for OXPHOS-derived ATP, such as endothelial cells, have far fewer copies of mtDNA.

However, when oocytes possess too few copies of mtDNA, they either fail to fertilise or arrest during preimplantation development. These developmentally incompetent oocytes can be rescued by mitochondrial supplementation at the time of fertilisation. The resultant blastocysts have gene expression patterns that are similar to blastocysts derived from normally fertilised oocytes possessing sufficient levels of mtDNA.

MtDNA is associated with a number of traits, which include tolerance to heat, growth and physical performance, meat and milk quality and fertility. Mitochondrial genomes can be clustered into groups known as mtDNA haplotypes. Pig fertility is directly related to a sow's mtDNA haplotype. A sow's mtDNA haplotype can influence litter size, the number of developmentally competent oocytes produced, and fertilisation and development rates. The use of assisted reproductive technologies, such as nuclear transfer, allow favourable chromosomal genetic traits to be mixed and matched with sought after mtDNA haplotype traits. As a result super breeds can be generated.

Keywords: mitochondrial DNA, fertilisation, development, copy number, haplotype

Serotonin is a regulator of maternal calcium homeostasis during lactation

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Calcium is the major mineral component of milk and colostrum. Circulating maternal calcium pools and dietary calcium are insufficient to support maternal physiology while simultaneously supporting milk synthesis. Dairy cows are particularly susceptible to the demand by the mammary gland for calcium due to the amount of milk they produce. This creates an enormous challenge to maintain maternal calcium concentrations within a normal physiological range. Therefore they must rely on mobilization of calcium from bone to support maternal calcium homeostasis. Approximately 50% of dairy cows will succumb to subclinical hypocalcemia and 5-10% will suffer from the clinical form of the disease, milk fever, which has major economic and animal health and production impacts. Additionally, regardless of disease state, dairy cows will lose approximately 13% of bone calcium during the first 2 months of lactation. Serotonin (5-HT) has emerged as a regulator of mammary gland physiology over the last decade, modulating several aspects of milk synthesis and mammary gland involution, and has independently been shown to influence bone metabolism. More recently, using several mammalian models, we have demonstrated the importance of 5-HT in regulating calcium mobilization from bone during lactation, as well as modulating calcium transport into the mammary gland from the circulation. Our research shows that 5-HT is critical for the expression of key mammary gland calcium transporters and pumps, as well as the production of parathyroid hormone related-protein (PTHrP), which is the hormone necessary for the induction of calcium mobilization from bone during lactation. In dairy cows, 5-HT concentrations are positively correlated with calcium and PTHrP status on day 1 of lactation. Furthermore, we have demonstrated that 5-HT concentrations fluctuate over the course of an entire lactation, and decrease substantially at parturition, compared to pre-partum concentrations. The drop in 5-HT at parturition closely mirrors the drop in circulating total calcium concentrations that also typically occur during this time. Finally, using rodent models, we have demonstrated that 5-HT increases calcium transport in the mammary gland during lactation, and also increases osteoclast size and numbers in bone tissue to increase maternal calcium concentrations. Combined, our research implicates the importance of 5-HT for the regulation of maternal calcium homeostasis, as well as for the secretion of calcium into milk and colostrum.

Keywords: serotonin, calcium, mammary gland

Effect of trans-10, cis-12 CLA on mammary lipid synthesis

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Milk fat is an economically important part of dairy production and is responsive to season, cow physiological state, time of day, diet composition, and rumen fermentation. Diet-induced milk fat depression (MFD) has been intensely studied by multiple generations and remains a common problem observed under both intensive and extensive management. The biohydrogenation theory established that MFD is caused by an inhibition of mammary synthesis of milk fat by specific bioactive fatty acids produced during ruminal biohydrogenation of unsaturated fatty acids. Trans-10, cis-12 conjugated linoleic acid (CLA) is the best studied of the bioactive fatty acids in the cow. Trans-10, cis-12 CLA has also been extensively studied in other experimental models, including the growing mouse, but care should be taken in interpretation of experiments that used drastically higher doses than commonly used in the cow. Trans-10, cis-12 CLA dose dependently decreases milk fat yield in the cow, but only explains a portion of the depression observed during diet-induced MFD. The mammary gland rapidly responds to CLA with decreased milk fat observed within 12 h and maximal response achieved in approximately 3 d. Both preformed and de novo synthesized pathways are decreased, but de novo is decreased to a greater extent especially with higher doses of CLA. Few changes are observed in whole animal metabolism and physiology, but CLA decreases mammary lipogenic capacity. Molecular investigations in the cow have predominantly focused on transcriptional regulation and CLA decreases expression of key lipid synthesis enzymes and lipogenic transcription factors in the mammary gland. Our investigations have established that expression of sterol response element-binding protein 1 (SREBP1) and thyroid hormone responsive Spot 14 (S14) are decreased and some elements of the endoplasmic reticulum stress pathway are increased. Furthermore, functional roles of SREBP1 and Spot 14 have been validated using transgenic mouse models and provide a deeper understanding of the regulation of milk fat synthesis. We have found little support for a role of LXRs or PPARs in CLA inhibition of milk fat synthesis. A better understanding of the impact on whole animal energetics is needed, but we have observed increased adipose tissue lipogenesis during short-term CLA treatment that could be explained by nutrients spared from milk fat synthesis. Trans-10, cis-12 CLA is arguably the best studied bioactive nutrient originating from the gastrointestinal microbiome that has clear real-world implications. The biology of CLA in the dairy cow continues to be a prolific area of research aided by diverse approaches by multiple groups, but key questions remain. Specifically, the direct interaction of CLA with signaling factors is not clear.

Keywords: conjugated linoleic acid, milk fat depression, lipogenesis

Supplementation of conjugated linoleic acid during the transition to lactation period improves milk production and reproductive efficiency

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The transition to lactation period in the dairy cattle lifecycle is the most metabolically challenging period and both milk production and reproduction are highly reflective of energy partitioning. Trans-10, cis-12 conjugated linoleic acid (CLA) has the potential to alter energy partitioning in lactating cows by marginally reducing milk fat synthesis. The objective of this study was to determine the effects of pre- and postpartum CLA supplementation on whole lactation milk production, body weight change, and reproductive performance in a commercial dairy setting.

Holstein cows in a robotic milking system were blocked by multiparous (mp) or primiparous (pp), and expected calving date, and randomly assigned to either a CLA group (mp n=100; pp n=39) or control group (mp n=98; pp n=38). Cows were supplemented with 100 g of lipid encapsulated CLA methyl esters (Lutrell Pure, BASF, Germany) mixed 50:50 with soybean meal to provide 10 g each of trans-10, cis-12 CLA and cis-9, trans-11 CLA via a robot mineral supplement unit. Supplementation was from -21 d precalving through 30 d in milk (DIM) for mp or 70 DIM for pp. Milk yield, fat, and protein concentration, and body weight were recorded daily and averaged by wk and reproductive data recorded. Data were analyzed using the MIXED procedure of SAS 9.4 with repeated measures. Treatment, wk, and treatment×wk were fixed effects with random effects of (cow)group. Means were considered different when $P<0.1$ and tended to differ when $P<0.15$.

Body weight changed over time but was not different by treatment or by treatment over time. Daily milk yield over 100 d was increased with CLA supplementation ($P=0.09$) with the greatest increase in milk production being 3.0 kg/d for mp (wk 5; $P=0.007$) and 3.9 kg/d for pp (wk 14; $P=0.001$) cows. Increased milk yield across 100 d was not sufficient to result in a difference in whole lactation milk yield. Supplementation with CLA during the transition period did not alter daily milk fat yield over the 100 d. Supplementation of CLA did not alter reproductive efficiency for mp cows. In pp animals, CLA supplementation decreased ($P=0.07$) DIM at conception and increased the chance of pregnancy (hazard's ratio 1.6). Supplementation of CLA during the transition to lactation period shifted energy partitioning to support greater milk production during the first 100 d without decreasing milk fat yield. Furthermore, the repartitioning of energy protected body weight and reproductive performance, despite increased milk yield.

Keywords: conjugated linoleic acid, transition cows, energy partitioning, robotic milking system

Altering prolactin concentrations in sows

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Prolactin is an important lactogenic and galactopoietic hormone. In swine, it was indeed demonstrated that inhibition of the prepartum peak of prolactin abolishes the onset of lactation, and that decreasing prolactin concentrations at any stage of lactation inhibits milk yield during that treatment period. Low circulating concentrations of prolactin were also associated with the agalactia syndrome in sows and suppression of prolactin secretion in late gestation inhibits mammary development. The essential role of prolactin in sows is therefore evident. It then becomes of interest to understand the various factors that can alter prolactin secretion, and these will be covered in the current presentation. Regulation of prolactin secretion is largely under the negative control of dopamine and dopamine agonists consistently decrease prolactin concentrations in sows. On the other hand, injections of dopamine antagonists can enhance circulating prolactin concentrations. Besides pharmacological agents, many other factors were also shown to alter prolactin concentrations in sows. The use of Chinese-derived breeds, for instance, leads to increased prolactin concentrations in lactating sows compared with standard European white breeds. Numerous husbandry and feeding practices may also have a potential impact on prolactin concentrations in sows. Factors such as provision of nest-building material prepartum, housing at farrowing, high ambient temperature, stress, transient weaning, exogenous thyrotropin-releasing factor, exogenous growth hormone-releasing factor, nursing frequency, prolonged photoperiod, fasting, increased protein and/or energy intake, altered energy sources, feeding high-fiber diets, sorghum ergot or plant extracts, were all studied with respect to their prolactinemic properties. Even though some of these practices did indeed affect circulating prolactin concentrations, none led to changes as drastic as those brought about by dopamine agonists or antagonists. It appears that the numerous factors regulating prolactin concentrations in sows are still not fully elucidated and that studies to develop novel applicable ways of increasing prolactin concentrations in sows are warranted.

Key words: dopamine, lactation, prolactin, regulation, sows

Quantification of nutrients absorbed, metabolized in the liver, and secreted into milk of lactating sows

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Nutrition of lactating sows is difficult to optimize because traits like milk yield, nutrient balances and mammary blood flow are difficult to quantify. In general, nutritional studies with lactating sows have hitherto focused on number and weight of piglets at birth and weaning and ignored which nutrients very mobilized from the sow`s body. However, milk production has highest priority for lactating sows and therefore body mobilization can be extensive to avoid a compromised milk yield. Ideally, feeding of animals should ensure that the transfer of nutrients to extra-hepatic tissues matches the nutrients required for production and reproduction (mainly milk in lactating sows). Using state-of-the-art techniques, we have quantified nutrients being absorbed and metabolized within lactating sows. Sows were surgically fitted with catheters to allow collection of blood from an artery and from portal and hepatic veins. In addition, a fourth catheter was inserted to allow infusion of para amino hippuric acid, which was used to quantify the blood flow in the portal and hepatic veins. Net portal-, net hepatic -, and net splanchnic fluxes were quantified to evaluate net absorption of nutrients from the GI-tract, liver metabolism of nutrients and transfer of nutrients to extra-hepatic tissues, respectively. In addition, sow milk was collected and analyzed and the milk yield was quantified using a recently developed mathematical model, which allowed the net splanchnic fluxes of nutrients to be compared with those being secreted into sow milk. Sows produced on average 6.7 and 12.7 kg/d at d 3 and 17 of lactation, respectively. In early lactation (d 3), the net splanchnic flux of glucose resembled the postprandial change in net portal flux, meaning that hepatic conversion of glucose was rather small. In contrast, at peak lactation (d 17), a constant and high net splanchnic flux of glucose was observed and did not change with time after feeding. Our data indicate that glycogen pools in the liver were replenish during the first 4 h after feeding, whereas hepatic glycogen was used to buffer the net splanchnic flux of glucose from 4 to 8 h after feeding to support milk production. Substantial amounts of alanine were cleared by the liver, whereas hepatic clearance of branched chain amino acids was minimal. Propionate and butyrate were metabolized by the liver with high efficiency, whereas acetate was scarcely metabolized and likely used for de novo fat synthesis in the udder. Efficiencies of using net splanchnic fluxes for milk production will be discussed.

Keywords: lactation, milk production, nutrient utilization, productivity, sow nutrition

New Concepts of the Central Control of Reproduction, integrating influence of Stress, Metabolic State and Season

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Gonadotropin releasing hormone (GnRH) is the primary driver of reproductive function and pulsatile GnRH secretion from the brain and causes the synthesis and secretion of luteinising hormone (LH), and follicle stimulating hormone from the pituitary gland. Recent work has revealed that the secretion of GnRH is controlled at the level of the GnRH secretory terminals in the median eminence. At this level, projections of kisspeptin cells from the arcuate nucleus of the hypothalamus are seen to be closely associated with fibres and terminals of GnRH cells. Direct application of kisspeptin into the median eminence causes release of GnRH. The kisspeptin cells are activated at the time of a natural 'pulse' secretion of GnRH, as reflected in the secretion of LH. This appears to be due to input to the kisspeptin cells from glutamatergic cells in the basal hypothalamus, indicating that more than one neural element is involved in the secretion of GnRH. Because the GnRH secretory terminals are outside the blood brain barrier, factors such as kisspeptin may be administered systemically to cause GnRH secretion; this offers opportunities for manipulation of the reproductive axis using factors that do not cross the blood-brain barrier. In particular, kisspeptin or analogues of the same may be used to activate reproduction in the non-breeding season of domestic animals. Whilst this method is successful in sheep, other species such as deer are less responsive to kisspeptin and long-acting analogues may be required in the latter. Metabolic status may also affect the secretion of reproduction and this could involve action of gut peptides. Neuropeptide Y and Y-receptor ligands have a negative impact on reproduction and NPY production is markedly increased in negative energy balance. Recent unpublished data show that GnRH secretory terminals express Y2 receptors and the direct application of peptide-YY (a gut hormone) can inhibit secretion of GnRH. This allows for correction of reproductive mitigation under negative energy balance by Y-receptor antagonists.

Another brain peptide that influences reproductive function is gonadotropin inhibitory hormone (GnIH). Work in sheep shows that this peptide acts on GnRH neuronal perikarya, but projections to the median eminence also allow secretion into the hypophysial portal blood and action of GnIH on pituitary gonadotropes. GnIH cells are upregulated in anestrus and infusion of GnIH can block the ovulatory surge in GnRH/LH secretion. Importantly, GnIH cells are activated during stress, with increased input to the GnRH cells. Since the secretion of GnIH into the portal blood is not increased, the stress effect is most likely effected at the level of GnRH neurons.

Keywords: GnRH, GnIH, stress, metabolic state, season

Impact of nutritional programming on growth, health and sexual development of bull calves

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A high postnatal feeding intensity improves short-term health status and constitution of calves. Moreover, nutritional stimuli during a sensitive period of development affect the long-term development of the adult organism. This phenomeon of metabolic programming permanently affects the release of hypothalamic neuropeptides controlling feed intake and long-term weight gain due to the plasticity of the regulatory system. Superior calfhood nutrition was found to augment gonadotropin secretion. Higher testosterone concentrations in week 10 of life in calves fed milk ad libitum in their first three weeks post natum compared to restrictively fed calves may reflect an enhanced sexual development of calves fed intensively before weaning. The impact of early pre-weaning feeding intensity on subsequent sexual development of bulls is still unclear.

Thus, we performed a respective study and fed 26 male Brown Swiss calves either restrictively or ad libitum for 4 wks. Calves offered milk ad libitum were further subdivided into a group consuming small amounts of milk and calves ingesting much milk. From wk 5 of life, all calves were fed restrictively with milk gradually decreased until weaning in wk 10. Concentrates and hay were offered free of choice. Starting at an age of 6 months, body weight and scrotal circumference were assessed biweekly. As soon as scrotal circumference reached 26 cm, semen was collected by electroejaculation every second week. Puberty was defined as > 50 million sperms in the ejaculate with > 10 % motility. As a second parameter indicating puberty, the age at which bulls achieved a SC of 28 cm was recorded. Within the study period, ad libitum calves with a high consuming a high amount of milk were on average almost 20-30 kg heavier compared to calves consuming small amounts of milk and calves fed restrictively. Scrotal circumference, however, did not differ between the groups at the respective points of time. Onset of puberty based on analysis of ejaculates did not differ between the groups irrespective of a considerable variance within each group. A SC of 28 cm was reached somewhat earlier in calves consuming a high amount of milk (252 d) compared to calves consuming small amounts of milk (271 d) and calves fed restrictively (268 d). In accordance with other studies, an increased milk intake within the first four weeks of life had long-lasting effects on growth but seems not to affect sexual development. By contrast, a high-nutrition diet fed between wk 2 and 31 was accompanied by a lower age at puberty and larger testes from wk 11 to wk 72 of life in another trial.

In conclusion, a high plane of nutrition during the first four weeks of life might be insufficient to induce a significant effect on the onset of puberty. Alternatively, the number of animals chosen in our study was inappropriate to demonstrate an effect of pre-weaning feeding intensity on subsequent sexual performance of bull calves due to the enormous inter-individual variation of parameters characterizing sexual development. Therefore, more studies are necessary to understand the effects of nutritional programming on sexual development in bull calves.

Keywords: nutritional programming, growth, sexual development, bull claves

Intra-pituitary mechanisms underlying the control of fertility: key players in seasonal breeding

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Recent studies have shown that in conjunction with dynamic changes in the secretion of gonadotrophin-releasing hormone (GnRH) from the hypothalamus, paracrine interactions within the pituitary gland play an important role in the regulation of fertility during the annual reproductive cycle. Morphological studies have provided evidence for close associations between gonadotrophs and lactotrophs and gap junction coupling between these cells in a variety of species. The physiological significance of this cellular interaction was supported by subsequent studies revealing the expression of prolactin (PRL) receptors in both the pars distalis and pars tuberalis regions of the pituitary. This cellular interaction is critical for adequate gonadotrophin output because, in the presence of dopamine, PRL can negatively regulate the luteinising hormone (LH) response to GnRH. Receptor signalling studies showed that signal convergence at the level of protein kinase C and phospholipase C within the gonadotroph underlies the resulting inhibition of LH secretion. Although this is a conserved mechanism present in all species studied so far, in seasonal breeders such as the sheep and the horse, this mechanism is regulated by photoperiod, as it is only apparent during the long days of spring and summer. At this time of year, the non-breeding season of the sheep coincides with the breeding season of the horse, indicating that this inhibitory system plays different roles in short and long day breeders. Whereas in the sheep it contributes to the complete suppression of the reproductive axis, in the horse it participates in the fine-tuning of gonadotrophin output preventing gonadotroph desensitisation. The photoperiodic regulation of this inhibitory mechanism is likely to rely on alterations in the folliculostellate cell population. Indeed, electron microscopic studies have recently shown an increased prevalence of this cell type together with up-regulation of gap junctions during the spring and summer. The association between gonadotrophs and lactotrophs could also underlie an interaction between gonadotrophic and prolactin axes in the opposite direction. In support of this alternative, a series of studies have demonstrated that GnRH stimulates PRL secretion in sheep through a mechanism that does not involve the mediatory actions of LH or follicle-stimulating hormone, and that this stimulatory effect of GnRH on the PRL axis is seasonally regulated. Collectively, these findings highlight the importance of intercellular communications within the pituitary in the control of gonadotrophin and prolactin secretion during the annual reproductive cycle in seasonal breeders.

Key Words: pituitary, luteinising hormone, prolactin, dopamine, seasonal breeding

Local immune system in oviduct physiology and pathophysiology: Attack or tolerance?

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The local immune system in the oviduct has a unique ability to deal with pathogens, allogeneic spermatozoa, and the semi-allogeneic embryo. To achieve this, it seems likely that the oviduct possesses an efficient and strictly controlled immune system that maintains optimal conditions for fertilization and early embryo development. The presence of a proper sperm/embryo-oviduct interaction in oviduct begs the question as to whether the local immune system in the oviduct exerts beneficial or deleterious effects on sperm. A series of studies has revealed that bovine oviduct epithelial cells (BOEC) are influenced by preovulatory levels of E2, P4 and LH to maintain a homeostatic microenvironment, via inhibition of pro-inflammatory Th1 responses that are detrimental to allogenic sperm and the semi-allogenic embryo. During pathological conditions, the mucosal immune system initiates a biphasic inflammatory response to the infection; at low concentrations, the bacterial lipopolysaccharide (LPS) induced a pro-inflammatory response with increased expression of TLR4, PTGS2, IL-1β, NFKB1 and TNFα. At higher concentrations, however, LPS induced a set of anti-inflammatory genes (TLR2, IL4, IL10 and PTGES). Moreover, BOEC can participate in antimicrobial processes through the secretion of acute phase protein, alpha 1-acid glycoprotein, which is partly regulated by ovarian steroids. Thus, BOEC respond to inflammatory stimuli with the secretion of this acute phase protein which, in turn, reduces expression of pro-inflammatory cytokine TNFα, contributing to an anti-infection process in the oviduct. Under physiological conditions, polymorphonuclear neutrophils (PMN) are existent in the oviductal fluid during preovulatory period in the bovine. Importantly, the bovine oviduct down-regulates sperm phagocytosis by PMN via prostaglandin E2 (PGE2) action. In addition, the major contraction-related substances, involving the angiotensin II-endothelin-1-PGE2 system, may fine-tune the local immunological environment of the bovine oviduct through regulation of PMN phagocytosis of sperm. Our recent results show that BOEC-sperm interaction adapts the PMN transcriptomic profile toward Th2 by suppression of pro-inflammatory cytokines (TNFα) and stimulation of anti-inflammatory cytokines (IL-10 & TGFβ).

In conclusion, the oviduct displays mucosal immunity that maintains an anti-inflammatory environment under physiological conditions. Moreover, the oviduct-sperm interaction regulates the phagocytic behavior and immunological responses of PMN, that exist in the oviduct, ensuring sperm quality prior to fertilization.

Key words: oviduct, immune cells, sperm, epithelium, cow

Early pregnancy in the mare: old concepts re-visited

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"Maternal recognition of pregnancy" (MRP) is commonly used to describe the ongoing embryo-maternal communication during early pregnancy that culminates in prevention of luteolysis and ensures the ongoing progestin support vital for embryo development. The conceptus-derived pregnancy recognition signal has not been identified in the horse. Although equine conceptuses produce substantial amounts of estrogens, evidence is lacking that estrogens are the pregnancy recognition signal in mares. Conceptus mobility is integral to MRP and is driven by conceptus-derived prostaglandin production. Cessation of conceptus mobility, referred to as fixation, is driven by an increase in conceptus size, increase in uterine tone and reduction in sialic acid content of the capsule.

Gene expression profiling of equine pre-implantation conceptuses revealed the expression of neuraminidase 2, also known as sialidase 2, an enzyme that cleaves sialic acid from polysaccharide chains. Quantitative RT-PCR showed increasing expression of neuraminidase 2 from Day 8 to Day 16 of development, whereas endometrial transcript abundance was negligible. NEU2 secreted by conceptuses in vitro proofed to be functionally active; it appears therefore, that the conceptus itself regulates the sialic acid content through the expression of neuraminidase 2. Gene expression profiling furthermore revealed that equine conceptus express increasing amounts of fibrinogen during early development, and western blot analysis confirmed secretion of fibrinogen into culture medium when conceptuses were cultured in vitro.

Upon immunohistochemistry, the acellular glycoprotein capsule of the conceptus showed particularly intense staining for fibrinogen. We hypothesize that conceptus-derived fibrinogen interacts with endometrial integrins to promote cessation of conceptus mobility and fixation. Indeed, NGS analysis of conceptuses and endometrial 16 days after ovulation revealed the integrin signaling pathway to be significantly enriched in both sample types. Real-time RT-PCR confirmed ITGAVB1 as the most abundant integrin receptor in endometrium; fibrinogen has the highest affinity for ITGAVB1 amongst the integrins receptors it binds to.

Lastly, we found the equine conceptus expresses increasing quantities of relaxin during pre-implantation development, with the endometrium expressing its corresponding receptors RFXP1 and RFXP4. In other species such as pig, mouse and human, relaxin is produced by the corpus luteum and is known to promote angiogenesis during early pregnancy. Preliminary data support the hypothesis that conceptus-derived relaxin promotes endometrial angiogenesis during early pregnancy in the horse.

In summary, substantial advances in understanding MRP in the horse are underway.

Keywords: equine, pregnancy, fibrinogen, relaxin, integrins, NEU2

The deleterious effects and the interaction of mastitis and heat stress on ovarian function in lactating cows: basic and applied aspects

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Reduced reproductive performance of lactating cows is highly associated with environmental and pathogenic stressors. This review summarizes the state of the art of the effects of acute or chronic heat stress (HS) and acute, clinical or chronic subclinical mastitis (intramammary infection; IMI) on ovarian function. It also offers various approaches to improving the fertility of cows under chronic HS and IMI. Comparing the two stressors reveals a similar mode of alteration in several functions of the follicle and its enclosed oocyte. Both HS and IMI cause a reduction in the preovulatory LH surge, with a more prominent effect in IMI cows, expressed by delayed LH surge and consequently delayed ovulation. Both stresses induce changes in follicular growth dynamics, reduce follicular steroidogenesis and disrupt follicular dominance. Unlike the similar effects of HS and IMI on follicular function, progesterone secretion by the corpus luteum is lower under chronic summer HS, but not under chronic subclinical IMI. Both stresses have been found to impair cytoplasmic and nuclear maturation of oocytes, subsequently associated with reduced embryonic development. Although the effects of acute stress (i.e., short-term HS and clinical IMI) on reproduction have been intensively studied, the long-term effects (i.e., seasonal HS and subclinical-chronic IMI) on fertility are much more important. The above findings have provided insights into the mechanism by which HS and IMI compromise fertility, which has enabled developing new strategies to mitigate these effects. For instance, treatment with GnRH and PGF2α to induce follicular turnover successfully improved conception rate in subpopulations of HS cows during the summer, in particular primiparous cows and cows with high body condition score. On the other hand, the 'Ovsynch' program, also based on the use of GnRH and PGF2α, has been shown to improve conception rate of subclinical IMI cows, most likely due to better synchronization of ovulation and AI timing. With respect to the corpus luteum, the deleterious effect of HS on its function has been mitigated by supplementing progesterone post-AI; conception rate was particularly improved in a subgroup of cows with postpartum uterine disease and with low body condition score. Treatment of IMI cows with exogenous progesterone has not yet been examined. In summary, similarities noted between the two stressors do not necessarily suggest a shared mechanism. On the other hand their additive deleterious effects on reproduction should not be ruled out. Accordingly, somatic cell count is higher in IMI cows during the summer, and their fertility is lower than that of their uninfected counterparts.

Keywords: heat stress, mastitis, ovarian function, bovine

Abstracts

Poster presentations

The immune system modulator α-1 acid glycoprotein inhibits insulin and IGF1 induced protein synthesis in C2C12 myotubes.

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Alpha-1 acid glycoprotein (AGP) has previously been demonstrated by our laboratory to be negatively correlated with growth rate in newborn piglets. However, a mechanism of action for AGP in growth has not been identified. Previous research has demonstrated that AGP can modify adipose tissue metabolism in swine by altering insulin action. The present study was undertaken to determine if AGP can modify muscle metabolism by examining protein turnover in the C2C12 murine muscle cell line. Cells were thawed from frozen stocks and propagated to confluency in 6 well tissue culture plates with DMEM containing 10% fetal bovine serum and antibiotics/antimycotics. Medium was changed to 5% horse serum in DMEM at confluency and cells were permitted to fuse into myotubes. Cells were used for experiments 4 days post fusion. Medium containing serum was removed from culture wells which were rinsed with serum free DMEM prior to addition of treatment media. Treatment media were comprised of serum free DMEM, 0.5% BSA, and 0, 0.1, 1.0 or 10.0 μg/mL murine AGP for the initial experiments. Treatment media were added to cultures for 1 h, then supplemented with 3H-thymidine/mL for an additional 2 h. Wells were washed three times with ice cold 10% trichloroacetic acid, with the last wash permitted to incubate with the cells overnight at 4°C. Cells were then scraped from wells and transferred to centrifuge tubes. Cells were centrifuged and supernatant removed. One mL of 0.5 M NaOH + 0.1% Triton X-100 was then added and tubes were incubated at 50°C for 1 h prior to counting. Treatment of C2C12 myotubes with AGP had no detectable effect on protein synthesis ($p > 0.05$; $n = 4$ trials). Insulin (10 nM) increased 3H-tyrosine incorporation by 27% ($P < 0.05$; $n = 3$ trials). However, addition of 1 μg AGP/mL medium reduced the insulin induced 3H-tyrosine incorporation by 56% ($P< 0.01$, $n = 3$ trials). Incubation with a higher concentration of insulin (1 μm) stimulated a 50% increase in 3H-tyrosine incorporation ($P < 0.01$, $n = 3$ trials). Addition of 1 μg AGP/mL medium did not affect the incorporation induced by 1 μM insulin ($P > 0.05$, $n = 3$ trials). Treatment with IGF1 (20 ng /mL) increased 3H-tyrosine incorporation by 53% ($P < 0.01$, $n = 3$ trials); while addition of AGP was associated with a 40% reduction in 3H-tyrosine incorporation that was induced by IGF1 ($P < 0.01$, $n = 3$ trials). These data indicate that AGP can indirectly reduce protein synthesis in skeletal muscle cells by altering the efficacy of physiological concentrations of insulin or IGF1.

Keywords: Alpha-1 acid glycoprotein, IGF1, insulin, myotube, protein synthesis

Effects of oral butyrate application on insulin signaling in various tissues of chickens

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Butyrate is widely used as a growth promoting feed additive in poultry and pig nutrition. Due to its epigenetic and receptor-mediated effects, butyrate can be a potent effector of carbohydrate metabolism by influencing the insulin signaling pathway. The aim of the present study was to investigate the effects of orally applied butyrate on certain insulin signaling proteins in different tissues of broiler chicken.

Ross 308 broilers were treated once daily with orally administered intra-ingluvial bolus of sodium butyrate (0.25 g/kg BW), or distilled water (control) following overnight starvation at day 20-24 of life. After slaughtering on day 24, tissue samples were collected from the liver, the abdominal and subcutaneous adipose tissue and from the gastrocnemic muscle. The expression level of insulin receptor (InsR), phosphatidyl-inositol-3-kinase (PI3K) and mammalian target of rapamycin (mTOR) were determined by Western blotting. In addition, plasma butyrate, glucose and insulin concentrations were measured prior to slaughtering.

With higher plasma butyrate concentration, the protein expression level of InsR was significantly decreased in the liver and in both examined adipose tissues of butyrate-treated animals; however, it was elevated in the muscle after butyrate treatment. PI3K expressions were affected by butyrate only in the liver, where moderate reduction could be observed. Butyrate decreased mTOR expression only in liver and subcutaneous adipose tissue. Further, blood glucose and insulin levels were significantly elevated by the orally administered butyrate.

These results suggest that intra-ingluvially applied butyrate could influence the carbohydrate metabolism through modulation of insulin signaling associated with higher peripheral blood glucose concentrations. Since the effect of butyrate was tissue-specific, it can be suggested that butyrate could act on glucose shifting among tissues by selectively increasing the glucose uptake of skeletal muscle. It can be concluded that butyrate is proved to be a potent effector of carbohydrate metabolism in chicken during the growing period, which could have special importance in growth performance and the development of carcass composition. However, the intra-ingluvial treatment with butyrate is not practicable in poultry production. Thus, the usefulness in modulation of insulin signaling of butyrate as feed supplement has to be proven by further studies.

Keywords: butyrate, insulin signaling, chicken

Regulation of insulin signaling proteins in adipose tissues of periparturient dairy cows

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The main physiological effect of insulin on adipose tissue is to promote glucose uptake and lipogenesis and to suppress lipolysis. The tensed metabolic condition of transition cows, arising due to energy deficit at the onset of lactation, is known to be associated with a reduced insulin sensitivity of adipose tissue, which is in support of energy mobilization and glucose shifting to the mammary gland. However, adipose cellular mechanisms to achieve this reduced insulin action are not well known. Therefore, this study aimed to describe the changes of expression and activation of signaling proteins involved in insulin transduction in subcutaneous and visceral adipose tissues of dairy cows around parturition. Twenty German Holstein cows were used to repeatedly collect subcutaneous (SCAT) and retroperitoneal adipose tissue (RPAT) biopsy samples at 42 days prepartum and at 1, 21, and 100 days postpartum. Protein expression and/or phosphorylation of key components of the insulin signaling pathway were detected by Western blotting. These included insulin receptor, phosphatidylinositol 3-kinase, protein kinase C ζ, protein kinase B, AMP-activated protein kinase, glucose transporter 4, fatty acid synthase, mammalian target of rapamycin and phosphodiesterase type 3. Expression data were analyzed for effects of periparturient time and adipose tissue localization by ANOVA. The expression of insulin receptor and most of its studied downstream signaling proteins were significantly lower at 1 day and at 21 days postpartum, compared with the prepartum state. At 100 days postpartum, expression of proteins was greater again, resembling prepartum levels. Not the insulin receptor, but a number of downstream signaling proteins had a greater abundance in SCAT than in RPAT. With regard to functional consequences, the current findings suggest that adipose tissues had a decreased capacity to respond to insulin during the early postpartum period, accounting for reduced insulin sensitivity. This can be identified as an essential mechanism in support of energy partitioning.

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Keywords: dairy cow, adipose tissue, transition period, insulin signaling

The insulin signaling cascade in the equine liver - acute response to insulin stimulation

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An impaired insulin signaling is a pathophysiological condition in the metabolic syndrome of horses. So far, the exact molecular localization of disruption of endocrine control is unknown. The objective of the study was to determine protein expression of components involved in the insulin signaling cascade and their extent of phosphorylation in equine liver as affected by variations in serum insulin concentration. Eleven warm blood horses were included in the study, age ranging from 4 to 24 years, body weight (bwt) from 440 to 695 kg and body condition score from 1.9 to 7.5. Horses were kept under standardized feeding and management conditions in the Clinic for Horses. Liver biopsies were collected under unstimulated basal conditions (B1) and under stimulated conditions. Stimulations were achieved by oral administration of glucose (naso-gastric intubation, 1 g glucose/kg bwt) with biopsy sampling 120 minutes after administration (B2) or with intravenous injection of glucose (150 mg/kg bwt) and insulin (0.1 IU/kg bwt) and biopsy sampling 12 minutes after injection (B3). This aimed to provoke variations in serum insulin concentrations. All horses underwent the three biopsies in a three week period with recovery and washout phases in between. In biopsy samples, concentration and extent of phosphorylation of key proteins of the insulin signaling cascade were semi-quantitatively determined using Western Blotting. While expression of signaling proteins did not differ in horses during different stimulations, there was a significant increase in extent of insulin receptor (InsR) phosphorylation comparing B1 with B3. Phosphorylated protein kinase B (PKB) also increased in B3 when compared with B1. However, AMP-activated protein kinase α (AMPK α) and phosphorylated AMPK α were not affected by different stimulations. The extent of phosphorylation was clearly related to serum insulin concentrations. Phospho-InsR was increased insulin concentration-dependently, but the inter-individual variation was high. The extent of phospho-PKB correlated well with the insulin concentration with only low inter-individual variations.

To conclude, the serum insulin concentration-dependent increase of phosphorylation of key components in insulin signaling appear to reflect activation of the cascade. However, endocrine responses of individual horses were quite variable, likely representing a spectrum of metabolic conditions regarding insulin sensitivity. Horses with a lower hepatic responsiveness of the insulin signaling cascade to stimulation may be on a higher risk to develop metabolic pathologies.

Keywords: horse, insulin signaling, liver

Suckling camels become low tolerant to glucose and less sensitive to insulin with the age

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Dromedary camels have evolutionary adaptations that enable them to live under extreme dry conditions. Despite consuming fibrous diets and producing glucose mainly by gluconeogenesis, the glycaemia of adults (90 to 125 mg/mL) duplicates that of true ruminants. Moreover, camels are known as poor insulinaemic and insulin resistant which seems to be related with water economy. With the aim of studying the metabolic changes induced by age, a total of 12 suckling camels (6 males and 6 females) at 2 ages: A (15 ± 3 d and 39.7 ± 1.8 kg BW; n = 6) and B (132 ± 19 d and 115 ± 5.8 kg BW; n = 6), were submitted to glucose tolerance tests (GTT) and insulin challenges (INSC). Glucose (0.25 g/kg BW, 50% solution) and insulin (4.6 µg/kg BW) were infused in the jugular vein after catheterization and fasting overnight. Treatments were randomly applied leaving 1 d of recovery after catheterization and between GTT and INSC. Blood samples were collected at 12 time-points (min –15 to 150) and plasma samples frozen (–20°C) until analysis by spectrophotometry (glucose, NEFA and BHBA) and bovine-ELISA (insulin). Basal glycaemia was lower in young camels (A vs. B: 121 ± 5 vs. 145 ± 3 mg/dL; $P < 0.01$), peaking at 5 min (A vs. B: 235 ± 6 vs. 290 ± 14 mg/dL; $P < 0.01$) and did not return to basal values in both camel groups after 150 min of GTT. Plasma insulin also peaked at min 5 in both groups, the greater values being those of young camels (A vs. B: 1.60 ± 0.63 vs. 0.41 ± 0.07 ng/mL, respectively; $P < 0.001$). Plasma NEFA decreased during GTT in both groups, reaching the minimum value at 20 min (0.37 ± 0.08 mmol/L, on average) followed by a slow increase thereafter. Regarding INSC, administration of insulin resulted in a parallel decrease of glycaemia in both groups (similar insulin sensitivity), the values of A being lower than those of B between 0 and 120 min ($P < 0.01$). Plasma NEFA decreased similarly in A and B camels during the first 20 min after INSC, increasing dramatically thereafter in group A, while steadied in group B. Plasma BHBA values were constant through the experiment and did not vary between groups. In conclusion, suckling camels increased their basal glycaemia with age, whereas they decreased their glucose tolerance, insulin secretion and insulin sensitivity, showing reduced signs of fat mobilization during glucose and insulin infusions.

Keywords: dromedary, glucose, insulin, hormonal challenge

Protein expression of free fatty acid binding receptor 2 in the liver of dairy cows with different lipid mobilization during late pregnancy and early lactation

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The free fatty acid binding receptor 2 (FFAR2), activated by short-chain fatty acids, is involved in the regulation of immune response, leptin secretion, adipogenesis and inhibition of lipolysis. It is expressed in adipose tissue, immune cells and intestine. FFAR2 knock out mice show reduced body fat mass and liver triglycerides. The FFAR2 expression is upregulated in the liver of rats fed a high fat diet. Here, we examined the protein expression of FFAR2 in the liver of dairy cows with different extent of lipomobilization during the peripartal period. In a previous study, multiparous German Holstein cows (2nd to 4th lactation, >10,000 kg/305 d) were fed a total mixed ration according to the recommendations of the German Society of Nutrition Physiology (GfE). Blood samples were taken weekly and liver biopsies were obtained at d −34, −17, +3, +18, and +30 relative to parturition as well as at slaughter (d +40). The samples were stored at -80 °C for further analysis. Selected animals were grouped according to their liver fat concentration in high (LFC > 30% in wet weight, n=5) or low (LFC < 30% fat in wet weight, n=6), or NEFA concentrations in high (NEFA > 700 mmol/L, n=6) or low (NEFA < 700 mmol/L, n=5). Protein expression of FFAR2 in liver biopsies was analysed by Western blotting and evaluated by repeated measures analysis using the mixed model of SAS. In animals grouped by NEFA concentrations or LFC, FFAR2 protein expression in liver increased after parturition ($P < 0.001$), but the effect was independent of high vs. low NEFA concentrations or LFC. The differential protein expression of FFAR2 with time indicates that this receptor could play an important role in liver metabolism of early lactating cows, although it may not have a relevant function in the regulation of hepatic lipid metabolism. An increment of short-chain fatty acids in plasma could be related, however, its regulation needs to be further elucidated.

Keywords: FFAR2, liver, dairy cows, lipid metabolism, short-chain fatty acids

Peripartal expression of free fatty acid binding receptor 1 in liver of dairy cows with differences in lipomobilization during early lactation

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The free fatty acid binding receptor 1 (FFAR1) binds medium and long-chain fatty acids and is involved in glucose stimulated insulin secretion and intestinal incretine release as shown in monogastrics. The receptor mRNA is expressed in different bovine tissues. In cows, highest mRNA abundance was detected in liver compared to adipose tissue, muscle and mammary gland. In mice the activation of FFAR1 alleviates hepatic steatosis. Excessive lipomobilization during early lactation of dairy cows is often associated with metabolic disorders linked with fatty liver and ketosis. The aim of the study was to analyze if individual differences in lipomobilization in early lactation of dairy cows are linked to the expression of FFAR1 in liver of late pregnancy vs. early lactation. For this purpose samples from multiparous German Holstein cows (2nd to 4th lactation, >10,000 kg/ 305 d) were used. The cows were fed a total mixed ration according to the recommendations of the German Society of Nutrition Physiology (GfE). Blood samples were taken weekly and liver biopsies were taken at d −34, −17, +3, +18, and +30 relative to parturition as well as at slaughter (d +40). The samples were stored at -80 °C for further analysis. For the study 13 cows were selected based on their beta-hydroxybutyric acid (BHBA) concentrations 2 to 3 wk post partum (pp) (high BHBA, > 1 mmol/L, n=8 vs. low BHBA, < 0.76 mmol/L, n=5). The FFAR1 protein was quantified by Western blotting. Data were analyzed by the mixed model of SAS using repeated measures analysis. Values for BHBA did not differ ante partum (ap) ($P < 0.05$) but were higher at d -30 in the high BHBA group ($P < 0.1$). The FFAR1 protein was not quantifiable in 3 high BHBA animals because of low band intensities. Animals with high BHBA concentrations pp had lower FFAR1 abundance at d -30, -17, +3, +40 ($P < 0.05$) and d +18 ($P < 0.1$). In conclusion, higher BHBA concentrations after parturition are associated with lower FFAR1 abundance at specific points in time pp and ap. Lower FFAR1 expression ap might be related to disturbances in lipid metabolism pp. However, mechanisms linking FFAR1 expression in liver of dairy cows with free fatty acid concentrations or ketone body formation must be disclosed in future studies.

Keywords: FFAR1, liver, dairy cow, BHBA, free fatty acids

Metabolic and inflammatory challenges affect cortisol secretion in dairy cows

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Cortisol in dairy cows is released episodically with a circadian rhythm. This study investigated the effects of long-term (56 h) manipulated metabolic states, i.e. manipulated plasma concentrations of glucose and BHBA, on the release of cortisol. Besides the concentration of cortisol, its pulsatile secretory pattern was studied in combination with an acute immune challenge through an intramammary LPS challenge.

Twenty-five mid-lactating dairy cows (parity: 3.1 ± 1.2, 28 ± 5 weeks post-partum; mean ± SD) were randomly assigned to one of four treatments: a hyperinsulinemic hypoglycemic clamp (HypoG, n = 6), a hyperinsulinemic euglycemic clamp (EuG, n = 6), infusion of β-hydroxybutyrate (HyperB, n = 5) or an infusion with physiological saline solution for the control group (NaCl, n = 8) for 56 h. Beginning at 48 h of infusion, an intramammary LPS challenge was performed. Blood samples for analysis of cortisol were taken every hour beginning 24 h after the start of infusions. Intra- and inter-assay CV for cortisol were 8.5 and 9.1%, resp. The cortisol profiles from 09:00 a.m. to 05:00 p.m. of the 2nd and 3rd day during infusions were analyzed using the PULSAR program designed for the evaluation of episodic hormone release. Data of cortisol concentration and characteristics of its pulsatile release were analyzed by the GLM procedure of SAS (Version 9.2), including treatment (HypoG, EuG, HyperB or NaCl), and day of infusion (without or with LPS treatment) as fixed effects. Differences between means were localized by Tukey's test at $P < 0.05$.

Different metabolic states induced by infusion treatments affected the characteristics of cortisol secretion (elevation of baseline (HypoG, HyperB) and decrease of peak length (HypoG)), while amplitude, peak interval, height, peak area, area under the curve (AUC) above baseline (AUCb) and the total AUC above the 0-line (AUCt) were not different between infusion treatments. The induced inflammatory response due to the intramammary LPS challenge for the last 8 h at simultaneously maintained infusion treatments diminished the pulsatile nature of cortisol release, while AUCb (and AUCt, respectively) was lowest for HypoG compared to HyperB and NaCl.

This study indicates that single metabolites (glucose, BHBA) and their availability or turnover (glucose) have a different impact on the regulation of cortisol secretion resulting in changes of its pulsatile release. Cortisol release during intrammammary inflammation was found to be higher in HyperB, EuG and NaCl compared to HypoG. This finding emphasizes the regulatory role of the current metabolic status on the cortisol release during inflammation.

Keywords: cortisol, pulsatility, secretion pattern, metabolism

Causes for culling in Swiss dairy cows – effects of metabolic load on longevity

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For the last decades, productive span of life in dairy cows decreased despite improved management conditions. Metabolic disorders, impaired udder health etc. occurring during early lactation account for culling in dairy cows. This study investigated if metabolic load in early lactation is associated to the age and life-time performance when animals reached thetime of culling.

In total, 232 multiparous (housed at 64 Swiss dairy farms) and metabolically stressed cows (milk fat-protein-ratio >1.5 in previous early lactation) entering at least their 3rd lactation were selected in 2007/08. Blood was sampled in week 4 pp and analyzed for NEFA, BHBA, and IGF-1. Age at culling, causes of culling, days lactating, and total life-time performance in milk yield were recorded. Animals were grouped by age at culling (group 1: parity 3+4, group 2: parity 5+6, group 3: parity 7+8, group 4: parity 9-17) and their metabolic load in early lactation determined in the field study as well as life-time performance and causes for culling were compared among groups.

There was no obvious difference in mean values and range of NEFA, BHBA [mmol/L] and IGF-1 [ng/mL] between groups differing in age at culling (group/mean/range min-max): NEFA (1/ 0.36/ 0.05-0.97), NEFA (2/ 0.33/ 0.05-0.83), NEFA (3/ 0.29/ 0.06-0.96), NEFA (4/ 0.33/ 0.09-0.80), BHBA (1/ 1.71/ 0.2-5.7), BHBA (2/ 1.39/ 0.3-5.5), BHBA (3/ 1.47/ 0.4-4.6), BHBA (4/ 1.67/ 0.4-4.9), IGF-1 (1/ 74.9/ 14.5-136.7), IGF-1 (2/ 70.8/ 26.5-135.8), IGF-1 (3/ 67.2/ 25.6-117.0), IGF-1 (4/ 63.5/ 23.6-160.8). Information about the reasons for culling of 206 cows was available. The main reasons for culling depended of the age at culling (age group 1: bad milkability, teat trauma, accident, acute liver failure, etc.; age groups 2 and 3: fertility; age group 4: senility). Life time performance increased with age of groups (group 1: 27`188 kg, group 2: 39`716 kg, group 3: 56`261 kg, group 4: 76`854 kg). Average milk yield per day of life increased with age at culling (group 1: 12.7 kg/d, group 2: 13.1 kg/d, group 3: 14.6 kg/d, group 4: 16.5 kg/d). Average milk yield per day in milk was lowest in groups 1 and 2 (23.8 kg/d and 23.7 kg/d, resp.) and was higher in groups 3 and 4 (24.2 kg/d, 24.7 kg/d, resp.).

Contrary to wide spread assumptions, a higher metabolic load in early lactation did not necessarily result in an earlier culling of dairy cows, although they might be more prone to metabolic disorders. Longevity was shown to have a positive impact on life-time performance of cows.

Keywords: longevity, robustness, metabolism

Long term effects of rearing intensity during early postnatal life on the development of the endocrine pancreas and on growth of the testes in Holstein-Friesian bull calves

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Long term effects of early postnatal development on growth and function of tissues and organs have been reported for various mammalian species. The objective of this study was to assess the impact of two rearing strategies during the neonatal period on the development of the insulin producing pancreatic ß-cells and the morphology of testes of Holstein-Friesian bull calves. During the first 3 weeks [wk] of life calves were fed either intensively (INT; ad libitum whole milk feeding; individual hutches; N=21) or according to an established restrictive rearing protocol (CON; 4 L whole milk/d during week 1 in hutches, 720 g/d milk replacer from days 8–21 in group pens; N=21). Thereby, the average daily gain was higher in INT-calves compared to CON calves during the first 3 wk of life ($p<0.001$). INT calves displayed increased blood glucose, insulin and IGF-1 concentrations in wk 3 of life compared with CON calves (all $p<0.05$). From d 22 of life, all calves were housed and fed similarly. Higher serum testosterone levels were analysed in INT calves in wk 10 of life compared with CON calves (0.26 vs. 0.17 ng/ml; $p=0.02$). At an age of 8 months all animals were slaughtered. Tissue from the medium body of the pancreas was removed and fixed in formaldehyde. By immunohistochemistry, the number of pancreatic islets of Langerhans per microscopic field of view was determined manually and the total area of insulin stained cells per photograph was obtained using a photomicroscope with an image processing software. In INT calves, more islets of Langerhans compared to CON calves were found (9.1 ± 0.3 vs. 7.8 ± 0.3 per microscopic field of view; n=6 -18 per calf; $p<0.01$). The total insulin stained area was larger in INT calves compared to CON calves (0.107 ± 0.005 mm2 vs. 0.084 ± 0.005 mm2; n=5 per calf; $p<0.01$). At slaughter testes of all animals were measured and weighed. The volume was determined by placing each testis into a measuring cup filled with water. No differences were found in measures, weights and volumes of the testes between both groups (all $p>0.05$).

In conclusion, the observed alterations in pancreatic insulin producing tissue imply that the rearing intensity during the neonatal period has long-term consequences. This finding supports the theory of a possible metabolic programming in intensively reared calves. Consequences for future performance particularly in dairy cows warrant further investigations.

Keywords: calves, pancreas, rearing intensity, testes

Performance, metabolic changes and endocrine regulation of growth in calves fed milk ad libitum after birth

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Potential for body growth in neonatal calves is not exhausted when milk is fed restrictively. Restricted milk feeding during the pre-weaning period is supposed to stimulate solid feed intake, but solid feed intake is generally very low during the first 4 wk of life. The objective of the study was to compare feed intake, growth performance, systemic and hepatic energy metabolism as well as endocrine growth regulation in calves with two different milk feeding levels. The hypothesis was tested that intensive milk feeding supports growth and metabolic and endocrine changes in blood and liver, and finally enhances body growth. Four d after birth, 28 Holstein × Charolais calves (male and female) were fed either 6 L milk replacer (MR; 125 g powder/L)/d for 8 wk (RES; n=14) or unlimited amounts of MR up to d 35 of life (AL; n=14) that was stepped down to 6 L/d afterwards. Concentrates and hay were available ad libitum for both groups. Blood samples were taken weekly for determination of plasma concentration of glucose, triglycerides, urea, insulin, IGF-I and IGF binding proteins (IGFBP). Calves were slaughtered at d 60 ± 2 and liver samples were taken for measurement of glycogen concentration and mRNA abundance of IGF-I, IGF-I- and insulin receptor, IGFBP, glucose-6-phosphatase, pyruvate carboxylase, cytosolic phosphoenolpyruvate carboxykinase (PCK1) and glucose transporter GLUT2 by quantitative real-time PCR. Data were analysed by the Mixed Model of SAS with feeding, sex and time (when relevant) as fixed effects. MR intake increased ($P < 0.001$) in AL to 14.5 ± 0.4 L/d in wk 5 of life, but did not change in RES calves. Concentrate intake increased equally ($P < 0.001$) in both groups from wk 4 onwards. BW was greater ($P < 0.001$) in AL than in RES calves. Plasma concentrations of triglyceride, glucose, insulin and IGF-I were higher ($P < 0.05$), and concentrations of urea and IGFBP-2 and -4 were lower ($P < 0.05$) up to d 35 of life in AL than in RES calves. Hepatic mRNA abundance of PCK1 was greater ($P < 0.05$) in AL than in RES calves, indicating possible influence of intensified milk feeding on gluconeogenesis. The hepatic somatotropic axis at slaughter was not influenced by milk feeding. Intensive milk feeding resulted in enhanced body growth and changes in metabolic and endocrine traits supporting anabolic metabolism, but had no long-lasting effects on systemic or hepatic growth regulation.

Keywords: calf, ad libitum feeding, energy metabolism

Colostrum versus formula feeding: effects on the mRNA abundance of genes related to protein synthesis and proteolysis in three different skeletal muscles of neonatal calves

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The rates of protein turnover are higher during the neonatal period than during any other stage of postnatal life. The mammalian target of rapamycin (mTOR) and the ubiquitin-proteasome system (UPS) are regarded as the major regulators of protein synthesis and protein degradation in muscle, respectively. The signals activating the mTOR pathway include amino acids (AA) and also insulin. We hypothesized that the expression of mTOR signaling and UPS-related genes in skeletal muscle of neonatal calves will be divergently affected by colostrum (COL) versus formula (FOR) feeding and will be related to the circulating concentrations of AA and insulin. Calves were fed COL (n=7) or milk-based FOR (n=7) up to 4 days (d) of life. Nutrient content (e.g. gross energy, crude protein and fat) in FOR and COL was similar, but FOR had lower concentrations of free branched-chain (BCAA) and total AA than colostrum. Blood samples were taken from d 1 to d 4 before morning feeding and on d 4 before and 2 h after feed intake. Muscle samples from M. longissimus dorsi (LD), M. masseter (M), and M. semitendinosus (S) were collected after slaughter on d 4 at 2 h after feeding. The preprandial concentrations of insulin, BCAA and total AA in plasma changed ($P \leq 0.01$) over time in both groups but did not differ between COL and FOR. Plasma insulin concentrations increased ($P < 0.05$) after feeding in both groups at d 4, but were higher ($P < 0.01$) in COL than in FOR. Plasma BCAA and total AA concentrations decreased ($P < 0.01$) in COL, whereas they increased ($P < 0.01$) in FOR after feeding, resulting in higher ($P < 0.05$) postprandial plasma BCAA and total AA concentrations in FOR than in COL. The mRNA abundance of mTOR (LD, $P = 0.03$; M and S, $P = 0.07$) and of ribosomal protein S6 kinase (LD, $P = 0.07$; M and S, $P < 0.01$) was greater or tended to be greater in COL than in FOR, respectively, whereas that of eukaryotic initiation factor 4E-binding protein was unaffected by diet. The mRNA abundance of ubiquitin activating (UBA1) and conjugating (UBE2G1) enzymes was not affected by diet, whereas that of UBE2G2 was greater (S, $P = 0.02$) or tended to be greater (LD, $P = 0.08$) in COL than in FOR. The mRNA abundance of atrogin-1 in LD and M muscles was lower ($P \leq 0.04$), whereas that of muscle ring-finger protein 1 was greater ($P = 0.02$) in COL than in FOR. The results indicate that colostrum feeding might stimulate muscle protein deposition in a muscle type specific manner, assumingly mediated by the postprandial rise in plasma insulin.

Keywords: mTOR, ubiquitin-proteasome system, muscle, calf

Growth hormone receptor (GHR) and Insulin-like Growth Factor 1 (IGF-1) expression in bovine primary hepatocytes: potential model for endocrine studies?

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The liver becomes resistant to GH during the transition period in dairy cattle. This is thought to be caused by decreased insulin. However, several other hormones (e.g. estradiol, triiodothyronine) are known to have an effect on the GHR-expression and -signal transduction. In order to study the influence of different hormones on the hepatic GHR, this study aimed in establishing a primary hepatocyte cell culture from arbitrary derived bovine liver, expressing a functional GHR. Therefore, the first aim was to establish a protocol to obtain primary hepatocytes. The second aim was to characterize their morphology and to measure mRNA expression of GHR1, IGF-1 and apoptosis markers: Bax, Bcl2-xL, Fas Ligand.

Cannulas were inserted in four vessels at the cut surface of the liver and fixed by tissue glue. A three-step-collagenase perfusion was performed until the extracellular matrix was abrogated. After washing, the viable hepatocytes were separated using Easycoll™. Viability of hepatocytes was proven by Trypan blue staining. The cells were seeded on collagen coated six-well plates. After 4 h adhesion, medium was replaced and the cells were either cultured (- fetal calf serum, 37°C, 5% CO_2) for 2 d in a monolayer or with a soft collagen overlayer (sandwich culture). After establishing this protocol, the monolayer procedure was repeated and samples for mRNA-expression were taken 20 min after slaughtering, after Easycoll™ purification and after 2 d (monolayer, n = 6 each).

The mean viability was 86 ± 7 % after purification. In the sandwich culture, the cells changed morphologically from roundish-spherical to polygonal hepatocyte like cell shape and build cell-cell contacts. The mRNA-expression of all apoptosis markers stayed low during the separation procedure, but increased after 2 d in monolayer culture ($P < 0.05$). The GHR-expression (mean ± SD, 48.4 ± 7.3) decreased after purification (30.9 ± 9.6; $P = 0.0155$), whereas IGF-1 expression stayed constant (2.87 ± 0.94 vs. 2.81 ± 1.10; $P = 0.9288$). Both, the GHR1A- (2.3 ± 1.2; $P = 0.0005$) and IGF-1-mRNA expression (0.75 ± 0.42; $P = 0.0268$) decreased further after 2 d cell culture (monolayer). These results show that a high viability of hepatocytes can be achieved from arbitrary derived liver. However, GHR1A and IGF-1 expression decreased. As parallel experiments in rat hepatocytes indicate, the GHR-expression increases again after 4 d in culture with a firm collagen overlayer; therefore further optimization of the primary cell culture is needed to ensure a functional GHR.

Keywords: growth hormone, IGF1, bovine hepatocyte

Responses of heat-stressed dairy goats to lipogenic and lipolytic signals

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Heat stress (HS) induces hormonal changes, but little is known about these changes in dairy goats. Eight multiparous Murciano-Granadina dairy goats (43.3 ± 1.6 kg BW; 2 ± 0.04 L/d; 81 ± 3 DIM) were kept in metabolic cages and randomly assigned to 2 climatic treatments according to a crossover design (two 28-d periods). Treatments were: 1) thermal neutral (TN; 15 to 20°C, 40 to 45% humidity, THI = 59 to 65), and 2) heat stress (HS, 12 h/d at 37°C and 40%, and 12 h/d at 30°C and 40%, THI = 86 and 77, respectively). Jugular silicon catheters were fitted, and insulin challenge, epinephrine challenge, and glucose tolerance test were done on different days. The insulin (4.6 µg/kg BW), epinephrine (2 µg/kg BW) and glucose (0.25 g/kg BW) solutions were administrated via the jugular catheter. Blood samples were collected at −30, −20, −10, 0, 5, 10, 20, 30, 45, 60, 90, and 120 min relative to the administration for analysis of plasma insulin, NEFA, and glucose concentrations. The DMI decreased ($P < 0.001$) by -29% as a result of HS treatment. Goats in both groups had similar blood NEFA after insulin injection, but NEFA values were greater ($P < 0.05$) in TN than HS goats after epinephrine administration. The HS goats secreted lower ($P < 0.05$) amounts of insulin than TN goats in response to the glucose tolerance test. In conclusion, body lipid tissue of HS goats became more resistant to lipolytic stimuli making them unable to mobilize body fat reserves despite the negative energy balance.

Keywords: heat stress, hormones, metabolism, dairy goats

Dairy cows selected for high RFI exhibited greater plasma cortisol response to an ACTH challenge during mid-lactation and the dry period

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The energy expended on stress responses can be a source of inefficiency in livestock production systems as this energy becomes unavailable for production. This experiment examined a physiological stress response in lactating and non-lactating dairy cattle selected based on residual feed intake (RFI) measured as growing calves (8 high RFI and 8 low RFI cows of 2nd and 3rd parity were used).

An adrenocorticotropin hormone (ACTH) challenge was conducted on each cow during both mid-lactation (122 ± 23 days in milk) and the non-lactating period (dry period; approximately 38 days post cessation of milking). Cows were housed in metabolism stalls for the challenge and were fed a diet of alfalfa cubes ad libitum for at least two weeks prior to the experiment (lactating cows were also offered an additional 6kg DM crushed wheat grain plus minerals fed at milking) and were fasted for 12 hours prior to the challenge. Starting at 0830 hours, ACTH (synacthen, 2µg/kg body weight) was infused via a jugular catheter and blood samples were collected at regular intervals pre- and post-infusion. Plasma was analysed for cortisol, non-esterified fatty acid (NEFA) and glucose concentrations.

The efficiency of conversion of feed into milk (the ratio of feed consumed to milk produced over the 7 days prior to the experiment) during mid-lactation was better (lower) in low RFI cows (0.86 vs. 1.02 kg/kg, P=0.032), although dry matter intake (DMI) did not differ due to RFI group in either mid-lactation or during the dry period (P>0.50). Low RFI cows exhibited a lower overall plasma cortisol concentration (P=0.035) than high RFI cows in both mid-lactation and the dry period (132 vs. 165 in mid-lactation and 123 vs. 131 nmol/L during the dry period for low and high RFI respectively) and this difference was greater during mid-lactation (P<0.001). Plasma NEFA concentrations were similar for high and low RFI cows, while average overall plasma NEFA concentrations were lower in mid-lactation compared to the dry period (160.0 vs. 300.9 µM for mid-lactation and the dry period respectively, P<0.001). Plasma glucose concentrations were consistently higher (P=0.02) in low RFI cows and higher during mid-lactation (4.7 vs. 4.3 mM) compared to the dry period (3.7 vs. 3.5 mM).

These data suggest that high RFI cows are more stress responsive than low RFI cows, supporting the concept that energy utilized for stress responses may be a source of energy wastage that contributes to loss of productivity in high RFI dairy cattle.

Keywords: RFI, ACTH, cortisol, efficiency and dairy cattle

Salivary cortisol as a biomarker for plasma cortisol during ACTH challenge and at variable salivary consistency

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Modern and sustainable dairy farming implies the consideration and improvement of animal welfare. Non-invasive sampling is crucial for unbiased results. The implementation of feasible biomarkers, however, must be robust to various physiological and environmental conditions.

Three experiments were performed to validate the suitability of saliva cortisol measurement: the first during stimulation of the adrenal glands, the second one during various events affecting the composition and consistency of saliva, and the third one to assess the rhythm of cortisol release during the development of inflammatory conditions. In exp. 1, ACTH (16 µg/100 kg body weight) was administered i.v. in 23 multiparous Holstein dairy cows in week 3 postpartum, and blood was sampled in parallel to saliva (collected by Salimetrics swabs) at 15 minutes intervals for 3 h relative to ACTH injection. Exp. 2 aimed to evaluate the effects of variable saliva consistency and composition on its cortisol concentration. Saliva and blood samples were taken in parallel from six cows before, during and after (5 and 15 minutes) drinking, feeding and ruminating. Exp. 3 is currently still running. Saliva and blood samples are taken at 2-minute intervals after an intramammary application of LPS leading to an inflammatory response with elevated concentrations of cortisol. Each saliva sample was taken immediately after the respective blood sample. Cortisol in saliva was measured by EIA (Salimetrics) and cortisol in blood plasma by RIA.

In exp. 1 and 2, the profiles of cortisol concentrations were similar in blood plasma and saliva. Saliva and blood cortisol concentrations were closely correlated during the ACTH challenge ($r = 0.75$) with significantly increased plasma cortisol concentrations following ACTH administration when compared to basal pre-injection values. Cortisol concentrations in plasma were consistently higher compared to saliva during the ACTH challenge. In saliva samples taken during different feeding and drinking actions, cortisol concentration in saliva was correlated to the cortisol concentration in blood. Saliva cortisol concentration was found not to be affected by salivary composition and consistency. This is an important finding emphasizing the power of saliva cortisol mirroring plasma cortisol for non-invasive stress estimation. In conclusion, saliva can be confirmed an ideal non-invasive substrate for robust cortisol determination in dairy cows, independent of physiological status and consistency of saliva.

Keywords: cortisol, saliva, dairy cow

A natural gain-of-function variant of the porcine glucocorticoid receptor has a major effect on HPA axis activity

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Glucocorticoids play a vital role in the maintenance of basal and stress-related homeostasis and influence productivity, health, and well-being of farm animals. Their production is under the control of the hypothalamic-pituitary-adrenocortical (HPA) axis. We performed genetic studies of the HPA axis in German Landrace pigs and showed that a natural substitution Ala610Val in the ligand binding domain of the glucocorticoid receptor (GRAla610Val) is a causal quantitative trait nucleotide for activity of the axis and consequently cortisol production. Functional studies of the receptor revealed that the GRAla610Val substitution increases its affinity and sensitivity to glucocorticoids, and thus most likely enhances negative feedback regulation of the HPA axis. In fact, homozygous carriers of the more sensitive Valine variant display about 1.5- to 2-fold reduction in parameters of HPA axis activity including expression of CRH gene in the hypothalamus, plasma ACTH and cortisol levels, adrenal weight and size of the adrenal cortex. The activity of the HPA axis is apparently persistently reduced by GRAla610Val, because we found significantly reduced cortisol levels (basal and stress-induced) in carriers of the substitution at all studied ontogenetic stages from first week post natum until slaughter at ~160 days post natum. We confirmed the large negative effect of the substitution on HPA axis activity (reduction of cortisol levels and adrenal weight at slaughter) also in German Large White and in a Pietrain×(German Large White×German Landrace) cross. Due to its large and robust effect the GRAla610Val substitution should be accounted for in future studies including parameters of HPA axis activity in pigs.

Examination of the effects of the GRAla610Val substitution on production traits yielded conflicting results; we found association of the substitution with increased fat and decreased muscle deposition in a population of commercial German Landrace pigs, however we could not replicate the association in other populations. Our current and ongoing studies concentrate on characterization of the effects of the GRAla610Val substitution on health and animal-welfare related traits, and on further elucidation of physiological and molecular mechanisms underlying its phenotypic consequences.

Keywords: HPA axis, glucocorticoids, cortisol, stress response, glucocorticoid receptor, causal mutation

The effect of diet and exercise on plasma hormone concentrations in horses measured before and after a standardized exercise test

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The effect of diet and exercise on plasma insulin and cortisol is well studied in horses, whereas little information exists on the plasma concentrations of ghrelin, leptin and IGF-1. This experiment was designed as a 4 x 4 Latin square with four experimental periods of 28 days each.

Four Norwegian Cold-blooded trotter horse geldings with a body weight (BW) of 542±17 kg were used in the study and were fed 4 iso-energetic diets. The dietary treatments were 2 fibre based diets: hay only and hay (85% of dry matter intake (DMI)) supplemented with molassed sugar beet pulp (mSBP) (15% of DMI), or two barley based diets of hay (68% of DMI) and barley (32% of DMI), and hay (68% of DMI), barley (26% of DMI) and mSBP (6% of DMI). The daily ration was divided into four meals and fed at 0600h, 0700h, 1600h and 2200h; barley and mSBP were fed at 06:00 h and hay at 0700h. Starch intake at 0600h was similar (2g starch/kg BW) for the 2 barley based diets.

Blood samples were collected on day 25 or 26 by jugular vein puncture into 10ml heparinized tubes before feeding the morning meal at 0600h, before a standardized exercise test (SET) (1000h), after the SET (1045h) and after recovery (1500h). The blood samples were centrifuged immediately after sampling at 3.000g for 10min and plasma was harvested and stored at -20°C for later analysis of plasma hormones. Plasma insulin, cortisol, leptin, ghrelin and IGF-1 were measured in plasma using radioimmunoassay methods.

Results were analysed as repeated measurements using the MIXED procedure in SAS, and effects were considered significant if $P<0.05$. An interaction between diet and time was present for plasma insulin ($P<0.01$). It increased after feeding the barley based diets but remained stable when feeding the fibre based diets. Insulin had returned to baseline values after exercise. Cortisol was affected by time ($P<0.001$) and by diet ($P=0.026$), increasing after exercise on all diets, and plasma concentrations were higher when feeding the barley based diets than the fibre based diets. Plasma leptin increased after the SET ($P=0.036$). However, there was no effect of diet or exercise on the plasma concentrations of ghrelin and IGF-1.

This study showed that time of sampling in relation to diet and exercise had no effect on the plasma concentrations of ghrelin and IGF-1, whereas exercise affected the plasma concentration of leptin. These results are important when evaluating results of hormone analyses in studies where diet and exercise cannot be controlled (e.g. in field studies).

Keywords: diet, exercise, hormones, horse

Metabolite dynamics during infusion of 5-hydroxytryptophan to multiparous dairy cows

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The transition from pregnancy to lactation is a metabolically demanding time for dairy cows. Due to copious milk production, dairy cows are susceptible to many transition-related disorders, including hypocalcemia. Hypocalcemia increases adverse health events and production losses, and negatively impacts animal welfare. Serotonin (5-HT) is critical for regulation of calcium (Ca) metabolism during lactation and is therefore a potential therapeutic target in the prevention of hypocalcemia.

Twelve multiparous (avg. lactation number 3.67 ± 0.43) Holstein dairy cows were intravenously infused for 5.75 ± 0.82 d pre-partum until calving, with either saline (n=6) or 1.0 mg/kg 5-hydroxytryptophan (5-HTP; n=6), the immediate precursor to 5-HT synthesis. Blood samples were collected immediately prior and following infusion, and at 2 h, 4 h, and 8 h post-infusion. Serum and plasma were harvested and total Ca, glucose, and non-esterified fatty acids (NEFA) were measured using colorimetric assays. All statistical analyses were performed in SAS using an ANOVA for repeated measures.

The d of infusion as determined by Area Under the Curve (AUC) had a significant effect on Ca (P = 0.0021), glucose (0.0595), and NEFA (P = 0.0001), with Ca highest on d 1, glucose lowest on d 2, and NEFA highest on d 3 of infusion. There was also a treatment by d interaction for NEFA (P = 0.0035). Within the infusion d, hour had an effect on Ca (P = 0.0304) and there was a treatment by hour interaction for Ca (P = 0.0005). Cows infused with 5-HTP had the highest Ca concentrations before infusion (2.878 ± 0.068), the lowest at 2 h (2.459 ± 0.063), recovering at 4 h and 8 h post-infusion, while saline cows' highest Ca was at 8 h (2.793 ± 0.093). Glucose concentrations tended to be affected by the hour of infusion (P = 0.1272). Hour had a significant effect on NEFA (P < 0.0001) as well as a treatment by hour interaction (P = 0.0005). NEFA peaked in 5-HTP cows immediately after infusion (416.483 ± 53.779) and were lowest at 4 h (182.006 ± 21.261), recovering by 8 h post-infusion. There was no significant change in NEFA concentrations in response to saline infusion. Taken together, these data suggest that infusion of 5-HTP pre-calving may improve Ca and NEFA mobilization required during the transition period to support milk synthesis and maternal metabolism.

Keywords: hypocalcemia, serotonin, calcium

Effect of medium-chain fatty acids on the plasma concentrations of metabolic hormones and metabolites in lactating cows

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Feeding medium-chain fatty acids (MCFA) to ruminants is well documented to modify the rumen environment such as exhibition of anti-protozoal activities and decrease of methane emission. However, there is limited information regarding the effects on metabolic traits, such as endocrine functions that regulate nutrient metabolism. The aim of this study was to determine the effect of calcium salts of MCFA (MCFA-Ca) on plasma hormone and metabolite concentrations in lactating cows.

Eleven multiparous Holstein cows (days in milk 70 ± 5) were used in a crossover design during each 14-day period. Diet administered included 1.5% (dry matter) MCFA-Ca (C8: 0–80%, C10: 0–20%) supplemented with a total mixed ration and without a supplement. To elucidate the metabolic traits, cows were fed diets with equal amounts of dry matter during each treatment. Periprandial blood samples were taken on day 14, and plasma concentrations of hormones (growth hormone, insulin-like growth factor 1, insulin, glucagon, and ghrelin) and metabolites (glucose, ketone bodies, non-esterified fatty acids, triglyceride, and total-cholesterol) were analyzed. Data were analyzed using the mixed model. The level of significance was set at $P < 0.05$.

Dry matter intake did not differ between treatments; however, milk yield tended to be lower ($P = 0.096$) and body weights were higher ($P < 0.05$) in cows fed an MCFA-Ca diet. MCFA-Ca increased plasma ghrelin and glucagon concentrations ($P < 0.05$). The postprandial decrease in plasma glucose and increase in ketone bodies was augmented by feeding MCFA-Ca ($P = 0.05$), suggesting that the ingested MCFA were rapidly β-oxidized and metabolized to ketone bodies, or produced energy in the liver. Increased plasma glucagon by MCFA may be due to low glucose levels. Plasma ghrelin concentration might be increased by MCFA ingestion, because conversion of ghrelin to its active form requires acylation by octanoic acid.

These results suggest that feeding MCFA alters nutrient availability and hormone secretion independent of dry matter intake, resulting in altered milk production and body restoration after calving.

Keywords: ghrelin, glucagon, medium-chain fatty acids

Effects on general health, energy status and calcium metabolism of a suspension of glucogenic precursors, vitamins and minerals supplemented to dairy cows immediately after calving

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A suspension of glucogenic precursors, vitamins and minerals (Reviva™) was supplemented to dairy cows immediately post-calving to evaluate the potential effects on energy status and calcium (Ca) metabolism as well as the overall health of the cow.

A total of 44 primi- and multiparous Holstein or Holstein x Friesian dairy cows completed the study, which started 3 weeks before their expected calving date and ended 4 weeks post-calving. Pre-calving the ration consisted of maize silage, hay, straw and dry cow concentrate. Post-calving cows received grass and maize silage, hay and concentrates. There were 22 cows per treatment with primiparous, 1st and 2nd lactation cows (as categorized at the beginning of the study) blocked within parity by expected calving date. Cows of parity 3 or greater were blocked by expected calving date. Cows were randomly assigned to the 2 treatments in their 7th month of pregnancy. The control group was offered 20 L of warm water (25-30°C); the treatment group 20 L of the aqueous suspension (25-30°C). Voluntary consumption was characterized into one of 3 categories; no consumption, partial consumption and full consumption. Of the control group, 4 cows (18%) did not voluntarily consume all of their water and the remaining quantities were drenched. Of the treatment group, all cows voluntarily consumed all of the aqueous suspension. Observations were analyzed as repeated measures with the MIXED procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC). The model included the fixed effect of treatment, treatment x time interaction, the random effect of block, and time as the repeated model statement.

There were no significant treatment effects on the general health parameters that were monitored, post-calving total dry matter intakes (control 18.8±0.7 kg/day vs treatment 17.9±0.7 kg/day; p=0.25) or milk yield (control 33.8±1.3 kg/day vs treatment 33.1±1.3 kg/day; p=0.60). Furthermore, after calving there was no significant treatment effect on serum insulin (control 0.46±0.05 µg/L vs treatment 0.42±0.05 µg/L; p=0.37). Post-calving serum Ca was significantly higher in the treatment group (2.27±0.04 vs 2.18±0.04 mmol/L; p=0.05), a difference significantly sustained for the first 48 hours post-calving. Most likely the Ca provided via the aqueous suspension increased the gastrointestinal Ca pool helping to maintain calcaemia during the critical period for Ca homeostasis. Serum magnesium (Mg) was significantly lower in the Reviva treatment (0.91±0.01 vs 0.93±0.01 mmol/L; p=0.03), which may reflect the known regulatory interaction between Ca and Mg.

Keywords: Ca, Mg, dairy cow

Effects of Sun Exposure and Intake of Pasture Grass on Serum 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 Concentrations and Milk Composition in Cows

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Dairy cows are housed in a cowshed for a major part of the day, and hence, they mainly rely on the feed for intake of vitamin D (vitD). As the in vivo contribution of sunlight-induced vitD is unclear in dairy cows, we investigated the effects of exposure to sun and of the intake of pasture grass on serum 25-hydroxyvitamin D3 [25(OH)D3] and 1,25-dihydroxyvitaminD3 [1,25(OH)2D3] concentrations and milk composition in cows.

Seven Holstein cows were subjected to 3 treatments that each for 2 weeks: (1) kept in a shaded cowshed in individual tie stalls (SHED); (2) outside in a paddock, which lacked pasture, for 5 h each day (PAD); (3) outside on pasture land for 5 h each day (PAS). Blood and milk samples were collected on the final day of each treatment to determine serum 25(OH)D3, 1,25(OH)2D3, Ca and iCa concentrations and milk 25(OH)D3 concentration. Intensity of solar radiation was measured by a weather observation network system.

Intensity of solar radiation was slightly higher under PAD conditions ($P < 0.1$) and was higher under PAS conditions ($P < 0.01$) than under SHED conditions. Serum 25(OH)D3 concentrations after the PAS treatment were higher than after PAD treatment ($P < 0.05$). Milk 25(OH)D3 concentration after PAD treatment was slightly higher than in the SHED group ($P < 0.1$). Serum Ca concentration in the PAS treatment was slightly higher than in the SHED and PAD treatment groups ($P = 0.1$). Serum 25(OH)D3 and iCa concentrations showed a significant positive linear correlation ($r = 0.841$; $P < 0.01$). Most mammals depend on sunlight to produce their required vitD. In the skin, UVB photons are absorbed by 7-dehydrocholesterol, which gets converted to vitD3. VitD3 is metabolized in the liver to 25(OH)D3 and then in the kidney to 1,25(OH)2D3, which contributes to maintenance of serum Ca and P levels. Our investigation indicated that sunlight was likely an important route for vitD synthesis in dairy cows, while intake of VitD2 from pasture grass contributed to increased serum 25(OH)D3 concentrations.

Our investigation suggested that sunlight and intake of pasture grass increases serum 25(OH)D3 concentration, but not serum 1,25(OH)2D3 concentration, in dairy cows.

Keywords: grazing, sunlight, pasture, 25-hydroxyvitamin D3, 1,25-dihydroxyvitaminD3

Shift of association between insulin serum concentration and phosphorylation of hepatic FoxO1 in dairy cows in transition period

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Forkhead box protein O1 (FoxO1) promotes the transcription of glucose-6-phosphatase (G6P) and cytosolic phosphoenolpyruvate carboxykinase (PCK1), genes involved in gluconeogenesis. In monogastric species, this transcriptional activity is inhibited by insulin-induced phosphorylation, thereby decreasing gluconeogenesis. The objective of this study was to investigate associations between plasma insulin concentration and expression of FoxO1and gluconeogenic enzymes as affected by lactation period and diet in dairy cows.

Twenty-one pluriparous German Holstein cows were fed with diets containing 0 g (C) or 24 g (N) niacin supplementation from -42 days related to calving (d-42) to d24. Each group was further divided and received diets with 30% (L) or 60% (H) of concentrate on dry matter basis from d-42 to d0. The dietary concentrate portion was set to 30% at d0 for all, and then increased up to 50% within 16 days for CL (n=5) and NL (n=5) and within 24 days for CH (n=5) and NH (n=6) to enhance lipid mobilization. Blood samples and liver biopsies were taken at d-42, d3, d21 and d100. Serum insulin concentration (Ins) was measured by radioimmunoassay. Protein expression of hepatic FoxO1 (FoxO1pro) and phosphorylated FoxO1 at serine 256 (pFoxO1) was measured semi-quantitatively by Western blotting and mRNA of hepatic FoxO1 (FoxO1rna), G6P, and PCK1 by real-time PCR. Associations between variables were examined by Pearson's correlation analysis. The significance level was set at $p<0.05$.

The patterns of examined associations varied between the lactation periods. No correlation was found at d-42 and d3. At d21, G6P correlated negatively with FoxO1pro and positively with pFoxO1-to-FoxO1pro-ratio (pFo/Fo). Noticeable, at d100, positive correlations of Ins–pFoxO1, PCK1–FoxO1pro and–FoxO1rna as well as G6P–FoxO1rna, and a negative correlation of PCK1– pFo/Fo were found. The examined associations were affected by diet only marginally. The observed shift in associations within the periparturient period reflected the suppressive effect of insulin on PCK1 transcription mediated by FoxO1at d100 only. The insulin action on the regulation of hepatic gluconeogenesis through phosphorylation of FoxO1 might thus be less in cows in late pregnancy and shortly after calving.

Keywords: insulin signaling, FoxO1, dairy cow, liver

Does overconditioning in dairy cows affect the performance of pancreatic β cells?

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Overconditioning in dairy cows is an important risk factor to develop a wide variety of health problems in the periparturient period. Since the endocrine pancreas plays a central role in the metabolic regulation, functional maladaptations that go along with overconditioning may play a pivotal role in these disorders.

The objective of the present study was to determine effects of overconditioning on the performance of the pancreatic β cells in dairy cattle.

The study was conducted in ten Holstein Friesian dairy cows in a variable body condition (normal- to overconditioned). During the dry period, animals were weekly monitored for body condition score (BCS) and back fat thickness (BFT), and blood samples were taken to determine the non-esterified fatty acid (NEFA) concentration. Two to 3 weeks before the expected calving date, cows underwent an intravenous glucose tolerance test (IVGTT) to measure the insulin secretory capacity of the pancreas. A glucose bolus was administered and blood samples were taken at 15 and 5 minutes prior to glucose infusion in order to determine basal levels, and at several time moments between 0 and 180 minutes after the glucose infusion to determine glucose (mg/dl) and insulin (µIU/ml) concentrations.

Based on the data obtained from the IVGTT, the elimination rates of glucose and insulin (ER% glu and ins) were determined. In addition, the area under the curve (AUC) of insulin for several time intervals was calculated using the trapezoidal rule, while the acute insulin response to glucose (AIRg) was determined using the MINMOD software program. Data were statistically analysed using SAS Enterprise Guide 9.4. Normality of the data was checked using the Kolmogorov-Smirnov test, and data were log-transformed if not normally distributed. Secondly, Pearson correlation coefficients (r) between BCS and BFT and the pancreatic insulin secretory capacity were calculated and an ANOVA was performed to evaluate differences in insulin secretory capacity between the normal- versus overconditioned group of animals (BCS ≤3.5 and >3.5 resp.). Significance and tendency were declared at $P<0.05$ and $0.05<P<0.10$, respectively. Results of the IVGTT revealed a tendency for a positive relation between the insulin secretory capacity of the pancreas and the condition of the animals. In addition, however, no significant effects of the condition status or NEFA-levels on the ER% ins and glu could be demonstrated.

It can be concluded that overconditioning in dairy cattle does affect the insulin secretory capacity of the pancreas in the periparturient period, but further research is needed to understand the underlying mechanisms.

Keywords: dairy cow, overconditioning, precalving, insulin secretion

Lactational estrus in sows

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Lactational estrus is expected in horses and desired in cattle, while the attitude to lactational estrus is ambivalent in swine production. To facilitate batch farrowing, estrus is synchronized by weaning. Here, lactational estrus will reduce the success of this strategy. Recently, lactational estrus is seen as a tool to increase nursing days for welfare matters, while keeping a high number of litters per sow and year. This investigation describes prevalence, effect and causes of lactational estrus in two commercial sow herds.

In two well managed production herds, 504/852 DanAvl hybrid sows were followed during lactation and until next farrowing. If lactational estrus was observed during the routine management, the sows were mated. A subset of 143/98 sows were bled at weaning. If serum progesterone exceeded 7 ng/L, the sow was assumed to have functional corpora lutea, and to have experienced lactational estrus.

In the two herds, 12/16 % of the sows were mated before weaning. Serum progesterone at weaning indicated, that an additional 13/10 % of the sows had been in estrus during lactation, indicating that 25/26 % of the sows had been cycling during lactation.

No sows were found cycling before 17/15 days. In order to nurse sows, 32/19% of the litters were transferred to another sow (two-step nurse) or were weaned (one-step nurse) to give room for a new litter at the sow. Estrus day four to seven after exchange of a litter explained 40 % of the sows in lactational estrus, if they were given a new litter more than nine days after farrowing. Sows in estrus during lactation were fed 9.2 /10 Feed Units/day, indicating that mainly healthy and high producing sows came into heat. In sows mated during lactation, the resulting litter size was not affected by short or long lactation period after mating. Mating during lactation resulted in 14.3/16.1 total born piglets, and 92/84 % of the sows farrowed after this mating. If serum progesterone level was high at weaning, then mating in the first estrus after weaning resulted in 15.8/17.9 total born piglets.

In both herds, approximately 25 % of the sows had lactational estrus. Despite being part of a trial, only half of these estruses were observed during lactation. Lactational estrus may be a frequent cause of sows not cycling at the expected day after weaning. Lactational estrus was not observed during the first 2 weeks of lactation. Lactational estrus was often caused by relocation of litters. Mating in case of lactational estrus resulted in normal fertility, but 1.5/2.0 fewer piglets were born in the succeeding litter than if mating the sow after weaning.

Lactational estrus can be frequent in highly productive sow herds. Sows in lactational estrus are highly fertile, but litter size will increase if mating is postponed to the following cycle.

Keywords: sow, lactation, estrus, prolactin

Possible action of interferon-tau on the neutrophil migration and function via interleukin-8 in the bovine corpus luteum during the early pregnancy

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Interferon-tau (IFNT), a well-known pregnancy recognition signal in ruminants, is secreted by embryonic trophoblast cells during the maternal recognition and acts within the uterus to prepare for pregnancy. IFNT was shown to act as an endocrine factor on the corpus luteum (CL) to induce refractory ability against the luteolytic action of PGF2□, thus IFNT may influence not only the uterine environment but also the CL in cows via local or peripheral circulation. In this study, we investigated qualitative changes in the CL of pregnant cows during the maternal recognition period (day 16) and the CL of non-pregnant cows.

Briefly, we selected day 16 after artificial insemination as the transitional phase to pregnancy, in comparison with the late phase of the luteal phase (day 16) during the estrous cycle. Ovariectomy was performed on day 16 of the estrous cycle (not inseminated, n=4) as well as on day 16 of pregnancy (n=5). The pregnancy was confirmed with the presence of an embryo within the uterus after slaughter of inseminated cows. The CL of pregnant animals had higher number of neutrophils, and the expression of interleukin (IL)-8 mRNA and its protein was higher as well as compared with the CL of non-pregnant animals. However, mRNA expression of CXCL6, another neutrophilic chemoattractant, did not differ between pregnant and non-pregnant cows. The number of macrophages and its chemokine CCL2 within the CL did not differ between pregnancy and non-pregnancy. Although IFNT did not affect progesterone secretion and neutrophil migration directly, IFNT stimulated IL-8 mRNA expression on luteal cells influenced the neutrophils, resulting in the increased migration of IFNT-activated neutrophils. Moreover, both IFNT-activated neutrophils and IL-8 increased progesterone secretion from luteal cells in vitro.

Our novel finding was the increase in neutrophils and IL-8 within the CL on day 16 of pregnant cows, suggesting the involvement of IFNT function within the CL toward establishment of pregnancy in cows. The present results imply that IFNT up-regulates neutrophil numbers and function via IL-8 on luteal cells in early pregnant CL, and that both neutrophils and IL-8, stimulated by IFNT, are associated with increase in progesterone concentrations during the maternal recognition period in cows.

Keyword: cow, corpus luteum, pregnancy, neutrophils, interferon tau

Evidence for upregulation of anti-inflammatory (Th2) cytokines in the bovine oviduct immunity by sperm-epithelial cell binding

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The oviduct provides an optimal condition for sperm survival and capacitation. Spermatozoa are allogeneic in the oviduct; however, they can survive in the oviduct up to two days without immunological attack. There is the evidence for existence of polymorphonuclear neutrophils (PMN) in the bovine oviduct fluid under physiological conditions. We hypothesized that binding between the bovine oviduct epithelial cells (BOEC) and spermatozoa could modulate the local immunological environment on the surface of epithelial cells in the oviduct, through induction of anti-inflammatory (Th2) cytokines permissive of sperm survival until fertilization.

Healthy oviducts were collected from the local slaughterhouse. BOEC were isolated and cultured. Sub-confluent BOEC monolayers were co-cultured with sperm after swim-up (105 sperm/ml) for 2 h to ensure sperm-BOEC binding. The supernatant containing dead and unattached sperm was removed, and rapidly replaced by fresh medium and the tissues were cultured for a further 3, 6, 12 or 24 h. BOEC monolayer cultures without sperm served as a control.

After 6, 12 or 24 h, there was up-regulation of microsomal prostaglandin E synthase-1 (mPGES-1) and Th2 cytokines (IL-10, TGFβ & IL-4) and down-regulation of Th1 cytokines (TNFα & IL-1β). Immunohistochemistry showed that bovine oviduct, especially the epithelial cells, clearly express proteins for major Th2 cytokines (IL-10 & TGFβ) in the isthmus and the ampulla, throughout the estrous cycle. PMN were isolated from blood and cultured for 4 h at a density of 1×105 cells/ml with BOEC supernatant (previously co-cultured with or without sperm for 24 h). Both types of BOEC supernatants suppressed the expression of genes for pro-inflammatory cytokines (e.g. TNFα), whereas only BOEC supernatant previously co-cultured with sperm stimulated anti-inflammatory cytokines (IL-10 & TGFβ). Moreover, BOEC supernatants with or without sperm stimulated PMN in PGE2 secretion and mPGES-1 gene expression.

In conclusion, our results indicate that the sperm binding to epithelial cells of the bovine oviduct optimizes the immunological environment that permits sperm survival until fertilization. Additionally, the sperm-oviduct binding appears to change PMN gene expression that further protects spermatozoa from phagocytosis, thus ensuring successful fertilization.

Keywords: oviduct, sperm, polymorphonuclear neutrophils, Th2, immunity

Ovarian and hormonal responses to single injection or continuous infusion of senktide, a neurokinin 3 receptor agonist, during the follicular phase in goats

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Recent studies suggest that neurokinin B (NKB) stimulates pulsatile gonadotropin-releasing hormone and luteinizing hormone (LH) secretion by action on its cognate receptor (NK3R). The aim of the present study was to investigate the effects of single injection or continuous infusion of a putative NK3R agonist (senktide) on the secretion pattern of LH and follicular dynamics in goats during the follicular phase of the estrous cycle.

Shiba goats were injected with 2 mg of prostaglandin F2α analogue (dinoprost) in the luteal phase of the estrous cycle, and treated with an intravaginal progesterone device for 10 days. At 12 h after the cessation of progesterone treatment, the goats received a single peripheral i.v. injection of senktide (200 nmol, n=4) or vehicle (n=4), or continuous i.v. infusion of senktide (20 nmol/min, n=6) or vehicle (n=6) for 6 h. Blood samples (2 ml) were collected every 10 min from -2 to 6 h to measure LH concentrations. Ovulation was monitored every 24 h until 96 h after treatment by transrectal ultrasonography.

A transient increase in LH concentration was observed immediately after single injection of senktide, reaching a peak 20-30 min after treatment, while the mean (± SD) number of LH pulses and mean LH concentration in the samples collected at 10-min intervals for 6 h after senktide injection (5.3 ± 0.5 pulses/6 h and 1.6 ± 0.9 ng/ml, respectively) were similar to measures in vehicle-treated goats (5.5 ± 0.6 pulses/6 h and 1.6 ± 0.7 ng/ml, respectively). Continuous infusion of senktide caused a sustained increase in LH secretion. The pattern of LH increase appeared to be due to an increase in the frequency of LH pulses, although discrete pulses could not be analyzed by means of statistical methods. Mean LH concentration was higher ($P < 0.001$) in agonist-treated goats than in vehicle-treated goats (2.8 ± 0.9 and 1.6 ± 0.9 ng/ml, respectively,). In 4/6 goats, LH concentrations reached a peak during the 6-h senktide infusion, and ovulation was observed at 48 h after the start of infusion without estrous behavior. In the remaining 2 senktide-treated goats and in all vehicle-treated goats estrus and ovulation occurred at 72 or 96 h after treatment.

These results suggest that continuous administration of senktide to goats during the follicular phase of the estrous cycle can cause sustained increase in LH and advance the time of estrus and ovulation.

Keywords: goats, luteinizing hormone, neurokinin 3, ovulation, senktide

Pulsatile and surge mode secretory responses of luteinizing hormone after progesterone treatment of different durations in ovariectomized goats

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We have reported that negative feedback effects of endogenous and/or exogenous hormones on the mechanism(s) that regulates pulsatile and surge mode secretion of luteinizing hormone (LH) are different in goats. The aim of the present study was to determine the effects of different durations of progesterone treatment on the profiles of pulsatile LH secretion and estradiol-induced LH surge. Ovariectomized Shiba goats received Silastic implants containing estradiol (Day 0), and were divided into three groups. The 7-day progesterone group (P7, n=4) received a subcutaneous implant of a Silastic packet (50 × 70 mm) containing progesterone from Day 0 to Day 7, and the 3-day progesterone group (P3, n=5) similar packets from Day 4 to Day 7. The control group (P0, n=4) received an implant of a blank Silastic packet from Day 0 to Day 7. Estradiol was peripherally infused for 36 h at a rate of 3 μg/h from 13 h after the removal of the Silastic packet in all the groups to evaluate the effects of the duration of progesterone treatment on LH surge. Blood samples were collected daily throughout the experiment for the analysis of steroid hormone levels, and at 10-min intervals for 6 h before the removal of the progesterone packet on Day 7 for analysis of pulsatile frequency of LH secretion. Blood samples were also collected every 2 h from −6 h to 48 h after the start of estradiol infusion. In the P7 and P3 groups, the mean plasma concentration of progesterone immediately increased and was maintained at the level of the luteal phase during the implant treatment. LH pulse frequency in the P7 (2.8±1.5 pulses/6h) and P3 (2.8±0.8 pulses/6h) groups was significantly ($P < 0.05$) lower than that of the P0 (6.3±0.5 pulses/6h) group, with no difference between the P7 and P3 groups. An LH surge occurred in all goats of the three groups. The peak time of LH surge from the start of estradiol infusion was significantly delayed in response to the administration period of progesterone (P7: 25.0±2.6 h, P3: 18.0±1.4 h, P0: 14.0±2.8 h; $P < 0.05$). There was no significant difference in the peak concentration and duration of LH surge among the three groups. These findings indicate that pulsatile LH secretion can be sufficiently suppressed by relatively short-term (3 days) treatment with progesterone in ovariectomized goats. On the other hand, the timing of estradiol-induced LH surge depends on the length of the preceding progesterone treatment period.

Keywords: goat, LH surge, negative feedback, progesterone

Measuring of fecal sex steroid hormones for diagnosis of anestrus in Egyptian cows

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Cattle fertility is negatively affected by postpartum anestrus. Postpartum anestrus can affect production if the calving interval is more than 365 days. Anestrus and low conception rates constitute the major causes of economic losses in cattle in Egypt. Measuring fecal concentrations of sex steroids is a non-invasive diagnostic method that would be informative in both dairy and beef cows. The aims of the present study were to detect the main causes of infertility in native Egyptian cows, and to compare the concentrations of the sex steroids in serum with those fecal samples.

Seventy eight native Egyptian cows (3-8 years old, with a mean body weight 355 ± 16.3 kg were used. The animals were divided into two groups; being fertile (n=6), or with a history of postpartum anestrus of more than one year (n=72). The animals were clinically examined for the causes of anestrus including case history, general inspection and genital examination. Blood and fecal samples were collected, in parallel, for the measurement of progesterone (P4) and estradiol (E2) using radioimmunoassay (RIA). In the infertile group, the causes of anestrus were found to be; ovarian inactivity (OI), prolonged luteal phase (PLP), and cystic ovarian degeneration (COD) with incidence of 47.2, 51.4 and 1.4 %, respectively. Data of COD is not shown, because only cow was diagnosed as COD. However, 70.6 % with OI and 67.6 % of cows with PLP, had a body condition score of < 3. For OI and PLP cases, mean (±SE) serum P4 concentrations were 0.2 ±0.2 and 3.29 ±2.3 ng/ml, respectively, and mean (±SE) fecal P4 concentrations were 14.4 ±18 and 326.5 ±2.3 ng/g, respectively. Mean (±SE) serum E2 concentrations were 2.5 ±2.3 and 1.4 ±1.8 pg/ml, respectively, and mean (±SE) fecal E2 concentrations were 0.2 ±0.2 and 0.2 ±0.2 ng/g, respectively. There was a highly significant correlation between serum and fecal P4 and the changes in fecal P4 concentrations were significantly different in cows with different causes of infertility.

In conclusion, the results indicated that anestrus in Egyptian cows is associated with low body condition score. OI and PLP are the main causes of anestrus in Egyptian cows, and that the fecal P4 assay can be used as a non-invasive diagnostic tool. Our methods were approved by the Animal Care and Use Committee of South Valley University, Faculty of Veterinary Medicine.

Keywords: cows, infertility, fecal, progesterone, anestrus

Milk progesterone profiles as possible predictors of success of insemination in dairy cows managed for an extended lactation

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The bovine estruos cycle can be described by changes in progesterone (P4) concentrations leading to the use of in-line measurements of milk P4 to detect estrus and the optimal day of insemination. Associations, between milk P4 and embryo survival, and between the follicular phase length have been defined by milk P4 concentrations and the success of insemination, have been described. The objective of the present study was to determine if milk P4 profiles can predict the success of insemination for cows managed for 16 months of lactation and inseminated around the 7th or 8th estrous cycle postpartum.

A total of 80 observations, around the 1st and 2nd inseminations, were analysed in 58 Holstein cows. The mean (±SEM) day of insemination was at 224 ± 17 days in milk. Progesterone profiles were analyzed for each cow, focusing on the cycles preceding the 1st and 2nd inseminations. The insemination success, day of estrus, and estrus duration (i.e., days when P4 < 3 ng/L) were recorded. The average P4 concentration of all the P4 peaks before the 1st insemination was used to define the 'P4 level' of each cow. Then, the peak preceding insemination was classified according to this 'P4 level' (above or below). The luteal phase (i.e., days between the day of the P4 peak and the day of estrus), the P4 at the peaks ('P4 peak'), and the duration of the peaks preceding inseminations were also recorded. The effects on the insemination success of 'P4 level', 'P4 peak', peak duration, estrus duration, number of estrus at 1st insemination, interval from calving to 1st estrus, interval between estruses, and luteal phase, were analysed with Student's t-test.

The parity had no effect on any of the estrus-related variables. The 'P4 level', 'P4 peak', peak duration, interval from calving to 1st estrus, interval between successive estrus periods, and luteal phases were similar for cows which became pregnant and cows which did not become pregnant. The number of estrus periods at 1st insemination was higher for cows with a positive pregnancy than for cows with a negative one (8.34 vs. 7.72 ± 1.34 estrus, P = 0.04) whereas there was no difference in day at 1st insemination (219 vs. 227 ± 17 days). Estrus duration was longer for cows that did tested pregnant than for cows that did test pregnant (7.03 vs. 5.91 ± 2.02 days, P = 0.02) but this effect was mainly observed in the 2nd part of the estrus (i.e., from estrus day to the last day where P4 < 3ng/L) and might consequently be due to the actual pregnancy.

To conclude, milk progesterone profiles are not good predictors of success of insemination. Estrus duration might be related to success of insemination, and that the data suggest that, in the same period of time, cows that have displayed the highest number of estrous cycles have the highest success of insemination.

Keywords: progesterone, milk, predictors, pregnancy

Vaginal discharge evaluated by Metricheck is related to metabolic status and subsequent reproductive performance in postpartum dairy cow

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The objective of this study was to investigate if the evaluation of vaginal discharge by using Metricheck® (MC) is a suitable indicator for metabolic disorders and subsequent reproductive performance in clinically healthy cows during the puerperal period. In 41 multiparous Holstein cows without any clinical signs of metritis, vaginal discharge was evaluated qualitatively (score 1 = clear or translucent mucus, 2 = mucus containing of white or off-white pus, 3 = discharge containing < 50% purulent material, 4 = discharge containing ≥ 50% purulent material and 5 = 4 with stench) by a MC device weekly from 2 to 6 wk post-partum (pp). Blood samples were collected twice weekly from 1 to 10 wk pp for analyses of metabolites such as protein metabolism and hepatic enzyme and progesterone (P4). Additional blood samples for 13,14-dihydro-15-keto- prostaglandin F2-alpha (PGFM) measurement were obtained on 7, 10, 13, 16 and 21 d pp. Intrauterine fluid and ovarian activity were assessed using ultrasonography weekly or twice weekly from 2 to 6 wk pp. Ovarian cycle was identified based on P4 and ultrasound examination profiles. Vaginal discharge was classified into 4 types as follows; cows with a score 1 at 5 and 6 wk pp (T1, n = 23), cows with a score ≥ 2 after having 1 (T2, n = 10), cows having a score 1 at 6wk pp (T3, n = 5), and cows never having a score 1 until 6 wk pp (T4, n = 3). As T1 was identified as the physiological discharge until 6 wk pp, statistical comparisons were made between T1 and other types. In cows of T2, serum urea nitrogen concentrations during the experimental period were higher ($P < 0.05$) and plasma PGFM concentrations on 13 d ($P = 0.07$) and 16 d ($P = 0.05$) pp tended to be higher than in cows of T1. In T3 and T4 cows, serum gamma-glutamyl transpeptidase and albumin levels during the experimental period were lower ($P < 0.05$) than in T1, as were plasma PGFM concentrations on 7 d ($P < 0.05$) and 10 d ($P < 0.05$) pp. The resumption of normal ovarian cycle was earlier ($P < 0.05$) and the pregnancy rate at 150 d pp was higher ($P < 0.05$) in T1 than other types although days to first AI and conception rate at first AI did not differ between involution types. In conclusion, cows with delayed recovery in vaginal discharge evaluated by MC showed lower efficiency of nitrogen in the rumen or hepatic dysfunction, and also delayed resumption of normal ovarian cycle and lower reproductive performance. Thus, disturbances in uterine health diagnosed by Metricheck® were related with metabolic status and subsequent reproductive functions in dairy cows.

Keywords: dairy cow, metabolic status, Metricheck, postpartum, uterus

Dairy cows in herbage-based feeding systems have a different metabolic load affecting resumption of ovarian cycle postpartum

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Due to topographic and climatic conditions, milk production in Switzerland is mainly based on herbage feeding with minimal use of concentrates. This study investigated the effects of a solely herbage-based diet on production, metabolic, and endocrine parameters of dairy cows, and determined the factors affecting the resumption of ovarian cycle postpartum (pp).

Twenty-three multiparous Holstein dairy cows (were divided into two groups according to their previous lactation yield (4679-10808 kg): a control (C, n=13) and a treatment group (nC, n=10) from week 3 antepartum until week 8 pp. While C received fresh cut herbage plus additional concentrate according to their estimated energy and nutrient requirements, no concentrate was fed to nC throughout the experiment. Milk yield and DMI were recorded daily. Blood samples were taken weekly and analyzed for IGF-1, glucose, NEFA, and BHBA. Milk progesterone (P4) concentrations were measured every 3 d in morning milk samples (skim milk) by radioimmunoassay and resumption of ovarian cyclicity was considered to have resumed when levels reached > 1 ng/mL.

Plasma NEFA and BHBA concentrations pp were higher ($P < 0.05$) in nC (0.82, 1.18 mmol/L) compared to C (0.55, 0.63 mmol/L,). Days to resumption of ovarian cycle was similar between 2 groups (C = 29.8 d, nC = 33.3 d), so the cows were divided into 2 sub-groups depending upon whether resumption was either earlier or later than the mean value; these were categorized as earlier ovulation (E-OV; C = 22.6 d, n = 7; nC = 21.4 d, n = 6) and delayed ovulation (D-OV; C = 38.3 d, n = 6; nC = 45.2 d, n = 4). In the C group, only BCS pp was lower in D-OV (2.81) compared to E-OV (3.21, $P < 0.05$). In the nC group, however, D-OV showed lower BW (571 vs. 697 kg in E-OV) and lower glucose concentration (3.5 vs. 3.6 mmol/L in E-OV + nC), higher BHBA (1.39 vs. 1.04 mmol/L in E-OV + nC) and NEFA concentration pp (0.89 vs. 0.76 mmol/L in E-OV + nC, $P < 0.05$), suggesting an energy deficiency/stress on lipid metabolism. Other metabolic and welfare-related parameters were similar between E-OV vs. D-OV.

In conclusion, on a herbage-based feeding system without supplementary concentrate, dairy cows experience a higher metabolic load, which is very likely to affect resumption of ovarian cyclicity.

Keywords**:** metabolic load, fertility, ovulation, herbage feeding, dairy cow

The influence of the housing environment on the sanitary status and sexual development of entire male pigs – Preliminary results

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Boar taint is the major issue for raising entire male pigs. Genetics and feeding are known to influence boar taint levels. Other factors such as health could also play a role by affecting the sexual development. Indeed, the immune system is able to modulate sexual hormones. The objective of the present study was to reveal the effect of a subclinical health challenge on the sexual development of boars, and therefore on boar taint. We tried to induce a weak chronic inflammation to mimic health problems in farms, thanks to a degraded sanitary environment.

Boars (n = 56) from a commercial breed were allocated at 139 (± 0.7) days of age (Day 0) and 80 (± 5.4) kg of liveweight to either "clean" (n = 32) or "dirty" (n = 24) rooms for 4 weeks. The "dirty" room was previously soiled by other pigs, was weakly ventilated and was not cleaned during the treatment period. The "clean" room was cleaned and disinfected before entry of the experimental pigs, cleaned every day, correctly ventilated and special clothes were used by staff entering the room. Blood was collected on Day 0 and 27 to measure numbers of granulocytes and lymphocytes, levels of the acute phase protein CRP and sex hormones (testosterone, estradiol) in plasma. On Day 27, a biopsy of fat was collected from the neck to measure boar taint compounds (androstenone, skatole, indole). Saliva was collected to establish variations of CRP and estrone. Cleanliness of boars was evaluated on a scale of 0 to 4 based on the percentage of the body soiled by feces (0: <10%; 4: 75-100%) and rectal temperature was measured weekly from Day 0 to 27.

Treatment had an effect on the cleanliness. Pigs on the "dirty" room were always dirtier during treatment ($P < 0.0001$). There was no treatment effect on any health parameters or on the levels of sex hormones ($P > 0.05$). Between Days 0 and 27, concentrations of sex hormones increased ($P < 0.0001$) in saliva and plasma indicating that the pubertal process of maturation was progressing ($P < 0.0001$). We found an effect of treatment on fat skatole (0.043 vs 0.13 μg/g of pure fat, $P < 0.0001$) and indole (0.030 vs 0.44 μg/g of pure fat, $P = 0.0005$) with higher values for the soiled boars. The correlation between fat androstenone and plasma estradiol levels was high on Day 0 ($r = 0.53$, $P < 0.0001$) and Day 27 ($r = 0.69$, $P < 0.0001$).

Our study did not reveal health differences with respect to the sanitary status of boars, so the stated hypothesis could not be tested. We did, however, confirmed a strong effect of a soiled environment on fat skatole levels. In addition, our data show that fat androstenone is dependent on the level of plasma estradiol measured just before or 4 weeks before.

Keywords: boar; sex hormones; health; puberty; housing

Effects of neonatal surgical castration or immunocastration on the adrenal axis of males pigs

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To explore the effect of the suppression of testicular hormones on the adrenal axis, we compared entire pigs (E, n = 18), pigs castrated early in life (SC, surgical castration at 5-6 days of age, n = 14) and pigs submitted to immunocastration around puberty (IC, vaccination against Improvac® at 88 and 117 days of age, n = 16).). Saliva samples were collected at 112 and 147 days of age at various times during the day (9:00, 11:00, 17:00, 23:00, 5:00 and 9:00). Blood samples were collected just before each vaccination and at 151 days of age. At slaughter (152 days of age), adrenals were collected, freed from surrounding tissue and weighed. For 11-12 pigs/experimental group, the adrenal cortex was frozen in liquid nitrogen and stored at -80°C until transcriptional activity analysis by the fluidigm technology (qPCR on cDNA).

Plasma testosterone level was lower in SC than in E and IC at 88 and 117 days of age and was lower in SC and IC pigs than in E pigs at 151 days of age ($P < 0.05$). A similar result was obtained for plasma estradiol except that level was similarly low in all groups at 88 days of age. Plasma cortisol and ACTH levels did not differ between groups at the three ages ($P > 0.1$). Salivary cortisol level was influenced by the time of day at both ages ($P < 0.001$) with the highest value at 6:00 and the lowest at 23:00. The treatment effect was not significant at 112 days ($P > 0.1$) contrarily to 147 days ($P < 0.01$) with a lower mean salivary cortisol level in E than in IC ($P < 0.04$) and C ($P < 0.09$) pigs. Adrenals were lighter in E than in SC pigs ($P < 0.05$) and intermediate in IC pigs. The levels of transcriptional activity of 11 genes essential to produce corticoids, DHEA or androstenedione (STAR, CYP11A1, CYP17, HSD3B1, CYP21, CYP11B), to control the cortisol-cortisone conversion (HSD11B1 and HSD11B2) and, of 3 cofactors (CYP5A, CYP5B, CYB5R3) were evaluated in the adrenal cortex. Transcript level of CYP11A1 was lower in E than in other pigs ($P < 0.05$) and that of CYB5R3 in IC than in other pigs ($P < 0.05$). A trend for a lower transcript level of STAR was observed in E compared to IC pigs. Therefore, the transcription of the CYP11A1 gene seems to be permanently influenced by the suppression of testicular hormones whereas the transcription of some genes (CYB5R3, STAR) is only transiently influenced and that of other genes (8 out of 11) is not influenced.

Overall, our data indicate a lower activity of the adrenal axis in entire than in surgically or immunologically castrated pigs, suggesting an inhibitory effect of sex hormones on the adrenal axis. The effect of sex hormones removal seems to depend on the time elapsed from castration.

Keywords: cortisol, testosterone, oestradiol, boar, castration

Changes within the somatotropic and thyreotropic axis during early pregnancy in dairy heifers

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It is well known that the local endometrial IGF-system is altered during a physiological cycle as well as during early pregnancy. Moreover, in humans it is known that human chorionic gonadotropin has a direct influence on the maternal thyroid metabolism, and induces an increase in free thyroxine (T4) concentrations mirrored by a lowering of thyroid-stimulating hormone (TSH) levels. However, in cattle, less is known about endocrine adaptions of the maternal metabolism towards early pregnancy. Therefore, the possible endocrine alteration of the somatotropic and thyreotropic axis during early pregnancy in Holstein Friesian heifers (n=19) were examined.

Blood samples of the day of ovulation (d0), d14 and d18 during pregnant and respective non-pregnant cycles were taken. The concentrations of progesterone (P4), estrogen (E), growth hormone (GH), insulin, insulin-like growth factor-1 and -2 (IGF1 & IGF-2), IGF-binding protein-2,-3,-4 (IGFBP2,-3,-4), TSH, T4 and triiodothyronine (T3), were analyzed via validated immunoassays and a repeated two-way ANOVA was performed.

As expected, P4 was higher and E was lower in p vs. np on d18 ($p<0.01$). IGFBP4 on d18 and TSH on d0 were lower in p compared to respective np cycles ($p<0.01$), and T3 (d18) tended to be lower in pregnant compared to non-pregnant cycle ($p=0.08$). Moreover, GH (d14 to 18) and IGF-2 (d0 to 14) increased, while IGF-1 (d0 to 14) and T3 (d0 to 18) decreased as the days of cycle proceeded ($p<0.05$).

These results indicate an influence of pregnancy on the IGFBP4 concentration in maternal blood. It can be speculated that this decrease might have a potential influence on bioavailability of IGF-1 for embryonic growth, as IGFBP4 is known as inhibitory binding protein. Moreover, clear cycle effect presumably originating from the sexual steroid hormone pattern on GH, IGF-1, IGF-2 and T3 were detected. This may relate to an endocrine adaption towards a potential pregnancy.

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Keywords: somatotropic axis, thyroid hormones, early pregnancy, heifer

Gestational Diabetes Mellitus (GDM) in the mare

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During pregnancy, partitioning of nutrients to the developing fetus is achieved by maternal insulin insensitivity and impaired glucose tolerance. Increased insulin secretion also occurs as a normal compensatory response. However, the pathophysiological state of GDM, characterized by pancreatic β cell failure associated with severe insulin resistance as noted in women, has not been fully described in other animals. For example, GDM has only been reported in dogs. Moreover the β cell defect of GDM is known to predispose women to future carbohydrate intolerance and diabetes. Here we provide evidence for GDM in mares.

Pregnant (*n=13)* mares were given either a high (*n=6*) or low energy (*n=7*) diet for the last trimester of pregnancy to achieve divergent body condition scores (BCS; scale 1-9). Insulin-modified, frequently sampled, intravenous glucose tolerance tests were performed in late gestation on days 290 and 320. Minimal model analysis was used to derive parameters such as insulin sensitivity (Si) and the acute insulin response to glucose (AIRg). In the subsequent breeding season, a cohort of these mares (n=4 per group) were retained and tested for ongoing glucose intolerance by oral GTT on day 325.

There were no significant differences in weights between mare groups in late gestation, but the diets resulted in different (P<0.001) BCS (7.2 ± 0.5 vs 3.3 ± 0.3). Insulin sensitivity in high BCS, 'overweight' mares was similar (Si 1.1 ± 0.4 $x10^{-4}$ $L.mU^{-1}.min^{-1}$) to those previously reported in mares as insulin insensitive. In low BCS, 'lean' mares, however, Si was lower (P<0.01) on day 290 (Si 0.2 ± 0.2), and markedly reduced (P<0.01) on day 320 (Si 0.04 ± 0.01) indicative of severe insulin resistance. Furthermore, the AIRg was similar between the mare groups on day 290, but reduced (P<0.01) in low BCS mares on day 320, indicative of β cell failure. In the subsequent breeding season, mares with previously low BCS displayed an ongoing β cell defect, with glucose intolerance and β cell dysfunction during an OGTT.

This study demonstrates that lean mares in late pregnancy develop glucose intolerance, severe insulin resistance, and β cell failure. Despite returning to normal BCS the same mares continued to exhibit glucose intolerance during their subsequent pregnancy. These changes are characteristic of GDM and provide novel evidence for this condition in horses.

Keywords: gestational diabetes, insulin, resistance, beta-cell failure, horse

DCA - National Centre for Food and Agriculture is the entrance to research in food and agriculture at Aarhus University (AU). The main tasks of the centre are knowledge exchange, advisory service and interaction with authorities, organisations and businesses.

The centre coordinates knowledge exchange and advice with regard to the departments that are heavily involved in food and agricultural science. They are:

Department of Animal Science
Department of Food Science
Department of Agroecology
Department of Engineering
Department of Molecular Biology and Genetics

DCA can also involve other units at AU that carry out research in the relevant areas.

SUMMARY

This report is the conference proceedings of the 8th International conference on Farm Animal Endocrinology, which took place in Billund, Denmark, 27-29 August 2015. The report gives the full program of the two-days conference in which 25 invited talks were presented in five scientific sessions; 1. Endocrine control of metabolism, 2. The gut: New critical control points for endocrine-immune metabolic targeting, 3. Animal health and stress: Consequences and strategies, 4. Endocrine control of lactation, and 5. Reproduction and Health. Besides abstracts of the 25 invited talks, the report also contains 38 abstracts submitted and presented as posters during the conference. Full papers of the invited talks will appear in Domestic Animal Endocrinology.