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Role of insulin in the development of metabolic and reproductive malfunctions of periparturient dairy cows

Theses of Ph.D. dissertation

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INTRODUCTION

Insulin is the most important antilipolytic, anabolic hormone with key function in the carbohydrate metabolism. Moreover, insulin is one of the key metabolic molecules which mediate the crosstalk between the hypothalamic-ovarian axis and has important role in the ovarian steroidogenesis. During the periparturient period in the dairy cows selected for high milk production the pancreatic β -cell insulin secretion is reduced, and the peripheral tissues sensitivity to the action of the insulin is decreased. In humans the release of cytokines, that occurs in association with obesity and inflammatory diseases (especially in those with endotoxemia), and the release of non esterified fatty acids plays an important role in the development of insulin resistance. Recent evidence exists that insulin resistance may have important role in some periparturient metabolic and reproductive malfunctions, such as displaced abomasum (*Pravettoni et al., 2004*), ovarian cyst development (*Opsomer et al., 1999*) or fatty liver (*Ohtsuka et al., 2001*). In dairy cow inflammatory diseases with intensive endotoxin/cytokine release (puerperal mastitis and metritis, clinical endometritis) are frequent complications in the puerperal phase. Our understanding on the relationship between periparturient metabolic disorders, insulin resistance and the poor reproductive performance in the modern dairy cow is limited, yet.

Challenge tests routinely used for the evaluation of insulin resistance comprise the evaluation of time related changes of the insulin- and glucose responses. However, these challenge tests are time consuming and labour intensive. A promising method developed to measure insulin sensitivity in epidemiological studies in human populations, the *revised quantitative insulin sensitivity index (RQUICKI)* has been evaluated in Holstein cows by *Holtenius and Holtenius (2007)* and *Balogh et al. (2009)*. RQUICKI implies the evaluation of the homeostatic energy balance based on plasma concentration of glucose, insulin and non-esterified fatty acids, therefore it would provide a more practical method to evaluate resistance in larger number of animals.

Feeding diets designed to increase plasma concentration of insulin may improve the postpartum ovarian activity (*Gong et al., 2002; Butler et al., 2000*). Also, energy supplementation may prevent the excessive insulin resistance by reducing lipid mobilisation and fatty acid eliberation. However, the type of energy supplement, the time of administration and the production trait of the animals may alter the results.

AIMS OF THE STUDIES

Our goal was to investigate the periparturient insulin pattern and insulin resistance in high lactating dairy cows in relation with some metabolic and reproductive malfunctions and to improve postpartum ovarian function through energy supplementations in cows under different management systems. The aims of the experiments were:

- (i) to determine the glucose-induced insulin responsiveness and the whole body-insulin sensitivity in cows showing different forms of periparturient ketone pattern with and without puerperal metritis, and the interrelation between results of these challenge tests with the revised quantitative insulin sensitivity check index (RQUICKI) and different metabolic and hormonal parameters in dairy cows (*Exp. 1.*);
- (ii) to study the effects of periparturient propylene glycol (PGL) supplementation (provided, as a pulverized product) on glucose-induced insulin responsiveness, whole-body insulin sensitivity, some metabolic and hormonal parameters, the time of the first postpartum ovulation and pregnancy rates in *TMR-fed* dairy cows (*Exp. 2.*);
- (iii) to investigate the effects of prepartum energy supplementation with cracked corn grain on postpartum milk production and reproductive performance in cows under a *pasture-based* dairy production system (*Exp. 3.*).

STUDIES

Experiment 1

Design

Thirty-one multiparous (parity: 3 to 5) cows producing mean \pm SEM: 8331 \pm 193 kg of fat corrected milk in the previous 305-day lactation were selected for the study on day 259-265 of gestation. Between day 1 and 7 after calving the uterine involution was checked once daily with vaginoscopy and rectal palpation, and also the rectal temperature was measured. If malodorous, reddish-brown, watery (putrid) vaginal discharge was present, animals were diagnosed as affected by *puerperal metritis (PM)*. Individual milk yield was recorded on d 7 and once between d 28-36 and d 60-70 after calving. Body condition score (BCS) was recorded at the time of inclusion in the study (d -18 to 25), on d 7 and between d 28-35 pp. Blood samples were taken regularly: on d 259-265 of gestation, again two times 7 days apart, subsequently once a day until d 7 after calving, and thereafter two times again, on d 25-35 and 60-70 of lactation for assaying the non-esterified fatty acid (NEFA), β -hydroxy-butyrate (BHB), insulin, insulin-like growth factor-I (IGF-I) and leptin. For monitoring the resumption of ovarian cyclicity milk samples were taken 2-3 days apart (three times a week) from d 8-10 after calving for 10-12 wk for progesterone determination.

Simultaneous *glucose (GTT)* and *insulin (ITT) tolerance tests* were performed between d 18-22 before delivery (e.g. late gestation), on d 7 after calving (e.g. early lactation), and again on d 60-70 of lactation. The initial blood collection with 10 min apart (t-10 and t0, respectively) was followed by a glucose¹ infusion in the *v. epigastrica superficialis* during an average time of 6 min. Blood samples were collected at 5, 15, 30, 45, 60, 75, 90, 120, 150, 180, and 210 min after finishing glucose infusion. After the blood collection in min 210 human recombinant insulin² was injected into the jugular vein. Further blood samples were collected at 240, 270, 300, 330, 360, 420 and 480 min after insulin injection. Metabolites and hormones were determined from basal samples and insulin and glucose from each sample. The rapid quantitative insulin check index³ (RQUICKI) was calculated from the serially collected blood samples.

¹ 0.5 g/kg body weight (Glucose 40% inf., Human Ltd., Gödöllő, Hungary)

² 0.1 IU/kg body weight (Humulin R inj., 40 IU/ml, Eli Lilly Co., Indianapolis, IN)

³ RQUICKI = 1/ [log (basal glucose) + log (basal insulin) + log (basal free fatty acid)]

Results and Discussion

Data of 3 cows showing moderate clinical signs of mastitis were excluded from the final analysis. Finally data from 28 cows were evaluated. In 6 of them puerperal metritis was diagnosed: the first pathognomonic signs (malodorous, reddish-brown, watery vaginal discharge with fever, anorexia and depression) were observed on d 2 to 6 after calving, and were still existing on day 7, when the GTT and ITT were conducted.

Based on the periparturient plasma BHB patterns and the occurrence of puerperal metritis the 28 cows could be classified into four groups. In 9 animals the BHB levels did not exceed the 1.20 mmol/L threshold value on the sampling days (*normoketone-mic* cows, NK), while in 7 cows were elevated only on the day of, and/or one day after calving (cows with *transient hyperketonemia*, tHK). In 6 cows, however, the ketone body elevation became significant 2 d before calving (BHB levels compared to those on day 18-22 before calving: $P < 0.05$), the BHB levels reached threshold value of 1.20 mmol/L on day -2 to 0 (day 0 = day of calving) and remained elevated through the first 7 d after calving, till the end of the intensive (once-a-day blood) sampling (cows with *continuous hyperketonemia*, cHK; $n=6$). In another 6 cows clinical signs of *puerperal metritis* were observed together with long-term hyperketonemia (cHK+PM).

The plasma *NEFA* profile followed almost the same pattern as BHB levels, except in cHK or cHK+PM cows, which presented early increases in plasma NEFA concentration. During late pregnancy the plasma *glucose* remained almost unchanged until d 3 before calving. A moderate decline was observed thereafter. On d 28-35 the plasma glucose returned to concentrations similar to those observed in early dry period in all cows. *Insulin* levels were usually high at the beginning of sampling, but presented a wide range of variation (7.0 to 25.0 pmol/L). Insulin started to decrease before parturition in all cows. In PM cows, insulin levels started to decrease more abruptly only on d 2-3 after calving, and reached the same level than their cHK mates on d 3-4 after parturition, i.e. at clinical manifestation of metritis. On d 28-35 after calving, plasma insulin levels reached the initial values in all cows except cHK. Plasma *IGF-I* levels started to decrease gradually from 3 wk before calving, and reached the nadir between d 3 and 7 after calving. Plasma *leptin* levels started to decrease before parturition in all cows, but remained higher throughout the study in NK cows. Cows with only transient HK also had low leptin concentrations.

Insulin area under the curve (AUC) and *maximal insulin response to glucose* (insulin peak) was significantly lower in early lactation than in late-pregnancy ($P < 0.001$),

reflecting decreased *insulin responsiveness*. The insulin response to GTT in the early lactation was further impaired in animals developing long term HK, with or without periparturient metritis, but not in cows with only transient increase in the BHB levels. Furthermore, the glucose decrease after insulin injection (*insulin sensitivity*) was lower in early lactation than in late pregnant or mid lactating animals. Likewise, more pronounced tissue unresponsiveness to insulin in continuously HK cows was reflected by the lower *insulin stimulated blood glucose decrease (ISBGR)*. There was no difference in the plasma NEFA dynamics in the blood between the cHK cows and the cHK+PM after parturition. It may be expected that hyperketonemic cows with severe clinical signs of PM should have even stronger evidence of insulin resistance, as a result of an additional effect of cytokine production to the insulin unresponsiveness. Insulin AUC and insulin peak after glucose- and the insulin-induced blood glucose level decrease numerically was lower during the early lactation in cows affected by PM than in cHK group without PM. However, these differences were not statistically significant at this number of animals.

There was a highly significant time effect on RQUICKI value ($P < 0.001$), and it was not affected in cows with different form of HK with or without PM ($P = 0.41$). The revised quick insulin sensitivity index (RQUICKI) originally developed to measure insulin sensitivity in human epidemiological studies has been evaluated in healthy Holstein cows (Butler et al., 2004; Balogh et al., 2008). However, only healthy cows were evaluated in these two experiments cited, and the periparturient changes in BHB pattern were not followed up. In the present study the cHK cows had decreased peripheral insulin sensitivity and glucose responsiveness to insulin, but there was no correlation between the RQUICKI and any of the measured parameters during the GTT and ITT. Moreover, the RQUICKI did not correlate with the BCS measured on d 7, d 25 or the BCS loss. In the present study a highly significant effect of time on RQUICKI was noted, which suggests that it is affected by the considerable changes of plasma metabolic and hormonal concentrations around the date of calving. These observations altogether suggest that the RQUICKI has a low discrimination power in diagnosing decreased insulin sensitivity in cows affected by various metabolic diseases. Additional studies are necessary to further assess the RQUICKI in dairy cows with different nutritional, metabolic and diseased conditions

The time of the first ovulation was recorded significantly later in hyperketonemic cows with or without PM, than in normoketonemic or only transiently HK cows (d

26.2±3.0, 34.7±4.0, 44.0±4.0, 50.1±5.0 for NK, tHK, cHK and cHK+PM, respectively; P=0.002). The time of first ovulation was negatively correlated with insulin AUC, insulin peak and ISBGR, indicating reduced insulin responsiveness in cows with delayed ovulation

Conclusions

In conclusion, we showed that pancreatic β -cell function and the biological potency of insulin is impaired in cows with long-term hyperketonaemia. Short-term elevations in plasma free fatty acids and BHB may not potentially induce further increase in peripheral tissue insulin resistance in the early lactation. Severe inflammatory diseases like puerperal metritis with intensive release of pro-inflammatory cytokines potentially further depress insulin secretion of the pancreatic β -cells and the whole body insulin responsiveness of the dairy cows, with long-term effects on metabolism and reproduction. However, further studies with larger number of animals are needed to improve these observations. The homeostatic model RQUICKI should be applied with cautions in the assessment of insulin sensitivity in dairy cows in different physiological and disease states.

Experiment 2

Design

On day 14 before the expected calving date 51 multiparous Holstein-Friesian cows were selected and assigned in two treatment group based on parity and the previous 305-d fat corrected milk (FCM) yield. From day 14 before the expected parturition until d 10 postpartum the PGL group received daily top-dressing of 350 g of pulverized propylene glycol (PGL; VMD Ltd, Budapest, Hungary; composition: 75 % propylene glycol, 25 % fumed silica⁴). Animals in the control (CTL) group did not receive PGL. In the supplemented group the daily 350 g PGL should provide an approximately 5.25 MJ of extra energy/day/animal, which is similar to the amount of PGL administered in previous studies that proved to be effective in improving metabolic status and/or reproduction

Blood sampling started after commencement of PGL treatment from the jugular vein on weeks -2, -1 before, and 1, 2, 3, 5, 7 after parturition for BHB, NEFA, insulin, IGF-I, 3,3',5-triiodothyronine (T₃), thyroxine (T₄) and glucose analysis. Blood samples were taken approximately within 3 hours after the morning feeding for metabolite and hormone analysis. Individual daily milk production was recorded beginning from d 10 postpartum until d 100 of lactation. The milk content of protein, lactose and fat were analyzed for each cow once a month.

Between d 7-10 after calving, a subset of 16 cows (CTL n=10; PGL n=6) were selected for *liver biopsy* and simultaneous *intravenous glucose (GTT) and insulin tolerance (ITT) tests*. The sampling procedures were carried out after the morning milking. Metabolites and hormones were determined from basal samples (t₀) and insulin and glucose were determined from each sampling time.

The time of first pp ovulation was determined by individual milk P4 profile from milk samples collected three times weekly from d 10 pp until d 100 pp. The Pre-Ovsynch protocol⁵ was used for synchronization of ovarian cyclicity from day 35 after calving. Cows were artificially inseminated (fixed time AI). Pregnancy was confirmed with rectal ultrasonography performed between d 35-40 after the AI. Non-pregnant

⁴ SiO₂; commercially available as HDKN20

⁵ Two prostaglandin F2 α injections at 14 days interval (500 μ g Cloprostenol, Estrumate®, Schering-Plough Animal Health, Union, NJ, USA), followed by GnRH (150 μ g Gonadorelin, Fertagyl®, Intervet, Angers, France), PGF2 α and GnRH injections on d 0, 7 and 9 after the second PGF2 α treatment.

cows after the first AI were inseminated and pregnancy rates were followed until d 150 after calving.

Results and Discussion

The daily 350 gr of propylene glycol supplementation provided top-dressed on the mondiet deliver about 14% of the daily energy requirement during the dry period and about 5% during early lactation. However, we did not measure the individual feed intake of the cows; therefore it cannot be excluded that the form of PGL absorbed on fumed silica reduced the palatability of the feed.

Daily average milk yield in the first 12 weeks of lactation and in milk protein and lactose content did not differ between treatments. On week 5 pp the average weekly milk yield was slightly higher in PGL than in CTL cows (33.1 ± 1.5 vs. 36.4 ± 1.4 kg/day; $P=0.1$). On d 7-10 there was no difference in BCS score between the CTL and PGL group (CTL_{BCS}: 2.9 ± 0.07 and PGL_{BCS}: 3.0 ± 0.01 ; $P=0.51$).

Plasma NEFA concentration started to increase on week 2 before parturition in both groups but there was no observable effect of treatment. Plasma BHB content started to increase after parturition in both groups and it was numerically higher in the CTL group on week 1 before- and after calving. However, these differences were not statistically significant. There was no effect of time or treatment on plasma glucose concentrations. Insulin level was significantly higher the PGL group during the supplementation prepartum ($P<0.01$), but not in the first week postpartu. Pulverized PGL supplementation had no effect on plasma T₃, T₄ and IGF-I levels. Hepatic total lipid content was not different between the CTL and the PGL group on d 7 after parturition (105.9 ± 48.9 vs. 76.7 ± 28.02 g/kg, $P=0.18$).

The supplementation had no effect on the pancreatic insulin response to glucose infusion reflected by insulin area under the curve (AUC). Glucose clearance rate had a tendency to be slower in the PGL group. However, the glucose disappearance must be interpreted cautiously, mostly in early lactation, when the glucose uptake of the mammary gland is increased. Similarly, the glucose response to exogenous insulin (ISBGR) had a tendency to be lower, indicating a more insulin sensitive state of the peripheral tissues compared to control group. Insulin resistant state can be exacerbated by elevated NEFA and ketone body levels in the blood as confirmed by the *Exp. 1*. Therefore by decreasing lipid mobilization with the means of glucogenic supplementation is an attractive alternative to augment the biological effect of insulin and increase glucose avail-

ability for peripheral tissues. However, in the present study PGL supplementation had no substantial effect on blood metabolites or on pancreatic insulin responsiveness.

There was no difference in the interval between calving to first postpartum ovulation between the CTL and PGL group (d 34.1 ± 12.9 vs. d 34.9 ± 14.5 , $P=0.98$). Pregnancy rate after the fixed time insemination was 10.7 % in the CTL and 17.6% in the PGL group ($P=0.45$). The number of pregnant cow till day 150 after parturition in the CTL and PGL group was 16.1% and 36% ($P=0.56$). The inconsistent effect of PGL administration on the time of first pp ovulation may be due to the differences in the dose and the method of PGL administration, and also in variances in the physiological state and body condition of the animals.

Conclusions

Manipulating the circulating insulin levels and the insulin sensitivity of the peripheral tissues by feeding glucogenic diet would be an attractive approach to reduce the negative energy balance and to enhance the postpartum ovarian activity in high yielding dairy cows. According to these data, however, we conclude, that feeding the current dry PGL preparation top dressed on the TMR from d 14 before calving till d 10 after calving had effect on the metabolic profile, insulin responsiveness, on time of first pp ovulation and on reproductive performance. The lack of notable effect of PGL supplementation may be explained by the allocation method of the PGL. On the other hand, economic benefits of PGL supplementation are questionable for dairy farms with adequate nutritional management, where – like the current conditions – animals are in appropriate energy balance.

Experiment 3

Design

Twenty multiparous Holstein cows with an average BCS of 2.5 ± 0.1 were selected from the experimental herd of the dairy farm research station of INIA La Estanzuela (Colonia, Uruguay). At day 21 before the expected calving date, cows were assigned to **Energy** group (n = 10) that received 3.5 kg/day of cracked corn grain 13.5 MJ each cow in individual feeders, and **Control** group (n = 10) without additional concentrate. Cows were offered also bales of hay of improved pasture (legumes and *Gramineae*) ad libitum, and both groups were kept in separate paddocks of improved natural pastures. After parturition, both groups were group-fed and received the same diet, that consisted in 4.0 kg/day of a commercial concentrate administered twice a day individually during milking time. This was complemented with 12 kg/day corn silage and the animals had access to a daily strip of improved pastures.

BCS was determined weekly from the beginning of the experiment until week 5 postpartum. Milk production was recorded daily and then averaged for each week. For determinations of milk composition (fat and protein content), a composite sample of four consecutive milking of each week was taken during the first 5 weeks postpartum. Blood samples were obtained weekly in the morning before the administration of concentrate from d -28 to d 35 (parturition: d 0) for assaying metabolites and metabolic hormones, and twice a week starting at the 2nd week postpartum for analyzing P4. Re-initiation of ovarian cyclicity was monitored by twice a week by transrectal ovarian ultrasonography and assaying P4 in the blood. Ovulation was determined by disappearance of the largest follicle followed by the formation of a corpus luteum which was confirmed by plasma P4 concentrations, from samples taken twice a week. The re-initiation of ovarian cyclicity was defined as the day when P4 increased from basal concentrations in two consecutive samples of >1.6 nmol/L or one sample of >3.2 nmol/L.

Results and Discussion

BCS was similar in both groups at the beginning of the trial, but from 7 d prepartum through remaining the experimental period, cows in the **Energy** group had higher BCS than cows in the **Control** group ($P < 0.01$), except for those observed at calving and at the first week postpartum, when BCS was similar in both groups. Milk production increased during the first 4 weeks of lactation, and was higher for the **Energy** group ($P < 0.05$). Milk protein and fat percentage decreased as the lactation progressed, and

there were no treatment-related differences ($P>0.5$). Information regarding the effect of energy supply during prepartum on milk production is conflicting; some studies proved an increase (*Overton and Waldron, 2004*) while others found no effect (*Holtenius et al., 2003; Roche et al., 2005*) in milk production. These differences may be attributed to the type of supplement, the way of administration and the quantity, as well as the production traits of the cows used in the different experiments. Another factor susceptible to increase milk production in the **Energy** group is the higher BCS and the resulting higher body fat mobilization as also reported by others (*Kokkonen et al., 2005*).

Prepartum concentrations of NEFA levels were similar in both groups; however, postpartum levels were higher in the **Energy** group. Cows in this group had a marked increase in NEFA after calving and showed a marked decrease from the first week postpartum. Cows in the **Control** group did not have a postpartum increase in NEFA concentration, but also showed a decrease during the postpartum period. The increase in the NEFA levels in the **Energy** group was associated with a drop in the BCS before and after parturition. This could have been due to an effect of the higher body fatness *per se* (*Chilliard et al., 2000*), and/or to a higher milk production. Since the **Control** group did not show an increase in this metabolite, we suggest that in this group there was not in NEB that is consistent with the lower milk production and loss of BCS. While **Energy** group BHB concentrations showed a significant increase after calving and maintained this level for the remaining period of the trial. From the third week postpartum BHB levels were similar in both groups

Plasma urea increased markedly in the **Control** group during the postpartum period while in the **Energy** group there was an increase up to the first week postpartum ($P<0.05$), to return to initial levels at the end of the trial. Urea decreased in both groups from the third week postpartum to the end of the trial.

While similar at the beginning of the trial, plasma insulin levels were consistently higher in **Energy** group. This treatment difference was due to a marked decrease of plasma insulin level before parturition in the **Control** as compared to the **Energy** group that maintained high levels during the postpartum period. This suggests that feeding easily fermentable carbohydrates during the prepartum transition period may deliver more glucogenic precursor to the liver and enhance insulin synthesis and/or release. On the other hand, the higher postpartum NEFA together with higher insulinemia in the **Energy** group could reflect higher insulin resistance due to higher body fatness (*Chilliard, 1999*), although this is true in relatively obese cows that was not the situation of

this study.

Mean *IGF-I* levels differed throughout the experimental period, there was also an effect of week of sampling ($P < 0.001$). Treatment difference was due to the higher *IGF-I* levels during the last week prepartum in the **Energy** group ($P < 0.01$). However, it decreased in both groups after calving, and those levels remained low throughout the experimental period.

Plasma *leptin* concentration was high during the prepartum period, dropped drastically after calving in both groups, and remained low for the rest of the trial. *Leptin* was higher in the treated cows during the first week prepartum ($P < 0.05$), but levels were similar in both groups during the postpartum period. There were no treatment differences in the circulating T_3 and T_4 levels.

Cows in the **Energy** group ovulated 12 days sooner than the **Control** group ($P < 0.05$). Interval from calving to first ovulation was 25.0 ± 3.7 days for the **Energy** and 37.4 ± 3.7 days for the **Control** group ($P < 0.05$). While all the cows in the energy group ovulated before 35 days postpartum, only 60% of those in the control group had the first ovulation before this period ($P < 0.05$). *Gong et al. (2002)* reported that the diet resulting in a higher insulin concentration reduced the interval from calving to first ovulation. The energy (cracked corn) supplementation on prepartum cows shortened the intervals from parturition to first ovulation, which is consistent with higher hormonal levels which have been reported to be related with reproduction in the prepartum period, although in the postpartum period only insulin levels were higher in the **Energy** group.

Conclusions

It was concluded that in lean cows kept under grazing conditions the energetic supplementation administered during the last three weeks precalving increased milk production and had a positive effect on the re-initiation of cyclic ovarian activity, which is consistent with a better EB (BCS), higher prepartum levels of insulin, *IGF-I* and *leptin*, and higher insulin levels during the early postpartum period.

NEW SCIENTIFIC RESULTS

The below results are thought to represent remarkable novelty value:

1. Long term hyperketonemia impairs the pancreatic insulin release and the whole-body glucose utilization in Holstein-Friesian cows. Only short term elevations in plasma free fatty acids and BHB may not potentially induce further increase in peripheral tissue insulin resistance in the early lactation (*Exp. 1.*).
2. Inflammatory diseases like puerperal metritis with intensive release of pro-inflammatory cytokines potentially further depress insulin secretion of the pancreatic β -cells and the whole body insulin responsiveness in dairy cows, with long term effects on metabolism and reproduction (*Exp. 1.*).
3. The homeostatic RQUICKI model developed for rapid and easy evaluation of insulin sensitivity in humans, and used previously in healthy cows should be applied only with cautions in dairy cows in different physiological and disease states (*Exp. 1.*).
4. Feeding a dry propylene glycol preparation absorbed on fumed silica, top dressed on the total mixed ration from d 14 before calving till d 10 after calving had no notable effect on the metabolic profile, insulin sensitivity, on time of first pp ovulation and on reproductive performance (*Exp. 2.*).
5. In lean cows kept under grazing conditions the energetic supplementation administered during the last three weeks precalving had a positive effect on the re-initiation of cyclic ovarian activity, which is consistent with a better BCS, higher prepartum levels of insulin, IGF-I and leptin, and higher insulin levels during the early postpartum period (*Exp. 3.*).

PUBLICATIONS RELATED TO THE PRESENT DISSERTATION

Full-text papers published in peer-reviewed journals in English

- **Kerestes M**, V. Faigl, M. Kulcsár, O. Balogh, J. Földi, H. Fébel, Y. Chilliard, G. Huszenicza. Periparturient insulin secretion and whole-body insulin responsiveness in dairy cows showing various forms of ketone pattern with or without perperal metritis. *Dom. Anim. Endocrin.*, 2009. 37. (4). 250-261
(last known IF: 2.165)
- Cavestany D, M Kulcsár, D Crespi, Y Chilliard, A La Manna, O Balogh, **M Keresztes**, C Delavaud, G Huszenicza, A Meikle. Effect of prepartum energetic supplementation on productive and reproductive characteristics, and metabolic and hormonal profiles in dairy cows under grazing conditions. *Reprod Dom. Anim.* 2009. 44. (4). 663 – 671. (last known IF: 1.526)
- Huszenicza Gy., **Keresztes M.**, Balogh O., Faigl Vera, Kátai L., Földi J., Lemo-niati, K., Kulcsár M. Periparturient changes of metabolic hormones and their clinical and reproductive relevance in dairy cows. Proc. XXV Jubilee World Buiatrics Congr., Budapest, July 6-11, *Magy. Áo. Lapja*, 2008. 130. (Suppl.) 1, 45-51. (last known IF:: 0.089)
- **Kerestes M**, Faigl V, Kulcsár M, Fébel H., Györffy A, Gaál T., Husvéth F, Bartha T, Mézes M, Tamási G., Gábor G., Szenci O, Huszenicza G. Effect of pulverized propylene glycol supplementation on periparturient metabolic profile and reproductive performance in Holstein-Friesian cows. *Vet. Med. Cz.*, submitted for publication (last known IF: 0.659)

Full-text papers published in peer-reviewed journals in Hungarian

- **Keresztes M**, Faigl V, Mézes M, Kulcsár M, Huszenicza Gy. Glükoneogenetikus takarmány-kiegészítők metabolikus és szaporodásbiológiai hatásai tejhasznú szarvasmarhában. Irodalmi áttekintés. *Magy. Áo. Lapja*, accepted for publication (last known IF: 0,089)

Poster or oral presentation on conferences:

- **Keresztes M.**, Crespi D., Kulcsár M., Meikle A., La Manna A., Faigl V., Balogh O., Delavaud C., Chilliard Y., Huszenicza Gy., Cavestany D.: Effect of prepartum energetic supplementation on milk yield, metabolism and reproduction in dairy cows under grazing conditions. *Proc. of 24th World Buiatrics Congress, Nice (France), 15-19 Oct., 2006*, oral presentation
- **Keresztes M.**, Faigl V., Györffy A., Langer D., Kulcsár M., Márton A., Gaál T., Fébel H., Bartha T., Tamás G., Szenci O., Huszenicza Gy. The effect of prepartum propylene glycol administration on metabolic and hormonal profile and on reproductive performances in Holstein-Friesian cows. *Magyar Buiatrikus Társaság XVIII. Nemzetközi Kongresszusa, Siófok, 2007. okt. 10-13*, oral presentation

- **Kerestes M.**, Faigl V., Győrffy A., Márton A., Langer D., Kulcsár M., Gaál T., Fébel H., Húsvéth F., Gábor G., Bartha T., Szenci O., Huszenicza G. The effect of periparturient propylene glycol administration on metabolism and on reproductive performance in Holstein-Friesian cows. 11th Annual conference of the European Society of Domestic Animal Reproduction (ESDAR), Celle, Germany. Abstract. *Reprod. Dom. Anim.*, 2007. 42. Suppl. 2. p. 47, poster presentation
- **Kerestes M.**, Faigl V., Kulcsár M., Huszenicza Gy. Insulin secretion in normal, ketonemic and in cows affected by puerperal metritis. 12th Annual conference of the European Society of Domestic Animal Reproduction (ESDAR), Utrecht, Netherland. Abstract. *Reprod. Dom. Anim.*, 2008. 43. Suppl. 5. p103, poster presentation

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