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**Abstracts of Short Communications** 

### Molecular mechanisms to control insulin sensitivity 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, which develops due to energy deficit at onset of lactation, is known to be associated with a reduced insulin sensitivity of adipose tissue in support of energy mobilization and glucose shifting to the mammary gland. However, understanding of the exact cellular mechanisms to achieve this reduced insulin action is still incomplete. 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  $\zeta$ , 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 insulin receptor, but a number of downstream signaling proteins were expressed at a greater amount in SCAT, compared with RPAT. Reduced insulin effect in the transition cow is known to be a consequence of decreased plasma insulin concentrations. But furthermore, the adipose tissue itself also expresses a decreased capacity to respond to insulin. This is achieved by down-regulating key signaling molecules during the early postpartum period, and is in accordance with the necessity of adipose mobilization to overcome energy deficit.

#### Acknowledgements

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# Trend to overcome the postpartum hormonal imbalance and oxidative stress in goat using *Trigonella foenum-graecum*

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Estrogen levels are sustained at elevated levels throughout the third trimester of pregnancy, and then drop dramatically during post-parturition period "estrogen withdrawal state". These fluctuations may contribute to postpartum stress, depression, and diseases. Fenugreek (Trigonella foenum-graecum L) is one of the oldest worldwide traditional medicinal plants. Fenugreek has many active compounds, including polyphenolic flavonoids, 4-hydroxyisoleucine, and trigonelline which possess multiple pharmacological effects, including hepatoprotective, antioxidant, hypocholesterolemic, anti-inflammatory and immunomodulatory properties. Also, it contains diosgenin, a steroidal saponin, which is used as lactation stimulator and to manufacture synthetic estrogen. Fenugreek has a long history as a breast enlarger promoting the growth of new breast cells and increasing the fullness of the breasts. This field study was aimed to evaluate the efficacy of Fenugreek to overcome postpartum stress and estrogen withdrawal state in goats. Nineteen postpartum goats  $(13 \pm 2 \text{ months})$  were allocated into two groups; 1) treated group (n = 10) received pulverized fenugreek seed at dosage of 2% in the diet for 4 weeks postparturition and 2) untreated control group (n = 9). Blood samples (plasma and serum) were collected at day 0 and every 7 days at 0700 hr and 1900 hr. Milk samples were collected daily at 0700 hr and 1900 hr. Plasma estradiol- $17\beta$ , progesterone, corticosterone and LH levels were determined at days 0, 7, 14, 21, and 28. Serum biochemical composition, tissue lipid peroxidation, and antioxidant biomarkers superoxide dismutase (SOD) and reduced glutathione (GSH) were analyzed at days 0, 14, and 28. Results revealed that plasma estradiol-17 $\beta$  and progesterone substantially increased after treatment, while plasma corticosterone levels significantly decreased ( $P \le 0.01$ ). Meanwhile, plasma concentrations of LH did not significantly change after treatment. Fenugreek supplementation significantly increased serum enzymatic antioxidant biomarker SOD and GSH levels compared with the control group. Furthermore, it significantly reduced lipid peroxidation. Milk production increase by 7% in treated group than the control. Besides, Fenugreek supplementation did not significantly affect ALT, AST and ALP enzyme activities and cholesterol levels in the serum. In conclusion, Fenugreek supplement proved to have a satisfactory efficacy in control of the oxidative stress and sudden drop in estrogen level postpartum, suggesting the usefulness of the supplement as a protective agent against postpartum stress and weakness in goats.

# The effects of chinese veterinary medicine preparation ChanFuKang on the endothelin and nitric oxide of postpartum dairy cows with qi-deficiency and blood stasis

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The research aimed to observe the effects of Chinese veterinary medicine preparation ChanFuKang on the vascular endothelial cells of postpartum dairy cows with qi-deficiency and blood stasis, and to explore the therapeutic mechanism of ChanFuKang. A total of 58 cows were divided into qi-deficiency and blood stasis treated and control groups, and healthy treated and control groups. The content of plasma endothelin (ET) and that of serum nitric oxide (NO) were measured before and after parturition, respectively. The contents of plasma ET decreased, while those of serum NO significantly increased in qi-deficiency and blood stasis control group compared with the healthy control group. The content of NO decreased on day 10 after parturition in qi-deficiency and blood stasis control group, but the content of ET was still higher than that in healthy control group. After treatment with ChanFuKang for 10 d, the NO content decreased, and there was no significant difference between qi-deficiency and blood stasis treated groups and healthy control group. In conclusion, ChanFuKang may be effective to improve the postpartum cows with qi-deficiency and blood stasis by increasing the contents of ET in plasma and decreasing that of NO in serum.

Key words: ChanFukang, dairy cow, endothelin, nitric Oxide, qi-deficiency and blood stasis.

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### Body condition correlates with results of ACTH-challenge in dairy cows

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High body condition at calving is a major risk factor for ketosis in early lactation of dairy cows. During stress episodes the hypothalamic-pituitary-adrenal axis (HPA-axis) is activated leading to release of cortisol. The reactivity of the HPA-axis as assessed by ACTH-challenge varies considerably between cows. In this study the relationship between HAP-axis reactivity and body condition was investigated. In 22 pluriparous German HF Dairy cows, kept in free stalls with cubicles and fed a typical TMR ration based on grass and corn silage and concentrate, an ACTH challenge was performed on day (d) 100 postpartum. Before and after injection of ACTH cortisol concentrations were measured in blood samples taken in short term intervals from indwelling jugular vein catheters. Baseline cortisol concentrations, the area under the cortisol concentration curve (AUC) and peak cortisol concentrations were used in a regression analysis (SAS statistical package) and correlated with subcutaneous fat mass (SC fat) assessed on d-42 prior to parturition, at d1, d21, d100 pp. Cows were also classified according the AUC cortisol results into high (HR; n = 7), intermediate (IR; N = 8), and low (LR; N = 7) 7) responder and fat mass was tested for differences by using ANOVA. Significant linear negative correlations were found between AUC and peak cortisol concentrations from ACTH challenge and SC fat at d-42, d1, d21 and d100. In HR compared to LR cows in average SC fat was significantly less at d-42, d1, d21 and d100 and gain prior to and loss of SC fat after parturition was lower. Cows with high reactivity of the HPA-axis are leaner during the whole transition period, gain less before and loose less SC fat after parturition. Dynamics in body condition appears to be closely correlated with HPA axis reactivity during the transition period in dairy cows.

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### Liver copper and serum ceruloplasmin concentrations in hyperketonemic pregnant ewes

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This study was accomplished due to evaluate the effect of induction of subclinical pregnancy toxemia (PT) and subsequent hyperketonemia in ewes on serum ceruloplasmin and liver copper concentrations. The experiment was performed on five pregnant native ewes aged 3-4 years, 45-50 kg weight with BCS of 3.5-4 on a 0-5 scale. The pregnancies of these ewes were confirmed with ultrasound examination. Blood and biopsy samples were taken from the jugular vein and 12th ICS, respectively before the induction of subclinical PT by food deprivation and after when serum  $\beta$ -hydroxybutyrate concentrations > 0.8 mmol/l and ewes were suffering from subclinical PT. Serum BHBA concentrations of ewes after induction of subclinical PT were significantly higher than before the induction (P < 0.01). Serum glucose concentrations of induced hyperketonemic ewes were significantly lower than before the induction of hyperketonemia (P < 0.01).

Serum ceruloplasmin concentrations of ewes after the induction of subclinical PT were significantly higher than before the induction (P < 0.01), but liver copper concentrations showed significant decrease after the induction of subclinical PT (P < 0.05). It is concluded that circumstances such as hyperketonemia, can increase ceruloplasmin concentrations and decrease liver copper concentrations. Although evaluation of serum ceruloplasmin concentrations in ewes is a routine procedure for estimation of copper status, its levels should be evaluated with caution during late pregnancy.

Key words: ceruloplasmin, hyperketonemia, ewe, liver copper.

### System for measuring blood calcium level in dairy cattle using wireless portable electrocardiograph

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We previously developed a non-invasive system for measuring the blood calcium (Ca) levels in cattle using a wired electrocardiogram (ECG) amplifier and a tablet PC, and applied to diagnosis of hypocalcemia based upon the strong positive correlation between the blood Ca concentration and the inverse of ST peak interval corrected according to Bazett's formula (STc: ST peak interval /SS peak interval<sup>0.5</sup>). In the present study, we have established a wireless portable system for measuring the blood Ca level aiming at the on-site application. A total of 894 Holstein cattle with normal ECG was used for analysis of blood Ca concentration, STc, days postpartum, age, calving number, body temperature, daily milk yield, hours post feeding and milking, and blood concentrations of P, Mg and K. A multiple regression analysis was conducted to obtain estimated regression equation of blood Ca concentration corresponding to days postpartum. In order to detect the abnormal ECG waveform, we created the algorithm to remove the noise from EMG and body movement, by superimposing the ECG waveforms on the S wave peak and averaging all the cardiac potential. These regression equations and algorithms were implemented in a system consisting of a wireless (Bluetooth®) ECG and a tablet PC. In cattle within three days postpartum, there is a strong correlation (r = 0.86) between blood Ca concentration and  $STc^{-1}$ . The correlation coefficient rose to 0.88 by adding calving number as the variable. However, in cattle after four days postpartum, the correlation was weak (r = 0.48); in case of hypocalcemia the similar regression equation was gained as in the cattle within three days postpartum, whereas in case of normal Ca levels no correlation was observed. The noise waveform was removed, and clear S, T and P wave peaks were obtained. Other peaks were recognized as abnormal waveforms. Altogether, this system is recommended to the use for cattle within the first three days postpartum. Addition of calving number or age into the estimated regression equation helped improve the estimation accuracy. The current wireless portable system also enables to cut out the abnormal waveform such as the bimodal or negative T wave, facilitating on-site use in the farm.

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# Isolation, identification, molecular characterization and epidemiological investigation of *Prototheca zopfii* from bovine mastitis

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Bovine mastitis, generally caused by a variety of pathogens including bacteria, fungi and algae, is the major cause of economic losses to the dairy industry. Prototheca zopfii has been considered as an important algal pathogen for the dairy cow mastitis. The aim of this research was to study the molecular characterization and epidemiology of P. Zopfii isolated from bovine mastitis in China. A total of 358 milk samples of bovine subclinical mastitis and 328 samples from environmental sources including 71 fecal, 76 feed, 83 drinking water and 98 bedding samples were involved. Routine morphological and biochemical methods as well as PCR assay based on 18s rDNA gene were used to identify P. zopfii; genotype-specific PCR assays and restriction fragment length polymorphism (PCR-RFLP) assays and susceptibility test were used to investigate the genotypic composition and resistance characteristics, respectively. A phylogenetic tree was constructed according to 18S SU rDNA and 28S SU rDNA of P. zopfii and its molecular epidemiology was analyzed. Seventy three P. zopfii isolates were detected, of which 47 (13.1%) from milk samples were assigned as P. zopfii genotype II; 19 (5.8%) and 7 (2.1%) isolates from environmental sources were identified as P. zopfii genotype I and genotype II by PCR-RFLP, respectively. Antibiotics susceptibility test showed that the studied isolates were only susceptible to few antibiotics and antifungal drugs including amphotercin B, nystatin, gentamycin, amikacin and streptomycin, Additionally, P. zopfii genotype I and genotype II had observed different resistance rates to various antibiotics and antifungal drugs. Phylogenetic analysis indicated that genotype I and genotype II isolates were grouped into two different clusters. P. zopfii genotype II isolates from environmental sources may not constitute an important risk factor to bovine mastitis. These results indicate that there is a higher isolation rate (10.6%) of P. zopfii from mastitis isolates, the organisms are showing high antibiotic resistance, P. zopfii genotype II from milk samples plays an important role in bovine subclinical mastitis.

Keywords: antibiotic susceptibility test, bovine mastitis, molecular characterization, genotyping, phylogenetic tree, *Prototheca zopfii*.

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# Subclinical mastitis in dairy cows in Kurunegala district of Sri Lanka: prevalance, associated risk factors and effects on reproductive performance

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Subclinical mastitis (SCM) causes huge economic losses to the dairy industry since it is difficult to detect due to absence of any visible clinical indications or changes in milk. The information on prevalence, associated risk factors of SCM is scanty in Sri Lanka. It has been suggested that mastitis affects reproductive performance of dairy cows. Therefore, this study was conducted to determine the prevalence, risk factors associated with SCM and the effects of SCM on reproductive performance of dairy cows in Kurunegala district. A total of 200 lactating cows in four large scale, NLDB (National Livestock Development Board) and eight small scale farms in Kurunegala district, during the period from April 2014 to August 2014 were selected for the study. Farms were visited during morning milking and California Mastitis Test (CMT) was conducted for each cow. Cows which gave a score of 2 or 3 for any of the quarter were considered as positive for SCM. Samples from infected animals were transported in ice to the laboratory of veterinary investigation center, Pannla for bacteriological analysis. Data on individual cows and herds were collected using a pre-tested questionnaire. Associated risk factors for SCM and the effect of SCM on reproductive parameters were separately analyzed by using binominal logistic regression. Kaplan-Meier survival function estimates were used to calculate crude associations of normal and SCM positive cows with median time from calving to Artificial Insemination (AI). Overall prevalence of SCM in the current study was 57.5%. Higher prevalence of SCM was detected in small scale dairy farms (68%) in comparison to NLDB dairy farms (56%). The most common pathogen was coagulase-negative staphylococcus aureus (87.09%) followed by E. coli (12.90%). The occurrence of SCM was higher in Jersey cows compared to crossbred cows (odds ratio (OR) = 27.84, P = 0.01). The prevalence was significantly higher in cows that produced a daily milk yield over 7 L compared to cows that produced less milk (P < 0.05). Farms that practiced machine milking showed a higher risk for having SCM compared to the farms that practiced hand milking or both (OR = 0.48, P < 0.01). Normal cows and infected cows did not show any significant difference for the reproductive parameters such as parity, calving interval and total number of AI (P > 0.05). Cumulative proportion inseminated against days from calving to AI in cows that had SCM was delayed by 15 days relative to normal cows (median d 79 and 64, P = 0.02). The results revealed that the prevalence of SCM is high in Kurunegala district and breed, milk yield and machine milking are the associated risk factors for SCM. SCM also affected the inseminated proportion of lactating cows in this area. Keywords: CMT; dairy cows; reproductive parameters; risk factors; subclinical mastitis.

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# Effects of preparturient exercise in an outdoor pen on periparturient energy condition and subsequent reproductive performance in dairy cows

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Suitable nutritional management during the dry period is necessary for minimizing postpartum negative energy balance in dairy cows. However, many cows in tie-stall dairy farms tend to be obese during late lactation, which complicates control of nutritional conditions after dry-up. This study evaluated the effects of exercise in an outdoor pen during the dry period on periparturient energy balance and subsequent reproductive performance in dairy cows. Eighteen multiparous Holstein cows were examined between 60 d prepartum to 28 d postpartum. The experimental group (n = 9) was allowed to exercise in an outdoor pen between 10:00 and 15:00 every day during the dry period; the control group (n = 9) was kept in tie-stalls during the entire experimental period. Body weight and body condition score (BCS), as well as concentrations of serum glucose, nonesterified fatty acids, and  $3-\beta$ -hydroxybutyrate were measured at 56, 28, and 7 d prepartum and 7, 14, and 28 d postpartum. The following were also recorded: the dates of first estrus, artificial insemination (AI), and conception; difficulty calving; time between parturition and placental expulsion; dairy milk yield; and milk progesterone levels to evaluate postpartum day of ovulation and resumption of ovarian cyclicity. BCS at 7 d prepartum was significantly lower in the experimental group  $(3.14 \pm 0.07)$  than in the control group  $(3.31 \pm 0.06)$ . BCS in the control group decreased continuously until 28 d postpartum but was lowest at 14 d in the experimental group. Ovarian cyclicity resumed normally in 6 and 7 cows in the experimental and control groups, respectively. First ovulation and insemination in the experimental and control groups occurred at 19.5  $\pm$  3.0 and 14.6  $\pm$  2.7 d postpartum and 79.8  $\pm$  6.1 and 57.4  $\pm$  2.4 d postpartum, respectively. Conception rates of first AI in the experimental and control groups were 85.7% and 42.9%, respectively; days open was decreased in the experimental group. No other parameters significantly differed between groups. These data suggest that preparturient exercise in dairy cows suppressed the abrupt decline of BCS around parturition and reduced days open during the next lactation.

# iTRAQ-based quantitative proteomic analysis of uterus tissue and plasma from dairy cow with endometritis

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To discover the molecular mechanism of endometritis in dairy cows, the proteomics of the uterine tissue and plasma from cows with endometritis were analyzed by quantitative proteomic. Some uterine tissue and the corresponding plasma were collected from dairy cows at the time of slaughter, and they were quickly frozen in liquid nitrogen. A patch of every tissue also was fixed into formalin. After brought them to lab, the fixed tissue was examined by pathological procedures, and the frozen tissue and the corresponding plasma with endometritis was selected as study sample. There were four normal control and four endometritis samples, and their corresponding plasma. Every set of tissue and plasma samples was mingled into one mixed sample in normal control and pathological groups, respectively. Lastly, two mixed control samples and two mixed pathological samples were processed for proteins extraction at BGI technology company (Shenzhen, China). The samples were labeled by iTRAQ, and detected by tandem mass spectrometry (MS/MS). Then the mass data were transformed into bio-informatics, and differentially expressed proteins (DEPs) were identified out and analyzed. DEPs also were enriched at bio-informatics notation including cluster, gene ontology (GO), and kyoto encyclopedia of genes and genomes (KEGG) pathways. There were 109 up-regulated proteins and 50 down-regulated proteins in uterine tissue, while 49 up-regulated proteins and 88 down-regulated proteins in plasma. The combined analysis showed that there were nine common proteins which were expressed in both tissue and plasma. For these proteins, three were up-regulated and two were downregulated in both of tissue and plasma; three proteins were up-regulated in tissue, but down-regulated in plasma; one was up-regulated in plasma, but down-regulated in tissue. Enrichment analysis of GO showed that inflammation primarily affected generation of cellular process, biological adhesion, metabolic process, regulation of biological process, response to stimulus, and single-organism process in both of uterine tissue and plasma. KEGG pathways analysis showed that pathways of staphylococcus aureus infection, phagosome, and complement and coagulation cascades were the most possible mechanism in pathogenesis. It may be concluded that iTRAQ technology can screen the common DEPs in tissue and plasma from dairy cows with endometritis. The study on the mechanism of endometritis may mainly focus on the activities of hydrolase and peptidase that played important roles in response of stimulus, stress, and immune regulation at extracellular region and plasma membrane.

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### Ovsynch enhances fertility of Holstein cows subsequent to treatment for uterine involution

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The objective of this study was to investigate the effect of Ovsynch program applied on Holstein cows subsequent to early postpartum  $PGF_{2a}$  treatment for uterine involution. A total of 2,059 lactating Holstein cows under early postpartum  $PGF_{2a}$  treatment, three i.m. injections of  $PGF_{2a}$  at 7, 14, and 21 DIM, were randomly divided into the treated (Ovsynch) and control (spontaneous estrus) groups. Cows in the treated group (n = 1,121) received Ovsynch program beginning at 45 DIM and at the same time heat detection was implemented. Artificial insemination (AI) was carried out in cows expressing estrus before the timed AI day of Ovsynch, while cows having no Graafian follicles on the timed AI day, delayed AI was carried out. Control cows (n = 938) were bred under the routine reproductive management after 45 DIM according to the a.m.-p.m. breeding rule, with occasional use of  $PGF_{2a}$  and GnRH when considered to be appropriate by the herd veterinarian and manager. Heat was detected by observing standing heat and by rectal palpation. Pregnancy diagnosis was performed by rectal palpation on d50 post-AI. The percentage of cows inseminated within 55 DIM was higher in treated group than control group (80.9% vs. 12.4%). Median days to first AI (55.9 vs. 66.2) and days open (81.8 vs. 91.0) were less for treated cows than for control cows, respectively. Pregnancy rates after the first AI were similar (33.2% vs. 35.7%) in the two groups even though treated cows were inseminated earlier after calving than control cows. In the treated group, during the execution of Ovsynch, a part of cows (11.1%) came into heat before timed AI day. On the day of timed AI most cows (68.5%) had a Graafian follicle, while 20.4% of the cows had no Graafian follicles. Pregnancy rates were similar (35.5% vs. 33.6% vs. 30.6%). In conclusion, by the application of Ovsynch program after early postpartum  $PGF_{2a}$  treatment for uterine involution, reproductive efficiency of Holstein cows is improved. And for some cows which follicular development is not synchronized under Ovsynch program, AI before or after fixed timed AI day can increase the pregnancy rate.

### Follow up of embryonic/fetal losses in different breeds of goats using real time B-mode ultrasonography

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The objectives of the current study were to characterize the timing of embryonic/fetal losses (EFL), and to identify the factors with which these losses were associated as well as to ensure whether relationship existed between serum progesterone (P4) and EFL. A total of 151 goats of different breeds (70 Zaraiebi, 42 Damascus, and 39 Ballade/Cross breeds) were evaluated by ultrasonography to monitor EFL during different stages of gestation (D19-22, D26-29, D33-36, D40-45, and D47-54 post breeding). Blood samples were collected at D20, and at each ultrasonographic scanning to clarify the changes of serum P4 concurrently with EFL. Results revealed that 45/109 goats (41.28%) were exposed to EFL either in the form of partial losses (18.35%), total losses (16.51%), or partial then total losses (6.42%). The ultrasonographic results showed a high incidence of EFL in Zaraiebi goats compared to others. Moreover, a high EFL% occurred in the period between D19-22 to D47-54 compared to the period between D47-54 to birth (11.76%). Age and goat parity had no significant effect on EFL % in all goats with special significance within each breed. Serum P4 at D20 did not show a significant difference between normal pregnant goats and goats exposed to EFL. Moreover, goats that experienced partial EFL showed mild non-significant (P > 0.05) reduction of serum P4 concomitant with EFL. On the contrary, in 70% of goats with total EFL, the P4 levels were sharply reduced (85.06%; P < 0.01) suggesting the probability of endocrine disruption of the corpus luteum. Moreover, 30% of total EFL goats showed low P4 reduction (24.90%;  $P \le 0.05$ ), which was considered to be this reduction is an effect rather than a cause of EFL. In conclusion, different factors such as breed, age, and parity were involved in the incidence of EFL in goats. Moreover, improvement of the goat management in the early stage of pregnancy is important to decrease EFL % in goats.

# Prevalence of prepartum negative energy balance and subclinical ketosis in the early lactation of dairy cows in peri-urban Kampala, Uganda

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Negative energy balance (NEB) within a few weeks before calving and subclinical ketosis (SCK) in the early lactation are the two major factors causing increased incidence of postpartum diseases, reduced milk production and impaired reproductive performance in dairy cows. Non-esterified fatty acid (NEFA) and ketones are reliable biomarkers of negative energy balance and metabolic disorders in the transition period. Objectives of this study were to show the prevalence of NEB in late dry period and postpartum SCK by measuring NEFA and  $\beta$ -hydroxybutyrate (BHBA), and to explore their adverse effects on health, production and reproduction in crossbred dairy cows. The herd metabolic monitoring was carried out in 2013 and 2014. During June to August, 2013, 34 farms in peri-urban Kampala were visited twice or thrice at 4 week intervals. Cattle within 30 d before due date (N = 47) and/or those within one month postpartum (N = 49) at each visit were enrolled into the monitoring program. Average milk yield per cow per day ranged from 5 to 20 L, majority being 5 to 10 L (41%) and 10 to 15 L (41%). In 2014, 31 farms in the same region were visited twice during a period from April to June. Thirty-five cows within one month postpartum were enrolled into the postpartum monitoring program. Blood samples were collected via the tail vein for the on-farm blood ketone test and for analyzing serum concentrations of NEFA. Cows diagnosed as having SCK were drenched with 300 mL propylene glycol for three days. Approximately 31% of 39 cows prepartum tested for NEFA had NEB. Prevalence of SCK within one month postpartum was 18.8% in 2013 and 13.9% in 2014. Cows with NEB prepartum had higher incidence of postpartum complications (P < 0.05) than those with normal EB. Cows with SCK and clinical ketosis postpartum tended to show a lower first breeding conception rate and longer interval from calving to conception (P < 0.10) than nonketoic cows. The study provides the first report on the prevalence of NEB and ketosis in crossbred dairy cows in Uganda, which were similar to the levels reported in Europe and Japan.

# Effects of intramuscular administration of vitamin e before transport on transport stress response of Holstein steer calves

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To develop labor-saving and effective acclimatization methods for grazing, we specifically examined transport, one of several major stressors at the beginning of grazing accompanied with a short-haul trip to public pasture lands. Our previous researches show that orally administered vitamin E (VE) supplementation before transport mitigates transport stress. Based on the earlier results, the present study assesses effects of single and repeated intramuscular administration of VE before transport. Nine Holstein steer calves, 5-7 months of age, were allocated three animals each to repeated treatment (R), single treatment (S), and control (C) groups using a  $3 \times 3$  Latin square design. The animals were kept tethered in a barn with basic feed comprising timothy hay and concentrated feed. A commercially available VE preparation was administered intramuscularly to group R calves at 10 mg tocopheryl acetate/kg BW/dose at 7 days and 1 day before transport. Saline and the VE preparation were administered in the same amounts to group S calves at each of the days, respectively. Saline alone was also given to group C calves. On the day of transport, the animals were transported by truck for 4 hr. Sampling was done at 7 days and 1 day before transport (d-7 and -1), immediately after the end of transport (4 hr), and 1, 3 and 7 days after the start of transport (d1, d3, and d7). Serum alphatocopherol increased rapidly until 4 hr and then decreased gradually in group S. The concentration of d-1 in group R was twice as high as d-7 (basal levels), although its time course resembled that of group S after the second treatment. It was higher in groups S and R than in group C until d3. Rectal temperature, plasma cortisol and peripheral blood neutrophil to lymphocyte ratio were highest at 4 hr, although these showed no differences among groups. Serum amyloid A was higher than that before transport in groups S and R over time from 4 hr to d3, and was higher in group R than in group C over time until d7. No enhanced PBMC cytokine production was observed from VE treatment, and peripheral blood phagocytosis showed only mild mitigation in reduction in group R. No difference in daily weight gain was apparent among groups after transport. In conclusion, high-dose intramuscular VE treatment might promote stress response and inflammation, perhaps because high-dose VE treatment before transport produced alpha-tocopherol biokinetics resembling those of oral supplementation, although this treatment exhibited less mitigation of transport stress.