



Changes of Body Condition Scores, Serum Biochemistry and Liver Triacylglycerol in Periparturient Holstein Friesian Dairy Cows Raised in a Small-Holder Farm

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ABSTRACT

Negative energy balance (NEB) inevitably occurs in periparturient dairy cows. Its consequences are related to reduced cows' performances. Most studies concerning the NEB are performed in dairy cows of large-scale farms, particularly raised under non-tropical climate. The current study aimed to investigate the changes in body condition score, serum biochemical parameters, and liver triacylglycerol (TAG) accumulation in periparturient Holstein Friesian dairy cows raised by a small-holder farm. In this regard, 10 healthy pregnant dairy cows in a small-holder farm were recruited for the study. At 4 weeks before and 1, 2, 4, and 8 weeks after calving, blood samples were collected for determination of glucose, non-esterified fatty acid (NEFA), β -hydroxybutyrate (BHBA), and insulin-like growth factor-I (IGF-I) concentrations. BCS was evaluated at 4 weeks before and 2 weeks after calving. Liver samples were collected 4 weeks before and 2 weeks after calving to determine TAG concentration. Results revealed that serum NEFA and liver TAG concentration were elevated postpartum. Serum BHBA concentrations increased postpartum and the concentration indicated that dairy cows entered NEB condition as type I ketosis with a longer period. Serum IGF-I concentrations and BCS did not differ between before and after calving. In conclusion, dairy cows raised under small-holder tropical conditions suffered from serious NEB, though the cows had low milk production, as compared with the commercial non-tropical condition.

Keywords: Blood biochemistry, Dairy cow, Liver triacylglycerol, Negative energy balance, Small-holder farm

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INTRODUCTION

Management of the periparturient period (3 weeks before and 3 weeks after calving) is the key strategy to determine milk production efficiency throughout the lactation period. In this phase, dramatic changes in rumen ecology, nutrient requirements, physiological events, and hormonal responses occur. The lipolysis process of adipose tissue is triggered most obviously before calving, corresponding to alteration of energy balance and reduction of serum glucose. Consequently, the elevation of serum non-esterified fatty acid (NEFA) and loss of body condition score (BCS) are observed in cows (Rukkwamsuk et al., 1999; Macrae et al., 2019). Thereafter, NEFAs enter the liver, and are broken down to acetyl-CoA before entering the citric acid cycle (CAC). In depletion of oxaloacetate in the CAC, acetyl-CoA is converted to ketone bodies, entailing acetoacetate, acetone, and β -hydroxybutyrate (BHBA), or the NEFA is re-esterified and accumulated within hepatocytes as triacylglycerol (TAG) so-called fatty liver (Rukkwamsuk et al., 1999). All metabolic changes, including high serum NEFA, high serum BHBA, and increased liver TAG, have negative effects on feed intake, milk production, reproductive performances, and health conditions (Raboisson et al., 2015; Garcia-Roche et al., 2019). Apart from biochemical changes, decreased serum growth hormone concentrations and insulin-like growth factor-I (IGF-I) impair mammary and reproductive functions (Duan and Xu, 2005; Murney et al., 2015).

Degrees and patterns of NEB responses of the cows are different across farms and geographical areas (Oetzel, 2007), leading to a bulk of studies in large-scale commercial farms, especially those under non-tropical climate. Various factors affecting NEB condition include nutritional management, cows' comfort, and heat stress condition. All of which differed depending on farming types and environmental conditions (Whitaker et al., 1999). This study aimed to evaluate changes in biochemical and hormonal parameters in relation to NEB in periparturient cows raised under a small-holder farm in Thailand.

MATERIALS AND METHODS

Ethical approval

All procedures were approved by the Institutional Animal Care and Use Committee (ACKU62-VET-098) of Kasetsart University, Thailand.

Animal selection

The study was carried out at a small-holder dairy farm in Nakhon Pathom province, Thailand. The DMS latitude longitude coordinates for Nakhon Pathom are 13°49'10.56"N, 100°2'39.37"E. In total, 10 healthy pregnant crossbred Holstein Friesian cows were recruited into this study. The average lactation number was 3.67, BCS ranged from 2.5 to 3.5, and average milk production in the previous lactation was 11.63 kg/cow/day. All cows were kept in a free stall area with free access to rice straw and water. The forage feeding was done twice a day with approximately 20 to 25 kg of mixed fresh corn cob and corn husk per cow, and the commercial concentrate was offered only in the afternoon (2.00 p.m.) at the rate of 1 kg concentrate per 2 kg of milk yields.

Sample collections and records

The BCS was recorded 4 weeks before the expected calving date and 2 weeks after calving in a 1 to 5 scale system. Milk yields were weighed weekly during the experimental period (from calving to 8 weeks after calving). The blood samples of all cows were obtained by venipuncture from the coccygeal artery or vein 4 weeks before the expected calving date and at 1, 2, 4, and 8 weeks after calving. Blood samples were left at room temperature for 30 min before centrifuging at 1200xg for 10 minutes. Subsequently, serum samples were harvested and stored at -20°C until the determination of the concentrations of glucose, BHBA, NEFA, and IGF-I. BCS and milk yields of all cows were recorded during the time blood samples were obtained. Liver samples were collected by percutaneous biopsy technique from all cows 4 weeks before the expected calving date and 2 weeks after calving. After collection, tissues were placed on filter paper for removing any connective tissues and blood clots. Thereafter, liver samples were transferred to a glass tube with physiological saline and kept in an icebox during transportation. The liver samples were weighed and stored at -20°C until analysis of TAG concentration, triplicate analyses were performed.

Sample analyses

Serum concentrations of glucose (Glucose, Erba mannheim®, UK), BHBA (Ranbut, Randox Laboratories Ltd., UK), NEFA (NEFA, Randox Laboratories Ltd., UK), and IGF-I (human IGF-I, Mediagnost®, Germany) were measured using the commercially available test kits as indicated. Liver concentrations of TAG were measured using the method described previously by van den Top et al. (1994). Briefly, liver tissues were dissolved overnight with 0.5 ml of KOH (20%) and then saponified with 1 ml of absolute ethanol. The reaction tubes were placed in a water bath at 37°C for 1 h. One milliliter of MgSO₄ (0.15 M) was added, followed by centrifugation at 1200xg for 10 min. Thereafter, supernatants were removed and 0.5 ml of KOH (0.5 M) in absolute ethanol was added. The sediment and the remaining ethanol were evaporated in a water bath at 100°C. Consequently, 2.0 ml of HCl (2 M) were added, and the tubes were placed in a water bath at 100°C for 2 hours. Titration with NaOH (5 M) was done until pH of 7.0 was attained. TAG concentrations in the supernatant were determined by a commercial kit (Triglyceride, Erba mannheim®, UK) and calculated into liver TAG concentrations, which were expressed as mg of TAG per gram of liver wet weight).

Statistical analyses

During the experiment, one cow was suffered from a serious illness, resulting in sudden death; therefore, data of these cows were excluded from the analyses. All data from the remaining 9 cows were analyzed for their normality using the Shapiro-Wilk test. Normally distributed data including blood glucose, NEFA, and BHBA concentrations were analyzed using sampling days as a repeated measure. Comparison of blood glucose, liver TAG, and serum IGF-1 concentrations between before and after calving were performed using paired t-test. Non-parametric analyses, where appropriate, of repeated measures ANOVA and paired t-test, were performed using Friedman test and Mann-Whitney U test, respectively. All analyses were conducted using the statistical software package STATA (version 13.0, Stata Corp., College Station, TX, USA).

RESULTS

General information of the studied cows (n = 9) is demonstrated in Table 1. The average BCS of cows before and after calving did not differ (2.69 vs 2.69). For individual cow's BCS, only 1 out of 9 (11.11%) cows lost BCS greater than 0.5 scores at 2 weeks postpartum, compared to 4 weeks prepartum. Average milk yields during the first 60 days of lactation were 6.31±3.4 kg/cow/day. Serum glucose concentration did not differ across sampling periods (Figure 1). NEFA

concentrations were significantly different between sampling periods (Figure 2). Average NEFA concentrations dramatically increased after calving and gradually decreased during sampling periods. For BHBA, average concentrations were 0.65 ± 0.078 , 1.40 ± 0.56 , 1.30 ± 0.39 , and 1.44 ± 0.634 mmol/L at 4 weeks before the expected calving date and at 1, 2, and 4 weeks after calving, respectively (Figure 3). When considered individually, the elevated serum BHBA concentrations above the baseline value in cows were reported as 62.5, 60.0, 33.3, and 25.0% at 4 weeks before the expected calving date, 1, 2, and 4 weeks after calving, respectively. Dairy cows in the experiment also demonstrated the elevation of liver TAG concentrations postpartum. According to previous studies, the TAG concentrations in this study did not reach moderate fatty liver conditions (Figure 4). Serum IGF-I concentrations before and after calving were 119.89 ± 48.16 and 72.65 ± 34.10 ng/ml, respectively (Figure 5). Regarding IGF-I concentrations, there was no difference before and after calving periods.

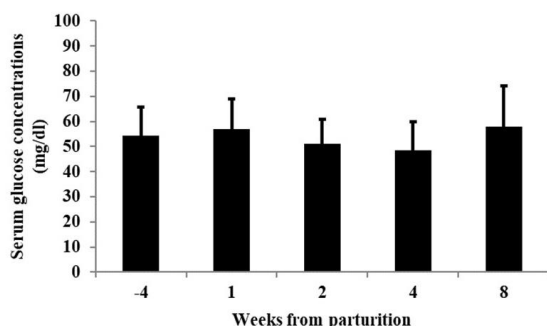


Figure 1. Serum glucose concentrations at 4 weeks before parturition and 1, 2, 4, and 8 weeks after parturition in periparturient cows (n = 9)

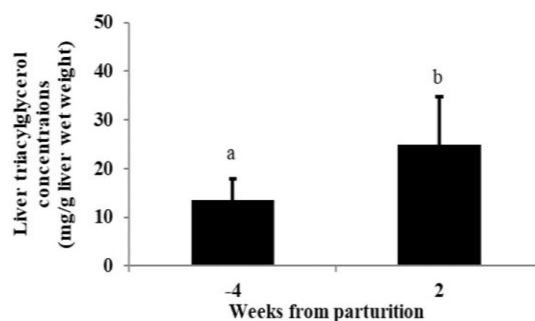


Figure 4. Liver triglyceride concentrations at 4 weeks before parturition and 2 weeks after parturition in periparturient cows (n = 9)

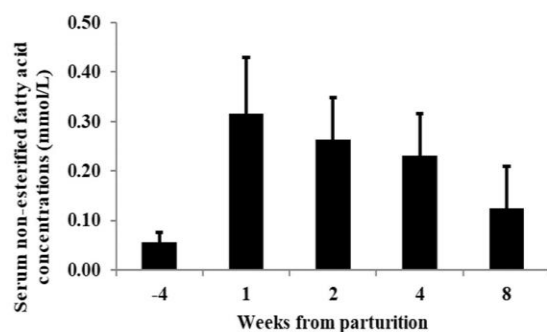


Figure 2. Serum non-esterified fatty acid concentrations at 4 weeks before parturition and 1, 2, 4, and 8 weeks after parturition in periparturient cows (n = 9)

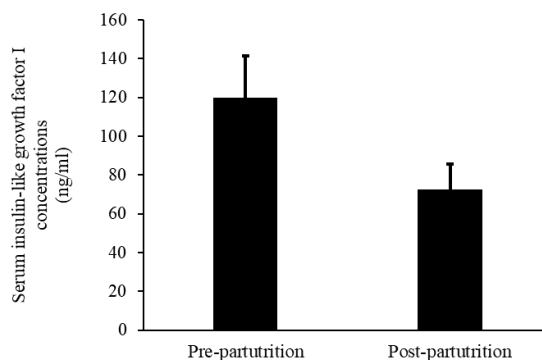


Figure 5. Serum insulin-like growth factor I concentrations prepartum and postpartum in periparturient cows (n = 9)

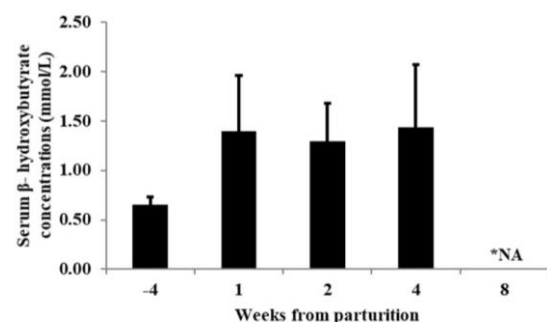


Figure 3. Serum β -hydroxybutyrate concentrations at 4 weeks before parturition and 1, 2, 4, and 8 weeks after parturition in periparturient cows (n = 9, NA: Not available)

Table 1. Average and standard deviation of lactation number, prepartum body condition score (at 4 weeks), postpartum body condition score (at 2 weeks), and milk yield (during the first 60 days of lactation) of periparturient cows (n = 9) during the experiment

| Item | Mean | SD |
|---------------------|------|------|
| Lactation number | 3.67 | 2.00 |
| Prepartum BCS | 2.69 | 0.17 |
| Postpartum BCS | 2.69 | 0.98 |
| Milk yield (kg/day) | 6.31 | 3.40 |

BCS: Body condition score, SD: Standard deviation.

DISCUSSION

Due to the unavailability of milk production data at small-holder dairy farm levels, it was not possible to compare average milk yields with previous milk yield records. However, dairy cows in the current study had far lower daily milk production throughout the lactation period when compared with the average milk yield (12.18 kg/cow/day) of Thailand (Aiumlamai, 2009). Average first 60-day milk yields were also lower than the previous average lactation milk yield of the farm (6.31 vs 11.63 kg/day). In this case, milk production did not reach the peak of lactation during the first 60 days in milk and this lactation yield tended to be lower than the previous lactation. The absence of the lactation peak and decrease in production compared with the previous lactation indicated that cows might confront problems during the periparturient period. In agreement with the BCS, the investigated cows had subtle changes of the BCS (prepartum vs postpartum), possibly resulted from very low milk production. It should be noted that reduction of BCS postpartum is a usual condition in dairy cows, and the cows who lose BCS greater than 0.5 scores postpartum are considered to face an NEB problem (Mulligan et al., 2006).

Average glucose concentrations were not in the hypoglycemic stage (>40 mg/dL, Mair et al., 2016). All NEFA concentrations in the current study were lower than a cut-off value for NEB condition (prepartum concentration <0.3 mmol/L, postpartum concentrations <0.7 mmol/L; McArt et al., 2013). Elevation of serum NEFA after calving, though not reach the NEB stage, was also reported in small-holder dairy farms in one study (Rukkwamsuk, 2010), however, the obtained results were inconsistent with the findings of a study conducted by Rukkwamsuk et al. (2006). In contrast to the findings of the current study, both studies found that average NEFA concentrations reached the NEB stage before calving (Rukkwamsuk et al., 2006; Rukkwamsuk, 2010). An increase in serum NEFA concentrations plus loss of BCS in periparturient cows resulted from lipolysis. The degree of both changes in the present study was quite low, therefore, lipolysis seemed to be limited.

Average BHBA concentrations indicated that cows were in the NEB stage throughout the sampling periods (normal cow prepartum <0.6 mmol/L, postpartum <1.0 mmol/L; McArt et al., 2013). However, some cows showed serum BHBA concentrations greater than 3.0 mmol/L, which could be considered as clinical ketosis stage. Average BHBA concentrations in the tropical and sub-tropical areas were reported by Whitaker et al. (1999), and data from many countries suggested that serum BHBA concentrations varied, concentrations were above the cut-off point of NEB in some countries. Prepartum average BHBA concentrations in many countries were not greater than 0.9 (0.3-0.9) mmol/L and 1.1 (0.4-1.1) mmol/L postpartum. In other studies, the percentages of cows with elevated BHBA concentrations above normal condition in high producing commercial dairy farms in non-tropical climate were reported as 26 % prepartum (>0.6 mmol/l, Chapinal et al., 2011); 31% (>1.0 mmol/l, Seifi et al., 2011), and 18-25% (>1.2 mmol/l, Ospina et al., 2010; Chapinal et al., 2011; Seifi et al., 2011; Suthar et al., 2013) postpartum. In the present study, average serum BHBA concentrations were higher than previous reports in a non-tropical climate with more milk production. These findings might be due to other management factors, such as heat stress conditions.

Liver TAG could be classified into the mild, moderate, and severe fatty liver disease at the concentrations of 0-50, 50-100, and > 100 mg per g of liver wet weight (Jorritsma et al., 2001). Increased liver TAG was reported in postpartum dairy cows in tropical areas (Rukkwamsuk et al., 2004) with non-indicated NEB from serum NEFA concentrations and absence of BCS loss postpartum as mentioned above.

Hyperketonemia so-called ketosis from NEB in peripartum cows reviewed by Oetze (2007) indicated two important types of ketosis. Type I ketosis is caused by feeding-inability to balance between nutrient intake and the requirement of fresh cows. This condition is dominated by a high ketogenic rate, resulting in a high degree of hyperketonemia around 3-4 weeks after calving, which is also related to the peak milk production period. The other type, type II ketosis or fat cow's syndrome is usually found 1-2 weeks after calving. High serum NEFA concentrations indicate fat mobilization process, which is the key diagnosis in this type of ketosis, and serum BHBA concentrations might not as high as type I ketosis. For dairy cows in the current study, elevations of serum NEFA and liver TAG concentrations were found but concentrations were still within normal ranges. Therefore, only a low grade of lipolysis occurred. Patterns of biochemical response to NEB were found with high serum BHBA concentrations during 1 to 4 weeks postpartum but there was no high degree of lipolysis process. Metabolic responses of the investigated cows were similar to type I ketosis; however, the time of hyperketonemia was expanded from 2 to 3 weeks to months after calving. A study conducted in a tropical area showed that dairy cows entered NEB condition immediately after calving 5-7 weeks postpartum, and milk production reached the peak performance 3-4 weeks after calving (Suadsong, 2012). In the present study, the cows might suffer from type I ketosis, however, it was not related to the time of peak milk production (3-4 weeks postpartum), failures to provide adequate nutrients or condition that could limit feed intake of the cows, which would result in enhancing the prevalence of ketosis right after calving. With a low degree of liver TAG, the liver can still metabolize NEFA into BHBA in response to energy demands. However, poor nutritional management could encourage cows to experience NEB earlier than we could expect at the peak milk production period. Consequently, hyperketonemia was demonstrated a month after calving in our experiment.

There was a strong negative correlation between serum glucose and BHBA concentrations (González et al., 2011). Decreased serum glucose concentrations at the hyperketonemia period were not found in our study, this might result from other stress stimuli for example heat stress or failure of cow comfort management in a small-holder tropical dairy farm (Mudro et al., 2005).

In mature animals, IGF-I was secreted mainly from the liver in response to growth hormone from the pituitary gland (Velazquez et al., 2008). The current study showed higher IGF-I concentrations compared with other studies both in tropical and non-tropical climate during prepartum (80-100 ng/ml, Kaewlamun, 2010) and postpartum (40-70 ng/ml, Kaewlamun, 2010; 69-73 ng/ml Castigliero et al., 2009) periods. High-producing dairy cows are likely to suffer from severe fatty liver disease, resulting in the impairment of liver function, including IGF-I and IGF-I binding protein production (Fenwick et al., 2008). The tropical area also has a longer day period, cows exposed to the long light period have higher serum IGF-I levels, compared to a shorter light period (Collier et al., 2008). This resulted in higher serum IGF-I concentrations in the current study that was performed under tropical climate, compared to a temperate area with a shorter day length.

CONCLUSION

Various biochemical responses, including elevated serum NEFA and increase liver TG accumulation were found but the degree of change did not reach the clinical stage. Serum BHBA indicated periparturient cows raised in a small-holder farm under tropical climate suffered from expanded type I ketosis 1-4 weeks postpartum. This study demonstrated that even with low milk production, the NEB problem should not be overlooked in small-holder tropical dairy farms.

DECLARATIONS

Authors' contribution

T. Rukkamsuk conceived, designed, and supervised the project. S. Triwutanon and T. Rukkamsuk performed the experiment and analyzed the data. Both authors interpreted the data, wrote, and critically revised the manuscript for intellectual content, and approved the final version. S. Triwutanon and T. Rukkamsuk had full access to all data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. All authors confirmed the final edition of the article and declared that they did not use any related data of this article on any other publications.

Competing interests

The authors declare that there is no conflict of interest.

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REFERENCES

- Aiumlamai S (2009). Dairy management, health, and production in Thailand. *International Dairy Topics*, 8: 11-13. Available at: <http://www.positiveaction.info/pdfs/articles/dt8.2p11.pdf>.
- Castigliero L, Grifoni G, Rosati R, Iannone G, Armani A, Gianfaldoni D, and Guidi A (2009). On the alterations in serum concentration of somatotropin and insuline-like growth factor 1 in lactating cows after the treatment with a little studied recombinant bovine somatotropin. *Research in Veterinary Science*, 87: 29-35. DOI: <https://doi.org/10.1016/j.rvsc.2008.10.012>
- Chapinal N, Carson M, Duffield TF, Capel M, Godden S, Overson M, Santos JEP, and LeBlanc SJ (2011). The association of serum metabolites with clinical disease during the transition period. *Journal of Dairy Science*, 94: 4897-4903. DOI: <https://doi.org/10.3168/jds.2010-4075>.
- Collier RJ, Miller M, McLaughlin CL, Johnson HD, and Baile C (2008). Effects of recombinant bovine somatotropin (rbST) and season on plasma and milk insulin-like growth factors I (IGF-I) and II (IGF-II) in lactating dairy cows. *Domestic Animal Endocrinology*, 35: 16-23. DOI: <https://doi.org/10.1016/j.domaniend.2008.01.003>
- Duan C, and Xu Q (2005). Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. *General and Comparative Endocrinology*, 142: 44-52. DOI: <https://doi.org/10.1016/j.ygcen.2004.12.022>.
- Fenwick MA, Fitzpatrick R, Kenny DA, Diskin MG, Patton J, Murphy JJ, and Wathes DC (2008). Interrelationships between negative energy balance (NEB) and IGF regulation in liver of lactating dairy cows. *Domestic Animal Endocrinology*, 34: 31-44. DOI: <https://doi.org/10.1016/j.domaniend.2006.10.002>.
- Garcia-Roche M, Casal A, Mattiauda A, Ceriani M, Jasinsky A, Mastrogiovanni M, Trostchansky A, Carriquiry M, Cassina A, and Quijano C (2019). Impaired hepatic mitochondrial function during early lactation in dairy cows: Association with protein lysine acetylation. *PLoS ONE*, 14: e0213780. DOI: <https://doi.org/10.1371/journal.pone.0213780>.
- González FD, Muiño R, Pereira V, Campos R, and Benedito JL (2011). Relationship among blood indicators of lipomobilization and

- hepatic function during early lactation in high-yielding dairy cows. *Journal of Veterinary Science*, 12: 251-255. DOI: <https://dx.doi.org/10.4142/jvs.2011.12.3.251>.
- Jorritsma R, Jorritsma H, Schukken YH, Bartlett PC, Wensing T, and Wentink GH (2001). Prevalence and indicators of post partum fatty infiltration of the liver in nine commercial dairy herds in The Netherlands. *Livestock Production Science*, 68: 53-60. DOI: [https://doi.org/10.1016/S0301-6226\(00\)00208-6](https://doi.org/10.1016/S0301-6226(00)00208-6).
- Kaewlamun W (2010). Effect of heat stress and beta-carotene supplementation on postpartum reproductive performance in dairy cow, PhD Thesis, Chulalongkorn University, Bangkok, Thailand. 145 p. DOI: <https://doi.org/10.14457/CU.the.2010.887>.
- Macrae AI, Burroughs E, Forrest J, Corbishley A, Russel G, and Shaw DJ (2019). Prevalence of excessive negative energy balance in commercial United Kingdom dairy herds. *The Veterinary Journal*, 248: 51-57. DOI: <https://doi.org/10.1016/j.tvjl.2019.04.001>.
- Mair B, Drillich M, Klein-Jöbstl D, Kanz P, Borchardt S, Meyer L, Schwendenwein I, and Iwersen M (2016). Glucose concentration in capillary blood of dairy cows obtained by a minimally invasive lancet technique and determined with three different hand-held devices. *BMC Veterinary Research*, 12: 34. DOI: <https://doi.org/10.1186/s12917-016-0662-3>.
- McArt JAA, Nydam DV, Oetzel GR, Overton TR, and Ospina PA (2013). Elevated non-esterified fatty acids and β -hydroxybutyrate and their association with transition dairy cow performance. *Veterinary Journal*, 198: 560-570. DOI: <https://dx.doi.org/10.1016/j.tvjl.2013.08.011>.
- Mudro P, Rehage J, Sallmann HP, Höltershinken M, and Scholz H (2005). Stress response in dairy cows related to blood glucose. *Acta Veterinaria Brno*, 74: 37-42. DOI: <https://doi.org/10.2754/avb.200574010037>.
- Mulligan FJ, Grady LO, Rice DA, and Doherty ML (2006). A herd health approach to dairy cow nutrition and production diseases of the transition cow. *Animal Reproduction Science*, 96: 331-353. DOI: <https://doi.org/10.1016/j.anireprosci.2006.08.011>.
- Murney R., Stelwagen K, Wheeler TT, Margerison JK, and Singh K (2015). The effects of milking frequency on insulin-like growth factor I signaling within the mammary gland of dairy cows. *Journal of Dairy Science*, 98: 5422-5428. DOI: <https://doi.org/10.3168/jds.2015-9425>.
- Oetzel GR (2007). Herd-level ketosis-diagnosis and risk factors. In: Proceedings of the 40th Annual Conference on Preconference Seminar 7C: Dairy Herd Problem Investigation Strategies 2007, Sep.16-19: Canada. American Association of Bovine Practitioners, pp. 67-91. Available at: <https://pdfslide.net/reader/f/herd-level-ketosis-diagnosis-and-risk-factors>.
- Ospina PA, Nydam DV, Stokol T, and Overton TR (2010). Associations of elevated nonesterified fatty acids and β -hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. *Journal of Dairy Science*, 93: 1595-1603. DOI: <https://doi.org/10.3168/jds.2009-2852>.
- Raboisson D, Mounié M, Khenifar E, and Maigné E (2015). The economic impact of subclinical ketosis at the farm level: Tackling the challenge of over-estimation due to multiple interactions. *Preventive Veterinary Medicine*, 122: 417-425. DOI: <https://dx.doi.org/10.1016/j.prevetmed.2015.07.010>.
- Rukkamsuk T (2010). A field study on negative energy balance in periparturient dairy cows kept in small-holder farms: Effect on milk production and reproduction. *African Journal of Agricultural Research*, 5: 3157-5163. DOI: <https://doi.org/10.5897/AJAR10.720>.
- Rukkamsuk T, Homwong N, Bumkhuntod W, Rohitakane P, and Sukcharoen R (2006). Negative energy balance in periparturient dairy cows raised in small-holder farms in Kamphaengsaen district, Nakhon Pathom province. *Kasetsart Journal (Natural Science)*, 40: 1000-1004. Available at: <https://li01.tci-thaijo.org/index.php/anres/article/view/243851/166467>.
- Rukkamsuk T, Rungruang S, and Wensing T (2004). Fatty liver in high producing dairy cows kept in evaporative cooling system in a commercial dairy herd in Thailand. *Kasetsart Journal (Natural Science)*, 38: 229-235. Available at: <https://li01.tci-thaijo.org/index.php/anres/article/view/242954/165789>.
- Rukkamsuk T, Wensing T, and Kurip TA (1999). Relationship between triacylglycerol concentration in liver and first ovulation in postpartum dairy cows. *Theriogenology*, 51: 1133-1142. DOI: [https://doi.org/10.1016/S0093-691X\(99\)80016-8](https://doi.org/10.1016/S0093-691X(99)80016-8).
- Seifi HA, LeBlanc SJ, Leslie KE, and Duffield TF (2011). Metabolic predictors of post-partum disease and culling risk in dairy cattle. *Veterinary Journal*, 188: 216-220. DOI: <https://doi.org/10.1016/j.tvjl.2010.04.007>.
- Suadsong S (2012). Alleviating Heat stress leads to improved cow reproductive performance. In *Milk production-an up-to-date overview of animal nutrition, management and health*, Ed., Chaiyabutr, N. London: InTech, pp. 309-330. DOI: <https://dx.doi.org/10.5772/50881>.
- Suthar SV, Canelas-Raposo J, Deniz A, and Heuwieser W (2013). Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. *Journal of Dairy Science*, 96: 2925-2938. DOI: <https://www.doi.org/10.3168/jds.2012-6035>.
- Van den Top AM, Wensing T, and Beynen AC (1994). The influence of calcium palmitate and oleate feeding on hepatic lipid metabolism in dry goats. *Journal of Animal Physiology and Animal Nutrition*, 72: 44-45. DOI: <https://doi.org/10.1111/j.1439-0396.1994.tb00369.x>.
- Velazquez MA, Spicer LJ, and Wathes DC (2008). The role of endocrine insulin-like growth factor-I (IGF-I) in female bovine reproduction. *Domestic Animal Endocrinology*, 35: 325-342. DOI: <https://doi.org/10.1016/j.domaniend.2008.07.002>.
- Whitaker DA, Goodger WJ, Garcia M, and Perera BMAO (1999). Wittwer F. Use of metabolic profiles in dairy cattle in tropical and subtropical countries on smallholder dairy farms. *Preventive Veterinary Medicine*, 38: 119-131. DOI: [https://doi.org/10.1016/S0167-5877\(98\)00118-4](https://doi.org/10.1016/S0167-5877(98)00118-4).