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ANIMAL REPRODUCTION

Relationship between energy balance and key blood metabolites in primiparous dairy cows at different calving conception interval

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ABSTRACT: This study identified the relationship between energy balance and some blood metabolites in primiparous dairy cows with different calving conception interval. Twenty primiparous Holstein-Friesian dairy cows were grouped according to the interval (days) from calving to conception into early-conception (early-c; <100 days), mid-conception group (mid-c; 100 – 150 days) or late-conception (late-c; >150 days) group; and evaluated on day -7 prior to parturition, and days 28, 56 and 90 postpartum. All groups presented positive energy balance, serum concentrations of HDL, urea-N and progesterone with no difference on days 28 and 56 of lactation. On day 90 of lactation all the cows presented negative energy balance; however, the early-c group presented a less intense ($P < 0.05$) net energy balance (-1.09 versus -2.15 and -1.86 Mcal/day), lower concentrations of serum urea-N (3.28 versus 4.22 and 4.88 mg/dL) and HDL cholesterol (0.84 versus 1.59 and 1.46 mmol/L) and greater serum concentration of progesterone (8.13 versus 4.54 and 1.83 ng/mL) than cows of mid-c and late-c groups, respectively. In conclusion, different from those usually observed for multiparous dairy cows, the energy balance during the first weeks of lactation was not a determining factor affecting the calving conception interval of primiparous dairy cows in the present study. Instead, the nutritional status and the serum concentration of urea-N and progesterone at a later stage, after 56 days of lactation, suggest having a role in delaying these cows' pregnant status.
Key words: conception, cholesterol, urea-N, progesterone.

Relação entre balanço energético e metabólitos sanguíneos em vacas l eiteiras primíparas com diferentes intervalos entre parto e concepção

RESUMO: O objetivo deste estudo foi avaliar a relação entre balanço energético e concentração sanguínea de alguns metabólitos relevantes durante o início da lactação em vacas leiteiras primíparas com diferentes desempenhos reprodutivos. Vinte vacas leiteiras holandesas primíparas foram agrupadas de acordo com o intervalo parto-concepção em precoce (<100 dias), intermediário (100 - 150 dias) e tardio (> 150 dias); e foram avaliados no dia -7 pré-parto, e nos dias 28, 56 e 90 dias pós-parto. Todos os grupos apresentaram balanço energético positivo, concentrações séricas de HDL, N-uréia e progesterona sem diferença nos dias 28 e 56 de lactação. Aos 90 dias de lactação todas vacas apresentaram o balanço energético negativo, porém o grupo de vacas precoce foi menos intenso ($P < 0.05$) (-1.09 versus -2.15 e -1.86 Mcal/dia), menor concentração sérica de N-uréia (3.28 versus 4.22 e 4.88 mg/dL) e colesterol-HDL (0.84 versus 1.59 e 1.46 mmol/L) e maior concentração sérica de progesterona (8.13 versus 4.54 e 1.83 ng/mL), que vacas dos grupos intermediário e tardio, respectivamente. Em conclusão, diferente do observado usualmente em vacas multíparas, o balanço energético durante as primeiras semanas de lactação não foi um fator determinante do intervalo parto concepção das vacas primíparas do presente estudo. De fato, a condição nutricional e as concentrações séricas de N-ureia e progesterona após 56 dias de lactação sugere ter impacto no atraso da concepção destas vacas.
Palavras-chave: concepção, colesterol, N-ureia, progesterona.

INTRODUCTION

Dairy cows are under negative energy balance (NEB) during the first weeks of lactation as a consequence of limited feed intake and increased milk production, which peaks 4 to 6 weeks after calving (LEDUC et al., 2021). The intensity of the NEB is dependent on the milk production, which is genetically determined, and energy intake, which is diet-dependent. During NEB, the use of body reserves of fat and protein is necessary to attend the demand for high milk yield (NIGUSSIE, 2018) causing a change in the metabolic and hormonal status (BARLETTA et al., 2017).

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Preview studies have consistently reported that the intensity of NEB may negatively affect the conception rates of dairy cows by increasing the incidence of early embryonic mortality and, consequently, the return of ovarian activity after calving (BRAGANÇA & ZANGIROLAMO, 2018). Negative energy balance consequences are probably similar to undernutrition and may manifest as delayed ovarian activity interfering in the pulsatile secretion of LH, which is essential for ovarian follicular development (IWASA et al., 2018). However, primiparous dairy cows usually did not reach their tissues growth plateau at the first lactation and presented lower reproductive performance after calving than multiparous dairy cows (LUCY et al., 1992; MEIKLE et al., 2004).

The nutritional and hormonal variables involved in dairy cows' reproductive performance were partially identified. For example, progesterone (P4) is a major hormone supporting early embryonic development, in which blood concentration is determined by the balance between P4 production, primarily by the corpus luteum (CL), and P4 metabolism, primarily by the liver (WILTBANK et al., 2014). In addition, most diets offered to cows during the early lactation contain excessive levels of rumen degradable protein, what leads to high levels of ammonia absorption from the rumen, increased urea production by the liver (NICHOLS et al., 2022), and increased blood urea concentration which negatively impacts embryo quality (MACHADO et al., 2020). In fact, the liver is an overcharged organ, particularly during the NEB period, when it has to process concomitantly nutrients coming from gut and from peripheral tissues (REYNOLDS, 1995). One previous study (CAPELESSO et al., 2019) reported that primiparous once-daily milked cows improve their energy status over twice-daily milked cows. Also, in this same study calving to conception interval did not differ between once- or twice-daily milking, but the interval from calving to first luteal activity tended to be shorter in once-daily milked cows.

However, the impact of the nutritional condition on blood metabolites and their relationship with the reproduction of primiparous dairy cows is not clearly understood. This study identified the relationship between energy balance and some blood metabolites in primiparous dairy cows with different calving conception interval.

MATERIALS AND METHODS

This study is correlated and used blood samples and nutritional data previously collected and reported by CAPELESSO et al. (2019). Twenty primiparous Holstein-Friesian cows were selected two weeks before the expected date of calving and blocked according to calving date, body weight (BW; 605 ± 67.2 kg, mean ± standard deviation), backfat thickness (3.6 ± 1.49 cm), and body condition score (3.6 ± 0.29, in a 1 to 5 point scale), and assigned to either once or twice-daily milking up to the 8th week of lactation. The cows were milked either at 6:00 a.m. or at 6:00 a.m. and 5:00 p.m. for once or twice-daily milking. After week 8, all cows were milked twice daily. All cows received the same diet throughout the experiment. Prior to the expected calving date (from -28 days), the cows were fed a total mixed ration (TMR), formulated to meet the requirements of heifers weighing 590 kg (NRC, 2001). In the postpartum period, the diet was formulated to achieve the requirements of cows weighing 520 kg and producing 30 kg of milk per day. Seventy percent of the estimated dry matter (DM) intake (NRC, 2001) was individually offered as TMR (approximately 15.2 ± 1.2 kg of DM/cow per day) after the morning milking. Following the afternoon milking, all cows grazed oat (*Avena sativa* L., var. INIA Halley) pasture on paddocks size adjusted to provide 12 kg of DM/cow per day. Intake of pasture and TMR was measured at weeks 1, 3, 4, 6, and 8 of lactation for 3 consecutive days per week. Individual milk yield was recorded automatically at each milking, using DairyPlan C21 (GEA Farm Technologies, Düsseldorf, Germany). The individual NEB was estimated at weeks 1, 3, 4, 6, and 8 of lactation as described (CAPELESSO et al., 2019). The milk production in Dairy cows is most profitable when conception occurs between 90 and 130 days postpartum (GIORDANO et al., 2011). Previous studies have analyzed cows conceiving by 100, 150, and beyond 150 days in milk (COOK & GREEN, 2016). For this reason, differently from CAPELESSO et al. (2019), the cows in our study were grouped accordingly to calving conception interval, as follows: early conception (early-c; n = 7) by 100 days in milk, mid-conception (mid-c; n = 8) from 101 to 150 days in milk and late conception (late-c; n = 5) group that conceived beyond 151 days in milk. This design allowed us to analyze the data (CAPELESSO et al., 2019) considering as the endpoint a successful pregnancy status.

Blood samples were collected from the jugular vein, in tubes without anticoagulant, on day -7 prior to parturition, and on days 28, 56, and 90 postpartum. The samples were kept at room temperature for 1 h, refrigerated at 4 ºC for 2 h, centrifuged (1000 × *g* for 10 min at 20 ºC) and stored at −20 ºC for further analysis. The N-urea in serum was determined by a colorimetry method using a commercial kit (Bioclin, MG, Brazil). The analysis of total and HDL cholesterol were performed by an enzymatic colorimetric method (Advia 1800, Siemens, Erlangen, Germany) and the LDL concentration was calculated by the Friedewald formula (SCHARNAGL et al., 2001). The progesterone analysis was performed through the direct chemiluminescence method using the Acridinium Ester Technology (Advia Centaur XP, Siemens, Erlangen, Germany). The number of days to cows return to cyclicity was recorded by visual observation of the standing estrus. Then, the cows were artificially inseminated and the number of artificial inseminations necessary to conception was also recorded.

For data analysis, cows were allocated into three different groups, according to the interval (days) from calving to conception: early-conception group (early-c; < 100 days), mid-conception group (mid-c; 100 – 150 days) and late-conception (late-c; > 150 days). The effect of group treatment on variables was tested by the ANOVA with repeated measures throughout the time. Differences between means were tested with Tukey-Kramer's HSD test. Data are presented as means ± SEM. Significant differences were stated at $P < 0.05$. Data that did not follow a normal distribution pattern (Shapiro-Wilk's test) were transformed to logarithms. Homogeneity of variance was tested with O'Brien and Brown-Forsythe's tests. All analytical procedures were performed with JMP software (SAS Institute, USA) with treatment group as main effect and cow replicate as a random variable in F-test. The effect of treatment was tested against each variable including milking frequency as co-variable.

RESULTS

Cows in the early-c group presented higher ($P < 0.05$) BCS (3.78) than cows of mid-c and late-c groups (3.25 for mid-c and 3.50 for late-c cows) on day 90, whereas no differences on BCS between groups were observed on days -7, 28 and 56 of lactation (Figure 1A). The energy balance (Figure 1B)

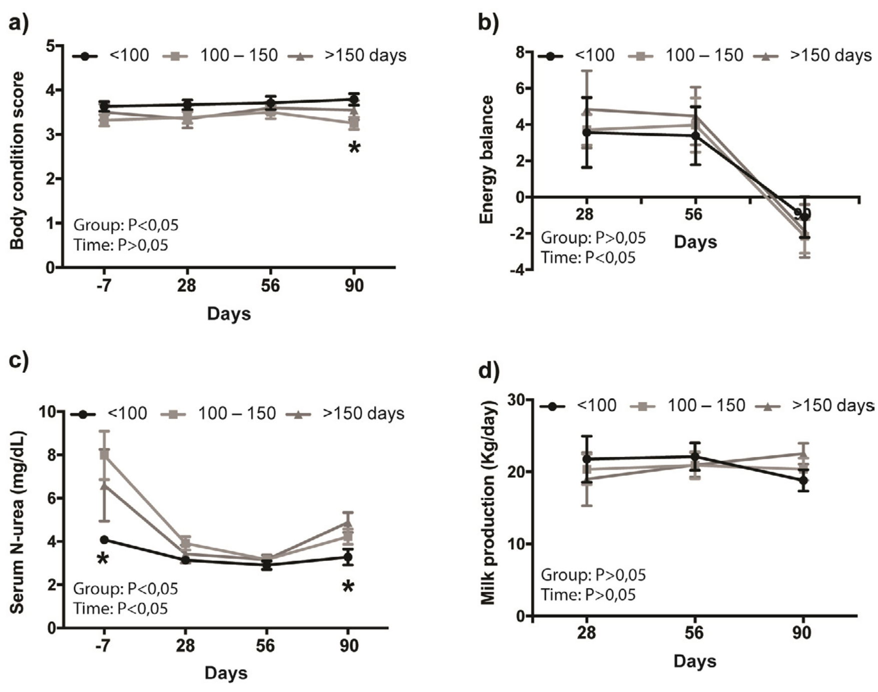

Figure 1 - Body condition score (A), energy balance (B), serum N-urea (C) and Milk production (D) of Holstein heifers allocated into one of three distinct groups according to calving to conception intervals: early-conception group (early-c; < 100 days), mid-conception group (mid-c; 100 – 150 days), and late-conception group (late-c; > 150 days). Data presented as mean ± standard error of the mean (SEM). Temporal differences are indicated by $P < 0.05$. * Indicates difference at $P < 0.05$ ($n = 20$).

was, on average of all groups, negative only at 90 days whereas positive energy balance values were observed in days 28 and 56 of lactation. However, on day 90 of lactation, the early-c group had a less intense NEB ($P < 0.05$) (-1.09 Mcal/day) than cows of other groups (-2.15 for mid-c and -1.86 Mcal/day for late-c). The cows presented no difference in days necessary to return to cyclicity (early-c: 31 days, mid-c: 30 days and late-c: 24 days) whereas the number of artificial inseminations (AI) was lower in early-c group (AI=1) when compared to mid-c group (AI = 2) and late-c group (AI > 3).

On day -7 prior to parturition, lower concentrations of serum N-urea ($P < 0.05$) were observed in cows of early-c group (4.08 mg/dL) when compared to the mid-c (7.98 mg/dL) and late-c groups (6.60 mg/dL), the same result observed on day 90 (3.28 mg/dL early-c; 4.22 mg/dL mid-c; 4.88 mg/dL late-c group) (Figure 1C). However, no differences between groups were observed in the concentration of serum N-urea on days 28 and 56 of the lactation. On day 90, but not in days -7, 28, and 56 of lactation, the HDL cholesterol concentration (Figure 2A) in serum of cows of the early-c group was lower ($P < 0.05$) (0.84 mmol/L) than in cows of mid-c (1.59 mmol/L) and late-c (1.46 mmol/L) groups. No differences were observed between groups for serum concentration of LDL cholesterol (Figure 2B) and total cholesterol (Figure 2C). Serum concentration of progesterone was similar for all groups on days -7, 28, and 56 of lactation (Figure 2D) and was higher in cows of the early-c group (8.13 ng/mL) than in cows of mid-c (4.54 ng/mL) and late-c (1.83 ng/mL) groups at 90 days of lactation.

DISCUSSION

Dairy cows frequently have severe NEB during the first 30 days of lactation, associated with longer intervals to first ovulation (ZHANG et al., 2021). However, the energy balance in cows of the present study (Figure 1B) was positive during the earliest lactation and, different from expected, it declined to a negative balance only about day 90

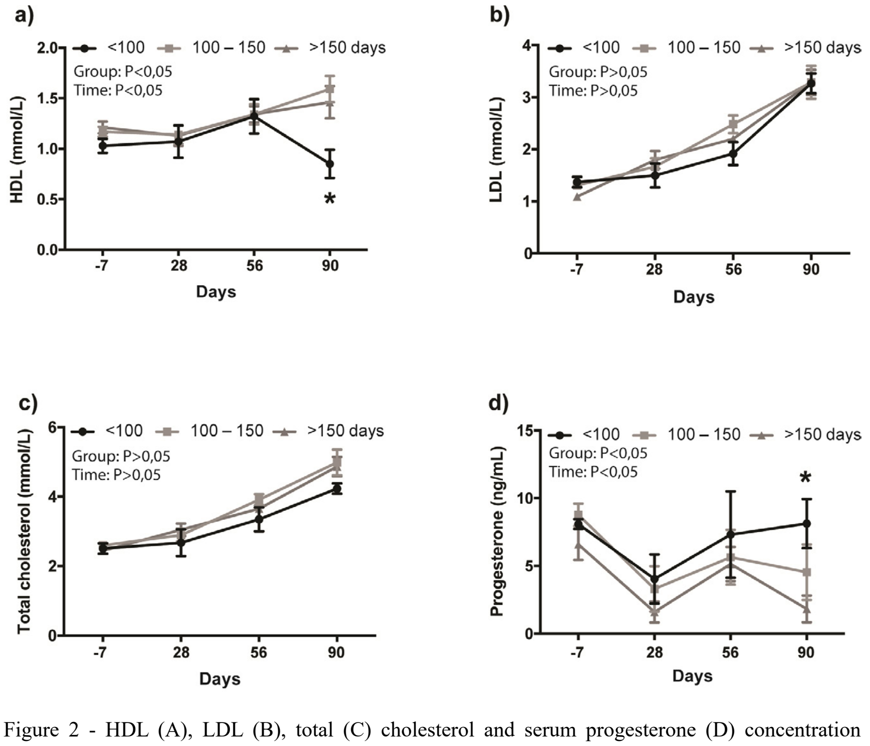

Figure 2 - HDL (A), LDL (B), total (C) cholesterol and serum progesterone (D) concentration of Holstein heifers allocated into one of three distinct groups according to calving to conception intervals: early-conception group (early-c; < 100 days), mid-conception group (mid-c; 100 – 150 days), and late-conception group (late-c; > 150 days). Data were presented as mean ± standard error of the mean (SEM). Temporal differences are indicated by $P < 0.05$. * Indicates difference at $P < 0.05$ ($n = 20$).

of lactation in all groups. These results suggested that the energy balance at early lactation was not determinant of the reproductive performance of primiparous cows and cannot explain the reason why mid-c and late-c cows did not get pregnant before 90 days. These results suggested that the pregnancy status of primiparous dairy cows in the present study were influenced by some factors occurring later, after the day 56 of lactation.

Between days 56 and 90 of lactation all cows experienced NEB, however, the NEB in this period was less intense in early-c group (Figure 1B). As consequence, these cows presented a better BCS (Figure 1A) at 90 days of lactation. In addition, at 90 days of lactation, cows of mid- and late-c groups showed increased serum urea-N concentration (Figure 1C). High serum N-urea concentration is inversely related to uterine pH and decreased pregnancy rates in lactating cows (BUTLER et al., 1996). Moreover, relatively high concentrations of serum N-urea may change the follicular, oviductal, and uterine environment of cows, impacting the viability and development of embryos (MACHADO et al., 2020). Increased urea-N concentration in blood may be a consequence of increased levels of degradable protein intake, excessive absorption of amino acids from gut or during NEB, when amino acids from tissues are deaminated into the liver of dairy cows (WRAY-CAHEN et al., 1997; KRAFT et al., 2011). Once mid-c and late-c cows presented a more intense NEB (Figure 1B) after 56 days of lactation, it is probable that these cows have mobilized more amino acids from peripheral tissues during this period than cows of the early-c group in the present study.

Progesterone plays a major role in the reproductive events associated with estrous cycle and the establishment and maintenance of pregnancy (COUTO et al., 2019). Cholesterol is present in low (LDL) or high-density (HDL) lipoproteins and is the precursor for biosynthesis of progesterone in ovarium (NISWENDER, 2002), which production is regulated by the development of the corpus luteum after the LH surge (WILTBANK et al., 2014) in addition to the constitutive production of progesterone by the large luteal cells (ARÉCHIGA-FLORES et al., 2019)). Moreover, HDL is the predominant lipoprotein present in bovine follicular fluid and it is the main responsible to stimulate progesterone production by luteal cells providing cholesterol substrate for luteal steroidogenesis (WILTBANK et al., 1989). However, in an apparent contrast, the serum concentration of HDL (Figure 2A) at 90 days was lower and the serum concentration of progesterone (Figure 2D) was higher in early-c group compared to mid-c and late-c groups. There is not a clear explanation for this difference. However, it is possible that blood extraction of HDL by ovarium and; consequently, progesterone synthesis, was increased of early-c cows at 90 days. In other way, increased blood concentration of progesterone in early-c cows at 90 days of lactation could also be consequence of decreased liver catabolism of this hormone by these cows or due to pregnancy in some cows prior to day 90.

Considering that the energy balance at early lactation may not be directly determinant of the reproductive performance of primiparous cows, it may be affected by impaired liver function, independent of what is the cause of liver injuries (ANGELI et al., 2019). High-production dairy cows in the early lactation have high levels of lipo mobilization, which can cause changes in liver function and metabolism, with urea being a blood indicator of liver function (GONZÁLEZ et al., 2009). In our study, we observed lower reproductive rates in the mid-c and late-c groups, which are the groups with the most severe negative energy balance and greater mobilization of body reserves.

However, differently expected, no differences were observed between groups for serum concentration of LDL cholesterol (Figure 2B) and total cholesterol (Figure 2C). In the early-c group, on Day 90, lower blood concentrations of HDL cholesterol (Figure 2A) and urea (Figure 1C) were observed, which may indicate lower levels of mobilization of body reserves. Therefore, we observed in the early-c group a better postpartum metabolic adaptation than in the mid-c and late-c group, which promotes a gradual loss of body condition score and the maintenance of metabolic health, ensuring the return of postpartum cyclicality and production of oocyte and corpus luteum quality that is required for successful insemination (CARDOSO et al., 2021).

CONCLUSION

The cows in this study were in negative energy balance only on day 90 postpartum. However, during this same period, they showed changes in metabolic variables and serum progesterone concentration. Cows that presented a decline in HDL (high-density lipoprotein) and N-Urea had an increase in serum progesterone concentrations and body condition score; and also, a shorter calving conception interval. These parameters are important indicators and may be used in animal selection for reproduction in the postpartum period and in the nutritional management of dairy properties.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

SS, AQA, VMP and GVK conceived and designed experiments. SS, AM, CC and JLPC performed the experiments, SS, CSA, CC carried out the lab analyses. SS, GVK, AM, CC and JLPC supervised and coordinated the animal experiments and provided field data. VMP performed statistical analyses of experimental data. SS, AQA, VMP and GVK prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The experiment was conducted at the Experimental Station of the Instituto Nacional de Investigación Agropecuaria (INIA), Estanzuela, Uruguay, in accordance with regulations of the use of animals in experimentation, education, and investigation established by the Ethics Commission on the use of experimental animals of INIA (Proc. Nº 2015.42).

REFERENCES

ANGELI, E. et al. Liver fatty acid metabolism associations with reproductive performance of dairy cattle. **Animal Reproduction Science**, v.208, 2019. Available from: <https://pubmed.ncbi.nlm.nih.gov/31405453/>. Accessed: Sept. 18, 2023. doi: 10.1016/j.anireprosci.2019.06.016.

ARÉCHIGA-FLORES, C. et al. Review: Function and regression of the corpus luteum during the estrous cycle. **AbanicoVet.**, v.9 p.1-21, 2019. Available from: <https://www.medigraphic.com/cgi-bin/new/resumenI.cgi?IDARTICULO=90917>. Accessed: Sept. 18, 2023. doi: doi.org/10.21929/abavet2019.924**.**

BARLETTA, R. V. et al. Association of changes among body condition score during the transition period with NEFA and BHBA concentrations, milk production, fertility, and health of Holstein cows. **Theriogenology**, v.104, p.30-36. 2017. Available from: <http://dx.doi.org/10.1016/j.theriogenology.2017.07.030>. Accessed: Nov. 11, 2022. doi: 10.1016/j.theriogenology.2017.07.030.

BRAGANÇA, L. G; ZANGIROLAMO, A. F. Strategies for increasing fertility in high productivity dairy herds. **Animal Reproduction**, v.3, p.256-260, 2018. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8382311/>. Accessed: Sept. 18, 2023.doi: 10.21451/1984-3143-AR2018-0079.

BUTLER, W. et al. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. **Journal of animal science**, v.74, n.4, p.858-865. 1996. Available from: <https://pubmed.ncbi.nlm.nih.gov/8728008/>. Accessed: Jul. 05, 2022. doi: 10.2527/1996.744858x.

CAPELESSO, A. et al. Reducing milking frequency in early lactation improved the energy status but reduced milk yield during the whole lactation of primiparous Holstein cows consuming a total mixed ration and pasture. **Journal of Dairy Science**, v.102, n.10, p.8919-8930. 2019. Available from: <https://doi.org/10.3168/jds.2019-16629> Accessed: Jul. 05, 2022. doi: 10.3168/jds.2019-16629.

CARDOSO, C. C. E. et al. Factors That Optimize Reproductive Efficiency in Dairy Herds with an Emphasis on Timed Artificial Insemination Programs. **Animals (Basel)**, v.11, 2021. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7912388/>. Accessed: Sept. 18, 2023. doi: 10.3390/ani11020301.

COOK, J. G.; M. J. GREEN. Use of early lactation milk recording data to predict the calving to conception interval in dairy herds. **Journal of Dairy Science**, v.99, n.6, p.4699-4706. 2016. Available from: <https://pubmed.ncbi.nlm.nih.gov/27040790/>. Accessed Jun. 02, 2022. doi: 10.3168/jds.2015-10264.

NATIONAL RESEARCH COUNCIL. **Nutrient Requirements of Dairy Cattle:** Seventh Revised Edition, 2001. Washington, DC: The National Academies Press, 2001, 408 p.

COUTO, S. R. B. et al. Impact of supplementation with long-acting progesterone on gestational loss in Nelore females submitted to TAI. **Theriogenology**, v.125, p.168-172, 2019. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0093691X18310355#:~:text=Conclusion,Under%20the%20conditions%20of%20this%20study%2C%20the%20use%20of%20long,both%20anestrous%20and%20cycling%20animals>. Accessed: Sept. 18, 2023. doi: doi.org/10.1016/j.theriogenology.2018.10.032.

GIORDANO, J. O. et al. An economic decision-making support system for selection of reproductive management programs on dairy farms. **Journal of Dairy Science**, v.94, n.12, p.6216-32. 2011. Available from: <https://doi.org/10.3168/jds.2011-4376>. Accessed: Jun. 05, 2022. doi: 10.3168/jds.2011-4376.

GONZÁLEZ, F. H. D. Ferramentas de diagnóstico e monitoramento das doenças metabólicas. **Ciência Animal Brasileira**, v.1, p.1-22, 2009. Available from: <https://revistas.ufg.br/vet/article/view/7662>. Accessed: May, 01, 2023.

IWASA T, et al. Effects of Low Energy Availability on Reproductive Functions and Their Underlying Neuroendocrine Mechanisms. **Journal Clinical Medicine**, v.7, p.166, 2018. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6068835/>. Accessed: Sept. 18, 2023. doi: 10.3390/jcm7070166.

KRAFT, G. et al. Adaptations of hepatic amino acid uptake and net utilisation contributes to nitrogen economy or waste in lambs fed nitrogen- or energy-deficient diets. **Animal**, v.5, p.678-690, 2011. Available from: <https://doi.org/10.1017/s1751731110002302>. Accessed: May, 01, 2023. doi: 10.1017/S1751731110002302.

LEDUC, A. et al. Effect of feed restriction on dairy cow milk production: a review. **Journal of Animal Science**, v.7, 2021. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8248043/>. Accessed: Sept. 18, 2023. doi: 10.1093/jas/skab130.

LUCY, M. et al. Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. **Animal Science**, v.54, n.3, p.323-331, 1992. Available from: <https://doi.org/10.1017/S0003356100020778>. Accessed: May, 22, 2022. doi: 10.1017/S0003356100020778.

MACHADO, A. F. et al. Effect of protein supplement level on the productive and reproductive parameters of replacement heifers managed in intensive grazing systems. **PLoS One**. v.15, 2020. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7540841/>. Accessed: Sept. 18, 2023. doi: 10.1371/journal.pone.0239786.

MEIKLE, A. et al. Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. **Reproduction**, v.127, n.6, p.727-737. 2004. Available from: <https://pubmed.ncbi.nlm.nih.gov/15175509/>. Accessed: Jun. 02, 2022. doi: 10.1530/rep.1.00080.

NICHOLS, K. et al. Review: Unlocking the limitations of urea supply in ruminant diets by considering the natural mechanism of endogenous urea secretion. **Animal**. v.16, 2022. Available from: <https://www.sciencedirect.com/science/article/pii/S175173112200088X>. Accessed: Sept. 18, 2023. doi.org/10.1016/j.animal.2022.100537.

NIGUSSIE, T. A Review on the Role of Energy Balance on Reproduction of Dairy Cow. **Journal of Dairy Research and Technology**. v.1, p.1-9, 2018. Available from: <https://www.researchgate.net/publication/335847755_A_Review_on_the_Role_of_Energy_Balance_on_Reproduction_of_Dairy_Cow>. Accessed: Sept. 18, 2023. doi: 10.24966/DRT-9315/100003.

NISWENDER, G. Molecular control of luteal secretion of progesterone. **Reproduction**, v.123, n.3, p.333-339. 2002. Available from: <https://pubmed.ncbi.nlm.nih.gov/11882010/>. Accessed: Jun. 07, 2022. doi: 10.1530/rep.0.1230333.

REYNOLDS, C. K. Quantitative aspects of liver metabolism in ruminants. In: VON ENGELHARDT, W. et al. **Ruminant physiology:** digestion, metabolism, growth and reproduction. Sttutgart: Ferdinand Enke Verlag, p.351-371.1995.

SCHARNAGL, H. et al. The Friedewald formula underestimates LDL cholesterol at low concentrations. **Clinical chemistry and laboratory medicine**, v.39, n.5, p.426-431. 2001. Available from: <https://pubmed.ncbi.nlm.nih.gov/11434393/>. Accessed: Jun. 13, 2022. doi: 10.1515/CCLM.2001.068.

WILTBANK, M. C. et al. Regulation of the corpus luteum by protein kinase C. II. Inhibition of lipoprotein-stimulated steroidogenesis by prostaglandin F2α. **Biology of reproduction**, v.42, n.2, p.239-245. 1989. Available from: <https://doi.org/10.1095/biolreprod42.2.239>. Accessed: May, 18, 2022. doi: 10.1095/biolreprod42.2.239.

WILTBANK, M. C. et al. Physiological and practical effects of progesterone on reproduction in dairy cattle. **Animal**, v.8, n.s1, p.70-81. 2014. Available from: <https://pubmed.ncbi.nlm.nih.gov/24703103/>. Accessed: May, 18, 2022. doi: 10.1017/S1751731114000585.

WRAY-CAHEN, D. et al. Hepatic response to increased exogenous supply of plasma amino acids by infusion into the mesenteric vein of Holstein- Friesian cows in late gestation. **British Journal of Nutrition**, v.78, p.913-930, 1997. Available from: <https://doi.org/10.1079/bjn19970209>. Accessed: Dec. 10, 2022. doi: 10.1079/BJN19970209.

ZHANG, F. et al. Plasma metabolite changes in anestrous dairy cows with negative energy balance identified using 1 H NMR technology. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.73, p.929-937, 2021. Available from: <https://www.scielo.br/j/abmvz/a/WqpbV7c8j5SFzXPFNsTKm6y/?lang=en> Accessed: Sept. 18, 2023. doi: https://doi.org/10.1590/1678-4162-12123.