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Biochemical profile in dairy cows with artificial induction of lactation[1]

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ABSTRACT.- Paiano R.B., Lahr F.C., Poit D.A.S., Costa A.G.B.V.B., Birgel D.B. & Birgel Junior E.H. 2018. **Biochemical profile in dairy cows with artificial induction of lactation.** *Pesquisa Veterinária Brasileira 38(12):2289-2292.* Departamento de Reprodução Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Avenida Prof. Dr. Orlando Marques de Paiva 87, São Paulo, SP 05508-270, Brazil. E-mail: renanpaiano@hotmail.com

The objective of this study was to determine the biochemical profile of dairy cows with induced lactation. For comparison, another group of normally calved cows was used as control. Lactation was induced in multiparous Holstein cows (*n=10*) with two norgestomet implants (3mg each implant) on day 1. The testing continued with intramuscular norgestomet (3mg/animal) on days 1, 3, 5, 7, 9, 11, 13 and 15. On days 1, 9, 16 to 18 and then every 14 days, bSTr (500mg/animal) was added. On day 16, the intravaginal implant was removed and intramuscular prostaglandin F2α (0.530mg/animal) and intramuscular estradiol benzoate (5mg/animal) were added. On days 16 to 18 dexamethasone (10mg/animal) was added, and from days 18 to 20 intramuscular metoclopramide (100mg/animal) was added. Milking began on day 19 of the induction. Blood was collected for a biochemical profile after 21 days in milk. It was found that urea and triglyceride concentrations were significantly higher in the induced cows ($P<0.05$). Therefore, it was concluded that the animals that had lactation induced did not present disorders related to the biochemical profile indicating that the hepatic function, renal function and lipidogram of the animals were not affected by the use of the drugs to induce lactation.

INDEX TERMS: Biochemical profile, dairy cows, hormonal induction, lactation, Holstein cows, cattle.

RESUMO.- [**Perfil bioquímico de vacas leiteiras submetidas a indução artificial de lactação.**] O objetivo deste estudo foi determinar o perfil bioquímico de vacas leiteiras com submetidas a indução de lactação. Para comparação, outro grupo de vacas que apresentaram parto normal foi usado como controle. A lactação foi induzida em vacas Holandesas (n=10) utilizando dois implantes de norgestomet (3mg cada implante) no dia 1. O protocolo continuou com a aplicação de norgestomet intramuscular (3mg / animal) nos dias 1, 3, 5, 7, 9, 11, 13 e 15. Nos dias 1, 9, 16 a 18 e depois a cada 14 dias, foi adicionado bSTr (500mg / animal). No dia 16, o implante intravaginal foi removido e adicionou-se prostaglandina F2a intramuscular (0,530 mg / animal) e benzoato de estradiol intramuscular (5mg / animal). Nos dias 16 a 18 foi adicionada dexametasona (10mg / animal) e dos dias 18 a 20 foi adicionada metoclopramida intramuscular (100 mg / animal). A ordenha começou no dia 19 da indução. O sangue foi coletado para mensuração do perfil bioquímico após 21 em leite. Verificou-se que as concentrações de ureia e triglicérides foram significativamente maiores nas vacas induzidas (P<0,05). Portanto, concluiu-se que os animais que tiveram a lactação induzida não apresentaram distúrbios relacionados ao perfil bioquímico, indicando que a função hepática, a função renal e o lipidograma dos animais não foram afetados pelo uso das drogas para induzir a lactação.

TERMOS DE INDEXAÇÃO: Perfil bioquímico, vacas leiteiras, indução hormonal, lactação, vaca Holandesa, bovinos.

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INTRODUCTION

The disposal of animals due to reproductive failures represents a great economic loss for dairy cattle, and one of the main factors for this is a non-pregnant cow at the end of lactation. Artificial induction of lactation is a tool that is based on the combination of synthetic hormones that mimic gestation in the final trimester and stimulates milk synthesis. This approach represents an excellent alternative for owners to maintain a genetically superior herd with good potential for milk production, avoid unproductive animals in the herd, generate new reproductive opportunities and increase profits (Magliaro et al. 2004, Freitas et al. 2010, Radavelli et al. 2016).

For more than 60 years, reports of use of lactation induction protocols have been described (Jewell 2002), including hormonal induction in heifers in the 1940s (Walker & Stanley 1941). However, the period of hormonal induction was too long, lasting about 180 days (Freitas et al. 2010), generating pain and discomfort in the animals (Pestano et al. 2015). Over time, the protocol was shortened to seven days due to a study by Smith & Schanbacher (1973), the cows produced 70% of the milk of a normal lactation. The protocols currently have duration of about 21 days, allowing the production of milk with normal solids content, although there is great variability in the milk induction response and milk production (Pestano et al. 2015).

The response to the reported lactation induction protocol was 100% in 98 cows used in the study by Mellado et al. (2006) and 78% in the study by Ramgattie et al. (2014). Based on milk production, a production volume of 77.2% was described from the previous lactation (Freitas et al. 2010), and 78% of the dairy production of cows that calved normally. (Mellado et al. 2006). The is a discrepancy in the data regarding the reproductive efficiency of the animals, whereby 41.4% of pregnant Holstein cows after lactation induction protocol performed in Brazil (Freitas et al. 2010), while Mellado et al. (2006) did not observe a difference between the proportion of pregnancies in induced dairy cows (71% of pregnancies) when compared to non-induced cows (75% of pregnancies) in a study carried out in Mexico.

Despite studies showing the efficiency of lactation induction protocols, no publications were found in scientific journals to evaluate the effects of its use on the metabolism of Holstein dairy cows. Therefore, the present study had the objective of evaluating the influence of lactation induction on the biochemical profile of Holstein cows.

MATERIALS AND METHODS

Cows and herd management. This study was conducted at the commercial dairy farm, located in Águas da Prata, State of São Paulo, Brazil. The cows were housed in free-stall barns and fed a total mixed ration twice daily. During the administration of hormonal protocol for induction lactation, cows received a diet formulated for dry period composed of 30kg corn silage, 0.6kg pre-dried oat, 1.0kg sorghum wet distillers grain, 1.9kg soybean meal, 0.02kg urea and 0.29kg anionic salt (Bovigold Beta prepartum®) per head, and was designed to contain 2.79% of ethereal extract, 14.53% of crude protein, 38.73% of neutral detergent fibre, 0.55% of calcium and 0.32 of phosphor.

With the beginning of milk production, all cows received the ration composed of 19.35kg corn silage, 2.0 kg tifton 85 haylage, 4.2kg corn grain, 3.4kg soybean meal, 3.0kg wet brewer grains, minerals and vitamins (Bovigold Beta postpartum®) and 3.3kg commercial concentrate per head, and was designed to contain 3.34% of ethereal extract, 17.76% of crude protein, 33% of neutral detergent fibre, 0.73% of calcium and 0.37% of phosphor. The cows were milked twice a day. A total of 20 multiparous (2 to 3 lactations and 3-4 years old) Holstein dairy cows that had the average milk yield 9,200kg in the previous lactation were included in the study. Two groups of cows were formed: 10 dairy cows with at least 45 days dry prior to the start of the hormonal protocol were artificially induced into lactation and 10 dairy cows naturally calved.

All animal procedures were approved by the Bioethics Committee of the School of Veterinary Medicine and Animal Sciences, University of São Paulo, São Paulo, Brazil (approval number: 8022150216/2018).

Hormonal protocol. The following hormonal protocol was used: Holstein cows (*n=10*) were implanted with two norgestomet 3mg each implant (Crestar, MSD Animal Health) on day 1. The testing continued with intramuscular norgestomet 3mg/animal (Crestar, MSD Animal Health) on days 1, 3, 5, 7, 9, 11, 13 and 15. On days 1, 9, 16 to 18 and then every 14 days, bSTr 500 mg/animal (Boostin, MSD Animal Health) was added. On day 16, the intravaginal implant was removed and intramuscular prostaglandin F2α 0.530mg/animal (Ciosin, MSD Animal Health) and intramuscular estradiol benzoate 5mg/animal (Fertilcare, MSD Animal Health) were added. On days 16 to 18 dexamethasone 10mg/animal (Azium, MSD Animal Health) was added, and from days 18 to 20 intramuscular metoclopramide 100mg/animal (Noprosil, Isofarma) was added as described by Silva et al. (2017). Milking began on day 19 of the induction.

Blood sampling and measurement of body condition score. Blood samples were collected at 21 days in milk (DIM) in the morning before feeding, from the middle coccygeal vein or artery puncture and stored in tubes without anticoagulant. Within 3 h of collection, blood samples were centrifuged at 2500rpm for 20 min. Serum samples were stored in Eppendorf tubes at -20°C until analysis measurements. Simultaneous with the blood collection, the body condition score (BCS) was measured by the same observer, using the five-point scale (Ferguson et al. 1994).

Measurement of biochemical samples. The concentration of total protein, albumin, globulins, urea, creatinine, β-hydroxybutyrate (BHBA), triglycerides, total cholesterol, gamma-glutamyltransferase (GGT) and aspartate aminotransferase (AST) were determined using commercial kits from Randox in an automatic biochemistry system (RX Daytona - Randox Laboratories).

Statistical analysis. The ANOVA was performed with the SAS software package (version 9.3 SAS/STAT, SAS Institute Inc., Cary, NC) using PROC GLIMMIX. To compare the means of biochemical parameters and BCS in the different groups (induced lactation vs. control), Tukey's test was used for detecting differences among means. Statistical significance level was regarded as $P<0.05$ and $0.05<P<0.1$ was considered to indicate a tendency toward a significant difference.

RESULTS

All continuous data are presented as least squares means ± SE. Biochemical concentrations and BCS values are shown in Table 1. Serum creatinine and triglyceride concentrations were significantly higher in induced cows ($P<0.01$). In the other variables, no statistically significant differences were found.

Table 1. Biochemical profile and body condition score from control cows and induced cows at 21 days in milk

Variables	Control cows	Induced cows
Total Protein (g/dL)	7.73±0.18	7.68±0.18
Albumin (g/dL)	2.92±0.12	3.23±0.11
Globulins (g/dL)	4.92±0.30	4.41±0.24
Urea (mmol/L)	4.01±0.31	4.89±0.31
Creatinine (mg/dL)	0.97±0.06[b]	1.30±0.06[a]
BHBA (mmol/L)	0.50±0.03	0.43±0.04
Triglycerides (mg/dL)	10.23±1.51[b]	14.33±1.52[a]
Cholesterol (mg/dL)	97.17±8.57	78.18±7.56
GGT (U/L)	15.11±2.97	22.11±2.98
AST (U/L)	66.11±6.55	71.22±6.56
BCS (points)	3.04±0.24	3.50±0.20

[a, b] Different lower case letters indicate difference by Tukey's test ($P \leq 0.01$).

DISCUSSION

Analysis of the results showed that there were no alterations in liver function due to lactation induction. The evaluation of GGT and AST enzyme activity were within the recommended limit for the species (Smith 2014). Measurement of the activity of GGT and AST enzyme contributed to the diagnosis of acute hepatic lesions (Russell & Roussel 2007).

The liver is responsible for the synthesis of proteins, and this functionality is dependent on an adequate nutritional status (González & Silva 2006). Albumin is responsible for the transport of amino acids and bilirubin (González 2000). The reduction of albumin concentration can be observed in animals with hepatic lesions, or in animals with inflammatory processes, being considered as a negative acute phase protein (Russell & Roussel 2007, Bertoni et al. 2015, Trevisi et al. 2015). Increased albumin concentration may occur due to dehydration (González 2000, Russell & Roussel 2007). No difference was observed in serum concentration of total protein, albumin and globulins. To the contrary, high concentrations of albumin and globulins were reported in sheep submitted to lactation induction protocol (Radavelli et al. 2016).

The urea concentration was within of biological range (Radostits et al. 2007). Urea is a product of nitrogen metabolism excretion, produced in the liver, and elevated urea values are suggestive of energy deficit, high protein in the diet or dehydration (González 2000, Roy et al. 2011). High values of urea concentration were observed in ketotic cows (İssi et al. 2016). On the other hand, Oliveira (2017) noticed a decrease in the concentration of urea when evaluating the effect of lactation induction on blood tests of crossbreed cows.

Control group showed lower blood creatinine concentration than those recommended by the literature (Radostits et al. 2007). Similar values to ours were observed by İssi et al. (2016) in control cows when compared to cows with ketosis and by Piccione et al. (2012) in dairy cows during the second week of lactation when compared to other stages of lactation, late gestation and dry period. The measurement of creatinine associated with serum urea assists in the evaluation of renal function, and creatinine is a more reliable parameter for renal function because it is eliminated exclusively through the kidneys unlike other nitrogen compounds that can be eliminated by the kidneys as well as mammary gland through milk (Smith 2009). Increased creatinine and urea values indicate that functionality of the nephrons is below 75% (Kaneko et al. 2008). Because the creatinine values were not elevated in the control group, the hypothesis of renal injury was ruled out. Low values of creatinine concentration can be caused due to starvation, low amount of protein in the diet and liver damage (Kaneko et al. 2008). This hypothesis was also ruled out because total protein values and liver enzyme activity were within normal range, indicating that low creatinine concentration in control group during postpartum was a normal finding.

The triglyceride concentration was higher in induced cows than the control animals, and this value was higher than physiological range (Radostits et al. 2007). Triglyceride measurement can be used to assess body energy resources (Nozad et al. 2014). Triglyceride values higher than measured in this study were observed in induced crossbreed cows (Oliveira 2017), and they associated this increase with the reduction of lipase lipoprotein activity in adipose tissue due to the use of estradiol during the protocol. The BCS reflects the energy balance of the animal (Jeong et al. 2015). Values similar to those obtained here were reported in cyclic postpartum cows (Jeong et al. 2015) and for induced crossbreed cows (Oliveira 2017).

According to the cholesterol concentration values, no difference was observed between the groups studied. The assessment of cholesterol helps in the diagnosis of energy balance (Sevinc et al. 2003). Metabolic and physiological adaptations related to the development of the mammary gland for milk production can cause hypocholesterolemia (Krajnicakova et al. 2003). Low serum cholesterol levels were observed in cows with puerperal ketotic (Djoković et al. 2013) and for cows in different reproduction periods (Piccione et al. 2012).

CONCLUSION

The data collected for this study indicates that the animals that had lactation induced did not present disorders related to the biochemical profile, indicating that the hepatic function, renal function and the lipidogram of the animals were not affected by the use of the drugs to induce lactation.

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