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Lactation induction protocol changes the milk composition and the biochemical profile of Holstein cows

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Abstract

Dairy cattle farming has made progress with improvements in its herds, combined with genetics, nutrition, management, health and the use of techniques that allow to increase production, as is the case with lactation induction protocols. However, due to the use of a wide variety of drugs, it's use can alter the composition of milk and the biochemical profile of animals. The objective of this study was to evaluate the composition of milk and the biochemical profile of Holstein cows submitted to a lactation induction protocol. Milk and blood samples were collected from five cows submitted to the lactation induction protocol. In the milk samples, the percentage of fat, protein, lactose and somatic cell count were analyzed and in the blood, the levels of the enzymes alanine aminotransferase and aspartate aminotransferase, total proteins, albumin, creatinine and urea were analyzed. There was a significant reduction in the levels of protein and lactose in milk ($p<0.05$), however, even with the reduction, the values were within the required parameters. Despite not showing a significant effect, values of aspartate aminotransferase were observed much higher than the reference values for the species, indicating impaired liver function. The use of the lactation induction protocol reduced the levels of protein and lactose in milk samples obtained between the 21st and the 30th days after the beginning of the protocol, however, even with the reduction, the values were presented, on the 30th day, within the required parameters. There were no differences between the levels of ALT, total proteins, albumin, urea and creatinine in the blood samples analyzed at the beginning and at the end of the induction protocol, however, the enzyme levels AST, were higher than the reference values for the species at D21, suggesting impaired liver function. Lactation induction is an important tool for milk producers, but more research must be conducted in order to guarantee the quantity and quality of milk produced in line with the health of the animals.

Keywords: dairy cattle; milk production; milk cows

INTRODUCTION

World milk production is around 816 million tons and the average annual consumption per inhabitant is 116.5 kg, and these numbers have been increasing every year (IFCN, 2019). In this scenario, it appears that production in Asia, Europe and America represents more than 90% of the world's total milk production (FAO, 2019). Among the production in America, Brazil stands out, since the country has the largest herd of cattle in the world, about 225 million head, being more than 24 million dairy cows, and is the 3rd largest country producing milk, accounting for more than 7% of the total milk produced in the world (Milk Yearbook, 2019).

Worldwide, and in Brazil, there is an increase in cow productivity and milk quality, due to modernization and specialization processes of dairy farms. These factors result in a better quality product for consumers, who value health and well-being. However, several factors must be considered in order to maintain this increasing productivity, among them, we point out the reproductive aspects. Reproductive failures are one of the main reasons for the disposal of animals from dairy herds, leading to losses in productivity, profitability and reduction in the reproductive efficiency of the herd (Milk Yearbook, 2019).

Thus, an alternative to reduce losses with the disposal of acyclic animals, heifers that repeat estrus successively or animals with fertilization problems is the use of a protocol to induce lactation (Freitas et al., 2010). The protocol promotes milk production, regardless of whether the cow has a pregnancy or conceives a calf.

There are several types of protocols, with different stages, hormones and moments of application, but most are carried out with a period between 19 to 21 days, with repetition, every 14 days, of bovine somatotropin (BST) (Pestano et al., 2015). The use of progesterone, prostaglandin, estrogen, somatotropin, dexamethasone, metoclopramide and other drugs, mimic the gestation and delivery of an adult animal and, thus, the cow starts milk production (Freitas et al., 2010). Although several studies have shown the efficiency of lactation induction protocols, there are few studies that have evaluated the composition of milk and/or the metabolic profile of cows submitted to the protocols. Freitas et al. (2010) evaluated forty Holstein cows, with previous reproductive problems, empty, with 2nd or more lactations, submitted to two types of lactation induction protocols and stated that the use of the protocols did not affect the milk composition. Paiano et al. (2018) evaluated the biochemical profile of ten Holstein cows that had induced lactation and reported that the animals had no disorders related to the biochemical profile, indicating that the liver function, kidney function and lipidogram of the animals were not affected by the use of drugs to induce lactation.

Due to the scarcity of research on the subject and given the importance of the dairy production chain in the global context, the objective of this study was to evaluate the composition of milk and the biochemical profile of Holstein cows submitted to lactation induction protocol.

MATERIALS AND METHODS

The study was conducted in 2019, on a dairy property located in southern Brazil. Data were collected from five Holstein cows (Table 1) submitted to the lactation induction protocol, maintained in the food and hygienic sanitary management adopted by the property. Protocol identification data, drugs used and dosages were also collected, which was based on injections, by intramuscular injection, of Estradiol Benzoate (Sincrodiol®, Ouro Fino, Cravinhos, São Paulo, Brazil), Progesterone (Sincrogest®, Ouro Fino, Cravinhos, São Paulo, Brazil), Sodium Cloprostenol (SincroCio®, Ouro Fino, Cravinhos, São Paulo, Brazil), Dexamethasone (Cortiflan®, Ouro Fino, Cravinhos, São Paulo, Brazil) and Bovine Somatotrophin - BST (Boostin®, MSD Saúde Animal, Cruzeiro, São Paulo, Brazil) (Table 2).

Table 1: Age, live weight and lactation order of Holstein cows submitted to the lactation induction protocol (n=5).

Animal identification	Age (months)	Live weight (kg)	Lactation order
1	53	480	2nd
2	68	550	3rd
3	65	655	3rd
4	18	600	1st
5	48	540	2nd

Table 2: Lactation induction protocol used and identification of blood and milk collection days.

Day	Activities
1	30mL Estradiol Benzoate + 2mL Progesterone + 500mg BST + blood collection
2 to 7	30mL Estradiol Benzoate + 2mL Progesterone
8	30mL Estradiol Benzoate + 2mL Progesterone + 500mg BST
9 to 14	20mL Estradiol Benzoate
15	500mg BST
16	2mL Sodium Cloprostenol
17 to 18	Interval and stimulation and adaptation to milking
19 to 20	40mL Dexamethasone
21	40mL Dexamethasone + milking start + blood and milk collection
22	500mg BST + milking + milk collection
23 to 30	Milking + milk collection

On day 1 (beginning of the protocol) and on day 21 (end of the protocol), blood samples were collected from the animals for analysis of the biochemical profile (Table 2). 4mL of blood were collected in a collection tube with a clot activator (BD Vacutainer®), in the caudal vein, with an adapter and needle for vacuum blood collection, 25mmx0.7mm (Labor Import®). The samples were sent for analysis of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), by the UV kinetic method, total proteins, by the colorimetric method, albumin, by photometric method and by urea and creatinine, by kinetics.

Between days 21 to 30, after the beginning of the lactation induction protocol, milking of the cows was started, with a bucket by the foot, and milk samples (10mL) were collected, in duplicate, in identified tubes, for carrying out the analyzes percentage of fat, protein, lactose and for Somatic Cell Count (SCC, x1000.mL^{-1}) (Table 2). The analyzes were performed at the Milk Analysis Laboratory, belonging to the State University of Maringá, Maringá, Paraná, Brazil.

Anaylses statistical: The variables were analyzed using the PROC GLM procedure of the SAS statistical program (2000), version 8.01, and the LSMEANS means test. Significance level ≤ 0.05 was considered.

RESULTS AND DISCUSSION

The result of the analysis of the average levels of fat, protein, lactose and SCC of milk samples collected, daily, between days 21 and 30 after the beginning of the lactation induction protocol (Table 3) showed a reduction in the values of all variables, however, only the percentages of protein and lactose in milk showed a significant reduction (p<0.05).

Table 3: Average values of fat, protein, lactose and somatic cell count (SCC) in milk samples collected from Holstein cows, from the 21st to the 30th day of the beginning of the lactation induction protocol (n=5).

Days after starting the protocol	Evaluated parameters (mean ± standard error)			
	Fat (%)	Protein (%)	Lactose (%)	SCC (x1000.mL^{-1})
21	5.24 ± 0.72	6.80 ± 0.44	10.18 ± 0.65	1.077 ± 210
22	7.20 ± 0.72	5.80 ± 0.44	8.60 ± 0.65	492 ± 210
23	5.50 ± 0.72	4.52 ± 0.44	6.74 ± 0.65	684 ± 210
24	5.70 ± 0.72	4.30 ± 0.44	6.28 ± 0.65	670 ± 210
25	4.66 ± 0.72	3.80 ± 0.44	5.48 ± 0.65	458 ± 210
26	5.74 ± 0.72	3.74 ± 0.44	5.58 ± 0.65	269 ± 210
27	6.10 ± 0.72	3.70 ± 0.44	5.44 ± 0.65	221 ± 210
28	5.78 ± 0.72	3.60 ± 0.44	5.30 ± 0.65	435 ± 210
29	5.20 ± 0.72	3.52 ± 0.44	5.20 ± 0.65	209 ± 210
30	4.58 ± 0.72	3.50 ± 0.44	5.20 ± 0.65	133 ± 210
Mean	5.57	4.33	6.40	465.28
p value	0.400	0.0001	0.0001	0.089

There were no differences (p>0.05) in most of the serum biochemical variables analyzed at the beginning (D1) and at the end (D21) of the induction protocol (Table 4). However, the observed levels of the enzyme AST showed differences (p>0.05) and were much higher than the reference values for the species (20 to 34 U.L^{-1}). On the other hand, serum levels of the enzyme ALT were within the expected limits for the species (14 to 38 U.L^{-1}) (Smith, 2014).

Despite significant differences in serum concentrations of total proteins and albumin (p>0.05), the levels of total proteins, both at the beginning and at the end of the protocol, as well as the levels of albumin at the end of the protocol, were slightly higher than the reference values (Russell and Roussel, 2007; Smith, 2014).

The urea and creatinine levels were also not affected by the use of the lactation induction protocol (P>0.05), however, the values obtained were lower than the reference (Russell and Roussel, 2007).

Table 4: Average serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total proteins (TP), albumin, urea and creatinine from Holstein cows, at the beginning (D1) and at the end (D21) of the lactation induction protocol (n=5).

Evaluated parameters	Mean values ± standard error at the beginning of the protocol (D1)	Mean values ± standard error at the end of the protocol (D21)	p value	Reference value for cattle
AST (U.L^{-1})	59.60 ± 14.96	121.20 ± 14.96	0.019	20-34
ALT (U.L^{-1})	33.40 ± 5.85	23.40 ± 5.84	0.261	14-38
TP (g.dL^{-1})	8.52 ± 0.16	8.00 ± 0.16	0.056	6.7-7.5
Albumin (g.dL^{-1})	3.52 ± 0.22	4.18 ± 0.22	0.067	3.0-3.6
Urea (mg.dL^{-1})	34.80 ± 4.17	31.80 ± 4.17	0.625	42.8-64.2
Creatinine (mg.dL^{-1})	0.62 ± 0.26	1.34 ± 0.26	0.093	1.0-2.0

DISCUSSION

In order to establish quality standards for milk, the Ministry of Agriculture, Livestock and Supply, in Brazil, published Normative Instruction N^{o} 76 (IN76), which regulates the identity and quality characteristics that refrigerated raw milk must present, pasteurized milk and type A pasteurized milk, as well as the procedures for collecting raw milk and transporting it in bulk (Brazil, 2018). The parameters established by IN76 to analyze quality include analysis of centesimal composition, SCC, plate count and research of antibiotic residues. Regarding the physical-chemical parameters, IN76 regulates as minimum fat content 3.0%, total protein 2.9%, anhydrous lactose 4.3% and CCS of, at most, 300,000 SC.mL^{-1} (Brazil, 2018). Thus, the results of the variables evaluated between the 21st and the 30th day after the beginning of the induction protocol, point out that, at the end, the expressed milk had levels adequate to Brazilian standards, showing that the protocol did not compromise its quality.

In fact, the levels of fat in milk can vary between 3.5 and 5.3% and its composition is basically triglycerides, which are responsible for the taste and palatability of dairy products, however, several factors can change fat contents, such as breed, time of year, geographical area, management, feeding and lactation stage of animals (Teixeira et al., 2018).

Despite the significant reduction, the protein levels observed in this study (3.50%) were within the reference range for Holstein cows, which is 3.20%. Proteins represent 3 to 4% of the solids found in milk (Teixeira et al., 2018).

As with protein, the levels of lactose, which is the main carbohydrate in milk, also showed a significant reduction between the 21st and 30th days after the start of the induction protocol. However, even with the reduction, its value, at the end of the protocol, was normal (5.20%). According to Teixeira et al. (2018), the percentage of lactose is approximately 5%, ranging from 4.7 to 5.2%. Lactose has great importance in the synthesis of milk, as it is the main osmotic factor responsible for controlling production, bringing water from the blood to the mammary gland. Therefore, the volume of milk produced and the amount of milk water depend on the amount of lactose synthesized in the mammary gland (Teixeira et al., 2018). The lower the amount of lactose in milk, the lower the income from dairy products in the industry.

The SCC was also not harmed by the use of the protocol, presenting levels adequate to the Brazilian legislation. Somatic cells are a set of cells present in milk, derived from the desquamation of the mammary epithelium, from milk secreting cells that are eliminated, and from defense cells of the animal's organism, macrophages, neutrophils and lymphocytes (Rangel et al., 2013). SCC is one of the main criteria for milk quality, and a high count indicates the presence of pathogenic microorganisms and chemical changes that compromise milk composition, industrial performance and food safety. Therefore, SCC indicates the health of the mammary gland of cows and signals significant losses in production and changes in milk quality (Rangel et al., 2013). The increase in SCC changes the composition of milk, reduces the levels of casein, fat and lactose, and affects the enzymatic activity and the clotting time, resulting in lower yield, productivity, quality and stability of dairy products (Teixeira et al., 2018). Therefore, reduced levels, such as those found in this study, are desirable.

Regarding the average serum levels of the metabolites evaluated, high values for AST (121.20 $U.L^{-1}$) were observed. AST or also known as oxalacetic transaminase, is used as an indicator of liver and muscle damage. Increases in AST can be observed in cases of infectious and toxic hepatitis, cirrhosis, biliary obstruction, fatty liver, hemolysis and selenium and/or vitamins deficiency (González and Silva, 2006). In ruminants, AST is a good indicator of liver function, so it is believed that the high doses of drugs and hormones used in the protocol are responsible for the results obtained in this study. However, it is noteworthy that the initial levels of AST were already high for the species, suggesting that the animals already had hepatic impairment, which was aggravated by the use of the protocol.

On the other hand, even with the indication of hepatic impairment, serum levels of the enzyme ALT, a hepatic specific enzyme, were observed, within the expected limits for the species (14 to 38$U.L^{-1}$) (Smith, 2014; Reece, 2017). In cases of hepatocellular degeneration or necrosis, their levels rise, if not observed in this study.

Regarding the serum values of total proteins and albumin, no significant differences were observed ($p>0.005$), however, the levels of total proteins, both at the beginning and at the end of the protocol, as well as the levels of albumin at the end of the protocol, were slightly higher than the reference values (Smith, 2014). Proteins perform various functions in the animal organism and undergo changes of clinical importance, especially in cases of inflammatory, bacterial, immunological, parasitic and metabolic processes (Smith, 2014). Among proteins, albumin is the most important protein, representing 40 to 60% of the total proteins in plasma. Its functions are to transport hydrophobic molecules such as bilirubin and fatty acids, in addition to nutrition and maintenance of blood osmotic pressure (Reece, 2017; Lehninger et al., 2019). Plasma levels of albumin are used as a parameter for the assessment of nutritional status and liver function, due to their relationship with protein supply and liver production, and hypoalbuminemia are common in cases of kidney, digestive and liver injuries, on the other hand, hyperalbuminemia is rarely observed, except in cases of dehydration or shock, where there is an excessive loss of water causing a hemoconcentration (Reece, 2017).

In order to evaluate the serum levels of total proteins and albumin in females submitted to lactation induction, Radavelli et al. (2016), as well as in our study, reported an increase in serum albumin concentrations in sheep submitted to the lactation induction protocol, however, Paiano et al. (2018) found no changes in the levels of total proteins and albumin in Holstein dairy cows subjected to lactation induction when compared to non-induced cows, however the values were lower than those found in this study, with the average protein levels being 7.68 $g.dL^{-1}$ and albumin of 3.23 $g.dL^{-1}$.

The urea and creatinine levels were also not influenced by the use of the lactation induction protocol, however, the values obtained were lower than the reference (Russell and Roussel, 2007). Urea is a product of nitrogenous excretion of protein metabolism and variations in serum levels can occur due to several factors, such as energy deficit, high protein content in the diet or dehydration (González and Silva, 2006; Roy et al., 2011) or when there is renal failure (Lehninger et al., 2019).

Creatinine, like urea, is a product of nitrogen degradation, originated from the breakdown of creatine, a substance present in muscle and involved in energy metabolism (Lehninger et al., 2019). However, measurement of urea associated with serum creatinine, assists in the assessment of renal function and increased values of creatinine and urea indicate that the functionality of the nephrons is below 75%, (Reece, 2017), a fact not observed in this study, even, highlights it was found that the initial urea levels were already below the reference values. Thus, we cannot infer that the reduction occurred and function of the protocol. Oliveira (2017) evaluated the effect of using the lactation-inducing protocol in crossbred cows and reported a reduction in urea levels, however, as in our study, Paiano et al. (2018) found no changes in serum urea levels of cows submitted to induction protocol.

CONCLUSIONS

The use of the lactation induction protocol reduced the levels of protein and lactose in milk samples obtained between the 21st and the 30th days after the beginning of the protocol, however, even with the reduction, the values were presented, on the 30th day, within the required parameters. There were no differences between the levels of ALT, total proteins, albumin, urea and creatinine in the blood samples analyzed at the beginning and at the end of the induction protocol, however, the enzyme levels AST, were higher than the reference values for the species at D21, suggesting impaired liver function. Lactation induction is an important tool for milk producers, but more research must be conducted in order to guarantee the quantity and quality of milk produced in line with the health of the animals.

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