

## Notas Científicas

### Metabolic attributes, yield and stability of milk in Jersey cows fed diets containing sodium citrate and sodium bicarbonate

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**Abstract** – The objective of this work was to evaluate the inclusion of sodium citrate and sodium bicarbonate in the diet of lactating Jersey cows, and its effects on the metabolic attributes, productivity and stability of milk. We evaluated urinary pH, levels of glucose and urea in blood, body weight, body condition score, milk yield, milk stability (ethanol test), and milk physicochemical properties of 17 cows fed diets containing sodium citrate (100 g per cow per day), sodium bicarbonate (40 g per cow per day) or no additives. Assessments were made at the 28<sup>th</sup> and 44<sup>th</sup> days. Supply of sodium citrate or bicarbonate has no influence on the metabolic attributes, productivity, body weight, and body condition score of the cows, neither on the composition and stability of milk.

**Index terms:** *Bos taurus*, additives, alcohol test, unstable nonacid milk.

### Atributos metabólicos, produtividade e estabilidade do leite em vacas Jersey alimentadas com dietas com citrato de sódio e bicarbonato de sódio

**Resumo** – O objetivo deste trabalho foi avaliar a inclusão de citrato de sódio e de bicarbonato de sódio na dieta de vacas lactantes Jersey e seus efeitos sobre os atributos metabólicos, a produtividade e a estabilidade do leite. Foram avaliados o pH urinário, os níveis sanguíneos de glicose e ureia, o peso e o escore de condição corporal, a produção e a estabilidade do leite (teste do álcool) e as propriedades físico-químicas do leite de 17 vacas, alimentadas com dietas com citrato de sódio (100 g por vaca por dia), bicarbonato de sódio (40 g por vaca por dia) ou sem aditivos. As avaliações foram feitas no 28<sup>o</sup> e 44<sup>o</sup> dias. O fornecimento de citrato ou de bicarbonato de sódio não influencia os atributos metabólicos, a produtividade, o peso e o escore de condição corporal das vacas, nem a composição ou a estabilidade do leite.

**Termos para indexação:** *Bos taurus*, aditivos, teste do álcool, leite instável não ácido.

Milk suitability for the dairy industry is usually tested in farms and at dairy plant platforms, relying on milk stability to the ethanol test. In order to be accepted by industries, milk should be stable in an alcoholic solution with at least 72°GL, otherwise it should be rejected (Brasil, 2011).

Besides high acidity caused by lactose microbial degradation, which renders milk unstable, other factors may influence milk stability in alcohol test, such as feed restriction (Zanela et al., 2006), lactation stage (Tsioulpas et al., 2007), and ruminal acidosis (Hernández & Ponce, 2005). This multifactorial feature of milk stability hinders the identification of its causes and, consequently, the proposition of solutions. Milk

producers tend to ignore this problem as most of them have restricted access to technological or scientific information about it. This scenario favors the adoption of empirical practices, such as the inclusion of reduced doses of sodium citrate and sodium bicarbonate in cows' diet, which allegedly would improve milk stability. Experimental and scientific information about this theme are not available and, therefore, these inclusions are being used with no theoretical background or experimental validation.

The objective of this work was to evaluate the inclusion of sodium citrate and sodium bicarbonate in the diet of lactating dairy cows, and its effects on metabolism-related parameters, body weight,

body condition score (BCS), and on yield and physicochemical traits of milk.

The study was carried out at Embrapa Clima Temperado, RS, Brazil. All procedures involving animals were approved by the animal welfare committee of the Animal Science Department, at the Universidade Federal do Rio Grande do Sul. Seventeen Jersey cows were used for the experiment with  $220 \pm 32$  days in milk, weighing  $420 \pm 62.4$  kg, and yielding  $12 \pm 2.3$  kg of milk per day. Cows were housed in a free-stall barn, individually fed, and milked twice a day, at 06:30 and 17:30 h. The trial was conducted on May and June. Average daily temperature and relative humidity during the experiment were  $15.45^\circ\text{C}$  and 88.12%, respectively.

Cows were assigned to a completely randomized design. The trial lasted for 44 days, from which 14 days were used for adaptation to the diet, and 30 days for data collection (experimental period). Before the start, and during the whole trial, each cow was fed the same basal diet (in dry matter, DM, per day), consisting of sorghum silage (8.38 kg), alfalfa hay (1.30 kg), soybean (1.3 kg), corn (0.9 kg) and rice bran (1.8 kg), which provided  $0.15 \text{ kg kg}^{-1}$  crude protein,  $0.68 \text{ kg kg}^{-1}$  TDN,  $0.06 \text{ kg kg}^{-1}$  calcium, and  $0.06 \text{ kg kg}^{-1}$  phosphorus. Dietary groups were: control ( $n=5$ ), which fed a standard diet without additive; bicarbonate ( $n=6$ ), fed basal diet with 40 g sodium bicarbonate per cow per day; citrate ( $n=6$ ), fed diet with 100 g sodium citrate per cow per day. Additives were mixed with the concentrate at the time of feeding. The choice of citrate and bicarbonate doses was based on the reported commercialized levels.

On the 28<sup>th</sup> and 44<sup>th</sup> days, cows were weighed and their body condition score (BCS) was evaluated on a 1 to 5 scale (Edmonson et al., 1989). Milk yield was registered daily during the whole trial, but only the values from days 28 and 44 were used in the analysis. Composite milk samples comprehended the mixture of milk samples collected automatically in the morning and evening milkings. Samples were analyzed for the contents of fat, protein, and lactose, using an infrared analyzer Bentley 2000 equipment (Bentley Instruments, Chaska, MN, USA). Somatic cell count (SCC) was determined by flow cytometry with Somacount 300. A subset of composite milk samples was analyzed for titratable acidity, expressed as grams of lactic acid per 100 mL, °D; and determinations were done for: pH, by potentiometry; density ( $\text{g L}^{-1}$ ),

using a lactodensimeter corrected for milk temperature at  $15^\circ\text{C}$ ; ethanol stability, by mixing, in a Petri dish, 2 mL milk with 2 mL alcohol solution, with ethanol concentrations ranging from 72 to  $80^\circ\text{GL}$ , until visual detection of clot formation (the result was considered as the minimal ethanol concentration that induced precipitation); and thermal stability, by boiling milk samples and checking for clot formation (results were expressed as absence or presence of clots). Clinical mastitis was monitored at every milking, with visual inspection of foremilk strips; subclinical mastitis was checked every fifteen days with the California Mastitis Test.

On days 28 and 44, urine samples were collected six hours after the feeding procedures, following spontaneous release or by vulvar massage. These samples were immediately analyzed for pH with a Hanna pH meter. On the same days, after the morning milking and before feeding procedures, blood samples were collected via jugular puncture in 10 mL non-heparinized vacutainers. Blood samples were cooled on ice and taken to a specialized laboratory to determine plasma concentrations of glucose by Trinder glucose oxidase (GOD) method, and urea via Trinder enzymatic method, both with the use of commercial kits (Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil).

Data obtained on days 28 and 44 were subjected to analysis of variance, considering a completely randomized design, testing the effects of diet, evaluation days, and the interaction between these factors as fixed effects. Evaluation day was considered as a repeated measure, and cows fed on diets were considered as random effect, using the mixed procedure of SAS, version 9.2 (SAS Institute, Cary, NC, USA). Values of SCC were transformed by  $\log_{10}$ . The boiling test values were considered as zero when no coagulation was observed after boiling, and one when coagulation occurred with boiling; their frequency was analyzed using Npar1Way procedure of SAS, version 9.2 (SAS Institute, Cary, NC, USA). The differences between the diets were detected by the Fisher test at 5% probability, and trends towards significance were discussed at 10% probability.

Inclusion of sodium bicarbonate and sodium citrate in the diet did not alter body weight, BCS, milk yield and composition, urinary pH, and blood parameters (Table 1). As the objective of the present experiment was to test the doses already used by milk producers which are allegedly efficient by the additive

companies for improving milk stability, the dietary cation-anion difference (DCAD, Na plus K minus Cl minus S; contents expressed in meq per 100 g) was not calculated prior to the study, but it was used to explain the effects (or its absence) of diet on cows' metabolism, since changes in DCAD may influence the animal's acid-basic status (Apper-Bossard et al., 2006), the osmotic pressure and, therefore, affect the enzyme and nerve function (Chan et al., 2005). The inclusion of bicarbonate and citrate slightly increased the DCAD, from +29.6 (control group) to +33.1 (bicarbonate group) and +38.2 meq per 100 g (citrate group).

Even though metabolic parameters like blood pH, pCO<sub>2</sub>, HCO<sub>3</sub>, and levels of beta-hydroxybutyrate and nonesterified fatty acids, among other parameters, were not evaluated, urinary pH can give an estimate of the animal's metabolic status (Del Claro et al., 2005). This variable was not altered by the inclusion of additives, suggesting that blood acid-base homeostasis may have not been altered. Along with urinary pH, metabolic profile may also be assessed by biochemical components in blood and their variations. For example: urea, representing protein metabolism; and glucose, indicator of the energetic metabolism (Wittwer & Contreras, 1980). Changes in DCAD may alter urea levels (Roche et al., 2005; Apper-Bossard et al., 2006),

but this was not the case in the present experiment, probably due to the low variation in DCAD between the three treatments. Lack of changes in glucose levels, due to manipulation of DCAD, was previously reported (Apper-Bossard et al., 2006; Wildman et al., 2007), such as in the present study.

Titrate acidity from all treatments stayed inside the normal range, 14 to 18°D (Brasil, 2011). Milk stability was similar between treatments (approximately 74°GL) and above the recommended values by Brazilian legislation (72°GL). However, milk with the stability level demanded by legislation can be discarded by industry, which is demanding levels of 78 to 80°GL (Fischer et al., 2012). Therefore, milk stability did not increase with the use of additives, which in some degree is corroborated by the lack of alteration in the metabolic parameters evaluated. The inclusion of sodium citrate and sodium bicarbonate to improve milk stability could be a reasonable alternative in cows with metabolic disorders, but further studies are required to prove this hypothesis.

The inclusion of sodium citrate and sodium bicarbonate, at the proposed commercial rates, into the diet of lactating healthy cows do not alter urinary pH, urea and glucose levels in blood, milk physicochemical properties and, more importantly, do not improve milk stability in the ethanol test.

**Table 1.** Mean values of production traits, milk yield and composition, urinary pH, and blood parameters from Jersey cows fed diets without additive (control) or with sodium bicarbonate (40 g per cow per day) and sodium citrate (100 g per cow per day).

Variable	Control	Bicarbonate	Citrate	p
Body weight (kg)	406	401	429	ns
Body condition score (1–5)	2.50	2.67	2.58	ns
Milk production (L per cow per day)	9.98	9.61	9.76	ns
Fat (g per 100 g)	5.73	5.57	6.15	ns
Protein (g per 100 g)	3.85	3.69	4.02	ns
Lactose (g per 100 g)	4.16	4.21	4.25	ns
Total solids (g per 100 g)	15.06	14.73	15.82	ns
Density (g L <sup>-1</sup> )	1,030.32	1,029.82	1,029.95	ns
Urinary pH	7.93	8.05	8.21	ns
Plasma urea (mg dL <sup>-1</sup> )	35.41	34.25	33.67	ns
Plasma glucose (mg dL <sup>-1</sup> )	59.91	59.75	61.67	ns
Milk stability (°GL) <sup>(1)</sup>	74.40	74.67	74.67	ns
Titrate acidity (°D)	17.90	17.00	16.16	ns
Milk pH	6.74	6.75	6.82	ns
Somatic cell count (cell mL <sup>-1</sup> )	942,200	557,000	674,833	ns

<sup>(1)</sup>Minimum ethanol concentration that induces coagulation of milk proteins. <sup>ns</sup>Nonsignificant by Fisher test, at 5% probability.

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Received on August 29, 2012 and accepted on April 29, 2013