

Stability of milk from dual purpose cows with different types of feeding

Hernández-Bautista Jorge¹; Rodríguez-Magadán Héctor M.¹; Salinas-Ríos Teodoro^{1*}, Pérez-León María I.²; Aquino-Cleto Magaly¹; Mariscal-Méndez Araceli¹

¹ Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma Benito Juárez de Oaxaca, Av. Universidad S/N, Ex-Hacienda 5 Señores, 68120, Oaxaca de Juárez, México.

² Instituto Tecnológico del Valle de Oaxaca, Tecnológico Nacional de México, Ex Hacienda de Nazareno, Agencia de Policía Nazareno Xoxo, Centro, 71230, Santa Cruz Xoxocotlán, Oaxaca, México.

* Correspondence: salinas980@hotmail.com

ABSTRACT

Objective: To establish milk stability using the alcohol test in cows with different feeding strategies in the tropical dairy system.

Design/methodology/approach: Ten crossbred Holstein × Zebu cows whose milk frequently tested positive to the alcohol test were housed in individual yards and distributed into two groups. Experiment 1. T1: cows consumed dry Pangola grass (*Digitaria eriantha*); T2: cows consumed green Maralfalfa grass (*Pennisetum violaceum*). Forage was provided *ad libitum*. Experiment 2. T1 cows were supplemented with a concentrate with higher crude protein and metabolizable energy percentage than T2 cows; this percentage was gradually increased up to 4.0 kg. Milk was subjected to alcohol testing. Variance analyzes were performed under categorical data models.

Results: Higher percentages of cases that tested positive for alcohol were observed in cows that consumed dry grass than in cows that consumed green grass ($P < 0.05$). The concentrate addition to diets based on dry or green forage reduced the positive case percentage.

Implications: A balanced diet improves milk stability.

Findings/conclusions: The improvement in the cows' nutritional value decreases the percentage of milk with positive results in the alcohol test.

Keywords: Forage, tropical dairy, milking, Abnormal Milk Syndrome.

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INTRODUCTION

In order to establish milk stability at the high temperatures to which it is subjected during its processing, a quick test has been established (Dumpler *et al.*, 2020). This test consists of adding equal amounts of milk and 68% ethanol (NMX-F-700-COFOCALEC-2012). If no clots appear in the milk, it is considered negative and suitable for its pasteurization; however, if coagulation and solid-liquid phase separation occur, it means that the result is positive and consequently milk is rejected. Nowadays, producers and collectors face a serious problem: most of the milk is rejected, because it tests positive for alcohol (Rodríguez-Magadán *et al.*,

2019). Several studies have been performed in order to identify what causes milk instability. Barchiesi-Ferrari *et al.* (2007) and other authors suggest that there is a correlation between the positive herds and the lower concentration of dry matter and high crude fiber. In this regard, Chávez *et al.* (2004) reported that the lower casein concentration of unstable milk does not affect the total protein; meanwhile, Reid *et al.* (2015) found that increasing protein and phosphorus in the diet results in lower milk stability at a 6.8 pH. Therefore, metabolic changes in lactating dairy cows aimed at maintaining the acid-base status of the blood in balance play an important role in keeping the stability of milk in face of ethanol (Martins *et al.*, 2015), since milk from cows with impaired acid-base balance is less stable, although these alterations do not always cause unstable milk (Fagnani *et al.*, 2014).

With regard to the genotype and time of the year, García *et al.* (2009) reported that the milk from Holstein cows is more stable than Girolando's; likewise, milk is more stable in the rainy season than in the dry season.

Production and cow body condition substantially improve in the rainy season, because cows eat green grass with a large number of sprouts and therefore obtain a better nutritional quality. On the contrary, in the dry season, cattle consume dry forage with higher fiber content and low nutritional concentration, which could influence milk stability. The purpose of this research was to determine the stability of the milk produced by cows managed in the tropical dairy system with different feeding strategies.

MATERIAL AND METHODS

The study was performed in the community of San José Manialtepec, Villa de Tututepec, in the coast region of the state of Oaxaca, Mexico (15° 58' 21" N and 97° 14' 40" W), at an altitude of 20 masl. The area has an average annual temperature of 28 °C and a 1,300-mm rainfall (INEGI, 2018).

Description of the production system

The experimental study was established in a tropical dairy production system in which calves were separated from their mothers the third day after birth and they were provided milk substitute for two months. Cows were fed with Tanzania (*Panicum maximum*) and Insurgente (*Brachiaria brizantha*) grasses, collected by grazing. Two milkings were performed per day (06:00 a.m. and 04:00 p.m.).

Description of the animal population and sample characteristics

Before the study was established, 11 milk samples (6 in the morning and 5 in the afternoon) were taken from each of the n=60 milked cows that constituted the herd; the aim was to identify those cows whose milk frequently tested positive for alcohol. The test consisted of mixing 1 mL of milk with 1 mL of 68% alcohol (NMX-F-700-COFOCALEC-2012); the resulting mix was immediately homogenized, and a reading was taken. The alcohol test was considered positive if coagulation and solid-liquid phase separation of the milk were observed (Carrera *et al.*, 2011). Subsequently, n=10 cows were selected because their milk production tested positive more frequently for alcohol. The specimens chosen were crossbred cows (Holstein × Zebu) with an average live weight of 369.08±44.70 kg. They

had been lactating between 3 and 6 months and showed a 2.5 average body condition (scale: 1 to 5). The study was divided into two experiments.

Experiment 1

The cows were organized into two groups by a completely randomized design. Group 1 (T1) was made up of n=5 cows consuming dry and grinded Pangola grass (*Digitaria eriantha*), while cows from group 2 (T2) consumed green and chopped Maralfalfa grass (*Pennisetum violaceum*). Each of the cows was housed in a 15×10 m individual yard, equipped with feeders and drinkers. The study was divided into three periods of 10 days each. The bromatological composition of the forages used in the experiment is shown in Table 1. Forage was provided *ad libitum*, twice per day—once in the morning (8:00 a.m.) and once in the afternoon (4:00 p.m.)— and a feeder reading was carried out.

Experiment 2

After experiment 1, the same 10 cows were distributed in a completely randomized design; the effect of using a concentrate as supplement was tested in cows consuming green and dry forage. T1 cows were supplemented with a concentrate with a higher crude protein and metabolizable energy percentage than those of T2 (Table 2), in order to observe if a balance in the nutritional value between the diets has an influence in milk stability. The test lasted 21 days; the concentrate was gradually increased until 4 kg were reached. For

Table 1. Bromatological composition of the forage used in the study.

Nutrient	Dry Pangola Grass 150 days to the cut	Green Maralfalfa Grass 60 days to the cut
Dry Matter, %	96.42	25.09
Ash, %	9.61	15.09
Neutral Detergent Fiber, %	46.27	42.00
Acid Detergent Fiber, %	41.85	38.43
Crude Protein, %	7.52	9.64

Table 2. Proportion of ingredients and nutritional composition of the formulas elaborated to supplement cows fed with dry and green grass.

Ingredients	T1	T2
Ground Corn, %	58.80	58.85
Molasses, %	7.10	7.10
Urea, %	1.60	1.55
Grass, %	6.0	15.50
Perennial soybean, %	25.50	16.0
Mineral Salt, %	1.0	1.0
Composition		
Crude Protein (%)	21.95	18.01
Metabolizable Energy (Mcal kg ⁻¹)	3.06	2.94
FC (%)	3.77	6.43

that purpose, it was divided into four periods: period 1 (day 1-3, supplemented with 1 kg); period 2 (day 4-6, supplemented with 2 kg); period 3 (day 7-9, supplemented with 3 kg); and period 4 (day 10-21, supplemented with 4 kg). In both studies, a milk alcohol test was carried out twice per day (in the morning and in the afternoon).

Data was subjected to a categorical analysis under a completely randomized model. In experiment 1, the fixed effects were the type of forage provided as well as the period. Meanwhile, in experiment 2, the fixed effects were the type of forage and the level of concentrate provided at different times. Statistical differences between case proportions were determined by Fisher’s exact test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Experiment 1

Throughout the three periods in which the study was divided, it was observed that, in the morning (Figure 1) and afternoon (Figure 2) milking’s, cows that consumed dry grass had a higher ($p < 0.05$) percentage of cases tested positive for alcohol than cows that consumed green maralfalfa grass.

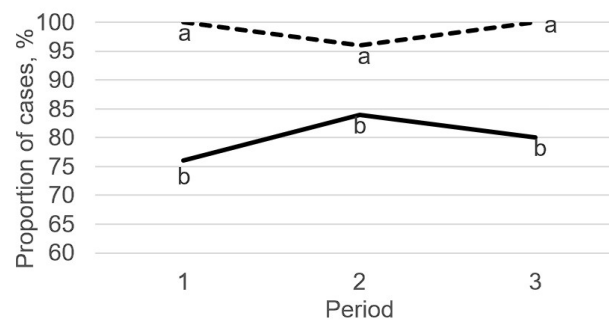


Figure 1. Percentage of cases tested positive for alcohol in milk from the morning milking, in dual-purpose cows fed with two types of forage. The dotted line ___ represents the cows that consumed dry forage (T1). The solid line ___ represents the cows that consumed green forage (T2).

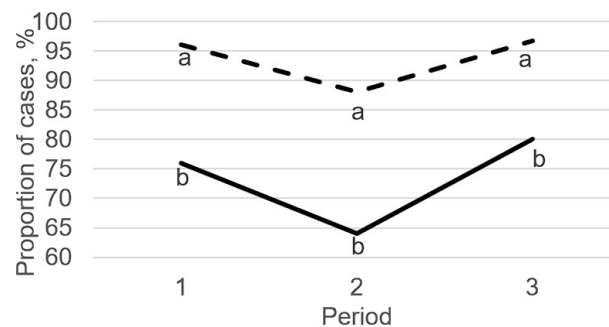


Figure 2. Percentage of cases tested positive for alcohol in milk from afternoon milking in dual-purpose cows fed with two types of forage. The dotted line ___ represents the cows that consumed dry forage (T1). The solid line ___ represents the cows that consumed green forage (T2).

Study 2

In the morning (Figure 3) and in the afternoon (Figure 4) milking's, the percentage of positive cases decreased as the concentrate amount provided increased, regardless of if the cows consumed dry or green grass. Up to a 3.0 kg consumption, cows that consumed dry grass ($p < 0.05$) had a higher percentage of cases that tested positive for alcohol; when both groups consumed 4.0 kg, the percentage of positive cases was similar ($p > 0.05$).

As a result of the assessment of the effect of body condition on the percentage of cases tested positive for alcohol, milk stability in studies 1 and 2 increases after the body condition reaches 3. However, when the body condition reaches 3.5, a significant decrease ($p < 0.05$) in cases tested positive for alcohol takes place. In both studies, a higher ($p < 0.05$) percentage of cases tested positive for alcohol in the milk obtained from the morning milking (Table 3).

García *et al.* (2009) and other authors showed that milk is more stable to the ethanol test in the rainy season than in the dry season. The results of this study contrast with the findings of Barchiesi-Ferrari *et al.* (2007) who reported that cases tested positive for alcohol decrease when the dry matter percentage of forage increases: green grass had less dry matter than dry grass and the positive cases percentage was lower in this study. The differences between this study and the study carried out by Barchiesi-Ferrari *et al.* (2007) comes from the fact that cows were grazing in an area where the lowest availability of

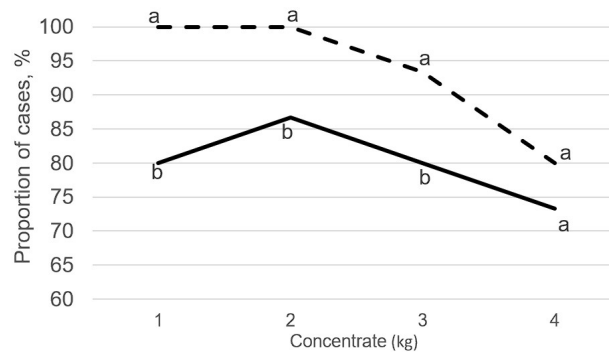


Figure 3. Percentage of cases tested positive for alcohol in the morning milking, in dual-purpose cows fed with two types of forage and supplemented with increasing levels of concentrate for 21 days. The dotted line _ _ _ represents the cows that consumed dry forage and concentrate (T1) and the solid line ___ represents the cows that consumed green forage and concentrate (T2).

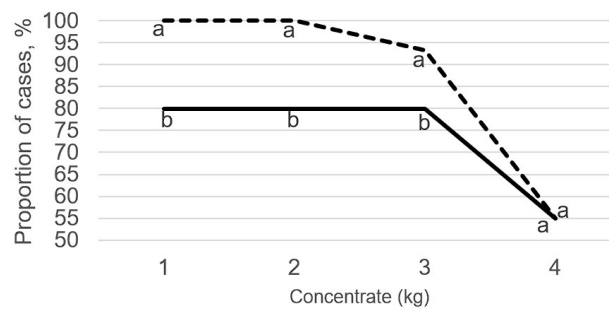


Figure 4. Percentage of cases tested positive for alcohol in the afternoon milking, in dual-purpose cows fed with two types of forage and supplemented with increasing levels of concentrate for 21 days. The dotted line _ _ _ represents the cows that consumed dry forage and concentrate (T1). The solid line ___ represents the cows that consumed green forage and concentrate (T2).

Table 3. Percentage of cases tested positive for alcohol in cow milk with different body conditions, at two milkings, in study 1 and 2.

Body Condition	Study 1	Study 2
2.25	91.67 ^a	91.27 ^a
2.5	95.87 ^a	88.10 ^a
3.0	94.92 ^a	85.71 ^a
3.5	78.75 ^b	55.95 ^b
Milking Hours		
Morning	91.33 ^a	82.38 ^a
Afternoon	84.00 ^b	69.52 ^b

In the studies, different letters ^a and ^b between rows are statistically different ($p \leq 0.05$).

matter occurs in the dry season; therefore, the highest percentage of cases tested positive is due to lower food consumption. In this study, cows were housed individually and had 24 hours access to forage; consequently, cows were fed green forage, resulting in a lower percentage of positive cases.

In this regard, Marques *et al.* (2011) report that changes in diet modify the concentration of those milk constituents that are related to its stability; consequently, when cows are subjected to a restricted feeding, the stability of milk to the ethanol test decreases (Barbosa *et al.*, 2012). Milk instability increases along with the crude fiber percentage (Barchiesi-Ferrari *et al.*, 2007). Therefore, the higher percentage of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in dry grass could have influenced milk stability. Harper and McNeill (2015) reported that higher NDF and ADF modify digestibility (Riaz *et al.*, 2014) and intake.

Interestingly, as more concentrate is added to the feed, milk stability increases. Likewise, the milk of cows that consume green forage is more stable, even when up to 3.0 kg of supplement are provided; when the concentrate supplementation increases to 4.0 kg, the milk stability of both groups behaves similarly. This behavior can be explained as follows: the concentrate consumed by the cows fed with green forage had a lower protein and energy proportion than the concentrate consumed by cows fed with dry grass. Consequently, at that concentrate level, the consumed nutrients amount was balanced.

By increasing temperature and heat treatment time, the instability of milk casein increases (Yang *et al.*, 2014). Chávez *et al.* (2004) determined that milk samples that remained stable when subject to alcohol tests have a higher casein amount without changing the total protein; meanwhile chlorine, sodium, and potassium concentrations are lower than in stable milk samples. As a result of the downwards trend in cases tested positive for alcohol as the concentrate addition increases, carrying out a physiological analysis about the maximum inclusion level in which the maximum milk stability is achieved would be an interesting subject to pursue.

CONCLUSIONS

The reduction of cases tested positive for alcohol is related to the improvement in forage quality. Increasing the amount of concentrate added to the cow diet provides a greater

stability to alcohol test; therefore, diets based on forages with a low nutritional value require a greater concentrate amount. Further studies about the feeding of grazing cows and its effects on the stability to the alcohol test are required.

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