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Effect of Silvopastoral Systems on the Correction of the Abnormal Milk Syndrome in Ecuador

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ABSTRACT

Background: Silvopastoral systems (SPS) improve the quality of cow's milk. **Aim.** To evaluate the effect of SPS on milk compositional quality and the correction of abnormal milk syndrome (AMS) under the production conditions of the Andean region of Ecuador. **Methods:** Agroecosystems (AES) 1 and 2 contained pastures and trees; Alnus and Acacia, respectively; AES 3 contained pastures only. A total of 35 milk mixes were taken from each AES, and milk composition was determined, along with a diagnosis for AMS. The effect of the year and AES was determined using a multifactorial ANOVA with interaction and the Bonferroni test to compare means by year and its interaction with AES, and the Duncan test according to AES. **Results:** In AES 1 and AES 2, AMS was not diagnosed, whereas in AES 3, AMS was diagnosed in 33.33% to 78.57% of samples. The percentage of fat, cryoscopic point, and milk density were higher ($p < 0.05$) in AES 1 and AES 2. Protein and non-fat solids were higher in the third year and in AES 1 and AES 2 with AES and year interaction. **Conclusion:** In AES 3, without trees, over the three years of study, more than 30% of milk samples were diagnosed with AMS, whereas in AES 1 and AES 2, AMS was not diagnosed.

Keywords: agroecosystems, milk, cow, fat percentage, density, cryoscopic point (Source: AGROVOC)

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INTRODUCTION

Abnormal Milk Syndrome (AMS) is a set of alterations in the physicochemical properties of milk, which cause disturbances in the processing of dairy products, their yields, and their final quality (Ponce, 2009; Romero, 2012).

AMS is associated with physiological, metabolic, and/or nutritional disorders, including poor body condition, metabolic acidosis, and low ruminal pH, which affect the mechanisms of milk synthesis and secretion at the mammary gland level (Hernández and Ponce, 2005; Ponce, 2009). These disorders were diagnosed in dairy herds with mineral, protein, and energy deficiencies; in Cuba (García-Díaz *et al.*, 2011) and in Ecuador (Balarezo *et al.*, 2020; 2021).

In the municipality of Pupiales, department of Nariño, Colombia, 48% of the 25 milk samples analyzed presented AMS criteria. The cows in these herds consumed 1 kg/cow/day of Itacol concentrate, water at will, 160 g of mineralized salt at 17%, but very little (Romero, 2012).

Another factor that favors AMS occurrence is heat stress, which causes a decrease in cell efficiency and blood flow to the udder, with low water availability for milk synthesis and reduced production and alteration of the total solids in milk (Ponce, 2009).

According to López *et al.* (2017), to reduce heat stress and simultaneously increase the forage supply in animal feed, farmers incorporate trees into their pastures and promote silvopastoral systems (SPS). This practice reduces metabolic acidosis and weight loss in animals; cows maintain milk production levels and milk quality (Sánchez *et al.*, 2018).

SPS have a positive impact because animal performance is better due to the higher and better quality of food, plant performance is high due to the availability of nutrients in the soil from better moisture retention and shade (Murgueitio *et al.*, 2014). There is also a greater amount of macro and microfauna in the soil, capable of degrading organic matter, mineralizing it and making it available for plant species that interact in the system (Silverman *et al.*, 2015).

Milk quality and composition depend on the origin or the way the animals' feed is managed. In SPS, milk is of higher quality, richer in essential fatty acids, vitamins, and beneficial antioxidants compared to supplemented and housed animals (Murgueitio *et al.*, 2016).

Despite the advantages of SPS, they are still not widespread in the Latin American region; mainly due to low knowledge of the technologies and lack of appropriate policies to stimulate the implementation of sustainable production systems in dairy farms (Chará *et al.*, 2017), as well as the lack of conclusive scientific information about the effect of SPS on milk compositional quality and AMS correction.

The purpose of this experiment was to evaluate the effect of SPS on milk compositional quality and AMS correction in the production conditions of the Andean region of Ecuador.

MATERIALS AND METHODS

Time-space frame

The experiment was conducted in dairy herds between 2020 and 2022 in the "El Carmelo" parish, Tulcán canton, Carchi province, Ecuador. It is located in the hydrographic area 230, from 0° 39' 33" N and 77° 36' 20" W to 0° 38' 55" N and 77° 36' 25" W, with an altitude ranging from 2916 to 3006 meters above sea level. (INAMHI, 2023).

The research scenario covers 54.4 hectares. The soil is of the Andisol order, with organic matter ranging from 10 to 25%, pH between 5.5 and 6.5, water retention between 20 and 100%, and effective depth between 20 and 70 cm. The relief has slopes ranging from 10 to 40%; soil organic carbon between 15 and 25%, cation exchange capacity between 15 and 25 meq 100 g⁻¹ of soil. Calcium predominates in the exchange complex, and nitrogen varies from medium to high values (Franco, 2016). The main limitation of soils in the Andean region of Ecuador is acidity (Calva and Espinosa, 2017).

Precipitation ranged between 892.50 and 1317.50 mm, and the average, maximum, and minimum temperatures during the study were 12.12, 16.49, and 8.06 °C, respectively (INAMHI, 2023).

Characteristics of Agroecosystems (AES)

Three AES were implemented in a completely randomized experimental design. The predominant grasses in the three AES were: Kikuyo (*Pennisetum clandestinum* L.), Ryegrass (*Lolium perenne* L.), Holcus (*Holcus lanatus* L.), and White Clover (*Trifolium repens* L.). AES 1 and 2 contained grasses and trees; *Alnus acuminata* H.B.K (Alder) in AES 1, and *Acacia melanoxylon* R.Br. (Acacia) in AES 2.

AES 3 contained only grasses and was considered the control. The trees in AES 1 and AES 2 were established in December 2019, with a planting density of 1000 trees ha⁻¹, in double rows, with a spacing of two meters, following the contour lines. To protect the young trees from damage caused by the cows, double electric fences were established, which also served to divide the paddocks.

Restricted time grazing was used for 18 hours a day. No protein-energy concentrate was administered, but mineral salts were supplemented orally *ad libitum*. The cows were milked mechanically twice a day, from 5:00 – 7:00 am and 3:00 – 5:00 pm. Artificial rearing of the calf was employed starting from the third day of birth.

Experimental design

Between 9 and 12 cows were assigned randomly per AES, from a total of 29 and 34 animals. The animals met the following criteria: Holstein cows aged between three and four years, with a body condition score at calving (BCC) between 3.0 and 4.0 points on a five-point scale, were in the third and fourth lactation with an average milk production of 10 ± 2 L cow⁻¹ day⁻¹, clinically healthy, and not receiving medical treatment.

Measurements

Health Status

The general health status was determined at the beginning and monitored throughout the study, using the functional invariants of the clinical method (Cuesta *et al.*, 2007). The animals were dewormed and vaccinated according to the area's schedule.

Body condition

Body condition (BC) was estimated through physical examination of the animals, which included inspection and palpation, classifying it on a scale of 1-5 points with divisions of 0.25 between them according to the methodology proposed by French *et al.* (2020). Because there were few animals in the AES studied, BC was determined only at the time of calving (BCC).

Determination of the Physicochemical Parameters of Milk

A total of 105 composite milk samples were taken, 35 from each AES (9 in the first year, 14 in the second, and 12 in the third). They were obtained from the refrigerated bulk tanks of each AES. The composition in fat, crude protein, non-fat solids, freezing point, and density was determined using the EKOMILK BOND Ultrasonic Milk Analyzers (BULTH 2000, Bulgaria), following the manufacturer's procedures. This equipment has an accuracy of $\pm 0.1\%$, $\pm 0.2\%$, $\pm 0.2\%$, ± 0.015 °C, and ± 0.0005 g/cm for fat, non-fat solids (NFS), protein, freezing point, and density, respectively.

Diagnosis of AMS

AMS was diagnosed according to the classification criteria of cow's milk established by Ponce (2009).

Statistical processing

Descriptive statistics (DS) were calculated for all variables in the milk composition. The effect of the year and AES on the milk components was determined using a multifactorial analysis of variance (ANOVA) with interaction. The means of the components according to the year and its interaction with AES were compared using the Bonferroni test (1936), and according to AES using the Duncan test (1955).

Statistical processing was done using the Statgraphics Centurion version XV. II (Statistical Graphic Corp., USA, 2006) package.

RESULTS AND DISCUSSION

Milk Composition

Table 1 composition in the AES. According to the classification criteria of cow's milk established by Ponce (2009) under production conditions in Cuba. However, these are common for dairy farming and the dairy sector in other scenarios as they are based on milk quality criteria and herd management and sanitary aspects (Hernández and Ponce, 2005).

In AES 3, in the first year, the percentage of samples meeting AMS criteria based on fat and density were 44.44 and 33.33%; in the second year, 78.57 and 42.86%, and in the third year 75 and 33.33%, respectively. The NFS compatible with AMS were 21.43% and 33.33% in the third year. In AES 1 and AES 2, no milk samples compatible with AMS were detected.

Table 1. Milk composition ($\bar{x}\pm SD$) and percentage of samples meeting AMS criteria in the three AES and years studied

Indicators	LCS	AES 1 (n=9)		AES 2(n=9)		AES 3(n=9)	
		$\bar{x}\pm SD$	C AMS,	$\bar{x}\pm SD$	C AMS,	$\bar{x}\pm SD$	C AMS,
First year							
Fat (g%)	3.53	3.85 - 0.19	0.00	4.04 \pm 0.07	0.00	3.52 \pm 0.14	44.44
CP (g%)	2.90	3.23 \pm	0.00	3.27 \pm 0.04	0.00	3.28 \pm 0.06	0.00
NFS (g%)	8.15	8.45 \pm	0.00	8.57 \pm 0.17	0.00	8.66 \pm 0.16	0.00
Freezing Point (m)	540	571.19 \pm	0.00	573.22 \pm	0.00	569.39 \pm	0.00
Density (g cm ³⁻¹)	1.028	1.030 \pm	0.00	1.029 \pm	11.11	1.028 \pm	33.33
Second year							
		AES 1 (n=14)		AES 2 (n=14)		AES 3 (n=14)	
Fat (g%)	3.53	4.06 \pm	0.00	4.11 \pm 0.20	0.00	3.49 \pm 0.15	78.57
CP (g%)	2.90	3.29 \pm	0.00	3.27 \pm 0.02	0.00	3.19 \pm 0.01	0.00
NFS (g%)	8.15	8.65 \pm	0.00	8.58 \pm 0.07	0.00	8.36 \pm 0.18	21.43
Freezing Point (m)	540	579.14 \pm	0.00	574.82 \pm	0.00	570.39 \pm	0.00
Density (g cm ³⁻¹)	1.028	1.030 \pm	0.00	1.030 \pm	0.00	1.028 \pm	42.86
Third year							
		AES 1 (n=12)		AES 2 (n=12)		AES 3 (n=12)	
Fat (g%)	3.53	4.04 \pm	0.00	4.01 \pm 0.15	0.00	3.50 \pm 0.07	75.00
CP (g%)	2.90	3.36 \pm	0.00	3.31 \pm 0.03	0.00	3.22 \pm 0.03	0.00
NFS (g%)	8.15	8.88 \pm	0.00	8.68 \pm 0.10	0.00	8.11 \pm 0.34	33.33
Freezing Point (m)	540	576.86 \pm	0.00	576.47 \pm	0.00	565.37 \pm	0.00
Density (g cm ³⁻¹)	1.028	1.031 \pm	0.00	1.030 \pm	0.00	1.028 \pm	33.33

Legend: LCS: Limit for AMS criteria (Ponce, 2009). C AMS, % Percentage of samples meeting AMS criteria. CP: Crude protein NFS: Non-fat solids. AES: Agroecosystem.

Although AMS is established by the occurrence of alterations in most milk components and not because one or only some of them are altered (Hernández and Ponce, 2005; Ponce, 2009), the results of this investigation indicate that there is AMS presence in AES 3, where there are objective conditions that can provoke it.

In AES 3, the average BCC of the cows was below three, which limits the energy available for mammary epithelial tissue and affects the synthesis and secretion of milk components, primarily casein, lactose, and the main implicated macrominerals, mainly phosphorus and magnesium (Ponce, 2009).

Additionally, the nutrient balances showed that in AES 3, there is a negative energy balance and a higher CP excess, which increases blood urea nitrogen (BUN) (Balarezo *et al.*, 2020; 2021). This leads to a rise in milk urea nitrogen (MUN) and increases AMS cases (Ponce, 2009).

Correction of AMS with SPS

Table 2 shows the comparison of means for the percentage of fat, freezing point, and milk density according to AES and year. Note that the three indicators were higher ($p < 0.05$) in AES 1 and AES 2 compared to AES 3. No differences ($p < 0.05$) in quality indicators were observed concerning the study year.

Table 2. Comparison of means of fat, freezing point, and milk density according to AES and years

	n	Fat (g%)	Freezing point (m °C)	Density (g cm ³)
AES				
		\bar{X}	\bar{X}	\bar{X}
AES 1	35	3.99 ^a	575.73 ^a	1.03 ^a
AES 2	35	4.05 ^a	574.83 ^a	1.029 ^a
AES 3	35	3.50 ^b	568.38 ^b	1.028 ^b
	\pm SE	0.02	0.19	0.00013
Year				
		$\bar{X} \pm SE$	$\bar{X} \pm SE$	$\bar{X} \pm SE$
1	27	3.80 \pm 0.03 ^a	571.27 \pm 1.34 ^a	1.02 \pm 0.00 ^a
2	42	3.89 \pm 0.02 ^a	574.78 \pm 1.07 ^a	1.02 \pm 0.00 ^a
3	36	3.85 \pm 0.02 ^a	572.90 \pm 1.16 ^a	1.02 \pm 0.00 ^a

^{ab}Different letters in the superscripts of each variable in the same column within each source of variation indicate significant differences * $p < 0.05$; Duncan, (1955). AES: Agroecosystem.

Protein and NFS (

Table 3) were higher in the third year and in AES 1 and AES 2 with AES and year interaction,

showing that the parameters were higher in these AES in the second and third years when AES 1 and AES 2 were more consolidated.

Table 3. Comparison of protein and non-fat solids ($\bar{X}\pm\text{SE}$) of milk mixtures from the three AES and study years

AES	n	Protein (g%)	NFS (g%)	
		$\bar{X}\pm\text{SE}$	$\bar{X}\pm\text{SE}$	
AES				
AES 1	35	3.30±0.006 ^a	8.66±0.034 ^a	
AES 2	35	3.29±0.006 ^a	8.61±0.034 ^a	
AES 3	35	3.23±0.006 ^b	8.38±0.034 ^b	
Year				
1	27	3.26±0.007 ^b	8.56±0.038 ^a	
2	42	3.25±0.005 ^b	8.53±0.030 ^a	
3	36	3.30±0.006 ^a	8.56±0.033 ^a	
Year-AES interaction				
AES	Year	n	$\bar{X}\pm\text{SE}$	$\bar{X}\pm\text{SE}$
1	1	9	3.23±0.012 ^{cde}	8.45±0.066 ^{bc}
	2	14	3.29±0.010 ^b	8.65±0.053 ^{ab}
	3	12	3.36±0.011 ^a	8.88±0.057 ^a
2	1	9	3.27±0.012 ^{bcd}	8.57±0.066 ^{bc}
	2	14	3.27±0.010 ^{bcd}	8.58±0.053 ^{bc}
	3	12	3.31±0.011 ^b	8.68±0.057 ^{ab}
3	1	9	3.28±0.012 ^{bc}	8.66±0.066 ^{ab}
	2	14	3.19±0.010 ^e	8.36±0.053 ^{cd}
	3	12	3.22±0.011 ^{de}	8.11±0.057 ^d

^{ab}Different letters in the superscripts of each variable in the same column within each source of variation indicate significant differences * $p<0.05$; (Bonferroni, (1936) for year and year-AES interaction, and Duncan, (1955) for AES). AES: Agroecosystem.

AMS can be corrected with formulations of additives capable of stabilizing and activating ruminal function and general metabolism; additionally, with the inclusion of adequate fiber levels in the diet (Ponce, 2009).

Accordingly, the implementation of SPS in AES 1 and AES 2 allows cows to consume fibrous foods that prevent ruminal and metabolic acidosis; thus, they constitute a proposal to partially solve AMS and a scientific contribution of this research for these production conditions, opening a new field of study for future research.

The results of this work indicate that milk production with cows in SPS with Acacia and Alder modified the percentages of fat and NFS, and milk density, corroborating what was published by

other authors who found a positive effect of multi-associated systems of trees and grasses on milk compositional quality (Hernández and Ponce, 2005; Ponce, 2009).

However, they differ from what was published by Jordan *et al.* (1995), who supplied *Leucaena leucocephala* with consumption levels exceeding 50% of the ration and observed no modifications in the percentage of fat, crude protein, and total solids in the milk. The difference may be motivated by the different tree species, the percentage of consumption established and fixed in the consulted work; additionally, in this research, the animals were fed at will.

Fat, protein, and NSF milk values in AES 2 are higher than those published in Holstein cows in Nariño, Colombia, fed with a ration containing 28.8% Acacia meal; the values of these indicators were 2.83 g%, 2.75 g%, and 7.75 g%, respectively (Jaramillo and Jiménez, 2000). The lack of correspondence may be motivated because in the consulted work the animals consumed flour with very fine particles, which can decrease propionate production, leading to decreased lactic acid and glucose levels. This stimulates insulin production, which reduces the release of fatty acids and consequently, reduces the fat percentage in milk (Suárez, 2020).

The lower fat milk percentage in AES 3 may be motivated by the cows consuming less fiber, resulting in shorter rumination time and saliva production, which decreases rumen pH and volatile fatty acids production, affecting the milk fat percentage. The decrease in rumen pH also reduces crude protein in the diet (Ponce, 2009).

Additionally, SPS reduces cows' body temperature by about 3 to 5 °C, so heat stress and its effects can be mitigated with multi-associated systems of trees and grasses (Murgueitio *et al.*, 2016). However, since this research was conducted in a cold climate, heat stress should not have caused differences in fat, protein, non-fat solids, freezing point, and density content (Navas, 2010; Murgueitio *et al.*, 2014).

The differences in dairy components can be explained by dietary changes that originate with SPS, considering that the most important factor influencing the compositional quality of milk is nutrition (Bajramaj *et al.*, 2017; Suárez, 2020). AES 1 and AES 2 improve the quality and nutrient supply in the diet, providing more precursors for milk fatty acids, protein, and total solids compared to AES 3.

Moreover, in SPS, animals can better utilize forage mixtures and increase their intake, thus obtaining a greater quantity of higher-quality nutrients (Murgueitio *et al.*, 2016). In some studies with dairy cows consuming SPS pastures, changes were found in the nutrient content of milk, particularly fat, protein, non-fat solids, and total solids (Hernández and Ponce 2004; Urbano *et al.*, 2006).

The results of this research are consistent with those obtained in the Colombian Amazon foothills with intensive SPS with *Tithonia diversifolia*, which increased ($p < 0.05$) milk production, NFS, and total solids per animal per day (Rivera *et al.*, 2015).

Higher crude milk protein levels in AES 1 may be caused by an excess of CP and energy deficit in the herd diet, consequently increasing BUN and MUN in cows. In diets with these characteristics, the capacity of rumen microorganisms to use NH_3 that reaches the liver via the bloodstream is saturated, where this organ converts it into urea, so NH_3 excesses lead to BUN and MUN rises (Correa and Cuéllar, 2004; Butler, 2013).

Although milk protein content is not very sensitive to variations in food quality within certain ranges, 76% of the nitrogen in milk is found in its proteins, mainly caseins, 18% in whey proteins, and 6% in non-protein nitrogen (Hernández and Ponce, 2004). Therefore, any MUN rise leads to increases in milk proteins; however, in this research, the opposite effect occurs, as AES 3 has lower protein content in the milk, an aspect that should be studied in depth.

The NFS levels (Table 3) are higher ($p < 0.05$) in AES 1 and AES 2 compared to AES 3. A similar situation was demonstrated in Holstein, Siboney de Cuba, and Holstein x Zebu crossbred cows under Cuban production conditions, where NFS levels were higher in herds maintained in SPS with *Leucaena leucocephala* compared to those fed on grasses, mainly star grass (Ponce, 2009).

AMS can be corrected with the inclusion of adequate fiber levels in the diet (Ponce, 2009). In this regard, the implementation of SPS in AES 1 and AES 2 allowed cows to consume fibrous foods that prevent ruminal and metabolic acidosis and increase BCC, thus constituting a proposal to partially solve AMS and a scientific contribution of this research for these production conditions, opening a new field of study for future research.

CONCLUSION

SPS including Acacia and Alnus trees, in the conditions of the Andean region of Ecuador, improved milk composition quality, while none of the milk samples were compatible with AMS; whereas in agroecosystems without trees, more than 40% of them met AMS criteria.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: HRBR, JRGD, ENA; data analysis and interpretation: JRGD, HRBR, ENA; redaction of the manuscript: JRGD, HRBR, ENA.

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.