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Research article

Incidence and effects of subacute ruminal acidosis and subclinical ketosis with respect to postpartum anestrus in grazing dairy cows

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ABSTRACT

Subclinical Ruminal Acidosis (SARA) and Subclinical Ketosis (SCK) are two of the most prevalent metabolic diseases of dairy cows, with impacts on reproductive performance. There is scarce literature about these diseases in dairy regions in Colombia. In 29 randomly selected herds in Pasto, Colombia, 249 dairy cows were followed weekly for two months postpartum to determine: 1) incidence risk of SARA and SCK; and 2) effects of SARA and SCK on the occurrence of postpartum anestrus (PA) at two months. Samples from ruminal liquor and blood were obtained one time per cow during the first week postpartum to determine presence of SARA (pH < 5.6) and SCK (1.0–2.9 mmol/L of blood Beta-Hydroxy-Butyrate), respectively. PA diagnosis was determined with ultrasound. Pregnancy risks at 30 and 60 days post-breeding (and assumed embryo losses between these days) were determined. Risk factors associated with PA were estimated through a mixed multi-level multivariable logistic regression model, adjusting for clustering of cows within herds. The incidence risks of SARA and SCK were 23.3% and 46.2%, respectively. Simultaneous occurrence of SCK and SARA (SCASCK) was present in 5.2% of the cows. In the final multivariable model, the occurrence of SARA (Odds Ratio: OR = 39.4), SCK (OR = 47.4) and SCASCK (OR = 68.5) was associated with increased odds of PA. Feeding a transition period diet was associated with reduced odds of PA (OR = 0.15). Second parity cows had significantly lower odds of PA than first parity cows (OR = 0.21). In conclusion, inadequate pre-partum and postpartum nutritional management of the herds increased the occurrence of SARA and SCK, which had adverse effects on reproductive performance.

1. Introduction

The energy requirements associated with insufficient food intake after calving is a key factor in the reproductive efficiency of the dairy cow (Raboisson et al., 2014). During the early post-partum period, metabolic and hormonal changes produce immunomodulation alterations and predisposition to disease (metabolic, uterine, infectious, etc.). These metabolic conditions can affect the ovarian function and the development of the embryo in the uterine lumen, causing infertility through prolonged calving-to-conception intervals, and long periods of lactation with subsequent economic losses (Daros et al., 2017; Rutherford et al., 2016). Within the metabolic diseases, SARA and SCK contribute to reduced reproductive performance, due to strong relationships with the energy status of the animals. Both conditions can be sub-clinically present, making them more difficult to be diagnosed, treated, and controlled, with subsequent herd fertility problems (Chaidate et al., 2014; Shin et al., 2015).

Bovine ketosis is the result of an imbalance between energy demand and requirements, produced by increased fat mobilization and hepatic ketogenesis. Subclinical ketosis in dairy cattle frequently occurs between two and seven weeks post-partum as milk production typically rises (Esposito et al., 2014; Tatone et al., 2016). Most affected cows can

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recover with or without treatment but resulting losses in the productive potential of each affected animal can be substantial. If the disease remains undetected and untreated, there can be substantial adverse effects on milk productivity (Vanholder et al., 2015) and lower reproductive efficiency, along with an increased incidence of several other diseases (Raboisson et al., 2014; Youssef et al., 2017).

There is variability in the diagnosis, treatment, and prognosis of SARA (Oetzel, 2004; Plaizier et al., 2008). For example, the current definition and ruminal pH threshold for SARA diagnosis varies among studies (Abdela, 2016; Krause and Oetzel, 2006; Plaizier et al., 2008). However, SARA typically occurs when the cow presents low ruminal pH (5.2–6.0) on a daily basis, and for several hours per day (Abdela, 2016; Oetzel, 2004; Ural, 2017).

Currently, in many dairy herds world-wide, milk production can appear to be temporarily increased by overfeeding grain, due to dietary requirements of energy and fiber. These requirements are not easily met in high-producing dairy cows (Plaizier et al., 2008), especially for early lactation cows because their energy expenditure typically exceeds the energy consumed (Negative Energy Balance) and predisposes them to SCK (Esposito et al., 2014). Starchy and low-fiber diets can be offered to increase the intake of energy, to improve milk production, and to reduce subclinical ketosis occurrence. However, these low-fiber diets can increase the risk of SARA (Plaizier et al., 2008).

Tropical dairy herds over the last few decades have improved the productivity of dairy cows substantially, making these diseases an issue. Specialized dairy production in Colombia is conducted in high altitude lands ranging from 2400 to 3200 m where the predominant breed is Holstein and its crosses. Milk is harvested through manual and/or mechanical milking, directly in the barn or on the paddocks (Munera et al., 2018). However, as milk yield increases, meeting a cow's dietary nutrient needs becomes challenging under these management conditions. There is scarce literature about SARA and SCK in dairy regions in Colombia.

The objectives of the present study were to determine: 1) the incidence risk of SARA and SCK from 1-7 days in milk (DIM); and 2) the effects of SARA and SCK on postpartum anestrus (PA) at 60 days postpartum in dairy cows from Colombia. Our hypothesis was that SARA and SCK would be positively associated with PA.

2. Materials and methods

2.1. Study population and experimental design

The present study was carried out between March and August of 2015 (n = 249) in the region of Pasto, Colombia, which is one of the main areas for specialized dairy production in the country. The herd selection criteria were as follows: voluntary participation of the producers, management conditions representative of the geographical area, accessibility to the farm by vehicle, not having dual purpose breeds, and having cow identification practices and cow records. A random sample of 29 herds that met the criteria was obtained for the study.

The herds were located at an altitude ranging from 2,600 to 3,129 m, with temperatures between 3 to 16 °C. The feeding system in the area consisted of permanent rotational grazing of pastures. The period of rotation through these pastures varied from 25 to 45 days according to the pasture size and fertilization type (Astaíza Martínez et al., 2017; Munera et al., 2018). Energy supplementation for lactating cows occurred twice per day (Vallejo Timarán et al., 2017).

2.2. Cow sampling and information recording

In each selected herd, all eligible cows were entered into the study based on the following inclusion criteria: specialized dairy breeds (e.g. Holstein), seven days postpartum or less, normal calving, and clinically healthy. Herds were visited weekly to select fresh cows in the first week postpartum (1–7 days postpartum) and rumen and blood samples were taken once on this first cow contact and tested for pH and BHB. Briefly, samples of ruminal liquor were obtained via medial ruminocentesis and tested for their pH through a digital pH meter (C1457 Hecotrax™). Blood samples were also obtained via coccygeal venopuncture and tested for beta-hydroxybutyrate (BHB) through a portable device (Optium Xceed™ Blood Glucose and Ketone Monitoring System).

Information about grazing and type of supplementation of the cows within each herd was collected through a questionnaire. Topics in this questionnaire included: 1) grazing on natural pastures (Kikuyu grass - *Pennisetum clandestinum*), improved pastures (Rye grass - *Lolium hybridum*) or improved pastures with legumes (Giant Red clover - *Trifolium pratense*); 2) supplementation with dairy concentrate, corn silage and/or agricultural by-products (e.g. post-harvest residues of potatoes or carrots); and 3) transition period feeding (if cows receive the milking diet prior to calving). Additional information related with cow parity and the average daily milk yield during the first 60 days in milk was also obtained.

The physiological reproductive status was determined through transrectal ovarian ultrasound at approximately 20, 30, 40 and 60 days postpartum. Cows were classified as having estrus activity or being anestrus (Peter et al., 2009). Pregnancy diagnosis was performed in cows at approximately 30 and 60 days post-breeding, with embryo loss being defined as pregnant at 30 days but not pregnant at 60 days.

2.3. Data management and analysis

The statistical analysis included: a) herd variables; average milk production (≥22 L/day or ≤21 L/day), average parity, transition feeding and type of diet (grazing, supplementation); b) disease variables; SARA and/or SCK during the first week postpartum, and PA at 60 days; and c) fertility variables; first ovulation day (20, 30, 40), pregnancy risks (30 and 60 days post-breeding), and embryo loss.

Subacute ruminal acidosis (SARA) was defined as a cow without clinical signs of indigestion or ruminal acidosis, with ruminal liquor pH < 5.6, considering a normal pH reference value ranges between 5.6 – 7.0 (Abdela, 2016; Plaizier et al., 2008). Subclinical ketosis (SCK) was defined as a cow without clinical signs of ketosis, with blood BHB levels between 1.0 and 2.9 mmol/L, considering a normal blood BHB reference value is <1.0 mmol/L (Oetzel, 2004). A cow with postpartum anestrus (PA) was defined as not having a corpus luteum and no follicles >8 mm throughout the 60 days postpartum.

Descriptive statistical analyses included proportions of cows with first ovulation at 20, 30, 40 and 60 days postpartum, pregnancy at 30 and 60 days post-breeding, and embryo loss. Incidence risks for the relevant postpartum diseases (SARA, SCK, and PA) were calculated as the number of cows with the condition divided by the number of cows entered in the study. We are assuming that the cows did not have any of these conditions while they were dry when energy requirements are low and grain feeding is limited, allowing the use of incidence risk.

To determine the effect of SARA and SCK on the occurrence of PA, model-building strategies included an initial bivariate simple logistic regression analysis ($P < 0.15$) between each independent variable (herd and disease variables) and PA as the dependent variable. Variables that met the P-value criterion were eligible for entry into a two-level multivariable mixed model logistic regression (MMLR) using backward selection. Herds were included as the random part of the model to account for clustering of cows (the lowest level of hierarchical data) within herds. Milk production and parity were considered as potential confounding variables. Due to the potential interaction between SARA and SCK, a new variable (SCASCK) was created for the logistic model. The SCASCK variable was grouped into four levels as follows: 1 = cows without SARA and SCK; 2 = cows with only SCK; 3 = cows with only SARA; and 4 = cows with both SARA and SCK. Model goodness-of-fit was determined by Hosmer and Lemeshow's test for the simple logistic model, and by the pseudo R^2 for the final multilevel logistic model. The proportion of variance explained by herd was calculated using the latent response approach (Herd Variance/(Herd Variance + $\pi^2/3$).

Although not a primary objective of the study, additional MMLR models (controlling for confounding by variables in the final model) were also built using initial bivariate simple logistic regression analyses ($P < 0.15$) between each independent variable (herd and disease variables) and pregnancy risks at 30 days post-breeding, pregnancy risks at 60 days post-breeding, and embryo loss risk between these pregnancy checks as dependent variables.

2.4. Ethics statement

This research was performed in compliance with the experimental practices and standards approved by the ethics committee of the Research and Postgraduate Studies Department of the University of Nariño, Colombia (Approval ID: CED009) and all efforts were made to minimize animal suffering.

3. Results

3.1. Descriptive statistics

There were 249 cows in 29 herds enrolled in the study. Table 1 shows information about variables included in the statistical analysis. In general, the study cows were in 3 + lactation producing at least 22 kg of milk per day. The majority of cows consumed fresh Kikuyu grass pasture, in a rotational system, which is available throughout the day, and this system was supplemented with carbohydrates (commercial dairy concentrate preparations, corn silage and/or agricultural byproducts) during milking twice a day (in the morning and the afternoon).

There were eight different types of diets present across the herds, based on the combinations of the three types of pastures (Kikuyu, Rye grass, Rye grass + legumes) and three types of supplements (dairy concentrate, corn silage, and/or agricultural by-products). The combinations of these diets included: I (Kikuyu grass only); II (Kikuyu grass + agricultural by-products); III (Kikuyu grass + dairy concentrate); IV (Kikuyu grass + dairy concentrate + agricultural by-products); V (Rye Grass + legume + agricultural by-products); VI (Rye Grass + legume + dairy concentrate + corn silage); VII (Rye Grass + dairy concentrate); and VIII (Rye Grass + dairy concentrate + agricultural by-products + corn silage). The frequency of these "Diet" combinations can be found in Figure 1.

The incidence risks for SCK and SARA for 1–7 days in milk were 46.2% and 23.3%, respectively. The simultaneous occurrence of SCK and SARA (SCASCK) was found in 5.2% of the animals. The incidence risk of PA at 60 days in milk was 42.4%.

Each of the combinations of diets had different SCK and SARA incidence risks, as observed in Figure 1. Cows that were grazing in Kikuyu grass only, or Kikuyu grass with agricultural by-products or dairy concentrate supplementation had the highest incidence of SCK (>40%), with SARA incidence <10%. Conversely, the incidence of SARA was highest (>40%) when cows grazed in Rye grass or Rye grass + legume, when receiving supplementation with agricultural by-products (>70%) or some other supplementation (>40%). SCASCK incidence risk among the study cows was highest (11–24%) in herds grazing in Rye grass and supplemented with corn silage.

Cows with SARA had first ovulation risks of 6, 14, 31% and 36% by 20, 30, 40 and 60 days, respectively, which were lower percentages compared with cows without the disease (Table 2). Similarly, lower percentages of first ovulation risk at 20, 30, 40 and 60 days occurred in cows with SCK than without SCK (Table 2). The incidence of postpartum anestrus in the study was 64% and 66% for cows with SARA and SCK, respectively, which were significantly higher than the 22% and 36% for cows without the diseases, respectively (Table 2).

Table 1. Distribution of independent variables amongst 249 cows from 29 dairy herds in Colombia in 2015.

Variable	Levels	Frequency	%
Parity	1	35	14
	2	76	31
	3	51	20
	4	37	15
	>5	50	20
Transition diet fed	Yes	23	9
	No	226	91
Milk production average for 0–60 days	≥22 L.	139	56
	≤21 L.	110	44
Pasture type	Rye Grass	60	24
	Rye Grass + Legumes	33	13
	Kikuyu Grass	156	63
Supplementation feeds	Dairy Concentrate	159	64
	Agricultural By-Products	103	41
	Corn Silage	42	17
Metabolic Variables	SARA + for 1–7 days postpartum	58	23
	SARA - for 1–7 days postpartum	191	77
	SCK + for 1–7 days postpartum	115	46
	SCK - for 1–7 days postpartum	134	54
Reproductive Variables	Anestrus - at 60 days postpartum	106	57
	Anestrus + at 60 days postpartum	143	43
	Pregnancy + at 30 days post-breeding	105	42
	Pregnancy - at 30 days post-breeding	144	58
	Embryo loss – between 30-60 days post-breeding	195	78
	Embryo loss + between 30-60 days post-breeding	54	22
	Pregnancy + at 60 days post-breeding	91	37
	Pregnancy– at 60 days post-breeding	158	63

SARA = Subacute ruminal acidosis.
SCK = Subclinical ketosis.

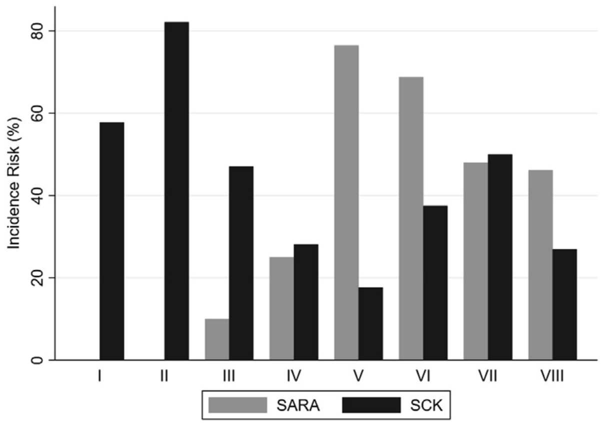

Figure 1. Distribution of the cow-level incidence risk of SCK and SARA, by diet characteristics in 249 cows from 29 dairy herds in Colombia in 2015. I= Kikuyu grass only (*n* = 45 cows in 5 herds). II = Kikuyu grass + agricultural byproducts (*n* = 28 cows in 2 herds). III = Kikuyu grass + dairy concentrate (*n* = 51 cows in 6 herds). IV = Kikuyu grass + dairy concentrate + agricultural by-products (*n* = 32 cows in 5 herds). V = Rye Grass + legume + agricultural by-products (*n* = 17 cows in 3 herds). VI = Rye Grass + legume + dairy concentrate + corn silage (*n* = 16 cows in 1 herd). VII = Rye Grass + dairy concentrate (*n* = 34 cows in 4 herds). VIII = Rye Grass + dairy concentrate + agricultural by-products + corn silage (*n* = 26 cows in 3 herds).

The pregnancy risks on days 30 and 60 post-breeding were also lower in cows with metabolic disorders (Table 3). Only 24% of cows with SARA were pregnant at 60 days post-breeding with an embryo loss of 14% between 30 and 60 days post-breeding, compared with 40% of cows being pregnant at 60 days post-breeding, with an embryo loss of only 3% in cows without SARA. Embryo loss was 7% in SCK cows, with only 23% of cows pregnant to 60 days post-breeding.

3.2. Bivariate statistical analysis on factors of postpartum anestrus occurrence

Results of the bivariate logistic regression analyses between the independent variables and the outcome anestrus are shown in Table 4. Parity, average daily milk production, diet, transition period, SARA, and SCK were statistically significantly associated with anestrus ($P < 0.15$). To build the multivariable model, a directed acyclic graph (DAG) was sketched (Figure 2) based on the results of the bivariate analyses to identify the explanatory variables and potential confounders possibly associated with the exposure of interest.

3.3. Multivariable logistic regression on factors of postpartum anestrus occurrence

The initial mixed multivariable logistic regression model included the following variables: transition feeding, parity, average milk production SARA and SCK. Through a backward elimination process, the final model included transition feeding, parity, SARA and SCK. Due to an interaction between SARA and SCK, a new variable (SCASCK) was created for the logistic model; variable SCASCK was grouped as follows: 1 = Cows without SARA or SCK; 2 = Cows with only SCK; 3 = Cows with only SARA; and 4 = Cows with both SARA and SCK. For the cow-level mixed multivariable logistic regression model, milk production and parity were considered as potential confounding variables for the outcome of PA and therefore were explored for confounding in the model.

In the final model (Table 5), the occurrence of SARA (Odds Ratio: OR = 47.4), SCK (OR = 39.4) or concomitance of both diseases (OR 68.5) increased the odds of PA occurrence. Second parity cows had significantly lower odds of PA than first parity cows (OR = 0.21). In the herds, transition period feeding was associated with lower odds of PA (OR = 0.15). The Hosmer and Lemeshow's test was 0.10 and the pseudo R^2 of this final model was 0.42. However, the proportion of the variance explained by the herd effect was only 6.4% (0.227/3.517).

3.4. Multivariable logistic regression on factors of pregnancy risks and embryo loss

Bivariate statistical analysis for pregnancy risk at 30 days post-breeding (DPB) as the dependent variable, found associations ($P < 0.15$) with transition diet, type of diet, and SCK. The MLRM found that the occurrence of SARA (OR = 0.27) reduce the odds of pregnancy at 30 DPB whereas transition diet (OR 7.34), Type-IV diet (OR = 11.3) and Type-V diet (OR = 23.3) were associated with increased odds of

Table 2. First ovulation (and anestrus) risks in 249 cows in 29 herds in Colombia in 2015, by disease status.

First ovulation		Day 20		Day 30		Day 40		Day 60		Day 60 Anestrus	
		Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.
SARA	Yes	6%	3	14%	8	31%	18	36%	21	64%	37
	No	14%	27	24%	45	53%	102	64%	122	36%	69
SCK	Yes	5%	6	8%	9	23%	26	34%	39	66%	76
	No	18%	24	33%	44	70%	94	78%	104	22%	30

* Anestrus = Cows with no ovulation by day 60 postpartum.
SARA = Subacute ruminal acidosis.
SCK = Subclinical ketosis.

Table 3. Pregnancy risks in 249 cows in 29 herds in Colombia in 2015, by disease status.

Disease/Outcome		Pregnancy Risk (Day 30)	Embryo Loss Risk (between 30-60 days)	Pregnancy Risk (Day 60)
		Yes	Yes	Yes
SARA	Yes	38%	14%	24%
	No	43%	3%	40%
SCK	Yes	31%	7%	23%
	No	52%	6%	47%

SARA = Subacute ruminal acidosis.
SCK = Subclinical ketosis.

Table 4. Bivariate analysis between postpartum anestrus and predictor variables in 249 cows from 29 dairy herds in Colombia in 2015.

Variables		Odds Ratio	Std. Err.	*P*-value	95% Conf. Interval	
					LL	UL
Transition Feeding		0.336	0.184	0.047	0.114	0.985
Kikuyu Grass		0.570	0.233	0.169	0.255	1.270
Rye Grass		1.860	0.858	0.178	0.753	4.595
Rye Grass + Legumes		1.158	0.689	0.805	0.360	3.719
Dairy Concentrate		1.352	0.584	0.485	0.579	3.154
Agricultural By-Products		0.709	0.292	0.405	0.316	1.590
Corn Silage		1.713	0.908	0.310	0.605	4.846
Diet	I	baseline		0.149*		
	II	1.629	1.162	0.494	0.402	6.593
	III	1.173	0.710	0.792	0.357	3.845
	IV	0.682	0.472	0.582	0.175	2.652
	V	0.713	0.606	0.691	0.135	3.770
	VI	3.100	2.610	0.179	0.595	16.147
	VII	2.610	1.743	0.151	0.704	9.667
	VIII	1.562	1.122	0.534	0.382	6.385
Parity	1	baseline		0.001*		
	2	0.201	0.098	0.001	0.077	0.525
	3	1.340	0.613	0.522	0.546	3.288
	4	7.727	4.675	0.001	2.360	25.295
Dairy production average		2.030	0.615	0.020	1.120	3.679
SCASCK neither		baseline		<0.001*		
SARA		67.593	45.677	0.001	17.975	254.169
SCK		64.420	48.794	0.001	14.597	284.289
SARA + SCK		153.172	150.825	0.001	22.234	1055.209

LL = Lower Limit; UL = Upper Limit.
I = Kikuyu grass only.
II = Kikuyu grass + agricultural byproducts.
III = Kikuyu grass + dairy concentrate.
IV = Kikuyu grass + dairy concentrate + agricultural by-products.
V = Rye Grass + legume + agricultural by-products.
VI = Rye Grass + legume + dairy concentrate + corn silage.
VII = Rye Grass + dairy concentrate.
VIII = Rye Grass + dairy concentrate + agricultural by-products + corn silage.
SARA = Subacute ruminal acidosis.
SCK = Subclinical ketosis.
* Global p-value for entire categorical variable.

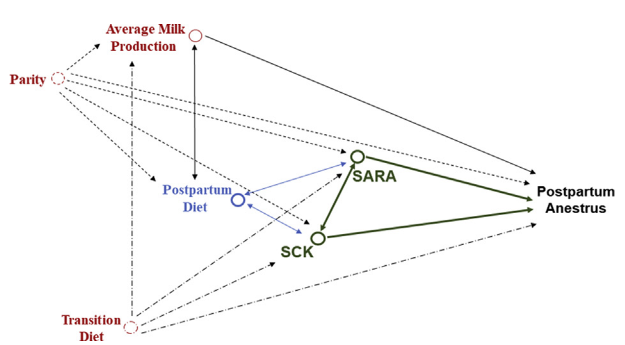

Figure 2. Directed acyclic graph (DAG) with the explanatory variables and potential confounders related with occurrence of SARA, SCK and postpartum Anestrus (PA) in 249 cows from 29 dairy herds in Colombia in 2015. Postpartum Anestrus = Outcome; Green arrow = Exposure; Blue arrow = Ancestor of exposure; Red color font words = Potential confounders; Dotted and black arrows = Adjusted variables.

pregnancy at 30 DPB (Table 6). Type-IV diet was Kikuyu Grass + dairy concentrate + agricultural by-products and Type-V diet was Rye Grass + legume + agricultural by-products. No variables were confounders for pregnancy risk at 30 DPB in the final model. The Hosmer and Lemeshow's test was 0.47 and the pseudo R^2 of this final model was 0.15. The proportion of the variance explained by the herd effect was only 4.8% (2.823/57.898).

For pregnancy risk at 60 days post-breeding, bivariate statistical analyses found associations ($P < 0.15$) with transition diet, type of diet, parity, SARA, SCK and SCASCK. The MLRM analysis showed that the occurrence of SARA (OR = 0.12) reduced the odds of pregnancy at 60 DPB whereas transition diet (OR = 6.93), Type-IV diet (OR = 8.31), and Type-V diet (OR = 39.8) were associated with increased odds of pregnancy (Table 6). There were no confounding variables for pregnancy risk at 60 DPB in the final model. The Hosmer and Lemeshow's test was 0.35 and the pseudo R^2 of this final model was 0.18. The proportion of the variance explained by the herd effect was 6.36% (3.45/54.28).

For embryo loss risk between 30 and 60 days post-breeding, bivariate statistical analysis found associations ($p < 0.15$) with pasture type, parity and SARA. MLRM found that after controlling for the confounding effects of pasture type, only SARA (OR = 4.78) was associated with higher odds of embryo loss in the final model (Table 7). No other variables were significant in the final model. The Hosmer and Lemeshow's test was 0.57 and the pseudo R^2 of this final model was 0.07. The proportion of the variance explained by the herd effect was only 3.93% (0.506/12.706).

Table 5. Final multivariable mixed logistic regression model of nutritional/cow characteristics and metabolic disorders associated with anestrus to 60 days in milk in 249 cows from 29 dairy herds in Colombia in 2015.

Pseudo $R^2 = 0.42$

Variables	Odds Ratio	Std. Err.	P	95% Conf Interval	
				LL	UL
Transition diet	0.155	0.101	0.004	0.043	0.560
Parity					
1	baseline		0.001*		
2	0.215	0.132	0.013	0.064	0.719
3	1.171	0.725	0.798	0.347	3.946
4	3.349	2.520	0.108	0.766	14.640
5	0.467	0.288	0.218	0.139	1.566
SARA-SCK					
No SCK or SARA	baseline		<0.001*		
SCK	47.475	32.113	<0.001	12.609	178.7
SARA	39.409	31.651	<0.001	8.165	190.2
SARA + SCK	68.501	69.919	<0.001	9.265	506.4
Intercept	0.065	0.047	<0.001	0.016	0.270
Herd Variance	**Estimate**	**Std. Err**		**LL**	**UL**
	0.227	0.345		0.012	4.446

LL = Lower Limit; UL = Upper Limit.
SARA = Subacute ruminal acidosis.
SCK = Subclinical ketosis.
SCASCK = Interaction variable of Subacute ruminal acidosis and Subclinical ketosis.
* Global p-value for entire categorical variable.

4. Discussion

In the present study, the incidence risk for SARA, defined as ruminal pH < 5.6 between 1 to 7 DIM was 23.3%, which was higher than the prevalence of 14% and 16% reported in Polish (pH ≤ 5.6; between 40 and 150 DIM) and Greek (pH ≤ 5.5; 10 to 90 DIM) high-yielding dairy cows, respectively (Kitkas et al., 2013; Stefańska et al., 2017). However, other studies have found similar or higher prevalence of SARA, such as 27.6% (pH ≤ 5.5; 3 to 20 DIM) and 26.3% (pH ≤ 5.5; 5 to 90 DIM) reported in the northeast of Iran (Tajik et al., 2009) and Egypt (Nasr et al., 2017), respectively, while the incidence reported in Canada (pH ≤ 5.5; 3 to 20 DIM) of 26% (Plaizier et al., 2008). According to Krause and Oetzel (2006) (Krause and Oetzel, 2006), the incidence of SARA increases as cows consume diets containing higher proportions of grain.

In the present study, the incidence risk of SCK (46.2%) was lower than the reported prevalence of 59–68% (BHB >1.2 mmol/L; 7 to 42 DIM) in an Iranian study (Asl et al., 2011) but similar to a study in Holland of 47.2% (BHB 1.2–2.9 mmol/L; 7 to 14 DIM) (Vanholder et al., 2015). Conversely, in grazing dairy production systems from Colombia, Cundinamarca (≥1.2 to <3.0 mmol/L; 1 to 42 DIM) and Brazil (BHB >1.2 mmol/L; 3 to 21| DIM), the incidence of SCK was only 25.3% and 20%, respectively (Daros et al., 2017; Garzon Audor and Oliver Espinosa, 2018). Other studies from different countries (Argentina and Europe) have also reported lower prevalences ranging from 10.3% to 39% (Berge and Vertenten, 2014; Garro et al., 2014; Suthar et al., 2013).

The SCK incidence risk showed some variation, depending on the diet characteristics, but also the postpartum period in which the samples were taken, and the sensitivity and specificity of the test employed to establish the ketonemia. The high SCK incidence risk reported in the present study could also be explained from the high fiber proportion present in the *Kikuyu* pasture. However, herds that received energetic supplementation had a high incidence risk of SARA which can be explained in three ways: 1) what supplements they ate; agricultural by-products can have a high proportion of soluble carbohydrates; 2) how much supplements they ate; supplementation was not offered solely based on the animal requirement (i.e. market prices led to higher feeding of cheaper agricultural by-products in season); and 3) what forages were fed; cows grazing in *Rye-Grass* had less fiber than cows on Kikuyu grass. On the other hand, our study found that the transition diet and diets Type IV and V diet were positively associated with the pregnancy risk at 30 and 60 DIM.

Independently of the pasture type, the common characteristic of this diet was the use of agricultural by-products (plus dairy concentrate or legumes addition). Future research should include ration analyses (diet composition and amount of the ration), which is needed to understand the point in which these diets can increase the reproductive performance with minimal adverse effects, mainly a low incidence of SARA/SCK.

This study found a concomitant occurrence of SCK and SARA in 5.2% of the study cows. However, one study from Thailand reported an inverse relationship between ruminal pH and serum beta-hydroxybutyrate (Chaidate et al., 2014). Dairy producers and nutritionists face the difficult challenge of providing foods that optimize energy intake and total milk yield without causing SARA or SCK (Krause and Oetzel, 2006). Further information should be collected about this relationship.

Cows that do not receive a nutritional transition period could have: a) a prolonged Negative-Energy Balance with a strong probability to suffering SCK; and b) a postpartum rumen with less adaptation to diets with high proportions of carbohydrates, with a strong probability to suffering SARA. In our study, feeding a transition diet also reduced the occurrence of PA. Based on an attributable fraction calculation, the SCK incidence risk found in our study can explain a large part of the occurrence of PA (67%), with 66% of the SCK animals having PA at 60 days postpartum compared to 22% in cows without SCK (Table 2). It is reported elsewhere that the probability of pregnancy was reduced by 20% in cows diagnosed with SCK in the first or second week postpartum (Walsh et al., 2007). Furthermore, compared with non-SCK cows, SCK cows can exhibit a lower peak estrus activity and shorter duration of the first postpartum estrus, delayed calving to first estrus interval, and longer parturition to first insemination interval, and therefore prolonged calving to conception interval (Rutherford et al., 2016).

In our study, the multivariate analysis showed no effect of SCK over the embryo loss and the pregnancy risks (30 and 60 days post-breeding). Our results contrast with a meta-analysis performed to determine the association between elevated β-hydroxybutyrate in transition dairy cows and reproductive performance (Abdelli et al., 2017). That study found

Table 6. Multivariable logistic regression model of nutritional/cow characteristics and metabolic disorders associated with pregnancy risk to 30 and 60 days post-breeding (DPB).

Pregnancy risk	Independent Variables	Odds Ratio	Std. Err.	P	95% Conf Interval	
					LL	UL
Pseudo $R^2 = 0.15$						
30 DPB	Transition diet	7.348	4.014	<0.001	2.518	21.441
	Diet					
	I	baseline		0.005*		
	II	1.373	0.747	0.560	0.472	3.990
	III	1.382	0.626	0.474	0.568	3.361
	IV	11.322	6.367	0.000	3.759	34.092
	V	23.395	18.441	0.000	4.990	109.675
	VI	2.474	1.819	0.218	0.585	10.457
	VII	0.994	0.544	0.992	0.340	2.907
	VIII	2.479	1.406	0.109	0.815	7.535
	SARA	0.279	0.129	0.006	0.112	0.692
	Intercept	0.366	0.123	0.003	0.189	0.710
Pseudo $R^2 = 0.18$						
60 DPB	Transition diet	6.938	3.766	<0.001	2.394	20.108
	Diet					
	I	Baseline		<0.001*		
	II	1.319	0.726	0.625	0.442	3.886
	III	1.574	0.723	0.323	0.639	3.876
	IV	8.317	4.544	<0.001	2.850	24.268
	V	39.814	33.314	<0.001	7.722	205.279
	VI	0.925	0.881	0.935	0.142	5.983
	VII	0.728	0.444	0.604	0.220	2.406
	VIII	3.050	1.794	0.058	0.962	9.664
	SARA	0.121	0.068	<0.001	0.040	0.365
	Intercept	0.327	0.113	0.001	0.166	0.645

LL = Lower Limit; UL = Upper Limit.
I = Kikuyu grass only.
II = Kikuyu grass + agricultural byproducts.
III = Kikuyu grass + dairy concentrate.
IV = Kikuyu grass + dairy concentrate + agricultural by-products.
VI = Rye Grass + legume + dairy concentrate + corn silage.
VII = Rye Grass + dairy concentrate.
VIII = Rye Grass + dairy concentrate + agricultural by-products + corn silage.
* Global p-value for entire categorical variable.

Table 7. Multivariable logistic regression model of nutritional/cow characteristics and metabolic disorders associated with embryo loss risk between 30 and 60 days post-breeding (DPB).

Pseudo $R^2 = 0.07$

Variables	Odds Ratio	Std. Err.	P	95% Conf Interval	
				LL	UL
Pasture Type					
RG	Baseline		0.860*		
RG + LG	1.149	0.976	0.870	0.217	6.073
Kikuyu	0.898	0.690	0.889	0.199	4.049
SARA	4.787	3.436	0.029	1.172	19.551
Intercept	0.032	0.014	<0.001	0.013	0.078

LL = Lower Limit; UL = Upper Limit.
SARA = Subacute ruminal acidosis.
RG = Rye Grass.
LG = Legumes.
* Global p-value for entire categorical variable.

that the risk of pregnancy at the first insemination was 0.62 and a hazard risk for time to pregnancy was 0.77 for cows with high BHB (Abdelli et al., 2017), indicating that high BHB led to lower risk of pregnancy at a given DIM. However, the same meta-analysis failed to clearly conclude that there was an association between estrus cyclicity and high BHB (Abdelli et al., 2017).

Moreover, the results of the present study found that SARA increases the odds of embryo loss and reduces the odds of pregnancy at both 30 and 60 days post-breeding. It has been suggested that in cows suffering from SARA, different factors affecting the gastrointestinal barrier integrity, such as epithelial damage due to acidic pH of the ingesta, proliferation of the ruminal papilla epithelium due to high amount of carbohydrates in the diet, and the high rumen osmolality (Rodríguez-Lecompte et al., 2014). While the ruminal pH is low, cell lysis of gram-negative bacteria increases the concentration of lipopolysaccharide (LPS) in the rumen (Zhao et al., 2018). Furthermore, the ruminal content of LPS in cows with grain-induced SARA is significantly higher compared with control cows (Rodríguez-Lecompte et al., 2014; Zhao et al., 2018). Consequently, swelling and erosion of the ruminal papillae, local damage and inflammation, and LPS translocation with a systemic inflammatory response can occur (Rodríguez-Lecompte et al., 2014; Zhao et al., 2018). The physiology of ovarian tissues is affected directly by the presence of bacterial endotoxins such as LPS, cytokines such as interleukin (IL-1) and tumor necrosis factor-alpha (TNFα) and inflammatory mediators (pathogen-associated molecular patterns, or PAMPs). This effect consists of the reduction in the pre-ovulatory LH surge induced by estradiol, GnRH secretion, and the pituitary response to GnRH pulses (Sheldon et al., 2008; Williams, 2013). Granulosa cells can elicit an innate immune response against bacterial cell wall components such as LPS, increasing oxidative stress and the expression of inflammatory mediators (IL-1β, IL-6, IL-8, and TNFα) and reducing oocyte quality (Bromfield and Iacovides, 2017). It has been reported that diseases of the reproductive tract and other body systems of both infectious and noninfectious etiology, all contribute to reproductive failure (Gilbert, 2019). Cows diagnosed with either clinical or subclinical diseases occurring during early lactation delay first postpartum ovulation, resulting in extended periods of postpartum anovulation causing anestrus, which in turn exerts long-lasting effects on fertility in dairy cows, including the lack of spontaneous estrus, reduced pregnancy per artificial insemination, and increased risk of pregnancy loss (Ribeiro et al., 2013; Santos et al., 2016).

Average milk production during the first 2 months postpartum was associated with PA in the bivariate analysis but did not remain in the final model. Parity and average milk production were correlated ($P = 0.002$), and therefore both were unlikely to remain significant in the final model. Average milk production would likely affect what and how much forages and supplements were fed. However, milk production data were based on verbal reports provided by the farmers for the day prior to the farm visits, with daily fluctuations in milk production not being captured. Therefore, daily milk production was dichotomized to minimize measurement error bias. As such, parity was deemed to be a more reliable variable than the dichotomized daily milk production and was retained for the final model.

One of the limitations of this research was the number of herds and cows included. A larger number of herds and animals should be included for future research projects in order to obtain more information about subgroups of nutritional management practices and the extent that they impact these metabolic conditions. The knowledge of this information would allow better establishment of specific nutritional interventions to reduce metabolic disease occurrence and postpartum anestrus. Another limitation of the study is the lack of composition information about the diets (e.g., Energy Mcal; Crude protein percentage; Dry matter amount). However, the project budget did not allow for these analyses. Future research should include ration analyses.

Finally, it is essential to consider the presentation of other causes of reproductive impairment in future research. Many other factors may be related to postpartum anestrus and the mechanisms around the occurrence of this condition: postpartum anovulatory conditions prior to anestrus at 60 days postpartum (e.g. follicular cyst, persistent corpus luteum); serum changes in progesterone and estradiol concentrations; additional postpartum events such as immunosuppression or subclinical hypocalcemia; and other concomitant diseases, such as postpartum uterine infections.

5. Conclusions

Inadequate prepartum and postpartum nutritional management of the study herds increased the occurrence of SARA and SCK during the first 60 days postpartum and increased the risk of anestrus at 60 days postpartum, along with other adverse effects on reproductive performance. Feeding a transition diet is an essential tool to adapt the rumen flora to feed supplements and to improve reproductive performance. The present study is the first report of SARA and SCK incidence in dairy cows in this dairy region of Colombia.

Declarations

Author contribution statement

D. Vallejo-Timaran: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

J. Astaiza-Martínez: Conceived and designed the experiments; Wrote the paper.

J. VanLeeuwen, J. Reyes-Velez; J. Maldonado-Estrada: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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