

1 **The effect of diet restriction on raw milk stability - A meta-analytic approach**

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6 SUPPLEMENTARY FILE

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35 **Supplementary Material and Methods**

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Initially, a systematic review of the literature was carried out in order to identify the factors that affect the stability of raw bovine milk in the alcohol (ethanol) test. In this systematic review (data not yet published), 35 factors capable of altering milk stability were identified, with animal feed restriction being the second most studied factor (n = 9) in studies that address factors that affect milk stability.

43 *Research methods to identify studies*

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Then, the PICO of the following study resulted in: POPULATION (population) - milk, being samples of individual animals and/or whole milk, on the farm; INTERVENTION (intervention/interest) - related to milk stability, to unstable non acid milk (UNAM), to alcohol or alizarol or ethanol testing, heat; CONTEXT (context) - the factors that have already been mentioned that can negatively affect milk stability.

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50 *Data extraction*

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Data extraction was performed by transcribing the information of the original articles in an Excel spreadsheet, organized, according to variables, in columns containing: article code, identification of the first author, temperature, breed of animals, number of animals, days in milk (DIM), time of year of experiment, type of system (confined or semi-confined or grazing + supplemented), type of forage fed to animals (silage, hay, feed or concentrate), amount of diet expressed as dry matter (DM) received by animals in both treatments (restriction diet or control diet with 100% of nutritional requirements), number of days of adaptation (pre-restriction), number of days in restriction, number of days of recovery (post-restriction), mean of stability (°GL) used in the alcohol (ethanol) test to coagulate milk samples, SCC and percentages of milk solids.

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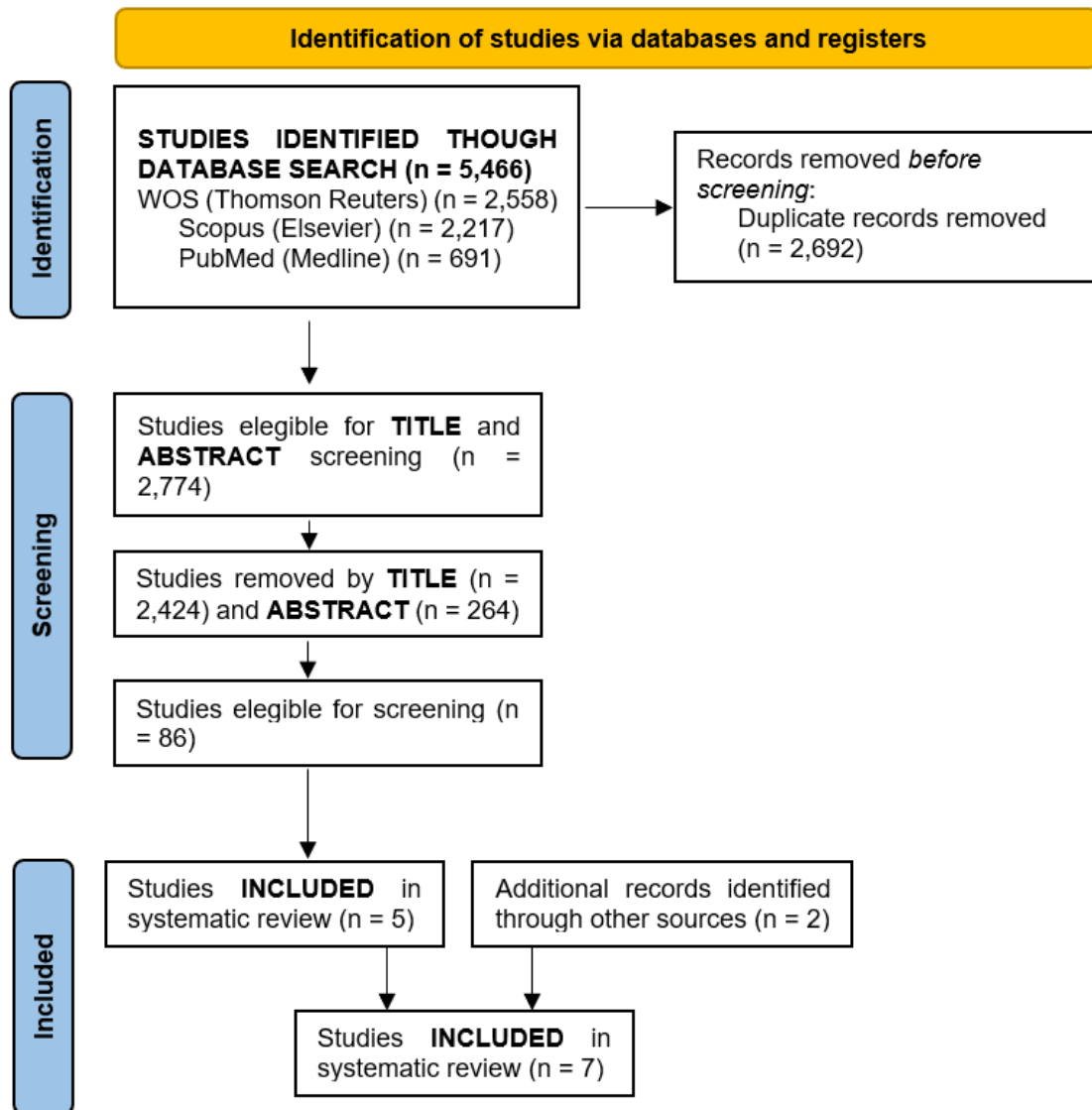
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Codes with qualitative clustering criteria were used in the analytical models. In this item, the main codes were applied to characterize feeding (for example, control or restricted feeding). Other codes were used to consider the variability between all the compiled experiments (for example, the effect of the study or trial).

69 **Supplementary Figure S1.** Diagram showing the search flow and selection of the
70 studies.



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Title	Reference	Restriction level	Access to raw data
Effect of reducing daily herbage allowance during early lactation on composition and processing characteristics of milk from spring-calved herds	Gulati <i>et al.</i> , 2019	Restricted amount of supplement	No
Behaviors associated with cows more prone to produce milk with reduced stability to ethanol test due to feeding restriction	Stumpf <i>et al.</i> , 2016	50% of the diet	Yes
Milk traits of lactating cows submitted to feed restriction	Gabbi <i>et al.</i> , 2015	40 to 50% of the diet	Yes
Feeding restriction impairs milk yield and physicochemical properties rendering it less suitable for sale.	Fruscalso <i>et al.</i> , 2013	50% of the diet	Yes
Severe feed restriction increases permeability of mammary gland cell tight junctions and reduces ethanol stability of milk	Stumpf <i>et al.</i> , 2013	50% of the diet	Yes
Electrophoretic characterization of proteins and milk stability of cows submitted to feeding restriction	Barbosa <i>et al.</i> , 2012	40% of the diet 30% nutrients	Yes
Unstable nonacid milk and milk composition of Jersey cows on feed restriction	Zanela <i>et al.</i> , 2006	40% of the diet	Yes
Effect of three types of diet on the appearance of metabolic disorders and their relationship with changes in milk composition in Holstein Friesian cows	Hernández and Ponce, 2005	20 and 50% of the diet	No
Milk instability as a result of a increase calcium ion content	Seekles and Smeets, 1952	Did not evaluate feed restriction levels, only used argument for discussion of results	-

86 **Supplementary Table S2.** Evaluation of articles that did not meet the criteria for
 87 analysis

Reference	Restriction levels	Justification
Gulati <i>et al.</i> , 2019	Restricted supplementation no nutritional requirements via diet	Authors did not perform the alcohol test to evaluate the stability but the coagulation time test to heat and we did not obtain access to the raw data.
Stumpf <i>et al.</i> , 2016	50% of the diet	Same database used by Stumpf <i>et al.</i> , 2013. Removed to avoid duplication of data
Hernández and Ponce, 2005	20 and 50% of the diet	We did not have access to the raw data.
Seekles and Smeets, 1952	Did not present an experiment evaluating restriction levels	He only used the food restriction factor to explain that during the period of the Second World War thousands of animals in France were under periods of food restriction and this may be a cause of decreased milk stability, and we didn't get access to the raw data either.

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105 **Supplementary Table S3.** Characteristics of articles included in the joint raw data
 106 analysis

Origin	Type of study	Reference	Level of feed restriction*	N° of individual observations (n)
Expert opinion	Dissertation	Schmidt, 2015	50%	71
Systematic review	Article	Gabbi <i>et al.</i> , 2015	30%	83
Systematic review	Article	Stumpf <i>et al.</i> , 2013	50%	24
Systematic review	Article	Fruscalso <i>et al.</i> , 2013	50%	59
Systematic review	Article	Barbosa <i>et al.</i> , 2012	40%	31
Systematic review	Article	Zanela <i>et al.</i> , 2006	40%	69
Expert opinion	Experiment	Zanela, 2006	20%	59

107 *Percentage of diet restriction to animals expressed as % of the total dry matter of the total diet

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124 **Supplementary Table S4.** Number of observations and descriptive statistics of the
 125 main responses available in the database

Variables	N total	Quartile		
		25	50	75
Milk production, kg/day	404	10.1	13.0	17.0
Stability, % ethanol	404	70.0	76.0	78.0
Acidity, °D ¹	328	15.0	16.0	17.0
pH	234	6.67	6.72	6.79
Density, g/L	231	1029.0	1030.0	1030.9
Freezing temperature, °C	113	-0.55	-0.54	-0.53
Fat content, g/100 g	379	3.51	4.08	4.81
Protein content, g/100 g	379	3.03	3.38	3.65
Lactose content, g/100 g Total	379	4.34	4.48	4.65
solids, g/100 g	379	12.1	12.9	13.8
Somatic cell count (x 1000), n°	346	35	90	272

126 ¹to convert acidity to g of latic acid/100 g of milk, divide per 100.

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128 Discussion

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130 *Quality analysis of studies*

131 It is worth to notice the way the different studies described the feed composition and
 132 management. Some offered a complete diet, composed of silage and concentrated in the
 133 trough with access to pastures (Zanela *et al.*, 2006) or silage, concentrate and hay in the
 134 trough with access to pastures (Barbosa *et al.*, 2012), other supplemented grazing cows
 135 with concentrate (Fruscalso *et al.*, 2013). Also, to enhance the possibility of adequate
 136 comparisons between studies, we calculated the amount of diet dry matter fed as the
 137 control diet and the restriction diet, as some studies reduced the supply of energy or
 138 protein (Schmidt, 2015) or remove only the concentrate of the restriction diet (Zanela,
 139 2006). In relation to what was previously published by Gabbi *et al.* (2015), the present
 140 study increased the levels of feed restriction from 0, 40 and 50% to 0, 30, 40 and 50%.

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142 References

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144 **Barbosa RS, Fischer V, Ribeiro MER, Zanela MB, Stumpf MT, Kolling GJ,**
 145 **Schafhäuser JJ, Barros LE and Egito AS** (2012) Electrophoretic characterization
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149 impairs milk yield and physicochemical properties rendering it less suitable for
150 sale. *Scientia Agricola* **70**(4), 237-241.

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152 **Neto AT, Schmidt FA and Fischer V** (2015) Milk traits of lactating cows
153 submitted to feed restriction. *Tropical Animal Health and Production* **48**(1), 37-43.

154 **Gulati A, Galvin N, Kennedy E, Lewis E, McManus JJ, Fenelon MA and Guinee**
155 **TP** (2019) Effect of reducing daily herbage allowance during early lactation on
156 composition and processing characteristics of milk from spring-calved herds.
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158 **Hernandéz R and Ponce P** (2005) Effect of three types of diet on the appearance of
159 metabolic disorders and their relationship with alterations in the composition of
160 milk in Holstein Friesian cows. *Zootecnia Tropical* **23**(3), 295-310.

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162 recuperação da instabilidade do leite ao teste do álcool. 2015. Dissertação de
163 mestrado. Centro de Ciências Agroveterinárias da Universidade do Estado de Santa
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174 **Zanela MB, Fischer V, Ribeiro MER, Barbosa RS, Marques LT, Stumpf W and**
175 **Zanela C** (2006) Unstable nonacid milk and milk composition of Jersey cows on
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