Journal MVZ Cordoba
2020; 25(2):e1708.
https://doi.org/10.21897/rmvz.1708

Original

Animal welfare assessment on pasture-based dairy farms in Savanna of Bogota, Colombia

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Received: August 2019; Accepted: February 2020; Published: July 2020.

ABSTRACT

Objectives. The aim of this study was to determine an overall on-farm animal welfare score (WS) for dairy farms using a protocol involving animal- and management/resource-based variables; and to identify the variables that contributed the most to the variance for the WS. **Materials and methods**. Twenty-five pasture-based dairy farms in Savanna of Bogota - Colombian high tropic, were visited twice. During each visit, a total of 15 animal- and 13 management/resource-based variables were evaluated. For each variable, a herd-level negative cut-off point was established to determine compliance. Based on compliance, a WS was determined (from 0 to 100) and farms were classified as 'excellent', 'enhanced', 'acceptable', or 'non-acceptable'. A linear regression model was used to evaluate the association between variables at the herd-level with the WS. **Results.** Overall, median WS was 82 (min = 67; max = 97). Two farms were classified as 'excellent' (WS = 94.5), 20 as 'enhanced' (WS = 82.5), and 3 as 'acceptable' (WS = 68). Among all variables, the noncompliance regarding subclinical mastitis, hind-legs and udder dirtiness score, ventral line wounds, *Haematobia irritans* fly counts, and condition/cleanliness of pre-milking holding area was significantly associated with a 5.6, 3.6, 6.7, 5.1, 6.5, and 6.1 points reduction in the WS, respectively (R^2 = 0.84). **Conclusions.** Welfare level found across farms was good and encouraging. Nonetheless, six variables were identified as the main contributors to an unfavorable result. Strategies for improvement could focus on these variables to be able to reach a welfare level of 'excellence'.

Keywords: Benchmarking; cattle; tropical; well-being (*Source: MeSH*).

RESUMEN

Objetivos. Determinar un puntaje de bienestar animal (PBA) a nivel de hato para ganaderías lecheras utilizando un protocolo que involucrara variables basadas en los animales y en manejo/recursos, e identificar las variables que más contribuyen a la varianza del PBA. **Materiales y métodos.** Veinticinco fincas lecheras basadas en pastoreo (Sabana de Bogotá – trópico alto colombiano) fueron visitadas dos veces. Durante cada visita, 15 variables basadas en el animal y 13 basadas en manejo/

How to cite (Vancouver).
Medrano-Galarza C, Zuñiga-López A, García-Castro F. Animal welfare assessment on pasture-based dairy farms in Savanna of Bogota, Colombia. Rev MVZ Cordoba. 2020; 25(2):e1708. https://doi.org/10.21897/rmvz.1708

ISSNe: 1909-0544

recursos fueron evaluadas. Para cada variable se estableció un punto de corte negativo a nivel de hato para determinar el cumplimiento. En función del cumplimiento, se determinó un PBA (de 0 a 100) y las fincas se clasificaron como 'excelente', 'buena', 'aceptable' o 'no-aceptable'. Se usó una regresión lineal para evaluar la asociación entre variables a nivel de hato con PBA. **Resultados.** La mediana de PBA fue de 82 (mín=67; máx=97). Dos fincas fueron clasificadas como 'excelentes' (PBA=94.5), 20 como 'buenas' (PBA=82.5) y 3 como 'aceptables' (PBA=68). Entre todas las variables, el incumplimiento con respecto a mastitis subclínica, calificación de suciedad (patas y ubre), heridas en la línea ventral, recuentos de mosca *Haematobia irritans* y condición/limpieza del área de espera pre-ordeño se asociaron significativamente con 5.6; 3.6; 6.7; 5.1; 6.5 y 6.1 puntos de reducción en el PBA, respectivamente (R^2 = 0.84). **Conclusiones.** El nivel de bienestar encontrado fue bueno y alentador. No obstante, se identificaron seis variables que contribuyeron a un resultado desfavorable. Las estrategias de mejora podrían centrarse en estas variables para alcanzar un buen nivel de bienestar.

Palabras clave: Bienestar; evaluación comparativa; ganado; trópico (*Source: MeSH*).

INTRODUCTION

Colombia is the fourth largest milk-producing country in South America, surpassed by Brazil, Mexico, and Argentina, and the Colombian cattle industry is growing (1,2). The latest available national census data showed that between 2014 and 2015 there was an increase of 3% in the number of cattle farms (from 497008 it went to 512103) (3), and 6% of these herds were farms oriented exclusively to milk production (dairy farms) (4). In 2016, dairy farms in Colombia produced 45% of the total milk production (3192 million liters), the remainder was produced by dual purpose farms (4). In addition, the majority (81%) of the farms in the country are small farms (<50 animals/farm) (2). In Colombia, milk is produced in high and low tropic regions, corresponding to regions where dairy and dual-purpose farms are located, respectively. The main dairy zones in the high tropics are the departments of Antioquia, Boyacá, Cundinamarca, and Nariño. Free trade agreements have opened new markets for Colombia to export dairy products, mainly powder milk, butter, yogurt, and cheese, to countries like Panama, Peru, United States and Russia. However, to be able to gain and maintain access to other competitive markets, the Colombian dairy industry will need to meet international quality standards that take into account animal welfare good practices.

Although not as strong and fast as in the EU and UK, animal welfare is strengthening in Latin American countries (5). One reason for this is the implementation of the regional animal welfare strategy in the Americas that supports the implementation of the World Organization for Animal Health (OIE) animal welfare standards and guidelines (5,6). This regional strategy has become the prime mover of change and improvement of production systems in the region, especially those addressed to small producers (6). In Latin American countries, awareness regarding animal welfare has mainly increased by showing producers the positive relationship between poor welfare, economic losses, and unsustainability (7). In addition, among initiatives to promote farm animal welfare in this region, it has been shown that the development of best management practices guidelines has been a useful tool to promote animal welfare in Brazil, Uruguay, and Chile (7).

In Colombia, García et al (8) published in 2012, the first study that attempted to adjust the European Welfare Quality assessment scheme (9) to the conditions of Colombian dairy farms in the high tropic. However, to date most of the work done in cattle welfare assessment has been done in transportation and slaughter, but to our knowledge there is few published researches about welfare assessment at the farm level specifically done on dairy farms. The Colombian Institution of Agriculture (ICA) is under the development of legislation and animal welfare guidelines to fulfill the international animal welfare mandate of the OIE (5), as well as to strengthen national legislations such as the Decree 616 (2006) and the Resolution 3585 (2008). Consequently, there is a need for research investigating welfare indicators throughout the production chain that generates information that can be used to build those guidelines and address legislation in Colombia. Therefore, the aims of this study were 1) to determine an overall on-farm animal welfare score for pasture-based dairy farms in Savanna of Bogota - Colombian high tropic - using an assessment protocol that involved animal-, management- and resource-based variables; and 2) to identify the variables that contributed the most to the variance for the welfare score across farms.

MATERIALS AND METHODS

This observational, longitudinal, cross-sectional study was reviewed and approved by the Agrosavia Scientific and Ethics Committee and the Colombian Ministry of Agriculture and Rural Development (agreement no. 1810). This study was conducted in Savanna of Bogota, Cundinamarca (eastern ranges of the Colombian Andes - high tropic), which average altitude is 2550 m. The average annual temperature is 14°C, but temperature can range from 0 to 24°C.

Enrollment of farms and farm visits. A convenience sample of 25 commercial dairy farms was selected based on location (<1 h drive from the Research Centre Tibaitatá - Agrosavia, Mosquera, Cundinamarca, Colombia; 4.697618, -74.204568), representativeness of the system (i.e., semi-extensive pasture-based systems), and willingness to participate in the present study. Each farm was visited 2 times, once per season (dry and wet season). Dry season visits occurred between June and August 2013 (accumulated precipitation = 98.9 mm); and wet season visits occurred between October and December 2013 (accumulated precipitation =231.1 mm). Before each visit, producers or farm workers were contacted by telephone to arrange farm visits.

Measurements. During each visit, animal- and management/resource-based measurements (Table 1 and 2, respectively) were evaluated. These measurements were extracted from the Welfare Quality protocol (9) but modified and adjusted to the local conditions (8); additionally, other variables relevant to Colombian dairy farms were added to the protocol (8).

Animal-based measurements description. Table 1 summarizes the animal-based measurements scoring charts used in this study. Body condition score (BCS) was assessed using a 5-point scale with 0.25-point increments (10). Cows were then classified as thin if BCS < 2.5, ideal if BCS ≥ 2.5 and ≤ 4, and obese if BCS > 4. The hygiene of the cows (dirtiness score) was assessed by evaluating 2 body areas of each animal, hind-legs and udder, using a 4-point scale (11). Hair loss and visible wounds were evaluated using a 4-point scale (12) with some modifications regarding the anatomical areas evaluated, which were: hocks, knees, flanks, hook bones, pin bones, tail, udder, and belly.

Fly count was done by directly observing cows during pasture time (between 1100 and 1300 h approximately). Specifically, horn and stable flies (*Haematobia irritans* and *Stomoxys calcitrans*, respectively) were counted by walking around each animal at a distance of 1 m approximately from the cow to avoid disturbing them. If fly counts per cow were < 25, flies were counted one by one; if fly counts exceeded 25, flies were counted in groups of 5 (i.e., 25, 30, 35, etc.) (13,14).

The prevalence of subclinical and clinical mastitis and lameness was determined by counting the number of milking cows presenting each disease and dividing that number by the total number of cows in the milking herd at the day of the visit. To diagnose subclinical mastitis, we used a 5-point scale based on the California Mastitis Test (CMT) (15,16). Clinical mastitis was diagnosed using visual inspection for abnormalities in the milk (e.g., clots and flakes) or changes in udder appearance (e.g., swelling and redness of quarter). Lameness was diagnosed assessing the gait score of each cow either when the cow was walking inside the parlor or when it was leaving the parlor after milking using a 5-point scale (17). Ultimately, gait scored was dichotomized such that a score < 3 indicated absence of clinical lameness and a score ≥ 3 indicated the presence of clinical lameness. The frequency of cows suffering other diseases such as eye cancer and photosensitivity during the day of the visit was recorded as well.

Behavioral variables were assessed during pasture and milking time. Flight distance of cows was measured on pasture. The observers tested cows as they walked towards the herd (18). Each tested cow was approached slowly (one step every second approximately) and with a decameter we measured the distance between the observer and the place where the cow was standing before it stepped to the side or moved away. If the cow allowed the observer to approach and touch it, the flight distance was equal to 0 m. Reactivity of cows during milking was assessed, from the moment the cow arrived to its milking place until milking machine was removed and the cow left, using a temperament 4-point scoring system adapted from Grandin (19) (Table 1).

Table 1. Outcome animal-based variables classified by Freedoms, scoring charts and negative cut-off points used for the evaluation of welfare of milking cows.

Freedom[1]	Variable	Scoring system						NCP[2]	
		0	1	2	3	4	5	IL	HL
Freedom of hunger and thirst, by ready access to a diet to maintain full health and vigor	BCS[3]	_____	Emaciated, thurl prominent and saw-toothed spine	No fat pad on pins, corrugations from short ribs visible 3/4 way from tip to spine	Line from hooks to pins forms a flattened V, hooks rounded	Sacral and tail-head ligament not visible	All boney prominences well rounded	< 2.5 or > 4	≥ 15%
Freedom of discomfort, by providing a suitable environment including shelter and a comfortable resting area	Cow dirtiness score, hind legs and udder	_____	Entire area clean, with no dirt	Less than half of the area was covered with dirt	Half or more of the area was covered with dirt	Entire area was covered by a layer of dirt	_____	≥ 3	≥ 15%
Freedom of disease, pain and injures, by prevention or rapid diagnosis and treatment	Integument wounds	No swelling, no hair missing	No swelling or minor swelling, bald area < 1 cm	Medium swelling and or lesion on bald area of 1 to 2.5 cm	Major swelling or a bald area > 2.5 cm	_____	_____	≥ 2	≥ 15%
	H. irritans[4]	_____	_____	_____	_____	_____	_____	≥ 150	≥ 15%
	S. calcitrans[4]	_____	_____	_____	_____	_____	_____	≥ 25	≥ 15%
	Subclinical mastitis, CMT[5]	Mixture remains liquid, no evidence of precipitate	Trace. Slight precipitate. Best seen by tipping; disappears with continued movement	Distinct precipitate but not tendency toward gel formation	Mixture thickens immediately, moves toward center	Gel forms and surface becomes convex	_____	≥ 2	≥ 15%
	Clinical mastitis	Negative	Positive	_____	_____	_____	_____	1	> 3%
	Lameness, gait score	_____	Smooth and fluid movement	Imperfect locomotion but ability to move freely not diminished	Capable of locomotion but ability to move freely is compromised	Ability to move freely is obviously compromised	Ability to move is severely restricted, most be vigorously encouraged to move	≥ 3	≥ 10%
Freedom of stress and prolonged fear, by providing enough space, proper facilities and the company of the animal's own kind	Flight distance	_____	_____	_____	_____	_____	_____	≥ 5 m	≥ 15%
	Reactivity during milking	_____	Standing stationary, calm, no movement	Slightly restless, slight calm movement of tail/feet	Uneasy, head, body, tail and feet movement	Continuous, very vigorous movements, animal may kneel/fall	_____	≥ 3	≥ 15%
Freedom to express natural behavior, by ensuring conditions which avoid mental suffering	Time waiting in holding area	_____	_____	_____	_____	_____	_____	≥ 120 min	≥ 15%

[1]The five freedoms and five provisions; [2]Cut-off points established at the individual- and the herd-level that meant unacceptable levels of each evaluated variable
[3]Body condition score; [4]Fly counts: *Haematobia irritans*, flies/cow and *Stomoxys calcitrans*, flies/cow; [5]California Mastitis Test
NCP= Negative cut-off point; IL= Individual-level; HL= Herd-level.

All animal-based measurements previously mentioned were assessed in a maximum of 20 milking cows randomly selected from the milking herd except for measurements related to disease frequency, holding time prior milking, reactivity during milking, and hygiene (all milking cows were included).

Management/resource-based measurements description. Table 2 summarizes the scoring charts used in this study. The condition and cleanliness of water trough and its surrounding area, feeders (i.e., buckets where concentrate is given to cows during milking), field (field where milking cows were kept on the day of the visit), trails (i.e., used by cows to go from the field to milking parlor in those farms without portable milking on pasture), pre-milking holding area, and milking parlor were assessed using a 3-point scale. Additionally, it was determined whether the field where cows were kept had shade (natural or artificial) and drinking water available for the animals. The time cows had to wait prior to be milked in the holding area was recorded for each individual animal as well as the level of noise at the milking parlor cows and workers were exposed to, using a sound meter (407750, Extech Instruments, Nashua, NH, USA). Ambient sound readings were done every 15 minutes throughout the milking process.

Table 2. Outcome management/resource-based variables classified by Freedoms, their scoring charts and negative cut-off points used for the evaluation of welfare of milking cows.

Freedom[1]	Variable	Scoring system				NCP[2]
		0	1	2	3	HL
Freedom of hunger and thirst, by ready access to a diet to maintain full health and vigor	Water trough / feeders' condition	_____	Good: optimal state, neither broken nor patched, easy access	Regular: acceptable state, possibly broken or patched but does not affect the availability of water or may cause a wound on the animal while it is drinking or eating, easy access	Bad: deplorable state, damaged that may be causing problems of availability or could generate a wound on the animal (i.e., with nails), hard access	≥ 3
	Water trough / feed bunk cleanliness	_____	Clean trough and clean water - absence of fungus, algae, soil, paper, stones, nails or any object; same for feeders	Partially dirty, trough is dirty, but water is looks clean and fresh; in the case of feeders no more than half of the feeder with an accumulation of old food and/or hard crust	Dirty trough and dirty water; in the case of feeders more than half of the feeder with accumulation of old food	≥ 3
	Drinking water availability	No water available	Adequate	_____	_____	0
	Area surrounding water trough	_____	Good: dry area without puddles or mud accumulation	Regular: area with small puddles and mud	Bad: area flooded and/or muddy	≥ 3
Freedom of discomfort, *by providing a suitable environment including shelter and a comfortable resting area*	Trail / paddock conditions and cleanliness	_____	Good: regular surface, absence of obstacles and good drainage - absence of mud and puddles; no presence of polluting material (e.g., plastic, wire)	Regular: not very regular surface, presence of an obstacle - ups and downs, stones, bags, wood, wire or other metallic structures; acceptable drainage (little mud and puddles)	Bad: totally irregular surface, presence of many obstacles - ups and downs, stones, bags, wood, wire or other metal structures (abundant presence of polluting material); poor drainage (presence of mud and puddles)	≥ 3
	Shade availability	No shade available	Shade available	_____	_____	0
	Holding area / milking parlor conditions and cleanliness	_____	Good: non-slip floor, no holes or open grids, clean area before starting milking, free of mud and accumulated fecal matter	Regular: slippery floor or floor with holes or open grids, partially dirty	Bad: slippery floor with gaps and open grids, area without cleaning prior to milking, mud and accumulated fecal matter	≥ 3
	Level of noise[3]	_____	_____	_____	_____	≥ 80 dB

[1]The five freedoms and five provisions; [2]Cut-off points established at the herd-level that mean unacceptable levels of each evaluated variable; [3]Average level of noise calculated using all readings done throughout the milking process in each visit for each farm
NCP=Negative cut-off point; HL= Herd-level.

The assessment of all measurements started at 1100 and finished at 1700 h. All measurements were performed by 2 observers. During the dry and wet season visits, a total of 816 and 588 milking cows, respectively, were assessed for disease frequency, holding time prior milking, reactivity during milking, and cow hygiene; and 414 and 366 of these milking cows, respectively, were assessed for the rest of variables while on pasture.

Data management and statistical analysis. Data were transcribed from recording paper-sheets into Microsoft Excel (Microsoft Corp., Redmond, WA, USA) for data cleaning and screening. Two farms withdrew from the study after the first visit, thus for the analysis, these two farms only had data for the visit done during the dry season. All data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

To determine the level of welfare (i.e., the overall on-farm welfare score, WS) for each farm we used a combination of methodologies and concepts proposed by the Welfare Quality (EU) (9). All variables that we evaluated were grouped into 3 main categories: fundamental, intermediate, and basic (Table 3). The classification of the variables was done according to their level of importance to ensure an adequate level of welfare, which was determined by a panel of experts (5 dairy scientists from government and academia). For each variable, we specified a negative cut-off point, i.e., a value indicative of an unacceptable level for each variable. Specifically, for all animal-based measurements the negative cut-off point was established at the individual- and herd-level (prevalence was calculated using those negative cut-off points; Table 1). For example, looking at cow dirtiness score the individual-level negative cut-off point was 3 or above, then based on the score given to each cow, cows were grouped as below or above this cut-off point. Based on this, a prevalence of dirty cows per farm was calculated as the number of cows above the cut-off point divided the total number of cows assessed. This prevalence must be below the herd-level negative cut-off point (15%) to be acceptable (compliance). For all management/resource-based variables, the negative cut-off point was established at the herd-level (Table 2). For example, looking at water trough condition, the herd-level negative cut-off point was 3 or above, thus a farm should be scored under 3 to be acceptable.

Based on the results for each variable (each variable must have an acceptable level, i.e., below the negative cut-off point, to be able to get the points assigned to it, otherwise it gets assigned zero points; Table 3), a final classification (WS) was done where each farm was categorized as 'excellent', 'enhanced', 'acceptable', and 'non-acceptable' (Table 3) (9).

Table 3. Classification of animal-, management/resource-based variables based on their level of importance to ensure an adequate level of welfare of milking dairy cows.

Category	Variables	PA[1]
Fundamental	Body condition score[a]	5
	Integument wounds[a]	5
	Horn fly: *Haematobia irritans*[a]	5
	Stable fly: *Stomoxys calcitrans*[a]	5
	Flight distance[a]	5
	Prevalence of	
	Clinical mastitis[a]	5
	Lameness[a]	5
	Eye cancer[a]	5
	Down cows[a]	5
	Photosensitivity[a]	5
	Cleanliness of water trough[b]	5
	Drinking water availability[b]	5
	Level of noise during milking[b]	5
Intermediate	Feet dirtiness score[a]	3
	Udder dirtiness score[a]	3
	Prevalence of subclinical mastitis[a]	3
	Restless behavior during milking[a]	3
	Condition and cleanliness of trails[b]	3
	Condition and cleanliness of paddock[b]	3
	Shade availability[b]	3
	Condition and cleanliness of holding area[b]	3
	Condition and cleanliness of milking parlour[b]	3
	Waiting time in holding area[b]	3
Basic	Bald areas (integument with hair loss)[a]	1
	Water through conditions[b]	1
	Feeders conditions[b]	1
	Cleanliness of feeders[b]	1
	Area surrounding water trough[b]	1
	Total points[2]	100

[1]Each variable must have an acceptable level (i.e., below the negative cut-off point) to be able to get the points assigned to it otherwise it gets assigned zero points.; [2]Overall on-farm milking cow welfare score: Excellent (90 to 100 points), Enhanced (70 to 89 points), Acceptable (40 to 69 points), and Non-acceptable (≤ 39 points).; [a]Animal-based variables; [b]Management/resource-based variables
PA=Points assigned.

Descriptive statistics calculated included percentages, medians, and inter-quartile range (1st and 3rd quartiles - IQR). Farm size was categorized based on the median number of milking cows across farms, leaving farm size as a dichotomous variable [small (≤ 20 milking cows) vs. large farms (> 20 milking cows)]. Homogeneity of variance and normality of continuous variables were evaluated graphically and using Anderson-Darling test, respectively. Chi-squared (or Fisher's exact) and Kruskal Wallis tests were used to evaluate differences in categorical and continuous variables, respectively, between dry and wet season visits, and small *versus* large farms.

Linear regression model (PROC MIXED in SAS) was used to evaluate the association between animal-based, resource- and management-based variables at the herd level with the overall on-farm WS given to each farm. Each variable was dichotomized based on the negative cut-off point established at the herd-level, where 0 indicated compliance and 1 indicated noncompliance. Any variables having an association with WS at $p<0.20$ (univariable analysis) were offered to the multivariable model. A variable was considered a confounder when the difference between coefficients of the full model and reduced model was >20%. The model was reduced using backward elimination and variables remained in the model when $p<0.1$. Homoscedasticity was evaluated in the final model by plotting the standardized residuals against the predicted values of the outcome. Normality of residuals was tested using Anderson-Darling test. The R^2 was calculated in SAS using the formula

$R^2=(1-SSE/SST)$

Where SSE indicated the sum of squared estimate of errors and SST indicated the total sum of squares. This coefficient of determination was calculated to describe the amount of variance of the WS (outcome) that is explained by the variables.

RESULTS

The median (IQR) herd size for small and large farms was 12 (9 to 15) and 38 (28 to 66) milking cows, respectively. Among small farms (n=12), five farms milked their cows manually (two farms in a designated fixed area and three farms at the pasture where cows were grazing) and seven farms used mechanic milking systems (five farms used a conventional milking parlor and two farms used a portable milking system to milk cows on pasture). Within large farms (n=13) only one farm milked cows manually in a designated fixed area, two farms used a portable mechanic milking system, and 10 farms used a conventional mechanic milking parlor. All farms used a mix of breeds, but the predominant breed was Holstein (81%), followed by crossbred cattle (7.4%, Holstein x Jersey and Brown Swiss x Holstein crossbred), Normand (5.8%), and Jersey (3.1%) among others. The average number of farm workers and milkers per farm was 3±1 and 2± 1 people, respectively. The most common education level among workers was elementary school (64%), followed by secondary (32%), 4% of workers were technicians.

Animal-based variables. Table 4 summarizes the median herd-level prevalence and range across farms for each of the assessed animal-based variables. It was found that median BCS of milking cows at different stages of lactation was 3.0 [IQR, cows in the first-third of lactation (1 to 60 days in milk, DIM): 2.5 to 3.0, n=141; in the second-third (61 to 240 DIM): 2.5 to 3.0, n=345; third-third (241 to 305 DIM): 2.75 to 3.5, n=100; and with more than 305 DIM: 3.0 to 3.5, n=99]. The anatomical site where more hair loss and integument wounds were most present was on the belly (at the level of the umbilicus - ventral line; Figure 1). In 8 (6 large and 2 small farms) out of the 25 farms the prevalence of cows with wounds in the ventral line exceeded 15%. In 8 (3 small and 5 large) out of the 25 farms the prevalence of cows with hair loss in the ventral line exceeded 15%. In the other sites where there was evidence of wounds or baldness, the affected population did not exceed 2% and 3%, respectively.

Regarding the presence of hematophagous flies, on average it was found that a cow had 56±64 *Haematobia irritans* (min=0, max= 326, n=773) and 9±18 *Stomoxys calcitrans* (min=0, max=136, n=772). In six farms (3 small and 3 large), more than 15% of the cows evaluated had *Haematobia irritans* counts ≥ 150 flies/cow. Similarly, six farms (5 small and 1 large) were above the acceptable cut-off point for *Stomoxys calcitrans* counts (<15% of the herd had 25 flies/cow).

Table 4. Summary of herd-level prevalence of animal-based variables by farm size and all farms grouped.

Variables	Median herd-level prevalence, % (range[1])			
	Farm size[2]		p-value[3]	All farms
	Small	Large		
Body condition score, < 2.5	0 (0 to 20)	2.5 (0 to 9)	NS	3.3 (0 to 20)
Wounds ventral line	1.5 (0 to 25)	11 (0 to 72)	*	5 (0 to 72)
Hair loss ventral line	1.2 (0 to 34)	6 (0 to 57)	NS	3 (0 to 57)
Fly counts/cow				
H. irritans ≥ 150 flies	1 (0 to 72)	5 (0 to 42)	NS	2.4 (0 to 72)
S. calcitrans ≥ 25 flies	3 (0 to 78)	0 (0 to 54)	NS	0 (0 to 78)
Dirty hind legs[4]	32 (6 to 50)	20 (10 to 33)	**	25 (6 to 50)
Dirty udder[4]	8 (0 to 30)	5 (0 to 21)	NS	7 (0 to 30)
Diseases				
Clinical mastitis	0 (0 to 7.4)	1 (0 to 7)	*	0 (0 to 7.4)
Subclinical mastitis	46 (0 to 59)	27 (2 to 53)	NS	28 (0 to 59)
Lameness	0 (0 to 43)	0 (0 to 8)	NS	0 (0 to 43)
Down cow	0	0 (0 to 4)	NS	0 (0 to 4)
Eye cancer	0 (0 to 43)	0 (0 to 4)	NS	0 (0 to 43)
Photosensitivity	0 (0 to 17)	0 (0 to 4)	NS	0 (0 to 17)
Flight distance ≥ 5 m	0 (0 to 12)	0 (0 to 8)	NS	0 (0 to 12)
Reactivity during milking[5]	0 (0 to 10)	1 (0 to 9)	NS	0 (0 to 10)

[1]Range: minimum and maximum; [2]Farm size: small (≤ 20 milking cows) and large farms (> 20 milking); [3]NS = no significant; *p<0.1 (tendency); **p<0.05; [4]Dirty scored per cow ≥3= dirty; [5]Reactivity score ≥3= uneasy/reactive

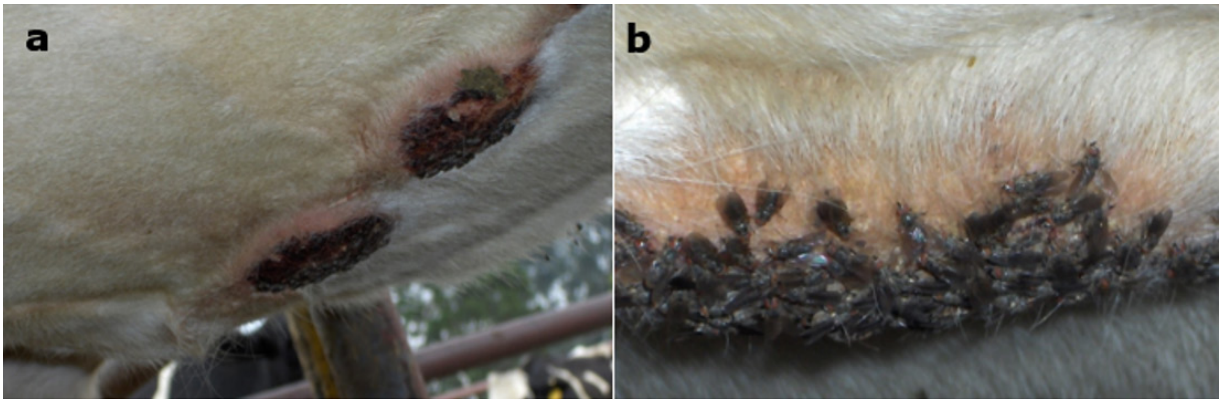

Figure 1. a) Integument wound by the ventral line of a milking cow; b) Hematophagous fly *Haematobia irritans* feeding by the ventral line of a cow. Source: F. E. García-Castro.

Dirtiness cow score was acceptable (dirtiness score = 1 and 2) in the majority of cows evaluated regarding hind legs and udder (77%, n=1048 and 93%, n=1257; respectively). Nineteen farms (10 small and 9 large) exceeded the established limit for the prevalence of cows with dirty feet, while six farms (4 small and 2 large) exceeded the limit regarding prevalence of cows with dirty udder (i.e., prevalence < 15%).

In relation to prevalence of diseases, 21 farms (10 small and 11 large) exceeded the established limit for the prevalence of subclinical mastitis (i.e., <15%) and 3 farms exceeded the established limit for the prevalence of clinical mastitis (≤3%). The prevalence of lameness was greater than 10% in 4 farms. Herd-level prevalence of down cows was less than 15% in all farms. Similarly, the herd-level prevalence of eye cancer and photosensitivity in all farms but 1 was lower than 15%. When looking at cows behaviour, the median flight distance among all cows evaluated across the 2 visits (n=715) was 2.5 m (ranged from 0 to 9 m). No farm had more than 15% of their herd with flight distances ≥5 m. Regarding restless behavior during milking, cows were mostly calm (91%), 6.5% of the animals were restless, 2% very

restless and 0.5% aggressive. No farm presented more than 15% of their animals in or above the 'very restless' category (score 3 or 4). Farm size tended to have an effect on wounds on the ventral line and clinical mastitis (Table 4).

Management- and Resource-based Variables. We found that cleanliness of the water trough was classified as good (score 1) in most farms among the 2 visits (36%). The number of farms with water trough classified as dirty (score 3) did not differ by farm size. Seven farms (3 small and 4 large) had water trough classified as dirty (4 farms had this score in both visits). The presence of water was adequate in most of the farms (n = 22), without differences by size. However, 3 large farms had no available water for milking cows when visited in the dry season. Only 4% (n = 1) and 8% (n = 2) of the farms evaluated had a poor condition regarding water troughs and feeders, respectively. In addition, 2 farms had a bad perimeter around the water troughs and 3 farms presented a poor state of cleanliness of the feeders. Across visits, the condition and cleanliness of trails was classified as good in 42% of the farms, as regular in 37%, and as poor in 21% of the farms. The condition of the field was found in a good state in 28% of the farms, regular in 68%, and poor in 4%. Only 16% of the farms (n = 4) had shade available always for the cows in the field. No differences were found by between farm sizes.

Regarding milking facilities and management, the condition and cleanliness of the holding area prior milking and milking area did not differ between farm sizes. The former was scored as good in 52% of the farms, regular in 28%, and poor in 20%, while the milking area was good in 60%, regular in 28%, and poor in 12% of the farms. When looking at the level of noise during milking, the median (IQR) level of noise was 70 dB (63 to 75 dB). In addition, the median (IQR) time that cows had to wait to be milked (in the pre-milking holding area) was 38.5 min (18 to 62 min). None of the farms had more than 15% of their milking cows waiting 2 hours or more to be milked.

Overall on-farm welfare score. Overall, median WS was 82 (IQR: 75 to 87; min = 67; max = 97). Specifically, two farms were classified as 'excellent' (median WS: 94.5; min = 92; max = 97), 20 farms as 'enhanced' (median WS: 82.5; min = 72; max = 89), and 3 farms as 'acceptable' (median WS: 68; min = 67; max = 68). No farm was classified as 'non-acceptable'. In multivariable analysis, we found that noncompliance regarding subclinical mastitis, hind legs and udders dirtiness score, wounds, *Haematobia irritans* counts, and condition/cleanliness of the holding area prior milking were associated with a decreased overall WS (Table 5). These variables explained 84% of the variance in the WS (R^2 = 0.84).

Table 5. Linear regression model of variables associated with the overall on-farm welfare score[a] (R-squared = 0.84).

Variables	Coefficient	SE	df	p-value
Subclinical mastitis compliance[1]				
No	-5.64	2.12	18	0.015
Yes	Referent			
Hind legs dirtiness compliance[2]				
No	-3.60	1.92	18	0.076
Yes	Referent			
Udder dirtiness compliance[2]				
No	-6.71	1.87	18	0.002
Yes	Referent			
Wounds compliance[3]				
No	-5.13	2.09	18	0.025
Yes	Referent			
Haematobia counts compliance[4]				
No	-6.56	2.30	18	0.010
Yes	Referent			
Holding area compliance[5]				
No	-6.18	2.10	18	0.008
Yes	Referent			
Intercept	94.6	2.39	---	---

[a]Overall on-farm welfare score: continuous outcome, where 90 to 100 points = Excellent, 70 to 89 = Enhanced, 40 to 69 = Acceptable, ≤ 39 = Non-acceptable; [1]Prevalence of cows with subclinical mastitis <15%; [2]Prevalence of cows with dirty legs or udders (dirty score ≥ 3) < 15%; [3]Prevalence of cows with wounds in ventral line < 15%; [4]Prevalence of cows with ≥ 150 *Haematobia* fly counts < 15%; [5]Conditions/cleanliness of holding area prior milking scored as good/clean (<3).

General differences between dry and wet season visits. The main differences found between seasons were related to wounds and dirtiness score. The number of cows with wounds on the ventral line was significantly higher during the dry season compared with wet season (20% vs. 12%, respectively; p=0.001). Furthermore, we found that a higher percentage of cows had hind-legs and udder classified as dirty (dirtiness score 3 and 4) during the wet season compared with the dry season (hind-legs: 41% vs. 10%, p<0.001; udder: 16% vs. 2%, p<0.001).

DISCUSSION

Overall, the level of cow welfare found across farms was good, motivating, and encouraging because most farms were considered 'enhanced', two farms were considered 'excellent', and no farm was classified as 'non-acceptable'. These results are comparable to findings from similar studies done in the Latin American countries such as Costa Rica (20) and Chile (21), where 55% and 47% of farms were classified as 'enhanced', respectively, and 0 farms were 'non-acceptable'. Conversely, our findings contrast results from a recent Mexican study, where all farms were classified as 'acceptable', although none were considered 'non-acceptable' (22). On the other hand, we identified an important number of variables with a high percentage of noncompliance across farms (subclinical mastitis, hind-legs and udder dirtiness, ventral line wounds, *Haematobia irritans* fly counts, and condition/cleanliness of pre-milking holding) that together explained 84% of the variance in the overall welfare score; therefore, contributing in greater proportion to an unfavorable WS, far from excellence, when classifying farms. Similarly, in dairy Argentinean (23) and Mexican farms (22) other researchers found subclinical mastitis and, cows' dirtiness and wounds, respectively, as variables of impact on the welfare of cows.

Among animal-based variables significantly associated with the overall WS, the one with the least compliance among farms was the prevalence of subclinical mastitis. In this study, we found a median farm prevalence of 28%, which is lower compared with findings of other studies done in Colombia (39.5% in Antioquia (24) and 54.3% in Norte de Santander (25)) and in the Northwest of Argentina (48.5% (23)). Despite of being lower than previous studies, prevalence of subclinical mastitis in this study (in 84% of the farms) exceeded the maximum recommended value for dairy farms (15%) (16). Having found a high prevalence of subclinical mastitis on farms triggers an alarm about possible errors made during the milking routine and parlor hygiene (26). In addition, mastitis is one of the most important diseases in dairy cattle, having negative effects not only on production but also on welfare, as it is considered one of the most painful diseases in dairy cows (27).

However, little is known about the effects of subclinical mastitis on cows' welfare and its relationship with pain. Peters et al (28) found that nociceptive thermal threshold tended to be lower among cows with subclinical mastitis compared with healthy cows, concluding that subclinical mastitis might be a welfare issue. In Argentina, Suárez et al (23) found that the risk of culling was 5.4 times higher for cows with subclinical mastitis compared with healthy cows. More research in this area is needed to determine the impact on cow welfare.

The second animal-based variable with low compliance among farms was dirtiness of hind legs and udder. In this study, median farm prevalence of cows with dirty legs and udder was 25% and 7%, respectively, which was similar to findings from Garcia et al (8), who did an observational study between 2008 and 2009 in the same dairy region of Colombia. The dirtiness of cows in our study was lower than values reported in a similar study in Mexico (where > 80% of cows were found with dirty legs and udder (22)), which authors mentioned that producers do not perceive this as a problem.

It is important to consider the risk associated with having dirty animals and the presentation of mastitis. Hind legs hygiene has been found to be correlated with udder hygiene, which is also positively correlated with the presentation of mastitis (29) and with CMT scores (23). Sant'Anna and Paranhos da Costa (11) found a significant positive effect of the level of dirt of hind legs and udder on somatic cell count, which would affect the quality of the milk, milk price, and cows' welfare.

Other animal-based variables with low compliance were the prevalence of wounds and hematophagous flies, specifically *Haematobia irritans*. Regarding wounds, 32% of the farms had more than 15% of the milking herd with lesions on the ventral line. The cause of this type of lesions was unknown. However, we

hypothesized that it could be a filarial dermatitis caused by *Stephanofilaria stilesi*, **which is known to cause lesions at the umbilical** and to be injected into the skin of cows when the fly *Haematobia irritans* feeds (30). Regarding flies, in our study, 24% of farms had more than 15% of their milking herd with *Haematobia irritans* counts above 150 flies/cow.

The presence of flies in dairy cattle is a stressor that affects not only production but the comfort of animals. A high number of flies on a cow directly affects the expression of their natural behavior. Wolley et al (14) found a positive correlation between flies' presence and cattle behavioural responses, showing increased tail head and legs movements, skin twitches, and standing up and grouping (i.e., bunching) to protect their body from being bitten. Similar results were found by Vitela-Mendoza et al (31) in addition to finding an increased stress (higher levels of cortisol) due to fly presence. Our findings remark the need for implementing integrated management plans to control ectoparasites in dairy farms.

Among management- and resource-based variables, it was found that the noncompliance regarding conditions and cleanliness of the holding area prior milking was a variable significantly associated with a decreased in the overall WS. Garcia et al (8) found that on average, 30% of farms had a dirty holding area, slightly similar to our findings. Additionally, DeVries et al (32) found that the longer the time spent standing in the holding area, the poorer the udder hygiene. In an observational study in Brazil, it was found that the risk of metritis was 2,1 times higher for dairy cows on farms with dirty holding areas (33). Therefore, if this area is in bad conditions, like we found in our study, the risk of udder dirtiness and consequently, mastitis, and metritis, could be higher among farms with noncompliance.

Regarding relevant differences between seasonal visits, we found that during the wet season cows were dirtier (dirtier legs and udder) compared with the dry season, which was similar to Sant'anna and Paranhos da Costa (11) findings. DeVries et al (32) state that lying area hygiene is likely to influence udder dirtiness, while manure management, frequency of cleaning of alleys, among others are likely factors to influence legs dirtiness score. When cows are kept on pasture-based farms, conditions of paddocks and trails during increased precipitation are hard to control, thus puddles and mudflows are easily formed, which could be the cause of increased dirtiness during the wet season.

Conclusions and future implications. For the most part, the welfare level of pasture-based dairy farms in Savanna of Bogota, Colombia was good, as most farms were classified as 'enhanced'. This means that there is room for improvement, mainly regarding milking routine, cleanliness of cows, and control of ectoparasites, therefore, opportunities to achieve excellence. There are many strengths in terms of animal welfare of pasture-based dairy systems, which are the most common type of cattle farming in Colombia. Cows live in a natural environment, with more space, freedom of movement, and generally, with a comfortable natural surface for resting and lying down. However, strategies to mitigate possible negative factors associated to an outdoor environment, such as mudflats on paddocks, trails and milking areas, and presence of flies, should be implemented. Finally, in Latin America, citizens' concern about animal welfare is increasing, associating welfare standards not only with their ethical purchase of food products but with food safety and good quality (34). Thus, they are willing to pay more for welfare friendly products (34,35). Consequently, the improvement of on-farm welfare level should be a milestone in the strategic development of the Colombian dairy industry.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

This research was funded by the Ministry of Agriculture and Rural Development of the government of Colombia and was part of the project "Development and application of technologies to improve meat and milk production and quality" – Agenda of research and rural development of the Colombian Corporation of Agricultural Research AGROSAVIA. We kindly thank all producers who participated in this study.

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