Milk quality of dual-purpose cows supplemented with biological fish silage (*Pterygoplichthys sp.*) as a protein source

Calidad de leche de vacas doble propósito suplementadas con ensilado biológico de pez diablo (Pterygoplichthys sp.) como fuente proteica

Qualidade do leite de vacas de duplo propósito suplementadas com silagem biológica de peixe (Pterygoplichthys sp.) como fonte protéica

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Abstract

Background: Devilfish (*Pterygoplichthys sp.*) is a pest of high impact in aquaculture production systems. Through a biological fermentation process, it could be used as a source of protein for dairy cows. However, milk palatability and smell could be limiting factors. **Objective:** to evaluate the quality of milk from cows supplemented with biological fish silage (*Pterygoplichthys sp.*) as a protein source. **Methods:** The treatments (T) evaluated were T1, 0% biological fish silage; T2, 10% biological fish silage; and T3, 20% biological fish silage. Twelve randomly selected cows were used in a Latin square experimental design, in which three treatments were tested with all of the cows during three time periods. Each period lasted 20 days (15-day adaptation period and 5-day experimental phase). Milk was analyzed for physicochemical, microbiological, sanitary condition and sensory characteristics. Analyses of variance were performed for all the response variables. **Results:** No significant differences for physicochemical variables were found among the treatments studied. Differences were observed in microbiological and sanitary variables among treatments, but values were in the range for high quality milk standards (<100,000 CFU mL^{-1} aerobic mesophilic bacteria, and <400,000 somatic cells mL^{-1}). In the sensory analyses, panelists did not detect strange odors nor fishy taste or odor in the milk of any of the treatments. **Conclusion:** Biological fish silage can be included up to 20% as a protein source in supplements for lactating cows.

Keywords: *cow milk; devil fish; dual purpose cows; feed supplement; fish silage; milk quality; protein source; protein supplement.*

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Resumen

Antecedentes: el pez diablo (*Pterygoplichthys sp.*) es una plaga de alto impacto en los sistemas de producción acuícolas. A través de los procesos de fermentación biológicos podría ser utilizado como fuente de proteína en los suplementos para vacas en producción. Sin embargo, el sabor y olor a pescado en la leche pudiera ser una limitante. **Objetivos:** evaluar la calidad de la leche de vacas suplementadas con ensilaje biológico de pez diablo como fuente de proteína. **Métodos:** los tratamientos (T) evaluados fueron T1, 0% de ensilaje biológico de *Pterygoplichthys sp.*; T2, 10% de ensilaje biológico de *Pterygoplichthys sp.*; y T3, 20% de ensilaje biológico de *Pterygoplichthys sp.*. Se utilizaron doce vacas seleccionadas al azar en un diseño experimental de cuadrado latino. Cada período tuvo una duración de 20 días (período de adaptación de 15 días y fase experimental de 5 días). La leche fue analizada para determinar su condición fisicoquímica, microbiológica, sanitaria y sensorial. Se realizaron análisis de varianza para todas las variables. **Resultados:** no se encontraron diferencias significativas entre los tratamientos estudiados para las variables fisicoquímicas las cuales estuvieron dentro de los estándares de leche de mayor calidad. Se observaron diferencias en las variables microbiológicas y sanitarias entre los tratamientos, pero los valores estuvieron dentro del rango para los estándares de leche de mayor calidad (<100.000 UFC ml^{-1} de bacterias mesofílicas aeróbicas y <400.000 células somáticas ml^{-1}). Los análisis sensoriales no detectaron olores extraños, ni olor ó sabor a pescado en la leche de los tratamientos estudiados. **Conclusiones:** se puede incluir hasta 20% de ensilaje biológico de *Pterygoplichthys sp.* como fuente de proteína en los suplementos de vacas en producción.

Palabras clave: *calidad de la leche; ensilado de pescado; fuente de proteína; leche de vaca; pez diablo; suplemento alimenticio; suplemento proteico; vacas doble propósito*.

Resumo

Antecedentes: o peixe cascudo (*Pterygoplichthys sp.*) é uma praga de alto impacto nos sistemas de produção aquícola. Por meio de processos de fermentação biológica poderia ser usado como fonte de proteína em suplementos para vacas em produção, porém o sabor e cheiro de peixe no leite podem ser uma limitação. **Objetivos:** avaliar a qualidade do leite de vacas suplementadas com silagem biológica de peixe cascudo como fonte de proteína. **Métodos:** os tratamentos (T) avaliados foram T1, 0% da silagem biológica de *Pterygoplichthys sp.*; T2, silagem biológica a 10% de *Pterygoplichthys sp.*; e T3, silagem biológica a 20% de *Pterygoplichthys sp.* Doze vacas selecionadas aleatoriamente foram utilizadas em um delineamento experimental de quadrado latino. Cada período durou 20 dias (período de adaptação de 15 dias e fase experimental de 5 dias). O leite foi analisado para determinar sua condição físico-química, microbiológica, sanitária e sensorial. Análises de variância foram realizadas para todas as variáveis. **Resultados:** não foram encontradas diferenças significativas entre os tratamentos estudados para as variáveis físico-químicas, os valores estavam dentro dos mais altos padrões de qualidade do leite. Observaram-se diferenças nas variáveis microbiológicas e sanitárias entre os tratamentos, mas os valores estavam dentro dos limites para os mais altos padrões de leite de qualidade (<100.000 CFU ml^{-1} de bactérias mesofílicas aeróbicas e <400.000 células somáticas ml^{-1}). As análises sensoriais não detectaram odores estranhos, nem cheiro ou sabor de peixe no leite dos tratamentos estudados. **Conclusões:** a silagem biológica de peixes pode ser incluída em até 20% como fonte de proteína em suplementos de vacas em produção.

Palavras-chave: *alimentos protéicos; fonte de proteína; leite de vaca; peixe cascudo; qualidade do leite; silagem de peixe; suplemento de proteína; vacas de duplo propósito.*

Introduction

Milk yield in dual-purpose cattle production systems is limited by the low availability and poor quality of pastures, so the use of energy and protein supplements is required to increase production (Garduza-Arias *et al.*, 2013). Protein is the most deficient and expensive nutrient, hence the importance of developing economically feasible technologies to produce foods using local resources. Devilfish (*Pterygoplichthys sp.*) is a pest with high environmental and social impact in aquaculture production systems (Pérez and Iglesias, 2016). It can be included as a protein source in supplements for lactating cows through anaerobic fermentation using lactic bacteria and carbohydrates (biological silage). However, the resulting fishy taste and smell in milk could limit its use.

Milk is one of the most important foods for humans. Milk quality involves several components (total solids, proteins, fats, vitamins, and minerals), and absence of disease-causing bacteria, chemical residues and inhibitors, especially antibiotics and unpleasant odors and flavors (Park *et al.*, 2013). It is well known that the type of food ingested by dairy cows can affect milk composition. Supplementation with fish-based ingredients may cause changes in milk fatty acids composition and aromatic compounds that could affect milk sensory and physicochemical characteristics. Therefore, the objective of this study was to evaluate the effect of biological silage of fish as a source of protein on the microbiological, sanitary, physicochemical and sensory characteristics of milk.

Materials and methods

Geographic location of the study area

The study was conducted in a cattle operation in the community of Vicente Guerrero, in Jalpa de Méndez, Tabasco, Mexico (18° 25' N and 18° 04' W, at 10 m altitude). The climate is warm and humid, with abundant rains during summer, when average high and low temperatures fluctuate between 30.5 and 22.5 ºC, respectively. Annual rainfall is between 1,500 and 2,000 mm.

Animals, treatments and experimental design

Twelve cross-bred cows (Zebu x Holstein and Zebu x Swiss) were selected, considering the body weight and days in lactation. Liveweight was 457.08 ± 43.61 kg, and the number of days of lactation was 45.08 ± 13.88 d. The cows were randomized in a Latin square design, in such a way that three treatments were tested on all the cows during three periods of time. The columns comprised the cows and the rows were periods.

Each period lasted 20 d (15 d for the adaptation phase, and 5 d for the experimental phase). The treatments (T) evaluated were T1, 0% fish silage (0FS); T2, 10% fish silage (10FS); and T3, 20% fish silage (20FS). The protein source in the control treatment was soybean meal, which was substituted by biological fish silage in the treatments.

Biological fish silage

Fresh fish caught (*Pterygoplichthys sp.*) were washed, decapitated, and ground in a Rayken RKP2000B C/Motor Husky 6.5 HP grinder (Rayken, Puebla, Mexico). Thereafter, a mixture of 60% fish, 20% molasses, and 20% microbial inoculum was prepared. The fermentation lasted 15 d in a hermetically sealed 200 L plastic tank (Figure 1). The mixture was stirred in the mornings of the first 5 days.

The microbial inoculum was previously obtained by fermentation in a liquid state medium consisting of 4% soybean meal, 4% rice bran, 15% molasses, 0.5% mineral salts, 0.5% urea, 0.3% magnesium sulfate, 5% natural yogurt (Yoplait®), and 70.7% water in a 200 L tank. The mixture was stirred four times per day. After three days, microbial inoculum traits were: pH 4.1, 81.8% moisture, 24.9% crude protein, and log10 8.97 colony forming units (CFU) of lactic acid bacteria per mL, and was ready to be used in the preparation of fish silage.

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Figure 1. Fermentation tank used to prepare the biological fish silage.

Preparation of supplements (treatments)

The supplements were prepared with the ingredients shown in Table 1. Once the ingredients were mixed, they were packed in 30 kg plastic bags to be preserved anaerobically. Supplements were formulated to be isoproteic (18% CP), and isoenergetic (2.8 Mcal kg^{-1} DM) using the "Solver" software from Microsoft Excel.

Chemical analysis of the supplements (treatments)

Dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash were determined following Latimer´s (2016) methods. Degradation of dry matter (DDM) was determined following the methodology described by Naranjo and Cuartas (2011).

Animal handling

The cows were milked twice a day (5:00 h and 16:00 h) using a milking machine. At each milking, each cow was offered the respective supplement (3 kg wet basis) treatment. After milking, the cows were kept in an enclosure where they had free access to water and sorghum silage, which contained 27.67 ± 1.5% DM, 8.9 ± 0.91% CP, 61.1 ± 3.6% NDF, 35.1 ± 3.09% ADF, and 8 ± 1.0% ash.

Milk sampling

In the experimental phase (day 15-20), three milk samples of different size were taken, according to analysis to determine milk quality. The first, for microbiological and sanitary analyses on the first day, a second for sensory tests on the third day, and a third for physicochemical analyses on the fifth day. All samples were kept refrigerated at 4 °C before analysis. For the microbiological analysis, 100 ml milk was collected by manual milking into Whirl-Pak® bags (Sigma, St Louis, MO, USA). For the sensory tests, the milk samples (from four cows per treatment) were taken by mechanized milking and bottled in 5-L glass jars. For the physicochemical analysis, 100 ml milk samples were taken into Whirl-Pak® bags by individual mechanical milking.

Table 1. Ingredients (%) used to make the supplements (wet basis for the treatments).

Ingredients	0% fish silage	10% fish silage	20% fish silage
Fish silage	0.0	10.00	20.00
Ground corn	40.33	39.26	38.19
Soybean meal	17.00	11.29	5.58
Wheat bran	28.17	24.95	21.73
Molasses	5.00	5.00	5.00
Microbial inoculum	5.00	5.00	5.00
Urea	0.50	0.50	0.50
Magnesium sulfate	0.50	0.50	0.50
Mineral salts	2.50	2.50	2.50
Common salt	1.00	1.00	1.00
Calculated chemical composition			
Dry mater (%)	84.30	79.89	75.49
Crude protein (%)	18.00	18.00	18.00
ME (Mcal kg^{-1} DM)	2.80	2.80	2.80

ME= Metabolizable energy.

Microbiological, sanitary and physicochemical analysis of milk.

The presence of *Salmonella spp* was assessed with the Ridascreen® *Salmonella* kit. (R-Biopharm AG®, Darmstadt, Germany). Aerobic mesophilic bacteria, total coliforms, and *Escherichia coli* were counted using the kits RIDA® Count total, RIDA® Count coliform, and RIDA® Count E. coli (R-Biopharm AG®, Darmstadt, Germany), respectively. Somatic cells (SC) were counted with DeLaval® cell counter DCC (DeLaval®, International AB, Tumba, Sweden), and the presence of antibiotic residues in milk was determined with the Delvotest® SP-NT kit (DSM® Food Specialties, Delft, Netherlands). Physicochemical composition of milk was determined with the Lactoscan® Milk Analyzer (Milkotronic® LTD, Nova Zagora, Bulgaria).

Sensory evaluation

Sensory evaluation of milk was conducted in the Sensory Evaluation Laboratory (DACA-UJAT) by eight trained panelists. The panelists were trained with samples of freshly milked and pasteurized milk and whole commercial milk. The results of each session subjected to analysis of variance (ANOVA) to detect differences between judges. Once the analysis did not indicate differences between judges, the treatments were evaluated. Prior to sensory evaluation, milk was pasteurized (65 ºC for 30 minutes), and stored at 5 ºC before use.

During each evaluation period, triangle-type discriminatory tests were conducted to evaluate differences among the three treatments (0FS vs 10FS, 0FS vs 20FS and 10FS vs 20 FS). In addition, a quantitative descriptive analysis (QDA) was performed to evaluate the intensity of sensory attributes of the samples of each treatment. The descriptors evaluated were those reported by Citalán *et al.* (2016), and “fish smell” and “fish taste” as additional descriptors. Descriptors and definitions are shown in Table 2. Those descriptors had been analyzed and discussed in a working session with the judges to clarify doubts and define the terms by consensus, as shown in Table 2.

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Table 2. Definitions of attributes generated by quantitative descriptive sensory analysis.

Sensory characteristics	Descriptors	Definitions of attributes	Anchor terms
Appearance	Appearance	Characteristic color, appearance and consistency of whole cow's milk.	Poor- optimal
	Characteristic color	Opalescent, white or yellowish-white color characteristic of whole cow's milk	Poor- optimal
Odor	Characteristic smell	Characteristic smell of whole cow's milk	Zero - extreme
	Strange odor	Uncharacteristic odor from whole milk	Zero - extreme
	Herbal odor	Scent of freshly cut grass	Zero - extreme
	Fruity odor	Fruit scent	Zero - extreme
	Sour or fermented odor	Related to a bitter, cheesey and slightly butyric odor, similar to baby vomit	Zero - extreme
	Fishy odor	Fishy odor	Zero - extreme
Flavor	Characteristic flavor	General term associated with the own notes of dairy products made with whole cow's milk	Poor- optimal
	Whole milk	Related to the taste of commercial packaged cow's milk	Poor- optima
	Cows milk	Related to freshly milked cow's milk	Poor- optimal
	Milk cream	Smell of fresh milk cream	Zero - extreme
	Strange	Uncharacteristic taste of milk	Zero - extreme
	Sweat	Basic sensation or taste of sucrose dissolved in water	Zero - extreme
	Medicinal	Chemical flavor	Zero - extreme
	Methalic	Chemical sensation on the tongue, associated with iron, copper, and/or silver spoons	Zero - extreme
	Rancid	Stale flavor	Zero - extreme
	Lack of freshness	Flavor of "cardboard" or associated with the packaging material. Related to the impression that the product has absorbed odors and flavors from other products during storage	Zero - extreme
	Bitter	Basic taste whose reference is caffeine dissolved in water. Relating to notes associated with fermented milk or broken protein	Zero - extreme
	Hay or fodder	Taste of silage, dried alfalfa, dried grains, or livestock feed	Zero - extreme
	Cooked milk	Notes of boiled milk	Zero - extreme
	A milk fat	Aromatics associated with milk fat	Zero - extreme
	Fish	Fishy flavor	Zero - extreme
Mouthfeel	Consistency	Related to movement and product on the tongue and perception in the mouth as a result of fat and dissolved solids content	Poor- optimal
	Thick consistency	Related to the perception of a high content of fat and dissolved solids that decreases the fluidity of the product on the tongue and in the mouth	Zero - extreme
	Fluid or watery consistency	Related to a greater fluidity or movement of the product in the mouth as a result of a watery and bland consistency due to a lower content of dissolved solids and fat in the product	Zero - extreme

Preparation and evaluation of samples for the descriptive test

Samples (40 mL) were served in glasses coded with three-digit random numbers. The panelists were instructed to evaluate the samples in the order of the attributes on a 10 cm non-structured linear scale ranging from null to optimal or null to intense. Intensity was indicated by placing a mark on the unstructured 10 cm linear scale, anchored with the terms "poor" and "optimal" or "null" and "intense" at each end. The answers were quantified by measuring the distance in centimeters from the extreme left to the mark indicated by the panelist. All samples were kept at 12 ºC for evaluation. Between samples, the judges drank natural water to eliminate effects from the previous sample.

Statistical analyses

An analysis of variance and Tukey tests of multiple comparisons of means were performed for each of the microbiological composition, sanitary, physicochemical and sensory (QDA) milk variables using SAS® 9.4 (2017). To normalize the data, values of aerobic mesophilic bacteria and somatic cells were transformed to Log10, and the values of total coliforms were transformed to Log10 [Y+1]. The data from the discriminatory tests were analyzed by counting the number of correct answers and comparing them with the minimum number required to establish a significant difference between the samples of the triangular test at a significance level of 0.05 (Roessler *et al.*, 1978).

Results

Chemical analysis of the supplements

Food supplements used in this study (Table 1) were calculated to be isoprotein (18 % PC), and isoenergy (2.8 Mcal kg^{-1} DM). However, when evaluating the chemical composition, the PC content of the 0FS treatment was higher ($p<0.05$) compared to treatments 10FS and 20FS (Table 3), the moisture content had a linear increase ($p<0.05$) in relation to the percentage of fish silage inclusion in the supplement, and treatment 20FS had the highest ($p<0.05$) percentage of ash relative to 10FS and 20FS; in all treatments, pH was less than 4, with no differences between treatments, due to the addition of 5% of the microbial inoculum in all treatments.

Feed intake

There were significant differences in supplement intake ($P<0.05$) on dry basis. Intake was highest for cows with 0% fish silage and lowest for cows that consumed the 20% treatment (Table 4). According to the total consumption of supplements on a dry basis, the consumption of devil fish silage was 0.469 and 0.908 kg $animal^{-1}$ d^{-1} for the 10FS and 20FS treatments, respectively.

Table 3. Chemical composition, and pH of supplements.

Treatments	Moisture (%)	Protein (%)	Ash (%)	pH
T1: 0% fish silage	13.31^{c}	18.54^{a}	7.83^{b}	3.60^{a}
T2: 10% fish silage	16.56^{b}	17.07^{b}	8.27^{b}	3.66^{a}
T3: 20% fish silage	20.28^{a}	16.77^{b}	9.22^{a}	3.79^{a}
SE±	1.220	0.225	0.050	0.103

Means within the same column with different superscript letters ($^{a, b}$) are statistically different ($P<0.05$).

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Table 4. Supplement intake on wet and dry basis.

Supplement intake	Fish silage			SE±
	0FS	10FS	20FS	
Wet base (kg cow d^{-1})	5.86^{a}	5.62^{a}	5.7^{a}	0.176
Dry basis (kg cow d^{-1})	5.08^{a}	4.69^{b}	4.54^{c}	0.223

Means within the same column with different superscript letters ($^{a, b}$) are statistically different (P < 0.05).

Milk analysis

Aerobic mesophilic bacteria were less than 100,000 CFU mL^{-1} for all the treatments. The number of coliforms was also low (Table 5). Neither *Salmonella* nor *E. coli* were detected in any sample. Low numbers of somatic cells were found, without significant difference for the three treatments (P<0.05). There were no residues of antibiotics or antimicrobials in milk from the animals studied, according to the Delvotest (Table 5). No significant differences (P>0.05) were found among treatments for contents of fat, protein, lactose, or non-fat solids (Table 5). Milk fat content was >31 g L^{-1} in the three treatments.

Sensory analysis of milk

The triangle sensory test did not reveal significant differences (P>0.05) between milk samples from 0FS and 10FS. However, there was a significant difference (P≤0.05) comparing 0FS vs 20FS; and between the 10FS vs 20FS (Table 6).

Table 5. Indicators of microbiological, sanitary and physicochemical quality of milk from cows supplemented with fish silage.

Indicators	Fish silage			Mexican norms
	0FS	10FS	20FS	
Microbiological indicators				
Aerobic mesophilic bacteria, CFU ($1x10^5$) mL^{-1}	0.048^{a}	0.016^{b}	0.014^{b}	≤1^{x}
Total coliforms CFU^{-1} g	4.67	3.16	2.13	≤10^{y}
E. coli MPN*	0	0	0	≤3^{y}
Salmonella spp.	Absent	Absent	Absent	Absent^{y}
Sanitary indicators				
Somatic cells, SC ($1x10^5$) mL^{-1}	0.316^{ab}	0.186^{b}	0.46^{a}	≤4^{x}
Antimicrobials, Delvotest	Negative	Negative	Negative	Negative^{x}
Physical indicators				
Density, g mL^{-1}	1.032	1.031	1.031	≥1.029^{z}
Freezing point, °C	-0.59	-0.60	-0.59	-.51 y -.54^{z}
Water	0	0	0	____
Chemical indicators				
Fat, g L^{-1}	39.2	34.0	36.8	≥30^{z}
Non-fat solids, g L^{-1}	90.4	92.0	90.2	≥83^{z}
Protein, g L^{-1}	34.1	34.6	34.0	≥30^{z}
Lactose, g L^{-1}	49.5	50.5	49.4	≥43 y ≤52^{z}

Means within the same column with different superscript letters ($^{a, b}$) are statistically different (P< 0.05).
*MPN= Most probable number; ^{x}NMX-F-700-COFOCALEC-2012; ^{y}NOM-243-SSA1-2010; ^{z}NOM-155-SCFI-2012.

Table 6. Number of correct answers with the triangle test during evaluation of milk from cows supplemented with biological fish silage.

Treatments	Number of correct answers/total number of questions	*P-Value
0FS vs 10FS	28/75	>0.05
0FS vs 20FS	44/75	<0.05
10FS vs 20FS	42/75	<0.05

*(P<0.05) according to Roessler *et al.* (1978).

In the quantitative descriptive analysis, 25 out of 26 of the descriptors did not differ statistically among treatments (Figure 2). The descriptor "creamy milk taste" of the QDA was significantly more intense in the samples from 20FS, relative to the intensity observed in 10FS and 0FS. The grade for typical milk taste was high (≥8) for the 3 treatments. Other descriptors such as "strange odor" or "fish odor" had very low grades (≤1) for all the treatments.

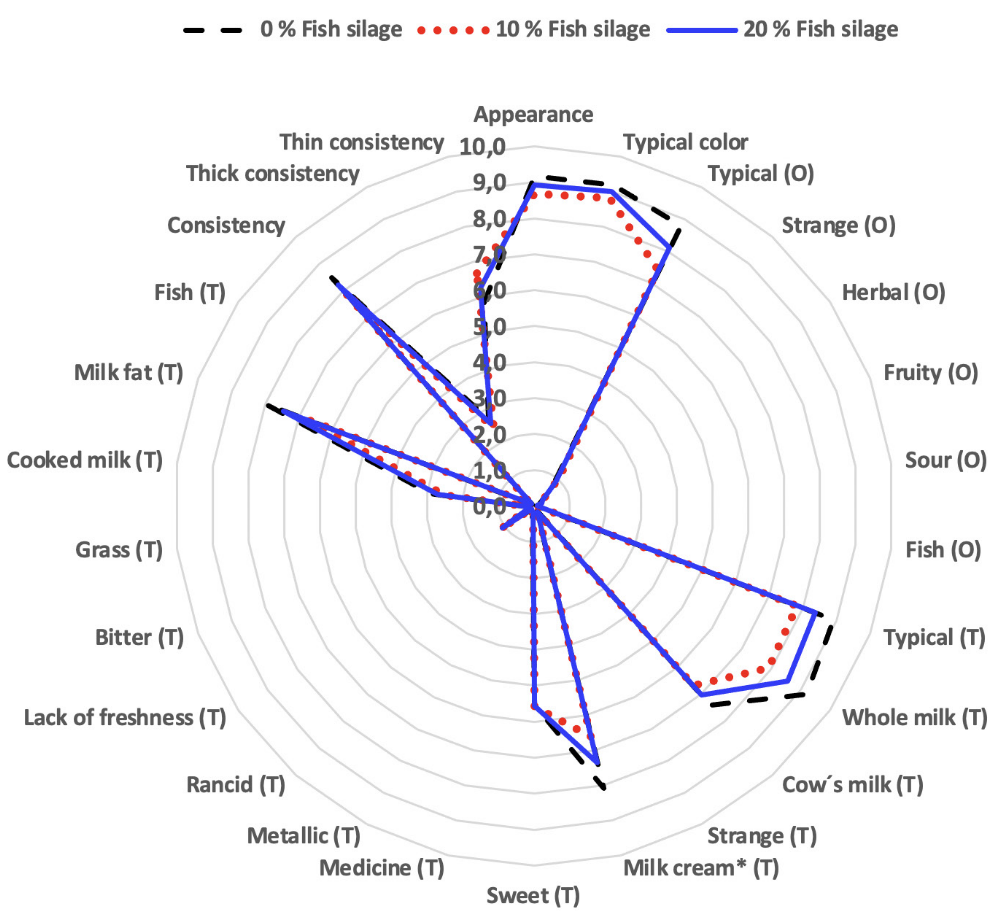

Figure 2. Sensory characteristics of milk from cows supplemented with biological fish silage as source of protein. (O) Odor; (T) Taste; (*) Statistical difference (P<0.05).

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Discussion

Perez (1995) reported that fish silage intake increased milk yield of Holstein cows in Cuba; however, that researcher did not include information on milk quality assessment. Milk of all the treatments in the present study was classified as "grade A" because it had <100.000 CFU mL^{-1} of mesophilic microbes, it had a low number of somatic cells, and no antimicrobials were detected in the Delvotest (Table 5). This quality is generally associated with good dairying farming practices during milking and sampling. Fat in the 3 treatments was higher than 32 g L^{-1}. Avramis *et al.* (2003) reported that Holstein cows fed on maize and fish meal as feed supplements produced milk that contained 24.3 g L^{-1} fat, which is lower than that found in our study. Cows fed on diets supplemented with fish meal usually reduce milk fat content and total production of dairy fat (Wright *et al.*, 2003). Spain *et al.* (1995) indicated that the percentage and yield of milk fat decrease with a higher intake of fish meal. Keady *et al.* (2000) reported that the higher the quantity of fish oil in the diet, the lower the percentage of fat and protein in the milk. In our study, the percentage of fat and protein in milk were not affected by the inclusion of fish silage. It is important that the supplements used to feed cows are associated with high-fat content in milk because fat it is one of the most economically valued components of dairy products. The price of milk increases with higher fat and protein contents (Wolter *et al.*, 2004).

Protein content in the milk from the three treatments was within the levels specified in CLASS A, because it contained >31 g L^{-1} (NOM-155-SCFI-2012, NMX-F-700-COFOCALEC-2012). Avramis *et al.* (2003) reported that Holstein cows fed a fish meal-based supplement produced milk with 33.2 g L^{-1} protein. When fish oil is included in the diet, protein content tends to be lower than that produced with other diets (Fatahnia *et al.*, 2008).

Avramis *et al.* (2003) stated that one of the main disadvantages of fish-based feed for ruminants is the fishy taste it produces in milk or meat, which is associated with supplements that contain fatty acids from ingredients such as fish oil or meal. However, we demonstrated that supplementation with biological fish silage as the protein source did not generate fish odor or fish taste in milk. Moreover, there were no differences between treatments associated with the other sensory attributes evaluated in the ADC (Figure 2), except for the "creamy milk taste". Shingfield *et al.* (2003) reported that fish oil in dairy cow diets contributes to changes in the composition of fatty acids in the milk. These changes in fatty acid composition could be related to a higher intensity of the "creamy milk taste" descriptor perceived by the judges. Avramis *et al.* (2003) did not find differences in the analyses of quality and taste between the milk from Holstein cows supplemented or un-supplemented with 60% herring (*Clupea* spp.) meal. The Holstein cows had been supplemented with fish meal as 4.5% of their total dry-matter intake.

Descriptors related to negative effects that supplementation of fish ingredients could have on sensory characteristics of milk, such as fish taste or odor, were included in the QDA. Chilliard *et al.* (2001) stated that changes in dairy cow diets could change milk taste, color, vitamin content, oxidative stability, proteins, and processing costs. Bragaglio *et al.* (2015) reported that milk from cows fed fish oil (protected or unprotected) was more susceptible to oxidation, which affects sensory properties. Indeed, oxidation of polyunsaturated fatty acids produces a complex mixture of volatile products of secondary oxidation, some of which produce unpleasant flavors. However, in our study, no increase or significant change in unpleasant or strange odors or flavors was observed; panelists did not perceive fish odor or taste in any of the evaluated samples (Table 6). Otherwise, no difference was observed relative to the consistency or general aspect of the product (Figure 2).

In conclusion, fish silage can be used up to 20% as a source of protein in supplements to produce high-quality milk. The fish silage in the

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supplement did not affect the microbiological, sanitary, physicochemical or sensory characteristics of the milk.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

Raquel Castillo carried out the experiments with the cows, collected and analyzed samples in the laboratory for her master's thesis and wrote the manuscript. Adolfo Bucio, supervised the microbiological and physicochemical analysis of milk, and did the English writing. Rosa Salinas planned and carried out the sensory test. Emilio Aranda contributed with the laboratory analysis of feeds. Francisco Izquierdo contributed to statistical analysis. Jesús Ramos directed the general research and the analysis of results.

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