

DIET AND BIOCLIMATIC CONDITIONS ON PRODUCTION AND MILK QUALITY¹

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¹Received: 14/11/2017. Accepted: 09/02/2018.

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ABSTRACT: It was aimed to analyze the productive performance of lactating cows on isoprotein fed diets, at differentiated environmental conditions. Eight Holstein cows were used, grouped in two 4 × 4 balanced Latin squares design. The treatments were evaluated in 2 × 2 factorial designs: sources of roughage (corn silage - CS plus concentrate, and the combination of corn silage with sugarcane - CSSC, 1:1 on DM, plus concentrate) and distinct environment (with= WS and without= OS, fan and nebulizers system= Sfn). The estimation of dry matter intake (DMI), productive performance and physicochemical parameters of milk were evaluated. There was no interaction effect of environment factors and source of forage. There was significant effects for the source forage factors, where the DMI for the CSSC based diet was higher than the CS based diet in the effect of forage (4.22 vs. 4.06% BW, and 22.3 vs. 21.7 kg/d, respectively, P≤0.05), but with similar milk production correcting 3.5% fat (23.01 vs. 22.62, CSSC and CS; 22.85 vs. 22.78 kg/day, WS and OS, respectively, P≥0.05). The feed efficiency and conversion was similar in both factors (102.8 vs. 104.7% and 0.99 vs. 1.0, CSSC and CS; 102.5 vs. 104.9% and 1.0 vs. 0.99, WS and OS, respectively, P≥0.05). The benefit-diet cost ratio was higher for CS-based diet than for CSSC (7.44 vs. 6.97, P≤0.05). There were effects only in the forage factor for CP milk (3.26 vs. 3.23%, P≤0.05), lactose (4.54 vs. 4.49%, P≤0.05) and urea nitrogen in milk (23.21 vs. 20.71 mg/dL, P≤0.05) and the superiority arising from the CSSC-based diet in comparison to the CS diet. There was higher for T and THI (28.1 vs. 23.6°C and 75.1 vs. 71.1, respectively, P≤0.05), and lower RH to 2:00 pm (47.7 vs. 64.5%, P≤0.05). The linear score showed negative correlations with DMI, milk production, lactose and urea nitrogen (-0.36, -0.69, -0.44 and -0.32, P≤0.05, respectively). The use of the diet based on CSSC proposed increases in DMI and milk quality without affecting production, but with smaller benefit-diet cost ratio. The use of the environmental cooling system did not improve the production, qualitative parameters of milk and thermal comfort of dairy cows under the conditions evaluated.

Key words: environment, roughages, intake, nutrition, ruminant.

DIETAS E CONDIÇÕES BIOCLIMÁTICAS SOBRE A PRODUÇÃO E QUALIDADE DO LEITE

RESUMO: Objetivou-se avaliar o desempenho produtivo de vacas em lactação alimentadas com dietas isoproteicas, em condição diferenciada de ambiência. Foram utilizadas oito vacas da raça Holandesa, agrupadas em dois quadrados latinos 4 × 4 balanceados. Os tratamentos foram avaliados em esquema fatorial 2 × 2: fontes de forragens (silagem de milho - SM e concentrado, e combinação de cana-de-açúcar com silagem de milho - CSM, 1:1 na MS, e concentrado) e ambiente distintos (C= com e S= sem, sistema de ventilador e nebulizadores= vn). Avaliou-se a estimativa da ingestão de matéria seca (IMS), desempenho produtivo e parâmetros físico-químicos do leite. Não houve efeito de interação de fatores de ambiência e fonte de forragem. Houve efeitos significativos para o fator fonte de forragem, onde o DMI para a dieta baseada em CSM foi maior do que a dieta baseada em SM (4,22 vs. 4,06% do PC, e 22,3 vs. 21,7 kg/dia, P≤0,05), mas com produção de leite

corrigido para gordura semelhante (23,01 vs. 22,62, CSM e SM; 22,85 vs. 22,78 kg/dia, Cvn e Svn, respectivamente, $P \geq 0,05$). A conversão e eficiência alimentar foram semelhantes para efeito de forragem e de ambiente (102,8 vs. 104,7% e 0,99 vs. 1,0, CSM e SM; 102,5 vs. 104,9% e 1,0 vs. 0,99, Cvn e Svn; respectivamente, $P \geq 0,05$). A relação benefício-custo da dieta foi maior para a dieta baseada em SM do que para CSM (7,44 vs. 6,97, $P \leq 0,05$). Houve efeito significativo somente para o fator forragem para PB do leite (3,26 vs 3,23%, $P \leq 0,05$), lactose (4,54 vs 4,49%, $P \leq 0,05$) e nitrogênio uréico do leite (23,2 vs 20,7 mg/dL, $P \leq 0,05$) e a superioridade decorrente da dieta baseada em CSM em comparação com a dieta SM. Houve maior temperatura ambiental e índice temperatura umidade (28.1 vs. 23.6°C e 75.1 vs. 71.1, respectivamente, $P \leq 0,05$) e menor umidade relativa do ar as 14:00 h (47.7 vs. 64.5%, $P \leq 0,05$). O escore linear do leite apresentou correlação negativa com IMS, produção de leite, lactose e nitrogênio uréico (-0,36, -0,69, -0,44 e -0,32 $P \leq 0,05$, respectivamente). O uso da dieta a base da CSM aumentou a IMS, alterou a qualidade do leite sem afetar a produção, mas com menor relação benefício-custo da dieta. O uso do sistema de resfriamento ambiental não melhora os parâmetros produtivos, qualitativos do leite e conforto térmico das vacas leiteiras nas condições avaliadas.

Palavras-chave: ambiente, consumo, forragem, nutrição, ruminantes.

INTRODUCTION

Sustainable animal production implies the use of resources aimed at increasing the efficiency of the system, with lower cost and without environmental and social damage. In this context, additional roughage produced in order to meet the demand for food in times of pasture scarcity is essential for the maintenance of ruminants, which was implicitly mentioned by Guyader et al (2016) and Wilkinson and Lee (2017).

Corn silage is the forage most used, however the cost is limiting for all categories of animals. An alternative would be the use of sugarcane mixed with corn silage, mainly because it is forage of lower cost, high dry matter production and widely used in ruminant production. Oliveira et al. (2012) states that sugarcane has achieved great interest from farmers because of the high dry matter production at a low production cost per unit area in comparison to corn silage.

Studies by Biondi et al. (1978) mentioned that the partial substitution of corn silage for sugarcane in the diets of lactating cows caused a linear reduction in milk production, but affirm if properly adjusted the diet could replace up to 50% of corn silage. Magalhães et al. (2004) found a linear decrease in dry matter intake and in milk production without changing the composition of the milk by using a mixture of sugarcane/corn silage. Accordingly, Pires et al. (2010) also showed better milk production results and concentrations of volatile fatty acids by animals fed a diet based on the mixture of sugarcane/corn silage (50:50% DM).

With that same focus, Campos et al. (2001, 2002, 2004a) had already confirmed that the mixture of sugarcane/corn silage (50:50, %DM) promoted an improved fermentation pattern in the ruminal fluid and *in vitro* digestibility of DM, due to the soluble carbohydrates and starch in amounts that did not cause damage to the *in vitro* environment. And, Canizares et al. (2014) showed a quadratic response for DMI, with a minimum point of 2.14 kg/day for a 68.04% inclusion of sugarcane in replacement of corn silage in the diet of dairy goats. However, it did not affect NDF intake, feed efficiency, rumination efficiency and rumination number to diet with roughage:concentrate ratio of 40:60%.

However, the quality of the diet is not the only factor that interferes in the production of dairy cows. There are other factors related to the environment in which they are being managed. According to Yousef and Johnson (1985), the main reason for the decline in milk production in warmer weather is the reduction in food intake by the animal in order to minimize the thermal imbalance and to maintain homeothermy. Kadzere et al. (2002) mentions that an increase in food intake results in an increase of heat and the animal's organism thus requires thermoregulatory mechanisms effective enough to maintain body temperature in a thermoneutral zone and physiological homeostasis. Wheelock et al. (2010) states that thermal stress in animals increases their need for maintenance due to an increase in energy needed for heat loss through sweating and breathing.

Under these conditions, more balanced diets of

a higher quality combined with the improvement of thermal comfort, using factors such as fans and nebulizers, would be an important tool in minimizing energy expenditure in order to maintain homeothermy and improve production efficiency.

This work was carried out with the goal of evaluating the productive performance of lactating cows on isoprotein fed diets. The diets were based on corn silage or a mixture of sugarcane with corn silage and concentrates, which were formulated to meet the production needs and the quality of milk under different environmental conditions, as well as the study of correlations.

MATERIAL AND METHODS

Experiment location and conditions

The experiment was conducted in Nova Odessa, SP, Brazil, at the coordinates 22°33'02" South latitude and 47°38'05" west longitude at an altitude of 550 m. The region has an average annual temperature of 22°C and rainfall of 1200 mm with prevailing southeast winds.

During the period of data collection, from November to February, there was 571 mm of rainfall. The average maximum temperature was 30.9°C and the minimum was 18.3°C. The dry bulb temperature (DBT) and relative humidity (RH) collected by a weather station, as a standard local reference ranged from 23°C to 25°C and 57% to 74%, respectively.

Animals and installations

Eight Holstein cows were used (137 ± 10 days in milk, 20 ± 3.0 kg/day) with a mean weight of 534 (± 42) kg. They were grouped into two lots and housed in free stall confinement adapted with a center partition made from a waterproof canvas in order to separate the environment, with (WS) fan and nebulizers system (Sfn) and without (OS) Sfn, respectively. The installation was divided into eight stalls by chains, four stalls for each environment. The space for the animals was 5.0 x 2.20 m, and had both troughs and automatic waterers and access to two beds made with fine washed sand for resting.

The *Freestall* confinement type used had the following dimensions and characteristics: 36 m length and 12 m in width, sides open, east-west orientation, a circulation corridor of 2.9 m, ceiling height of 3.80 m, gable roof covered with clay tile and rustic concrete flooring.

The nebulization system was set 2.5 m above the centerline, between the feeders and sand

bed. It consisted of PVC tube, with five nebulizer nozzles spaced at 1.8 m. The flow of water in the sprinkling line was 30 L/hour where there was Sfn. The nebulizers operated intermittently, with automated activation every 7 minutes and working for 1 min at a time to prevent floor flooding. The fan had a diameter of 0.9 m, with a 1/4 HP (horse power) motor and a flow of 300 m³/hour and 495 RPM (rotation per minutes). It had the capacity to produce air movement of up to 2.5 m/s at the height of the animal's back. The temperature sensor, located in a neutral area and connected via cable to the operation center, activated the automated ventilation and nebulization when the temperature reached 22°C and turned off when it was lower.

The entire experimental procedure was approved by the Animal Experimentation Committee, Institute of Zootecny IZ/APTA, Ministry of Agriculture and Supplies of Sao Paulo, Brazil, under the number 089.

Characterization of the environmental properties

During the experimental period, the dry bulb temperature (environmental temperature= ET, °C) and relative humidity (RH) via thermo-hygrometer data logger, Text-171®, metallic, installed in the central portion of each experimental area, were registered. Subsequently, the index of temperature and humidity (ITH) was calculated as according to Johnson (1980), dew point temperature (DPT) and enthalpy (kcal/kg dry air). Was adopted the classification system proposed by Renaudeau et al. (2012) in which THI<68 no heat stress, heat stress threshold 68 to 71, mild-moderate heat stress 72 to 79, moderate-severe stress 80 to 89, severe heat stress 90 to 98.

It was defined that temperatures below 21°C (cold), between 21 and 27°C (comfort) and higher than 27°C (hot) as according to Igono et al. (1992). And, according to a thermoneutral zone (zone of thermal comfort) proposed by Johnson et al. (1976), critical enthalpy occurs when the T is equal to or greater than 24°C and the RH is equal to 76%. Meteorological data was collected, such as environmental temperature and relative humidity during the experimental phase, in order to characterize the environment.

Experimental diets

The diets were established in accordance with the chemical composition of forages (Tables 1 and 2) and to meet the demands of producing 40 kg of milk to explore the genetic potential of animals and

Table 1. Chemical composition of forages and concentrates ingredients used for the initial formulation of the diet.

Nutrients	Corn silage	Sugarcane chopped	Extruded corn	Wheat bran	Whole cottonseed	Soybean meal
DM, %	30.10	34.50	87.11	88.31	66.13	87.69
MM, % DM	3.95	1.64	1.24	5.48	3.87	6.57
CP, % DM	8.77	1.63	11.39	16.41	24.35	54.18
EE, % DM	1.99	0.89	4.36	4.02	20.13	2.89
NDF, % DM	58.61	50.55	22.32	46.79	50.66	14.82
ADF, % DM	35.18	32.90	15.99	14.77	46.99	10.58
LIG, % DM	4.60	6.18	1.54	4.64	15.72	1.01

DM= dry matter; MM= ash; CP= crude protein, EE= ether extract; NDF= neutral detergent fiber; ADF= acid detergent fiber; LIG= lignin.

Table 2. Proportional relationship of the ingredients in the diet, in % and kg dry matter, the total concentrate, of roughage:concentrate ratio and chemical composition of experimental diets.

Ingredients	Experimental diets			
	CS-based		CSSC-based	
	%	kg	%	kg
Corn silage	55.1	9.6	24.1	4.8
Sugarcane chopped	-	-	23.3	4.7
Mix concentrated ¹	33.5	5.9	42.6	8.5
Whole cottonseed	11.4	2.0	9.9	2.0
Total concentrate offered	44.9	7.8	52.6	10.5
Roughage:concentrate ratio (R:C)	1.23	-	0.90	-
Nutrients				
Crude protein	18.1	-	18.3	-
Ether extract	4.6	-	4.2	-
Neutral detergente fiber	45.6	-	40.5	-
Acid detergent fiber	27.4	-	23.6	-
Non- fibrous carbohydrates	25.9	-	31.2	-
Nutrients digestible total	69.6	-	68.3	-
Net energy, Mcal/kg DM	1.72	-	1.65	-
Calcium, g	0.48	-	0.60	-
Potassium, g	0.32	-	0.32	-
Phosphorus, g	1.17	-	1.14	-

¹Composition of mix concentrated: extruded corn, 45%; soybean meal, 26.5%; wheat bran, 19.5%; lime, 2.5%; sodium bicarbonate, 1.0%; urea, 2.5%; ammonium sulfate 0.5%; mineral salt, 1.5%, salt (sodium chloride), 1.0%. CSSC= corn silage plus sugarcane, 1:1, % DM, plus concentrate (R:C= 47.4:52.6%); CS= corn silage plus concentrate (R:C= 55.1:44.9%).

the quality of milk. The treatments were: CS + WS; CS + OS; CSSC + WS; CSSC + OS; where CS= corn silage plus concentrate, diet based, CSSC= combination of corn silage and sugarcane, 1:1 based on DM%, plus concentrate, diet based; WS= with fan and nebulizers, OS= without fan and nebulizers. The treatments were evaluated in 4 × 4 Latin square, double and simultaneous, in 2 × 2 factorial design, with the following factors: sources of forage and distinct environment conditions.

The experiment began 137 days post-calving. Testing took 80 days, with all animals passing through the four different treatments. Each treatment had 11 days of adaptation to the diets and 9 days of data collection, a total period of 20 days. Diets were established according to the NRC (2001) to meet the requirements of cows producing 40 kg milk/day, with the goal of exploring the genetic potential of animals and milk quality. The productive properties that were evaluated, were the voluntary dry matter intake (DMI, % body weight= BW and kg/day), milk production, production efficiency and the physico-chemical properties of milk. The daily diet cost was estimated for both total daily intake and per kg of DMI. It was also estimated the benefit- diet cost ratio, which is the ratio of milk production to the cost of the diet.

Food management

This consisted of two feeding schedules. In the first period (8:00 am) the total amount of the diet for the day was provided and in the second period (2:00 pm), the revolving of the diet in the trough served to stimulate consumption amongst the animals. The fresh sugarcane was chopped daily and mixed with corn silage (50:50% in DM) and concentrate, at a roughage:concentrate ratio 47.4:52.6%, on a DM basis, maintained along the experiment. The corn silage was produced on the state farm in a trench silo. Corn silage was harvested daily in the silo and offered to the cows maintaining the ratios of 55.1:44.9% roughage:concentrate throughout the experiment.

The amount of DM provided was adjusted weekly, ensuring a minimum of 10% of remnants so as not to limit the voluntary consumption of the diets. The voluntary intake of dry matter (DMI, kg/d) and the milk yield (kg/day) was measured daily and recorded during sampling times. At the end of each 9-day period, the animals were weighed with the intention of estimating feed efficiency, feed conversion and voluntary dry matter intake in relation to the percentage of body weight (%BW).

Processing and Sampling of food

Food samples, composed individually for the period, were collected on a daily basis and both the diets and the roughage were evaluated. They were dehydrated at 55°C in a forced air circulation kiln for 72 hours and then subsequently milled in a knife mill to particle sizes of 1 mm. We conducted the following chemical analysis: dry matter (DM), ash, crude protein (CP), ether extract (EE), according to Campos et al. (2004b), and the fibrous components of the cell wall: neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin and 72% H₂SO₄, according to Robertson and Van Soest (1981), Van Soest et al. (1991).

The proportions and chemical composition of forage and concentrates used in the experimental diets are presented in Tables 1 and 2.

Milking management and milk collection

The animals were milked twice daily (7:00 am and 3:00 pm) in milking machines with a Tandem type structure, with the individual milk production registered. Milk production was corrected to 3.5% milk fat, according to the Sklan et al. (1992) method. During the two daily milking's, individual milk samples were collected within each evaluation period and for five consecutive days. These were placed in 60 mL plastic bottles, with the addition of two tablets Bronopol (2-bromo-2-nitropropane-1,3-diol) and forwarded to the Clinic Milk - ESALQ/USP for estimates of physicochemical parameters. The estimates of the concentrations of fat, crude protein, lactose and total solids of milk (raw milk) were performed by using the infrared absorption equipment (Bentley 2000®, Bentley Instruments, 1995a). The concentration of milk urea nitrogen (mg/dL) was evaluated by the enzymatic method and spectrophotometric trans-reflectance (ChemSpec® 150, Bentley Instruments, 1998). The somatic cell count (SCC) was determined by flow cytometry with the equipment Somacount® 300 (Bentley Instruments, 1995b). The linear score milk (LS) was issued by the Clinical Laboratory Milk - ESALQ/USP, routinely, obtained by transformation of SCC (LS= [Log² (SCC/100)] + 3) as reported by Ali and Shook, (1980).

Statistical Analysis

Initially, the data was analyzed for homogeneity variance and normality of residuals, to meet the assumptions of variance analysis. When necessary, data transformations were conducted for each

variable and the models were added in foot note tables.

The dependent variables analyzed were: voluntary dry matter intake (kg/day and %BW, body weight), feed conversion, feed efficiency, milk production, milk production corrected to 3.5% fat and milk quality (crude protein, fat, lactose, total solids, urea nitrogen, somatic cell count and linear score milk). Data was analyzed using PROC GLM of SAS (SAS, 2005), according to design 4 × 4 Latin Square double and simultaneous, in 2 × 2 factorial designs, according to the following mathematical model:

$$Y_{ijk} = \mu + A_i + P_j + D_k + e_{ijk};$$

Where:

μ = constant common for each observation;

A_i = *i*th animal effect (*i* = 1, ..., 4);

P_j = *j*th period effect (*j* = 1, ..., 4);

D_k = *k*th diet effect (*k* = 1, ..., 4);

e_{ijk} = residual error, assumed independent and identically distributed in a normal distribution, with a zero mean and a variance of δ^2 .

The treatment means were compared using the command LSMEANS and Tukey tests and F, at a 5% probability. Studies were also performed using Pearson correlations amongst the variables tested.

The independent variables were analyzed, using times repeat measure, in 2 × 2 factorial designs (two environment and two time collection data). The objective was the characterization of the environment in the presence or absence of nebulizers and fan and time collection data to ascertain whether there were indeed significant differences between the environments evaluated. These independent variables do not suffer the effects of diets, but can interfere with animal performance.

RESULTS

It was found by the ANOVA results that the models analyzed were significant, isolated effects factors was observed in the variables analyzed. However, there was not interaction effect by forage and environment condition (Table 3).

For the production variables there was significant effects for both the environment and forage (Tables 3 and 4), where the DMI for the CSSC based diet was higher than the CS based diet in the effect of forage (4.22 vs. 4.06% BW and 22.3 vs. 21.7 kg/d, $P \leq 0.05$), but with milk production correcting 3.5% fat similar (23.01 vs. 22.62, CSSC and CS; 22.85 vs.

22.78 kg/day, WS and OS, respectively, $P \geq 0.05$). The feed efficiency and conversion was similar in both forage effect and environment (102.8 vs. 104.7% and 0.99 vs. 1.0, CSSC and CS; 102.5 vs. 104.9% and 1.0 vs. 0.99, WS and OS, respectively, $P \geq 0.05$). There was only significant effect for the dietary forage source factor for the total daily cost of the diet ingested, cost per kg DMI/day and benefit-diet cost ratio (Table 5). The CSSC-based diet presented higher costs than CS diet (US\$ 3.30 vs. 3.04 and US\$ 0.149 vs. 0.141, $P \leq 0.05$). Whereas, there was a better benefit-diet cost ratio of the diet based on CS compared to CSSC (7.44 vs. 6.97, $P \leq 0.05$).

For the qualitative variables of the milk there was effects only in the forage for CP (3.26 x 3.23%, $P \leq 0.05$), lactose (4.54 vs. 4.49%, $P \leq 0.05$) urea nitrogen (23.21 vs. 20.71 mg/dL, $P \leq 0.05$) and the superiority arising from the CSSC-based diet compared to the CS diet, (Table 3), and other similar variables.

Through the results presented in Table 6, it is shown that there was an effect of the factors isolated for RH, T, and THI variables to distinct environmental conditions (WS and OS) and in two times collection data (9:40 am and 2:00 pm). For the variables DPT and H, there was an interaction effect of the environment conditions and data collection factors (Table 5).

There was higher effects climatic data to 2:00 pm than 9:40 am for T and THI (28.1 vs. 23.6°C and 75.1 vs. 71.1, respectively, $P \leq 0.05$), and lower RH to 2:00 pm (47.7%, $P \leq 0.05$), Table 6. In the afternoon, 2:00 pm, in the presence of Sfn, there was higher DPT and H (18.00 vs. 14.4°C, 76.1 vs 72.1 kcal, $P \leq 0.05$) than in the absence of Sfn (Table 5). While in the absence of Sfn, there was a greater accumulation of DPT in the morning, 9:40 am, (17.6 vs. 14.4°C, $P \leq 0.05$) and H was similar in the two sampling times ($P \geq 0.05$). But, in the presence of Sfn, there was a greater H at 2:00 pm than at 9:40 am (76.1 vs. 70.8 kcal, $P \leq 0.05$) (Table 3).

As for the correlation study of productive variables and the qualitative of milk there were several significant and negative coefficients (Table 4). A verified fact for the CP of milk versus milk production ($r = -0.58$, $P \leq 0.05$) and other variables to milk as fat, total solids, SCC and LS versus milk production ($r = -0.37$, -0.38 , -0.12 , and -0.69 , respectively, $P \leq 0.05$), being positive only for lactose and urea nitrogen ($r = 0.28$ and 0.28 , respectively, $P \leq 0.05$). Meanwhile, the CP of the milk showed a high correlation index with fat content ($r = 0.62$,

Table 3. Mean values of production variables and milk quality due to the effects of forage and environment, and their significances.

Production variables	Forage		Environment		SE	<i>P-value</i>		
	CSSC	CS	WS	OS		For	Env	F×A
DMI, % BW	4.22 ^a	4.06 ^b	4.14	4.15	0.03	0.0010	0.9111	0.7446
DMI, kg/d	22.30 ^a	21.68 ^b	22.02	21.96	0.180	0.0198	0.7942	0.4264
Milk prod, kg/d	21.75	21.06	21.48	21.38	0.140	0.1494	0.8621	0.1918
Fat milk C, kg/d	23.01	22.62	22.85	22.78	0.161	0.3322	0.4428	0.1507
Feed efficiency, %	102.8	104.7	102.5	104.9	1.030	0.1905	0.088	0.2635
Feed conversion	0.99	1.00	1.01	0.99	0.010	0.6806	0.1218	0.2492
Diet cost, \$/d *	3.30 ^a	3.04 ^b	3.17	3.16	0.018	<0.0001	0.5614	0.9288
Diet cost, \$/kg DMI/d*	0.149 ^a	0.141 ^b	0.145	0.145	0.001	<0.0001	0.8927	0.4284
Benefit-diet cost ratio	6.97 ^b	7.44 ^a	7.24	7.24	0.070	<0.0001	0.2036	0.3763
Milk quality variables								
Fat, %	3.90	3.90	3.88	3.92	0.035	0.9754	0.3255	0.9253
Crude protein, %	3.26 ^a	3.23 ^b	3.26	3.24	0.009	0.0057	0.0798	0.7489
Solids, %	12.66	12.59	12.60	12.65	0.044	0.2530	0.4427	0.8113
Lactose, %	4.54 ^a	4.49 ^b	4.52	4.51	0.016	0.0194	0.9046	0.2378
Urea nitrogen, mg/dL	23.21 ^a	20.71 ^b	21.90	22.03	0.282	<0.0001	0.7614	0.3458
Linear score milk	4.20	4.33	4.25	4.24	0.126	0.3404	0.9421	0.9421
SCC (×10 ³ Cells/mL)	455.3	652.1	589.2	518.2	57.70	0.3404	0.9421	0.1346

^{a,b}: Mean values followed by differ letters rows, superscript in the same variable and within each factor were significantly different by Tukey test ($P \leq 0.05$).

Factor: forage (CSSC= corn silage + sugarcane, 1:1, % DM, plus concentrate; CS = corn silage plus concentrate); environment (WS= with fan and nebulizers system, OS = without fan and nebulizers system). DMI = dry matter intake, kg/day; Fat milk C = corrected milk 3.5% fat; SE = standard errors; *P-value* = probability.

Transformed data for the purpose of statistical analysis ($Y = \text{variable}$): SCC= somatic cell count, $\text{Log}_{10}(Y)$; Milk prod.= milk production, $Y^{1.5}$. * Data expressed in US dollar.

$P \leq 0.05$), milk total solids ($r = 0.86$, $P \leq 0.05$) and median relationships with SCC and LS ($r = 0.26$ and 0.32 , $P \leq 0.05$, respectively). For milk total solids both crude protein and fat were the elements that showed the highest correlation coefficients ($r = 0.86$ and 0.83 , $P \leq 0.05$ respectively).

DISCUSSION

The ITH for the two environments was found to be in the range of alert conditions, reaching a critical level of stress, according to Igonon et al. (1992), Du Preez (1990ab) and Johnson et al. (1976) for enthalpy. According to Renaudeau et al. (2012)

the cows are classified as mild-moderate heat stress 72 to 79.

The increase of DMI, as much in %BW as well as kg/day, in the diet based on CSSC (Table 3), happened to answer the nutritional requirements of the cows and the lower NDF content (40.5 vs. 45.6%) and a higher content of NFC (31.2 vs. 25.9%) (Table 2). However, this increase in DMI, for forage effect did not imply a significant increase in milk production (21.8 vs 21.1 kg/day, $P = 0.1494$ or milk 3.5% fat, 23.0 vs. 22.6 kg/day, $P = 0.3322$) for both diets (Table 3). Study carried out by Cattani et al. (2017), evaluating the total replacement of corn silage by sorghum silage corrected with

Table 4. Correlation of mean values of production and nutritional quality of milk.

Variables	DMI	Milk prod.	Fat	CP	Lactose	Total sol.	SCC	Urea N.
Milk prod.	0.38644							
Prob > F	<0.0001							
N°	158							
Fat	-0.01176	-0.36627						
Prob > F	0.8849	<0.0001						
N°	154	155						
CP	-0.05286	-0.57686	0.61840					
Prob > F	0.5095	<0.0001	<0.0001					
N°	158	159	156					
Lactose	0.19627	0.27855	-0.08468	0.03160				
Prob > F	0.0201	0.0008	0.3216	0.7089				
N°	140	141	139	142				
Total sol.	0.07478	-0.37755	0.82633	0.85830	0.17985			
Prob > F	0.3551	<0.0001	<0.0001	<0.0001	0.0328			
N°	155	156	154	157	141			
SCC	-0.12027	-0.44300	0.44802	0.25969	-0.22605	0.36338		
Prob > F	0.1615	<0.0001	<0.0001	0.0020	0.0123	<0.0001		
N°	137	138	136	139	122	137		
Urea N.	0.38501	0.27984	-0.04576	-0.14289	0.02608	-0.04553	-0.19744	
Prob > F	<.0001	0.0004	0.5706	0.0715	0.7580	0.5712	0.0198	
N°	158	159	156	160	142	157	139	
Linear score	-0.35898	-0.69355	0.33260	0.31633	-0.44116	0.22925	0.84488	-0.31683
Prob > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0049	<0.0001	<0.0001
N°	150	151	148	152	134	149	131	152

DMI= dry matter intake of diet; Milk prod.= milk production; CP= crude protein, Total sol.= Total solid milk; Urea N.= milk urea nitrogen milk; Linear score= LS= linear score milk, SCC= Somatic cell count.

ingredients concentrated in diets of lactating cows, did not find differences in the milk yield and production efficiency. According to Grant (1997), the amount and particle size of forage in the diet interacts with non-forage fiber sources to determine the net impact on the rate of ruminal digestion and passage rate of fiber. It is probable that this effect occurred in the CSSC diet, where there was a distinct fiber from roughages and concentrates.

However, the CSSC-based diet presented a higher cost and a lower benefit-diet cost ratio (Table 3), probably due to the higher proportion of concentration used to correct the diet by the inclusion of sugarcane (Table 2).

Accordingly, it can be said that the quality of the CSSC diet was sufficient to provide similar results in production and milk quality, especially for fat, crude protein, lactose and total solids. However,

Table 5. Mean values of environmental parameters obtained during the experimental phase.

Environment	Time, h		SE	<i>P-value</i>		
	9:40	14:00		Env	Time	E×T
	DPT,* °C					
WS	17.5 ^{A,a}	18.0 ^{A,a}	0.660	0.0300	0.0770	0.0109
OS	17.6 ^{A,a}	14.4 ^{B,b}	0.660			
	H, kcal/kg dry air					
WS	70.8 ^{A,b}	76.1 ^{A,a}	1.01	0.099	0.0038	0.0187
OS	71.5 ^{A,a}	72.1 ^{B,a}	1.01			

^{a,b}: Mean values followed by differ letters rows, superscript in the same variable and within each factor were significantly different by Tukey test ($P \leq 0.05$).

^{A,B}: Mean values followed by differ letters column, superscript in the same variable and within each factor were significantly different by Tukey test ($P \leq 0.05$).

DPT = dew point temperature, H = enthalpy, energy in the environment. Factor: Env = Environment (WS = with fan and nebulizers system, OS = without fan and nebulizers system); E×T = statistical interaction (Env × Time collection data); SE = standard errors; *P-value* = probability.

* Mean values original, but data transformed for the purpose of statistical analysis (Y variable): $DPT = DPT^{1.5}$.

Table 6. Mean values of environmental parameters obtained during the experimental phase.

Variables	Environment		Time, h		SE	<i>P-value</i>		
	WS	OS	9:40	14:00		Env	Time	E×T
RH, %	57.9	54.3	64.5 ^a	47.7 ^b	1.73	0.1496	<0.0001	0.0926
T, °C	25.7	26.0	23.6 ^b	28.1 ^a	0.256	0.4605	<0.0001	0.8579
THI	73.3	72.9	71.1 ^b	75.1 ^a	0.280	0.3640	<0.0001	0.1297

^{a,b}: Mean values followed by differ letters rows, superscript in the same variable and within each factor were significantly different by Tukey test ($P \leq 0.05$).

RH= relative humidity of the air; T= ambient temperature; THI= temperature and humidity index. Factor: Env= Environment (WS= with fan and nebulizers system, OS= without fan and nebulizers system); E×T= statistical interaction (Env × Time collection data); SE= standard errors; *P-value*= probability.

the difference (0.9%) in the crude protein content of milk (3.26 vs 3.23%, $P \leq 0.05$) for both diets, was probably due to the SCC (Table 3) presented which was always higher for the CS-based diet. However, there is still no consensus in the literature, because according to the report by Leitner et al. (2004), the lactose content of milk decreases with the infected milk and found that the increase in the SCC was potentially correlated with the proteolysis of milk casein and therefore a reduction in milk. Summer et al. (2012) found that sheep milk with high SCC was responsible for increasing the milk whey proteins content and low lactose milk content, however, found no significant change in the CP content of milk. However, these authors, in numerical terms, verified there was a difference of 5.41 vs 5.13 g/100 g for low and high SCC, respectively. Le Maréchal

et al. (2011) reported that, in general, there is an increase in the concentration of protein compounds associated with immune and inflammatory response and a decrease in the endogenous milk protein such as casein.

Another possible explanation for the increase in crude protein content of the milk may be a reflection of the total concentrate added in the CSSC-based diet to meet the nutritional requirements of the animals. This in turn led to an increase of 10.8% of urea nitrogen in the milk in the CSSC based diet compared to the CS diet (23.21 vs 20.71 mg/dL, $P \leq 0.05$, respectively). Similarly, Benchaar et al. (2014) observed a better N use efficiency for diets based on corn silage, which was reflected in greater N use efficiency in milk production. According to Grant (2005), mean values above 16 mg/dL of urea

nitrogen in the milk is indicative of a deficiency in the fermentation of non-fiber carbohydrates, an excess of crude protein in the diet and/or imbalance between the availability of energy and nitrogen in the rumen. Migliano et al. (2016) evaluating the use of sugarcane-based diets with high and low concentrations of CP (13 vs. 14.8%) observed higher urea nitrogen in the milk from diet based with 14.8% CP content, and similar DMI, milk production and efficiency of dietary nitrogen.

The cows fed the CS based diets, produced milk numerically higher in SCC than the animals that received the diet based on CSSC ($P \geq 0.05$). By transforming SCC in the milk linear score (LS), which is the most practical way to visualize the loss of milk production due to mastitis, it was found that the LS in numerical term provided from the animals fed CS based diets (4.33 vs 4.20, $P \geq 0.05$) than those that received a diet based on CSSC, respectively (Table 3). For Coldebella et al. (2004) losses arising from inflammation of the mammary gland are definite, for every one unit increased on the natural logarithm scale in the SCC range. In studies conducted by Summer et al. (2012) with individual sheep milk, it was found that the SCC significantly affected the quality of milk.

Another event observed is that for treatments based on CS and CSSC, for forage effect, where a diet had been established for producing 40 kg milk/day, there was lower dry matter intake than that estimated by the NRC (2001), it was closer to 25 kg DM per day. Probably, there was an overestimation of DMI because the program does not have sugarcane in its database to create these equations. Thus, the deficit obtained was 3.3 kg DM for the animals fed CS based diets and 2.7 kg DM for the CSSC (Table 2). This deficit was probably caused by an inability of the animals to ingest the DMI because of the rumen filling effect or the organic acid content of the corn silage, which probably limited the intake. There was no lack of food in the trough, considering that there was a safety margin so that at least 10% remained in the trough.

In general, the thermal stress in high producing dairy cows results in the reduction of roughage intake and rumination time. Fuquay et al. (2011) mentioned that cows subjected to heat stress are at a higher risk for ruminal acidosis due to decreased contractility of the rumen. This decrease in the number and intensity of ruminal contractions, which may be related to the reduction in the concentration of volatile fatty acids in the rumen, has a negative effect on the production of saliva,

leading to a decrease in ruminal pH and, according to Malafaia et al. (2011) this condition leads to a decrease in fiber digestibility by the lower activity of fibrolytic rumen bacteria.

According to Wheelock et al. (2010) animals in heat stress, have a need for increased maintenance to dissipate heat, in values of 25 to 30%. On the other hand, Brosh et al. (1988) cite that ruminants adapted to hot conditions are able to maintain their intake next to the requirement for maintenance or in periods of moderate growth.

However, in the presented conditions the environment did not interfere in the DMI, milk production and feed efficiency or conversion (Table 3). Probably, the relative humidity (RH), dew point temperature (DPT) and enthalpy (H) caused thermal discomfort in the cows (Table 6). For Armstrong (1994), relative humidity can cause thermal discomfort if it is associated with high temperatures, damaging the processes of body heat dissipation. However, in the present study (Table 3) the feed efficiency, both for effect in the forage and in the environment, were not changed, remaining similar (103.2 vs 104.2 and 102.7% vs 104.5%, $P \geq 0.05$, respectively). This shows that in the conditions evaluated, the use of nebulizers and fans does not alter the production efficiency of the cows by virtue of the animals being physiologically adapted to environmental conditions.

In regards to the correlations of milk production versus the CP, fat, total solids, SCC and LS all showed negative correlation indices ($P \leq 0.05$) and this shows that they are parameters that are more likely linked to genetic potential and sanitary management than to environment and diet (Table 4). However, the levels of lactose and urea nitrogen ($r = 0.28$ and 0.28 , respectively, $P \leq 0.05$) were directly proportional, with influences of 28% in milk production and are lastly variables that respond directly to the diet of the animals. Cunha et al. (2008) also obtained negative correlation between SCC and milk production, while between SCC, percentages of fat and crude protein they were positive. Summer et al. (2012) reported that the individual milk from sheep with high SCC provided lower lactose content (4.05 vs. 4.60%) and casein (4.28% vs. 3.91) than with low SCC, respectively.

Kitchen (1981) mentions that when the lactose content is below 4.69% it may be indicative of mastitis. So, in this work, despite not having presented SCC differences ($P \geq 0.05$), it can be inferred that the cows revealed infections caused by mastitis, by the lactose content that was below 4.69%

(4.54 vs 4.49%, $P \geq 0.05$, Table 3) mainly in the basic treatment of CS diet, in which there was a higher SCC in numerical terms ($P \geq 0.05$). This fact is proved by the negative correlation of LS with the lactose content in milk ($r = -0.44$). Thus the higher the LS in the milk the lower the concentration of lactose in milk. Silva et al. (2000) also obtained a lactose content of 4.61% and negative correlation for the LS with the lactose ($r = -0.34$). These authors, also compared data for mastitic milk and non-mastitic and the results showed lower concentrations of lactose in mastitic milk (an average of 4.49% for mastitic milk and 4.68% for the non-mastitic milk, $P < 0.0001$). They explained that this reduction is to be expected, as the infection of the mammary gland leads to the destruction of secretory tissue thus reducing the capacity of the synthesis of the gland and consequently reducing lactose production. Furthermore, in clinical cases, the swelling of the gland may limit the glucose supply to the gland, further limiting the production of lactose.

The positive correlations of CP in the milk with fat and milk solids probably relate to the structural forms of the composition of fats that are mainly composed of fat layers, proteins and water, which are components of total solids milk. SCC and LS interfere positively in CP and this is an indication that the bacteria that makes up the mastitic milk contributes in the crude protein levels in milk, 26% and 32%, respectively. According to Jaeggi, et al. (2003), the increase of proteolytic activity, associated with an increase in SCC was due to the proteolytic enzyme activity, such as plasmin or others derived from proteases somatic cells.

The negative correlations of LS with DMI, milk production, lactose and urea nitrogen are an indication that the higher the LS the lower the DMI, milk production, lactose and urea nitrogen will be (Table 4). Thus, it can be said that the reduction of the DMI from the diets evaluated, milk production, the lactose content and the concentration of milk urea nitrogen is explained, respectively, 36, 69, 44 and 32% in the increase of the LS of the milk, as a response to the possible mastitis presented by the cows evaluated. This is checked with the index negative correlation ($r = -0.44$) of milk production and SCC. This index reflects a 44% reduction in milk production with an increase of the SCC from the milk (Table 4).

The high correlation index of LS with SCC ($r = 0.84$) indicates that these variables are similar (84%), through the origin of the LS, and that 16% are consequences of sample variations (Table 4).

Silva et al. (2000) reported that to transform the value of the SCC by log function in LS average milk limits the action of very high values of SCC above the average, thus the sample space is more representative.

The high correlation levels of total milk solids with CP and fat (0.86 and 0.83, $P \leq 0.05$, respectively), show the genetic potential of the cows to respond to the main diets used, especially when considering the fat and lactose variables of the milk. However, milk production ($r = 0.39$, $P < 0.0001$) was directly connected to DMI diets evaluated at 39% (Table 4).

CONCLUSION

The use of a diet based on the mix of sugarcane with corn silage (1:1 in DM) and concentrate, increases dry matter intake and changes the milk quality without affecting production. But, it increases the diet cost and provides less benefit-diet cost ratio. The use of the environmental cooling system did not improve the productive, qualitative parameters of milk and thermal comfort of dairy cows under the conditions evaluated. The high correlation of the linear-score milk with the productive performance and milk quality evidences an important parameter in the management of dairy cows.

ACKNOWLEDGEMENTS

The current study was financially supported with grants 2005/50926-0 Sao Paulo Research Foundation (FAPESP), SP, Brazil.

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