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Performance of Dairy Cows Supplemented with By-Pass Fat under Heat Stress Conditions

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Abstract

The objective of this study was to determine the effect of supplementation with a protected fat source on the productive response, metabolic environment and physiological indicators in Holstein cows under heat stress conditions during a 12-week experimental period. Thirty Holstein cows were distributed in 15 blocks by parity (2.0 ± 1.1), days in milk (182 ± 80) and milk production (29.4 ± 5.7 kg·day^{-1}) at the beginning of the trial and randomly assigned within each block to the following treatments (diets): SPF: supplementation with protected fat or WPF: without supplementation with protected fat. All the cows were kept in a dry-lot where they were given a partial mixed ration (PMR) *ad libitum* while in the milking parlor they received individual supplementation depending on the treatment. The SPF diet contained 4.0 kg·day^{-1} concentrate in pellet form + 0.6 kg·day^{-1} ground corn grain + 0.7 kg·day^{-1} protected fat, while the WPF diet was similar to that offered in SPF, but the protected fat was isoenergetically replaced by ground corn grain. The fat supplement contained fats of animal and vegetable origin and microencapsulation was used for its preparation. Total dry matter and metabolic energy intakes were similar ($p > 0.05$) between treatments. Fat corrected milk (4% FCM) production was higher ($p = 0.04$), while energy corrected milk and fat productions tended ($p = 0.06$) to be higher in cows from the SPF group, without effects ($p > 0.05$) on the rest of the milk production and composition parameters. These results could be attributed to an improvement in the efficiency of the use of the energy consumed. Protected fat supplementation neither modified the metabolic profile, nor reduced the respiratory rate and body temperature of heat-stressed cows. Future research is needed to explain this latter result.

Keywords

Dairy Cattle, By-Pass Fat, Heat Stress

1. Introduction

Heat stress negatively affects milk yield, generating significant annual economic losses in the global dairy industry [1] [2]. In the central dairy area of Argentina, high temperatures and relative humidity during the summer months, generate heat stress in dairy cows, affecting their productive behavior [3]. The temperature-humidity index (THI) is used to monitor daily environmental conditions; being values ≥ 72 indicative of heat stress situations [4].

Heat stress reduces dry matter intake (DMI), rumination activity and nutrient absorption, and increases maintenance requirements [5], resulting in decreased energy availability for milk production. Together, these changes cause heat-stressed cows to go into negative energy balance, regardless of the stage of lactation [6].

The decrease in nutrient intake is one of the main causes of reduced production [7]. However, a decreased dry matter intake does not fully explain the reduction in milk production of heat-stressed cows. Only 35% to 50% of the decrease in milk production would be explained by the reduction in nutrient intake [8] [9]. According to [10], in animals subjected to heat stress, metabolic changes related to nutrient partitioning occur in order to prioritize the maintenance of euthermia, which would be the main responsible for the reduction of animal performance during the summer months.

One of the dietary strategies to balance this energy deficit is to increase the energy density of the diet through fat supplementation [11]. Fats are utilized with higher efficiency for milk production and have lower heat increment than nutrients like starch and fiber [6]. However, the addition of fats rich in unsaturated fatty acids (FA) can greatly affect ruminal fermentation, causing a reduction in the digestibility of non-lipid energy sources [12]. In this context, supplementation with a protected fat source (which is not altered at the ruminal level) would ensure an energy supply without the heat increase produced by fermentation [13].

The objective of this study was to determine the effect of supplementation with a protected fat source on the productive response, metabolic environment and physiological indicators (body temperature and respiratory rate) in Holstein cows under heat stress conditions.

2. Materials and Methods

2.1. Experimental Site, Animals and Treatments

The trial was conducted at the dairy farm of the Rafaela Experiment Station of the National Institute of Agricultural Technology (INTA) (Santa Fe, Argentina,

31˚12'S, 61˚30'W), starting on January 6th, 2021 and lasting for 12 weeks (2 weeks of pre-experimental period, 1 week of habituation to lipids and 9 weeks of data collection). The experimental protocol was evaluated and approved by the Institutional Committee for the Care and Use of Experimental Animals of the Santa Fe Regional Center of INTA (CICUAE-CERSAN).

Thirty Holstein cows were distributed in 15 blocks by parity (2.0 ± 1.1), days in milk (182 ± 80) and milk production (29.4 ± 5.7 kg·day^{-1}) at the beginning of the trial and randomly assigned within each block to the following treatments (diets): SPF: supplementation with protected fat or WPF: without supplementation with protected fat. All the cows were kept in a dry-lot (with access to shade and free access to water) where they were given after a.m. milking a partial mixed ration (PMR) *ad libitum* (26.0% corn silage, 33.2% alfalfa silage, 8.5% ground corn, 18.1% soybean meal, 5.2% soybean expeller and 9.0% alfalfa hay) while in the milking parlor they received differential supplementation depending on the treatment. The SPF diet contained 4.0 kg·day^{-1} pelleted concentrate + 0.6 kg·day^{-1} ground corn grain + 0.7 kg·day^{-1} of protected fat, distributed individually in equal parts in each milking shift, while the WPF diet was similar to that offered to SPF group, but the protected fat was replaced isoenergetically by ground corn grain (equivalence: 1 kg DM fat = 2 kg DM corn). The fat supplement (96% DM, 84.2% EE, 15.2% NFC, 0.6% ash) contained animal and vegetable fats and microencapsulation was used in the manufacturing process. The composition of fatty acids (FA) was 32.0% palmitic, 33.4% stearic, 6.9% oleic, 19.4% linoleic, 3.0% linolenic and 5.3% others. Before the start of the trial, the degree of protection of the fat supplement was determined, for which the impact of adding it on ruminal fermentation was evaluated through an in vitro digestion system [14].

During the pre-experimental period, the cows received the WPF diet. Milk production and composition during this period were used as a covariate. All cows were equipped with neck transponders for automatic recording of daily milk production on an individual basis (ALPRO version 6.60/DeLaval, Tumba, Sweden).

2.2. Measurements

Ambient temperature (AT) and relative humidity (RH) measurements were recorded daily at three different times (0900 AM, 0300 PM and 0900 PM) by the Agrometeorological Station of Rafaela Experiment Station. The temperature-humidity index (THI) was used to monitor environmental stress conditions. The following equation was used for THI calculation [15]:

$$THI = (1.8 \times Tdb + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times Tdb - 26),$$

where Tdb is dry bulb temperature.

2.2.1. Diet Chemical Composition

Representative samples of the concentrate, the PMR, and the ingredients that

composed it were taken every 7 days. All samples were dried in an oven with forced air circulation at 65˚C to constant weight to determine the DM content and grinded in a Wiley mill (1 mm mesh). The content of ashes [16], crude protein [17], neutral detergent fiber (NDF; [18]), acid detergent fiber (ADF; ANKOM Technology Method 5-2011 validated by [19]), acid detergent lignin (ADL; [19]), ether extract (EE; [20], modified for automated extract) and *in vitro* DM digestibility (IVDMD; two-stage fermentation technique by [21]) was determined. Lipid metabolizable energy (ME) content was calculated based on the equations from NRC (2001). The content of ME of the PMR and of the concentrate was estimated according to the formula: ME (Mcal kg MS^{-1}) = 3.608 * IVDMD. The net energy for lactation (NEL) was calculated as 64% of the ME [22].

2.2.2. Milk Production and Composition

Milk production was measured individually and daily by the milk measurement system DeLaval ALPRO (DeLaval International AB, Tumba, Sweden), considering averages by week. Milk composition was evaluated from individual samples collected weekly. Two milk subsamples were taken from each cow in consecutive milkings (morning and afternoon) using milk meters (DeLaval International AB, Tumba, Sweden), then a single individual sample (pool) weighted by the respective production was obtained. In each composite sample the content of fat, total protein, lactose, total solids, non-fat solids and urea was determined by infrared spectrophotometry (MilkoScan™ Minor; FOSS Electric, Hilleroed, Denmark) according to the standard method [23] Fat-corrected milk (4% FCM) was calculated according to [24] and energy-corrected milk (ECM) as proposed by [25].

Individual aliquots of milk (100 ml) were collected in the 6th week of the data collection period and were stored at −24˚C for the subsequent determination of the FA profile. The fatty acid methyl esters (FAME) were formed by transesterification with methanolic potassium hydroxide solution as an interim stage before saponification [26]. The FA composition in milk was determined by gas chromatography with a Shimadzu (GC 2014) chromatograph equipped with an automatic injector (AOC-20i auto injector Shimadzu) and a flame ionization detector (SFID1) as stated in [27].

2.2.3. Body Weight and Body Condition Score

Cows were individually weighed with an electronic scale every 14 days after the morning milking. In conjunction with the weighing, body condition score (BCS) was determined by two independent observers using a 5-point scale (1 = extremely thin and 5 = extremely fat) with 0.25 increments [28] and the value analyzed was the average result of both evaluators.

2.2.4. Plasma Concentration of Metabolites and Hormones

Blood samples were obtained by puncture of the coccygeal vein after the morning milking, every 2 weeks. Blood was collected in tubes containing sodium heparin (5 U/ml). Plasma was obtained by centrifugation (2000 × g for 15 min at

4˚C) and stored at −24˚C until analysis of glucose (Enzymatic glycemia, Wiener Laboratory, Rosario, Argentina), urea (Uremia, Wiener Laboratory, Rosario, Argentina), insulin, growth hormone (GH) and insulin-like growth factor (IGF-I) was carried out as described in [29]. Beta-hydroxybutyrate (βHBA) was determined in whole blood with a FreeStyle Optium ketone test (Abbot Diabetes Care Ltd., Witney, UK).

2.2.5. Physiological Parameters

Measurement of vaginal temperature (˚C) was used to determine core body temperature. Compared with rectal temperature, the use of digital thermometers to monitor vaginal temperature can reduce the disruption of animal behavior and provide continuous measurements of core body temperature [5]. Vaginal temperature was monitored using intravaginal data loggers (DS1922L Thermochron iButton Device; Maxim Integrated, San Jose, CA) inserted into a modified, blank (progesterone-free) internal drug release device (Zoetis, Florham Park, NJ) according to [30].

Respiratory rate (breaths/minute) was measured 3 times/week (Monday, Wednesday, and Friday) at 3 times/day (07:30 h AM, 02:30 h PM and 06:30 h PM) by counting the number of flank movements in a 15-s period and multiplying this by 4 to determine breaths per minute.

2.2.6. Dry Matter Intake

The individual daily concentrate intake was determined by the difference between offered and rejected throughout the trial. The individual PMR intake was determined by the difference between offered and rejected during the 5th week of the experimental period, for which the cows were housed in individual pens. The total DM intake was calculated as the sum of concentrate and PMR DM intake.

2.3. Statistical Analysis

The results referring to milk production and composition, BW, BCS, and plasma metabolites and hormones were analyzed according to a randomized complete block design with repeated observations in time adjusted by covariate ($\alpha = 0.05$). The following model was used:

$$Y_{ijkl} = \mu + T_i + B_j + A(B)_{k(j)} + W_l + (T \times W)_{il} + Cov + E_{ijkl},$$

where:

Y_{ijkl} = dependent variable, μ = general mean, T_i = treatment effect, B_j = block effect, $A(B)_{k(j)}$ = random effect of animal nested to block, W_l = sampling week effect, $(T \times W)_{il}$ = effect of treatment interaction × sampling week, Cov = covariate and E_{ijkl} = residual error.

The intake and feed efficiency data were analyzed using a model with a classification criteria (treatment):

$$Y_{ijk} = \mu + T_i + B_j + A(B)_{k(j)} + E_{ijk},$$

where:

Y_{ijk} = dependent variable, μ = general mean, T_i = treatment effect, B_j = block effect, $A(B)_{k(j)}$ = random effect of animal nested to block and E_{ijk} = residual error.

All statistical analyzes were performed using the MIXED procedure of the SAS statistical package (2010). Trend was considered $0.05 < p < 0.10$.

3. Results and Discussion

3.1. Diet Chemical Composition

The average values of the chemical composition of the PMR used in the trial are presented in Table 1. The chemical composition of the pelleted concentrate was 92.2% ± 0.8% DM, 13.2% ± 0.6% CP, 28.2% ± 2.4% NDF, 14.4% ± 1.3% ADF, 3.4% ± 0.3% EE, 13.0% ± 0.6% ash and 71.5% ± 1.4% IVDMD.

The DM content of PMR was within the range commonly used in high-producing dairy herds (40% to 60% DM; [31]). The contents of NDF and energy of the PMR were within the ranges recommended by the NRC (2001) [22] for cows in mid-lactation, that is, 33% - 35% NDF and 1.55 - 1.60 Mcal·NEL·kg·DM^{-1}, while the protein content (20.2%) was above the recommended range (16% - 17% CP; [22]). However, the final protein concentration of the experimental diets (18%) was slightly higher than the indicated for cows in mid-lactation.

3.2. Dry matter and Energy Intake

PMR and total DMI were similar ($p > 0.05$) between treatments, while concentrate DMI was significantly higher in the control group (Table 2). This latter result is because the cows in the control group received +0.8 kg·day^{-1} of concentrate due to the type of trial design (isoenergetic concentrates).

Table 1. Chemical composition of the PMR offered to Holstein cows supplemented with protected fat (SPF) (0.70 kg·day^{-1}) or without protected fat (WPF) during the summer months.

Parameter	Values[1]
DM, %	42.2 ± 2.0
IVDMD, %	67.0 ± 1.0
CP, % DM	20.2 ± 1.1
NDF, % DM	33.8 ± 1.6
ADF, % DM	22.1 ± 1.3
ADL, % DM	4.4 ± 0.4
EE, % DM	3.6 ± 0.3
Ash, % DM	7.7 ± 0.5

[1]Values expressed as the average ± standard deviation. DM = dry matter; IVDMD = in vitro dry matter digestibility; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin, EE = ether extract.

Table 2. DM and NEL intake in Holstein cows supplemented with protected fat (SPF) (0.70 kg·day^{-1}) or without protected fat (WPF) during the summer months.

Intake	Treatment[1]		SEM	p-value
	SPF	WPF		
DM, kg·day^{-1}				
PMR	16.90	16.49	0.36	0.43
Concentrate[2]	4.83	5.60	0.04	<0.01
Total	21.73	22.09	0.36	0.49
NEL[3], Mcal·day^{-1}				
PMR	26.15	25.54	0.55	0.46
Total	35.86	35.39	0.57	0.57
FCE[4]				
4% FCM/DMI	1.25	1.17	0.02	<0.01
ECM/DMI	1.24	1.16	0.02	0.01
4% FCM/NEL intake	0.76	0.73	0.01	0.07
ECM/NEL intake	0.75	0.72	0.01	<0.10

[1]Values expressed as least square means (LSMeans) and the standard error of the LSMeans (SEM). [2]SPF concentrate: 4.0 kg·day^{-1} pelleted concentrate + 0.6 kg·day^{-1} ground corn grain + 0.7 kg·day^{-1} protected fat; WPF concentrate: 4.0 kg·day^{-1} pelleted concentrate + 2.1 kg·day^{-1} ground corn grain. [3]Estimated NEL values for SPF concentrate, WPF concentrate and PMR: 2.01, 1.76 and 1.55 Mcal·kg·DM^{-1}, respectively. [4]Feed conversion efficiency.

Decreases in concentrate DMI when using supplemental fats in rations have been associated with a lower rate of intake and size of each meal [32], which would be a relevant problem in dairies where the concentrate is supplied for a limited time in the milking parlor, as in this study. Australian researchers [33] included increasing doses from 0% to 40% of free FA (as feed) in the concentrate fed to grazing dairy cows twice daily at each milking, observing decreases in concentrate intake when the fat concentration was higher than 22%. They concluded that the negative effects observed were a consequence of the concentration rather than the amount of fat supplied. In the present study, in agreement with that reported by [33], the inclusion of protected fat in the experimental concentrate was 14% and did not affect its palatability.

The inclusion of fat supplements in the diet is generally associated with a reduction in DM intake in thermal neutral cows [34], but this effect is commonly not observed in heat-stressed cows [35]. Coincidentally, in this study, the total DM intake was similar between treatments (**Table 2**).

The estimated total NEL intake was similar between treatments (**Table 2**), in agreement with [32], who reported that the inclusion of fat supplements on TMR-based diets would have a null or slightly positive effect (5% - 6%) on ener-

gy intake in thermal neutral cows.

In the present study, a possible negative effect of the fat supplement on DM and NEL intake could not be detected.

Cows receiving by-pass fat produced significantly more than 4% FCM and ECM per kilogram of total DMI and tended to produce more 4% FCM and ECM per Mcal of NEL intake (**Table 2**). Similar results were obtained in previous studies [11] [13] [36] and are in agreement with [37] suggestion that increasing diet energy density by supplementation with non-fermentative nutrients might improve the conversion efficiency of feed to milk in heat-stressed cows.

The energy conversion efficiency (ECM Mcal NEL^{-1}) was 0.030 higher in the cows of the SPF group. This difference was above 0.012, which is the value of the potential improvement in energy use efficiency when fat replaces dietary carbohydrate and is added to 3% of dietary DM [11], as was observed in the present study.

3.3. Milk Production and Composition

The existing information on the productive response of dairy cows supplemented with fat under heat stress conditions is limited (reviewed by [35]). An analysis of the results of the reviewed studies (n = 8) indicated a positive average effect of protected fat on milk production (+1.16 kg·day^{-1}, $p < 0.01$), FCM (+1.22 kg·day^{-1}, $p = 0.03$) and fat (+0.08 kg·day^{-1}, $p = 0.01$) for average intake levels of 0.57 ± 0.21 kg·day^{-1}, with no significant effects on protein content (+0.03 g·$100g^{-1}$, $p = 0.47$) and production (+0.01 kg·day^{-1}, $p = 0.62$). In addition, a trend to a higher fat content was observed in the supplemented cows (+0.14 g·$100g^{-1}$, $p = 0.08$).

In this study, milk production was similar between treatments, while ECM production tended ($p = 0.06$) to be higher (+1.0 kg·day^{-1}) in cows supplemented with protected fat (**Table 3**).

The inclusion of protected fat in the rations for dairy cows would increase the efficiency of energy use for milk production due to a decrease in energy losses in the form of methane, direct use of long-chain FA for milk fat secretion and a higher efficiency of ATP generation from long-chain FA rather than acetate [38] [39]. In this sense, it has been postulated that the contribution of 8% of the absorbed energy requirement in the form of protected lipids predisposes to achieve a better global productive response of the dairy cow [40] [41]. The total ME requirement calculated according to [22] for a cow of 653 kg of body weight, with a body weight gain of 0.526 kg·day^{-1}, producing 26.6 kg of milk with 4.04% fat, 3.32% of protein and 5.04% of lactose (average values obtained in the SPF group during the experimental period) and under heat stress conditions would be 56 Mcal·cow^{-1}·day^{-1}. Taking into account that the intake of protected lipids recorded in the SPF group was 0.672 kg DM·cow^{-1}·day^{-1} and assuming that their energy value is 6.3 Mcal·kg·DM^{-1} [22], the amount of ME contributed by the lipids in SPF group can be calculated as 4.23 Mcal·cow^{-1}·day^{-1}, which is equivalent

Table 3. Production and composition of milk in Holstein cows supplemented with protected fat (SPF) (0.70 kg·day^{-1}) or without protected fat (WPF) during the summer months.

Variable	Treatment[1]		SEM	p-value[2]		
	SPF	WPF		Treat	S	Treat × S
Milk, kg·day^{-1}	26.6	26.2	0.36	0.38	<0.01	0.99
4% FCM, kg·day^{-1}	26.3	25.2	0.40	0.04	<0.01	0.70
ECM, kg·day^{-1}	26.2	25.2	0.43	0.06	<0.01	0.59
Fat						
%	4.04	3.83	0.10	0.12	<0.01	0.84
Kg·day^{-1}	1.05	0.99	0.02	0.06	<0.01	0.75
Total protein						
%	3.32	3.32	0.03	0.92	<0.01	0.90
kg·day^{-1}	0.87	0.86	0.02	0.78	<0.01	0.83
Lactose, %	5.04	5.03	0.02	0.66	<0.01	0.47
Urea, mg·100ml^{-1}	34.2	34.6	0.43	0.48	<0.01	0.03

[1]Values are expressed as least square means (LSMeans) and the standard error of the LSMeans (SEM). [2]Effects of treatment (Treat), sampling (S) and treatment × sampling interaction (Treat × S). 4% FCM = 4% fat corrected milk; ECM = energy corrected milk.

to 7.55% of the total ME requirement. This latter value was slightly lower than that proposed by [40] and [41] in order to improve the global productive response in dairy cows.

When correcting milk yields for the same fat content (4% FCM), differences between treatments were detected. The supply of protected fat significantly increased the production of 4% FCM (+1.1 kg·day^{-1}) compared with the control group (**Table 3**). This increase was very similar to the average response observed in experiments conducted under heat stress conditions (+1.22 kg·day^{-1}). As cows had similar total NEL intake (**Table 2**), the increase in 4% FCM yields (**Table 3**) after fat feeding were not apparently explained by higher energy absorption.

Fat supplementation did not significantly modify milk fat content (**Table 3**), but it tended ($p = 0.06$) to increase fat production (+0.06 kg·day^{-1}). This increase was similar to that observed in studies conducted under heat stress conditions (+0.08 kg·day^{-1}). The net effect of supplementation with protected lipids on the concentration and production of butterfat depends on the balance between increased uptake of dietary FA by the mammary gland and decreased *de novo* synthesis [32]. In this study, the increase ($p = 0.02$) in preformed FA content (>C17:0) recorded in lipid-supplemented cows (42.5 vs. 39.7 g 100 g·FA^{-1}, for SPF and WPF, respectively) would not have compensated the reduction ($p < 0.01$) observed in the content of FAs synthesized *de novo* (C4:0 - C15:1) with respect to the control group (22.0 vs. 24 .6 g 100 g·AG^{-1}, for SPF and WPF, re-

spectively), since the fat content of the milk was not significantly modified.

Neither the protein concentration nor the amount of protein secreted was affected by the treatments (Table 3), in agreement with the average null effect observed on these parameters in studies carried out under thermal stress conditions. The absence of negative effects on the protein content of milk is an important aspect since this parameter determines the price of milk and affects the speed and quality of coagulation for subsequent transformation into cheese.

Lactose content was not affected by lipid supplementation (Table 3), a result compatible with the similar milk production observed between treatments. A reduction in the availability of fermentable carbohydrates in the rumen could be expected with the isoenergetic replacement of corn grain by lipids in the SPF concentrate. However, the average concentrations of urea in milk were similar between treatments (Table 3), a result consistent with the similar concentrations of urea in plasma observed (Table 4). The average values of urea in milk (Table 3) were very close to the upper limit of the range (21.4 - 34.2 mg·100ml^{-1}) proposed by [42], which would reflect a high efficiency of N use and a low excretion of N.

3.4. Body Weight, Body Condition and Plasma Concentration of Metabolites and Hormones

Supplementation with protected fat did neither affect any of the parameters associated with the variation of body reserves evaluated, nor did it modify the metabolic profile of the cows (Table 4).

The absence of treatment effect on BW and BC was compatible with the similar levels of βHB observed (Table 4). Apparently, in the cows of the SPF group,

Table 4. Body weight, body condition and plasma concentration of metabolites and hormones in Holstein cows supplemented with protected fat (SPF) (0.70 kg·day^{-1}) or without protected fat (WPF) during the summer months.

Variable	Treatment[1]		SEM	p-value[2]		
	SPF	WPF		Treat	S	Treat × S
BW (kg)	646.0	646.0	3.79	0.97	<0.01	0.99
BC	3.11	3.08	0.03	0.32	<0.01	0.07
Glucose (g·l^{-1})	0.62	0.63	0.01	0.32	<0.01	0.45
Urea (g·l^{-1})	0.43	0.43	0.01	0.48	<0.01	0.79
βHB (mmol·l^{-1})	0.44	0.45	0.02	0.86	<0.01	0.17
GH (ng·ml^{-1})	0.96	1.09	0.11	0.39	<0.01	0.89
IGF-I (ng·ml^{-1})	134.0	133.0	5.65	0.87	<0.01	0.99

[1]Values expressed as least square means (LSMeans) and the standard error of the LSMeans (SEM). [2]Effects of treatment (Treat), sampling (S) and treatment × sampling interaction (Treat × S). BW = body weight; BC = body condition; βHB = beta hydroxybutyrate; GH = somatotrophin; IGF-I = somatomedin C.

the energy consumed was not directed to a differential accumulation of body reserves, but was diverted to the production of 4% FCM and ECM (**Table 3**). Similar results were obtained by [36] when replacing energy in the form of starch (corn grain) with energy in the form of lipids (calcium salts of fatty acids) in the diet of high-producing cows in mid-lactation and under heat stress conditions.

Uremia and glycemia were similar between treatments (**Table 4**), a result compatible with the similar values of urea and lactose observed in milk (**Table 3**). Fat supplementation had no consistent effects on circulating glucose concentrations [43]. However, as fat replaced starch in the concentrate, a decrease in the entry of propionic acid to maintain hepatic glucose synthesis could be expected. Fatty acids absorbed from the by-pass fat may have also contributed to maintaining glycemia by reducing total (CO_2) or partial (to NADPH2) glucose oxidation [38].

In cattle, the GH/IGF-I system plays a critical role in the control of lactation [44]. Effects of fat feeding on plasma GH concentration are highly variable, which depends on the sources of fat supply and the physiological state of the animals [45]. In this study, GH concentrations were not affected by fat feeding to dairy cows in mid-lactation, a result consistent with the similar milk yields observed (**Table 3**).

No differences in IGF-I plasma concentrations were observed (**Table 4**). The lack of effects on IGF-I concentrations is consistent with the similar GH plasma concentrations (**Table 4**) and energy intake (**Table 2**) recorded in both treatments, since these two parameters and hepatic production of IGF-I would be positively correlated [46].

It can be concluded that in the present study, the increase in production observed in the supplemented group could not be explained by changes in the hormonal profile of the animals.

Plasmatic metabolites and hormones such as glucose, non-esterified fatty acids (NEFA), insulin, GH and IGF-I act as dynamic indicators of energy balance [47]. Decreases in plasma glucose, insulin, and IGF-I concentrations and increases in NEFA and GH circulating levels are observed in cows with a negative energy balance [48]. Taken together, the results obtained in this study (similar plasma concentrations of glucose, βHBA, GH and IGF-I) suggest that supplementation with by-pass fat in replacement of corn as an energy source did not affect the energy metabolism of dairy cows in mid-lactation.

3.5. Physiological Parameters

The daily average THI during the data collection period was 74.0 (range 80.8 to 68.6), which was 2.0 units above the value of 72 which was determined as being critical for milk production [49] [50].

The average daily respiratory rate and the one recorded at 2:30 p.m. were significantly higher in the cows of the SPF group, without differences at the rest of the times recorded (**Table 5**).

Table 5. Respiratory rate (breaths/minute) and body temperature (˚C) in Holstein cows supplemented with protected fat (SPF) (0.70 kg·day^{-1}) or without protected fat (WPF) during the summer months.

Variable	Treatment[1]		SEM	p-value[2]		
	SPF	WPF		Treat	S	Treat × S
Respiratory Rate						
07:30 h AM	48.4	45.4	1.10	0.06	<0.01	0.90
02:30 h PM	82.5	75.1	2.30	0.02	<0.01	0.24
06:30 h PM	70.3	68.9	1.88	0.60	<0.01	0.10
Average	66.9	62.9	1.43	<0.05	<0.01	0.83
Body temperature						
07:30 h AM	38.4	38.2	0.09	0.14	<0.01	0.21
02:30 h PM	39.2	38.9	0.10	0.06	<0.01	0.17
06:30 h PM	39.4	39.1	0.10	0.10	<0.01	0.01
Average	38.9	38.6	0.10	0.04	<0.01	<0.01

[1]Values expressed as least square means (LSMeans) and the standard error of the LSMeans (SEM). [2]Effects of treatment (Treat), sampling (S) and treatment × sampling interaction (Treat × S).

No significant differences in body temperature between treatment groups at any of the recorded times during the experimental period were observed (Table 5). Treatment × sampling interaction was detected for average daily body temperature (Table 5). During the first 39 days of the data collection period, the average body temperature was significantly higher ($p < 0.05$) or tended to be higher ($p < 0.10$) in the cows of the SPF group, without differences in the rest of the period.

Most of the experiments reviewed by [35] reported little or no difference in body temperatures and respiratory rates. In fact, only one report, in line with our results, indicated that cows fed additional fat had increased body temperatures and respiratory rates, even though the estimated metabolic heat production was lower compared with cows in the control group [36].

Feeding fat does not appear to be an appropriate nutritional strategy to improve heat balance in heat-stressed cows. To explain these results, [35] suggested that it could be possible that small reductions in a thermal load would be difficult to detect at limited time points, although these minor changes would accumulate over time into a significant improvement. Consequently, the authors recommend measuring body temperatures in heat-stressed cows with a continuous thermometer system, such as the one used in the present study.

4. Conclusion

Under this study conditions, *i.e.*, cows in mid-lactation under heat stress and a

diet based on PMR, the replacement of fermentable energy in the rumen (starch, provided by the corn grain) by non-fermentable energy (protected fat) improved solids-corrected milk yield and fat yield, with no effect on milk chemical composition. Since the increase in production was obtained without an apparent increase in energy intake or endogenous energy mobilization, the quality of the energy provided in the concentrate (lipids vs. starch) seems to have contributed to increasing the efficiency of energy use. Supplementation with protected fat did not reduce the respiratory rate or body temperature of the cows. Future research is needed to explain the mechanisms by which fat supplementation does not appear to improve heat balance in heat-stressed cows.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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