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Original Article
Özgün Araştırma

The effect of different particle sized rations and live yeast supplementation on energy profile in dairy cows in heat stress

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Abstract: The aim of the present study was to determine the effect of adding different sized feed particles and live yeast culture (LY) to the rations of dairy cattle on their metabolic energy balance and some blood parameters under heat stress conditions. In this study, four Holstein-Fresiean dairy cattle (600 ± 25 kg of BW) in the middle of the lactation, which gave multiple births, were used. The effect of LY supplementation on the live weight (BW) change and on feed consumption was not determined. However, it was observed that the non esterified fatty acids (NEFA), beta hydroxybutyric acid (BHBA) and glucose levels of dairy cattle decreased significantly, while the urea level increased with the supplementation of LY to the ration (P<0.05). It was determined that the dry matter (DM), organic matter (OM) and neutral detergent fiber (NDF) consumption of dairy cattle under heat stress increased (P<0.05) by decreasing the particle size in the ration, while the consumption of non fiber carbonhydrate (NFC) was not affected (P >0.05). In addition, it was observed that NEFA and BHBA in the sera of dairy cattles decreased (P <0.05) significantly with the decrease in the particle size in the ration. Considering the data obtained from the present study, it can be said that reducing the particle size and supplementing of LY in the rations of dairy cattle under heat stress ameliorate the metabolic energy balance.

Keywords: Energy balance, heat stress, live yeast, particle size

Isı stresindeki süt ineklerinde farklı rasyonların enerji profili üzerindeki etkisi

Özet: Bu çalışmanın amacı, ısı stresi şartlarında süt sığırlarının rasyonlarına farklı yem parçacık büyüklüğü ve canlı maya kültürü (CMK) ilavesinin metabolik enerji dengesi ve bazı kan parametrelerine etkisini belirlemektir. Çalışmada birden fazla doğum yapmış laktasyonun ortasında dört adet Holstein-Fresiean süt sığırı (600 ± 25 kg CA) kullanıldı. Rasyona CMK katkısının canlı ağırlık (CA) değişimi ve yem tüketimine etkisi saptanmamıştır. Bununla birlikte rasyona CMK ilavesi ile süt sığırlarında esterleşmemiş yağ asidi (NEFA), beta hidroksi bütirik asit (BHBA) ve glukoz düzeyinin önemli derecede düştüğü, üre düzeyinin ise yükseldiği saptanmıştır (P<0.05). Rasyonda parça büyüklüğünün düşürülmesi ile ısı stresi altındaki süt sığırlarında kuru madde (KM), organik madde (OM) ve nötral deterjan lif (NDL) tüketiminin arttığı (P<0.05), lif olmayan karbonhidrat (LOK) tüketiminin ise etkilenmediği (P<0.05) belirlenmiştir. Ek olarak rasyonda parça büyüklüğünün düşmesi ile süt sığırlarında serumda NEFA ve BHBA'nın önemli derecede düştüğü (P<0.05) gözlemlenmiştir. Bu çalışmadan elde edilen veriler göz önüne alındığında, ısı stresi altındaki süt sığırlarında rasyon parça boyutunun küçültülmesi ve CMK ilavesinin metabolik enerji dengesini iyileştirdiği söylenebilir.

Anahtar kelimeler: Canlı maya kültürü, enerji dengesi, ısı stresi**,** parçacık boyutu

Introduction

Stress represents the body's response to stimuli that disrupt homeostasis and often have deleterious effects (Koubkova et al., 2002; Patel et al., 2016). Animal metabolism shows many neuro-endocrine responses, which can alter biochemical reactions to cope with stress. To determine the change in physiological mechanisms during stressful conditions, it is a good way to examine the plasma or serum levels of the enzymes that direct the reactions. Because the enzymatic activities of the animal under stress and the animal in the comfort zone are different (Patel et al., 2016).

Heat stress (HS) is one of the greatest challenges to improve production for dairy farms in tropical and subtropical regions. As it is known, increased

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body heat accumulation in dairy cows primarily appears when the ambient temperature is above 25°C. Dairy cows change their feeding behaviour such as decreasing dry matter intake (DMI), to reduce body heat production. However, this change causes a decrease in dairy production. Many studies have demonstrated that dairy cows exposed to HS had lower milk yield (Das et al., 2016), poor milk quality (Kadzere et al., 2002), and depressed reproductive efficiency (Khan et al., 2013). Therefore, there are many studies to alleviate the detrimental effects of HS on the production of dairy cows. However, the determination of effective methods for alleviating the negative effects of heat stress is also important.

Microbial additives, especially live yeast (LY) has been widely used in dairy rations under HS conditions. The main purpose of using LY in dairy rations is to enhance rumen flora and improve feed efficiency in dairy cows exposed to HS. Schingoethe et al. (2004) reported that supplementing 60 g/cow/day LY (*Saccharomyces cerevisiae*) improved feed efficiency defined as ECM/DMI but no differences in milk production in dairy cows exposed to HS. In another study, Bruno et al. (2009) stated that dietary 30 g/day LY (*Saccharomyces cerevisiae*) increased milk production (average +1,1 kg/day) in the hot season.

The physical form of dairy rations has a critical role in preventing either normal rumen functions or dairy production. Although reduction in chewing activity, saliva flow, and rumen pH with decreasing ration particle size in dairy cattle has been reported in many studies (Nørgaard 1983; Cassida and Stokes 1986). It was demonstrated that dairy cattle had higher DMI when offered a ration consists short particle size (DeVries et al., 2005). However, no study investigates the responses of dairy cattle when fed with different ration particle size under HS conditions. Although decreased DMI is the main detrimental effect of HS on dairy production, it was suggested that reduced DMI is responsible for only about 35% of the decrease in milk production, and the direct effects of heat on energetic metabolism may be the main problem that limits dairy production (Wheelock et al., 2010). Therefore, further studies are needed which investigate the effects of nutritional strategies on energy status in dairy cattle under HS conditions. The current study was conducted to compare the effects of two different nutritional strategies on metabolic profiles in dairy cows exposed to HS.

Material and Methods

Experimental Design and Animals

The study was conducted from June 10 to September 1, 2022, at the research farm of Adnan Menderes University, Faculty of Agriculture, located in Aydin, Türkiye (37°45′ N, 27°45′ E). Cows were kept in individual pens (20m^2) and were milked 2 times daily at 07:30 and 19:30. Local ambient temperature (^{o}C) and relative humidity (%) were recorded every 60 min with Hobo U12-013 (Onset, Bourne, USA). The temperature-humidity index (THI) was calculated using the following formula of THI= Temperature (^{o}C) + 0,36 x Dew point (^{o}C) +41,2 (Yousef, 1985).

Four multiparous Holstein-Friesian dairy cows (in midlactation, 600 ± 25 kg BW) were randomly assigned according to a 2x2 factorial arrangement in a 4x4 Latin square design to investigate the effects of dietary LY supplementation (0 or 1 g/d/cow) and two different ration peNDF contents in dairy cows under HS conditions. The total length of the trial was 84 d, with the 4 experimental periods consisting of 14 d of adaptation to treatments and 7 d of sample collections.

Experimental diets and Feeding

In the present study, 4 lactating cows were fed with 4 different rations: ration consisting of high particle size (HPS), ration consisting of high particle size with 1g/d/cow LY (Levucell® Sc 10 Metıtan® CNCM I-1077 (10x10^9 cob/g) (HPS+LY), ration consisting of short particle size (SPZ), ration consisting of short particle size with 1g/d/cow LY (Levucell® Sc 10 Metıtan® CNCM I-1077 (10x10^9 cob/g) (SPS+LY). To prepare rations consisting of different particle size alfalfa hay was chopped in two different choppers; a forage chopper for 1-2 cm, and a vertical feed mixer equipped with 8 blades for 5-9 cm.

Rations were adjusted to meet the nutrient requirements for Holstein dairy cattle (NRC 2001) (Table 1). Cows were fed 2 times daily at 08.00 and 20.00 h with free access to drinking water. The forage and concentrate of the rations were weighed and manually mixed for 7-8 minutes. During the morning feedings 200 ml water contained LY (1g) was drunk the cows in groups HPS+LY and SPS+LY.

Physical and chemical analyses of Total-Mixed Rations (TMR)

Penn state particle separator was used to determine particle size distributions of TMRs (Heinrichs and Kononoff 2002).

For chemical compositions, samples of TMR were ground 1 mm by using a lab grinder. DM, CP, ether extract (ee), and ash contents of the samples were analyzed according to (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were assayed according to (Van Soest et al., 1991) using an Ankom 200/220 Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Non-fiber carbohydrates (NFC) were calculated using the following formula of NFC.

NFC, % = 100- (NDF, % + CP, % + Ash, % + ee,

Table 1. Ingredients and chemical compositions of the experimental diets

Ingredients, %DM	High particle size	Short particle size
Forage	50	50
Corn silage	31,4	31,4
Alfalfa hay (coarsely chopped)	18,6	-
Alfalfa hay (finely chopped)	-	18,6
Concentrate	50	50
Corn grain	16,9	16,9
Barley grain	8,9	8,9
Sunflower meal	12,6	12,6
Cottonseed meal	6,3	6,3
Soybean meal	2,1	2,1
Wheat bran	1,3	1,3
Limestone	0,9	0,9
Sodium bicarbonate	0,5	0,5
Salt	0,4	0,4
Premix*	0,1	0,1
Chemical composition		
DM, %	56,5	56,5
CP, %	16,4	16,4
EE, %	3,2	3,2
NDF, %	36,8	36,8
ADF, %	21,9	21,9
NFC, %	36,2	36,2
Ash, %	7,4	7,4
Ca, %	0,71	0,71
P, %	0,43	0,43
NE_l, Mcal/kg **	1,45	1,45
Particle size distributions (DM basis)		
>19 mm	7,64	1,35
8-19 mm	29,57	27,21
<8 mm	62,77	70,42
$Pef_{8,0}$	31,71	22,34
$peNDF_{8,0}$	11,16	7,81

Each kilogram contains 1.300.000 IU vitamin A, 260.000 IU vitamin D3, 3.000 mg vitamin E, 120.000 mg niacin, 5.000 mg Mn, 5.000 mg Fe, 5.000 mg Zn, 1.000 mg Cu, 15 mg Co, 80 mg I, and 15 mg Se
** Calculated using NRC (2001) recommendations.

Analysis of Blood

Blood samples from all cows were collected at 14:00 on the 21 st days of the experimental periods. The blood serum was kept at -20°C until it was examined for biochemical parameters. Serum glucose, triglyceride, total cholesterol, Non-esterified Fatty Acids (NEFA) and b-hydroxybutyrate (BHBA) levels were analyzed by spectrophotometric method in an autoanalyzer (Biosystems S.A, Barcelona, Spain) with commercial test kits (Biosystems, Spain).

Statistical Analysis

The SPSS (version 22.0 Armonk, NY) procedure for General Linear Model (GLM) was performed to determine effects of experimental treatments on BW, nutrient intakes and blood parameters. Data were presented as means ± standard deviation and $P<0.05$ was accepted as statistically significant.

Results

Environmental Conditions

Average weather conditions in experiment periods were presented in Table 2. Throughout the experiment, the ambient temperatures ranged from 19.5 to 39.7 °C with average 29.3 °C. During the experiment, average THI was above 76.5 in all experimental periods.

Table 2. Temperature (°C), relative humidity (%), and temperature humidity index of the experiment periods.

Environmental Conditions	Experimental Time
Temperature means, °C	**29,3**
Minimum	19,5
Maximum	39,7
Relative humidity means %	**46,7**
Minimum	23,5
Maximum	74,0
THI means	**76,8**
Minimum	66,4
Maximum	83,00

Effects of dietary LY supplementation and ration particle size on nutrient intakes

Effects of dietary LY supplementation and ration particle size on nutrient intakes (kg DM/BW) were presented in Table 3. In the current study BW was not affected by treatments. However, intakes of DM, OM and NDF as percentage of BW were statistically higher in cows fed with SPS (P<0.05). Dietary LY supplementation did not affect the nutrient intakes. Additionally, there was no interaction between the groups.

Table 3. Effects of dietary LY supplementation and ration particle size on nutrient intakes (kg/BW).

	Particle size[a]		LY supplementation[b]		SEM	P-value		
Item	HPS	SPS	LY_N	LY		PS	LY	PSxLY
BW	611,5	611,1	611,0	611,6	3,150	0,934	0,891	0,912
DMI/BW	3,16	3,20	3,18	3,18	0,020	*	0,992	0,914
OMI/BW	2,85	2,91	2,87	2,89	0,017	*	0,650	0,909
NFCI/BW	1,511	1,508	1,510	1,509	0,007	0,814	0,932	0,338
NDFI/BW	1,035	1,063	1,046	1,052	0,013	*	0,856	0,532

[a] HPS: High particle size, SPS: Short particle size
[b]LY_N: 0 g/day live yeast, LY: 1g/d/cow live yeast (Levucell® Sc 10 Metıtan® CNCM I-1077 (10x10^9 cob/g)
*= P<0.05

Effects of dietary LY supplementation and ration particle size on biochemical parameters

Table 4 shows the effects of treatments on blood serum NEFA, BHBA, glucose, triglyceride total cholesterol and urea concentrations. Both feeding with SPS and LY supplementation reduced serum NEFA and BHBA concentrations (P<0.05) in dairy cows in heat HS. Additionally, cows fed with SPS had lower urea and higher glucose concentrations compared to cows fed with HPS. However, serum glucose, triglyceride and total cholesterole concentrations were not affected by experimental treatments. No interaction was observed between the ration particle size and dietary LY supplementation for serum NEFA, BHBA, glucose, triglyceride and total cholesterole concentrations.

Table 4. Effects of treatments on blood serum NEFA, BHBA, triglyceride, glucose and urea concentrations.

	Particle Size[a]		LY Supplementation[b]		SEM	P-Value		
Item	HPS	SPS	LY_N	LY		PS	LY	PSxLY
NEFA (mmol/L)	0,234	0,218	0,242	0,210	0,046	*	*	0,203
BHBA (mmol/L)	0,546	0,414	0,491	0,369	0,029	*	*	0,089
Triglyceride (mg/dL)	7,40	7,33	7,10	7,63	0,67	0,948	0,581	0,080
Glucose (mg/dL)	40,57	41,28	39,17	45,68	2,79	0,860	*	0,811
Total Cholesterole (mg/dL)	162,12	143,11	158,15	147,08	12,91	0.318	0.556	0,056
Urea (mg/dL)	23,44	22,86	25,12	19,68	1,53	0,592	*	0,141

[a] HPS: High particle size, SPS: Short particle size
[b]LY_N: 0 g/day live yeast, LY: 1g/d/cow live yeast (Levucell® Sc 10 Metıtan® CNCM I-1077 (10x10^9 cob/g)
*= P<0.05

Discussion

It has been demonstrated that the critic THI point at which the effects of heat stress begin to appear for dairy cattle is 68 (Collier et al., 2012) or 72 (Armstrong 1994). In the current study, the recorded THI average was above 76.5. Therefore, it can be said that cows were exposed to severe HS in all experimental periods.

Heinrich and Kononoff (2002) suggested that optimal part, the optimal particle size distributions of TMR was 2 to 8 percent for long particles (>19 mm), 30 to 50 percent for medium particles (8-19 mm), 30 to 50 percent for short particles (1.18-8 mm) and less than 20 percent for fine (<1.18 mm). In the present study, the particle size distribution of HPS ration (Table 3) was similar to this suggestion. However, SPS ration had lower long particles (1.34 %

of DM), and higher fine particles than guidelines for TMR particle size by Heinrich and Kononoff (2002).

In the current study, BW changes of cows were not affected by any experimental treatments (Table 3). The effects of different ration particle size on dairy cow performance in hot seasons were not found in the literature reviews. However, Beauchemin and Yang (2005) declared that peNDF contents of ration had no effect on BW in dairy cows, but they did not give any information about environmental conditions. Similar to our study, Dehghan-Banadaky (2013), reported that dietary LY supplementation ($15x10^9$ cob/g) did not affect the BW changes in dairy cows under HS.

Allen (2000) and Kononoff and Heinrichs (2003) investigated effects of ration particle size on DMI in dairy cows and stated that reduction in ration particle size increased the DMI in dairy cows. Our findings in the current study were similar to these previous reports. Because DM intakes as percentage of BW were significantly higher in cows fed SPS (Table 3). The passage rate which was faster in cows, fed with lower peNDF content ration (Allen 2000) may be associated with higher DMI in cows fed with SPS. Additionally, feed sorting may be another explanation for differences in DMI. DeVries et al. (2005) and Miller-Cushon et al. (2019) observed that cows under HS conditions selected against long particles of rations. In the present study, decreasing particle size of ration may prevent the feed sorting in dairy cows exposed to HS. NDF and NFC intakes (Table 4) also support this explanation. Because NDF intakes which can represent forage form of TMR were higher in cows fed SPS while NFC intakes were similar between the groups. However, in the present study serum glucose, triglyceride and total cholesterol concentration changes were not affected by the experimental treatments.

In the present study, no statistical difference was observed between triglyceride and total cholesterol concentrations in sera obtained from different ration groups in cows under HS ($P>0.05$). In the previous studies on this subject, it was stated that HS caused an increase in the concentrations of NEFA and BHBA (Moore et al., 2005; Garner et al., 2017; Kumar et al., 2017), which are energy metabolites, and a decrease in glucose concentration (Calamari et al., 2011; Abeni et al., 2007). It is stated that this effect occurs as a result of adipose tissue mobilization, glucose oxidation and gluconeogenesis pathways (Ronchi et al., 1999; Rhoads et al., 2009; Wheelock et al., 2010; Shah et al., 2020), which may occur as a result of negative energy balance due to HS. In the present study, cows fed with SPS had lower serum NEFA and BHBA concentrations than cows fed with HPS ($P<0.05$). This can be attributed to the higher DM and thus higher energy intake of the cows fed with SPS compared to the other group (Table 3). It is known that high dietary energy has a positive effect on the energy balance of the metabolism in the negative energy balance status. It has been reported that the use of amino acids as an energy source in dairy cows under HS conditions is higher than in dairy cows under normal conditions (Ronchi et al., 1999; Abeni et al., 2007). In the presented study, although DM intake was not affected by LY supplements, serum NEFA, BHBA and urea concentrations were lower and glucose concentrations were higher in cows fed with LY. This result can be associated with the positive effect of LY supplementation on ruminal flora protein synthesis (Bruno et al., 2009; Miller-Webster et al., 2002) and nutrient digestibility (Perdemo et al., 2020).

Conclusion

THI data obtained from the study show that severe heat stress conditions occur for dairy cows in the summer months in Aydın province. On the other hand, it is possible to say that reducing the particle size of the ration in dairy cows increases DM consumption and improves metabolic energy balance under heat stress conditions. Although the addition of LY to the ration had no effect on DM intake, a ameliorate effect on lipid and energy metabolites was observed. As a result, it has been suggested that feed ration prepared as SPS+LY in dairy cows under heat stress can create better energy performance and thus reduce the negative effects of heat stress more. However, more comprehensive studies on this application are needed.

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Ethical statement: All procedures of this experiment were managed according to the ethical approval by the local committee of Aydın Adnan Menderes University, Türkiye (protocol number: 64583101/2022/018).

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