Kafkas Univ Vet Fak Derg
18 (1): 115-120, 2012
DOI:10.9775/kvfd.2011.5143

RESEARCH ARTICLE

The Effects of Additional Glycerol in Different Feed Form on Dairy Cows [1]

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[1] *This study was supported by TÜBİTAK (The Scientific and Technological Research Council of Turkey), Research Grand: TÜBİTAK-TOVAG 106O360*

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Makale Kodu (Article Code): KVFD-2011-5143

Summary

This research was conducted to determine the impact of diet supplementation with glycerol in form of mash or pelleted feed on milk yield, milk composition, live weight and changes in body condition scoring and the effect of on blood plasma glucose, nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) concentrations of dairy cows. In middle lactation stage, Holstein dairy cows (n = 94) were divided into three main groups: (1) control (n = 40); (2) mash concentrated feed+ glycerol group (n = 27); and (3) pelleted concentrated feed + glycerol (n = 27). Glycerol is added into concentrated feed at the rate of 5%. The study was lasted 69 d. There was not a significant difference among groups in terms of milk yield, body weight and plasma glucose, NEFA and BHBA concentrations. In the last day of study, milk solid non fat (SNF) were higher and milk urea-N content were lower in pelleted feed+ glycerol group (P<0.05).

Keywords: *Cow, Glycerol, Milk yield, Milk composition, Plasma NEFA*

Süt İneklerinde Farklı Yem Formları İle Gliserol İlavesinin Etkileri

Özet

Bu çalışma, gliserinin süt ineklerine toz veya pelet formdaki yemler içinde verilmesinin süt verimi, sütün kompozisyonu, canlı ağırlık ve vücut kondisyon skorundaki değişimler ile kan plazmasında glikoz, NEFA ve BHBA konsantrasyonları üzerine etkisini incelemek amacıyla yapılmıştır. Araştırmada laktasyonun ortalarında bulunan süt inekleri (n = 94), kontrol (n = 40), toz yem + gliserol (n = 27), pelet yem + gliserol (n = 27) olmak üzere üç gruba ayrılmıştır. Gliserol konsantre yemlere %5 oranında ilave edilmiştir. Araştırma 69 gün sürdürülmüştür. Gruplar arasında süt verimi, canlı ağırlıklar ve plazma glikoz, NEFA ve BHBA konsantrasyonları açısından önemli bir farklılık gözlenmemiştir. Çalışmanın son günü, peletlenmiş gliserinli yem tüketen grupta sütte yağsız kuru madde oranı daha yüksek, süt üre-N'u içeriği daha düşük bulunmuştur (P<0.05).

Anahtar sözcükler: *İnek, Gliserol, Süt verimi, Süt kompozisyonu, Plazma NEFA*

INTRODUCTION

Glycerol is a product of the processing of fats for the chemical industry and for biodiesel production [1]. The availability of glycerol as a substrate for glucose production also can arise as a result of carbon recycling and may be an important gluconeogenic precursor as the cow adapts to lactation. Glycerol can be converted to glucose in the liver and enters the glucogenic pathway at the level of dihydroxyacetone phosphate and 3-phosphoglyceraldehyde [2-4].

Recent data [5] suggest that glycerol may be an important glucose precursor only during the transition period. The transition period of dairy cows, ranging from three weeks prepartum to three weeks postpartum, is a period marked with large changes in metabolic demands due to parturition and lactogenesis [4,6,7]. Ketosis and fatty liver are two metabolic disorders related to energy metabolism that occur in varying frequency and severity during the transition period of the dairy cow [8-10]. As a result of

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these metabolic disorders increased concentrations of nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA). Gluconeogenic supplements have been reported to decrease NEFA and BHBA, and increase blood glucose during the transition period [8-10]. Early studies [11-13] showed that added glycerol to cows diets during the transition period increased appetite, milk production and plasma glucose concentrations and decreased plasma ketone bodies. But in the further periods of lactation, there is no study of glycerol usage in dairy cow feeding.

The objective of this study was to determine the effect of glycerol on milk production, milk composition, body weight, body condition score and some blood parameters on dairy cows in middle lactation period.

MATERIAL and METHODS

Experimental Design, Animal Care, and Treatments

Experimental procedures were approved by the Selcuk University, Veterinary Faculty Ethics Commission.

Ninety-four lactating Holstein cows housed in free stalls at the a private dairy cow farm which has a capacity of 480 dairy cows and fed a basal lactating cow diet as a TMR balanced to meet NRC [14] guidelines for 650-700 kg Holstein dairy cows producing 30 kg of milk/d with 3.5% of milk fat. The base ration contained corn silage 15 kg, wheat straw 2.5 kg, alfalfa hay 5 kg, wet sugar beet pulp 4 kg and concentrated feed 10.5 kg *(Table 1)*. Cows were grouped to milk production and parity and randomly divided into three main groups: (1) control mash concentrated feed (n = 40); (2) mash concentrated feed + glycerol group (n = 27); and (3) pelleted concentrated feed + glycerol (n = 27) *(Table 2)*. Crude glycerol (4.14% methanol, 0.68% EE, 4.05% ash, 1.96%, pH degree is 5.49) is added in concentrated feed at the rate of 5%. The experimental period lasted 69 days which consist of 9 days adaptation period. Body weight and milk yield were recorded at each milking and body condition score was scored by 3 trained individuals based on a 5-point scale [15]. At 0, 30, and 60 d of the experiment, 12 cows were randomly selected from each group for determination body weight and body condition score.

Sample Collection and Laboratory Analyses

- Feed analyses

Samples of the corn silage, wheat straw, dehydrated alfalfa, sugar beet pulp and concentrate mix were dried by the means of oven until to obtained the constant weigh and determined for dry matter content [16].

- Milk sampling and analyses

Cows were milked twice daily and milk samples were taken on morning of the d 0, 30, 60 by sampling kit in the milking system during milking and bronopol (2-bromo-2-nitropropane-1.3 diol) is used in order to avoid milk spoilage. Milk samples were analyzed for dry matter, fat, solid non fat, protein, lactose and milk urea nitrogen

Table 1. *Components and chemical composition of concentrate*
Tablo 1. *Konsantre yemin bileşimi ve kimyasal kompozisyonu*

Ingredients	g/kg
DDGS [1]	150
Corn gluten feed	135
Sunflower meal	125
Corn	115
Barley	100
Lentil	100
Cottonseed meal	100
Wheat bran	75
Molasses	66
Limestone	15
Salt	10
Vegetable oil	8
Vitamin and mineral premix	1
Calculated analysis	
Metabolisable energy (kcal/kg)	2631
Dry matter	875.0
Ash	89.9
Crude protein	187.6
Crude Fat	43.5
Calcium	9.7
Total phosphorus	5.3

[1] ***DDGS:*** *Dehydrated Distillers Grains with Solubles*

Table 2. *Characteristics of cows in study*
Tablo 2. *Çalışmada kullanılan ineklerin özellikleri*

Item	Control	Mash Feed + Glycerol	Pelleted Feed + Glycerol
Number of cows (n)	40	27	27
Days of lactation at the beginning of study	113±9.7	113±16.3	111±10.2
Average lactation numbers	2.30±0.19	2.48±0.22	2.59±0.25
Milk yield before grouping, l/day	26.7±0.63	26.4±0.87	27.1±0.87

(MUN) by means of Foss Milkoscan FT120.

- Blood sampling and analyses

Blood samples were collected three hours later after feding in the morning on d 0, 30, 60. Samples were taken into vacotainers containing EDTA K_3 by venipuncture. Plasma was analyzed for BHBA, NEFA and glucose concentrations by spectrophotometric method by using test kits (Randox, RANBUT, NEFA, GLUC-PAP). (Randox Laboratories Ltd, Antrim, UK)

Statistical Analyses

Data were subjected to one-way analysis of variance using SPSS [17] software. Significant effects of dietary treatments on experimental groups were evaluated with the Duncan test. Statements of statistical significance are based on a probability of P<0.05.

RESULTS

Dry Matter Intake, Body Weight, Body Condition Score and Milk Yield

On days 1-30, 31-60, 1-60, dry matter intake was same between the treatment and control groups (22.75 kg/cow/day). Supplementing lactating cow diets with glycerol + mash or pelleted concentrated feed did not affect body weight, body condition score and milk yield (P>0.05) *(Table 3, 4)*

Milk Composition

The data of milk composition of the groups are shown in *Table 4*. There were no difference in milk DM, fat and lactose between the groups on days 0, 30 and 60, however there were difference in non solid fat (d 0, 60), protein (d 0) and MUN (d 60) between the groups (P<0.05).

Plasma BHBA, NEFA and Glucose

Table 5 shows the effect of Glycerol with different applications on plasma BHBA, NEFA, glucose concentrations of lactation cows. Plasma BHBA, NEFA and glucose concentrations did not differ significantly (P>0.05) among the three experimental groups during the experimental period.

DISCUSSION

The addition of glycerol with mash or pelleted concentrated feed to the diets did not affect the body weight and body condition score. DeFrain et al.[18] reported that addition glycerol to the diets did not affect body weight and body condition score of dairy cows in transition period. Also Ogborn [4] reported that postpartum body weight and body condition score were not affected by the incorporation of glycerol in the postpartum diet. In another study [19] Cows fed diets containing 10 and 15% glycerol gained more weight than containing 0 or 5% glycerol but body condition scores did not affect with glycerol feeding.

Some studies have shown that the milk yield did not affect with the glycerol addition to diets [4,18,20]. DeFrain et al.[18] reported no effect of feeding glycerol on milk yield between 14 d prior to expecting calving to 21 d postpartum. Also Ogborn [4] reported that drenching glycerol for the first 5 d of lactation did not affect milk yield or milk fat percentage during the first 63 d of lactation. In the present study, there were no difference in milk DM, fat and lactose between the groups on days 0, 30 and 60. Also there were differences in non solid fat (d 0, 60), protein (d 0) and MUN (d 60) between the groups. At the last day of the study, it is observed that non solid fat content increased and MUN content of the milk decreased in the group which consume pelleted concentrated feed and glycerol. The changes of energy and protein metabolism in rumen affect MUN content of milk. MUN is an indicatior of how much of microbial NH_3 is used in protein synthesis and to control the energy balance of the ration [21,22]. Decreased MUN suggest greater N efficiency. However, bacterial N content of rumen fluid decreased by glycerol [23]. Ogborn [4] reported that milk fat, protein and MUN were not affected by feeding glycerol

Table 3. *Body weight and body condition score of cows* [1]
Tablo 3. *İneklerin, canlı ağırlık ve kondüsyon skorları* [1]

Item	Control	Mash Feed + Glycerol	Pelleted Feed + Glycerol	P
Body Weight, kg				
Beginning of the experiment	672.0±15.9	680.1±21.0	698.0±19.1	0.610
30. day of the experiment	683.1±19.0	692.2±16.2	691.0±11.8	0.907
Last day of the experiment	672.8±23.1	679.2±17.3	680.3±12.3	0.857
Body Condition Score				
Beginning of the experiment	3.10±0.04	3.06±0.04	2.97±0.04	0.073
30. day of the experiment	3.05±0.04	3.08±0.05	3.08±0.05	0.862
Last day of the experiment	3.03±0.03	3.10±0.06	3.07±0.04	0.519

[1] Data are mean values for 12 cows for each groups
Probability that treatment means are not different (P>0.05)

***Table 4.** Milk yield and milk composition of groups, l/day [1]*
***Tablo 4.** Gruplara gore süt verimi ve sütün kompozisyonu, l/day [1]*

Item	Control	Mash Feed + Glycerol	Pelleted Feed + Glycerol	P
Milk Yield (day)				
1-30	25.53±0.61	24.89±0.79	26.97±0.90	0.178
31-60	24.65±0.57	25.46±0.92	26.29±1.14	0.379
1-60	25.09±0.58	25.18±0.85	26.64±1.01	0.321
Milk Composition (day)				
Dry matter, %				
0	11.41±0.18	11.49±0.20	11.74±0.16	0.408
30	12.06±0.27	11.47±0.25	11.85±0.24	0.280
60	12.20±0.18	11.91±0.27	12.26±0.21	0.473
Non Solid Fat, %				
0	8.19^{b}±0.08	8.37^{ab}±0.09	8.51^{a}±0.05	0.016
30	8.38±0.11	8.27±0.11	8.58±0.08	0.109
60	8.43^{b}±0.11	8.44^{b}±0.12	8.83^{a}±0.07	0.023
Fat, %				
0	3.13±0.13	3.03±0.03	3.18±0.18	0.764
30	3.58±0.23	3.09±0.19	3.18±0.23	0.276
60	3.67±0.15	3.38±0.21	3.37±0.2	0.531
Protein,%				
0	2.75^{b}±0.07	2.97^{a}±0.09	2.96^{a}±0.05	0.046
30	3.03±0.09	2.89±0.08	3.08±0.07	0.259
60	3.07±0.08	3.14±0.07	3.30±0.08	0.101
Laktose, %				
0	4.62±0.04	4.59±0.04	4.70±0.03	0.140
30	4.52±0.04	4.56±0.05	4.64±0.04	0.089
60	4.52±0.06	4.46±0.08	4.69±0.04	0.079
MUN, mg/100 ml				
0	19.59±0.52	20.29±0.79	18.93±0.39	0.265
30	21.41±0.62	20.92±0.57	22.02±0.64	0.126
60	21.99^{a}±0.57	20.64^{ab}±0.95	19.32^{b}±0.68	0.039

***MUN:** Milk urea nitrogen*
[1] Data are mean values for 40 samples for control, 27 samples for each mash feed + glycerol and pelleted feed + glycerol
[a–b] Means within the same row bearing different superscripts differ significantly (P<0.05)

during either the prepartum or postpartum periods. Also DeFrain et al.[18] found that glycerol administration as a dietary supplement does not significantly increase milk components during early lactation. In another research [19] found that milk composition was unaffected by glycerol feeding with the exception that MUN decreased.

Administration of gluconeogenic supplements such as glycerol and propylene glycol have been reported to decrease NEFA and β-hydroxybutyrate and increase blood glucose in transition period [8,24-26]. But in the present study, glycerol treatments did not affect glucose, NEFA or BHBA concentrations of dairy cows in middle lactation period. Also some researches [4,18] reported that glycerol addition to diets did not significantly affect plasma concentrations of glucose, NEFA, and BHBA during the transition periods. Some studies have shown that the BHBA concentrations of the dairy cows in the transition period decreases with addition glycerol to diet or water [25,26]. On the contrary, at the end of the study, plasma BHBA concentrations were found high (>1200 µmol/l) and this is an indication subclinic ketosis that defined as circulating concentration BHBA greater than 1200 µmol/l [27]. The reason why it is high BHBA concentration in the middle of the lactation period is not understood. NEFA concentration is one of the parameters for determining metabolic profile of dairy cows. Leblanc et al.[28] reported that in the dairy cows whose plasma NEFA concentrations is above 0.500 mmol/l the risk of catch metabolic disease is 3,6 times much possible In the present study, there were no difference on NEFA concentrations

***Table 5.** Plasma BHBA, NEFA and glucose concentrations of cows [1]*
***Tablo 5.** İneklerin plazma BHBA, NEFA ve glikoz konantrasyonları [1]*

Day	Control	Mash Feed + Glycerol	Pelleted Feed + Glycerol	P
BHBA, µmol/l				
0	669.4±95.0	609.1±107.1	661.5±91.4	0.895
30	607.9±45.6	588.1±36.7	539.7±26.4	0.410
60	1316.9±84.5	1161.4±59.2	1303.3±71.3	0.275
NEFA, mmol/l				
0	0.228±0.06	0.194±0.03	0.217±0.04	0.871
30	0.330±0.07	0.256±0.05	0.309±0.05	0.633
60	0.254±0.03	0.233±0.04	0.240±0.03	0.901
Glucose, mg/100ml				
0	75.07±5.00	69.92±3.72	72.33±3.78	0.690
30	60.36±2.74	64.62±3.17	64.38±2.98	0.524
60	73.11±3.19	76.15±2.46	76.87±4.18	0.706

***BHBA:** β-hydroxybutyrate, **NEFA:** Nonesterified fatty acids*
[1] Data are mean values for 40 samples for control. 27 samples for each mash feed + glycerol and pelleted feed + glycerol groups
Probability that treatment means are not different (P>0.05)

between the groups and NEFA concentrations were found below critic levels that Leblanc et al.[28] reported. BHBA, NEFA and glucose concentrations of the dairy cows in the middle of the lactation period cannot be compared with the findings of previous studies because there are currently no other published data regarding the effect of glycerol addition on BHBA, NEFA and glucose concentrations of dairy cows at mid lactation.

Application of crude glycerol to the diets of 5% did not significantly affect milk yield, milk composition, body weight, body condition score, plasma concentrations of glucose, NEFA, and BHBA in mid lactation dairy cows. Based upon this result, glycerol supplementation with mash or pelleted concentrated feed to dairy cows in mid lactation is not useful as a glucogenic supplement. But it can be said that in the case of crude glycerol cost being cheaper than compound feed cost it can be used as an alternative to grain as an energy source in dairy cow diets, and in case of its cost being high it would not be advantageous to add glycerol in diets.

REFERENCES

1. Schroder A, Sudekum KH: Glycerol as a by-product of biodiesel production in diets for ruminants. *Proceedings of the 10th International Rapeseed Congress*, Canberra, Australia, 26-29 September, 1999.

2. Overton TR: Substrate utilization for hepatic gluconeogenesis in the transition dairy cow. *Proceedings, Cornell Nutrition Conference for Feed Manufacturers*. Cornell University, Ithaca, NY, 20-22 October, pp. 237-246, 1998.

3. Drackley JK, Overton TR, Douglas GN: Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during peri-parturient period. *J Dairy Sci*, 30, 100-112, 2001.

4. Ogborn KL: Effects of method of delivery of glycerol on performance and metabolism of dairy cows during transition period. *MSc Thesis*, Cornell University, 2006.

5. Reynolds CK, Burst B, Lupoli B, Humphries DJ, Beever DE: Visceral tissue mass and rumen volume in dairy cows during the transition from late gestation to early lactation. *J Dairy Sci,* 87, 961-971, 2003.

6. Grummer RR: Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *J Anim Sci*, 73, 2820-2833, 1995.

7. Drackley JK: Biology of dairy cows during the transition period: The final frontier? *J Dairy Sci*, 82, 2259-2273, 1999.

8. Grummer RR: Etiology of lipid metabolic disorders in periparturient dairy cows. *J Dairy Sci*, 76, 3882-3896, 1993.

9. Arslan C, Tufan T: Geçiş dönemindeki süt ineklerinin beslenmesi I. Bu dönemde görülen fizyolojik, hormonal, metabolik ve immunolojik değişiklikler ile beslenme ihtiyaçları. *Kafkas Univ Vet Fak Derg*, 16, 151-158, 2010.

10. Arslan C, Tufan T: Geçiş dönemindeki süt ineklerinin beslenmesi II. Bu dönemde görülen metabolik hastalıklar ve besleme ile önlenmesi. *Kafkas Univ Vet Fak Derg*, 16, 159-166, 2010.

11. Johnson R: The treatment of ketosis with glycerol and propylene glycol. *Cornell Vet*, 44, 6-21, 1953.

12. Fisher LJ, Erfle JD, Satter LD: Preliminary evaluation of the addition of glucogenic material to the rations of lactating cows. *Can J Anim Sci*, 51, 721-727, 1971.

13. Linke PL, DeFrain JM, Hippen AR, JardonPW: Ruminal and plasma responses in dairy cows to drenching or feeding glycerol. *J Dairy Sci*, 87, 343, 2004.

14. NRC-National Research Council: Nutrient Requirements of Dairy Cattle. 7th rev. ed., National Academy Press, Washington DC, 2001.

15. Wildman EE, Jones GM, Wagner PE, Boman RL, Troutt Jr HF, Lesch TN: A dairy cow body condition scoring system and its relationship to selected production characteristics. *J Dairy Sci*, 65, 495-501, 1982.

16. AOAC: Official Methods of Analysis. 15th ed., Association of Official Analytical Chemists, Arlington, VA, 1990.

17. SPSS 15.0: Statistical software package for the social sciences SPSS, Int., USA, 2006.

18. DeFrain JM, Hippen AR, Kalscheur KF, Jardon PW: Feeding glycerol to transition dairy cows: Effects on blood metabolites and lactation performance. *J Dairy Sci*, 87, 4195-4206, 2004.

19. Donkin SS, Koser SL, White HM, Doane PH, Cecava MJ: Feeding value of glycerol as a replacement for corn grain in rations fed to lactating dairy cows. *J Dairy Sci*, 92, 5111-5119, 2009.

20. Fisher LJ, Erfle JD, Lodge GA, Sauer FD: Effects of propylene glycol or glycerol supplementation of diet of dairy cows on feed intake, milk yield and composition, and incidence of ketosis. *Can J Anim Sci*, 53, 289-296, 1973.

21. Kirchgessner M, Kreuzer M, Roth-Mailer DA: Milk urea and protein content to diagnose energy and protein malnutrition of dairy cows. *Arch. Animal Nutrition*, 36, 192-197, 1986.

22. Spohr M, Wiesner HU: Monitoring herd health and milk production by means of the extended milk production performance. *Milchpraxis,* 29, 231-236, 1991.

23. Kijora C, Bergner H, Gotz KP, Bartelt J, Szakacs J, Sommer A: Research note: Investigation on the metabolism of glycerol in the rumen of bulls. *Arch Tierernahr,* 51, 341-348, 1998.

24. Nielsen NI, Ingvartsen KL: Propylene glycol for dairy cows: A review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. *Anim Feed Sci Technol,* 115, 191-213, 2004.

25. Chung YH, Rico DE, Martinez CM, Cassidy TW, Noirot N, Ames A, Varga GA: Effects of feeding dry glycerin to early postpartum Holstein dairy cows on lactational performance and metabolic profiles. *J Dairy Sci,* 90, 5682-5691, 2007.

26. Osborne VR, Odongo NE, Cant JP, Swanson KC, McBride BW: Effects of supplementing glycerol and soybean oil in drinking water on feed and water intake, energy balance, and production performance of periparturient dairy cows. *J Dairy Sci*, 92, 698-707, 2009.

27. Duffield TF, Sandals D, Leslie KE, Lissemore K, McBride BW, Lumsden JH, Dick P, Bagg R: Efficacy of monensin for the preventation of subclinical ketosis in lactating dairy cows. *J Dairy Sci,* 81, 2866-2873, 1998.

28. LeBlanc SJ, Leslie KE, Duffield TF: Metabolic predictors of displaced abomasum in dairy cattle. *J Dairy Sci*, 88, 159-170, 2005.