EFFECT OF GLYCEROL ON MILK YIELD, ITS QUALITY AND BLOOD PARAMETERS OF HOLSTEIN COWS

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ABSTRACT

The aim of this study was to investigate the effect of adding glycerol at different levels (T1 = control treatment without glycerol, T2 = 75 ml and T3 = 150 ml glycerol) on daily milk yield, milk components and some blood parameters of Holstein cows during the period of 60 days. Milk yield increased significantly (P≤0.05) at 15th day of the experiment, and highly significant (P≤0.01) at 30th and 60th days of the experiment in T3 group. Percentage of milk protein and lactose increased (P≤0.01) in T2 and T3 groups and the percentage of milk fat in the T3 group decreased at day 60 of the experiment. The blood glucose concentration increased (P≤0.01) in T2 and T3 groups at 30 and 60 days of the experiment. Triglyceride concentration increased (P≤0.01) in the blood of T2 and T3 treated cows at day 60. The concentration of cholesterol, NEFA and BHBA decreased (P≤0.01) in T3 treated cows at day 60 of the experiment. The concentration of phosphorous and magnesium were also affected as a result of glycerol treatment. It can be concluded that the addition of dietary glycerol 150 ml / day enhanced milk yield, protein and lactose percentage, and improved blood parameters by increasing blood glucose and reducing NEFA and BHBA concentrations of Holstein cows.

Key words: Pure glycerol, Milk production, NEFA, BHBA.
***Part of Ph.D. dissertation of the 1st Author.**

تأثير الكليسيرول في انتاج الحليب ونوعيته ومعاييرالدم لابقار الهوشتاين

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المستخلص

اجريت هذه الدراسة بهدف معرفة تأثير اضافة الكليسيرول بمستويات مختلفة (T1 = معاملة السيطرة بدون كليسيرول و T2 = 75 مل و T3 = 150 مل كليسيرول) في انتاج الحليب ومكوناته الرئيسة وبعض معاير الدم لابقار الهولشتاين. ازداد انتاج الحليب معنوياً (P≤0.05) عند اليوم 15 من بدء التجربة وعالي المعنوية (P≤0.01) عند اليوم 30 و60 من التجربة لدى ابقار المعاملة T3. زادت نسبة بروتين ولاكتوز الحليب معنوياً (P≤0.01) لدى ابقار المعاملتين T2 و T3 وانخفضت نسبة دهن الحليب لدى المجموعة T3 عند اليوم 60 من التجربة. ازداد تركيز كلوكوز الدم معنويا (P≤0.01) في المعاملتين T2 وT3 عند اليوم 30 للتجربة. عند اليوم 60 من التجربة ارتفع تركيز الكلوكوز و Triglyceride معنوياً (P≤0.01) في دم ابقار المعاملتين T2 وT3 وانخفض معنوياً (P≤0.01) تركيز كل من الكوليسترول و NEFA وBHBA في المعاملة T3. نستنتج ان اضافة الكليسيرول الى العليقة بكمية 150 مل/يوم في تغذية الابقار يعزز من انتاج الحليب ونسبتي بروتين ولاكتوز الحليب وحسن من معاير الدم من خلال زيادة كلوكوز الدم وخفض تراكيز NEFA و BHBA لدى ابقار الهولشتاين.

الكلمات المفتاحية: كليسيرول نقي, انتاج الحليب, NEFA, BHBA.
***البحث مستل من اطروحة الدكتوراه للباحث الاول.**

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INTRODUCTION

Milk is a necessary food for the body because it contains important nutrients from minerals and vitamins (2), and the milk production is one of the most important economic characteristics of cows (40, 3). In recent years, increased the demand for primary feed materials, especially those equipped with energy (corn, oilseeds, etc.), and this has led to an increase in their prices globally, making feeding ruminants more expensive and thus increasing production costs (1, 22). Also, high -producing cows are more susceptible to ketosis when they reach the peak milk yield as a result of low energy, resulting from low feed intake or low dieting energy source (36). All these reasons encouraged the related studies to search for alternative feed sources with lower costs and good energy sources (7). Glycerol is one of the alternative feed energy sources, which has been widely used in recent years in the diet of ruminant animals, with lesser cost than corn and oilseeds that characterized by high energy source (30, 39, 48). Moreover, in ruminants glycerol enter into the liver for gluconeogenesis, reducing the need of propionate required for glucose synthesis (30). Glycerol is generally obtained from oils and fats of plant and animal sources by esterification, hydrolysis or saponification processes of triglycerides (15). Studies indicated that glycerol prevents metabolic problems in cows during the transition period (3 weeks pre- and 3 weeks post- partum) (38), as well as increases animal energy and reduces free fatty acids responsible for ketosis (25, 30). Valencia et al (46) reported that adding glyce-rol to the diet of dairy cows (1500 g/day/cow), leads to an increase milk production by 14.2% and milk protein by 2.1%. Similar, Kupczynski et al (31) reported the importance of using glycerol in the diet of ruminants to reduce production costs by replacing it with feed grains or using it as a preventive agent against metabolic problems and ketosis. Other studies indicated that the effect of glycerol on the performance and productivity of cows depends on the level and purity of glycerol added to the feed (19, 26, 45). Despite those and other studies that indicated the importance and role of glycerol in enhancing cow productivity, they did not indicate the qccurate level of dietary glycerol (33). The aim of this study was to study the effect of adding different levels of dietary pure glycerol on the production and components of milk and some blood parameters.

MATERIALS AND METHODS

Experimented animals and design

This study was conducted at the Al-Salam Private Station For Dairy Cows (Latifia, 25 km south of Baghdad), for the period from 11/11/2020 to 12/1/2021. Fifteen Holstein dairy cows (4^{th} lactation season) were used 7 days post-partum, randomly divided into three equal groups. All three groups were fed with a standardized concentrated diets (Table1) at a rate of 4 kg daily divided into two meals, at the morning and evening. The roughage feed (dry and green) was introduced ad libitum during the experimental period. Also, mineral salt blocks were introduced to all animals and the water was constantly available throughout the experiment Glycerol was added to the concentrate diet for the morning meal of the cows for 60 days, and the treated groups were as follows: First group was the control (T1) without glycerol added to the diet, the second group (T2) including added 75 ml of glycerol, while in the third group (T3), 150 ml of glycerol was added. The Measurements of milk and blood were made at the 15^{th}, 30^{th} and 60^{th} days of the experiment. The glycerol (99% purity) used in the experiment was a liquid form and produced by the Spanish company (PanReac AppliChem).

Table 1. Constituents of the experimental concentrate diet

Ingredients	%
Wheat bran	40
Barley	20
Sunflower meal	15
Corn	11
Soybean meal	10
Salt	1.5
Calcium carbonate	2
Vitamins and Premix	0.5
Total	100%

Milk yield and components

Milk yield of the experimental cows was estimated at 15^{th}, 30^{th} and 60^{th} days of the experiment using a two-line portable automatic milking machine (Turkish-made). Measurements of milk components (fat, protein and percentages lactose) were carried out as a sample of milk was taken for each

cow from the morning milking (50 ml) and examined using the apparatus for analyzing the components of milk, type Milkotester, (Bulgarian-made).

Blood sampling and assay

Blood samples (10 ml) were collected via Jugular venipuncture at the morning (before feeding) on the same days as the milk samples taken. Glucose concentration was measured by oxidase method, using kit of BIOLABO, France. Cholesterol concentration was measured by enzymatic methods, using kit of BIOLABO, France. Triglyceride concentrati-on was measured by Enzymatic hydrolysis associated with oxidative reaction, using kit of BIOLABO, France. Non-esterified fatty acids (NEFA) was measured by Enzymatic Colorimetric Method, using kit of MyBioSource ,USA. β-hydroxybutyrate acid (BHBA) concentra-tion was measured by competitive inhibition enzyme immunoassay, using kit of Cayman Chemical. USA.

Statistical analysis

The experimental data were analyzed using Complete Random Design (CRD) to study the effect of treatments influencing the studied traits, using SAS program (43). The significant mean were compared using Duncan's multiple range test (17).

Mathematical Model

$Yij = \mu + Ti + eij$

Yij: Value of the view j of the treatment i

μ: The general mean of the trait

Ti: Effect of treatment i (0, 75 or 150 ml/d of glycerol).

Eij: Random error which is naturally distributed at an average of zero and a variation of σ^2e

RESULTS AND DISCUSSION

Milk yield and components

The current results (Table 2) showed a significant increase (P≤0.05) in the average milk yield at day 15 of the experiment in T3 cows (9.15 ± 0.12 kg) compared to T1 cows (8.60 ± 0.15 kg), while it T2 cows (8.75 ± 0.15 kg) did not significantly different withe both T1 and T3 cows. In the same context, the milk yield of the T3 group increased (P≤0.01) on the 30th and 60th days of the experiment, which was 10.30 ± 0.16 and 12.20 ± 0.12 kg respectively, compared with the groups T1 and T2, as the T1 group recorded the lowest average of milk yield on the 30th and 60th days of the experiment, namely 9.25 ± 0.13 and 10.15 ± 0.10 kg respectively, and the milk yield of the T2 groups 9.40 ±0.06 and 11.15 ±0.13 kg respectively (Table 2). On the other hand, the current results revealed that there were no significant differences among the cows of the three groups in the percentage of milk components (fat, protein and lactose %) on the 15th day of the experiment (Table 2). The percentage of milk fat was not affected by glycerol treatment at the day 30 of the experiment, while it increased significantly (P≤0.01) in the group T3 (3.04 ± 0.10%) compared to T1 and T2 groups (3.78 ± 0.12 and 3.53 ± 0.12% respectively) at day 60th of the experiment. In addition, there were no significant differences between T1 and T2 cows in the percentage of milk fat at day 60 of the experiment (Table 2). Similarly, the percentage of milk protein was significant (P≤0.05) greater in T2 group on day 30 of the experiment, namely 3.12 ± 0.07%, followed by the T3 group being 3.04 ± 0.07%. The lowest protein percentage was in T1 group, namely 2.83 ± 0.08% (Table 2). At day 60 of the experiment, the percentage of milk protein was significantly increased (P≤0.01) in T2 and T3 groups (3.18 ± 0.02 and 3.31 ± 0.11% respectively) compared to T1 cows (2.89 ± 0.06%). Moreover, the percentage of milk lactose increased (P≤0.01) in T2 and T3 cows at day 30 and 60 of the experiment compared with those of T1 cows (Table 2).

Table 2. Milk yield and constituents of Holstein cows supplemented with dietary glycerol at different days of the experiment (Mean ± SE).

Item	Groups[1] T1	T2	T3	Level of significance
Day 15th of the experiment				
Milk production (Kg)	8.60 ±0.15^{b}	8.75 ±0.13^{ab}	9.15 ±0.12^{a}	P≤0.05
Fat %	3.75 ±0.20	3.72 ±0.11	3.68 ±0.18	NS
Protein %	2.77 ±0.10	2.76 ±0.08	2.84 ±0.11	NS
Lactose %	4.14 ±0.04	4.21 ±0.10	4.34 ±0.09	NS
Day 30th of the experiment				
Milk production (Kg)	9.25 ±0.13^{b}	9.40 ±0.06^{b}	10.30 ±0.16^{a}	P≤0.01
Fat %	3.80 ±0.18	3.73 ±0.12	3.65 ±0.18	NS
Protein %	2.83 ±0.08^{b}	3.12 ±0.07^{a}	3.04 ±0.07^{ab}	P≤0.05
Lactose %	4.22 ±0.03^{b}	4.49 ±0.04^{a}	4.594 ±0.09^{a}	P≤0.01
Day 60th of the experiment				
Milk production (Kg)	10.15 ±0.10^{c}	11.15 ±0.13^{b}	12.20 ±0.12^{a}	P≤0.01
Fat %	3.78 ± 0.12^{a}	3.53 ± 0.12^{a}	3.04 ± 0.10^{b}	P≤0.01
Protein %	2.89 ±0.06^{b}	3.18 ±0.02^{a}	3.31 ±0.11^{a}	P≤0.01
Lactose %	4.19 ±0.06^{b}	4.58 ±0.04^{a}	4.69 ±0.07^{a}	P≤0.01

Groups[1]: T1(0 glycerol), T2 (75 ml glycerol), T3(150 ml glycerol).

The increasing in milk yield with glycerol supplementation may be attributed to the ability of ruminants, including dairy cows, to convert glycerol into glucose, whether through its direct transmission via the blood to the liver, which is used in the process of gluconeogenesis (30), or by increasing the synthesis of propionate when glycerol is digested by rumen bacteria into volatile fatty acids (37), The increase in glucose contributes to an increase in the energy needed for milk yield (16, 23), and this is in line with a study conducted on Holstein cows by Valencia et al (46), whose observed an increase in the milk produced by 13.3% when 1500 g/glycerol /cow was added, the returned this increasing of milk produced of glucose-generating glycerol to increases the availability of energy for milk production. Glucose is one of the main components of milk, and more than 70% of the glucose synthesized inside the body of cows is used to produce milk (32, 34, 50). The increase in milk protein may be attributed to the ability of glycerol to improves the efficiency of nitrogen utilization by ruminal microorganisms and this leads to an increase in the synthesis of microbial proteins, which can increase milk protein content (14), or may be attributed to the effect of glycerol on increasing the insulin concentration in the blood, which has a positive role in increased milk protein synthesis (24, 49). The increase in milk protein may be attributed to the fact that glycerol enhances the microbial protein by increasing the energy available to microorganisms inside the rumen, as well as improving the efficiency of nitrogen use in the rumen (28, 42). Using of pure glycerol in feeding dairy cows enhances milk production and its protein content (6, 9). The increase in the percentage of milk lactose may due to the fact that glycerol increases the synthesis of glucose in the liver, which in turn increases the production of lactose in the mammary gland, as glucose in the blood is the main source for the synthesis of milk lactose (27). Also, the reason for this may be due to the increase the energy resulting from glycerol intake, and this enhances the synthesis of milk lactose consequently (46). This result is in agreement with the study of Zacaroni and Souto (50), whose reported that the glycerol supplementation in ruminants feeding enhances production on milk lactose. The decrease in the percentage of milk fat as affected by adding of glycerol may be attributed to the effect of glycerol on reducing the rate of acetate formation inside the rumen, which is one of the substrates of milk fat synthesis. Moreover, an increasing the animal's energy reduces fat mobilization and its dissolution from fatty tissues, resulting in a

decrease in milk fat percentage (6, 8, 40). This is consistent with the study of Kolif (29) who showed that adding glycerol to cows' feeding has a positive effect in increasing milk yield and decreasing milk fat synthesis through reducing the proportion of acetate production with the increasing the proportion of propionate production. Furthermore, the reason behind the decreasing in milk fat can be attributed to the increase in milk production, as there is an inverse relationship between the quantity of milk yield and the proportion of milk fat (5).

Blood parameters

Table (3) revealed that there were no significant differences among the three groups in the concentrations of plasma glucose, cholesterol, Non-esterified fatty acids (NEFA) and β-hydroxybutyrate acid (BHBA) at 15th day of the experiment, except for triglycerides (TG), which significantly (P≤0.05) increased in blood of T3 compared with T1 and T2 cows. The concentration of the plasma TG in cows of the T1, T2 and T3 at 15th day of the experiment were 11.21 ± 0.25, 10.92 ± 0.47 and 12.43 ± 0.35 mg/dL, respectively. In addition, the TG concentration was significantly increased (P≤0.05) at day 30 in T3 (15.28 ±0.43 mg/dL) compared to T1 and T2 cows (13.62 ±0.42 and 13.73 ±0.32 mg/dL, respectively), which did not differ significantly. At the 60th day, there was a highly significant (P≤0.01) increase in the triglyceride concentration in T2 and T3 cows (15.13 ±0.34 and 15.43 ±0.51 mg/dL, respectively) compared to T1 cows (12.69 ±0.33 mg/dL), however, the differences between the latter two groups lacked significance. The current results showed a highly significant increase (P≤0.01) in glucose concentration of T2 and T3 cows compared with T1 cows on day 30 and 60 of the experiment, namely 68.06 ±1.35, 72.15 ±1.16 and 74.61 ±1.29 mg/dL for T1, T2 and T3 respectively. At day 60 of the experiment were, 67.31 ±1.35, 76.73 ±1.83 and 77.61 ±1.31 mg/dL respectively (Table 3). At 30th day of the experiment, there was a significant decrease (P≤0.05) in the plasma concentration of NEFA cows of the T2 and T3 groups, being 0.409 ± 0.013 and 0.414 ± 0.02 mmol/L, respectively, compared with cows of those T1 group (0.518 ± 0.038 mmol/L), however, there were no significant differences between T2 and T3 groups in plasma concentration of NEFA at the time mentioned above. At the 60th day there was a highly significant (P≤0.01) decrease in the NEFA concentration in cows of T2 and T3 groups compared to T1 cows, namely 0.515 ±0.054, 0.360 ±0.018 and 0.321 ± 0.020 mmol/L, for T1, T2 and T3 respectively. On the other hand, non-significant differences were observed in BHBA concentration among the groups on the 30th day of the experiment. At 60th day of the experiment, the BHBA concentration was significantly (P≤0.01) decreased in T3 cows (0.688 ± 0.03 mmol/L) compared with cows of T1 and T2 (0.834 ±0.03 and 0.793 ±0.03 mmol/L, respectively), while there were non-significant differences between T1 and T2 cows in BHBA concentration at the mentioned above period (Table 3). Cholesterol concentration was not affected by the glycerol treatment on the 30th day of the experiment, but it decreased (P≤0.01) in T2 and T3 compared to T1 cows on the 60th day, however, non-significant differences were noticed between T2 and T3 cows (Table 3). The high concentration of glucose in T2 and T3 groups may be due to the fact that the dietary glycerol is mostly absorbed directly by the rumen epithelium or the small intestine and transported through the bloodstream to the liver where the enzyme glycerol kinase converts it to glycerol-3-phosphate used to stimulate glucose formation in the liver through the process of gluconeogenesis (31, 41), or due to the fermentation of glycerol by rumen bacteria to propionate, which is transferred to the liver to convert to Succinyl-CoA, which enters the Krebs cycle to turn into pyruvate phosphoenol to form glucose through the process of Gluconeogenesis (35). This result is consistent with the study of Torres et al (45) who reported that glycerol increases blood glucose concentration by 2.5%, and also the study of Goff and Horst (21), who indicated that giving milk cows with an amount of 1, 2 or 3 liters of glycerol results in an increase in blood glucose by 16%, 20%, and 25%, respectively, as glycerol is one of the precursors that form glucose from the process of gluconeogenesis in the liver (21). In

addition to other studies, which showed the positive effect of glycerol in increasing blood glucose concentration in dairy cows (11, 46, 47). The increase in the plasma concentration of TG of glycerol-treated cows was consistent with the increase in glucose synthesis, which enhanced the animal's energy, which positively affected the increase in liver activity in the synthesis of TG and its excretion outside the liver as VLDL (34), or it may be due to the inclusion of glycerol other reasor metabolism process that may result in TG synthesis, as glycerol is the backbone of triglycerides (4). In addition, the increase of glucose in the blood, is positively correlated with the concentration of TG (10, 13), as glucose is an important source of carbon in the synthesis of fatty acids (18).

Table 3. Plasma glucose, cholesterol, triglyceride, NEFA, BHBA of Holstein cows supplemented with dietary glycerol at different days of the experiment (Mean ± SE).

Item	Groups[1] T1	T2	T3	Level of significance
Day 15th of the experiment				
Glucose (mg/dL)	66.32 ±1.14	65.07 ±1.55	67.41 ±2.07	NS
Cholesterol (mg/dL)	95.13 ±7.57	100.08 ±4.40	96.59 ±3.99	NS
Triglyceride (mg/dL)	11.21 ±0.25^{b}	10.92 ±0.47^{b}	12.43 ±0.35^{a}	P≤0.05
NEFA[2] (mmol/L)	0.635 ±0.04	0.588 ±0.02	0.561 ±0.02	NS
BHBA[3] (mmol/L)	0.976 ±0.06	0.930 ±0.03	0.844 ±0.03	NS
Day 30th of the experiment				
Glucose (mg/dL)	68.06 ±1.35^{b}	72.15 ±1.16^{a}	74.61 ±1.29^{a}	P≤0.01
Cholesterol (mg/dL)	120.31 ±4.22	111.27 ±3.70	113.39 ±3.59	NS
Triglyceride (mg/dL)	13.62 ±0.42^{b}	13.73 ±0.32^{b}	15.28 ±0.43^{a}	P≤0.05
NEFA (mmol/L)	0.518 ±0.03^{a}	0.409 ±0.01^{b}	0.414 ±0.02^{b}	P≤0.05
BHBA (mmol/L)	0.905 ±0.02	0.884 ±0.03	0.815 ±0.03	NS
Day 60th of the experiment				
Glucose (mg/dL)	67.31 ±1.35^{b}	76.73 ±1.83^{a}	77.61 ±1.31^{a}	P≤0.01
Cholesterol (mg/dL)	132.34 ± 3.75^{a}	116.68 ± 3.74^{b}	105.39 ± 3.85^{b}	P≤0.01
Triglyceride (mg/dL)	12.69 ±0.33^{b}	15.13 ±0.34^{a}	15.43 ±0.51^{a}	P≤0.01
NEFA (mmol/L)	0.515 ±0.05^{a}	0.360 ±0.01^{b}	0.321 ±0.02^{b}	P≤0.01
BHBA (mmol/L)	0.834 ±0.03^{a}	0.793 ±0.03^{a}	0.688 ±0.03^{b}	P≤0.01

Groups [1]: T1(0 glycerol), T2 (75 ml gycerol), T3(150 ml glycerol).
NEFA[2]: Non-esterified fatty acids.
BHBA[3]: β-hydroxybutyrate acid

The decrease concentration of cholesterol affected by the addition of glycerol may be due to an increase in the body's energy through the positive effect of glycerol in promoting the process of gluconeogenesis (34), since the availability of glucose is elevated, animals are minimally mobilized their body fat for generating energy thus lowering blood cholesterol (44). This result is consistent with the study of Ezequiel et al (20), which showed that adding glycerol to the diet of Holstein cows lowers blood cholesterol concentration. The T1 and T2 groups reduced the level of plasma NEFA addition to the decrease in the level of plasma BHBA in T2 group. This may be due to the effect of glycerol on increasing glucose production and improving the animal's energy, which results in a reduction in the breakdown of fats from tissues (30). The fatty responsible for exporting NEFA to the blood, as well as the low concentration of BHBA in the blood that comes from NEFA (34), or the reason may be due to the animal's increased energy that enhances the liver's activity in absorbing NEFA from the blood and its esterification into triglycerides and then It is released outside the liver as VLDL (12, 34). It is noteworthy that the correlations coefficient between serum BHBA concentrat-ion and NEFA concentration is r = 0.850 (11). This result is consistent with the study of Kupczynski et al (30), conducted on Holstein cows, by given 300 ml / day of pure glycerol, and the result was a decrease in the concentration of NEFA and BHBA and an

increase in the serum concentration of triglycerides, indicating the positive effect of glycerol on reducing the level of NEFA and BHBA (47). Similarly, Torres et al (45) reported that glycerol increases the serum concentration of glucose in the blood by 2.5% and reduces the concentration of NEFA by 5.6%.

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