EFFECT OF GLYCEROL ON PERFORMANCE AND SOME BLOOD CHARACTERISTICS OF HOLSTEIN CALVES

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ABSTRACT

This research was to study the effect of adding glycerol at different levels (T1 = control treatment without glycerol, T2 = 75 ml and T3 = 150 ml glycerol) in the body weight, body measurements and some blood characteristics of Holstein calves from 60 to 120 days of calves age. Body weight increased (P≤0.05) in T3 calves at 80 and 100 days of age, also body weight increased in T2 and T3 calves at 120 days of age. T2 calves were significantly superior (P≤0.01) in average total gains from 60 to 120 days of age. Significantly increased (P≤0.05) at the ages of 100 and 120 days of: Wither height in T3 calves, Body length in T2 calves and Heart girth in T2 and T3 calves. Blood glucose concentration increased (P≤0.05) and blood NEFA concentration decreased (P≤0.01) in T2 calves at 80 days of age. At the age of 100 and 120 days, the concentration of blood glucose was increased (P≤0.01 and P≤0.05) and the concentration of NEFA and BHBA decreased (P≤0.01) in calves of the two treatments T2 and T3. We conclude that the addition of glycerol (75 and 150 ml/day) in the feeding of calves increases body weight, promotes growth and improves blood characteristics by increasing blood glucose and reducing NEFA and BHBA concentrations.

Key words: glycerol, holstein calves, weight and measurements body, NEFA, BHBA.

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تأثير الكليسيرول على الاداء وبعض صفات الدم لعجول الهوشتاين

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المستخلص

الهدف من الدراسة الحالية هو لمعرفة تأثير اضافة الكليسيرول بمستويات مختلفة (T1 = معاملة السيطرة بدون كليسيرول و T2 = 75 مل و T3 = 150 مل كليسيرول) على النمو وبعض صفات الدم لعجول الهولشتاين. ازداد وزن الجسم معنوياً (P≤0.05) لدى عجول المعاملة T3 عند عمر 80 و 100 يوماً, فضلاً عن زيادة وزن الجسم لدى عجول T2 و T3 عند عمر 120 يوماً. عجول T2 تفوقت معنوياً (P≤0.01) في معدل الزيادة الوزنية الكلية من عمر 60 الى 120 يوماً. هنالك زيادة معنوية (P≤0.05) عند عمر 100 و 120 يوماً كل من ارتفاع الجسم لدى عجول T3 وطول الجسم لدى عجول T2 و محيط الصدر لدى عجول T2وT3. ازداد تركيز كلوكوز الدم (P≤0.05) وانخفض تركيز NEFA الدم (P≤0.01) لدى عجول T2 عند عمر80 يوماً, أما عند العمر 100 و 120 يوماً فقد تفوق معنوياً (P≤0.01 وP≤0.05) تركيز كلوكوز الدم وانخفض معنوياً (P≤0.01) تركيز NEFA و BHBA لدى عجول المعاملتين T2 و T3. نستنتج ان اضافة الكليسيرول (75 و 150 مل/يوم) في تغذية العجول يزيد من وزن الجسم ويعزز النمو ويحسن من صفات الدم من خلال زيادة كلوكوز الدم وخفض تراكيز NEFA و BHBA.

الكلمات المفتاحية: كليسيرول, عجول الهولشتاين, وزن وقياسات الجسم, NEFA, BHBA.

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INTRODUCTION

The purpose of obtaining high production efficiency of milk and meat, it is necessary in the first place to pay attention to calves, because they are the nucleus of the herd, through which optimal production can be reached (15, 24), and to obtain healthy and natural growth with high resistance. For diseases of calves , attention should be paid to nutrition, which is the source of animal energy (2, 13, 28), and in recent years, the demand for primary feed materials, especially those equipped with energy (corn, oilseeds, etc.), has increased, and this has led to an increase in their prices globally, which has made feeding ruminants, including calves, more expensive and thus increasing producti-on costs (1, 3), these reasons encouraged researchers and workers in the field of dairy cows to find alternative fodder sources that are rich in energy, have a lower cost, and do not compete with human food (6, 19). Glycerol is one of the alternative feed energy sources, which has been widely used in recent years in the diet of farm animals, especially in ruminant feed, as one of the equipped sources of energy at a lower cost than corn, oilseeds and other feed materials that are used as an energy source (24, 39,45), which is an alcoholic sugar that has the property of forming glucose in ruminants (23, 25), and some studies have indicated the possibility of using glycerol mainly as an energy supplement in feeding ruminants without any negative effect on rumen fermentation. (8, 30 43). Glycerol is generally obtained from plant and animal sources through esterification, hydrolysis or saponification processes of triglycerides, as glycerol is the backbone of triglycerides, in addition, crude glycerol is largely produced by biodiesel production (12). There are a number of studies that have indicated that glycerol, which is added as a nutritional supplement in dairy cattle feed, or replaced with a certain percentage of corn, or added to water, or given directly orally, leads to increased milk production and growth, improves metabolism and digestion of feed materials, enhances body energy, blood and other characteristics that show the positive effect of glycerol on dairy cattle (12, 18, 21, 25, 35, 41). Despite these studies, there are a few studies and research that dealt with the issue of the effect of glycerol on newborn calves (24). One of these studies indicated that adding glycerol to rehydration solutions given orally has a therapeutic role for calves suffering from metabolic disorders through the positive effect of glycerol on glucose, which increases its concentration in the blood, as a result of the rapid absorption of glycerol by the digestive system of calves, and this is a good indicator of the animal's body energy (36), and in the same context, Maciel et al. (29) reported that glycerol added to calves' diet enhances the amount of feed intake and improves the performance and growth of calves and rumen development, which results in the possibility of weaning calves at an early age. The aim of the study is to investigate the effect of adding glycerol at different levels to the feed on weight, body measurements and some blood characteristics in Holstein calves.

MATERIALS AND METHODS

1- Animals, treatments and experimental design: This study was conducted at the Al-Salam Station For Dairy Cows, Private sector (Latifia, 25 km south of Baghdad), for the period from 20/1/2021 to 30/3/2021. Fifteen Holstein calves of 60 days of age and average weight 59-64 kg were used, randomly and equally divided into three treatments, each treatment comprising five calves. All calves for the three treatments were fed with a standardized concentrated diets (Table1) at a rate of of 3% of the live body weight of the calves divided into two morning and evening meals, and roughage feed was provided free (dry and green) for the duration of the experiment(60 day), also mineral salt cubes were left in front of all animals and the water was constantly available throughout the experiment. Glycerol was added to the concentrated feed for the morning meal of the treatment cows for 60 days, and the treatments were as follows: First treatment was the control (T1) no glycerol was added to their feed, the second treatment (T2) was added 75 ml of glycerol and the third treatment(T3) was 150 ml of glycerol. The measurements were made for the studied traits at the 60, 80, 100 and 120^{th} days of the age calves. The glycerol (99% purity) used in the experiment was a liquid form and produced by the Spanish company (PanReac AppliChem).

Table 1. Formulation of the concentrated feed used in the experiment

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%

2- Body weight and measurements

Calves were weighed and body measurements taken on days 60, 80, 100 and 120 for calves age, the measurements were conducted according to the method of Mirzaei et al. (34) and included: withers height (distance from base of the front feet to the withers), body length (distance between the points of shoulder and rump), heart girth (circumference of the chest) and body barrel (circumference of the belly before feeding).

3- Blood samples

Blood for the experiment was collected from an external jugular vein (V. Jugularis externa) dose 10 ml, in the morning (before feeding) at the 80,100 and 120 days for calves age, then the blood was drained into yellow tubes, which were vacuum and contain a gel that prevents clotting and separates the serum (produced by the Jordanian AFCO Company), After completing the process of drawing blood, the tubes containing the blood samples were placed in a cooler box, and then the box was transferred to the laboratory for analysis, which was carried out according to the following:

- Glucose concentration was measured by oxidase method (44), using kit of BIOLABO, France (Glucose GOD- PAP, Cat. No. 02160)
- Cholesterol concentration was measured by enzymatic methods (4), using kit of BIOLABO, France (Cholesterol CHOD PAP)
- Triglyceride concentration was measured by Enzymatic hydrolysis (16) associated with oxidative reaction (44), using kit of BIOLABO, France (Triglyceride GPO Method)
- Non-esterified fatty acids (NEFA) was measured by Enzymatic Colorimetric Method (10), using kit of MyBioSource ,USA (Cat. No. MBS255698).
- β-hydroxybutyrate acid (BHBA) concentration was measured by competitive inhibition enzyme immunoassay (32), using kit of CaymanChemical.USA (Colorimetric Assay Kit, Item No. 700190).

4- 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 (42) and the averages of the coefficients were compared using the polynomial (14) to estimate the significant differences between the treatments and means.

RESULTS AND DISCUSSION

Calves performance

The results of the study showed that there were no significant differences in body weight among the calves of the three treatments T1(0 Glycerol), T2(75 ml Glycerol) and T3 (150 ml Glycerol) at the 60th day of the calves age (Table 2). The weights of T3 (150 ml Glycerol) calves were significantly ($P \leq 0.05$) superior to the weight of T1 calves at day 80 and 100 of age, as the mean weight of T3 calves was 74.48 ±1.03 and 88.81 ±0.92 kg, respectively, and the average weight of T1 calves 70.50 ±0.69 and 84.46 ±0.83 kg respectively, while the weights of T2 calves recorded an average of 71.44 ± 1.10 and 87.69 ± 1.01 kg respectively, between calves of T1 and T3 at the same period (Table 2). Body weight increased ($P \leq 0.05$) in T2 and T3 calves (101.14 ± 0.52 and 99.90 ± 1.20 kg, respectively) compared with T1 calves (95.82 ±1.11 kg) on day 120 of age calves, and there are no significant differences in body weight between T2 and T3 calves at the same period (Table 2).

Table 2. Body weight (BW) for calves fed with different amounts of Glycerol from 60 days of age calves to 120 days

Treatment	Average BW ± standard error (Kg)			
	60 days of age calves	80 days of age calves	100 days of age calves	120 days of age calves
T1 (0 Glycerol)	61.23 ±1.01	70.50 ±0.69 b	84.46 ±0.83 b	95.82 ±1.11 b
T2 (75 ml Glycerol)	60.43 ±1.06	71.44 ±1.10 ab	87.69 ±1.01 ab	101.14 ±0.52 a
T3 (150 ml Glycerol)	62.34 ±0.82	74.48 ±1.03 a	88.81 ±0.92 a	99.90 ±1.20 a
Level of s.g	NS	*	*	*

NS: No significant, * (P≤0.05)
The averages with different letters within the same column significantly differ between them

Table 3. Average total gains for calves fed with different amounts of Glycerol from 60 days of age calves to 120 days

Treatment	Average BW ± standard error (Kg)
Control (0 Glycerol)	35.59 ±0.67 b
T1 (75 ml Glycerol)	38.71 ±0.70 a
T2 (150 ml Glycerol)	35.56 ±0.86 b
Level of s.g	**

** (P≤0.01): The averages with different letters within the same column significantly differ between them

Table (3) shows the average total gains were higher (P≤ 0.01) for T2 calves which were 38.71 ±0.70 kg compared with T1 and T2 calves which were 35.59 ±0.67 and 35.56 ±0.86 kg, respectively, from 60 to 120 days of age, and there were no significant differences in the average total gains between the calves of T1 and T3 for the same period. The results of the study indicated that there were no significant differences in the wither height, body length, heart girth and body barrel among the calves of the three treatments (T1, T2, and T3) at the age of 60 and 80 days, allso the body barrel at the 100th day of age calves. However, wither heigh increased (P≤0.05) in T3 calves at 100 and 120 days of age (92.41 ±1.19 and 94.66 ±1.26 cm, respectively) compared with T1 calves (88.56 ±0.96 and 89.40 ±0.96 cm, respectively), while it was recorded T2 calves (91.88 ±0.76 and 92.92 ±0.63 cm respectively) averaged between T1 and T3 at the same period (Table 4). In addition, the results of the study indicated a significant superiority (P≤0.05) in the body length of T2 calves compared with T1 at the ages of 100 and 120, while the T3 calves recorded an average body length between T1 and T3 calves at the ages of 100 and 120 day, the body length at 100 days of age calves of the T1, T2 and T3 was 82.11 ± 1.02, 85.94 ± 0.84 and 83.78 ±1.21 cm, respectively, and at 120 days of age 86.04 ± 0.99, 90.66 ± 0.58 and 88.95 ± 1.36 cm, respectively (Table 4). Heart girth increased (P≤0.05) in T2 and T3 calves at 100 and 120 days of age compared to T1 calves, and there were no significant differences in heart girth between T2 and T3 calves, as the heart girth of calves T1, T2 and T3 at 100 days of age was 90.12 ±0.79, 93.30 ±1.02 and 93.58 ±0.86 cm respectively, and at 120 days of age was 94.14 ±0.78, 98.46 ±1.00 and 97.82 ±1.62 cm, respectively, also, body barrel increased (P≤0.05) in calves T2 at 120 days of age which was 104.66 ±1.10 cm, compared with calves T1 which was 98.52 ±0.65 cm, and the calves of T3 recorded an average in body barrel which was 102.79 ±1.16 cm, between calves of T1 and T2 at the same period (Table 4).

Table 4. Wither height, body length, heart girth, and body barrel for the calves fed different amounts of Glycerol from 60 to 120 days of age calves

Item	Treatment T1 (0 Glycerol)	T2 (75 ml Glycerol)	T3 (150 ml Glycerol)	Level of s.g
Wither height (cm)				
60 days	84.36 ±1.39	83.82 ±1.52	85.26 ±1.12	NS
80 days	86.26 ±1.05	86.00 ±1.08	88.62 ±1.32	NS
100 days	88.56 ±0.96 b	91.88 ±0.76 ab	92.41 ±1.19 a	P≤0.05
120 days	89.40 ±0.96 b	92.92 ±0.63 ab	94.66 ±1.26 a	P≤0.05
Body length (cm)				
60 days	78.16 ±1.28	79.14 ±1.19	79.02 ±0.99	NS
80 days	79.58 ±0.91	81.10 ±1.22	82.91 ±1.11	NS
100 days	82.11 ±1.02 b	85.94 ±0.84 a	83.78 ±1.21 ab	P≤0.05
120 days	84.04 ±0.99 b	88.66 ±0.58 a	86.95 ±1.36 ab	P≤0.05
Heart girth (cm)				
60 days	86.02 ±1.43	84.78 ±1.42	87.42 ±1.12	NS
80 days	88.28 ±1.01	87.80 ±1.14	90.86 ±1.07	NS
100 days	90.12 ±0.79 b	93.30 ±1.02 a	93.58 ±0.86 a	P≤0.05
120 days	94.14 ±0.78 b	98.46 ±1.00 a	97.82 ±1.62 a	P≤0.05
Body barrel (cm)				
60 days	87.52 ±1.16	86.84 ±1.33	90.06 ±0.99	NS
80 days	90.32 ±1.04	92.06 ±1.40	93.88 ±1.50	NS
100 days	93.02 ±0.96	96.90 ±1.14	97.68 ±1.62	NS
120 days	98.52 ±0.65 b	104.66 ±1.10 a	102.79 ±1.16 ab	P≤0.05

NS: No significant, * (P≤0.05)
The averages with different letters within the same column significantly differ between them

In view of the present results, the increase in body weight and average total gains when glycerol is added to the concentrated diets of calves may be attributed to the positive effect of glycerol in enhancing the microbial efficiency of the rumen , stimulation of rumen epithelium development, greater diets intake and increasing the energy of the animal (5, 9). This result is consistent with the study of Ramos and Kerley (40) and Gunn et al. (17), which were conducted on calves, who reported that the addition of glycerol in the feed of calves increases the daily weight gain and final body weight, the researchers attributed the reason for this to that the addition of glycerol it has enhanced rumen fermentation and improved rumen growth, which reflected positively on body weight. The significant increase in body height, body length and heart girth in calves treated with glycerol may be attributed to increased body weight and improved growth of calves (9, 20), as body weight is positively correlated with body height (r = 0.51), body length (r =0.54) (38) and heart girth (r =0.334) (22, 26). This result is consistent with the study of Maciel et al. (29), which showed that adding glycerol to calves' diet increases body height, body length and heart girth because they have a positive relationship with body weight.

Blood characteristics

Table (4) shows that the glucose concentration was increased (P≤0.05) at day 80 of age in T3 calves (93.94 ±2.31 mg/dL) compared with T1 calves (88.07 ±1.14 mg/dL), and calves of T3 recorded an average rate of glucose concentration (91.82 ±1.81 mg/dL) between T1 and T2 calves. Also, at days 100th and 120th of calves age, the glucose concentration increased (P≤0.01 and P≤0.05, respectively) in the calves of T2 and T3 compared with calves of T1, and there were no significant differences between the calves of T2 and T3 at the same period, the glucose concentration of the calves of the three treatments T1, T2 and T3 at day 100 of age was 76.15 ± 2.52, 89.87 ±2.93, 88.26 ±3.33 mg/dL respectively, and at day 120 it was 69.47 ±1.92, 77.54 ± 1.80, and 75.01 ± 1.51 mg/dL sequentially, and there were no significant differences in glucose concentration between T2 and T3 calves for the same period. The concentration of NEFA was affected by the glycerol treatment, as it significantly decreased ($P \leq 0.01$) in calves of T2 and T3 compared with T1 calves at days 80, 100 and 120, and no significant differences appeared between calves of T2 and T3 at the same period, the NEFA concentration in calves of treatments T1 and T2 The T3 at day 80 was 0.282 ± 0.02, 0.206 ± 0.02 and 0.201 ± 0.02 mg/dl respectively, at day 100 it was 0.177 ± 0.01, 0.110 ± 0.02 and 0.114 ± 0.02 mg/dl, respectively, and at day 120 it was 0.269 ± 0.02, 0.184 ±0.02 and .176 ±0.02 mg/dL, respectively (Table 4). The results of the study showed a significant decrease ($P \leq 0.01$) in BHBA concentration in T2 and T3 calves at days 100 of age (0.287 ± 0.01 and 0.273 ± 0.02 mg/dl, respectively) and at days 120 (0.355 ± 0.02 and 0.360 ± 0.02 mg/dl, respectively) compared with T1 calves which concentration of BHBA at day 100 was 0.360 ± 0.02 mg/dl and at day 120 it was 0.432 ± 0.01 mg/dl, and there were no significant differences in BHBA concentration between T2 and T3 calves at the same period (Table 4). Table (4) shows that there were no significant differences in the concentration of cholesterol and triglycerides in the blood between the three treatments calves, T1, T2 and T3 at days 80, 100 and 120 of the calves age . Also, the concentration of BHBA in the blood of the three treatments calves was not affected by the difference in glycerol treatment at day 80 of calves age. Increased blood glucose concentration in calves treated with glycerol may be caused by the absorption of most of the glycerol entering the rumen by rumen epithelium and its transport by the blood to the liver, which uses it in the process of gluconeogenesis that leads to an increase in glucose in the blood (46). This result is in agreement with the study of Anan et al. (5), in which it was shown that giving calves (4-2 months old) amounts of 50, 100 and 200 ml/glycerol increases blood glucose concentration, due to the fermentation of glycerol by rumen bacteria mainly to propionate (27), which is a major precursor to glucose formation in ruminants (31), as propionate correlates positively (r = 0.48) with blood glucose concentration (18), in addition to the result of the study of Barros et al. (7), which showed that adding glycerol to the diet of young bulls enhances the concentration of blood glucose as a result of the fermentation of glycerol by rumen bacteria to propionate that is transmitted through the bloodstream to the liver to convert to Succinyl-CoA that enters The Krebs cycle turns into Pyruvate Phosphoenol to form glucose by Gluconeogenesis. The decrease in the level of Non-esterified fatty acids (NEFA) and β-hydroxybutyrate acid (BHBA) in the blood of calves affected by the addition of glycerol in their feed may be due to the improvement of the animal’s energy, which may reduce the breakdown of fats from adipose tissue, which is one of the main sources of NEFA, as well as the decrease in the concentration of BHBA sourced from NEFA (24, 37). The decrease in the concentration of NEFA in the blood may be due to the positive effect of glycerol in improving the animal’s energy, which may enhance the activity of the liver in increasing the absorption of NEFA from the blood and its esterification into triglycerides and then its release outside the liver in the form of VLDL, which results in a decrease in the concentration of NEFA and then a decrease in BHBA formation (21). The decrease in the concentration of NEFA and BHBA was consistent with the increase in blood glucose concentration as a result of their association (NEFA and BHBA) in a negative relationship with glucose concentration (r = -0.362 and r = -0.77, respectively) (18, 10). This result is consistent with the result of the study of Burakowska et al. (9), which showed that adding glycerol to the starter diet of Holstein calves reduced the concentration of both NEFA and BHBA in the blood of calves. Kholif (24) reported that despite the few studies that dealt with the issue of the effect of glycerol on calves, they did not clearly show the effect of glycerol on the concentration of NEFA and BHBA in the blood of calves

Table 4. Concentration the Glucose, Cholesterol, Triglyceride, NEFA, BHBA in blood of Holstein calves fed diets supplemented with glycerol at 80, 100 and 120 day of age calves

Item	Treatment			Level of significance
	T1 (0 Glycerol)	T2 (75 ml Glycerol)	T3 (75 ml Glycerol)	
80th day of age calves				
Glucose (mg/dL)	88.07 ±1.14 [b]	93.94 ±2.31 [a]	91.82 ±1.81[ab]	P≤0.05
Cholesterol (mg/dL)	80.04 ±3.64	79.63 ±4.43	80.00 ±4.36	NS
Triglyceride (mg/dL)	29.66 ±2.13	32.81 ±1.88	31.98 ±2.34	NS
NEFA[1] (mmol/L)	0.282 ±0.02[a]	0.206 ±0.02[b]	0.201 ±0.02[b]	P≤0.01
BHBA[2] (mmol/L)	0.304 ±0.02	0.289 ±0.02	0.282 ±0.2	NS
100th day of age calves				
Glucose (mg/dL)	76.15 ±2.52[b]	89.87 ±2.93[a]	88.26 ±3.33[a]	P≤0.01
Cholesterol (mg/dL)	74.84 ±2.92	68.13 ±4.15	69.73 ±3.55	NS
Triglyceride (mg/dL)	32.12 ±1.03	35.24 ±1.45	34.66 ±1.19	NS
NEFA (mmol/L)	0.177 ±0.01[a]	0.110 ±0.02[b]	0.114 ±0.02[b]	P≤0.01
BHBA (mmol/L)	0.360 ±0.02[a]	0.287 ±0.01[b]	0.273 ±0.02[b]	P≤0.01
120th day of age calves				
Glucose (mg/dL)	69.47 ±1.92[b]	77.54 ±1.80[a]	75.01 ±1.51[a]	P≤0.05
Cholesterol (mg/dL)	81.19 ±3.00	74.05 ±2.79	76.21 ±3.17	NS
Triglyceride (mg/dL)	28.50 ±1.74	31.63 ±1.15	30.99 ±1.13	NS
NEFA (mmol/L)	0.269 ±0.02[a]	0.184 ±0.02[b]	0.176 ±0.02[b]	P≤0.01
BHBA (mmol/L	0.432 ±0.01[a]	0.355 ±0.02[b]	0.360 ±0.02[b]	P≤0.01

NS: No significant, * (P≤0.05), ** (P≤0.01)

The averages with different letters within the same column significantly differ between them

NEFA[1]: Non-esterified fatty acids., BHBA[2]: β-hydroxybutyrate acid.

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