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The Effect of Replacing Corn with Glycerol and Fibrinolytic Enzymes on the Productive Performance of Lactating Goats

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ABSTRACT

The increase in bio-ethanol industry has created a need for alternative to corn for ruminants. In the other side there is increase in availability of glycerol, a primary co-product material of biodiesel production. The objective of this study was to investigate the effect of partial replacing of corn with glycerol in diets fed to lactating goats. Twelve lactating Nubian goats were fed a base diet (T_1), diets containing 9% glycerol (on DM basis) (T_2) and diet containing 9% glycerol plus commercial enzymes 4 g kg^{-1} DM (on DM basis) (T_3) for 84 days. The experimental diets T_2 and T_3 decreased butyric acids concentration and acetate:propionate ratio in rumen liquor in relation to T_1, the concentration of propionic acid was increased in T_2 and T_3 compared with T_1. Replacing corn by glycerol (T_2) decreased apparent nutrients digestion coefficients Dry Matter (DM), Organic Matter (OM), Crud Protein (CP), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) comparing with other treatments (T_1 and T_3). Milk production was 1581, 1174 and 1610±77.6 g h^{-1} day^{-1} and FCM was 1774, 1030 and 1648±115.9 g h^{-1} day^{-1} for T_1, T_2 and T_3, respectively, Milk composition was not altered by glycerol feeding plus fibrinolytic enzymes (T_3) except that milk total protein was decreased from 4.6 24 to 3.5%. While, replacing corn by glycerol (T_2) decreased values of milk composition compared with control diets (T_1). The results indicated that glycerol is a suitable replacement for corn grain with adding fibrinolytic enzymes in diets for lactating goats and that it may be included in rations to a level of at least 9% of dry matter without adverse effects on milk yield or milk composition.

Key words: Glycerol, corn, digestibility, enzymes, milk, milk protein rumen liquor, rumen acids

INTRODUCTION

The high global demand for energy led to an increasing production and trade of biofuel, especially liquid fuels for transportation, in order to substitute fossil energy sources, to enhance energy security and to respond to greenhouse gas emissions (Walter *et al*., 2008; Heinimo and Junginger, 2009). This led to an increasing competition for raw materials for food, feed and fuel usage and the disposability of more by-products. For example, starch and vegetable oils are converted into bioethanol and biodiesel during the production process, one of the by-products of this process is glycerol. Glycerol is an essentially structure component of triglycerides and phospholipids and its glucogenic properties are well established (Cori and Shine, 1935). Glycerol enters the metabolic pathway to glucose at a different step than other glucogenic precursors (Leng, 1970). The

glycerol component can be converted to glucose by the liver or kidneys (Krebs and Lund, 1966) to provide energy for cellular metabolism. Glycerol has been shown to be an effective treatment against ketosis in dairy cattle (Leng, 1970). Cows fed glycerol lost less body weight and remained in more positive energy balance (DeFrain *et al*., 2004), Several researchers (Schroder and Sudekum, 1999; Mach *et al*., 2009) estimated the energy value of glycerol in beef and they have concluded to be similar to that of corn grain. Therefore, glycerol could be used as an energetic ingredient in animal diets replacing cereals. The inclusion of glycerol in animal nutrition has been studied in several species and reports indicate that moderate inclusions do not compromise animal performance (Lammers *et al*., 2008; Mach *et al*., 2009).

Many *in vitro* and *in vivo* studies carried-out to investigate glycerol as feed on rumen fermentation and concluded that glycerol increase total volatile fatty acids, propionate and butyrate concentrations and decreases the molar ratio of acetate: propionate concentration (Schroder and Sudekum, 1999; Goff and Horst, 2001; Farkasova *et al*., 2008; Abo El-Nor *et al*., 2010). The objective of the present study is to find alternative source energy could be used in ruminant diets by evaluating the effects of substituting corn by glycerol with some biological additives (Fibrinolytic enzymes) on productive performance of lactating goats.

MATERIALS AND METHODS

This study was carried out to investigate the effect of replacing corn by glycerol alone or glycerol with a biological additive comparing with the control diet on the productive performance of lactating Nubian goats.

Digestibility and lactation trails: Twelve lactating Nubian goats ranging from 35-40 kg were assigned randomly into three experimental groups (four animals each) using Latin Square design. The experimental extended for 84 days after 7 days of parturition and consisted of three equal experimental periods (28 days each). The goats were fed individually according to nutrients requirement recommendations of NRC (1981). The dietary forage: concentrate ratio was 38:62. Forage source was Egyptian clover (*Trifolium alexanderinum*) (Table 1). The experimental

Table 1: Ingredient and nutrient composition of diets

Item	T_1	T_2	T_3*
Ingredient (% of DM)			
Egyptian clover	38.00	38.00	38.00
Corn, ground	30.00	18.00	18.00
Wheat bran	12.00	12.00	12.00
Bean, cracked	18.00	21.00	21.00
Glycerol	-	9.00	9.00
Mineral/vitamin	2.00	2.00	2.00
Chemical composition (%)			
Crud protein	15.93	15.90	15.90
Ether extract	6.12	5.05	5.05
Acid detergent fiber	21.79	19.62	19.62
Neutral detergent fiber	29.39	27.17	27.17
Hemicellulose	7.60	7.55	7.55
Ash	6.03	4.95	4.95
Net energy for lactation (kcal kg^{-1})	1.73	1.73	1.73

*Enzymes (15000 cellulase enzyme unit, 187500 protease enzyme unit, 300000 α-amylase enzyme unit, 15000 β-amylase enzyme unit, 75×10^8 CFU of *Bacillus subtilis*) add at rate of 4 g kg^{-1} DM

treatments were: control fed on 38% Egyptian clover and 62% Concentrate Feed Mixture (CFM) without glycerol (T_1); glycerol group fed as control except replacing 30% corn with glycerol (T_2) and the third group (T_3) were fed as glycerol group with adding enzymes (4 g kg^{-1} DM). Diets were offered twice daily at 08:00 and 16:00 h. water was available continuously.

At the last day of each experimental period, rumen liquor samples were collected from each animal at zero, 3 and 6 h post-morning feeding by stomach tube. The samples were strained through two layers of cheese cloth and were immediately used for determination of ruminal pH. Rumen liquor samples were stored in glass bottles after adding metaphosphoric acid (15%) and stored at deep freeze (-18°C) for analysis of ammonia and volatile fatty acids fraction. Feces grab sample method was used and silica as internal marker was applied for determining the apparent digestibility. Feces grab samples were collected at 07:00 h for three successive days from each animal. Solution of 10% H_2SO_4 were added to the representative samples then dried in oven at 70°C for 24 h. The dried feces samples from each animal were mixed and stored at -18°C for chemical analysis of DM, OM, CP, NDF, ADF and ash. The animals were handily milked twice daily at 07:00 and 19:00 h during the last three days of each experimental period. After each milking, sample of each animal represents a mixed sample of constant percentage of the evening and morning yield and stored at (-18°C) for further analysis of milk constituents.

Chemical analysis

Feed and feces samples analysis: The dry matter contents of feed and faeces were determined by oven-drying for 4 h at 105°C according to AOAC (1990) method No. 930.15. Ash analysis was conducted at 550°C for 4 h based on the AOAC (1990) method No. 942.05. Nitrogen was measured using a mixed catalyst Kjeldahl method AOAC (1990); method No. 988.05). The crude protein content was calculated by multiplying nitrogen by 6.25. Ether extract was determined by the Soxhlet method with petroleum ether as a solvent following AOAC (1990) method No. 963.15. The TMR samples were also analyzed for ADF (method 973.18c; AOAC (1990) and NDF (Van Soest *et al*., 1991) using α-amylase (A3306; Sigma Chemical Co., St. Louis, MO) and sodium sulfite corrected for ash concentration adapted for an Ankom 200 fiber analyzer (Ankom Technology, Fairport, NY).

Rumen liquor samples analysis: Samples for VFA analyses were prepared and analyzed as described by Bush *et al*. (1979). Ammonia samples of rumen liquor were centrifuged at 2000X g at 4°C for 10 min and the supernatant was acidified with 0.5 mL of 0.1 N HCl then analyzed for NH_3 by a Bio-Diagnostic kit (Bio-Diagnostic, Cairo, Egypt) using a spectrophotometer (T80 UV/Vis spectrophotometer, PG Instruments Ltd., England).

Milk samples analysis: Milk samples were analyzed for Total Solids (TS), Solids Not Fat (SNF), Total Protein (TP), fat and lactose using infrared spectroscopy (Bentley 150, Infrared Milk Analyzer, Bentley Instruments, USA).

Statistical analysis: Data were statistically analyzed using the ANOVA procedures of SAS according to procedures outlined by Snedecor and Cochran (1982), significant level was 0.05.

RESULTS

The ingredients and chemical composition of the experimental diets are presented in Table 1. Dietary ADF, NDF and ether extract content decreased as proportion of glycerol in diets. Dietary CP and net energy for lactation (NEL) content were similar among experimental diets.

Rumen fermentation parameters: Rumen pH values for the experimental treatments through different sampling times showed that replacing corn by glycerol tends to slightly decrease rumen pH comparing with control (T_1) (Table 2). These results indicated that there were insignificant ($p>0.05$) differences among the overall mean of rumen pH in different treatments being 6.42, 6.43 and 6.31 for T_1, T_2 and T_3, respectively. The molar proportion for acetate decreased ($p<0.05$) with feeding glycerol (T_2) recorded the lowest value (44.56%) compared with T_1 (46.96%), while T_3 increased the value of acetic acid value (47.93%). The pattern of propionic acid concentration had showed that T_2 recorded the highest value (33.1%) then T_3 (28.43%) then T_1 (26.33%). The acetate to propionate ratio decreased ($p<0.05$) with the T_2 diet relative to the T_1 and T_3 diet. The molar proportions for butyrate decreased with feeding glycerol (T_2 and T_3) compared with T_1, the recorded values for butyric acid concentration were 23.66, 21.33 and 22.23% for T_1, T_2 and T_3, respectively. The overall means of NH3 concentration were lower significantly ($p<0.05$) in T_2 compared to T_1 and T_3 being 12.33, 8.83 and 10.2 100 mg mL^{-1} for T_1, T_2 and T_3, respectively.

Table 2: Effect of experimental diets on ruminal parameters

		Treatments		
Parameters	Sampling time (h)	T_1	T_2	T_3
pH	0	6.90	6.80	6.80
	3	5.96	5.82	5.90
	6	6.40	6.67	6.24
Average pH		6.42	6.43	6.31
Acetic acid (%)	0	44.10	43.90	54.70
	3	48.90	45.30	41.60
	6	47.90	44.50	47.50
Average acetic acid (%)		46.96^a	44.56^b	47.93^a
Propionic acid (%)	0	23.60	32.00	22.40
	3	29.20	34.40	35.50
	6	26.20	32.90	27.40
Average propionic acid (%)		26.33^c	33.10^a	28.43^b
Acetate:propionate ratio		1.78^a	1.35^b	1.69^a
Butyric acid (%)	0	21.20	23.10	20.20
	3	26.20	19.10	21.70
	6	23.60	21.80	24.80
Average butyric acid (%)		23.66	21.33	22.23
Ammonia (100 mg mL^{-1})	0	6.00	5.80	5.60
	3	17.00	11.80	16.80
	6	14.00	8.90	8.20
Average ammonia (100 mg mL^{-1})		12.33^a	8.83^c	10.20^b

T_1: Total mixed ration consist of 62% concentrate feed mixture (CFM) and 38% roughage (Egyptian clover), T_2: Replacing 30% of diet corn with crude glycerol, T_3: Replacing 30% of diet corn with crude glycerol plus 4 g kg^{-1} DM enzymes, Means in the same row with different superscripts are significantly differ at $p<0.05$

Table 3: Effect of experimental diets on nutrients digestibility

	Treatments		
Digestibility (%)	T_1	T_2	T_3
Dry matter	72.74±1.02^a	69.42±1.11^b	73.53±1.22^a
Organic matter	72.94±1.53^b	70.66±1.63^b	77.36±1.99^a
Crud protein	76.24±1.92^b	72.59±1.79^c	78.95±2.01^a
Ether extract	68.04±1.10^b	69.61±1.04^{ab}	71.51±1.30^a
Neutral detergent fiber	56.48±1.98^b	52.86±2.15^c	61.05±2.31^a
Acid detergent fiber	59.81±2.60^a	48.92±3.01^b	57.85±2.96^a
Hemicellulose	60.84±0.47	61.35±0.50	61.63±0.38

T_1: Total mixed ration consist of 62% concentrate feed mixture (CFM) and 38% roughage (Egyptian clover), T_2: Replacing 30% of diet corn with crude glycerol, T_3: Replacing 30% of diet corn with crude glycerol plus 4 g kg^{-1} DM enzymes, Means in the same row with different superscripts are significantly differ at $p<0.05$

Table 4: Effect of experimental diets on milk yield and composition

	Treatments		
Parameters	T_1	T_2	T_3
Milk Yield (g h^{-1} day^{-1})	1581.30±83.00^a	1174.80±72.90^b	1610.10±76.80^a
4% FCM (g h^{-1} day^{-1})	1774.30±99.80^a	1030.10±110.1^b	1648.40±117.9^a
Milk composition (%)			
Total solids	14.60±0.41^a	12.90±0.290^b	13.20±0.330^{ab}
Total protein	4.60±0.17^a	3.70±0.190^b	3.50±0.120^b
Fat	4.80±0.15^a	3.40±0.220^b	4.70±0.210^a
Lactose	4.49±0.17	5.30±0.190	5.30±0.120
Solids not fat	9.79±0.19	9.50±0.320	9.10±0.170
Ash	0.70±0.02	0.50±0.060	0.50±0.020
Total solids	230.70±11.17^a	151.50±16.17^b	212.50±12.17^a
Milk constitnents yields (g)			
Total protein	72.74±3.97^a	43.47±8.620^b	56.35±6.32^{ab}
Fat	75.90±6.25^a	39.94±5.380^b	75.67±7.310^a
Lactose	71.00±5.36^b	62.26±4.100^b	85.34±3.200^a
Solid not fat	154.80±6.94^a	111.60±8.660^b	146.50±7.0200^a
Ash	11.07±0.86^a	5.87±1.030^b	8.05±0.890^{ab}

T_1: Total mixed ration consist of 62% concentrate feed mixture (CFM) and 38% roughage (Egyptian clover), T_2: Replacing 30% of diet corn with crude glycerol, T_3: Replacing 30% of diet corn with crude glycerol plus 4 g kg^{-1} DM enzymes, Means in the same row with different superscripts are significantly differ at $p<0.05$

Digestibility coefficients: The results of Table 3 showed a significant ($p<0.05$) decrease in digestion coefficients of DM, OM, CP, NDF and ADF of T_2 compared with T_1, while there was no significant differences ($p<0.05$) between T_3 and T_1 in DM, ADF and hemicellulose digestibility, while adding fibrinolytic enzymes to glycerol diet (T_3) significantly ($p<0.05$) increased OM, CP, NDF and Ether Extract (EE) digestibility comparing with T_1.

Milk yield and composition: Goats fed glycerol (T_2) produced lower amount of milk and FCM ($p<0.05$) during the feeding period as goats fed a diet containing no glycerol (T_1) or containing glycerol with fibrinolytic enzymes (T_3) (Table 4). Milk composition was altered to be significantly reduced ($p<0.05$) in response to glycerol feeding except for lactose ($p>0.05$). Milk total solids, total

protein, SNF and fat were decreased ($p<0.05$) in case T_2 which replaced corn by glycerol compared with control (T_1) while adding fibrinolytic enzymes has a significant effect on the milk composition of T_3.

DISCUSSION

These results indicated that there were insignificant ($p>0.05$) differences among the overall mean of rumen pH in different treatments being 6.42, 6.43 and 6.31 for T_1, T_2 and T_3, respectively. T_2 recorded the lowest value through 3 hrs post-feeding which reflect the high fermentation potential of glycerol in the rumen and produced more propionate which contributes a decrease in pH value. Roger *et al.* (1992) reported that the addition of glycerol to the in vitro media greatly inhibited the growth and cellulolytic activity of rumen bacteria and fungi. Paggi *et al.* (2004) reported that the cellulolytic activity of ruminal extract was reduced as glycerol concentration in rumen cultures increased from 50 to 300 mmol. The reduction in the molar proportion of acetate and acetate to propionate ratio in T_2 was consistent with the reduction in NDF digestibility. Studies that have reported reduction in NDF digestibility have also reported reductions in acetate concentration and acetate to propionate ratio (Ribeiro *et al.*, 2005; Castillejos *et al.*, 2006). The decrease in NH3 in the rumen of glycerol groups might be due to the reduction of proteolytic activity of rumen microorganisms (Paggi *et al.*, 1999) who found that adding glycerol reduced proteolytic activity by 20%, the glycerol has three hydroxyl groups, but unlike VFA and to a lesser extent lactic acid, glycerol lacks a hydrophobic chain. This lack would make the medium where glycerol is dissolved less suitable for the activity of the proteolytic enzymes. This latter effect of the structure of glycerol would predominate over its effect on the solubility of the substrate rendering, as a balance, a depressive effect on the proteolytic activity of rumen microorganisms. Glycerol also reduced the proteolytic activity of bovine rumen fluid by about 20% when concentrations of glycerol in the medium (Paggi *et al.*, 1999). Glycerol in excess of 1% in bacterial culture media (*in vitro*) inhibited growth and cellulolytic activity of *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* Roger *et al.* (1992).

The tendency for a reduction in NDF digestibility 183 was matched by reductions in OM or DM digestibility. These data contrast with previous observations of Donkin *et al.* (2009) which reported a notable increase in DM, OM and nitrogen digestibility with addition of glycerol to lactating cow diets at 15% of DM.

The reduction of milk yield and FCM values might be due to replacing corn by glycerol and its negative effects on rumen microflora (as microbial protein) and activity and the reduction in DM, OM, CP, NDF and ADF digestion coefficients, while, in T_3 adding fibrinolytic enzymes to glycerol enhance the animals' response which clears in the return of nutrients digestion value of DM, OM, CP, NDF, ADF and EE compared to the control diet (T_1). The reduction of rumen micro-flora biomass and activity caused the significant reduction in nutrients digestion coefficients especially NDF, ADF and CP which lead the observed reduction in milk yield and milk constituents' reduction mainly milk total solids, fat and protein. While the slight increase ($p>0.05$) of lactose percent in case of inclusion of glycerol in the diet might be due to propionic acid which is the main volatile fatty acid derived from glycerol, supporting the glycogenic role of glycerol in ruminants (Johns, 1953; Garton *et al.*, 1961).

CONCLUSION

From the results of these experiments it is clear that replacing corn by glycerol at relatively high level (9% of DM) required addition of the fibrinolytic enzymes to avoid the negative effect of glycerol on the digestion and productive performance of lactating goats.

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