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Research Article
Nano-Dispersed Glycerol as an Alternative Energy Source for Ruminant Nutrition

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Abstract

Background and Objectives: The growth of the ethanol industry has increased the need for alternatives to corn in lactating cow diets. Glycerol has the potential to replace corn in the diet. Due to high potential fermentation of glycerol in the rumen, the study aimed to investigate the efficiency of encapsulation and nano-dispersed techniques to protect glycerol from ruminal degradation and fermentation. **Materials and Methods:** The current study was carried out using batch culture technique using 9 treatments as follow: Control diet (50% concentrate: 50% roughage), G20: replacing 20% of corn in control diet with glycerol, G30: replacing 30% of corn in control diet with glycerol, G40: replacing 40% of corn in control diet with glycerol, B20: replacing 20% of corn in control diet with glycerol encapsulated beads, B30: replacing 30% of corn in control diet with glycerol encapsulated beads, B40: replacing 40% of corn in control diet with glycerol encapsulated beads, N20: replacing 20% of corn in control diet with nano-dispersed glycerol solution, N30: replacing 30% of corn in control diet with nano-dispersed glycerol solution and N40: replacing 40% of corn in control diet with nano-dispersed glycerol solution. **Results:** The results showed a negative effect of using crude glycerol and encapsulated glycerol beads on ruminal digestibility and pH, while using nano-dispersed solution were similar to control diet. **Conclusion:** It could be concluded that the nano-dispersed glycerol solution might be more effective under rumen condition and fermentation.

Key words: Nano-dispersed glycerol, rumen fermentation, encapsulation, gas production, digestibility, lactating cow, ruminal degradation, ethanol industry

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

As ruminant production systems became increasingly intensified, economic assessment related to feeding became critical, as feed accounts for 30-70% of total production costs depending on activity and type of operation[1,2] reducing the profit margin for producers[3]. Energy is the most expensive component of ruminant diets and its price influenced due to use of corn, soybean and other grains for ethanol and biodiesel production. Crude glycerol is the main by-product generated from biodiesel production: Approximately 100 mL of crude glycerol are produced from each liter of biodiesel[4]. According to the Food and Drug Administration, glycerol is recognized as a safe feed ingredient in animal diets. The use of glycerol in livestock diets interest renewed due to the increased availability and favorable pricing of glycerol. Several studies involved cattle[5,6] and sheep[7] have been developed to determine the effects of glycerol from different sources on performance, meat and milk quality and digestibility of nutrients. Dietary glycerol seemed to be extensively fermented to propionate by ruminal bacteria[8,9], although level of feeding and method of delivery affected the amount of glycerol that escapes fermentation[10,11]. Despite the many features of using glycerol as alternative energy source, especially with raising of grains prices, different studies reported negative effects on rumen microbial population and fermentation pattern. *In vitro* fermentation of glycerol has shown that growth of cellulolytic bacteria such as *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* were inhibited when glycerol was added at 2-5% of the medium with cellobiose as the main substrate[12]. Due to the negative effects of using glycerol in the liquid form on the rumen fermentation process and rumen microflora, using crude glycerol as feed additives or substituting corn, the encapsulated form should be studied.

The main objective from this research project was to study the use of glycerol as alternative energy source or corn substituting in encapsulated form. Natural, bio-compatible, bio-degradable and non-toxic polymers were used as components of encapsulation process.

MATERIALS AND METHODS

Preparing glycerol beads: For preparing alginate glycerol beads, sodium alginate solution was prepared by dissolving sodium alginate in distilled water to get a final concentration of 2.5% (w:v). Alginate/glycerol concentration chosen was 5% alginate/glycerol mixtures and these were achieved by adding glycerol to alginate solution (w:w). Glycerol beads formation was carried out according to the dripping method of Deladino *et al.*[13]. The mixture was poured into encapsulator system allowed to drip slowly into a beaker containing a 3% calcium chloride solution with gentle stirring. The beads were left for 1 h with gentle stirring for additional hardening of the beads. The beads removed, filtered, rinsed with distilled water and left for air-drying at room temperature. Following drying, the beads were stored in containers for subsequent measurements.

Preparing nano-dispersed glycerol: The nano-dispersed solution of glycerol was prepared by mixing 50:50 of glycerol/distilled and then using an ultrasonic processor (SONICS Vibra Cell) for 20 min, pulse 20 sec, rest 5 sec, ampl 80% as followed by El-Sherbiny *et al.*[14].

In vitro incubation done at Dairy Science Department, National Research Centre during summer 2018 (July-August 2018) and carried outaccording to Khattab *et al.*[15]. The experimental diets consisted of 50:50 concentrate: roughage ratio (on DM basis). The experimental diets were: control diet (50% concentrate: 50% roughage), G20: replacing 20% of corn in control diet with glycerol, G30: replacing 30% of corn in control diet with glycerol, G40: replacing 40% of corn in control diet with glycerol, B20: replacing 20% of corn in control diet with glycerol encapsulated beads, B30: replacing 30% of corn in control diet with glycerol encapsulated beads, B40: replacing 40% of corn in control diet with glycerol encapsulated beads, N20: replacing 20% of corn in control diet with nano-dispersed glycerol solution, N30: replacing 30% of corn in control diet with nano-dispersed glycerol solution and N40: replacing 40% of corn in control diet with nano-dispersed glycerol solution.

Rumen fluid was collected from 3 ruminal cannulated Holstein dairy then mixed and squeezed through a 4 layers cheese cloth under continuous flushing with CO_2 and immediately transported to laboratory at 39°C. Each treatment was tested in 8 replicates (the experiment was repeated twice) accompanied by blank vessels (no substrate). About 400 mg of milled substrate was added to the incubation vessels of 100 mL capacity. Each vessel was filled with 40 mL of the incubation medium. Then the treatments were incubated at 39°C for 24 h. The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample. After 24 h digestion, the samples were transferred into test tubes and centrifuge for 1 h in order to obtain the residues which was then filtered and the residues dried at 65°C for 24 h. The dry residues were weighed and digestibility calculated using the equation as follows:

$$\text{IVDMD (\%)} = \frac{\text{Initial DM input} - \text{DM residue} - \text{Blank}}{\text{Initial DM input}} \times 100$$

Samples analysis: Samples of fermenter fluid were analyzed for pH and NH3-N. Substrates and substrate residues after 48 h of incubation were dried at 70°C and analyzed for the amount of DM (DM digestibility) according to AOAC[16]. The NH_3-N concentration was determined as described by Khattab *et al.*[15].

Statistical analysis: Data were statistically analyzed using GLM procedure of SAS software (Version 9.2). Significant differences between means of treatments were carried out by the Duncan's test and the significance threshold was set at $p<0.05$.

RESULTS AND DISCUSSION

Effect of experimental diets on *in vitro* ruminal pH: The average pH values of different experimental treatment groups were within the normal range favorable of cellulytic bacteria activity (pH. 5-7) except for encapsulated glycerol beads which recorded values under 5.0 (Fig. 1).

The pH values decreased post incubation which reflect the activity of rumen microflora. The results of different nano-dispersed glycerol treatments enhanced rumen pH values compared with glycerol beads treatments. Glycerol beads treatments which recorded the lowest values of pH under 5.0 reflected the high fermentation potential of glycerol in the rumen and produced more propionate which contributes a decrease in pH value, while other treatments pH values were above 5.7 which indicated a better digestion of cellulolytic materials[17].

Effect of experimental diets on *in vitro* ruminal ammonia: Average mean values of ruminal ammonia concentrations (μmol L^{-1}) in the different experimental shown in Fig. 2. The values of NH_3 concentration were lower in G40, B20 and B30 compared with control, while G20, G30, B40, N20, N30 and N40 increased the ammonia values as compared with control. The decrease in NH_3 in the rumen of G40, B20 and B30 groups might be due to the high release of encapsulated glycerol which affected on reduction of proteolytic activity of rumen micro-organisms[18]. Paggi *et al.*[18] found that adding glycerol reduced proteolytic activity by 20%, glycerol had three hydroxyl groups, but unlike VFA and to a lesser extent lactic acid, glycerol lacks a hydrophobic chain. This lack made the medium where glycerol is dissolved in are less suitable for the

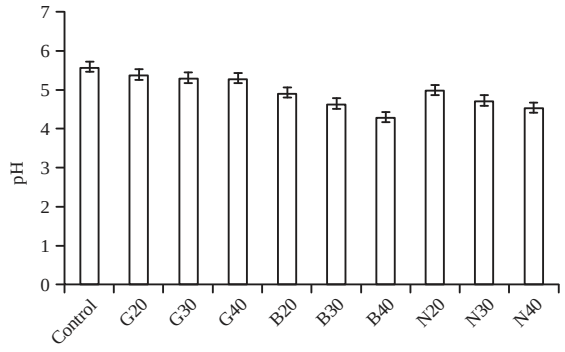

Fig. 1: Effect of experimental diets on *in vitro* ruminal pH

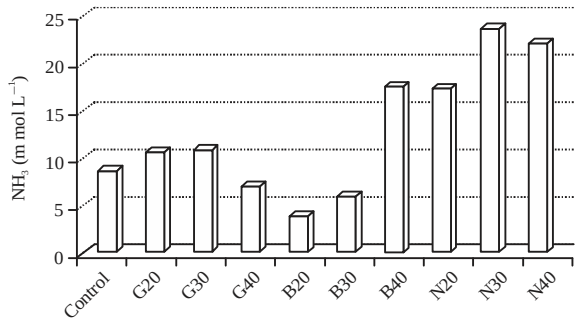

Fig. 2: Effect of experimental diets on *in vitro* ruminal ammonia concentrations

activity of the proteolytic enzymes. This latter effect of the structure of glycerol pre-dominated over its effect on the solubility of the substrate rendering, as a balance, a depressive effect on the proteolytic activity of rumen micro-organisms.

Effect of experimental diets on *in vitro* ruminal dry matter digestibility: The effects of the experimental diets on dry matter disappearance percent (DMD) presented in Table 1. Substituting corn by glycerol encapsulated beads (B20, B30 and B40) decreased DMD%, however, replacing corn with nano-dispersed glycerol solution (N20, N30, and, N40) enhanced DMD compared with control.

This reduction in case of replacing of corn with glycerol encapsulated beads (B20, B30, and, B40) on the diet may be due to inhibition of the growth and activity of cellulolytic bacteria and fungi on the rumen[12,19].

These results were disagreeing with previous *in vitro* experiment using continuous culture, Abo El-Nor *et al.*[6] stated that replacing 30% of corns' diet with glycerol had no significant effect on DMD. Avila *et al.*[20] reported linear increase with relatively higher levels of glycerol in diets. These differences in results may be due to the differences of starch contents in different diets used in those experiments.

Table 1: Effect of experimental diets on DM disappearance (%) gas production

Parameters	Control	G20	G30	G40	B20	B30	B40	N20	N30	N40
DM Dig.	57.60^{d}	60.04^{c}	60.00^{c}	60.33^{bc}	51.58^{e}	51.00^{e}	53.21	62.79^{a}	60.77^{abc}	62.51^{ab}
Total GP	150^{ef}	166^{cd}	167^{c}	170^{c}	198^{b}	204^{ab}	209^{a}	159de	155^{ef}	157^{ef}

Effect of experimental diets on *in vitro* gas production: Gas production results presented in Table 1. The results showed that replacing corn diet with different crude glycerol ratio (G20, G30, G40, B20, B30 and B40) increased gas production compared with control. The highest values of gas produced from rumen fermentation recorded for encapsulated glycerol beads (B20, B30 and B40), while, using nano-dispersed glycerol solutions (N20, N30 and N40) reduced gas production to the normal range as noted in control.

It well known that there was a negative correlation between gas production and cell wall contents (NDF and ADF) which tended to reduce the microbial activity which affected by glycerol addition to diets[6].

CONCLUSION

The current results indicated that glycerol encapsulated had no potential protection for glycerol from ruminal degradation and fermentation. However, the nano-dispersed glycerol solution was more effective under rumen condition and fermentation.

SIGNIFICANCE STATEMENT

This study showed that encapsulation technique was not effective in protecting glycerol from ruminal fermentation, but in other hand nano-dispersed technique could be more effective as protection technique under ruminal fermentation conditions. This study will help to investigate more effective protection techniques to improve utilization of different feedstuffs and maximize the rumen performance.

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