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IN-VITRO EVALUATION OF DIETS CONTAINING CRUDE GLYCEROL AS A SUBSTITUTE FOR CORN WITH SOME ADDITIVES

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SUMMARY

In vitro studies were carried out to investigate the effect of using crude glycerol (biodiesel co-product) as a substitute for yellow corn with some feed additives. Corn comprising 30% of the whole diet was substituted by crude glycerol at four levels (0, 25, 50 and 75%) with three types of additives; buffer ($NaHCO_3$& MgO), dry yeast (*Saccharomyces cerevisiea*) and fibrolytic enzymes. *In vitro* dry matter disappearance (IVDMD), organic matter disappearance (IVOMD) and total gas production (GP) were studied. Crude glycerol substitution at 25% significantly increased (P<0.05) IVDMD, IVOMD and GP values, while 50 and 75% glycerol significantly decreased (P<0.05) IVOMD and GP values compared with control. No significant difference was observed for IVDMD value when crude glycerol was included at 50% of yellow corn. Addition of 4 g/kg DM of either fibrolytic enzymes or dry yeast significantly increased (P<0.05) IVDMD by 6.5 and 6%, IVOMD by 6 and 4% and GP by 15.8 and 11.5%, respectively. On the other hand, buffer addition had no effect on measured parameters. Using high level of fibrolytic enzymes or dry yeast (6 g/kg DM) significantly increased (P<0.05) IVDMD, IVOMD and GP values for 25 and 50% glycerol compared with the lower level (4 g/kg DM). In conclusion, crude glycerol in combination either with 6g/kg DM fibrolytic enzyme (ALLZYME™) or dry yeast (*Saccharomyces cerevisiea*) could replace up to 50% of the yellow corn equivalent to 15% of the whole dietary DM.

***Keywords**: Crude glycerol, fibrolytic enzymes, dry yeast and buffer.*

INTRODUCTION

Grains, specially yellow corn, are mainly used in ruminant, poultry and fish rations, resulting in great competition with human diets especially in Egypt. The feed gap between requirements and production forced the nutritionists to use alternative feed resources. Glycerol which is a biodiesel energy co-product could be used as an alternative energetic feed resource that is not needed for further purification in food, pharmaceutical, and cosmetic industries (Thompson and He, 2006).

Recently, Donkin *et al.* (2009) indicate that glycerol can replace corn grain as much as 15% in diets for dairy animals. Abo El-Nor *et al.* (2010) observed that corn substitution with glycerol at low level (36 g/kg DM) had no adverse effect on digestibility of DM, NDF and ADF compared to control. In the contrast, Khattab *et al.* (2012) found that substitution of 30% of corn in the feed mixture by glycerol significantly decreased (P<0.05) in *vitro* dry matter and organic matter disappearance.

Early studies concluded that glycerol was entirely fermented to propionate (Johns, 1953 and Garton *et al.*, 1961) which in turn, serves as a glucogenic precursor, supplying 32 to 73% of the glucose demands in ruminants (Seal and Reynolds, 1993). In this context, Carvalho *et al.* (2011) studied the effect of glycerol inclusion at 110 g/kg of DM in the diet of dairy cows during the dry period on ruminal fermentation pattern where they found an increase in the proportion of propionate and butyrate at the expense of acetate.

Some exogenous feed enzymes that contain fibrolytic activities may help in enhancing fiber digestion in the rumen (Kung *et al.*, 2000), which could lead to improve feed conversion efficiency. In dairy goats, Khattab *et al.* (2012) stated that feeding glycerol with 4 g/kg DM fibrolytic enzyme improved nutrients digestibility of DM, OM, CP, NDF, ADF and milk production compared to feeding glycerol without additives. On the other hand, Haddad and Goussous (2005) and Kholif and Khorshed (2006) found that digestibility of OM, CP and CF were significantly increased with yeast addition to goats or lambs rations at 2.5 or 3 g/h/d. Beside that buffers in dairy rations have been recommended primarily to avoid depression of milk fat content; control ruminal acidity and to avoid reduction in dry matter intake (Doepel and Hayirli, 2011).

The aim of this *in-vitro* study was to: (1) Evaluate the substitution of corn with different levels of crude glycerol. (2) Use of some feed additives with glycerol to enhance IVDMD, IVOMD and gas production in comparison to corn.

MATERIALS AND METHODS

Experimental design:

This study was carried out at Regional Center for Foods and Feeds (RCFF), Agricultural Research Center (ARC), Giza, Egypt. Crude glycerol (biodiesel co-product) was used as a substitute for yellow corn at four levels (0, 25, 50 and 75%) to determine the *in vitro* dry matter disappearance (IVDMD), *in vitro* organic matter disappearance (IVOMD) and total gas production (GP). Substitution levels were carried out with three additives included; buffer ($NaHCO_3$& MgO), dry yeast and fibrolytic enzymes. Additive levels were 4 g/kg DM either for dry yeast or fibrolytic enzymes and 0.5% $NaHCO_3$ + 0.5% MgO of dietary DM. Dry live yeast *Saccharomyces cerevisiea*: 1 x 10^{10} cell/gram (Pro-Bio-Fair™) and fibrolytic enzymes (ALLZYME™SSF containing per gram: 300 standard phytase units, 700 protease unit, 40 carboxymethyl cellulase units, 100 xylanase units, 200 beta glucanase units, 30 fungal amylase units, and 4000 pectinase units). Another *in-vitro* study was carried out to investigate the effect of two levels (4 and 6 g/kg DM) of two additives (dry yeast and fibrolytic enzymes) with two glycerol substitution levels (25 and 50%).

In vitro incubation:

Piston-pipettes (glass syringes, 100 ml volume) with capillary attachment, were used (5 piston-pipettes per each treatment) to determine IVDMD, IVOMD and GP. The procedures of the *in vitro* technique were carried out according to Menke *et al*. (1979). About 200 mg of samples (on DM basis) were weighed and placed into piston-pipettes. Then 30 ml of rumen liquor medium-mixture (one part of liquor was mixed with two parts of the medium in a woulff-bottle kept at 39°C in a water bath and stirred by a magnetic stirrer under CO_2 atmosphere) were added using an automatic pipette. Any gas bubbles in the syringes were removed, the plastic clip on the silicon tube closed, the position of the piston recorded, and the syringes placed in the incubator for 48 hrs.

Rumen liquor was obtained from males mature sheep fed 50% clover hay and 50% concentrate mixture. Whole rumen content was obtained before the morning feed using a stomach rubber tube. Rumen liquor was squeezed through four layers of gauze and the fluid was kept in a pre-warmed thermos flask. The experimental rations are presented in Table (1).

Table (1): Feed ingredients of experimental rations.

Item	Experimental rations			
	G0	G25	G50	G75
Ingredient, % of DM				
Yellow corn	30	22.5	15	7.5
Soybean meal	9.3	10.2	11.1	12.3
Cottonseed meal	6.6	9.3	11.7	13.5
Wheat bran	11.1	7.5	4.2	1.2
Glycerin	0	7.5	15	22.5
Minerals	1.5	1.5	1.5	1.5
Vitamins	1.5	1.5	1.5	1.5
Egyptian clover hay	40	40	40	40

Analytical procedures:

Chemical analyses of samples were carried out according to the methods of AOAC, (2012). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al*. (1991).*In vitro* DM and OM disappearances and GP were determined according to Menke *et al*. (1979).

Statistical analysis:

Data were statistically analyzed by the least square procedure of the General Linear Model Program of SAS (2009) according to procedures outlined by Snedecor and Cochran (1982). Fixed models, one, two and three-ways analysis of variance, were used. Firstly, one way analysis was used to evaluate crude glycerol substitution without additives on IVDMD, IVOMD and GP, according to the following model:

$$\mathbf{y_{ij} = \mu + S_i + e_{ij}}$$

Where $\mathbf{y_{ij}}$ is the jth sample of the ith substitution level, $\mathbf{\mu}$ isthe overall mean, $\mathbf{S_i}$ is the fixed effect of the ith substitution level (i = 1, 2, 3, 4; 1 = 0%, 2 = 25%, 3 = 50% and 4 = 75%) and $\mathbf{e_{ij}}$ is the random error assumed to be normally and independently distributed.

Secondly, two-way analysis of variance was used to examine the effect of substitution levels, different additives and the interactions between them, according to the following model:

$$\mathbf{y_{ijk} = \mu + S_i + A_j + (S.A)_{ij} + e_{ijk}}$$

Where$\mathbf{y_{ijk}}$, $\mathbf{\mu}$, $\mathbf{S_i}$ and $\mathbf{e_{ij}}$ are the same as the one way model while $\mathbf{A_j}$ is the fixed effect of the jth additive (j = 1, 2, 3; 1 = buffer, 2 = dry yeast and 3 = fibrolytic enzymes) and $\mathbf{(S.A)_{ij}}$ is the interaction between the ith substitution level and the jth additive.

Thirdly, three-way analysis of variance was used to examine the effect of substitution levels, different additives, additive level and the interactions between them, according to the following model:

$$\mathbf{y_{ijkn} = \mu + S_i + A_j} + L_k + \mathbf{(S.A.L)_{ijk} + e_{ijkn}}$$

All terms where the same as the previous module except that the substitution levels were two (25% and 50%) and the additives were also two (dry yeast and fibrolytic enzymes) in addition to $\mathbf{L_k}$: the fixed effect of the kth additive level (k = 1, 2; 1= 4 g/kg DM and 2 = 6 g/kg DM) and $\mathbf{(S.A.L)_{ijk}}$: the interaction between the ith substitution level, the jth additive and the kth additive level.

Procedures of Duncan's New Multiple Range Test according to Duncan's, (1955) Least Square Means according to; Steel and Torrie, 1980 and Tukey's multiple comparison test according to honestly significant difference (HSD) were used to asses significance (P<0.05) of differences among means.

RESULTS AND DISCUSSION

Chemical composition and fiber fractions of experimental rations:

Results of chemical composition (Table 2) indicated that contents of most nutrients were similar in all experimental rations. However, a slight increase in CF content and a decrease in NFE content were observed with rations contained crude glycerol. This could be attributed to the linear increases in cottonseed meal and the decreases in yellow corn proportions with increasing glycerol substitution level which was made to formulate iso-nitrogenous rations.

Likewise, fiber fractions were almost the same in all experimental rations except, G75 ration that contained the highest ADF and cellulose contents which may be as a result of high cottonseed meal proportion. In the contrast, Khattab *et al.* (2012) recorded decreases in ADF and NDF contents with rations contained glycerol.

Effect of glycerol level:

Regarding crude glycerol substitution level (Table 3), replacement of 25% yellow corn by crude glycerol had significantly higher IVDMD, IVOMD and GP values being 57.27%, 61.96% and 32.18

ml/200 mg DM compared with control and other substitution levels (50 and 75%). No significant effect was observed for IVDMD value at 50% crude glycerol compared with control (54.14% and 55.14%, respectively). Meanwhile, the values of IVOMD and GP (58.56% and 27.70 ml/200 mg DM) were significantly decreased (P<0.05) compared with control (60.39% and 30.11 ml/200 mg DM). The 75% replacement had the lowest IVDMD, IVOMD and GP values being 52.54%, 56.50% and 24.98 ml/200 mg DM, respectively.

Data showed that increasing concentrations of crude glycerol more than 25% of corn or 7.5% of DM (G50 and G75) was associated by a decrease of IVOMD and GP values. This result could be attributed to the inhibitory effect of glycerol on ruminal fungi (Roger *et al*., 1992) or cellulolytic bacteria (Abo El-Nor *et al*., 2010). Moreover, Abo El-Nor *et al.* (2010) found that substitution of corn with glycerol at low level (15%) had no negative effect on digestibility of DM, NDF and ADF using continuous fermentor compared to control. However, replacing corn by glycerol at 30 and 45% was noted to reduce digestibility of NDF and ADF compared to control (Khattab *et al*., 2012 and Avila-Stagno *et al.*, 2014). In contrast, Donkin *et al.* (2009) found that apparent digestibility of DM and OM were increased compared with control when 5, 10 and 15% of corn grains were substituted by glycerol in lactating dairy cows.

Table (2): Chemical composition of experimental rations.

Item	Experimental rations			
	G0	G25	G50	G75
DM, %	92.27	92.11	91.15	90.39
DM basis, %				
OM	92.34	92.17	92.53	92.48
CP	15.59	16.26	16.18	15.97
CF	9.49	9.99	10.03	11.42
EE	2.81	3.91	3.72	3.56
NFE	64.45	62.01	62.60	61.53
Ash	7.66	7.83	7.47	7.52
Fiber fractions				
NDF	21.78	21.43	19.53	22.31
ADF	12.17	12.74	13.76	15.88
ADL	3.30	3.34	4.39	3.12
Hemicellulose	9.61	8.69	5.77	6.43
Cellulose	8.87	9.40	9.37	12.76
Lignin	2.81	3.25	3.60	2.60

Table (3): Effect of glycerol levels and different additives on *in vitro* dry matter and organic matter disappearance (%) and total gas production (ml/200 mg DM).

Item	IVDMD	IVOMD	GP
Replacement levels			
Control (G0)	55.14^{b}	60.39^{b}	30.11^{b}
G25	57.27^{a}	61.96^{a}	32.18^{a}
G50	54.14^{b}	58.56^{c}	27.70^{c}
G75	52.54^{c}	56.50^{d}	24.98^{d}
±SE	0.51	0.54	0.72
Additives			
Without	53.07^{b}	58.07^{b}	27.05^{b}
Buffer	53.10^{b}	57.58^{b}	26.40^{b}
Fibrolytic enzymes	56.53^{a}	61.32^{a}	31.34^{a}
Dry yeast	56.39^{a}	60.43^{a}	30.17^{a}
±SE	0.51	0.54	0.72

Means in the same row with different superscripts are significantly different (P<0.05).

Effect of additives type:

Regarding type of additives (Table 3), the addition of 4 g either from fibrolytic enzymes or dry yeast /kg DM had a positive significant (P<0.05) effects on IVDMD (56.53% and 56.39%) and IVOMD (61.32% and 60.43%), and GP (31.34 and 30.17 ml/200 mg DM) compared with no additive treatment (53.07%, 58.07% and 27.05 ml/200 mg DM, respectively). However, buffer addition did not affect significantly IVDMD, IVOMD and GP values (53.10%, 57.58% and 26.40 ml/200 mg DM, respectively).

The positive effect of fibrolytic enzymes might be explained by creating a stable enzyme-feed complex that protects free enzymes from proteolysis in the rumen (Kung *et al.,* 2000). Several potential modes of action have been proposed, included: 1) increasing the microbial colonization of feed particles (Yang *et al.,* 1999), 2) enhancing attachment and /or improve access to the cell wall matrix by ruminal microorganisms which result in accelerating the rate of digestion (Nsereko *et al.* 2000) , 3) enhancing the hydrolytic capacity of the rumen due to added enzyme activities and/or synergy with rumen microbial enzymes (New bold, 1997 and Morgavi *et al.,* 2000) and 4) enzymes were able to degrade complex substrate to simpler ones, allowing a faster ruminal microbial colonization and fermentation (Colombatto *et al.,* 2003).

The positive effect of dry yeast might be explained by the increase in the population and/or activity of rumen cellulolytic bacteria as mentioned by Erasmus *et al*. (1992) and Newbold *et al*. (1995) and also might increase ciliate protozoa number which represent more than 90% of rumen fibrolysis activity (Kamel *et al.,* 2004 and Tripathi and Karim, 2011). It was mentioned also that yeast provides rumen microflora with vitamins or other growth factors or by scavenging oxygen entered the rumen (Chaucheyras-Durand *et al*., 2008) and might increase proleolytic bacteria counts (Yoon and Stern, 1996 and Tripathi and Karim, 2011).

Results concern fibrolytic enzyme and yeast addition are coincide with those obtained by Haddad and Goussous (2005), Kholif and Khorshed (2006) and Khattab *et al.* (2012).

In the contrary, Gomez-Alarcon *et al*. (1990) and Hristov *et al.* (2010) found that adding yeast at 3 g/h/day to rations of Holstein cows did not detecte significant effects on digestibility of DM, OM, CP, NDF and ADF compared with control.

Results concerning buffer addition are in agreement with those obtained by Doepel and Hayirli (2011) who demonstrated that cows fed diets containing 20% steam-rolled wheat with sodium bicarbonate had no significant effect on the digestibility of DM, CP, ADF, and NDF compared with control diet.

The effect of interaction between glycerin levels and different additives on *in vitro* dry matter and organic matter disappearance and total gas production (Table 4) Indicated that using dry yeast as additive when 25% of corn replaced by crude glycerol had the highest (P<0.05) IVOMD and GP values (63.52% and 34.25 ml/200 mg DM, respectively) compared with 50% replacement level of corn (59.60% and 29.07 ml/200 mg DM, respectively) or 75% replacement level (56.07% and 24.41 ml/200 mg DM, respectively). Meanwhile, addition of fibrolytic enzymes had almost the same effect on IVOMD and GP values when corn replaced by crude glycerol either at 25% (63.12% and 33.72 ml/200 mg DM, respectively) or 50% (63.00% and 33.56 ml/200 mg DM, respectively), but the values were significantly decreased (P<0.05) with 75% glycerol (58.05% and 27.02 ml/200 mg DM, respectively).

The addition of dry yeast or fibrolytic enzymes at low level of corn substitution by crude glycerol (25%) could enhance digestibility, while with the higher substitution level (50%) fibrolytic enzymes could be used as the best additive to improve digestibility and alleviate glycerol negative effects. These results are paralleled with those reported by Yang *et al*., (1999) and Kung *et al*. (2000) that exogenous feed fibrolytic enzymes could enhance fiber digestion in the rumen. In this context, Khattab *et al.* (2012) detected insignificant differences between goats fed glycerol with fibrolytic enzymes (4 g/kg DM) and those fed control diet in digestibility of DM, ADF and hemicellulose.

Effect of additives level:

The effects of using two levels of fibrolytic enzymes and dry yeast (4 g and 6 g/kg DM) at 25% and 50% glycerol on *In vitro* dry matter and organic matter disappearance and gas production (Table 5) indicated that using high level of fibrolytic enzymes or dry yeast increased IVDMD, IVOMD and GP for 25% and 50% glycerol level. The highest IVDMD, IVOMD and GP values recorded when high level of fibrolytic enzymes (6 g/kg DM) was added either with glycerol at 25% (59.52%, 64.37% and 35.37 ml/200 mg DM, respectively) or at 50% (59.27%, 64.05% and 34.95 ml/200 mg DM, respectively) and when high level of dry yeast (6 g/kg DM) was added at 25% glycerin (60.49%, 64.14% and 35.07 ml/200 mg DM, respectively).

Values of IVOMD and GP of 50% glycerol diet were higher (P<0.05) at high level of fibrolytic enzymes (64.05% and 34.95 ml/200 mg DM, respectively) than those at high level of dry yeast (61.23% and 31.23 ml/200 mg DM, respectively). The same results obtained by Khattab *et al.* (2012) who stated that substituting 30% of corn by crude glycerol with fibrolytic enzymes addition significantly increased IVDMD and IVOMD compared with glycerol alone, without significant difference compared with control.

Table (4): Effect of interaction between glycerol levels and additives on *in vitro* dry matter and organic matter disappearance (%) and total gas production (ml/200 mg DM).

Item	IVDMD	IVOMD	GP
G0			
Without	53.88^{cd}	59.58^{bcde}	29.04^{bcde}
Buffer	52.52^{def}	58.33^{cdef}	27.39^{cdef}
Fibrolytic enzymes	57.95^{ab}	61.11^{abcd}	31.06^{abcd}
Dry yeast	56.19^{abc}	62.53^{ab}	32.94^{ab}
G25			
Without	55.83^{bc}	61.34^{abc}	31.36^{abc}
Buffer	56.30^{abc}	59.85^{bcde}	29.40^{bcde}
Fibrolytic enzymes	58.06^{ab}	63.12^{a}	33.72^{a}
Dry yeast	58.89^{a}	63.52^{a}	34.25^{a}
G50			
Without	50.27^{f}	53.56^{g}	21.10^{g}
Buffer	52.87^{def}	58.06^{def}	27.05^{def}
Fibrolytic enzymes	56.46^{abc}	63.00^{a}	33.56^{a}
Dry yeast	56.94^{ab}	59.60bc^{de}	29.07^{bcde}
G75			
Without	52.29^{def}	57.81^{ef}	26.70^{ef}
Buffer	50.69^{ef}	54.07^{g}	21.77^{g}
Fibrolytic enzymes	53.66^{cd}	58.05^{def}	27.02^{def}
Dry yeast	53.53^{cde}	56.07^{fg}	24.41^{fg}
±SE	1.02	1.08	1.43

Means in the same row with different superscripts are significantly different (P<0.05).

Table (5): Effect of fibrolytic enzyme and dry yeast levels with 25 and 50% glycerol replacement on *in vitro* dry matter and organic matter disappearance (%) and total gas production (ml/200 mg DM).

Glycerol level	Additive	Additive level	IVDMD	IVOMD	GP
25%					
	Fibrolytic enzymes	L	58.06^{bcd}	63.12^{ab}	33.72^{ab}
		H	59.52^{ab}	64.37^{a}	35.37^{a}
	Dry yeast	L	58.89^{abc}	63.52^{ab}	34.25^{ab}
		H	60.49^{a}	64.14^{ab}	35.07^{ab}
50%					
	Fibrolytic enzymes	L	56.46^{d}	63.00^{b}	33.56^{b}
		H	59.27^{ab}	64.05^{ab}	34.95^{ab}
	Dry yeast	L	56.94^{cd}	59.60^{d}	29.07^{d}
		H	59.71^{ab}	61.23^{c}	31.23^{c}
±SE			0.74	0.45	0.59

Means in the same column with different superscripts are significantly different (P<0.05).

Low: 4 g/kg DM; High: 6 g/kg DM.

CONCLUSION

Substitution of 25 or 50% yellow corn by crude glycerol had the potential to improve *in vitro* DM and OM disappearance and GP when combined either with fibrolytic enzyme (ALLZYME™) or dry yeast (*Saccharomyces cerevisiea*) and could be used as an alternative energy source in ruminant rations.

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التقييم المعملى لعلائق تحتوى على الجليسرول الخام كإحلال من الأذرة مع بعض الإضافات

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تم إجراء عدة تجارب معملية *in vitro* لدراسة تأثير استخدام الجليسرول الخام كبديل للأذرة الصفراء مع بعض إضافات الأعلاف. تم استخدام الجليسرول الخام بأربع مستويات إحلال (صفر، 25، 50 و75%) من الأذرة الصفراء مع ثلاث إضافات وهى: منظم الحموضة (بيكربونات الصوديوم وأكسيد المغنسيوم)، الخميرة الجافة (*Saccharomyces cerevisiea*) والإنزيمات المحللة للألياف (ALLZYME™) لدراسة معامل إختفاء كلاً من المادة الجافة والمادة العضوية معمليًا وإنتاج الغاز. أوضحت النتائج أن استخدام 25% جليسرول خام أدى الى زيادة قيم معامل إختفاء المادة الجافة،المادة العضوية معمليًا والغاز الناتج (P<0.05)، بينما مستوى 50 و 75% أدى الى حفض قيم معامل اختفاء المادة العضوية معمليًا والغاز الناتج معنويًا (P<0.05). لم يكن هناك فرق معنوى بين قيم معامل إختفاء المادة الجافة معمليًا عند إحلال الجليسرول الخام بنسبة 50% من الذرة الصفراء. أيضًا تبين من النتائج أن إضافة 4جم/كجم مادة جافة سواء من الإنزيمات المحللة للألياف (ALLZYME™) أو من الخميرة الجافة أدى الى زيادة معامل إختفاء المادة الجافة بنسبة 6.5 و6%، ومعامل إختفاء المادة العضوية بنسبة 6 و4% و إنتاج الغاز بنسبة 15.8 و11.5% على التوالي. بينما لم يؤثر استخدام منظم الحموضة على أي من المقاييس السابقة. أوضحت النتائج أن استخدام المستوى المرتفع من الإنزيمات المحللة للألياف والخميرة الجافة (6 جم/كجم مادة جافة) أدى إلى زيادة معنوية (P<0.05) لقيم معامل هضم كلاً من المادة الجافة و المادة العضوية وإنتاج الغاز لكلاٍ من نسبتي الإحلال بالجليسرول الخام 25 و50% من الأذرة الصفراء مقارنة باستخدام المستوى المنخفض من الإضافات (4 جم/كجم مادة جافة).

تشير النتائج الى إمكانية استخدام الجليسرول الخام كمصدر بديل للطاقة في علائق المجترات بنسبة تصل إلى 50% من الأذرة الصفراء سواء مع اضافة الإنزيمات المحللة للألياف (ALLZYME™) أو الخميرة الجافة (*Saccharomyces cervisiea*).

الكلمات الدالة: الجليسرول الخام ، الإنزيمات المحللة للألياف، الخميرة الجافة و منظمات الحموضة.