EFFECT OF USING SOME FIBROLYTIC ENZYMES IN THE RATION ON LACTATING GOATS PERFORMANC

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SUMMARY

The present study was carried out at farm and laboratory of Animal Production Department- Faculty of Agriculture, Fayoum University, Egypt. Fibrolytic enzymes were evaluated throughexperiments conducted by using nine of lactating baladi goats after 20 days of parturition and divided into three groups, three animals per each group. The first group was fed 50% concentrate feed mixture , 10% Egyptian clover and 40% wheat straw (Control ration). The second group was fed control ration supplemented with Asperozym(locally produced cellulase enzyme) at level of 1000 unit of cellulase enzyme /kg DM intake (R1). The third group was fed control ration supplemented with Phytabex plus ® (commercial cellulolytic enzyme source) at level of 1000 unit of cellulolytic enzymes /kg DM intake. (R2). The results revealed that Asperozym was superior to Phytabex plus ® for improving feed digestion and milk production by goats.. There were significant ($P \leq 0.05$) increases in fat corrected milk yield (4 % fat) and fat percentage of (R1) and (R2) compared to control ration. Feed conversion of DM, SV and TDN was decreased significantly ($P \leq 0.05$) with control ration compared to R1 and R2 rations. Results of some blood serum analysis showed that no side effect of using the tested cellulolytic enzymes on lactating goats. From economical point of view, the best ration was R1.

Keywords: *cellulase enzyme, digestibility, milk yield and its composition, lactating goats.*

INTRODUCTION

The problems of feeding roughage directly to farm animals are in general, low protein content, high crude fiber, low digestibility coefficients and containing some anti-nutrients factors such as tannins and alkaloids (Kholif*et al*., 2005). Thus, to increase digestibility of these crop residues, it is important to destroy the compact nature of this lignocellulosic tissues and reduce the deleterious effects of the anti-nutrients factors.There are main reasons for using enzymes as livestock feed supplements: 1) to break down anti-nutritional factors; 2) to increase the availability of starches, proteins and minerals enclosed within fiber-rich cell walls; and 3) to break down specific chemical bounds in raw materials which are not usually broken down by the animals' own enzymes (Sheppy, 2001).Many researchers demonstrated that, supplementing rations of dairy animals with fibrolytic enzymes can improve feed utilization and animal performance by enhancing fiber degradation *in vitro* (Gado*et al.,* 2009, Rodrigues *et al.,* 2008 and Azzaz*et al,* 2012).

Addition of exogenous enzymes to animal rations can improve feeding values by increasing feed intake and improving fibre degradation (Salem *et al*., 2015 and Valdes *et al*., 2015). Some studies showed that enzyme addition increased nutrient digestibility and increased milk production of dairy animals (Khattab*et al.*, 2011, Kholif*et al*., 2012, , Salem *et al*., 2015, Silva *et al*., 2016, and Upadhaya*et al*., 2016), but others showed only weak effects on animal performance (Ballard *et al.* 2003and Reddish and Kung, 2007). Therefore, this study was carried out to investigating the impact of adding these enzymes to lactating goats ration on nutrients digestibility, milk yield and composition, feed conversion and some blood parameters were conducted .Also, simple economical evaluation of the tested rations was considered.

MATERIALS AND METHODS

The present study was carried out at farm and laboratory of Animal Production Department, Faculty of Agriculture, Fayoum University, Egypt.

Enzyme source:Asperozym,**(local cellulase enzyme)** waslaboratory produced **by Aboul – Fotouh*et al*.(2016)** from *Asperigillusniger*and each (g) of it contains 240 cellulase units.Phytabex plus® ,a commercial cellulolytic enzyme source produced by EN BIO. TECH CO., LTD – China and purchased from the company of IBEX International LTD (United Kingdom). Each (g) of it contains 500 unit of cellulase.

Digestibility and lactation trials:

Experimental animals:

Nine of lactating baladi goats (in their 2^{th} to 4^{th} lactation seasons) and weighed 20 ± 1 kg in average .After 20 days of parturition were randomly assigned into three groups, three animals per each tested ration (R) using complete randomized design. The experimental period was 45days.

The tested rations:

The goats were individually fed rations of concentrate: roughage at ratio of 1:1 on DM basis. The first animal group was fed on ration of 50 % concentrate feed mixture (CFM), 10% Egyptian clover and 40% wheat straw (Control ration).The two cellulase enzymes were supplemented to the rations at the optimum rate which recommended from the *in vitro* experiment (**Aboul – Fotouh*et al*.,2016)**.Where,the second group (R1) was fed control ration supplemented with Asperozym at 1000 unit of cellulase enzyme/kg DM, while the third group (R2) was fed control ration supplemented with Phyabex plus® (Commericial enzyme) at 1000 unit of cellulolytic enzymes /kg DM .Animals were fed to cover their nutritional requirements according to N.R.C (1985). The compositionof tested rationsare shown in Table (1).

Table (1): Composition of the tested rations of lactating goats (on DM basis).

Item	The tested rations		
	Control	R1	R2
Concentrate feed mixture	50	50	50
Wheat straw	40	40	40
Egyptian clover	10	10	10
Cellulase enzyme	----	1000 unit of Asperozym/kg DM	1000 unit of phytabex plus®/kg DM

Formulation of concentrates feed mixture on DM basis was 55% yellow Corn , 21.5% wheat bran , 20% soya bean meal , 3.5% feed Additives (feed additives composed of 1.5% limestone,0.5% dicalcium phosphate, 0.2% yeast, 0.3% bicarbonate, 0.5% premix and 0.5% NaCl.)

Digestibility trial:-

Digestibility trial was performed at the end of the lactation experiment, the nutrient digestibilities and feeding values were determined using acid insoluble ash (AIA) technique of VanKeulen and Young (1977). Feces samples were collected daily per each animal for seven days, dried over night at 60 °C in hot air oven, weighted, ground through 1mm screen, then complete drying was undertaken at 105 °C for 3 hrs and weighted and stored in tight bottles for chemical analysis.

Milk yield:

The technique of hand milking was used to estimate milk yield. Goats have been milked twice daily at 6:00 am and 6:00 pm by milking one teat while, the other one was lift to lamb for suckling according to Farag (1979). Daily milk yield and Total milk yield were recorded for each animal in the experiment for two weeks after preliminary period (24 days).

Methods of analysis:

Feeds and feces analysis

Chemical analysis of feed stuffs and feces samples were carried out according to methods ofA.O.A.C . (1995).the nitrogen free extract (NFE) was calculated by difference. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent Lignin (ADL) were determined in feeds and feces according to Goering and Van Soest (1970).

Milk samples and analysis:

Daily milk samples (50 ml each) were collected at 6:00 am and 6:00 pm and mixed for each animal in the experiment. It were kept frozen at (-20 C) until the chemical analysis were executed. Milk composition were determined by Ekomilk ® analyzer (KAM98-2A USA).

Fat corrected milk (4% FCM) was calculated by using the following equation according to Gaines (1928).

Blood samples: Blood samples were collected before starting of lactation trial and at the end of the experiment before morning feeding. Serum urea was measured according to Richard *et al.* (2011). Serum glucose (SG) was measured according to Howanitz and Howantiz (1984).Serum creatinine was measured according to Spierto*et al.* (1979).Total cholesterol was quantified by colorimetric method according to Burtis*et al*. (2006).Serum aspartate aminotransferase (AST) and alaninaminotransferase (ALT) were determined by using test kits according to Reitman andFrankel (1957).

Statistical analysis:

Statistical analyses were conducted by the general linear model procedure adapted by SPSS (2007) according to the following model:$Y_{ij}=\mu+T_i+e_{ij}$**Where Y_{ij}, is the dependent variable, μ is the overall mean,T_i, is the effect of treatment and e_{ij}, is the residual error. Duncan's multiple test (Duncan, 1955) was carried out to separate among means.**

Simple economical evaluation:

Economical returns of the tested rations were calculated at the time of the experiment (May, 2016) assuming that the price of one kg of raw milk was 9 L.E. The cost of one ton DM of CFM , Egyptian clover and wheat straw were 2800, 250 and 800 L.E., respectively.Also price of one kg of Asperozym was 50 L.E.and price of one kg of phytabex plus ® was 200 L.E.

RESULTS AND DISCUSSION

Chemical composition of feed ingredients: The chemical composition and cell wall constituents (DM basis) feed ingredients are shown in Table (2). The chemical composition indicated a comparable DM composition of all ingredients. Wheat straw showed the highest levels of crude fiber and ash content and lower content of CP compared toEgyptian clover and concentrate feed mixture. Also, wheat straw showed higher levels of NDF and cellulose contents compared to other feed ingredients.

Digestibility and nutritive values: Data of Table (3) clearly show that, both of rations supplemented with Asperozym (R_1) and Phytabex plus® (R_2) significantly ($P\leq0.05$) increased DM, OM and CF digestibility compared to the control ration. Ration supplemented withAsperozym (R_1) was superior significantly ($P\leq 0.05$) to control ration regarding NFE digestibility. On the other hand, no significant differences were found between Asperozym and phytabex plus rations concerning OM,CP,CF and EE digestibilties.

Such findings are in favor with other studies which reported increase in total tract digestibility of DM and OM, following treatment with fibrolytic enzymes (Gado *et al.*, 2009, Azzaz *et al.*, 2012, Kholif *et al.*, 2012, Salem *et al*., 2015, Aguirre *et al*., 2016, Silva *et al*.,2016, Morsy*et al*., 2016 and Upadhaya *et al*., 2016).

Exogenous fibrolytic enzymes would be expected to increase total tract digestibility by increasing the rate of ruminal digestion of the potentially digestible NDF fraction (Yang *et al.*, 1999).

The nutritive values of the experimental rations are shown in Table (3).Ration supplemented with Asperozym (R_1) significantly ($P\leq 0.05$) increased SV % compared to control ration. Ration supplemented with Phytabex plus® (R_2) increased SV% but not significantly differences was found with control ration.

Table (2): Chemical composition of feed ingredients (on %DM basis).

Item	CFM	wheat straw	Egyptian clover
	Chemical composition, %		
OM	96.64	82.57	83.8
CP	16.73	3.84	17.67
EE	4.86	0.66	1.64
CF	4.83	42.52	25.77
NFE	70.22	35.55	38.72
Ash	3.36	17.43	6.97
	Cell wall constituents, %		
NDF	16.13	69	38.86
ADF	5.54	54	17.92
ADL	0.86	20.3	4.96
Hemicellulose	10.59	15	20.94
Cellulose	4.68	33.7	12.96

Hemicellulose = NDF-ADF, Cellulose = ADF-ADL, CFM: concentrate feed mixture

Table (3): Effect of Cellulolytic enzymes on digestion coefficients and nutritive values of the tested rations fed to goats.

Item	Control	R1	R2	± SE
	Nutrient digestibilities (%)			
DM	63.69^{c}	67.01^{a}	65.89^{b}	0.67
OM	68.52^{b}	72.63^{a}	71.53^{a}	0.86
CP	61.07	62.61	61.91	1.47
CF	64.42^{b}	67.74^{a}	68.36^{a}	0.70
EE	68.98	71.52	70.00	0.76
NFE	73.30^{b}	76.25^{a}	74.93^{ab}	0.58
	Nutritive values:			
TDN (%)	65.73	68.74	67.63	0.78
SV (%)	56.53^{b}	60.99^{a}	58.22^{ab}	0.64
DCP (%)	7.13	7.67	7.23	0.17

Average in the same row having different superscripts are differ significantly (P≤0.05) for a, b and c.

This may be attributed to accumulation of a large amount of readily fermentable carbohydrate which liberated due to action of cellulolytic enzymes on cellulose and pectin of rations. On the other hand, digestible crude protein (DCP) and total digestible nutrients (TDN) of rations supplemented with cellulolytic enzymes were insignificantly higher than control ration. Digestible crude protein (DCP) was not affected by cellulolytic enzymes rations. Our results are in the same trend with those obtained by Knowlton *et al*. (2002) and Muwalla *et al*. (2007). They mentioned that, apparent protein digestibility was not significantly affected by fibrolytic enzymes treatment.

Lactating goats performance:

Milk yield and its composition: Data of Table (4) showed that, there were no significant (P≤0.05) differences among control and cellulolytic enzymes rations in actual milk yield. Control ration recorded the lowest milk yield**.** Generally, adding Asperozym or phytabex plus® to lactating goats ration increased milk yield and its compositions compared to control ration. Concerning 4% fat corrected milk, there were significant (P≤0.05) increases in fat corrected milk yield and fat percentage of (R1) and (R2) compared to control ration. These results confirmed those obtained by Beauchemin *et al*. (1999) they found that, actual

milk production was not affected significantly (P<0.05) by fibrolytic enzyme supplementation; However production of 4%FCM tended to be higher (P<0.05) for cows fed supplemental enzyme than control cows. These findings may reflect the effect of exogenous enzyme which attributed to the larger amount of fibre digested in the rumen to provide more acetate for fatty acid synthesis. On the other hand, Bowman *et al*. (2002) reported that,despite the increase in total tract feed digestion, the response in milk production was not observed with fibrolytic enzymes supplementation to diets of dairy Holstein cows. Such differences may reflect the effect of animal breed.

Table (4): Effect of cellulolytic enzymes supplemented rations on lactating goat's performance.

Item	Rations			± SE
	Control	R_1	R_2	
Average actual milk yield (g / head / day).	339.44	385	373.3	39
Total milk yield (kg / head 45day) /	15.27	17.33	16.80	2.17
Average 4% Fat corrected milk yield (g /head /day)	275.29^{b}	348.62^{a}	340.82^{a}	33.14
Milk compositions % :				
Total solids	10.84	11.66	11.48	0.93
Fat	2.74^{b}	3.37^{a}	3.42^{a}	0.12
SNF	8.10	8.29	8.06	0.11
Total protein	2.98	3.32	3.03	0.07
Lactose	4.49	4.31	4.38	0.06
Ash	0.63	0.66	0.65	0.01
Average daily feed intake/head:				
DM, kg	0.743	0.756	0.757	0.21
SV, Kg	0.42	0.46	0.44	0.04
TDN, kg	0.49	0.52	0.51	0.11
DCP, g	52.98	57.99	54.73	0.82
Feed conversion*:				
DM/ kg/kg milk	2.70^{a}	2.17^{b}	2.22^{b}	0.31
SV/ kg/kg milk	1.53^{a}	1.32^{b}	1.29^{b}	0.18
TDN/ kg/kg milk	1.78^{a}	1.49^{b}	1.50^{b}	0.23
DCP/ g/g milk	0.19	0.17	0.16	0.08

Average in the same row having different superscripts are differ significantly (P≤0.05) for a and b. Each value is amean of 3 samples., Fed conversion was calculated depend on daily 4% fat corrected milk.*

Feed conversion:Result of daily feed intake in (Table, 4) for lactating goats clearly showed that, no significant differences were found between the tested rations.Peter*a et al*. (2015) investigated that, no effects observed of exogenous fibrolytic enzymes supplementation on Dry matter intake of dairy cows during different stages of lactation .Results of daily feed conversion in Table (4) for lactating goats clearly showed that, feed conversion of DM, SV and TDN of control ration was significantly (P≤ 0.05) decreased compared to (R_1) and (R_2) rations .On the other hand, there were no significant differences were detected in feed conversion of DCP between the tested rations. Also, there were insignificant differences between R1 and R2 regarding feed conversion. Azzaz, (2009) found that, diets supplemented with cellulolytic enzymes efficient for feeding than control diet of lactating zaraibi goats.

Some blood serum parameters:

Effect of the cellulolytic enzymes on serum urea concentration of lactating goats received the tested rations are shown in Table (5). Urea is the principal end product of nitrogen metabolism in ruminants. It is synthesized in the liver and extract in glomerular. The values of serum urea were 30.67, 32.33 and 34 (mg/dl) for control, R1 and R_2, respectively

Serum creatinine of lactating goats received the tested rations are shown in Table (5).). There wereno significantdifferences between the tested rations in serum creatinine. Salem *et al.* (2015) found that, feeding horses a high fiber diet with exogenous fibrolytic enzyme supplementation no effects (P<0.05) were observed for blood creatinine.

Aspartate aminotransferase (AST) of lactating goats received the tested rations are shown in Table (5). There were insignificant differences among the rations in the overall means of serum AST. Such finding indicated that, no side effect was found regarding using the tested cellulolytic enzymes in lactating goats rations. Azzaz *et al*. (2012) found that, insignificant differences (P<0.05) among the rations of lactating goats which contained cellulolytic enzyme compared to the control ration. This finding may suggest the obtained results.

Alanin aminotransferase (ALT) of lactating goats received tested rations are shown in Table (5). There were insignificant differences among all groups in the overall means of serum ALT concentration. Kholif (2006) found that, animals fed on fibrolytic enzymes or fungi treated silage had no significant increase in serum ALT concentration. Such findings indicated that experimental animals were in good health.

Table (5): Effect of cellulolytic enzymes supplemented rations on some blood parameters of lactating goats.

Item	Rations			± SE	Normal range
	Control	R1	R2		
Urea, mg/dl	30.67	32.33	34	0.71	10-50
Creatinine, mg/dl	0.73	0.73	0.70	0.04	0.7-1.5
AST, U/dl	32.44	31.86	32.38	2.39	8-40
ALT, U/dl	26.33	24.36	25	1.15	5-30
Glucose, mg/dl	68.25	69	71.67	3.57	48-76
Cholesterol, (mg/dl)	67.33	69.33	68.67	2.40	65-136

Each value is mean of 3 samples

Serum glucose of lactating goats received the tested rations are shown in Table (5). There were insignificant differences among all groups in the overall means of serum glucose. These results are similar with those obtained by Kholif (2006) who found that animals fed on fibrolytic enzymes or fungi treated silage had no significant increase in serum glucose concentration. Farther, Azzaz *et al.* (2012) found that, cellulases addition to rations of lactating goats was not significantly affected plasma glucose concentration.

Serum total cholesterol of lactating goats received the tested rations are shown in Table (5). There were insignificant differences among all groups in the overall means of serum cholesterol and they within the normal range (65-136 mg dl) as stated by (Boyd, 2011). These results are similar with those obtained by Kholif *et al.*(2012), who found that, animals fed on fibrolytic enzymes had no significant effect in serum cholesterol

Simple economical evaluation of the tested rations:

The economical evaluation of the tested rations fed to lactating goats are presented in Table (6).The best net revenue (L.E/45d / head) was recorded for lactating goats fed ration supplemented with Asperozym (R1) followed by lactating goats fed control ration then lactating goats fed ration supplemented with Phytabex plus®. The cost of feed consumed for lactating goats fed ration supplemented with Phytabexplus® was higher than the other tested rations becauce of the price of commercial enzyme was higher . Azzaz (2009) found that, diets supplemented with cellulolytic enzymes economically better than control diet for feeding lactating zaraibi goats. The supriorety of R1 regarding net revenue may explain the lower cost of produced enzyme (Asperozym) compared to phytabex plus ®.

Table (6): Simple economical evaluation of cellulolytic enzyme supplemented rations of lactatinggoats.

Item	Rations		
	Control	R_1	R_2
Milk yield (kg/head/45d)	15.27	17.33	16.80
Dry matter consumed (kg / head /45d)	33.44	34.02	34.04
Price of one kg DM of the ration, L.E*	2.08	2.28	2.48
Cost of feed consumed (L.E / head / 45d)	69.56	77.57	84.42
Total revenue, L.E*	137.43	155.97	151.2
Net revenue, L.E**	67.87	78.4	66.78
Relative percentage of net revenue	100	115.5	98.39

**, Total revenue, L.E= Milk yield (kg 45day) × 9.0 L.E (price of one kg goats milk (L.E./h/45d)*

***, Net revenue (L.E./h/45d) = Total revenue) - Cost of feed consumed (L.E./h/45d).Head*

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تأثير إستخدام بعض الإنزيمات المحللة للألياف فى العليقة على أداء الماعز الحلابة

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أجريت هذه الدراسة فى مزرعة ومعمل قسم الإنتاج الحيوانى بكلية الزراعة – جامعة الفيوم. هدفت هذه الدراسة إلىمعرفة تأثير إضافة إنزيم تم إنتاجه معمليا ومقارنته بإنزيم تجارىإلى علائق الماعز الحلاب على هضم المواد الغذائية ، إنتاج اللبن ومكوناته ، كفاءة تحويل الغذاء و بعض مكونات سيرم الدم كما أجرى تقييم إقتصادى بسيط للعلائق المختبرة .حيثتم إستخدام تسعة رؤوس منالماعز البلدى الحلاب (تتراوح مواسم الحلابة من الموسم الثانى إلى الرابع بمتوسط وزن ٢٠ ± ١ كجم)بعد مرورعشرينيوم من الولادةوقسمت عشوائيا الى ثلاثة مجموعات كل مجموعة بها ثلاثة حيوانات . المجموعة الأولى تم تغذيتها على ٥٠ % مخلوط علف مركزة و٤٠ % تبن قمح و١٠ % برسيم مصرى (عليقة المقارنة) . المجموعة الثانية (**R1**) تم تغذيتها على عليقة المقارنةمضاف اليها إنزيم الإسبيروزيمالمنتج معمليا بمعدل ١٠٠٠وحدة إنزيمية / كجم مادة جافة بينما المجموعة الثالثة (**R2**) تم تغذيتها على عليقة المقارنةمضاف إليها إنزيم فيتابكس بلسالتجاريبمعدل ١٠٠٠وحدة إنزيمية / كجم مادة جافة . تمت دراسة تأثير إضافة هذه الإنزيمات على آداء الماعز الحلاب . وأوضحت النتائج مايلى:أظهرتالعلائق المضاف إليها كل من الإسبيروزيم و الفيتابكس بلس زيادة معنويةفى معدل هضم المادة الجافة ، المادة العضوية و الألياف الخام بالمقارنة بعليقة المقارنة.. العليقة المضاف اليهاالإسبيروزيم زادت معنويا من معدل هضم المستخلص الخالى من النيتروجين بالمقارنة بعليقة المقارنة.كما أوضحت نتائج العليقة المضاف إليها الإسبيروزيم زيادة معنوية فى معادل النشا بالمقارنة بالعليقة المضاف إليها الفيتابكس بلسوكذلك عليقة المقارنة. لوحظ وجود زيادة معنوية فى الدهن % وكذلك فى محصول اللبن المعدل عند ٤ % دهن للعليقتين (الإسبيروزيم والفيتابكس بلس) بالمقارنة مع عليقة المقارنة. أظهرت عليقة الكونترول أقل قيمة بدرجة معنوية فى كفاءة تحويل الغذاء من حيث المادة الجافة ، المركبات الكلية المهضومة و معادل النشا مقارنة بعليقة الإسبيروزيم وعليقة الفيتابكس بلس . لوحظ عدم وجود إختلافات معنوية فى محتوى سيرم الدم بين جميع العلائق المختبرة لجميع قياسات الدم .

تحسنت القيمة الاقتصادية للماعز المغذى على العليقة المضاف لها الإسبيروزيمالمنتج محليا مقارنة بالماعزالمغذى على العليقة المضاف لهاالفيتابكس بلسالتجاريوكذلك الماعزالمغذى على عليقة المقارنة.

يستنتج من هذه الدراسة ان إنتاج إنزيم الإسبيروزيم محليا يساهم فى خفض تكاليف الإستيراد.كما أن إضافة الإنزيم المنتج محليا والإنزيم التجارى فى علائق الماعز البلدى الحلاب أدت إلى زيادة فى معدلات هضم اغلب العناصر الغذائية فضلا عن أنها حسنت معنوياًمنإنتاج اللبن.