Effect of Diet Supplemented with Cellulase Enzymes on Lactating Goats Performance, and Milk and Cheese Properties

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Abstract: The effects of feeding lactating goats on diet supplemented with two cellulase enzyme preparation on serum parameters, milk production and composition, cheese yield, cheese fatty acid profile and cheese properties were studied. Six Baladi lactating goats, of 3 years 24 ± 0.8 kg body weight, after 42 days of parturition were assigned randomly into three groups of 3×3 Latin Square design with 30 days interval periods. Goats were fed on total mixed ration without enzyme (control) or supplemented with 3.08 U/kg DM Asperozym® (ASP) or supplemented with 1.54 U/kg DM Tomoko® (TOM). Increased ($P < 0.05$) milk yield was obtained with goats fed on ASP or TOM than control. However, no effects ($P > 0.05$) were noted on blood serum parameters, milk composition and properties of resultant soft cheese. Improved cheese content from unsaturated fatty acids with decreased long chain fatty acids was obtained with ASP. From obtained result, it could be concluded that supplementing lactating goats' diet with cellulase enzyme improved milk yield, and cheese fatty acids profile, without adverse effects on health of lactating goats, milk composition and resultant soft cheese properties.
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1. Introduction

In Egypt, there is an acute shortage of conventional feed stuffs for livestock feeding (Khattab *et al*., 2013). The big feed gap between the requirements and the available sources forced the feeding planners and nutritionists to look for non–conventional resources, where there is no competition with human. Such as agricultural by–products are available around the year, but are not efficiently used. The problems of feeding agricultural by–products (lignocellulosic materials) to farm animals are in general, low protein content, high crud fiber, low digestibility coefficients and containing of anti-nutrients factors such as tannins and alkaloids (Kholif *et al*., 2005; Khattab *et al*., 2011; Alsersy *et al*., 2015). Thus, to increase digestibility of these lignocellulosic materials, it is important to destroy the linkage between cellulose, hemicellulose and lignin or destroy the compact nature of the tissue. There have been attempts to do that by mechanical, chemical or biological treatments (Abd El-Tawab *et al*., 2015; Abdel-Aziz *et al*., 2015; Alsersy *et al*., 2015; Togtokhbayar *et al*., 2015) and feed additives (Azzaz *et al*., 2013; Elghandour *et al*., 2014; Morsy *et al*., 2014).

Cellulose biodegradation by cellulases, produced by numerous microorganisms is very important in several agricultural byproduct treatment processes (Salem *et al*., 2015a,b; Valdes *et al*., 2015). Enzymes supplementation to ration is often accompanied by increased feed intake, which may partly be due to increased palatability of the diet due to sugars released by pre-ingestive fiber hydrolysis (Khattab *et al*., 2011; Salem *et al*., 2013). However, post-ingestive enzyme effects, such as increased digestion rate and extent of digestion, may increase hydrolytic activity in the rumen to reduce gut fill and enhance feed intake (Krueger *et al*., 2008; Gado and Salem, 2009). Direct-fed enzymes can also enhance microbial colonization of feed by increasing numbers of ruminal fibrolytic microbes (Morgavi *et al*., 2000 and Nsereko *et al*., 2000; Elghandour *et al*., 2015) to increase rate of degradation of fiber in the rumen (Giraldo *et al*., 2008), rumen microbial protein synthesis (Yang *et al*., 1999 and Nsereko *et al*., 2002) and for rumen digestibility. Positive effects of adding exogenous enzymes to ruminant diets have been reported for lactating dairy cows and growing cattle (Khattab *et al*., 2011; Salem *et al*., 2013). Dairy cows fed forage treated with a fibrolytic enzyme additive consumed more feed and produced 5–25% more milk (Stella *et al*., 2007 and Gado *et al*., 2009), improved the energy balance of transition dairy cows and increased milk production in small ruminants (Stella *et al*., 2007; Kholif and Aziz, 2014). A commercial exogenous enzyme mixture (ZADO®), prepared from anaerobic bacterium, has been shown to improve ruminal fermentation, N balance and nutrient digestibility (Gado *et al*., 2007), daily gain (Valdes *et al*., 2015) as well as milk yield (Gado *et al*., 2009; Khattab *et al*., 2011; Salem *et al*., 2013). Abd El-Aziz *et al.* (2012) found that diet supplemented with fibrolytic enzymes had no effect on milk yield and composition for lactating buffalos.

The research on the effect of cellulose enzymes on the white cheese yield, composition and cheese sensory evaluation are rare. Therefore, the objective of this study was to determine effects of two cellulase enzyme preparations Asperozym® and Tomoko® on blood serum parameters, milk production and composition, fatty acid profile, soft cheese yield, and cheese properties of lactating goats in mid-lactation.

2. Materials and methods

2.1. Enzyme preparation

Two enzyme products were used in the current study; Tomoko® and Asperozym®. Tomoko® is a commercial enzymes source of Biogenkoji Research Institute – Japan was used. The enzyme product was made from *Aspergillus Awamori* (3×10^6 cells/g) including 1000 unit/g of acidic protease, 30 unit/g of pectinase, 25 unit/g of xylanase, 20 unit/g of α-amylase, 10 unit/g of phytase, 5 unit/g of glucoamylase and 4 unit/g of cellulase as provided from the manufacture. Asperozym® is a local enzyme produced from *Asperigillus niger* in Dairy Animal Production Lab., Dairy Science Department, National Research Centre, with 770 unit of cellulose per kg.

2.2. Experimental animals and management

The present study was conducted at a private experimental farm in Om Dinar, Embaba, Giza, Egypt. Six lactating Baladi goats of 3 years 24 ± 0.8 kg body weight. After 42 days of parturition, goats were assigned randomly into three groups of two animals each using 3×3 Latin square design with 30 days interval periods. Goats were individually fed according to NRC (1981) recommendations plus a 15% margin. The used total mixed ration (TMR) was composed from concentrate: grounded date stone: berseem clover at 50: 10: 40 on DM basis. The concentrate feed mixture, grounded date stone and berseem clover chemical composition is shown in Table 1. Goats were fed on the TMR without enzymes (control), or with 3.08 U/kg DM Asperozym® (ASP) or with 1.54 U/kg DM Tomoko® (TOM). Goats were offered the TMR two times daily at 0800, 1600 h. The enzymes were introduced to each animal with concentrates before feeding. Dry matter intake was measured during the last 5 days of each period. Water was available continuously.

2.3. Blood sampling

At the last day of each experimental period, 10 ml of samples was collected after 4 h post morning feeding in heparinized plastic tubes and then centrifuged at 3000 rpm for 20 min (IEC centra-4R, International Equipment Co., USA). The plasma were separated at once by long pastier pipette, and stored at -20°C for further analysis. Total protein, albumin, globulin alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, glucose, triglyceides and cholesterol were determined according to the instruction manual of manufacture (Biodiagnostic, Dokki, Giza, Egypt).

2.4. Sampling and analysis of milk

Goats were milked twice daily at 0900 and 1700 h during the last three days (i.e. d-28, d-29, d-30) of each experimental period. Milk samples were pooled within goat, relative to production, to obtain one composite milk sample per goat and frozen at −20°C until analyzed for milk fatty acids profile and soft cheese manufacture. Milk samples were stored at 4°C with a preservative (bronopol-B2) until analyzed for total solids, proteins fat, ash and lactose.

2.5. Cheese manufacturing

The collected milk from each group was pooled and used for analysis and manufacturing of soft cheese according to Fahmi and Sharara (1950). All milk batches were heated to 75°C and then cooled to 38°C. The mixed starter culture (1% w/w) of *Lactococcus lactis* ssp. *Lactis and Lactococcus lactis* ssp. *Cremoris* (Chr. Hansen's Lab., A/S Copenhagen, Denmark) and salt (4%w/w) were added to cheese milk, and appropriate amount of rennet was added to achieve coagulation in 120 min. After coagulation, curd was cut and transferred to mould and left to rest overnight at room temperature. Cheese blocks were cut, weighted and analyzed.

2.6. Chemical analysis

Total solids, fat, total protein (N×6.38) and ash content of both milk and soft cheese were determined according to AOAC (2007) respectively. Milk lactose contents were calculated. pH values were measured using a digital pH meter (HANNA, instrument, Italy).

2.7. Cheese yield and recovery

Cheese yield was calculated as the weight of finished cheese divided by the weight of milk used. The recovery percentage of fat and protein were calculated according to Mahran *et al.* (1999).

2.8. Sensory evaluation of cheese

Cheese samples were judged by a panel taste of 15 staff members of the Dairy Science Department, National Research Center, Egypt. The cheese was scored for appearance (15 points), body and texture (35 points) and flavor (50 points) as suggested by ADSA (1987).

2.9. Statistical analysis:

Data obtained from this study were statistically analyzed by SAS (2004) according to procedures outlined by Snedecor and Cochran (1982). The Duncan's multiple range tests were used for data of the experiment to test the significance among means.

3. Results and discussion

3.1. Plasma parameters

Goats fed on ASP and TOM showed a slight increase in plasma globulin, ALT, AST and glucose ($P > 0.05$), with decreased plasma albumin, albumin/globulin ratio, cholesterol and triglycerides ($P > 0.05$) concentrations. However, no changes in plasma total proteins among all treatments (Table 2). The results of blood plasma parameters are in line with that obtained by Farahat *et al.* (2007).

3.2. Milk yield and composition

Increased milk yield (g/d) ($P < 0.05$) were obtained with ASP and TOM. However, no differences ($P > 0.05$) were noted in milk yield between ASP and TOM (Table 3). A similar results were obtained by Titi and Lubbadeh (2004) and Khattab *et al.* (2011) when the enzymes were fed to goats and sheep. Gado *et al.* (2009) reported a 7-15% higher in milk production of dairy cows fed fibrolytic enzymes. They explained the improvement as being due to increased nutrient digestibility and microbial protein synthesis. Alsersy *et al.* (2015) reported the use of enzyme additives improved efficiency of microbial protein synthesis is a result of enzyme action on the forage structural polysaccharides, altering the rate of ruminal degradation of structural carbohydrates and the provision of a suitable ruminally degradable nitrogen source. Moreover, Valdes *et al.* (2015) reported that the supplementation of exogenous enzymes (10 g/day), improved their DM and OM digestibility with a positive impact on the use of N and microbial protein synthesis.

The same trend was observed in the daily production of total solids, fat, protein, lactose, ash and solids not fat, which were significantly higher in ASP and TOM than in C, reflecting the higher milk yields. However, there was no significant difference in milk composition (%) produced by ASP, TOM and control ($P > 0.05$). These results are in line with the study of Titi and Lubbadeh, (2004) when reported no effect of feeding enzyme to dairy goats on the concentration of all milk components. From these observations, we hypothesized that the supplementing goat's diets with cellulase enzymes had no effect on soft cheese yield and composition.

3.3. Cheese yield, composition and sensory evaluation

Decreased soft cheese yields, with no significant differences ($P > 0.05$), were obtained with ASP and TOM, than those produced from control (Table 5). Moreover, slight decreased ($P > 0.05$) protein recovery was observed in soft cheese produced from ASP *versus* TOM and control. Inversely, slight increases ($P > 0.05$) in total solids, fat and proteins contents were observed for soft cheese produce from both ASP and TOM compared with that produced from control (Table 5). In the same time, no much difference ($P > 0.05$) was observed in appearance, flavor, and total scores among all treatments (Table 6). In addition, no significant difference was found in body and texture of resultant cheese, even if body and texture score was numerically higher in cheese produced from both ASP and TOM than in that produced from control.

The insignificant differences in soft cheese yield and cheese composition and its sensory evaluation between treatments are normal, which agreed with the results of Kholif *et al.* (2009, 2010). This insignificant difference between groups in this matter may be due to the insignificant differences between groups concerning milk composition used in manufacturing of soft cheese in this experiment. The body and texture score may be correlated with high total solids and protein content obtained in the current study.

3.4. Fatty acids profile in cheese

Cheese fat produced from ASP had increased content from short chain fatty acids (SCFA), medium chain fatty acids (MCFA) and unsaturated chain fatty acids (USFA), but lower in long chain fatty acids (LCFA) and saturated chain fatty acids (SFA) compared with control. Individually, cheese fatty acids from ASP was increased in C4, C10, C12:1, C14:1 and C18:2n6cis, but decreased in C14, C18:2n6trans and C18:3n3 compared with control; the difference being significant only with C18:3n3 ($P < 0.05$). These results confirm that the effect of cellulolytic enzymes on the diets, which enhance digestibility and metabolism (Alsersy *et al.*, 2015; Valdes *et al.*, 2015). Also, cheese fat from TOM showed lowered C_{14} and C_{18}, with increased C_{10} and SCFA compared to control. However, other fatty acids were similar among treatments. A similar observation was found by Abd El-Aziz *et al.* (2012) in milk fat produced from lactating buffaloes fed different rations supplemented with fibrolytic enzymes.

Table 1. Chemical analysis of feed ingredients (%, on dry matter basis).

Item	CFM[1]	Berseem clover	Date stone
Dry matter	89.7	19.3	91.7
Chemical composition			
Organic matter	89.2	89.2	95.5
Crud protein	13.7	12.1	3.00
Ether extract	2.70	3.40	7.60
Crud fiber	11.3	34.3	28.8
Nitrogen free extract	61.5	39.4	56.1

[1]CFM: concentrate feed mixture consisted of 50% corn, 25% cotton seed meal, 22% wheat bran, 2% limestone and 1% NaCl

Table 2. Blood plasma parameters concentrations of lactating goats fed diet unsupplemented or supplemented with Asperozym® or Tomoko® (n=6).

Plasma parameter	Diets[1]				
	Control	ASP	TOM	SEM	*P*-value
Total protein (g/dL)	10.62	10.70	10.17	0.69	ns
Albumin (g/dL)	6.07	5.26	5.35	0.36	ns
Globulin (g/dL)	4.55	5.44	4.82	0.56	ns
Albumin/globulin ratio	1.42	1.01	1.21	0.28	ns
Urea (mg/dL)	36.25	37.74	37.60	1.25	ns
ALT (IU/L)	67.16	69.96	69.99	9.46	ns
AST (IU/L)	50.79	59.55	65.72	12.27	ns
Glucose (mg/dL)	90.20	93.13	99.20	9.36	ns
Cholesterol (mg/dL)	196.1	181.7	195.1	12.32	ns
Triglycerides (mg/dL)	229.5	199.5	213.6	22.19	ns
Creatinine (mg/dL)	1.03	1.05	1.04	0.02	ns

[1]Control, goats fed on TMR; ASP, goats fed TMR supplemented with Asperozym® (3.08 U/kg); TOM, goats fed on TMR supplemented with Tomoko® (1.54 U/kg).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; ns, not significant.

Table 3. Milk yield (g/d) and composition (%) of lactating goats fed diet unsupplemented or supplemented with Asperozym® or Tomoko® (n=6).

Item	Diets[1]				
	Control	ASP	TOM	SEM	*P*-value
Yield					
Milk	989^{b}	1134^{a}	1165^{a}	48.0	*
Total solids	122.7^{b}	145.3^{a}	145.2^{a}	6.30	*
Fat	36.21	40.28	40.01	1.21	ns
Protein	35.03	40.55	39.29	1.52	ns
Lactose	42.41	50.22	53.57	1.40	ns
SNF	86.50	104.98	105.23	2.39	ns
Ash	10.67	12.24	12.70	0.22	ns
Milk composition					
Total solids	12.41	12.81	12.46	0.68	ns
Fat	3.66	3.55	3.43	0.12	ns
Protein	3.54	3.58	3.37	0.15	ns
Lactose	4.29	4.43	4.60	0.14	ns
SNF	8.75	9.26	9.03	0.23	ns
Ash	1.08	1.08	1.09	0.02	ns

[1]Control, goats fed on TMR; ASP, goats fed TMR supplemented with Asperozym® (3.08 U/kg); TOM, goats fed on TMR supplemented with Tomoko® (1.54 U/kg).
SNF, solids not fat; ns, not significant; *, significant at 5% level.

Table 4. Cheese yield and composition (%) of lactating goats fed diet unsupplemented or supplemented with Asperozym® or Tomoko® as cellulase enzymes.

Items	Diet[1]				
	Control	ASP	TOM	SEM	*P*-value
Cheese yield	28.17	26.48	26.12	1.32	ns
Fat recovery	92.64	93.44	94.09	2.17	ns
Protein Recovery	87.17	85.51	88.49	3.04	ns
Cheese composition					
Total solids	31.05	32.05	32.57	1.02	ns
Fat	12.08	12.50	13.00	0.52	ns
Protein	10.97	11.48	11.51	0.46	ns
Lactose	3.55	3.66	3.69	0.33	ns
Ash	4.45	4.41	4.37	0.28	ns
pH	6.51	6.55	6.51	0.03	ns

[1]Control, goats fed on TMR; ASP, goats fed TMR supplemented with Asperozym® (3.08 U/kg); TOM, goats fed on TMR supplemented with Tomoko® (1.54 U/kg).
ns, not significant

Table 5. Fatty acids profile (g/100 g total fatty acids) of milk cheese fat of lactating goats fed diet unsupplemented or supplemented with Asperozym® or Tomoko®.

Fatty acids	Diet[1]				
	Control	ASP	TOM	SEM	*P*-value
C4:0	1.04	1.21	1.00	0.38	ns
C6:0	1.69	1.93	1.79	0.51	ns
C8:0	1.92	2.37	2.94	1.43	ns
C10:0	8.33	10.25	9.75	4.29	ns
C12:0	2.83	3.49	2.95	1.22	ns
C14:0	8.06	6.83	6.71	2.75	ns
C14:1	0.33	1.88	0.16	1.70	ns
C15:0	0.78	0.39	0.72	0.20	ns
C16:0	25.75	23.25	25.57	4.13	ns
C16:1	1.21	1.00	0.95	0.86	ns
C17:0	0.79	0.31	0.98	0.64	ns
C18:0	19.78	18.15	17.78	7.51	ns
C18:1n9 trans	22.04	24.06	23.73	5.49	ns
C18:1n9 cis	3.50	3.37	3.56	0.78	ns
C18:2 n6 trans	0.62	0.25	0.32	0.75	ns
C18:2 n6 cis	0.76	1.26	0.76	0.37	ns
C18:3 n6	0.30	0.45	0.12	0.36	ns
C18:3 n3	0.23^{ab}	0.16^{b}	0.25^{a}	0.04	*
C20:0	0.13	0.12	0.14	0.02	ns
SCFAs C4-8	4.64	5.51	5.73	1.98	ns
MCFAs C10-15	19.54	22.45	19.57	7.57	ns
LCFAs C16-20	75.86	72.59	74.86	8.36	ns
SFAs	71.07	68.13	70.33	7.37	ns
USFAs	28.97	32.42	29.97	7.53	ns

[1]Control, goats fed on TMR; ASP, goats fed TMR supplemented with Asperozym® (3.08 U/kg); TOM, goats fed on TMR supplemented with Tomoko® (1.54 U/kg).
SCFAs, short chain fatty acids; MCFAs, medium chain fatty acids; LCFAs, long chain fatty acids; SFAs, saturated chain fatty acids; USFAs, unsaturated chain fatty acids, NS, not significant; *, significant at 5% level.

Table 6. Sensory evaluation of soft cheese of lactating goats fed diet unsupplemented or supplemented with Asperozym® or Tomoko® as cellulase enzymes

Items	Diets[1]				
	Control	ASP	TOM	SEM	*P*-value
Appearance	8.70	8.42	9.03	0.18	ns
Flavor	44.43	43.58	43.75	0.36	ns
Body and texture	36.60	38.02	38.12	0.73	ns
Total Score	89.73	90.2	90.90	0.91	ns

[1]Control, goats fed on TMR; ASP, goats fed TMR supplemented with Asperozym® (3.08 U/kg); TOM, goats fed on TMR supplemented with Tomoko® (1.54 U/kg).
NS, not significant.

4. Conclusion

Results showed that feeding lactating Baladi goats on diet with supplemented with cellulase enzyme improved milk yield, and cheese fatty acids profile. However, no effects were obtained on milk composition and resultant soft cheese properties. Moreover, goats health was not adversely affected due to enzymes supplementation.

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