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Research Article
Effect of Cellulase and Tannase Enzymes Supplemention on the Productive Performance of Lactating Buffaloes Fed Diets Contain Date Palm Fronds

[1]A.M. Abd El Tawab, [1]M.S.A. Khattab, [2]H.M. El-Zaiat, [1]O.H. Matloup, [3]A.A. Hassan, [1]M.M. Abdou, [3]M.S. Khalel and [3]M.H. Yacout

[1]Department of Dairy Science, National Research Center, Dokki, Giza, Egypt
[2]Department of Animal Production, Faculty of Agriculture, Alexandria University, Alexandria, Egypt
[3]Animal Production Research Institute, Ministry of Agriculture, Dokki, Giza, Egypt

Abstract

Objective: This study was carried out to investigate the effect of supplementing diets containing date palm fronds with cellulase and tannase enzymes. **Materials and Methods:** Fifteen lactating buffaloes were randomly assigned into three groups (five animals each) using complete random design and the experimental diets were T1 group fed 50% (CFM), 30% Egyptian clover and 20% rice straw, T2 group fed 50% (CFM), 20% clover, 15% rice straw and 15% date palm fronts and T3 group fed T2 diet plus 4 g kg^{-1} DM of enzymes. **Results:** The results showed that DMI decreased in T2 compared with T1 and T3. However, enzymes supplementation significantly ($p<0.05$) increased DM, CP, EE, NFE, NDF, ADF and cellulose digestibility compared with T2. Also, T3 decreased ($p<0.05$) CP, CF, ADF and cellulose digestibility compared with T1. Blood plasma of animals fed T2 diets recorded the lowest ($p<0.05$) values for glucose, total protein and globulin compared with other groups. But, T2 group was insignificantly ($p>0.05$) decreased for albumin and urea compared with T3 group. While, there were no significant ($p>0.05$) differences for cholesterol, AST and ALT among groups. Milk yield and energy corrected milk were significantly ($p<0.05$) increased with enzymes supplementation to diet (T3) compared with T2. The increases of milk yield and energy corrected milk were 6.24 and 2.58%, respectively for T3 compared with T1. While, T2 decreased milk yield and energy corrected milk by 12.61 and 20.31% compared with T1. There were no differences ($p>0.05$) between experimental treatments in total solids. While, there were significant ($p<0.05$) decrease in fat and protein between T2 and other groups. Solids not fat and ash were significant ($p<0.05$) increase with T2 and other groups. **Conclusion:** It could be concluded that cellulase and tannase enzymes supplementation to diet could enhance the performance of lactating buffaloes.

Key words: Date palm fronds, cellulase enzyme, tannase enzyme, diet, nutrients digestibility, milk production, milk composition, lactating buffalo

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Corresponding Author: A.M. Abd El Tawab, Department of Dairy Science, National Research Centre, P.O. Box 12622, Dokki, Giza, Egypt
Tel: 20201111947046 Fax: 20233370931

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

Date palm fronds are one of agricultural by-product which can be used for livestock. Moreover, date palm fronds have been used as feedstuff in diets[1,2]. El Hag and Al Shargi[3] found that chopped date palm fronds palatable for ruminants. Also, Mahgoub *et al.*[4] reported that used ground date palm fronds success as a component of concentrate formulated and used for sheep feeding. Problems of feeding agricultural by-products by ruminants are regarded to its low protein, high content of cellulytic components, lower nutrients digestibility coefficients and high content of crude fiber such as cellulose and anti-nutrients factors such as tannins[5]. Cellulase enzyme can be effective additive with agricultural by-product to produce simple glucose units[6]. Sujani and Seresinhe[7] found that fibrolytic enzymes play a direct role in animals feeding by improved digestion in ruminants. Moreover, Nussio *et al.*[8] reported that adding enzyme mixture just before to feeding was effective as forage treatment than adding by 1-3 days before feeding. On the other hand, tannase enzyme can hydrolyzes tannins substrates such as tannic acid, propyl gallate, methyl gallate, epicatechin gallate, digallic acid and epigallocatechin gallate releasing gallic acid[9,10]. Abd El Tawab *et al.*[11] suggested that using tannase enzyme as a feed additive for lactating goats' diets decreased tannins contents and improved nutrients digestibility, milk yield. The objectives of this study were to investigate the effect of using cellulase and tannase enzymes on degradation of cellulose and tannins of diets and its effect on feed intake, nutrients digestibility, nutritive values and blood plasma metabolites and milk yield and composition of lactating Egyptian buffalos.

MATERIALS AND METHODS

Enzyme sources: Cellulase and tannase enzymes produced from anaerobic bacteria (*Clostridium butyricum*). Each g of enzyme mixture contains 5179 IU g^{-1} of cellulase and 866 IU g^{-1} of tannase was used.

Animals and diets: Fifteen lactating Egyptian buffaloes at 3rd-5th season of lactation and weighed (550±35 kg) were divided randomly assigned into three groups (five animals each) using complete random design. The experimental periods were extended for 90 days started 2 week after parturition and consisted of four equal periods. Buffaloes were individually fed amounts equal 3% of live body weight on experimental diets which consisted of 50:50 concentrate: Roughage ratio (on DM basis). The T1 group fed on 50% CFM, 30% Egyptian clover and 20% rice straw, T2 group fed on 50% CFM, 20% clover, 15% rice straw and 15% date palm fronts and T3 group fed T2 diet plus 4 g kg^{-1}DM of enzymes. Buffaloes were fed twice daily at 08:00 and 16:00 h. Dry matter intake recorded during the last seven days of experimental period. The fresh water was always available to animals.

Feed and fecal samples analysis: Samples of ingredients and rations were analyzed for Dry Matter (DM), ash, Crude Fiber (CF), Organic Matter (OM) and Ether Extract (EE) according to method of AOAC[12]. While Nitrogen Free Extract (NFE) was calculated by difference. The Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) contents were determined using the methods described by Van Soest *et al.*[13]. Total tannins were determined according to Hagerman and Butler[14].

Determination of nutrients digestion coefficients: Grab sample method was applied for determining the nutritive value and digestibility in which Acid Insoluble Ash (AIA) was used as an internal marker according to Khattab *et al.*[15]. Feces grab samples were collected manually at 07.00 for three successive days at the first three days of collection periods for each experiment. Representative feces samples were sprayed by 10% sulfuric acid solution and formalin, then dried in oven at 70°C for 24 h. The fresh feces samples for each animal were mixed well by equal weights then ground and stored in polyethylene bags for chemical analysis of DM, OM, CP, CF, NDF, ADF and ash.

Sampling and analysis of blood plasma: Blood samples were taken from each animal at the last day three of each experimental period. The samples were taken before morning feeding and at 4 h. post feeding. Each blood sample was withdrawn from the jugular vein into a heparinized tube and centrifuged at 4000 rpm/15 min, then blood plasma was separated into a clean dried glass vial and stored in deep freezer at -18°C for further analysis. Plasma glucose, total protein, albumin, cholesterol, urea, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by spectrophotometric ally measured (T80 UV/Vis spectrometer, PG Instruments Ltd., UK) according to the standard protocols of the suppliers.

Sampling and analysis of milk: The animals were hand milked twice daily at 06.00 and 18.00 during the last three days of each experimental period. Samples of milk were collected from each animal at morning and evening milking immediately. The representative sample of each animal was a

mixed sample of constant percentage of the evening and morning yield for total chemical analysis. Milk samples were analyzed for total solids, solids not fat, total protein, fat and ash using infrared spectroscopy (Bentley 150, Infrared Milk Analyzer, Bentley Instruments, USA). Energy Corrected Milk (ECM) was calculated according to Sjaunja *et al.*[16] as:

$$ECM\ (kg\ day^{-1}) = \frac{\text{Milk yield (kg day}^{-1}\text{)}\times[38.3\times\text{fat (g kg}^{-1}\text{)}+24.2\times \text{protein (g kg}^{-1}\text{)}+16.54\times\text{lactose (g kg}^{-1}\text{)}+20.7]}{3140}$$

Statistical analysis: Data were statistically analyzed according to a completely randomized design 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

The chemical compositions of the experimental diets are presented in Table 1. Results showed that inclusion of date palm fronds in diet decreased dry matter intake in T2 compared with T1. While adding enzymes in T3 increased DMI (Table 2).

Table 1: Chemical composition of diet

Parameters	CFM	Rice straw	Clover	Palm fronds
DM	89.58	90.44	19.63	87.74
OM	95.30	91.23	94.69	94.05
CP	15.84	3.73	12.75	4.12
CF	6.82	39.61	24.81	38.77
EE	2.76	1.07	1.17	1.89
NFE	69.88	46.82	55.96	49.27
NDF	31.64	74.79	42.73	72.38
ADF	20.49	50.51	25.37	52.31
ADL	5.38	13.68	6.85	14.55
Hemicellulose	11.15	24.28	17.36	20.07
Cellulose	15.11	36.83	18.52	37.76
Ash	4.71	8.77	5.31	5.95

Nutrients digestion coefficients: Effect of experimental treatments on nutrients digestibility showed in Table 2, generally, T2 group recorded the lowest (p<0.05) values for DM, CP, EE, NFE, NDF, ADF and cellulose digestibility compared with T1. Also, T3 decreased (p<0.05) CP, CF, ADF and cellulose digestibility compared with T1. Enhancing in nutrients digestibilities related to improve cellulase digestibility and reduce effect of tannins content by adding cellulase and tannase enzymes. These results agreed withprevious studies[5,17-22] who found that adding fibrolytic enzymes to animals diets led to a significant (p<0.05) increase of nutrients digestibility. Increasing fiber digestion by adding cellulase enzyme by many mechanisms byenhancing the rate of ruminal digestible fiber[17], adjustment in ruminal fermentation[23] and reducing digest viscosity[24]. It also improves colonization of ruminal bacterial cells and attachment with plant cell wall[25,26]. Wang *et al.*[25] and Khattab *et al.*[27] suggested that adding enzymes increased numbers of non-fibrolytic and fibrolytic bacteria which could reflected as an increase of microbial biomass which would provide polysaccharidase activity to digest feedstuffs. On the other hand, Makkar[28] reported that tannins compounds reduce protein degradability protein and other nutrients. Negative effect of tannins and phenolics compounds on digestibility has been related to their toxic effects on rumen microbes[29]. Abd El Tawab *et al.*[11] suggested that adding tannase enzyme to reducing tannins effect of dairy goats diets resulted to a significant increase in nutrients digestibility.

Blood metabolites: Effect of experimental treatments on blood metabolites are showed in Table 3, it was affected by addition of enzymes for Egyptian buffaloes diets. Generally, T2 group recorded the lowest (p<0.05) values for glucose, total protein and globulin compared with other groups. But, T2 group was insignificant (p>0.05) decrease for albumen and urea compared with T3 group. While, there were no significant (p>0.05) differences for cholesterol, AST and ALT among

Table 2: Effect of experimental treatments on nutrients digestibility

Parameters	Control	Palm fronds	Palm fronds+enzymes
Dry matter intake (kg h^{-1} day^{-1})			
Concentrate	5.00	5.00	5.00
Roughage	5.14	4.97	5.33
Nutrients digestibility (%)			
DM	65.83±0.315^{a}	57.05±0.695^{b}	64.26±0.354^{a}
CP	67.67±0.259^{a}	60.48±0.407^{c}	65.64±0.429^{b}
CF	59.49±0.505^{a}	53.61±0.649^{c}	56.81±0.531^{b}
EE	74.71±0.657^{a}	72.23±0.830^{b}	73.70±0.325^{ab}
NFE	65.56±0.274^{a}	62.99±0.531^{b}	65.19±0.353^{a}
NDF	61.92±0.545^{a}	56.76±0.793^{b}	60.28±0.401^{a}
ADF	59.17±0.467^{a}	53.37±0.426^{c}	56.81±0.421^{b}
Cellulose	62.29±0.166^{a}	56.95±0.556^{c}	60.00±0.350^{b}

^{a,b,c}Means with different in the same row are significant (p<0.05)

Table 3: Effect of experimental treatments on blood metabolites

Parameters	Control	Palm fronds	Palm fronds+enzymes
Glucose (mg dL^{-1})	85.62±0.355[a]	79.16±0.177[b]	85.15±0.780[a]
Total protein (TP) (g dL^{-1})	8.21±0.116[a]	6.49±0.260[b]	7.90±0.105[a]
Albumin (A) (g dL^{-1})	4.36±0.136[a]	3.97±0.055[b]	4.01±0.092[b]
Globulin (G) (g dL^{-1})	3.86±0.034[a]	2.52±0.205[b]	3.88±0.023[a]
Urea (mg dL^{-1})	42.17±0.381[a]	39.76±0.453[b]	41.11±0.345[ab]
Cholesterol (mg dL^{-1})	94.33±0.668[a]	91.40±1.093[a]	92.69±0.609[a]
GOT (U L^{-1})	33.00±0.577[a]	32.00±0.881[a]	32.00±0.333[a]
GPT (U L^{-1})	20.00±0.333[a]	20.00±0.333[a]	21.00±0.011[a]

[a,b,c]Means with different in the same row are significant ($p<0.05$)

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

Parameters	Control	Palm fronds	Palm fronds+enzymes
Milk yield (kg day^{-1})	7.85±0.269[a]	6.86±0.271[b]	8.34±0.230[a]
Energy corrected milk (kg day^{-1})	6.99±0.251[a]	5.57±0.224[b]	7.17±0.236[a]
Total solids	16.51±0.105[a]	16.49±0.095[a]	16.60±0.072[a]
Fat	6.65±0.032[a]	5.95±0.034[c]	6.43±0.069[b]
Protein	4.18±0.020[a]	3.96±0.028[c]	4.11±0.022[b]
Solids not fat	9.86±0.089[c]	10.54±0.079[a]	10.17±0.040[b]
Ash	0.79±0.007[b]	0.81±0.006[a]	0.79±0.006[b]

[a,b,c]Means with different in the same row are significant ($p<0.05$)

groups. Positive influence in T3 group compared with T2group related to enzymes supplementations. Enhancing in total protein may be related to improvising occurred in metabolic process as a response to cellulase and tannase enzymes additive and indicate that these buffaloes cover their protein needs. These results were agreed with Abd El Tawab *et al.*[11] who found that increase in plasma total protein in goats fed diets with tannase supplementation. Decrease ($p>0.05$) of plasma albumin for T2 may be due to the lowest crude protein digestibility, These results are in agreement with earlier studies[11,27,30]. Plasma glucose concentration was lowest ($p>0.05$) in T2 compared with T1 and T3. These results are agreed with other studies[11,22,31]. The decrease of plasma urea for T2 compared with T1 and T3. These results in agreement with previous studies[11,27,30] who found that serum urea concentration increased with enzymes supplementation compared with control. Also other researchers[32-34] found that reduced blood urea concentration in goats fed diets containing tannins. Plasma AST and ALT activity were in normal range[35] which is a good indicator for normal liver cells activity. Many studies found that AST and ALT activities were not affected by enzymes supplementation[11,27,30].

Milk yield and composition: Effect of experimental treatments on milk yield and composition are summarized in Table 4. Milk yield and energy corrected milk were significantly ($p<0.05$) increased with enzymes supplementation to diet (T3) compared with T2. The increases of milk yield and energy corrected milk were 6.24 and 2.58%, respectively for T3 compared with T1. While, T2 decreased milk yield and energy corrected milk by 12.61 and 20.31% compared with T1. These results are in agreement with Clark *et al.*[36] who found a positive relationship between blood glucose contents and milk yield. These enhancing may be due to improve of nutrients digestibilities of T1 and T3. Other studies[22,27,30] found that adding fibrolytic enzymes to animal's diets caused an increase in milk yield also, tannase enzyme supplementation in dairy goat's diets improved milk yield[11]. There were no differences ($p>0.05$) between experimental treatments in total solids. While, there were significant ($p<0.05$) decrease in fat and protein between T2 and other groups. These results are in agreements with previous experiments[11,27]. While, there were significant ($p<0.05$) increase sold not fat and ash T2 and other groups.

CONCLUSION

The results of the study showed that adding cellulase and tannase enzymes to lactating buffaloes diets containing palm date fronds led to a significant increase in nutrients digestibility and milk yield and improving animal's performance without any adverse effect on animal healthy.

SIGNIFICANT STATEMENTS

- This study was carried out to investigate adding mixture of cellulase and tannase enzymes to lactating buffaloes' diet and its effect on the milk production
- The experimental diets had no negative effect on animal health status
- Milk production were significant impacted by adding mixture of cellulase and tannase enzymes to the diets compared with using palm fronds in the diet

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