A NEW PECTINASE PRODUCED FROM *ASPERGILLUS TERREUS* COMPARED WITH A COMMERCIAL PECTINASE ENHANCED FEED DIGESTION, MILK PRODUCTION AND MILK FATTY ACID PROFILE OF DAMASCUS GOATS FED PECTIN-RICH DIET

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Abstract

Pectinase hydrolyses pectin and increases the utilization of agriculture byproducts as feeds for ruminants. A newly developed pectinase from *Aspergillus terreus* was compared with a commercially available pectinase at 600 IU/kg feed on feed utilization and lactational performance of Damascus goat fed orange byproduct silage and sugar beet pulp based diet (i.e., pectin-rich diet) for 12 weeks. Thirty (one week postpartum) Damascus goats stratified by previous milk production, body weight and parity were divided into three experimental groups. Does were fed a control diet containing concentrates, orange byproduct silage, sugar beet pulp and wheat straw at 50:20:20:10, respectively without a supplement (control treatment) or supplemented with a newly developed pectinase (new treatment) or commercial pectinase (commercial treatment). With similar feed intake, the new pectinase increased ($P<0.01$) nutrient digestibility and milk production efficiency more than the other treatments. Out of all the blood parameters, only serum glucose was affected by the treatments with the highest ($P=0.025$) value noted for the new pectinase. Similarly, the new pectinase increased daily milk production ($P<0.005$) and the concentrations of milk components compared to the other two treatments. Additionally, pectinase (both the commercial and new) inclusion increased ($P<0.05$) the concentrations of total conjugated linoleic acid and unsaturated/saturated fatty acids ratio, and decreased atherogenic index ($P=0.01$) compared with control treatment. It is concluded that the supplementation of the diet of lactating goats with pectinase at 600 IU/kg feed will enhance feed digestion and milk production. The newly developed pectinase performed better than the commercial pectinase.

Key words: exogenous enzymes, feed utilization, lactating goat, pectin, pectinase

There is an increasing interest in using agro-byproducts as alternative feeds for ruminants to minimize cost and reduce environmental pollution (Khatab et al., 2013; Kholif et al., 2017 b). Moreover, using byproducts as feeds diminishes dependence of livestock on grains that is also consumed by humans (Kholif et al., 2014; Olafadehan et al., 2020).

Orange pulp and sugar beet pulp are two examples of byproduct that can be used in feeding animals (Abd El Tawab et al., 2020). The ruminal microbiota can effectively derive nutrients from these structural carbohydrate materials (Alsersy et al., 2015; Alnaimy, 2017). The main limitation with orange pulp and sugar beet pulp is the high pectin contents (Alnaimy, 2017).

Pectin is widely distributed in plants and mainly consist of galacturonans and rhamnogalacturonans (Amin et al., 2019). Pectin is fermented in the rumen by *Butyrivibrio fibrisolvens*, *Prevotella ruminicola*, *Bacteroides ruminicola* and *Lachnospira multiparus* through the production of pectinolytic enzymes which are predominantly pectin lyases (Castillo-González et al., 2014). Ruminal microbiota produce pectinase in the rumen with *L. multiparus* as the major producer (Murad and Azzaz, 2011). Pectinases can be synthesized from bacteria, yeast and fungi; however, its production from fungi (e.g. *Aspergillus niger* and *Aspergillus terreus*) is the most common (Murad and Azzaz, 2011).

Pectinases initiate the breakdown of the glycosidic bonds in the galacturonic acid chains of the pectin rich materials (Murad and Azzaz, 2011; Amin et al., 2019). Utilization of microbial pectinases to treat agriculture byproducts as feeds for ruminants has recently gained significant interest (Azzaz et al., 2020 b). Supplementation with exogenous pectinases can improve feed utilization and animal performance (Murad and Azzaz, 2011). Pectinases have been used in animal feeding to decrease feed viscosity and increase nutrient absorption and utilization (Murad and Azzaz, 2011). Azzaz et al. (2013) reported that treating sugar beet pulp with fibrolytic enzymes rich in pectinase (30 IU/g) enhanced nutrient digestibility and improved milk production and composition of buffaloes.

Fibrolytic enzymes like cellulase and xylanase supplementation to ruminant have been extensively evaluated with few studies on pectinase supplementation (Aboul-Fotouh et al., 2016; Elghandour et al., 2016; Salem et al., 2016; Azzaz et al., 2019). Pectinase enzyme is currently used under very few circumstances due to the limited knowledge of the catalytic activities. Additionally, the low production and availability coupled with high prices limit its use in animal production. Pectinase can be produced utilizing available byproducts as substrates. The activity of commercial pectinase differs from that of the developed pectinase; therefore, a comparative study with the commercial pectinase is imperative. Therefore, the present experiment aimed to evaluate the supplementation of pectinase from two sources: a newly developed pectinase and a commercially available pectinase on feed intake and digestion, blood biochemistry, milk production and components and milk fatty acid profile of Damascus goat fed orange byproduct silage and sugar beet pulp based diet. We hypothesized that pectinase will hydrolyze the polysaccharides in the diet, especially pectin resulting in enhanced utilization of the pectin-rich diet.

Material and methods

Production of pectinase

A new pectinase was developed at the Dairy Science Department, National Research Centre (Egypt) from *Aspergillus terreus*. *A. terreus* was obtained from the Laboratory of Plant Pathology, National Research Centre (Egypt). *A. terreus* was tested to explore its ability to utilize pectin as main carbon source for production of pectinase enzyme. The submerged cultivation technique was used for pectinase production on static cultures, using the following medium (per L): 6 g NaCl, 1 g $(\text{NH}_4)_2\text{SO}_4$, 1 g K_2HPO_4 , 0.05 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g CaCl_2 , 4 g glucose and 10 g beet pulp powder. Medium pH was adjusted to 4.0 and was sterilized in an autoclave at 110°C for 15 minutes. Production conditions were compared to define the optimal ones, as different inoculum ratios ranging from 1–8% (v/v) were compared. Pectinase activity assay was compared after various incubation periods (24 – 144 h). Different initial pH values (pH = 3, 4, 5, 6, 7, and 8) were compared. Various inorganic N sources (ammonium sulfate and ammonium chloride) and organic N sources (meat extract, yeast extract, urea and peptone) were compared separately at an equivalent concentration of 0.33 g N/L media. The crude enzyme was precipitated employing ammonium sulfate at the saturation level of 75% concentration. Precipitated protein was collected by centrifugation at 10,000 rpm for 15 min at 4°C. The precipitate was re-suspended in 0.1 M citrate phosphate buffer (pH = 5).

Pectinase maximum production by *A. terreus* was obtained at pH 4 of the growth medium, ammonium sulfate as a sole nitrogen source, 4 days of incubation period and 7% of the inoculum size. The activities of the new and commercial pectinases were determined according to method of Buga et al. (2010). The IU of pectinase activity was defined as the amount of enzyme that produced one μ mole of D-galacturonic acid per minute at 40°C and pH of 5.0. The assay was based on the ability of the produced enzyme to hydrolyze citrus pectin to reducing sugars. The products (mainly D-galacturonic acid) were determined photometrically at 575 nm by measuring the resulting increase in the reducing groups using 3, 5-dinitrosalicylic acid.

According to the results of the *in vitro* trials (Azzaz et al., 2020 b), the maximum produced pectinase efficiency (%) for DM degradability was obtained at 600 IU/kg DM. Accordingly, 600 IU/kg DM of the new and commercial enzymes was used for the feeding trials.

Does, feeding and experimental design

A full description of the experimental protocols and animal management have been previously described in Kholif et al. (2019). Thirty lactating Damascus does, weighing 29.1 ± 0.4 kg were randomly assigned to three dietary treatments in a completely randomized design. Does were stratified according to parity (2 ± 1 parity), type of kidding (single or twins) and previous milk production (900 ± 5 g/d). Previous milk production was the average daily milk yield in the previous lactation prior to the experiment, and it was measured for 12 weeks. The does were in their first week of lactation and the kids were not removed from the dams. Does were individually housed in pens ($1.5 \text{ m}^2/\text{doe}$), with free access to water, and offered the

experimental diets, in the form of a total mixed ration, to meet their nutrient requirements according to NRC (2007) recommendations plus a 10% margin. Diets were offered *ad libitum* to ensure collection of orts and to allow maximum intake and to meet the needs of dams irrespective of the type of birth or number of kids produced per dam. The control diet consisted of 500 g of concentrate feed mixture, 200 g orange byproduct silage 200 g sugar beet pulp and 100 g wheat straw on DM basis. The concentrate portion had on per kg DM basis: 380 g corn, 250 g wheat bran, 120 g sunflower meal, 120 g canola meal, 100 g flaxseed meal, 10 g NaCl, 10 g limestone, 7 g vitamins and minerals, and 3 g antitoxins. The control diet in the present experiment was the same feed fed to the animals before starting the experiment.

Table 1. Chemical composition (g/kg DM) of ingredients, concentrate and control diet fed to lactating Damascus goats

	Concentrates feed mixture ¹	Orange by-product silage	Sugar beet pulp	Wheat straw	Control diet ²
Dry matter (g/kg fresh weight)	909	929	936	947	922
Organic matter	920	934	965	844	924
Crude protein	180	98	97	42	133
Ether extract	61	35	33	17	46
Non-structural carbohydrates	415	313	346	149	354
Neutral detergent fiber	265	488	489	636	391
Acid detergent fiber	143	384	323	510	264
Pectin	ND ³	425	251	ND	ND

¹Contained (per kg DM): 380 g corn, 250 g wheat bran, 120 g sunflower meal, 120 g canola meal, 100 g flaxseed meal, 10 g NaCl, 10 g limestone, 3 g antitoxins and 7 g vitamins/minerals (Containing per kg: 141 g Ca, 87 g P, 45 g Mg, 14 g S, 120 g Na, 6 g K, 944 mg Fe, 1613 mg Zn, 484 mg Cu, 1748 mg Mn, 58 mg I, 51 mg Co, 13 mg Se, 248000 IU vitamin A, 74000 IU vitamin D₃, 1 656 IU vitamin E).

²The basal diet based on concentrates feed mixture, orange byproduct silage, sugar beet pulp and wheat straw at 500:200:200:100, respectively.

³ND, not determined.

The control diet was formulated to be rich in pectin due to its contents of orange byproduct and sugar beet pulp. The goats were fed the control treatment supplemented with the newly developed pectinase at 600 IU/kg feed daily (new pectinase treatment; contains 253,340 IU/kg) or commercial pectinase (Polyzyme™, Zeus Biotech Limited, India; contains 240,000 IU/kg) at 600 IU/kg feed daily (commercial pectinase treatment). The enzymes were delivered individually in 100 g concentrate before morning feeding at 08:00 h to ensure complete intake. Table 1 shows the chemical composition of the ingredients, concentrate and control diet. Daily diet allocations were delivered in two equal portions at 08:00 and 16:00 h. The experiment was for 12 weeks, comprising of 4 weeks of adaptation to the new ration and enzyme supplementation and 8 weeks of measurements and samples collection. Feeds were sampled daily, composited weekly and dried at 60°C in a forced-air oven for 48 h and stored for chemical analyses.

Individual does were weighed on a digital multi-purpose platform scale monthly during the experiment.

Nutrient digestibility and chemical analyses

During the 5th and 12th weeks of the experiment, feed intake was recorded daily by weighing the feed offered and orts from the previous day. Details on samples collection and analyses were as previously described by Kholif et al. (2017 c). Acid insoluble ash was used as an internal indigestible marker (Sales and Janssens, 2003). Acid-insoluble ash contents of feeds and feces were determined gravimetrically after drying, ashing, boiling of ash in hydrochloric acid (HCl), filtering and washing of the hot hydrolysate, and re-ashing. Coefficients of digestion were calculated according to Ferret et al. (1999). Fecal grab samples were collected from goats, twice daily during the collection weeks (5th and 12th weeks) at 07:00 and 18:00 h, dried at 105°C in a forced-air oven for 12 h (AOAC, 1997), and pooled per goat. The composited fecal samples, feed and orts samples were ground to pass a 1-mm screen using a Wiley mill grinder (Arthur H. Thomas, Philadelphia, PA, USA), and retained for later determination of compositional contents.

Feed, orts, and fecal samples were analyzed for ash after heating samples in a muffle furnace at 550°C for 12 h (method ID 942.05), N using Kjeldahl method (method ID 954.01), and ether extract (EE) using diethyl ether in a Soxhlet extractor (method ID 920.39) according to AOAC (1997) official methods. Neutral detergent fiber (NDF) was determined by the procedure of Van Soest et al. (1991) without the use of alpha amylase but with sodium sulfite. Acid detergent fiber (ADF; method ID 973.18) was analyzed according to AOAC (1997) (method ID 973.18) after digestion with sulfuric acid and cetyl trimethylammonium bromide, and expressed exclusive of residual ash. Lignin was analyzed by solubilization of cellulose with sulfuric acid in the ADF residue according to Van Soest et al. (1991). Pectin concentration in the ingredients and control diet was analyzed according to Shelukhina and Fedichkina (1994). Non-structural carbohydrate ($1,000 - [\text{NDF} + \text{crude protein (CP)} + \text{EE} + \text{ash}]$), cellulose [$\text{NDF} - \text{lignin}$], hemicellulose [$\text{NDF} - \text{ADF}$], and organic matter (OM; [$1,000 - \text{ash}$]) were calculated.

Sampling and analyses of blood serum

Blood sampling and analyses have been previously described in Kholif et al. (2018 a). Blood samples were collected on the last day of each month via jugular vein. Blood samples (10 mL) were taken 4 h after feeding from the jugular vein of all goats into plain clean dry tubes without anticoagulants. Blood parameters were centrifuged at $4,000 \times g$ for 20 min. Serum was separated into 2-mL clean dried Eppendorf tubes and frozen at -20°C , until analysis using commercial kits (Stanbio Laboratory, Boerne, Texas, USA).

Milk sampling, composition and fatty acids analyses

Three weeks after parturition, does were milked weekly during the last three days of each week by hand twice daily at 09:00 and 21:00 h. Kids were separated from their mothers for 24 h during milk measurement and bottle fed with milk. After the end of the day, a subsample (10% of total milk yield) was taken and stored for analysis. Milk samples were analyzed for different components using infrared spectrophotometry (Milkotester LM2, Belovo, Bulgaria).

At the end of each month, fatty acids in milk were determined using methyl esters prepared by base-catalysed methanolysis of the glycerides (KOH in methanol) according to International Standards (International Standard ISO 15884-IDF 182. 2002. Brussels, Belgium: International Dairy Federation) on a Perkin-Elmer chromatograph (model 8420, Beaconsfield, Perkin Elmer, Beaconsfield, UK) equipped with a flame ionization detector. Gross energy content of the milk was calculated by using the equation of Tyrrell and Reid (1965): Milk energy content (MJ/kg) = $4.184 \times [(41.63 \times \text{fat (g/kg)} + 24.13 \times \text{protein (g/kg)} + 21.60 \times \text{lactose (g/kg)} - 117.2)/10000] \times 2.204$. Energy corrected milk (ECM) was calculated by using the equation of Sjaunja et al. (1991): ECM (kg/d) = milk (kg/day) $\times [38.3 \times \text{fat (g/kg)} + 24.2 \times \text{protein (g/kg)} + 16.54 \times \text{lactose (g/kg)} + 20.7]/3140$. Fat corrected milk (FCM, 4% fat) was calculated by using the equation of Gaines (1928): FCM = $[(0.4 \times \text{kg milk}) + (0.15 \times \text{kg milk} \times \text{fat \%})]$.

Statistical analyses

The experiment was arranged as a completely randomized design with repeated measures. Data for intake, apparent nutrient digestibility, weight changes, milk composition and blood profiles were subjected to ANOVA by using the PROC MIXED procedure of SAS (SAS Inst. Inc. Cary, NC, USA) with week as repeated measures and individual animal as the experimental unit.

The statistical model was: $Y_{ijk} = m + T_i + P_j + (TP)_{ij} + E_{ijk}$,

where:

Y_{ijk} is each individual observation for a given variable, m is the overall mean, T_i is the treatment effect, P_j is the period effect, $(TP)_{ij}$ is the interaction between treatment and period, and E_{ijk} is the residual error. Animal nested within treatment was considered as a random effect, while treatment was considered as the fixed effect. When the treatment F -test was significant at $P < 0.05$, means were compared by applying the probability of difference option of the least squares means statement. Means were also compared by using the orthogonal contrasts (i.e., control vs. the average of the new and commercial pectinase treatments; and new vs. commercial pectinase).

Results

Feed intake and digestibility

Pectinase inclusion did not affect feed intake (Table 2). The newly developed pectinase increased ($P < 0.01$) nutrient digestibility, and diet nutritive value more than the other two treatments. Similarly, the commercial pectinase enhanced ($P < 0.01$) nutrient digestibility compared with the control treatment.

Table 2. Dry matter intake, nutrient digestibility and nutritive value of the newly developed or commercial pectinase supplemented diets fed to lactating Damascus goats

	Diets ¹			SEM	Diet	P-value		
	control	commercial	new			period	diet × period	control vs. pectinase
Intake (g/d)	1177	1146	1169	62.9	0.936	0.969	0.157	0.801
Digestibility (g absorbed/kg ingested)								
dry matter	617 c	644 b	691 a	3.3	<0.001	0.166	0.147	<0.001
organic matter	647 c	670 b	714 a	4.9	0.002	0.230	0.235	0.003
crude protein	621 c	652 b	694 a	5.8	0.003	0.957	0.013	0.003
ether extract	637 b	700 a	699 a	3.1	<0.001	0.474	0.594	<0.001
non-structural carbohydrates	632 c	657 b	705 a	7.8	0.002	0.294	0.283	0.002
neutral detergent fiber	559 c	601 b	646 a	4.5	<0.001	0.933	0.830	<0.001
acid detergent fiber	561 c	611 b	661 a	3.3	<0.001	0.446	0.199	<0.001
Digestible nutrients and energy value ²								
total digestible nutrients (g/kg DM)	660 c	688 b	722 a	4.2	0.001	0.408	0.215	0.001
metabolizable energy (MJ/kg DM)	2.91 c	3.03 b	3.19 a	0.018	0.001	0.419	0.202	0.001
net energy of lactation (MJ/kg DM)	1.50 c	1.57 b	1.65 a	0.010	0.001	0.418	0.222	0.001
digestible crude protein (g/kg DM)	93.5 c	97.5 b	103.0 a	0.75	0.003	0.957	0.013	0.003

Means in the same row with different letters differ, $P < 0.05$.

¹The control diet based on (per kg DM): 500 g of concentrates feed mixture, 200 g orange byproduct silage, 200 g sugar beet pulp and 100 g wheat straw without addition of supplements (control treatment) or with addition of 600 IU of new pectinase/kg feed daily (new treatment), or 600 IU of commercial pectinase/kg feed daily (commercial treatment).

²Calculated according to NRC (2001).

Table 3. Blood serum parameters of lactating Damascus goats fed diets supplemented with the newly developed or commercial pectinase

	Diets ¹			SEM	P-value			
	control	commercial	new		diet	period	diet × period	control vs. pectinase new vs. commercial
Total protein (g/dL)	6.11	6.40	6.93	0.212	0.868	0.350	0.552	0.052
Albumin (g/dL)	3.30	3.45	3.55	0.118	0.348	0.516	0.644	0.186
Globulin (g/dL)	2.81	2.95	3.39	0.259	0.300	0.340	0.576	0.284
Albumin/globulin	1.25	1.30	1.12	0.138	0.629	0.443	0.579	0.790
Glucose (mg/dL)	62.7 c	68.8 b	72.9 a	1.16	0.025	0.452	0.462	0.034
Total lipids (mg/dL)	439	444	470	18.6	0.470	0.157	0.009	0.446
Glutamic-oxaloacetic transaminase (U/L)	30.8	34.3	33.5	1.91	0.414	0.420	0.657	0.204
Glutamic-pyruvic transaminase (U/L)	20.7	25.2	22.4	0.94	0.015	0.633	0.204	0.017

Means in the same row with different letters differ, P<0.05.

¹The control diet based on (per kg DM): 500 g of concentrates feed mixture, 200 g orange byproduct silage, 200 g sugar beet pulp and 100 g wheat straw without addition of supplements (control treatment) or with addition of 600 IU of new pectinase/kg feed daily (new treatment), or 600 IU of commercial pectinase/kg feed daily (commercial treatment).

Table 4. Body weight, milk yield and milk composition of lactating Damascus goat fed diets supplemented with the newly developed or commercial pectinase

	Diets ¹			SEM	P-value			
	control	commercial	new		diet	period	diet × period	control vs. pectinase new vs. commercial
Body weight								
initial weight (kg)	29.4	28.6	29.2	1.69	0.944	–	–	0.812
final weight (kg)	30.6	30.4	31.5	1.65	0.879	–	–	0.889
total change (kg)	1.21	1.71	2.29	0.319	0.085	–	–	0.059
weight change (g/d)	19.3	27.2	36.3	5.06	0.085	0.051	0.089	0.059
Milk production (g/d)								
milk	936c	996 b	1085 a	13.1	<0.001	0.017	0.379	<0.001
energy corrected milk (ECM)	860 c	973 b	1085 a	31.4	0.003	0.051	0.982	0.003
fat corrected milk (FCM)	859 c	946 b	1053 a	30.5	0.001	0.017	0.948	0.001
milk energy output (MJ/d)	2.68 c	3.04 b	3.40 a	0.099	0.003	0.048	0.983	0.003
milk components (g/kg)								
total solids	120 b	128 a	131 a	2.58	0.024	0.028	0.608	0.009
solids not fat	93.2 b	91.7 a	85.7 a	1.45	0.004	0.540	0.952	0.001
fat	34.7 b	36.7 a	37.8 a	0.81	0.047	0.001	0.233	0.026
protein	31.0	32.7	32.2	0.65	0.215	0.948	0.341	0.099
lactose	47.4 b	51.3 a	53.4 a	0.99	0.002	0.284	0.892	0.006
ash	7.35	7.67	7.70	0.220	0.461	0.203	0.239	0.221
milk energy content (MJ/kg)	2.86	3.05	3.12	0.081	0.082	0.006	0.409	0.033
Feed (milk) efficiency								
milk yield/intake	0.80 b	0.87 a	0.93 a	0.048	0.101	0.047	0.333	0.047
ECM yield/intake	0.73 b	0.85 b	0.93 a	0.054	0.004	0.031	0.137	0.007
FCM yield/intake	0.73 b	0.83 b	0.90 a	0.045	0.002	0.022	0.114	0.002

Means in the same row with different letters differ, $P<0.05$.¹The control diet based on (per kg DM): 500 g of concentrates feed mixture, 200 g orange byproduct silage, 200 g sugar beet pulp and 100 g wheat straw without addition of supplements (control treatment) or with addition of 600 IU of new pectinase/kg feed daily (new treatment), or 600 IU of commercial pectinase/kg feed daily (commercial treatment).

Table 5. Fatty acids profile (g/100 g total fatty acids) in milk of lactating Damascus goats fed diets supplemented with the newly developed or commercial pectinase

	Diets ¹			SEM	P-value				
	control	commercial	new		diet	period	diet × period	control vs. pectinase	new vs. commercial
C4:0	0.11	0.14	0.12	0.039	0.901	0.761	0.843	0.788	0.728
C6:0	0.25	0.16	0.23	0.053	0.491	0.647	0.515	0.447	0.370
C8:0	1.25	1.17	1.26	0.115	0.841	0.293	0.915	0.816	0.606
C10:0	7.20	7.40	7.28	0.221	0.818	0.636	0.570	0.623	0.714
C12:0	4.62	4.44	4.53	0.315	0.925	0.625	0.051	0.740	0.855
C13:0	0.11	0.12	0.10	0.008	0.210	0.841	0.666	0.676	0.096
C14:0	11.5	11.2	10.6	0.41	0.357	0.188	0.007	0.304	0.316
C14:1	0.23	0.26	0.24	0.088	0.971	0.881	0.903	0.853	0.887
C15:0	1.14	1.00	1.09	0.067	0.378	0.701	0.306	0.306	0.346
C16:0	30.6	29.5	29.7	1.07	0.764	0.253	0.683	0.484	0.937
C16:1	0.70	0.76	0.74	0.034	0.443	0.928	0.858	0.234	0.740
C17:0	0.56	0.55	0.53	0.109	0.977	0.951	0.105	0.867	0.901
C17:1	0.80	0.86	0.89	0.103	0.823	0.401	0.224	0.569	0.852
C18:0	9.46	9.48	9.64	0.742	0.983	0.050	0.790	0.916	0.889
C18:1 <i>n</i> 9 <i>C</i>	24.0	25.6	24.7	1.75	0.809	0.422	0.078	0.598	0.731
C18:1 <i>n</i> 9 <i>T</i>	0.51	0.60	0.70	0.354	0.932	0.645	0.810	0.759	0.851
C18:2 <i>n</i> 6 <i>c</i>	2.62	2.90	2.93	0.103	0.139	0.758	0.964	0.057	0.827
C18:2 <i>n</i> 6 <i>t</i>	0.24	0.20	0.31	0.112	0.781	0.413	0.507	0.912	0.506
C18:3 <i>n</i> 3	0.26	0.29	0.29	0.019	0.511	0.684	0.585	0.268	0.905
C18:3 <i>n</i> 6	0.21	0.24	0.25	0.029	0.636	0.023	0.732	0.362	0.938
C20:0	0.14	0.13	0.13	0.016	0.787	0.133	0.544	0.509	0.945
C20:1	0.13	0.12	0.16	0.031	0.683	0.097	0.529	0.819	0.419

C20:2	0.26	0.25	0.25	0.046	0.984	0.604	0.541	0.877	0.942
C20:4	0.48	0.44	0.42	0.139	0.945	0.147	0.845	0.757	0.922
C22:0	0.15	0.11	0.06	0.051	0.553	0.324	0.996	0.398	0.513
Total saturated fatty acids (SFA)	67.0	65.4	65.2	1.66	0.712	0.894	0.564	0.433	0.919
Total unsaturated fatty acids (UFA)	30.4	32.5	31.9	1.49	0.614	0.013	0.421	0.362	0.777
Mono unsaturated fatty acids	26.4	28.2	27.5	1.53	0.700	0.569	0.092	0.456	0.737
Poly unsaturated fatty acids	4.06	4.31	4.44	0.268	0.622	0.019	0.128	0.380	0.731
Total conjugated linoleic acid ²	2.85b	3.09 a	3.24a	0.011	0.036	0.812	0.812	0.201	0.585
Omega-6/omega-3 ratio	0.81	0.85	0.88	0.140	0.938	0.811	0.811	0.755	0.887
UFA/SFA ratio	0.45 b	0.50 a	0.49 a	0.023	0.019	0.126	0.126	0.045	0.873
Atherogenic index ³	2.66 a	2.43 b	2.43 b	0.082	0.010	0.666	0.222	0.040	0.996

Means in the same row with different letters differ, $P < 0.05$.

¹The control diet based on (per kg DM): 500 g of concentrates feed mixture, 200 g orange byproduct silage, 200 g sugar beet pulp and 100 g wheat straw without addition of supplements (control treatment) or with addition of 600 IU of new pectinase/kg feed daily (new treatment), or 600 IU of commercial pectinase/kg feed daily (commercial treatment).

²Total conjugated linoleic acid = trans-10, cis-12 C18:2 + cis-9, trans-11 C18:2.

³Calculated according to Ulbricht and Southgate (1991): atherogenic index = $(C12:0 + 4 \times C14:0 + C16:0) / \sum$ of UFA.

Blood serum measurements

Pectinase inclusion had no effect on the concentrations of serum total protein, albumin, globulin, albumin/globulin, total lipids, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) (Table 3). Treatments increased serum glucose ($P=0.025$) compared with the control treatment; however, the glucose concentration was higher ($P=0.029$) in the new pectinase compared with the commercial pectinase.

Milk yield, milk composition and fatty acid profile

The newly developed pectinase had the greatest effect on daily milk production ($P<0.001$), ECM ($P=0.003$), FCM ($P=0.001$) as well as the yields of milk components ($P<0.05$; Table 4). Additionally, the new and commercial pectinases increased ($P<0.05$) total solids, solids not fat, fat and lactose contents in the milk without affecting protein or ash concentrations.

Greater feed efficiency calculated as milk yield/intake, ECM yield/intake and FCM yield/intake were observed with the new pectinase followed by the commercial pectinase and the control treatment in that order.

Treatments did not affect the concentration of individual fatty acids in milk (Table 5). Also, both the new and commercial treatments did not affect total saturated fatty acids (SFA), total unsaturated fatty acids (UFA), omega-6/omega-3 ratio; however, they increased the concentrations of total conjugated linoleic acid (CLA; $P=0.011$) and UFA/SFA ratio ($P=0.023$), and decreased atherogenic index ($P=0.01$) compared with the control treatment.

Discussion

Reports on the inclusion of pectinase as a feed additive in the diet of ruminant are limited. Few number of studies evaluated the inclusion of enzymes mixture containing pectinase in ruminant feeding. Therefore, the present experiment will be compared with experiments that fed fibrolytic enzyme mixture containing pectinase.

Feed intake and digestibility

Consistent with our results, Morsy et al. (2016) reported no effect on feed intake of buffaloes that were supplemented with pectinase-rich (30 IU/g) exogenous enzymes. The absence of effect on feed intake may be explained based on the ability of the exogenous enzyme supplementation to accelerate availability of nutrients, without affecting rumen fill (Morsy et al., 2016). This assumption can be strengthened by the result of nutrient digestibility in the present experiment.

The main objective of feeding fibrolytic enzymes is to enhance fiber and other nutrients digestion (Rojo et al., 2015; Togtokhbayar et al., 2015; Vallejo et al., 2016; Kholif et al., 2018 b). Pectinases improved nutrient digestibility due to their ability to convert polysaccharides such as pectin, cellulose and hemicellulose, which are the major component of ruminant diets, into soluble sugars so that they can be used by ruminal microorganisms (Castillo-González et al., 2014) to enhance nutrient di-

gestion. Additionally, pectinases separate the middle lamella fibers of feed particles making them more degradable by ruminal microbes (Azzaz et al., 2020 b). Azzaz et al. (2013) reported that the increased nutrient digestibility with pectinase-rich enzyme supplementation in lactating buffaloes fed diet containing sugar beet pulp was due to the ability of pectinase to degrade complex compounds (cellulose and pectin) into simpler compounds through alteration of the structure of feeds, thereby increasing the surface area and microbial attachment with subsequent increase in fermentation. Similar to other fibrolytic enzymes, pectinase can reduce digesta viscosity and liberates nutrients, either by hydrolysis of non-biodegradable fibers or by liberating nutrients blocked by these fibers (Murad and Azzaz, 2011). Pectinase alters ruminal fermentation and enhances attachment and colonization of ruminal microbiota to the plant cell wall, as well as the synergism with endogenous enzymes in rumen fluid (Murad and Azzaz, 2011). Beauchemin et al. (2004) reported that enhancing nutrient digestibility with exogenous enzymes is not the main reason due to the relative low enzyme activity compared with ruminal activity, making the assumption that pectinase elevated numbers of cellulolytic and non-cellulolytic bacteria in rumen fluid, which elevates microbial biomass (Giraldo et al., 2008). Previous reports using fibrolytic enzymes preparation rich in pectinase have also shown that nutrient digestibility was increased (Morsy et al., 2016).

Better response noted by the new pectinase when compared with the commercial pectinase may be related to the activity of pectinase from different sources (Morsy et al., 2016; Azzaz et al., 2020 a). Morsy et al. (2016) compared two preparations of exogenous enzymes in the diet of lactating buffaloes and reported different responses on feed digestion.

Blood serum parameters

All blood metabolites determined in the present study were within the reference range for a healthy animal (Boyd, 1984). Pectinases did not affect the concentration of serum total protein, albumin or globulin revealing minimal effects on the nutritional status of the goats. Reported values for GOT and GPT concentrations were within the normal physiological range (Boyd, 1984; Pettersson et al., 2008). Pectinases did not affect the concentration of serum GPT or GOT indicating minimal effects on liver health and activity. Azzaz et al. (2013) reported that fibrolytic enzyme preparation containing pectinase did not affect the concentrations of serum GOT and GPT enzymes. Lack of effect on serum total lipids with pectinases is consistent with the results of Azzaz et al. (2013). Morsy et al. (2016) also reported no changes in serum total protein, albumin, globulin, urea, total lipids, cholesterol, GOT and GPT in their study.

The commercial and new pectinases increased serum glucose (which is an important nutrient indicator) by 9.8 and 16.4%, respectively. Enhancing nutrient digestibility especially OM, non-structural carbohydrates (NSC) and fibers with pectinase is the main reason (Kholif et al., 2017 a) for the observation. Increasing OM and NSC digestibility enhances ruminal propionate production and energy utilization, resulting in a high rate of glucose synthesis. Another reason might be a modified biohydrogenation at ruminal level caused by differences in microbiota.

Milk yield, milk composition and fatty acid profile

Pectinases increased production of milk (by 6.4 and 16%), ECM (13.1 and 26.1%) and FCM (10.2 and 22.6%) for commercial and new pectinases, respectively. Enhancing milk production without corresponding increase in feed intake resulted in increased feed/milk efficiency calculated as milk yield/intake (by 8.4 and 16.3%), ECM yield/intake (by 16.4 and 27.4%) and FCM yield/intake (by 13.7 and 23.3%) for the commercial and new pectinases. In a previous study, Azzaz et al. (2013) reported no change in milk production when lactating buffaloes were fed pectinase-rich diet. The energy status of animals, species, diet type, enzyme dose, and stage of lactation may be responsible for these differences. Knowlton et al. (2002) noted that the effect of fibrolytic enzymes on milk production is more pronounced in early lactation. Increased milk production with pectinase may be due to enhanced nutrient digestibility, especially as milk production followed the same trends observed in nutrient digestibility which increased energy available for milk production (Morsy et al., 2016).

As previously noted in nutrient digestibility, greater milk production noted for the new pectinase compared with the commercial pectinase could have been as a result of different pectinases from different sources (Morsy et al., 2016). Morsy et al. (2016) compared two preparations of exogenous enzymes in the diet of lactating buffaloes and reported different responses for milk production.

Pectinases increased the concentration of fat in milk by 5.8 and 9.1%, respectively for the commercial and new pectinases which could be as a result of enhanced fiber digestibility (Morsy et al., 2016). Theoretically, enhancing fiber digestion increases the production of ruminal acetate which is known to increase milk fat synthesis (Palmquist, 2006). Fredeen (1996) noted that changes in milk fat and lactose contents are primarily affected by nutrients. Morsy et al. (2016) also reported that supplementing lactating buffaloes with pectinase rich enzymes product increased milk fat content.

The commercial and new pectinases increased milk lactose by 8.4 and 12.7%, respectively, which may be related to enhanced OM and NSC digestibility which is well documented to enhance ruminal propionate production. Milk fatty acid profile is very sensitive to dietary manipulation (Kholif et al., 2019) but limited differences were noted in the present study. Greater concentrations of CLA (by 8.4 and 13.5%) and UFA/SFA ratio (by 9.8 and 8.2%), and decreased atherogenic index (by 8.7% for both) were noted for commercial and new pectinases, respectively. These parameters are widely used to evaluate the nutritional value of milk and the vital role they play in human health by contributing to either decreasing or increasing the chances of cardiovascular disorders (Abedi and Sahari, 2014).

Results indicate that pectinase supplementation affected ruminal biohydrogenation. More than 60% of milk fatty acids originated mainly from the diet or rumen microbial activities (Palmquist, 2006). Milk CLA are unique to ruminants and produced primarily by ruminal bacteria from partial biohydrogenation of linoleic acid. A secondary important source of CLA is the mammary gland and this is responsible for approximately 70% of total CLA in the milk (Corl et al., 2001). Increasing the concentration of CLA in milk is mainly related to the diets and manipulation of rumi-

nant's diet can result in up to a 10-fold increase in the concentration of CLA in milk (Kim et al., 2003). In the present study, the does were fed the same diet (supplemented with different enzymes) so differences in milk CLA were not caused by dietary manipulation. One can only speculate that greater CLA in the treatment groups was due to increased tissue synthesis of CLA in the mammary gland (Corl et al., 2001).

The present results suggest that ruminal fermentation was altered by the supplementation of pectinase which affected precursor availability for fatty acids synthesis. Morsy et al. (2016) reported that supplementing diets of buffaloes with exogenous enzymes, rich in pectinase (30 IU/g) increased poly UFA and mono UFA contents and decreased SFA content. Although they did not evaluate ruminal fermentation, they extrapolate that acetate and propionate were altered in the rumen.

Conclusion

Agricultural byproducts such as sugar beet pulp and orange byproduct silage can be used as animal feeds with the supplementation of pectinase to overcome the limitation associated with their high pectin contents. Pectinases enhanced the nutritive value of these materials as feeds for lactating goats at 600 IU/kg DM feed. The new pectinase showed promising results compared with the commercial pectinase. The newly developed pectinase enhanced nutrient digestion, milk production, blood chemistry and milk fatty acids profile without affecting blood biochemistry. However, additional studies, involving *in vitro* and *in vivo* evaluations, are recommended to investigate different levels of the newly developed pectinase on the performance of animals at different stages of production.

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