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Dose-Response Effect of Exogenous Enzymes Treatment of Tomato and Watermelon Crop Byproducts on *In vitro* Nutrient Degradability and Rumen Fermentation Kinetics

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Abstract | This study aimed to investigate the potential of exogenous enzymes treatment of tomato and watermelon crop byproducts (TCB and WCB, respectively) on gas production, rumen fermentation characteristics, and feed degradability using *in vitro* gas production method. Four different concentrations (0, 6, 12, and 24 mg/g) of ENZ were added with the substrate (TCB and WCB) inside the incubation tubes. Berseem hay substrate was used as a positive control. The results of chemical analyses of TCB and WCB showed that most of the nutrients are lower than those in berseem hay. The untreated WCB and TCB displayed a significant reduction in cumulative gas production (GP), microbial crude protein, short-chain fatty acid (SCFA), nutrient degradability, net energy (NE), and metabolizable energy (ME) contents. Still, they increased the partitioning factor value in comparison with berseem hay. However, increased GP, SCFA, ME, and NE with increasing ENZ levels were observed in both crop residues with a significant effect at the level of 24 mg/g. Also, the application of ENZ enhanced the degradation of dry matter (DM), crude protein (CP), and crude fiber (CF) compared with untreated WCB and TCB. All ENZ levels did not elicit any significant alterations in the ruminal pH, NH_3–N concentration, and protozoa count. Conclusively, the results suggest that treatment of crop residues with ENZ, especially at 24 mg/g DM, could have the potential to improve the efficiency of feed utilization fed to ruminants, as evidenced by better gas production, *in vitro* DM, CF, and CP degradability.

Keywords | Agricultural byproducts, Rumen fermentation, Tomato, Watermelon, ZADO®.

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INTRODUCTION

Byproducts from agriculture may be of importance not only for reducing nutrition costs but also to reduce environmental problems associated with byproduct accumulation (Al-Sagheer et al., 2019). Most of these biomaterials are not used and end up in municipal sites, where microbial breakdown and leachate production produce severe environmental challenges. Thus, the use of agricultural byproducts in ruminant nutrition has been virtually adopted as an approach for minimizing the expense of feeding and reducing the need to recycle wastes, which its disposal is costly (Omer and Abdel-Magid, 2015). Fibrous feeds contain high cellulose and hemicellulose content, making a complex with lignin and carbohydrates to decrease the carbohydrates' digestibility and decrease the utilization of ruminants from forages. Improvement of fibrous feeds digestibility is generally very desirable and leads to better animal productivity. Degradation of plant cell walls of high fiber feed in the rumen is possible principally because of the produced enzymes of rumen microflora (Krause et al., 2003). Numerous studies have focused on enhancing the efficiency of fibrous feeds degradation in the rumen using physical, chemical, and biological treatment such as yeast products, exogenous fiber degrading enzymes, and inoculants (Adesogan et al., 2019).

As an effective treatment strategy, exogenous enzymes have attracted worldwide growing interest and proved to be a useful tool to improve ruminant production (Beauchemin et al., 2019). The enhancement in the nutritive value of fibrous feeds by exogenous fiber enzymes treatment may be due to increasing attachment by rumen bacteria (Wang et al., 2001). Moreover, the enzymes act synergistically with the rumen microbes, which increases their ability to hydrolytic the fiber in the rumen (Beauchemin et al., 2004). ZAD® is a commercial product prepared from anaerobic bacteria, which by particular enzymes convert polysaccharides into monosaccharides (Salem et al., 2013). The ZADO® enzyme mixture contains α-amylase, xylanase, endoglucanase, and protease. This enzyme mixture has been used in previous studies to improve microbial crude protein synthesis, ruminal fermentation parameters, nutrient digestibility, and milk production (Gado et al., 2009, López et al., 2013, Salem et al., 2013).

Therefore, this *in vitro* study aimed to evaluate the ZADO® enzyme mixture's dose-response effect on the gas production, nutrient degradability, and rumen fermentation characteristics of two crop residues of tomato and watermelon. In particular, no study, to our knowledge, has considered the nutritional assessment of two highly available crops byproducts treated with exogenous enzyme mixture.

MATERIALS AND METHODS

The current study was accompanied by the Animal Nutrition Laboratory, Animal Production Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Plant byproducts and chemical analysis

Samples of tomato (*Solanum Lycopersicum*) and watermelon (*Citrullus lanatus*) crop byproducts (TCB and WCB, respectively) were manually collected from a private farm in Zagazig, Egypt. Plant byproducts were weighed, wholly cut, dried at 65 °C to about 90 % dry matter (DM), and finely powdered (1 mm). These dried samples were used for chemical analysis and *in vitro* experiment. The sample was analyzed for organic matter (OM), DM, crude fiber (CF), ash, ether extract, and crude protein (CP) according to the AOAC (2006). Estimate the concentration of elements in the sample by atomic absorption device (iCE 3000 Series AAS), according to Elmer and Conn (1982). Estimate the concentration of phosphorus element by element appreciation chromatography device, according to Marker (1992). The chemical and mineral composition of TCB and WCB compared with berseem hay are listed in Table 1.

Enzyme mixture and additive dose

The used exogenous enzyme (ZADO®) was kindly provided by Dr. Hany Gado, Professor of Animal Nutrition, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. ZADO® is a multi-enzyme powder (Patent No.: 22155, Cairo, Egypt), commercially available, manufactured by the Academy of Scientific Research and Technology in Egypt from *Ruminococcus flavefaciens* bacteria. The enzyme mixture has been examined for enzymatic activities and was found to contain (per g of enzyme) 61.5 units of α-amylase, 7.1 units of endoglucanase, 29.2 units of protease, and 2.3 units of xylanase activity. Four doses of exogenous enzymes (0, 6, 12, and 24 mg per g DM) were added with the substrate (TCB and WCB) inside the incubation tubes. Berseem hay substrate was used as a positive control.

In vitro incubations

The ruminal liquid was collected from a slaughtered cow from a slaughterhouse located at Zagazig, Sharkia Governorate, Egypt. Rumen liquids were immediately transported to the laboratory in pre-warmed (39 °C) isolate flasks and kept under anaerobic environments. The rumen fluid was filtered through four layers of cheesecloth and incubated at 39 °C in a water bath, and it was saturated with CO_2 until inoculation.

The buffer incubation medium (MB9) contained $CaCl_2$ (0.1g/l), NaCl (2.8g/l), $MgSO_4.7H_2O$ (0.1g/l), KH_2PO_4. H_2O (2g/l), and Na_2HPO_4 (6g/l). The rumen fluid was mixed with the MB9 media at the MB9 media ratio to rumen fluid of 2:1 (v/v). Thirty millimeters of mixed ruminal fluid was introduced into calibrated glass tubes, containing 200 mg of the byproducts + dose of ZADO® (ENZ), quickly closed by a gas release rubber stopper fitted with a tri-way valve coupled with a calibrated plastic syringe to measure the gas production. The volume of gas production was measured at 3, 6, 12, 24, 36, 48, and 72 h of incubation. The total gas volume was corrected from a blank tube. Three runs of gas production were conducted for all substrates. In each run as four blank bottles (without substrate) and six bottles for each treatment. Gas production kinetics were calculated following the model of Ørskov and McDonald (1979).

The contents of three tubes of each treatment were used for determining truly degraded dry matter (DMD) after 72 h of incubation by adding 30 mL of neutral detergent solution to each bottle and placed at 105 °C for three h. Then, each sample was filtered through pre-weighed Gooch crucibles, dried at 105°C for three h, and the residual DM weight was estimated (Blümmel et al., 1997). After that, it was used to estimate crude protein and crude fiber degradability (CPD and CFD), according to AOAC (2006). The concentration of NH_3-N, total volatile fatty acids (TVFA), and protozoa count were determined in the contents of another three tubes of each treatment. The protozoa count

Table 1: Analyzed nutrient and minerals contents of tested substrates.

	Berseem hay	Watermelon (whole plant byproduct)	Tomato (whole plant byproduct)
Chemical composition (% on DM basis)			
Crude protein	15.70	10.08	8.55
Crude fiber	25.18	36.39	26.90
Ether extract	4.15	2.40	2.04
Organic matter	88.30	79.09	82.18
Ash	11.70	20.91	18.82
Nitrogen free extract	43.27	30.22	43.69
Mineral composition (on DM basis)			
Calcium, %	1.45	1.02	0.073
Potassium, %	2.32	0.112	0.111
Phosphorus, %	0.262	0.008	0.006
Magnesium, %	0.290	0.032	0.024
Copper, ppm	15.18	0.822	0.646
Zinc, ppm	20.25	2.939	1.3416
Iron, ppm	178.2	42.69	17.99
Chromium, ppm	0.520	0.6154	0.4103

Table 2: Effect of various exogenous enzyme (ENZ) doses addition to watermelon crop byproducts (WCB) on cumulative gas production kinetics, predicted values, and fermentation parameter compared with berseem hay as a positive control

	Berseem hay	ENZ levels added to WCB substrate				SEM	P-value		
		0 g/kg^{-1}	6 g/kg^{-1}	12 g/kg^{-1}	24 g/kg^{-1}		T	L	Q
Gas production, ml/g DM									
3 h	37.00^{ab}	30.17^{b}	32.07^{b}	39.06^{ab}	48.13^{a}	1.58	0.0001	0.0001	0.200
6 h	59.25^{ab}	42.75^{c}	47.21^{bc}	53.56^{abc}	65.56^{a}	2.02	0.0001	0.0001	0.236
12 h	86.38^{a}	59.50^{b}	69.43^{ab}	77.83^{ab}	86.50^{a}	2.64	0.0001	0.0001	0.870
24 h	110.38^{ab}	95.54^{b}	102.79^{ab}	107.44^{ab}	119.13^{a}	2.42	0.009	0.0001	0.536
36 h	127.88^{ab}	119.88^{b}	128.00^{ab}	131.78^{ab}	143.38^{a}	2.45	0.015	0.0001	0.631
48 h	141.38^{ab}	132.88^{b}	137.00^{b}	140.94^{ab}	157.69^{a}	2.87	0.039	0.001	0.001
72 h	151.63^{ab}	143.71^{b}	147.00^{b}	148.89^{b}	168.44^{a}	2.85	0.032	0.002	0.108
a	18.63^{ab}	14.06^{b}	14.24^{b}	21.20^{ab}	32.89^{a}	1.88	0.003	0.0001	0.079
b	139.11	143.66	141.19	135.72	146.76	3.00	0.813	0.992	0.227
c	0.05	0.04	0.04	0.05	0.04	0.002	0.124	0.160	0.015
a+b	157.74	157.72	155.44	156.92	179.65	3.20	0.079	0.025	0.042
Predicted values									
SCFA, mmol /0.2g DM	0.62^{a}	0.42^{c}	0.45^{bc}	0.47^{bc}	0.51^{b}	0.011	<0.001	0.001	0.978
ME (MJ/kg DM)	5.54^{a}	4.14^{c}	4.39^{bc}	4.52^{bc}	4.75^{b}	0.080	<0.001	0.001	0.978
NE_L (MJ/kg DM)	3.18^{a}	2.56^{c}	2.69^{bc}	2.84^{bc}	2.99^{b}	0.059	<0.001	0.001	0.978
OMD, %	47.83^{a}	36.46^{c}	37.85^{bc}	38.58^{bc}	39.89^{b}	0.554	<0.001	0.001	0.978
MCP (mg/g DM)	751.38^{a}	605.34^{b}	638.61^{b}	665.80^{b}	660.47^{b}	11.87	<0.001	0.024	0.936
PF (mg TDOM/ml gas)	1.71^{b}	1.92^{a}	1.84^{ab}	1.80^{ab}	1.76^{ab}	0.019	<0.001	0.001	0.658
Fermentation parameters									
pH (24 h)	6.21	6.20	5.89	5.81	5.80	0.07	0.092	0.041	0.244

pH (48 h)	6.21	6.21	6.01	5.95	5.88	0.05	0.085	0.009	0.371
pH (72 h)	5.40	5.50	5.60	5.30	5.30	1.65	0.098	0.521	0.106
Ammonia-N, mg/dL	41.07	34.16	36.55	36.40	33.25	1.15	0.327	0.862	0.305
TVFA, ml eq./L	167.67	154.60	203.75	218.50	193.00	12.09	0.442	0.270	0.208
Protozoa count, 10^3/mL	164.83^{b}	198.00^{ab}	235.00^{a}	233.75^{a}	198.75^{ab}	8.19	0.038	0.855	0.037

Means in the same row bearing different letters differ significantly ($P < 0.05$); SEM indicates standard error of the mean; Probability of main effects of treatment (T), linear (L), and quadratic (Q); a = the gas production from the immediately soluble fraction (ml); b = the gas production from the insoluble fraction (ml); c = the gas production rate constant for the insoluble fraction b (h); a+b = potential gas production (ml); ME, metabolizable energy; NE_L, net energy lactation; OMD, organic matter degradability; PF, partitioning factor at 72 h of incubation; SCFA, short-chain fatty acids; MCP, microbial crude protein production; TVFA is the total volatile fatty acids.

was estimated microscopically after sample preparation in line with the method of Kamra et al. (1991). The TVFA concentration was estimated by the steam distillation method, according to Warner (1964). Ruminal NH_3-N concentration was detected according to the method described by Conway (1957). At 24 h of incubation, the partitioning factor (PF) was calculated as the ratio of OM (mg) degradability to the volume of gas production (in mL after 24 h) (Blümmel et al., 1997). Net energy lactation (NE_L, MJ/kg DM) and metabolizable energy (ME, MJ/kg DM) were calculated according to Menke and Steingass (1988). The calculation of the *in vitro* organic matter digestibility (OMD %) was accomplished according to Menke et al. (1979) equation. Short chain fatty acid concentrations (SCFA) were calculated according to Getachew et al. (2002). Microbial CP biomass production was estimated, according to Blümmel et al. (1997).

Statistical analysis

The statistical analysis of the *in vitro* results was conducted using the general linear model procedure (GLM) using SPSS 21 (Chicago, IL) software. The orthogonal polynomial contrast was applied to identify the linear, quadratic, and cubic effects of increasing exogenous enzyme levels. Tukey's test was used to test the significant differences (at $P < 0.05$) between the means.

RESULTS

Watermelon crop byproducts

Data presented in Table 2 exhibited no significant differences in gas production kinetics, TVFA, NH_3-N, pH, and protozoal count between untreated WCB and berseem hay. In contrast, a significant reduction in predicted NE, SCFA, ME, MCP, and OMD, but a significant increase in PF, were observed in the untreated WCB group compared to the berseem hay (Table 2). The addition of ENZ led to a linear increase ($P<0.001$) in cumulative gas production, gas production from a soluble fraction (a), and the predicted value of ME, NE, SCFA, and OMD with increasing dietary ENZ levels. The highest values were observed in diet fortified with ENZ at 24 g/ kg compared with un-supplemented WCB. All concentrations of the tested ENZ did not alter the ruminal NH_3-N, TVFA, pH, and protozoal count (Table 2).

At all incubation periods, DMD, CFD, and CPD for untreated WCB were significantly lower ($P<0.001$) than that for berseem hay (Table 3). However, the application of ENZ at 24 g/kg improved ($P<0.01$) the degradation of CF and CP after 72 h of incubation compared with untreated WCB (Table 2). Also, the highest ($P=0.001$) value of DMD was detected in WCB treated with 12 and 24 g/kg of ENZ after 24h but not at 48 and 72h of incubation (Table 3).

Tomato crop byproducts

As shown in Table 4, cumulative gas production (12-72h of incubation) was significantly ($P<0.001$) reduced in untreated TCB compared with berseem hay substrate. Also, a significant decrease in the predicted value of ME, NE, SCFA, MCP, and OMD, but a significant increase in PF, were detected in the un-supplemented WCB substrate compared to those in berseem hay. Incubation of TCB substrate with ENZ resulted in a linear increase in cumulative gas production (3-72h of incubation) with a significant effect at the level of 24 g/kg (Table 4). Predicted values of ME, NE, SCFA, MCP, and OMD showed a linear increase at the addition of ENZ to the incubation media containing TCB relative to the untreated one. Notably, the highest significant effect on the earlier parameters was evident at the level of 24 g/kg. Nevertheless, a linear decrease was recorded in the PF when the incubation media containing TCB was fortified with ENZ with a significant effect at 12 and 24 g/kg. The values of pH, NH_3-N, TVFA, and protozoa were not significantly altered by the ENZ treatment (Table 4).

A significant reduction in DMD (after 48 and 72h), CFD

Table 3: Effect of various exogenous enzyme (ENZ) doses addition to watermelon crop byproducts (WCB) on nutrient degradability after 24, 48, and 72 hours of incubation compared with berseem hay as a positive control.

	Berseem hay	ENZ levels added to WCB substrate				SEM	P-value		
		0 g/kg^{-1}	6 g/kg^{-1}	12 g/kg^{-1}	24 g/kg^{-1}		T	L	Q
Dry matter degradability (%) after									
24 h	39.00^{a}	32.97^{c}	34.48^{bc}	37.37^{ab}	37.42^{ab}	0.64	0.001	0.001	0.313
48 h	55.18^{a}	40.15^{b}	40.07^{b}	42.22^{b}	42.92^{b}	1.64	<0.001	0.911	0.217
72 h	59.96^{a}	44.89^{b}	47.83^{b}	50.68^{b}	50.98^{b}	1.21	<0.001	0.015	0.372
Crude fiber degradability (%) after									
24 h	21.14^{a}	6.21^{c}	6.75^{bc}	7.53^{bc}	9.83^{b}	1.51	<0.001	0.011	0.082
48 h	32.66^{a}	9.24^{c}	13.48^{bc}	18.77^{bc}	20.20^{b}	2.25	<0.001	0.004	0.525
72 h	36.98^{a}	10.30^{d}	20.2^{c}	27.70^{bc}	30.85^{ab}	2.20	<0.001	0.000	0.143
Crude protein degradability (%) after									
24 h	44.00^{ab}	38.88^{b}	43.35^{ab}	43.68^{ab}	46.48^{a}	0.88	0.041	0.007	0.579
48 h	58.41^{ab}	45.93^{c}	53.01^{bc}	58.04^{ab}	62.77^{a}	1.70	0.001	0.000	0.468
72 h	68.09^{a}	57.50^{c}	59.03^{bc}	59.80^{bc}	64.49^{ab}	1.04	0.001	0.093	0.103

Means in the same row bearing different letters differ significantly ($P < 0.05$); SEM indicates standard error of the mean; Probability of main effects of treatment (T), linear (L), and quadratic (Q).

Table 4: Effect of various exogenous enzyme (ENZ) doses addition to tomato crop byproduct (TCB) on cumulative gas production kinetics, predicted values, and fermentation parameter compared with berseem hay as a positive control

	Berseem hay	ENZ levels added to TCB substrate				SEM	P-value		
		0 g/kg^{-1}	6 g/kg^{-1}	12 g/kg^{-1}	24 g/kg^{-1}		T	L	Q
Gas production, ml/g DM									
3 h	46.38^{b}	36.88^{b}	51.75^{ab}	51.06^{b}	66.83^{a}	2.16	<0.001	<0.001	0.978
6 h	68.25^{ab}	51.44^{b}	65.80^{b}	63.72^{b}	85.25^{a}	2.51	<0.001	<0.001	0.472
12 h	102.63^{ab}	71.06^{c}	85.70^{bc}	84.11^{bc}	109.50^{a}	2.85	<0.001	<0.001	0.254
24 h	140.50^{a}	107.31^{b}	119.85^{ab}	119.33^{ab}	137.75^{a}	2.89	0.001	0.001	0.646
36 h	163.63^{a}	128.38^{b}	137.95^{b}	141.44^{ab}	162.92^{a}	3.18	0.001	0.001	0.328
48 h	178.00^{a}	138.44^{c}	147.25^{bc}	155.78^{abc}	170.50^{ab}	3.57	0.004	0.003	0.674
72 h	184.50^{a}	151.13^{b}	160.50^{ab}	167.50^{ab}	183.92^{a}	3.49	0.010	0.003	0.627
a	21.14^{b}	19.95^{b}	35.67^{ab}	36.19^{ab}	50.98^{a}	2.70	0.001	<0.000	0.792
b	157.77	140.70	133.05	146.27	143.25	3.49	0.358	0.492	0.647
c	0.05	0.04	0.04	0.04	0.04	0.002	0.542	0.987	0.632
a+b	178.91	160.65	168.72	182.45	194.23	4.26	0.113	0.006	0.806
Predicted values									
SCFA, mmol /0.2 g DM	0.62^{a}	0.46^{b}	0.54^{ab}	0.55^{a}	0.61^{a}	0.01	<0.001	<0.001	0.434
ME (MJ/kg DM)	5.52^{a}	4.36^{c}	4.91^{bc}	5.00^{ab}	5.39^{ab}	0.09	<0.001	<0.001	0.434
NE_L (MJ/kg DM)	2.90^{a}	2.05^{c}	2.45^{bc}	2.52^{ab}	2.81^{ab}	0.07	<0.001	<0.001	0.434
OMD, %	47.74^{a}	39.42^{c}	42.53^{bc}	43.07^{b}	45.27^{ab}	0.55	<0.001	<0.001	0.434
MCP (mg/g DM)	759.66^{a}	632.24^{b}	721.75^{a}	710.14^{a}	716.99^{a}	10.10	<0.001	0.010	0.073
PF (mg TDOM/ml gas)	1.71^{b}	1.89^{a}	1.75^{ab}	1.72^{b}	1.65^{b}	0.02	<0.001	<0.001	0.243
Fermentation parameters									
pH (24 h)	6.43^{a}	5.80^{b}	5.80^{b}	5.85^{b}	5.77^{b}	0.06	<0.001	0.877	0.369
pH (48 h)	6.42^{a}	6.10^{ab}	5.96^{b}	5.96^{b}	6.04^{b}	0.05	0.008	0.480	0.074
pH (72 h)	5.48	5.59	5.56	5.65	5.54	0.033	0.722	0.870	0.625
Ammonia-N, mg/dL	40.73^{b}	46.58^{ab}	46.90^{ab}	43.30^{ab}	52.80^{a}	1.39	0.041	0.165	0.102

TVFA, ml eq./L	165.00	223.25	218.25	208.25	246.60	10.39	0.196	0.496	0.347
Protozoa count, 10^3/mL	170.67^{b}	177.38^{ab}	241.88^{a}	164.13^{b}	170.90^{ab}	9.18	0.021	0.189	0.115

Means in the same row bearing different letters differ significantly (P < 0.05); SEM indicates standard error of the mean; Probability of main effects of treatment (T), linear (L), and quadratic (Q); a = the gas production from the immediately soluble fraction (ml); b = the gas production from the insoluble fraction (ml); c = the gas production rate constant for the insoluble fraction b (h); a+b = potential gas production (ml); ME, metabolizable energy; NE_L, net energy lactation; OMD, organic matter degradability; PF, partitioning factor at 72 h of incubation; SCFA, short-chain fatty acids; MCP, microbial crude protein production; TVFA is the total volatile fatty acids.

Table 5: Effect of various exogenous enzyme (ENZ) doses addition to tomato crop byproduct (TCB) on nutrient degradability after 24, 48, and 72 hours of incubation compared with berseem hay as a positive control.

	Berseem hay	ENZ levels added to TCB substrate				SEM	P-value		
		0 g/kg^{-1}	6 g/kg^{-1}	12 g/kg^{-1}	24 g/kg^{-1}		T	L	Q
Dry matter degradability (%) after,									
24 h	40.37	36.73	37.83	38.44	38.28	0.86	0.833	0.565	0.756
48 h	54.30^{a}	46.61^{c}	49.89^{bc}	50.95^{ab}	54.19^{a}	0.77	<0.001	<0.001	0.985
72 h	60.30^{a}	47.69^{b}	56.32^{a}	56.36^{a}	57.67^{a}	1.02	<0.001	0.019	0.117
Crude fiber degradability (%) after									
24 h	25.80^{a}	14.37^{b}	20.71^{a}	22.20^{a}	24.97^{a}	1.08	<0.001	<0.001	0.007
48 h	34.32^{ab}	25.54^{b}	31.49^{ab}	33.81^{ab}	35.88^{a}	1.21	0.031	<0.001	0.244
72 h	36.86^{ab}	30.36^{b}	34.23^{b}	36.07^{b}	47.67^{a}	1.66	0.003	0.001	0.351
Crude protein degradability (%) after									
24 h	46.75	46.34	49.19	50.54	53.37	0.90	0.057	0.012	0.099
48 h	60.41^{ab}	49.77^{b}	56.32^{ab}	59.12^{ab}	63.64^{a}	1.65	0.004	<0.001	0.844
72 h	67.26^{a}	55.35^{b}	64.15^{a}	64.15^{a}	65.84^{a}	1.04	<0.001	0.008	0.055

Means in the same row bearing different letters differ significantly (P < 0.05); SEM indicates standard error of the mean; Probability of main effects of treatment (T), linear (L), and quadratic (Q).

(after 24h), and CPD (after 72h) were evident in untreated TCB compared to berseem hay (Table 5). Treatment of TCB with ENZ displayed a linear increment in the DMD, CFD, and CPD. In particular, after 72h of incubation, a significant effect was apparent on the degradability of both DM and CP at 6, 12, and 24 g/kg of ENZ, while CFD was significantly affected at 24 g/kg (Table 5).

DISCUSSION

For enhancement of livestock productivity with minimal environmental impact, several nutritional strategies have been developed in recent years, like the use of plant byproduct and natural feed additives (Alsaht et al., 2014, Al-Sagheer et al., 2017, Ayyat et al., 2018). Herein, two types of crop residues treated with exogenous enzyme were assessed for the efficiency in enhancing feed degradability in ruminants. The results of decreased gas production of untreated TCB (12-72h of incubation) and WCB (6-12 h of incubation) is in line with the findings of Sallam et al. (2007). These authors found a significant decrease in gas production from linseed straw and rice straw compared to berseem hay. This finding might be related to the low feeding value of these crop residues. Haddi et al. (2003) noted a significant negative correlation between the rate of gas production and fiber contents (NDF and ADF) of the plant. The negative influence of fiber content on gas production might be related to the decreased ruminal microbes' activity through the lack of suitable environmental conditions for fermentation as incubation time progresses (Bakhashwain et al., 2010).

Our observations of increased gas production with increasing ENZ levels at all the incubation times in both crop residues are in harmony with numerous studies (Nsereko et al., 2002, López et al., 2013). This response might be due to the *Ruminococcus flavefaciens* content of the ENZ that stimulate ruminal microbial fermentation. Also, increased gas production up to 72 h of incubation period due to ENZ supplementation may reflect the increase in microbial numbers, and hence, the degradation efficiency of the ruminal microbes (López et al., 2013).

Herein, the untreated WCB and TCB showed significantly lower SCFA, MCP, OMD, DMD, CFD, CPD, and energy content, but higher PF compared with berseem hay. This observed decrease was probably because of low CP and high NDF, and ADL contents of tested crop byproduct (Bakhashwain et al., 2010). Parissi et al. (2005) reported

a positive link between ME or nutrient degradability and CP content. Also, there is a negative relationship between lignin, phenolics, and NDF with digestibility (Ammar et al., 2005). Similarly, the cell-wall content and assessed indicators like NE, OMD, SCFA, MP, and ME were considered negatively correlated, as reported by Parissi et al. (2005). The high protein lignification (nearly half protein content is associated with ADF) might clarify the low CP degradability (Ventura et al., 2009). Besides, Fondevila et al. (1994) showed that tomato byproducts have poorly degradable CP. Owing to the poor CP degradability and the great NDF lignification, the most degradable OM was non-structural carbohydrates (Ventura et al., 2009).

Our findings showed that ENZ addition to TCB and WCB resulted in a significant linear increase in SCFA, ME, NE, MCP, and OMD. Similarly, Gado et al. (2009) and Salem et al. (2013) have also shown that ENZ addition amplified SCFA amounts. Also, these results are comparable to those of Omer et al. (2009), who reported that ENZ supplementation to calves diets enhanced nutrient digestibility and rumen SCFA concentrations. Beauchemin et al. (2003) stated that ENZ supplementation augmented the digestible energy intake of ruminants fed diets rich in fiber content, and energy was the controlling nutrient in the diet. The linear increase of OMD, ME, MCP, and SCFA in TCB and WCB with the ENZ addition may have been because of amplified CFD and modify fermentation in the rumen (Nsereko et al., 2002). Additionally, the enhanced predicted parameters might also be due to increasing rumen microorganisms colonization to the cell wall of the plant (Wang et al., 2001, Nsereko et al., 2002). Also, the synergism between the ENZ and ruminal enzymes could be a potential ENZ mode of action (Morgavi et al., 2001). The enhanced DMD, CPD, and CFD due to ENZ addition to the incubation media containing TCB and WCB are in line with earlier studies that evaluated the same mixture of enzymes (Gado et al., 2009, Salem et al., 2013). This effect can be attributed to ENZ' ability to degrade the lignocellulose substrate complex into simple compounds that could alter its surface structure or weaken the chemical bond between lignocelluloses, making it easier to access ruminal microbial degradation or to promote ruminal microbial colonization and fermentation efficacy (López et al., 2013). Also, several modes of action are suggested, like the ability of ENZ to increase colonization of ruminal microbe on the feed particles surface (Yang et al., 2000). Also, ENZ capacity to augment colonization and enhance the entrance to the matrix surface via ruminal microbes to hasten digestion rate has also been suggested as a possible mode of action (Jalilvand et al., 2008). Moreover, ENZ has been reported to enhance the ruminal microbes hydrolytic potency because of enzyme activities or augmented synergism with enzymes of ruminal microbes (Morgavi et al., 2000). The obtained results suggest that the tested ENZ of *Ruminococcus flavefaciens* could have the potency to enhance ruminants' feed utilization efficiency, as evidenced by better gas production, *in vitro* DM, CP, and CF degradability.

CONCLUSION

This study is an attempt to improve the nutritional quality of two crop wastes (TCB and WCB) as feed for ruminants using a multi-enzyme feed additive resulted from *R. flavefaciens*. The low nutritional content of both crop residues resulted in dwindling in the *in vitro* gas production, nutrient degradability, and the energy content (ME and NE). However, WCB and TCB treatment with ENZ, especially at 24 mg/g DM, enhanced gas production and energy content and DM, CP, and CF degradation compared with untreated ones. Nevertheless, further studies are needed to be carried out to apply these results *in vivo*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

SMB, SAS, AAA conceived and designed the experiments. MMA, AAA performed the experiments, analyzed the data, and drafted the manuscript. SMB, SAS, AAA reviewed the manuscript and performed the final check. All authors read and approved the final manuscript.

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