Research Article



Influence of Fibrolytic Enzymes Supplementation on Lactation Performance of Ossimi Ewes

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Abstract | Fibrolytic enzymes can also enhance the utilization of feedstuff by increasing fiber digestion and degradation through increasing microbial communities in the rumen and reducing fluid viscosity thus this study aims to evaluate the effects of dietary fibrolytic enzymes supplementation on the performance of lactating Ossimi ewes. Twenty-one days postpartum 15 lactating Ossimi ewes (41±1 kg and at 3rd to 4th lactation season) were divided into three groups randomly (5 animals each). The 1st group (G1) was kept as control and fed on 50% concentrates feed mixture (CFM), 25% Egyptian clover (EC), and 25% rice straw (RS). The 2nd group (G2) was fed on a control diet with locally produced enzyme (LPE) at a concentration of 2g /kg DM. The 3rd group (G3) was fed on a control diet with commercial fibrolytic enzyme (POLYZYME*) at a concentration of 2g /kg DM. The results revealed increases (P ≤ 0.05) of crude fiber (CF) and crude protein (CP) digestibility in G2 and G3 diets compared to G1. The G2 and G3 increased (P ≤ 0.05) starch value (SV) and total digestible nutrient (TDN). Milk production yield (MPY) and 4% fat corrected milk (FCM) were increased (P≤0.05) in G2 and G3. Insignificant increases were recorded in milk compositions percentage among all the tested groups. Feed conversion of TDN and SV decreased (P ≤ 0.05) in G1 compared to G2 and G3 diets. Biochemical analysis revealed significant differences among all groups in overall means of glucose, where G3 was higher and all values within the normal range. From an economic point, the best net profit (L.E./head/63d) was recorded in G2, G3, and G1 diets.

Keywords | Lactating ewes, Digestibility, Milk yield, Milk composition, Blood serum

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INTRODUCTION

The increases in feed prices particularly grains and declines in costs of locally produced enzymes have promoted the interest in the use of enzyme additives in lactating animal diets for increasing the utilization of nutrients and ensuring profitability and sustainability of livestock production activities (Beauchemin et al., 2008). By using enzymes with fibrolytic properties in animal feed, an increase in ruminal energy utilization was recorded, as these products increase the number of enzymes that are available for digesting higher amounts of fiber in the rumen (Vinici et al., 2003).

Biological treatment of agricultural products by fibrolytic

January 2022 | Volume 10 | Issue 1 | Page 27

enzymes plays an important role in the degradations of cellulose, hemicellulose, and pectin, in turn; simplify those compounds which increase nutrient digestibility in animals supplemented with these enzymes (Aboul-Fotouh et al., 2016). Cellulase enzyme is a complex multi-enzyme system acts to hydrolyze cellulose from agriculture by-product for producing simple glucose units (Smith, 1996), while pectinase enzyme act for breaking down polysaccharide pectin structure present in the cell wall constituents into galacturonic acid monomers (El-Garhy et al., 2020a).

Several studies on enzymes that play an important role in lactating animals' diets reported an increase in yields of milk by 3–18% (Kholif et al., 2016, 2018; Morsy et al., 2016; Aboul-Fotouh et al., 2017; Azam et al., 2017; Tirado-

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Advances in Animal and Veterinary Sciences

González et al., 2018; Azzaz et al., 2019, 2020b; El-Garhy et al., 2020b). Thus, the current study aims to determine the influence of supplemented fibrolytic enzymes in lactating ewe's diet on digestibility of nutrients, milk yield, and composition, FCM yield, feed conversion, and some blood metabolites. In addition, an economic evaluation of the tested diets was carried out.

MATERIALS AND METHODS

ETHICS APPROVAL

Animal studies have been approved by the ethical committee. The research was performed following the ethical standard laid down in the 1996 declaration of Helsinki and its later amendments.

LOCATION, HOUSING, AND TIME OF THE EXPERIMENT

The current trial was performed at farm of Faculty of Agriculture while all analysis of this study were carried out at the laboratory of Animal Production Department, Fayoum University, Egypt, from January to March 2021. Ewes were kept outdoors with shelter during the day and housed in semi-open barn at night. They were maintained under the same managerial and environmental conditions. Ewes were healthy and clinically free of external and internal parasites. The lighting program by using light bulbs provided 13 h of light per day for each animal in the trial.

ANIMALS AND DIETS

Fifteen lactating Ossimi ewes at the 3^{rd} to 4^{th} season were used for the current study. The ewes were chosen based on their weight with an average body weight of 41 ± 1 kg. They were divided into 3 groups of five ewes randomly 21 days postpartum. The experiment was designed to last for 63 days.

The 1st group (control diet, G1) was fed on a diet of 50% concentrate feed mixture (CFM), 25% Egyptian clover (EC), and 25% rice straw (RS). The 2nd group (G2) was fed a control diet with locally produced enzyme (LPE) at a concentration of 2g /kg DM intake. The 3rd group (G3) was fed a control diet with a commercial fibrolytic enzyme (POLYZYME*) at a concentration of 2g /kg DM intake. The two pectinolytic enzymes were supplemented to the diets at the optimum rates as recommended by the manufacturer (IBEX international LTD). The CFM consisted of 55% yellow corn, 22.5% wheat bran, 20% soybean meal, 1% limestone, 0.5% dicalcium phosphate, 0.5% premix, and 0.5% NaCl on dry matter (DM) basis. The nutritional requirements of ewes were calculated according to NRC (1985) and fresh water was available freely during all the day time to the animals.

The locally produced enzyme (LPE) was extracted from *Aspergillus niger* which was grown onto sugar beet pulp media. Each gram contains 2500 units of cellulase and 450 units of pectinase. The POLYZYME[®] is a commercial enzyme that was purchased from IBEX International LTD company (United Kingdom), Egypt. Each gram contains 3000 units of cellulase and 500 units of pectinase.

SOURCE AND CHARACTERIZATION OF ENZYMES

The carboxymethyl-cellulase (CMC) and pectinase activities for the LPE and POLYZYME* enzymes were determined as recommended by Mandels et al. (1974) and Buga et al. (2010). Cellulase unit was determined as the quantity of enzyme that liberates reducing sugar at (1 μ mol/ml/min) under examination conditions (Miller, 1972). While pectinase unit was determined as the quantity of enzyme that produces 1 μ mol of D-galacturonic acid/ml/min at pH 5.0 and 40°C (Soares et al., 1999).

DIGESTIBILITY TRIAL

A digestibility trial was performed at the end of the lactation period. Digestibility of nutrients was determined using the acid insoluble ash (AIA) method as reported by Van Keulen and Young (1977). Samples of polled ewe's feces were combined daily for seven days. Feces were dried for 24 h at 70°C, crushed, dried for 3 hours at 105°C, weighed, and stored in bottles for the chemical analysis.

BLOOD SAMPLES

Two blood samples (5 ml) were taken from jugular vein of each animal in the trial through the last 2 days of each month of the experimental period (63 days) before morning feeding. A sample of blood was collected from the jugular vein into dry clean glass tubes per animal. Blood samples have been centrifuged for 15 minutes at 3500 rpm for serum separation. Serum was stored at -20°C for chemical analysis.

MILK PRODUCTION

The method of hand milking was used for calculating milk yield. Ewes were milked twice daily at 7:00 am and 7:00 pm by milking half udder while the other half udder lift for suckling young lambs as cited by El- Garhy et al. (2020b). Total milk yield and daily milk yield were estimated for each ewe in the trial period for 63 days. Six milk samples (300 ml each) were collected for each animal in the trial during the experimental period, milk samples were kept at (-20°C) until analysis of milk components immediately after the experiment is over.

SAMPLE ANALYSIS FEEDS AND FECES

Samples (250 g on DM basis) of concentrate feed mixture, Egyptian clover, and rice straw were collected for chemical



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analysis while there were seven samples of feces were collected for each animal at the end of the trial. Samples for each animal were polled and examined for the percentage of dry matter (DM), crude protein (CP) by using the macro Kjeldahl method, ether extract (EE), crude fiber (CF), and ash contents according to AOAC (2009). The nitrogenfree extract (NFE) was determined using the following formula:

Organic matter (OM) – (ether extract (EE) + crude protein (CP) +crude fiber (CF))

The cell wall constituents as neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined in feeds and feces concerning Goering and Van Soest (1970).

BLOOD SERUM

Commercial kits (Stanbio Laboratory, USA) were been used to analyze all blood serum metabolites according to the manufactures instruction and Spectrophotometer (T80 UV/VIS PG instrument Ltd, UK). The total protein that was determined by the biuret reaction as noted by the method of Armstrong and Carr (1964) and expressed as g/dl. Serum urea (mg/dl) was measured by using test kits according to Richard et al. (2011). Serum creatinine (mg/ dl) was determined by using test kits as reported by Spierto et al. (1979). Serum aspartate aminotransferase (AST, IU/L) and Alanine aminotransferase (ALT, IU/L) were measured by using test kits as mentioned by Reitman and Frankel (1957). Serum glucose (mg/dl) was determined at the wavelength of 500 nm by using test kits as investigated by Howanitz and Howantiz (1984). The concentration of serum cholesterol (mg/dl) was measured as investigated by Burtis et al. (2006).

MILK ANALYSIS

The total solids, protein, and ash of milk samples were determined by AOAC (2009). The milk fat samples were estimated by Gerber's according to Bradley et al. (1992). Solids not fat were calculated by the following formula:

Lactose content was calculated by difference by the following formula:

The 4% FCM was calculated by the equation of Gaines (1928) as the following:

Where; M= milk yield (gram/day), F=amount of fat= (milk concentrate feed mixture. Each value is a mean of 5 samples.

vield x fat %).

SIMPLE ECONOMIC EVALUATION

Simple financial returns of the tested diets were calculated as; the price of 1 kg of milk of the tested ewes was 12 L.E (Egyptian pound). The cost of 1 ton DM of CFM (93.56%) DM), EC (15% DM), and RS (91.8% DM) were 6000, 350, and 1450 L.E., respectively. Prices of 1 kg of LPE and POLYZYME[®] enzymes were 60 and 180L.E, respectively.

STATISTICAL ANALYSIS

The obtained data of nutrient digestibility, feed conversion, biochemical blood parameters, and milk compositions were statistically analyzed by one-way analysis of variance (ANOVA) to test the significance among means through the general linear model procedure reported by SPSS (2007) according to this model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where; Y_{ii} is observed traits, μ is the overall mean, T_{i} is the experimental group and e_{ij} is the random error. The significant differences among treatment groups were tested by using Duncan's Multiple Test (Duncan, 1955).

RESULTS AND DISCUSSION

CHEMICAL COMPOSITION OF THE EXPERIMENTAL DIETS

Chemical composition and cell wall constituents on DM basis of CFM, EC, and RS that were used in the current study are shown in Table 1. The chemical composition showed that RS was the highest levels of crude fiber, ash, NDF, ADF, hemicellulose, and cellulose compared to CFM and EC.

Table 1: Chemical composition of feed ingredients (on %DM basis).

Item	CFM	EC	RS
OM	96.64	82.50	77.45
СР	16.73	15.40	1.95
EE	2.86	2.70	1.39
CF	4.83	23.80	45.23
NFE	72.22	40.60	28.88
Ash	3.36	17.5	22.55
NDF	16.13	48.75	70.55
ADF	5.54	38.85	55.14
ADL	0.86	9.69	5.66
Hemicellulose	10.59	9.90	15.41
Cellulose	4.68	29.16	49.48

Hemicellulose= NDF-ADF; Cellulose= ADF-ADL; CFM,

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DIGESTIBILITY AND NUTRITIVE VALUES

The results of Table 2 showed that diets supplemented with LPE (G2) and POLYZYME® (G3) significantly $(P \le 0.05)$ increased CP and CF digestibility compared to (G1) control. There was a non-significant increase between all the tested diets concerning DM, OM, EE, and NFE digestibility. Moreover, a non-significant increase was recorded in G2 and G3 diets concerning CP and CF digestibility. Using enzymes with fibrolytic properties in animal feed can increase ruminal energy utilization as these products increase the number of enzymes that are available for digesting more fiber in the rumen (Vinici et al., 2003). Fibrolytic enzymes can enhance the utilization of feedstuff by increasing fiber digestion and degradation through increasing microbial communities in the rumen and reducing fluid viscosity of (Morgavi et al., 2000; Adesogan et al., 2014). The current results were nearly similar to those obtained by (Aboul-Fotouh et al., 2017; Arif et al., 2019; Khattab et al., 2019; El-Garhy et al., 2020b) who observed an increase in total tract digestibility of DM and OM when using fibrolytic enzymes in diets. The increased nutritive value of diets by the supplementation with LPE and POLYZYME® may be attributed to the availability of a readily great amount of fermentable carbohydrate liberated by the enzymatic action on cellulose and pectin of diets causing the improvement in nutrients digestibility. These results were following those obtained by El- Garhy et al. (2020b) who reported that dietary fibrolytic enzymes supplementation to lactating buffaloes contributed to significantly improved nutrients digestibility and nutritive value of the tested diets compared with animals of control.

Table 2: Impact of fibrolytic enzymes on digestion coefficients and nutritive values of the experimental groups of lactating Ossimi ewes.

The experimental groups							
Item	G1	G2	G3	± SE			
Nutrient digestibilities(%)							
DM	69.05	71.51	71.22	0.68			
OM	69.33	70.84	70.09	0.67			
СР	63.76 ^b	65.95ª	65.73ª	0.87			
CF	61.42 ^b	65.78ª	65.49ª	0.81			
EE	58.81	60.19	61.09	0.95			
NFE	68.81	70.26	69.36	0.53			
Nutritive values							
TDN (%)	61.16 ^c	64.93ª	63.22 ^b	0.55			
SV (%)	53.99°	57.71ª	56.02 ^b	0.54			
DCP (%)	8.03	8.68	8.21	0.13			

a, b, and c: Means in the same row with different superscripts are significantly different ($P \le 0.05$). Each value is a mean of 5 samples.

The nutritive values of the experimental groups as total digestible nutrients (TDN), digestible crude protein

Advances in Animal and Veterinary Sciences

(DCP), and starch value (SV) were revealed in Table 2. The supplemented diets (G2 and G3) significantly ($P \le 0.05$) increased SV and TDN compared to the G1 diet (control). Moreover, the diet of G2 significantly ($P \le 0.05$) increased TDN and SV% compared to the G3 diet. There were non-significant increases between all the experimental groups in DCP.

FEED INTAKE AND FEED CONVERSION

Daily feed intake of lactating Ossimi ewes in Table 3 revealed non-significant differences between the tested groups. Aboul-Fotouh et al. (2017); Khattab et al. (2019); El-Garhy et al. (2020b) mentioned that there are no effects on daily feed intakes of DM when enzymes were added to an animal's diet.

Table 3: Fibrolytic enzymes effect on feed intake and feedconversion in lactating Ossimi ewes.

Items	The exper	± SE		
	G1	G2	G3	
Average yield of 4% FCM (g/head/day)	535.83 ^b	678.81ª	706.92ª	25.42
Average daily feed intal	ke/head			
DM, g	1280	1310	1300	0.17
TDN,g	782.85	850.58	821.86	0.45
SV, g	691.07	756.00	728.26	0.30
DCP, g	102.78	113.71	106.73	0.20
Feed conversion *				
DM/ g/g milk	2.39	1.93	1.84	0.11
TDN/ g/g milk	1.46ª	1.25 ^b	1.16 ^b	0.04
SV/ g/g milk	1.29ª	1.11 ^b	1.03 ^b	0.16
DCP/ g/g milk	0.19	0.17	0.15	0.01

a and b: Means in the same row with different superscripts are significantly different ($P \le 0.05$). *, Feed conversion was determined depending on daily FCM. Each value is a mean of 5 samples.

Daily feed conversion of lactating Ossimi ewes in Table 3, revealed that TDN and SV in the diet of G1 were significantly (P≤ 0.05) decreased compared to G2 and G3 diets. In contrast, there were non-significant differences in feed conversions of DM and DCP between the tested diets. Moreover, there were non-significant differences concerning feed conversions between G2 and G3. The current results were consistent with the findings of Mohamed et al. (2013) who mentioned that adding exogenous fibrolytic enzymes in diets of lactating animals had decreased (P≤0.05) feed conversions compared to control not supplemented diets. El-Garhy et al. (2020b) found that adding enzymes to buffalo's diet decreased (P≤0.05) feed conversions compared. Moreover, the decrease in feed conversions by ewes supplemented with enzymes may be attributed to the improvement of the feed nutrients digestibility.



OPEN OACCESSAdvances in Animal and Veterinary SciencesTable 4: Influence of fibrolytic enzymes supplemented diets on some blood parameters of lactating Ossimi ewes.

Items	The experimental groups		± SE	Physiological range	References of physiological	
	G1	G2	G3			range
Total protein (g/dl)	7.2	6.4	6.6	0.27	6.1-7.5	Boyd (2011)
Creatinine (mg/dl)	1.11	1.17	1.12	0.04	0.7–1.5	
Urea(mg/dl)	34.5	33.92	33.75	0.87	10-50	Kaneko (1989)
AST (IU/L)	82	84	81	4.7	Up to 275	
ALT (IU/L)	19	25	22.7	1.66	30-5	
Glucose(mg/dl)	57.67°	63.33 ^b	67ª	1.23	48-76	Boyd (2011)
Cholesterol (mg/dl)	77	81	78	2.35	65–136	

a, b, and c: Means in the same row with different superscripts are significantly different ($P \le 0.05$). Each value is a mean of 5 samples.

BLOOD SERUM

Influence of LPE and POLYZYME® enzymes supplemented diets on serum total protein, creatinine, urea, AST, ALT, cholesterol, and glucose of lactating Ossimi ewes were shown in Table 4. The addition of fibrolytic enzymes to the ewes' diet showed significant (P≤ 0.05) increases in glucose concentration. The diets supplemented with POLYZYME® (G3) had increased $(P \le 0.05)$ serum glucose concentration compared to LPE (G2). Moreover, the diets which were supplemented with fibrolytic enzyme had recorded a reduction in total protein and urea concentrations compared to control. There were non-significant differences between all experimental groups in overall means of blood concept glucose and all values of serum biochemical parameters in the current study were within the normal range. Arif et al. (2019) reported that increasing the concentration of blood glucose may be attributed to the action of enzymes that release soluble sugars in animals fed on diets supplemented with the enzyme. Increasing the concentration of blood glucose in animals may be due to a high fermentable carbohydrate which increased concentrations of volatile fatty acids especially propionate which is the precursor of glucose synthesis as cited by El-Garhy et al. (2020b). The lower serum total protein and urea concentration in the current study may be attributed to high protein utilization by lactating Ossimi ewes supplemented with fibrolytic enzymes. El-Garhy et al. (2020b) reported reductions in serum urea concentrations that may be attributed to the use of urea for protein synthesis on the rumen hepatic pathway.

MILK YIELD AND COMPOSITION

Table 5 revealed that diets supplemented with enzymes (G2 and G3) significantly ($P \le 0.05$) increased milk yield and FCM yield compared to (G1) control. The control group (G1) showed the lowest milk yield followed by G3. The highest value was recorded in G2. In addition, there were non-significant increases in milk yield and FCM yield between groups supplemented with fibrolytic enzymes (G2 and G3). Furthermore, adding LPE increased milk yield by 25.56% and FCM by 26.68%, while adding POLYZYME^{*} increased milk yield by 24.74% and FCM yield by 31.9%.

In contrast, there was a non-significant increase in all the experimental groups in milk compositions percentage. While G2 and G3 diets significantly ($P \le 0.05$) increase milk components yield of total solids, SNF, fat, total protein, and lactose. There were non-significant increases in all the experimental groups in ash yield. Many kinds of research on enzymatic supplementation to lactating animals diets shown an increase in milk yields by 3-18% (Kholif et al., 2016, 2018; Morsy et al., 2016; Aboul-Fotouh et al., 2017; Azam et al., 2017; Tirado-González et al., 2018; Azzaz et al., 2019, 2020b; El-Garhy et al., 2020b). The results of 4% FCM yield were in agreement with the findings obtained by (Aboul-Fotouh et al., 2017; Arif et al., 2019; Khattab et al., 2019; El-Garhy et al., 2020b) who reported an increase in FCM yield for lactating animals fed fibrolytic enzymes compared with control animals. The current results may reflect the influence of fibrolytic enzymes as the current results reported greater amounts of digested fiber in the rumen that provide more acetate for fatty acid synthesis. Moreover, El-Garhy et al. (2020b) reported that the enhancement in milk yield may be attributed to the large amounts of nutrients absorbed in the rumen and gastrointestinal tract.

A SIMPLE ECONOMIC EVALUATION OF THE TESTED DIETS

Economic evaluations for the experimental groups are shown in Table 6. The net profit (L.E./head/63day) was increased for lactating Ossimi ewes fed on diets supplemented with LPE and POLYZYME® enzymes. The best net profit (L.E./head/63 days) was recorded by lactating Ossimi ewes fed a diet supplemented with LPE followed by those fed a diet supplemented with POLYZYME* then control diet. The cost of DM consumed by ewes fed on diet with adding POLYZYME® was higher than the other tested diets as the price of 1 kg of POLYZYME® was higher. Aboul-Fotouh et al. (2017) and El-Garhy et al. (2020b) mentioned that the economic values were increased for animals fed with diets supplemented with laboratory enzymes compared to commercial enzyme and control diets. The supremacy of diet (G2) regarding values of net profit may be due to the lower cost of LPE enzyme compared to POLYZYME®.



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 Table 5: Influence of fibrolytic enzymes supplemented diets on milk yield and composition of lactating Ossimi ewes.

Items	The experimental groups			± SE			
	G1	G2	G3				
Average milk yield (g/head/day).	457 ^b	573.8ª	570.1ª	22.33			
Average 4% Fat corrected milk yield (g/head/day)	535.83 ^b	678.81ª	706.92ª	25.42			
Milk compositions %							
Total solids	14.68	14.93	15.62	0.32			
Fat	5.15	5.22	5.60	0.15			
SNF	9.53	9.71	10.02	0.30			
Total protein	3.75	3.71	4.03	0.12			
Lactose	5.07	5.27	5.31	0.21			
Ash	0.71	0.73	0.68	0.02			
Milk components yield (g/head/day)							
Total solids	67.09 ^b	85.67ª	89.05 ^a	2.97			
Fat	23.54 ^b	29.95ª	31.93ª	1.24			
SNF	43.55 ^b	55.72ª	57.12ª	1.86			
Total protein	17.14^{b}	21.29ª	22.98ª	0.96			
Lactose	23.17 ^b	30.24ª	30.27ª	1.18			
Ash	3.24	4.19	3.88	0.17			

a and b: Means in the same row with different superscripts are significant ($P \le 0.05$). Each value is a mean of 5 samples.

Table 6: Fibrolytic enzymes supplemented diets effect on economic evaluation of lactating Ossimi ewes.

Item	The experimental groups		
	G1	G2	G3
Milk yield (kg/head/63day)	28.79	36.15	35.9
Dry matter consumed (kg / head /63day)	80.64	82.53	81.9
Fibrolytic enzymes (g/head/63day)	0	165.1	163.8
Price of one kg dry matter of the diet, L.E	4	4.16	4.47
Cost of feed consumed (L.E / head / 63 day)	322.56	343.32	366.09
Total profit, L.E*	345.5	433.8	430.8
Net profit, L.E**	22.94	90.48	64.71

*, Total profit, L.E= Milk yield (kg /head/63 days) × 12 L.E (the price of one kg ewes milk). **, Net profit (L.E./ head/63day) = Total revenue (L.E./ head/63day) - cost of feed consumed (L.E./ head/63day). Each value is a mean of 5 samples.

CONCLUSIONS AND RECOMMENDATIONS

Supplementation of diets of lactating Ossimi ewes with the LPE and POLYZYME[®] enzymes contributed to an increase of nutrient digestibility and significantly increased milk yield and FCM. In a simple economic study, the diet of LPE was the best one and all mean values of some blood serum metabolites were within the normal range.

NOVELTY STATEMENT

This study contributes to improving the nutrient digestibility, feeding values ,feed conversion and milk production of Ossimi ewes using locally and commercial fibrolytic enzymes.

AUTHOR'S CONTRIBUTION

GAM, MAA, OAH conceived and designed the experiment. GAM, MAA, OAH took a part in experimenting. GAM preparation, writing, and editing the manuscript.

STATEMENT OF CONFLICT OF INTEREST The authors have declared no conflict of interest.

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Advances in Animal and Veterinary Sciences

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