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ORIGINAL PAPER

Dietary Date Palm Leaves Ensiled with Fibrolytic Enzymes Decreased Methane Production, and Improved Feed Degradability and Fermentation Kinetics in A Ruminal In Vitro System

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Abstract

Purpose The present experiment aimed to evaluate date palm leaves (DPL) treated without or with fibrolytic enzymes as a feed for ruminants.

Methods The experiment employed an in vitro wireless gas production system to evaluate the dietary inclusion of DPL as sun-dried, DPL ensiled without or with fibrolytic enzymes for 45 days. The different forms of DPL replaced berseem hay (300 g/kg diet) at 0, 25, 50, 75 and 100% in the diet.

Results Dried DPL linearly decreased the asymptotic total gas production (GP), rate of methane (CH_4) and carbon dioxide (CO_2) production, and acid detergent fiber degradability, and increased the lag of total GP ($P < 0.05$). The ensiled DPL also linearly decreased ($P < 0.05$) the asymptotic total GP, asymptotic CH_4, asymptotic CO_2 production and the rate of CH_4 and CO_2 productions, but dry matter degradability and total volatile fatty acid (VFA) concentrations were unaffected. Date palm leaves treated with fibrolytic enzymes linearly decreased the asymptotic total GP, CH_4 and CO_2 productions, and the rate of CH_4 and CO_2 production. Ensiling of DPL with fibrolytic enzymes increased ($P < 0.05$) dry matter and fiber degradability and the concentrations of ruminal ammonia-N and total VFA.

Conclusion It is concluded that DPL treated with fibrolytic enzymes can replace berseem hay up to 100% in the diet to reduce CH4 production from ruminants. Ensiling with fibrolytic enzymes is recommended as a sustainable strategy to reduce environmental pollution and utilization of DPL.

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Graphical Abstract

Keywords Date palm leaves · Ensiling · Fibrolytic enzymes · Greenhouse gases · In vitro fermentation · Sustainability

Statement of Novelty

Under semi-arid and arid conditions, trees and shrubs such as date palm can be used as an adequate source of feed for goats and sheep to reduce feed cost. However, the low nutritive value of such materials limits their dietary use. Ensiling with fibrolytic enzymes can be used to enhance the nutritive value of date palm leaves and other agriculture byproducts before feeding to animals. Exogenous fibrolytic enzymes can alter the structure of the tissues and enhance nutrient digestibility, resulting in improved performance (daily gain or milk production). This may enhance farmers' gain and animal health. This is the first experiment to utilize date palm leaves after ensiling with fibrolytic enzymes to enhance the nutritive value of date palm leaves as an unconventional feed.

Introduction

Utilization of agricultural byproducts may be considered as a strategy to minimize environmental pollution, reduce cost of animal feeding, and broaden the feedbase reducing food-feed competition [1]. The inclusion of agricultural and industrial byproducts in the diets of animals, which sometimes contain neutraceutical properties, has shown beneficial responses on the ruminal fermentation and animal performance [2, 3]. However, upgrading the nutritive value of agricultural and industrial byproducts and judicious utilization are recommended for feeding to animals [4].

Egypt produces large amounts of agricultural byproducts without a significant utilization [4, 5]. One of the major crops produced in Egypt is date palm (*Phoenix dactilifera*) with about 14 million date palm trees yielding about 650,000 tons dry matter (DM) annually [1]. On average, about 20 kg of leaves are produced from each tree. The main problems of feeding date palm leaves (DPL) are the low nutritive value and high fiber contents with 5–16.5% crude protein (CP) and 42.7–72.4% neutral detergent fiber (NDF) [1, 6]. Recently, Hamdon et al. [7] reported that growing lambs fed DPL-based diets with/without direct-fed microbials improved live-weight gain, total feed intake and feed efficiency compared with the lambs fed a control diet (a concentrate feed mixture and wheat straw at a ratio of 60:40). Besides nutrient content, tree leaves contain many plant secondary metabolites, namely, polyphenolics, saponins and tannins, which exert both positive and negative effects on the ruminal fermentation and ruminant production depending upon the level of inclusion [8, 9]. Greater levels of inclusion in diets results in adverse performance due to lowered digestibility and adverse ruminal fermentation because polyphenolic compounds including tannins inhibit ruminal microbiota, especially fiber degrading bacteria [8, 10]. However, an optimum level of tannins in diets results in improved ruminal fermentation and ruminant performance due to inhibition of methanogenic archaea and protein degrading bacteria in the

rumen and formation of tannin-protein complex resulting in decreased methane (CH_4) production, protein degradation and ammonia-N (NH_3–N) concentration in the rumen [10, 11]. Along with many tree leaves, DPL are rich in polyphenolic compounds including flavonoids and tannins and possess antioxidant and radical scavenging activities [12]. Therefore, the use of optimum level of DPL may be beneficial in terms of decreasing methane CH_4 production and protein degradation in the rumen and health of animals.

Biodegradation of lignocellulose materials in the agricultural byproducts has been documented using exogenous enzymes [13, 14]. The most common methods of exogenous enzymes administration are direct feeding [15, 16] or pretreatment of feeds [17]. Administration of fibrolytic enzymes in diets of animals showed mixed results. However, the main finding of most experiments was the enhanced nutritive value of agricultural byproducts [4]. Fibrolytic enzymes alter ruminal fermentation, and enhance fiber digestibility through solubilizing dietary fiber, supplying readily fermentable nutrients to microorganisms, and increasing microbial enzyme activities and microbial attachment to feed particles in the rumen [18]. The dose of fibrolytic enzymes administration and feed type are major factors affecting the response to their administration [19]. High doses of fibrolytic enzymes can negatively affect the responses associated with their administration in diets of ruminants because high levels of exogenous enzymes may prevent binding of exogenous enzymes to substrates resulting in decreased rate of attachment by ruminal microbiota to feed particles [13, 20]. Such results can partially explain the inconsistencies between experiments [19].

We hypothesized that DPL and rice straw (RS) ensiled with fibrolytic enzymes may enhance the nutritional value of DP and RS silage, and their use in the complete diets may reduce greenhouse gases production and improve ruminal fermentation. Therefore, the objectives of the present experiment were to evaluate the nutritive value, in vitro biogas (total gases, $CH_4$and carbon dioxide (CO_2) production, and ruminal fermentation of DPL (dried, ensiled without enzyme or treated with fibrolytic enzymes) with inclusion of different levels of DPL in the diets by replacing berseem hay.

Materials and Methods

Ingredients, Ensiling and Diets

Fresh DPL were collected from different sites in the New Valley Governorate (Egypt) and sun-dried for 10 days [7]. Sun-dried rice straw was collected from local suppliers in Egypt. Date palm leaves, rice straw and vegetable/fruits byproducts (bought from local markets and based mainly on carrot roots, tomatoes, cabbage leaf and courgette at 1:1:1:1 DM weight) were individually ensiled under anaerobic conditions for 45 days using tightly closed plastic sheets [21]. Briefly, the chopped materials (i.e., DPL and rice straw) were spread individually with a solution containing 40 g/L of urea and 40 g/L of molasses. Before ensiling, moisture content was increased to reach about 35–40% and fibrolytic enzymes were added at 4 g/kg DM. The materials were packed into polythene bag silos (40 × 70 cm) and compressed manually to create anaerobic environment. Then, silo bags were sealed and kept indoors on a dry concrete floor. For DPL and rice straw, two types of treatments were employed: DPL and rice straw ensiled without fibrolytic enzyme or with fibrolytic enzymes (Polyzym, Zeus Biotech, India) at 4 g/kg DM. The enzyme product contained (per kg DM): 4×10^6 IU xylanase, 4×10^5 IU cellulase and 2×10^5 IU β-glucanase. For assessment of ensiling process, silage sample (200 g fresh weight) was mixed with 800 mL distilled water, homogenized for 3 min with a blender and filtrated through 4-layer cheesecloth. The filtrate was collected measurement of pH using a digital pH meter (Thermo Scientific, Orion Star™ A121, Beverly, MA, USA), NH_3–N according to AOAC [22], and volatile fatty acids (VFA) according to AOAC [22]. Aflatoxin (AF_1) concentration was measured in silage with the use of a Fluorometer, Series-4 (VICAM, USA) based on the methods described by AOAC [22]. A basal total mixed ration (TMR) was prepared to be used as a substrate, and contained (per kg DM): 500 g concentrates feed mixture, 100 g ensiled vegetable/fruits byproducts, 100 g chopped and non-ensiled rice straw and 300 g of berseem hay. Berseem hay was replaced with DPL (dry, ensiled without enzyme or ensiled with fibrolytic enzymes) at 25, 50, 75 and 100%. Nutrient contents of ingredients and TMR are shown in Tables 1 and 2, respectively.

In Vitro Fermentation and Biodegradation

Before the incubation process, the incubation medium containing buffer, macro-mineral, micro-mineral and resazurin solutions and distilled water were prepared according to Goering and Van Soest [23] and mixed in a volumetric flask using a magnetic stirrer and a hot plate set at 39 °C. A reducing solution of sodium hydroxide and sodium sulfide was added (2 mL) to the buffer shortly before rumen fluid addition. Thereafter, the ruminal inoculum (20 mL) and the buffer (80 mL) solution were mixed in each 250-mL bottle.

Rumen inoculum was collected from the rumen of three sheep from a local slaughterhouse at Cairo (Egypt). Before slaughtering, sheep were fed ad libitum a diet containing concentrate mixture, berseem hay and rice straw at 500:400:100 (DM basis), with free access to water. Rumen contents were taken in a plastic thermos maintained at 39 °C and transported to the laboratory where it was continuously flushed with CO_2. Upon arrival at the laboratory, the rumen

Table 1 Chemical composition (g/kg DM) of the ingredients used in the substrates for the in vitro study

	Concentrate feed mixture	Berseem hay	Ensiled vegetable/fruit byproducts	Date palm leaves			Rice straw		
				Dried without treatment	Ensiled without enzymes	Ensiled with enzymes	Dried without treatment	Ensiled without enzymes	Ensiled with enzymes
Dry matter	903	890	412	893	741	754	943	749	751
Organic matter	923	884	934	912	907	908	849	851	847
Crude protein	165	128	61	40	47	50	43	43	48
Ether extract	47	54	74	23	22	21	19	20	19
Nonstructural carbohydrates	414	224	428	271	276	346	159	167	229
Neutral detergent fiber	297	478	371	578	562	491	628	621	551
Acid detergent fiber	175	381	283	322	316	297	397	391	332
Cellulose	142	329	125	199	194	172	314	309	253
Hemicellulose	122	97	88	256	246	194	231	230	219
pH					4.3	3.8		4.8	4.5
Ammonia-N (g/kg of total N)					64	51		89	58
Total volatile fatty acids					73	89		82	88
Aflatoxin F_1 (μg/kg of DM)					3.2	3.0		3.0	3.1

Table 2 Ingredients and chemical composition of the experimental diets[a] (g/kg DM)

	Control	Dry date palm leaves				Date palm leaves ensiled without fibrolytic enzymes				Date palm leaves ensiled with fibrolytic enzymes			
	0%	25%	50%	75%	100%	25%	50%	75%	100%	25%	50%	75%	100%
Ingredient													
Berseem hay	300	225	150	75	0	225	150	75	0	225	150	75	0
Date palm leaves[b]	0	75	150	225	300	75	150	225	300	75	150	225	300
Rice straw[b]	100	100	100	100	100	100	100	100	100	100	100	100	100
Ensiled vegetable/fruit byproducts	100	100	100	100	100	100	100	100	100	100	100	100	100
Concentrate mixture	500	500	500	500	500	500	500	500	500	500	500	500	500
Chemical composition													
Dry matter	854	854	855	855	855	824	812	801	790	825	815	804	794
Organic matter	905	907	909	911	913	907	909	910	912	907	908	910	912
Crude protein	131	125	118	112	105	125	119	113	107	126	120	114	108
Ether extract	49	47	44	42	40	47	44	42	39	47	44	42	39
Nonstructural carbohydrates	333	336	340	343	347	338	342	345	349	349	358	367	377
Neutral detergent fiber	392	399	407	414	422	397	404	410	416	385	386	387	388
Acid detergent fiber	270	265	261	257	252	264	259	255	250	257	251	244	238
Cellulose	214	204	194	184	175	203	193	183	173	196	184	172	160
Hemicellulose	122	134	146	158	170	133	144	155	167	128	135	143	150

[a]Date palm leaves at different forms (dried, ensiled without enzymes or ensiled with fibrolytic enzymes) replaced the berseem hay at 0, 25, 50, 75 or 100%, DM basis

[b]Date palm leaves and rice straw were dried or ensiled without enzymes or ensiled with enzymes for the respective diets

fluid was filtered through two-layer cheesecloth to remove large feed particles, and the particulate materials were squeezed to obtain microbes attached to feed particles. The initial pH of the medium with inoculum was maintained at 6.8–6.9.

All replacement levels were tested in two 96-h incubation runs with 3 replicates per run. In each incubation run, 2 bottles containing inoculum without feed (blanks) were also included to establish baseline fermentation gas production (GP). The substrate of each TMR diet (1 g ± 10 mg) was weighed into filter bags (ANKOM F57; Ankom Technology, Macedon, NY, USA). The filter bags were then put into 250-mL ANKOM bottles (ANKOM^{RF} Gas Production System) fitted with an automatic wireless in vitro GP module (Ankom Technology, Macedon, NY, USA) with pressure sensors.

The gas volumes (mL) at standard pressure and temperature were calculated from the pressure of the accumulated gas in the bottles. The average baseline gas measured in the blank bottles was subtracted (blank corrected GP). Gas volumes were recorded at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, 72 and 96 h of incubation. At each incubation time, 5 ml of headspace gas was taken from each bottle and infused into a Gas-Pro detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK) to measure the concentration of CH_4 and carbon dioxide (CO_2).

After 96 h of incubation, the fermentation was stopped by swirling the bottles in ice for 5 min. After opening the bottles, the pH was measured immediately using a pH meter (Thermo Scientific, Orion Star™ A121, Beverly, MA, USA). The filter bags were removed from the bottles and dried in a forced air oven set at 55 °C for 48 h. Dry matter, NDF and acid detergent fiber (ADF) degradation were calculated by subtracting and the weight of the dried residue left after incubation from the initial weight of the dried substrate. Total GP (mL) and production of CH_4 and CO_2 are reported in relation to degraded DM, degraded NDF, and degraded ADF at 96 h of incubation.

Sampling and Analysis of Fermentation Variables

After 96 h of incubation, supernatant fluid samples (5 mL) from each bottle were collected in glass tubes for determination of NH_3–N and total and individual VFA concentrations. A subsample of 3 mL was preserved with 3 mL of 0.2 M hydrochloric acid solution for NH_3–N analysis according to AOAC [22]. Another subsample (0.8 mL) was mixed with 0.2 mL of metaphosphoric acid (250 g/L) solution for total VFA analyses by titration after steam distillation. Individual VFA were measured using a chromatography after processing 1.6 mL of strained rumen fluid with 0.4 mL of a solution containing 250 g of metaphosphoric acid as described previously [24].

Chemical Analysis of Substrates

Samples of ingredients and formulated TMR were analyzed for ash after burning the samples in a muffle furnace at 550 °C for 12 h (method ID 942.05), ether extract (EE) using diethyl ether in a Soxhlet extractor (method ID 920.39), and N using Kjeldahl method (method ID 954.01) according to AOAC [22] methods. The concentration of NDF was determined by the procedure of Van Soest et al. [25] without the use of alpha amylase but with the use of sodium sulfite. The content of ADF (method ID 973.18) was analyzed according to AOAC [22] (method ID 973.18), and was expressed exclusive of residual ash. Lignin content was analyzed by solubilizing cellulose with sulfuric acid in the ADF residue according to Van Soest et al. [25]. Non-structural carbohydrate (NSC), cellulose, hemicellulose, and organic matter (OM) were calculated.

Calculations and Statistical Analyses

For the determination of different gas production kinetic, total gas, CH_4 and CO_2 volumes (mL/g DM) were fitted using the NLIN procedure of SAS (2021, Version 9.4, SAS Inst., Inc., Cary, NC) according to France et al. [26] model as: $y = b \times [1 - e^{-c\,(t-Lag)}]$ where *y* is the volume of total gas, CH_4 or CO_2 at time *t* (h); *b* is the asymptotic total gas, CH_4 or CO_2 production (ml/g DM); *c* is the fractional rate of gas production (/h), and *Lag* (h) is the discrete lag time before any gas production.

Data were analyzed using the GLM procedure (SAS Inst. Inc. Cary, NC, USA) for a completely randomized design using the model:

$$Y_{ijk} = \mu + R_i + D_j + (R \times D)_{ij} + \varepsilon_{ijk}$$

where: Y_{ijk} is the observation, μ is the population mean, R_i is the ration type effect, D_j is the replacement level effect, $(R \times D)_{ij}$ is the interaction between ration type and replacement level, and ε_{ijk} is the residual error. Linear and quadratic contrasts were used to examine replacement level responses within each ration type. In addition, means also were compared using orthogonal contrasts (i.e., dried DPL without ensiling vs. ensiled DPL without enzymes, and ensiled DPL without enzymes vs. ensiled DPL with enzymes).

Results

Chemical Composition

Ensiling with or without fibrolytic enzymes increased CP concentration in ensiled DPL (Table 1). Administration

of fibrolytic enzymes increased NSC concentration and decreased the concentration of NDF, ADF, cellulose and hemicellulose in ensiled DPL and rice straw. The ensiled materials had good silage characteristics with pH values of 4.3 for DPL silage and 3.8 for DPL silage treated with fibrolytic enzymes. The ensiled rice straw was of relatively lower quality with pH values of 4.8 and 4.5 for ensiled without enzymes and with enzymes, respectively. Ammonia-N concentrations were lower than 10% of total N and aflatoxin F_1 level below 3.2 µg/kg of DM for all ensiled materials.

The inclusion of dried DPL in the TMR did not affect DM concentration; however, ensiling without or with fibrolytic enzymes decreased DM concentration (Table 2). Inclusion of dried DPL decreased CP concentration and increased NSC concentration in the TMR. The inclusion of ensiled DPL without fibrolytic enzymes increased NDF concentration, whereas the fibrolytic enzymes-treated DPL in TMR decreased NDF, ADF and cellulose concentrations while increased hemicellulose concentrations.

Biogas Production

In vitro GP (mL/g incubated DM) of TMR containing different levels of DPL in different forms is presented in Fig. 1. Diet × replacement interactions were observed ($P < 0.01$) for the rate of total gas and CH_4 productions (Table 3). Additionally, diet affected ($P < 0.05$) kinetics of total gas, CH_4 and CO_2 productions, while replacement level affected the asymptotic total GP, the rate of GP and the lag of CO_2 production.

Figures 2 and 3 show the in vitro rumen CH_4 and CO_2 production (mL/g incubated DM) of TMR containing different levels of DPL in different forms. As shown in Table 3, the TMR containing different levels of dried DPL linearly decreased the asymptotic total GP and the rate of CH_4 and CO_2 production, and increased the lag of total GP ($P < 0.05$). Replacing berseem hay with the ensiled DPL (without enzymes additives) linearly decreased ($P < 0.05$) the asymptotic total gas, CH_4, CO_2 production and the rate of CH_4 and CO_2 productions. Moreover, replacing berseem hay with the DPL treated with fibrolytic enzymes linearly decreased the asymptotic total gas, CH_4 and CO_2 productions, and the rate of CH_4 and CO_2 production.

Diet × replacement interactions were observed for CH_4 and CO_2 productions expressed as per unit of degraded DM (Table 4), while TMR type affected CH_4 and CO_2 production per unit of degraded NDF and ADF and proportional CH_4 production. The inclusion level affected CO_2 production per gram of degraded DM, degraded NDF and degraded ADF as well as proportional CH_4 and CO_2 productions. Without affecting CO_2 production (per unit of degraded DM, NDF, ADF) and proportional CO_2, replacing berseem hay with dried DPL linearly increased CH_4 production per unit of degraded DM and proportional CH_4 production, and decreased CH_4 production per unit of degraded NDF. Replacing berseem hay with the ensiled of DPL (without fibrolytic enzymes) linearly ($P < 0.01$) decreased CH_4 and CO_2 productions per unit of degraded DM, CO_2 production per unit of degraded NDF and CH_4 and CO_2 productions per

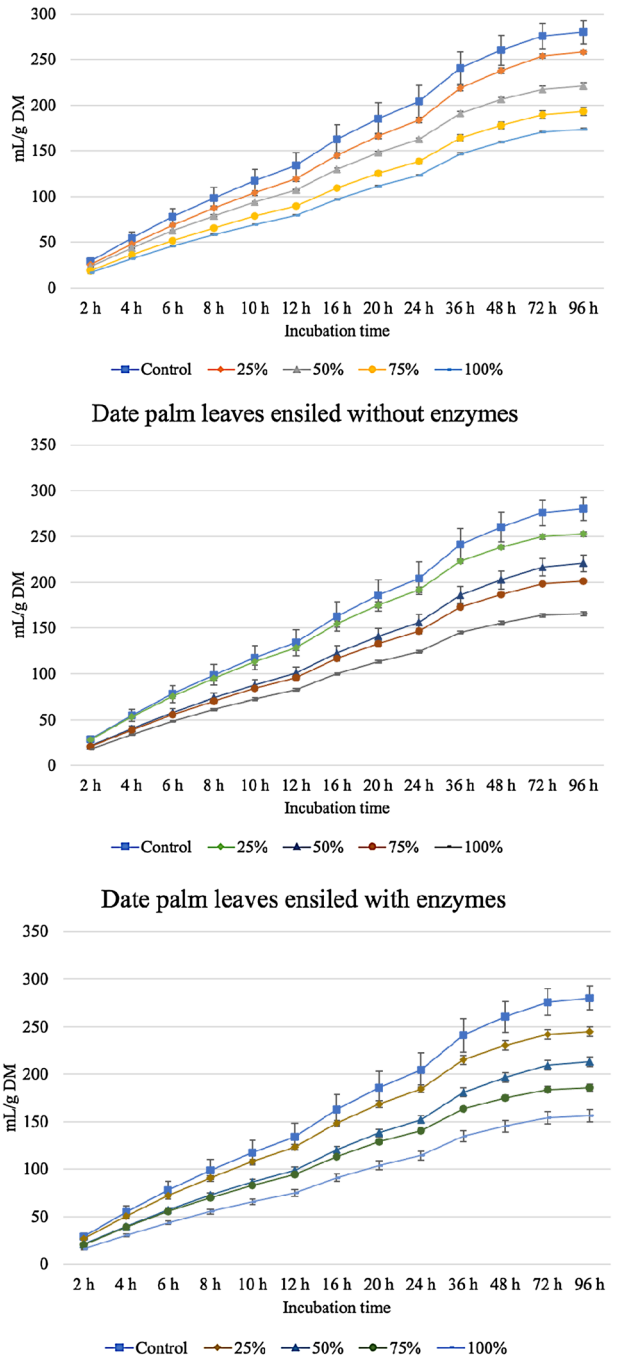

Fig. 1 In vitro rumen gas production (mL/g incubated DM) of TMR containing different levels of date palm leaves in different forms (P values: diet < 0.001, replacement level < 0.001, diet × replacement level = 0.231)

Table 3 Total gas, methane (CH_4) and carbon dioxide (CO_2) production kinetics of diets containing different levels of dried date palm leaves, or date palm leaves ensiled with or without fibrolytic enzymes

Ration[a]	Replacement level	Gas production (mL/g DM)[b]			CH_4 production (mL/g DM)[c]			CO_2 production (mL/g DM)[d]		
		b	*c*	*Lag*	*b*	*c*	*Lag*	*b*	*c*	*Lag*
Control	0	281.9	0.054	1.28	66.2	0.038	5.60	185.6	0.044	5.62
Dried date palm leaves	25%	260.5	0.051	1.66	65.7	0.031	6.52	185.2	0.027	6.54
	50%	222.1	0.055	1.86	63.1	0.033	6.64	147.6	0.028	6.63
	75%	194.6	0.052	1.79	66.3	0.027	6.55	123.1	0.033	6.69
	100%	175.2	0.051	1.87	62.2	0.029	6.31	108.7	0.028	6.22
	SEM	3.52	0.0014	0.107	2.36	0.0026	0.206	4.46	0.0024	0.341
	Linear	< 0.001	0.256	0.003	0.345	0.025	0.058	< 0.001	0.008	0.240
	Quadratic	0.280	0.393	0.052	0.898	0.322	0.108	0.398	0.009	0.054
Date palm leaves ensiled without enzymes	25%	253.4	0.059	1.80	51.9	0.036	6.78	174.8	0.032	6.48
	50%	222.5	0.050	1.41	53.8	0.030	7.28	154.3	0.030	6.34
	75%	202.5	0.054	1.78	57.2	0.029	6.77	133.2	0.033	6.53
	100%	167.1	0.057	1.54	54.9	0.030	6.19	100.1	0.031	6.03
	SEM	4.18	0.0018	0.121	1.39	0.0021	0.282	1.75	0.0026	0.113
	Linear	< 0.001	0.835	0.254	0.006	0.011	0.263	< 0.001	0.018	0.217
	Quadratic	0.859	0.259	0.156	0.001	0.191	0.003	0.001	0.046	0.201
Date palm leaves ensiled with enzymes	25%	245.5	0.058	1.30	61.5	0.019	5.63	169.3	0.035	5.79
	50%	214.7	0.052	1.48	65.9	0.024	6.20	144.3	0.029	6.27
	75%	186.4	0.059	1.70	62.9	0.027	5.89	119.4	0.033	6.08
	100%	157.0	0.054	1.64	56.4	0.032	6.73	93.7	0.031	5.37
	SEM	4.11	0.0014	0.162	2.41	0.0028	0.417	3.96	0.0029	0.332
	Linear	< 0.001	0.666	0.054	0.038	0.002	0.084	< 0.001	0.015	0.836
	Quadratic	0.310	0.470	0.851	0.253	0.637	0.649	0.236	0.056	0.079
SEM		3.14	0.0012	0.115	2.20	0.0024	0.305	3.75	0.0024	0.322
P value										
Diet		< 0.001	0.007	0.011	< 0.001	0.004	0.012	0.002	0.012	0.032
Replacement level		< 0.001	0.007	0.216	0.141	0.520	0.427	< 0.001	0.224	0.025
Diet × replacement level		0.158	< 0.001	0.086	0.201	0.007	0.210	0.091	0.724	0.869
Dried without ensiling vs. ensiled without enzymes		0.403	0.003	0.059	< 0.001	0.522	0.169	0.809	0.106	0.168
Ensiled without enzymes vs. ensiled with enzymes		< 0.001	0.379	0.204	< 0.001	0.002	0.003	0.003	0.770	0.174

[a]Date palm leaves at different forms (dried, ensiled without enzymes or ensiled with fibrolytic enzymes) replaced berseem hay at 0, 25, 50, 75 or 100%, DM basis

[b]*b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); *Lag* is the initial delay before gas production begins (h)

[c]*b* is the asymptotic methane production (mL/g DM); *c* is the rate of methane production (/h); *L* is the initial delay before methane production begins (h)

[d]*b* is the asymptotic carbon dioxide production (mL/g DM); *c* is the rate of carbon dioxide production (/h); *L* is the initial delay before carbon dioxide production begins (h)

SEM standard error of the mean

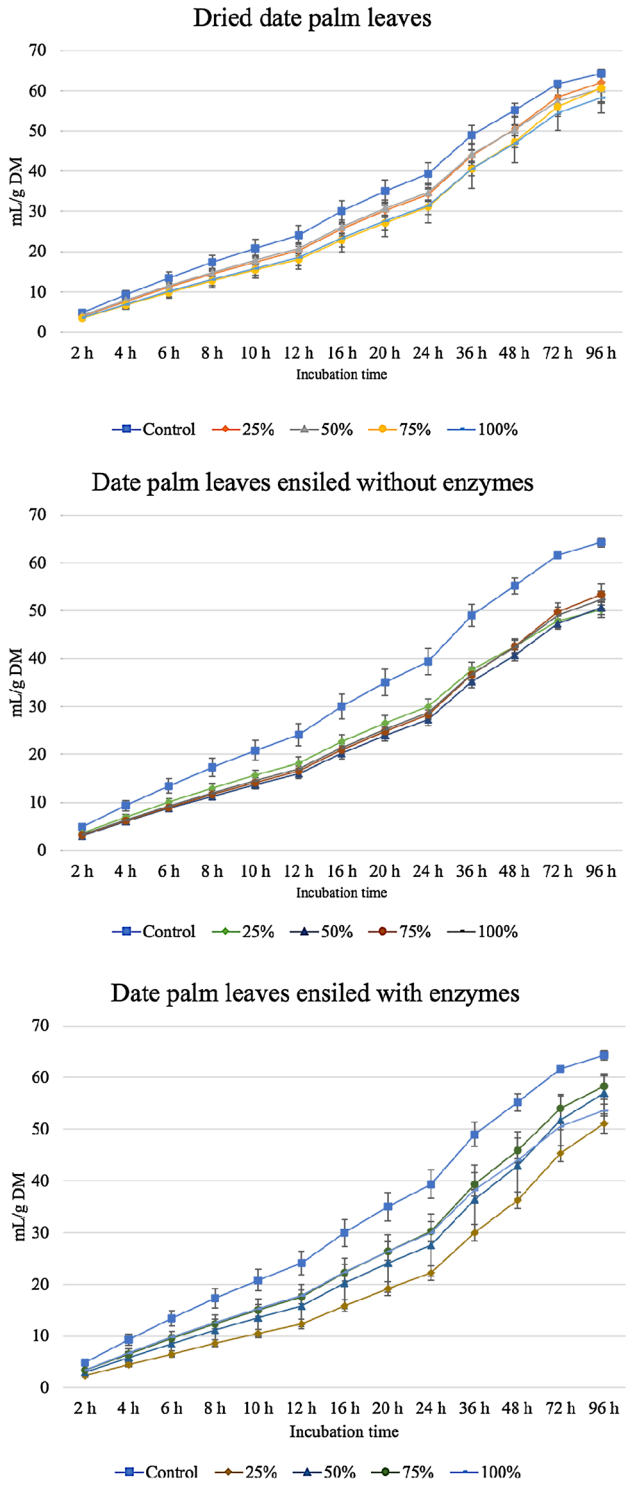

Fig. 2 In vitro rumen methane production (mL/g incubated DM) of TMR containing different levels of date palm leaves in different forms (P values: diet < 0.001, replacement level = 0.060, diet × replacement level = 0.052)

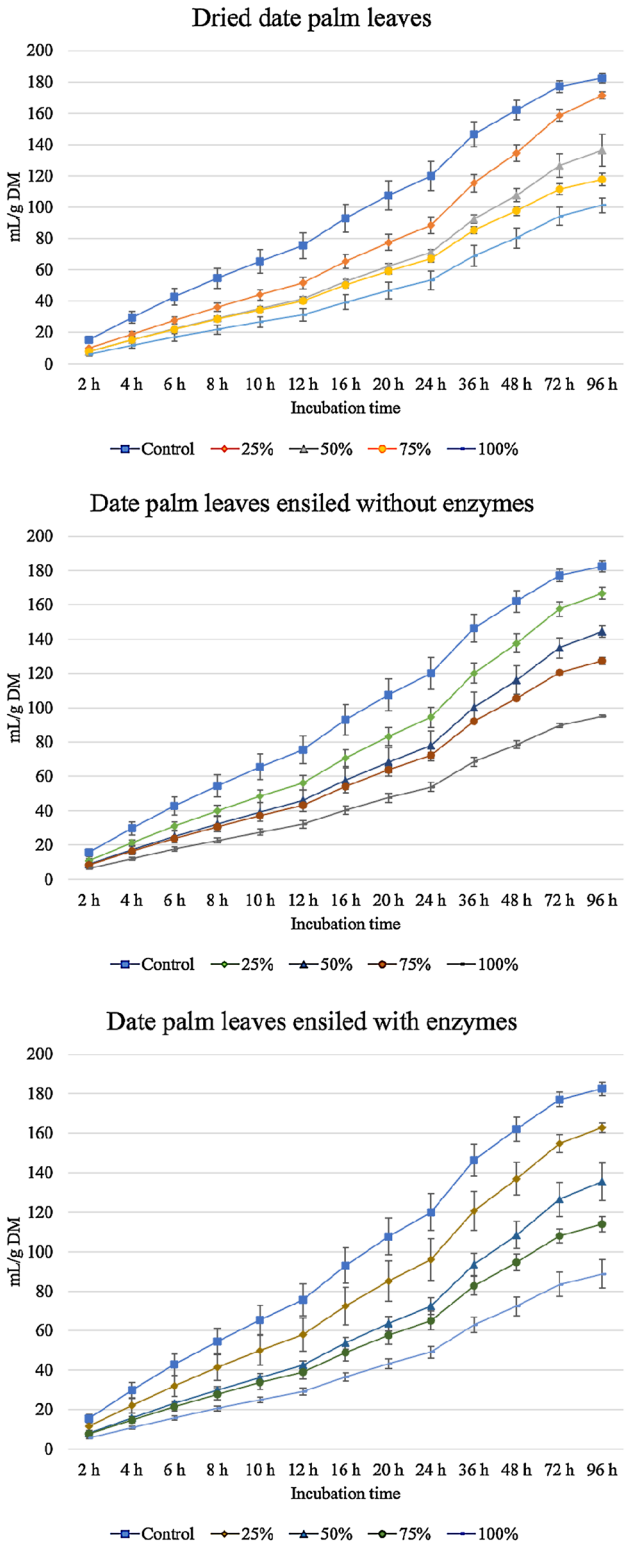

Fig. 3 In vitro rumen carbon dioxide production (mL/g incubated DM) of TMR containing different levels of date palm leaves in different forms (P values: diet = 0.002, replacement level < 0.001, diet × replacement level = 0.106)

Table 4 Methane (CH_4) and carbon dioxide (CO_2) production relative to degraded dry matter (DM), neutral detergent fiber (NDF) or acid detergent fiber (ADF) and as a percent of total gas production (at 96 h of incubation) for diets containing different levels of dried date palm leaves, or date palm leaves ensiled with or without fibrolytic enzymes

Ration[a]	Replacement level	mL/g degraded DM		mL/g degraded NDF		mL/g degraded ADF		Proportional production	
		CH_4	CO_2	CH_4	CO_2	CH_4	CO_2	CH_4	CO_2
Control	0	111.3	316.0	336.2	955.2	513.1	1458.4	23.0	65.2
Dried date palm leaves	25%	120.7	333.2	302.6	834.7	569.3	1573.1	24.0	66.3
	50%	122.3	275.0	284.6	641.0	526.8	1189.2	27.4	61.8
	75%	117.8	229.1	288.7	560.3	570.0	1105.4	31.4	60.9
	100%	127.9	222.9	282.2	490.6	623.2	1076.1	33.5	58.2
	SEM	4.15	29.20	12.87	29.84	38.77	79.65	0.93	5.37
	Linear	0.043	0.301	0.014	< 0.001	0.102	0.006	< 0.001	0.201
	Quadratic	0.769	0.338	0.144	0.084	0.594	0.968	0.424	0.445
Date palm leaves ensiled without enzymes	25%	91.2	302.4	268.7	886.7	417.4	1383.4	19.9	66.1
	50%	96.4	275.8	256.0	731.5	448.7	1283.1	22.9	65.5
	75%	95.5	228.2	260.7	621.0	445.2	1063.7	26.5	63.3
	100%	84.6	155.2	248.5	452.3	498.8	907.4	31.1	57.0
	SEM	3.19	10.31	19.80	53.22	20.08	59.11	4.68	4.96
	Linear	0.002	< 0.001	0.075	0.989	0.015	< 0.001	0.221	0.303
	Quadratic	0.359	0.008	0.296	0.006	0.115	< 0.001	0.205	0.113
Date palm leaves ensiled with enzymes	25%	84.9	270.7	103.0	831.5	260.8	1672.8	20.9	66.6
	50%	97.8	232.3	120.0	650.6	272.9	1220.4	26.8	63.5
	75%	103.0	202.2	128.0	566.8	290.5	1076.5	31.4	61.5
	100%	89.2	147.5	112.3	453.4	273.9	986.4	34.4	56.8
	SEM	2.15	7.56	2.64	33.14	12.76	51.95	0.78	5.43
	Linear	0.003	< 0.001	0.041	< 0.001	0.0266	< 0.001	< 0.001	0.777
	Quadratic	0.054	0.721	0.027	0.365	0.215	0.153	0.011	0.068
SEM		3.63	9.37	16.04	39.01	29.82	61.71	0.80	1.22
P value									
Diet		< 0.001	< 0.001	0.009	0.031	0.001	0.034	< 0.001	0.317
Replacement level		0.107	< 0.001	0.814	< 0.001	0.067	< 0.001	< 0.001	< 0.001
Diet × replacement level		0.043	0.032	0.957	0.761	0.522	0.057	0.120	0.557
Dried without ensiling vs. ensiled without enzymes		< 0.001	0.002	0.006	0.202	< 0.001	0.013	< 0.001	0.151
Ensiled without enzymes vs. ensiled with enzymes		0.776	0.002	0.825	0.009	0.029	0.525	< 0.001	0.267

[a]Date palm leaves at different forms (dried, ensiled without enzymes or ensiled with fibrolytic enzymes) replaced berseem hay at 0, 25, 50, 75 or 100%, DM basis

SEM standard error of the mean

unit of degraded ADF. The inclusion of DPL treated with fibrolytic enzymes linearly decreased ($P < 0.05$) CH_4 and CO_2 production per g degraded DM, CH_4 and CO_2 production per unit of degraded NDF, and increased proportional CH_4 production.

Degradability and Fermentation

Diet × replacement interactions were observed ($P < 0.05$) for ruminal pH and concentrations of ruminal NH_3–N total VFA, acetate and propionate (Table 5). The type of TMR affected ($P < 0.01$) the degradabilities of DM and ADF, and the concentration of NH_3–N, while replacement level affected the degradability of ADF, and the concentrations of acetate and butyrate ($P < 0.05$).

Replacing berseem hay with dried DPL did not affect the degradabilities of DM, NDF and ADF, the concentrations of ruminal NH_3–N, total and individual VFA as well as ruminal pH. Replacing berseem hay with the ensiled DPL increased ruminal pH without affecting ruminal total and individual VFA. Replacing berseem hay with fibrolytic enzymes treated DPL increased ($P < 0.05$) NDF degradability and the concentrations of ruminal NH_3–N, total VFA, acetate and propionate and decreased the concentration of butyrate ($P < 0.05$).

Table 5 Degradability and in vitro rumen fermentation profile of diet containing different levels of dried date palm leaves, or date palm leaves ensiled with or without fibrolytic enzymes

Ration[a]	Replacement level	Degradability[b]			Fermentation[c]		Volatile fatty acids[d]			
		DM	NDF	ADF	Ammonia-N	pH	Total	Acetate	Propionate	Butyrate
Control	0	57.8	49	46.6	11.53	6.06	50.8	30.9	11.0	8.9
Dried date palm leaves	25%	51.5	51.5	40.3	11.20	6.23	56.8	33.9	14.7	8.2
	50%	49.7	52.4	44	11.77	6.19	56.2	33.3	13.4	9.4
	75%	51.5	50.8	42	11.83	5.39	57.1	32.2	16.6	8.3
	100%	45.7	49	37.3	10.87	5.51	58.5	35.8	14.3	8.4
	SEM	4.73	1.43	2.13	0.449	0.292	4.27	3.48	2.77	0.40
	Linear	0.101	0.886	0.023	0.633	0.062	0.651	0.103	0.606	0.545
	Quadratic	0.488	0.061	0.649	0.318	0.461	0.620	0.084	0.126	0.668
Date palm leaves ensiled without enzymes	25%	55.4	48.6	45.9	11.23	5.60	54.1	31.4	13.8	8.8
	50%	52.5	49.2	43.5	10.90	6.13	58.5	35.3	14.5	8.7
	75%	56.1	50.1	47.2	10.77	6.43	54.9	33.0	13.8	8.1
	100%	61.2	51.5	41.1	10.40	6.46	53.8	33.4	12.8	7.6
	SEM	2.41	3.09	1.94	0.407	0.144	3.46	1.40	1.13	0.49
	Linear	0.303	0.553	0.175	0.085	0.010	0.222	0.214	0.394	0.083
	Quadratic	0.024	0.762	0.556	0.968	0.233	0.119	0.288	0.076	0.516
Date palm leaves ensiled with enzymes	25%	60.1	50.9	37.9	12.17	5.73	55.1	32.6	13.6	8.8
	50%	58.3	54	44.3	12.87	6.10	59.4	33.7	17.2	8.5
	75%	56.6	52.3	43.6	12.00	6.28	53.6	34.1	13.6	6.0
	100%	60.2	50.6	37.8	13.43	6.53	57.7	34.9	15.8	7.0
	SEM	0.98	0.84	1.57	0.337	0.064	1.36	1.03	0.82	0.82
	Linear	0.669	0.044	0.137	0.007	< 0.001	0.017	0.016	0.004	0.028
	Quadratic	0.501	0.109	0.821	0.010	0.002	0.068	0.541	0.028	0.983
SEM		1.77	2.18	1.86	0.404	0.202	1.43	1.27	0.76	0.58
P value										
Diet		< 0.001	0.330	0.0136	0.001	0.044	0.222	0.025	0.093	0.050
Replacement level		0.523	0.732	0.007	0.500	0.243	0.002	0.001	0.471	0.014
Diet × replacement level		0.057	0.903	0.311	0.007	0.002	0.003	0.006	0.006	0.236
Dried without ensiling vs. ensiled without enzymes		< 0.001	0.404	0.004	0.082	0.030	0.479	0.046	0.111	0.518
Ensiled without enzymes vs. ensiled with enzymes		0.022	0.141	0.025	0.002	0.991	0.300	0.479	0.037	0.077

[a]Date palm leaves at different forms (dried, ensiled without enzyme or ensiled with fibrolytic enzymes) replaced berseem hay at 0, 25, 50, 75 or 100%, DM basis

[b]Degraded substrate (mg/g DM), DM is dry matter, NDF is neutral detergent fiber, ADF is acid detergent fiber

[c]Ammonia-N (mg/dL)

[d]Volatile fatty acids concentration (mmol/L)

Discussion

Chemical Composition

Fibrolytic enzymes administration during ensiling increased NSC concentration, while decreased NDF, ADF, cellulose and hemicellulose contents in DPL and rice straw, which indicated the ability of fibrolytic enzymes to enhance the fiber biodegradation with the releases of soluble carbohydrate components [27]. Increased NSC concentration with ensiling releases soluble carbohydrates to provide ruminal microflora with additional energy for activity. Kholif et al. [4] observed that ensiling of wheat straw, corn stalks and sugarcane bagasse increased CP and decreased OM, NSC, and fiber contents. High fiber concentration locks up dietary nutrients and hinders their digestibility and utilization resulting in less nutrient utilization and high environmental pollution due to more nutrients passing out in the feces. Ensiling of agricultural byproducts with fibrolytic enzymes may result in preingestive enzyme-feed interactions enabling partial hydrolysis and bioutilization of fiber [4]. Feng et al. [28] noted that treatment with fibrolytic enzymes modifies plant cell wall structure and increases anaerobic fiber digestion.

The kinetics of total gas, CH_4 and CO_2 productions differed between TMR as a result of different nutrient concentrations in each TMR. Differences in fiber concentrations, fractions and structure are the main reasons [29], revealing that their productions are substrate-dependent [4]. The type of TMR and level of inclusion affected the kinetics of GP (e.g. the asymptotic GP, the rate of GP, the lag of CO_2 production, CH_4 and CO_2 production, and proportional CH_4 production) indicating the importance of identifying the better treatment and optimum inclusion level of treated DPL in the diets. Moreover, these observations confirm the previously noted effects of chemical composition of the diets on its nutritive value.

Gas Production

Gas production is a quick and acceptable indicator to test nutrient OM degradability, fermentability and microbial protein production [19]. In the present experiment, increasing levels of dried and ensiled DPL linearly decreased the asymptotic GP indicating negative effects on the nutritive value of diets. This observation can be confirmed with the observed delayed initiation of total GP (i.e. increased lag of total GP). These results may be related to the concentration and fractions of fiber in DPL compared to berseem hay. Even when the DPL were treated with fibrolytic enzymes, the asymptotic total GP, the rate of GP and the lag time of GP were not improved. In previous studies [17, 30], enzymatic treatment of poor quality forages enhanced microbial colonization to feed components at the initial phases in the rumen enabling a faster microbial growth. Elghandour et al. [31] observed a decrease in production of gases as the level of fiber increased in the diet.

Methane and Carbon Dioxide Production

Ruminal fermentation produces many gases; however, H_2, CO_2 and CH_4 are the main gases. Reduction of CO_2 and CH_4 emissions are essential from environmental aspects, as CO_2 and CH_4 exert global warming effects directly. Methane production is strongly related to the composition of the diet [32, 33]. Ruminal methanogenic archaea utilizes H_2 produced during OM degradation for CH_4 production. Methane production relates to the content of NSC in the fermented diets, which can be used as energy sources for rapid microbial growth. The inclusion of ensiled DPL without or with enzymes additives linearly decreased the asymptotic CH_4 and CO_2 production. The ensiling without fibrolytic enzymes was more effective to reduce CH_4 production, but produced more CO_2 compared to the ensiling with fibrolytic enzymes. Improving fiber degradability increases ruminal acetate and butyrate, which consequently increases H_2 availability for methanogenic archaea to produce more CH_4. Mao et al. [34] reported greater CH_4 production when cellulase and xylanases were added to rice straw. The amounts of fermentable carbohydrate and fiber in the diets determine the amount of CH_4 production [35]; however, the administration of fibrolytic enzymes may affect CH_4 production depending on the type and sources of enzymes, diet, and other factors such as the pH of fermentation and rumen microbial populations [17].

The decreased CH_4 production with the inclusion of ensiled DPL might also be related to the change in rumen microbial and methanogen population as will be discussed later; however, the ruminal methanogen microbial population change was not assessed in this study. Increased NDF degradability could increase H_2 production as fiber degrading bacteria are predominantly acetate producers and acetate production through anaerobic metabolism of glucose releases H_2, which subsequently results in greater CH_4 production [36]. However, even CH_4 production expressed per unit of NDF degradability was lower due to inclusion of DPL, more predominantly for treated DPL. This might indicate that lowered CH_4 production was not exclusively related to NDF degradability, but other factors, presumably, plant phenolics including tannins were responsible for this mitigation due to reduction of the methanogenic activities [11]. Treatment of DPL might quickly release more phenolic compounds in the in vitro ruminal conditions, which might exert greater inhibition effect on the methanogens. It will be

interesting to study the treated DPL in more details on the ruminal microbiota because it reduced CH_4 production, but improved fiber digestion, which is not frequently observed [36, 37]. Increasing levels of dried DPL linearly decreased the rate of CH_4 and CO_2 production, revealing the ability of DPL to be used as an environmentally friendly feeds to reduce greenhouse gases production from ruminants.

Degradability and Fermentation

Replacing berseem hay with the ensiled DPL did not affect ruminal total and individual VFA; however, the treatment with fibrolytic enzymes increased total VFA, acetate and propionate concentrations. These observations are paralleled with the observations of others [38–41] who found increased VFA, propionate and acetate productions due to addition of enzymes. The increased ruminal acetate concentration was paralleled with the result of the increased NDF degradability [40], but conversely to the results of ruminal CH_4 production. As previously noted, production of acetate releases H_2, which is used by methanogens to form CH_4 [42]. It is hard to explain the lowered CH_4 production while acetate was increased. But it may be related to inhibition of the archaeal populations due to the presence of phenolic compounds including tannins in the DPL [11].

Replacing berseem hay with fibrolytic enzymes-treated DPL increased NDF degradability, reveling a shift in the microbial population towards increasing the number of cellulolytic bacteria [18, 41, 43]. Increasing NDF digestibility may be a result of the hydrolytic effects of fibrolytic enzymes prior to incubation with ruminal microorganisms [44]. The increased NDF degradability indicates that feeding ruminant animals on diets containing enzyme treated DPL may increase intake and improve performance of animals due to increased supply of digestible energy to ruminants [7].

Conclusions

The inclusion of dried date palm leaves decreased total gas production; however, fibrolytic enzymes treatment lowered methane and carbon dioxide production. Additionally, the treatment of date palm leaves with fibrolytic enzymes increased neutral detergent fiber degradability. The observed interaction between ration type and inclusion level on enhancing ruminal fermentation indicates the synergy between these two factors. Replacing berseem clover hay with fibrolytic enzymes-treated date palm leaves silage up to 100% could be a valuable strategy for sustainable improvement of the environmental conditions through mitigation of methane and carbon dioxide emissions and utilization of waste biomass for animal feeding under the dietary conditions of this study. Further studies are needed to establish the efficacy of replacement of berseem hay with fibrolytic enzymes-treated date palm leaves silage in in vivo trials for production performance and greenhouse gas mitigation.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest There is no conflict of interest for the publication of this article.

Ethical Approval This study was conducted in compliance with the guide of Agricultural Research and Teaching of Federation of Animal Science Societies, Champaign, IL, USA.

Consent to Participate All authors agree to participate in the current work.

Consent for Publication All authors agree to publish the findings of the current research.

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