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Research Article

Influence of Replacing Corn with Levels of Treated Date Press Cake on *in vitro* Ruminal Fermentation, Degradability and Gas Production

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Abstract

Background and Objective: The use of food by-product in ruminant feeding is an important trend as it reduces feed manufacturing costs. The present experiment was aimed to evaluate replacing corn grain with date press cake (DPC) using the *in vitro* batch culture technique. **Materials and Methods:** Corn grain was replaced with DPC at 25, 50, 75 and 100% without or with fibrolytic enzyme (ZAD) at 2 mL kg^{-1} ration. Rumen fluid was obtained from three slaughtered sheep into 1 L flask and flushed with CO_2 until filtration alt laboratory at 39°C. Filtered rumen fluid was mixed with buffer solution at 4:1. After, 40 mL of inoculum was added to each bottle with about 400 mg of substrate and then flushed with CO_2 before incubating at 39°C for 24 h. At the end of incubation, total gas production, dry matter, organic matter and neutral detergent fiber degradability were estimated. Data were statistically analyzed as a completely randomized design using SAS Software. **Results:** No significant differences were observed between the control and other treatments in total gas production, ammonia nitrogen, total volatile fatty acids as well as organic and dry matter degradability. **Conclusion:** It was concluded that the treatment of 25% DPC instead of the corn grains with or without ZAD showed the best results relative to the other treatments.

Key words: Date press cake, *in vitro*, ruminal fermentation, ruminal feeding, residual solids, gas production, microbial biomass production

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

It is known that the press cake product is the residual solids after pressing the plant's raw material to extract the liquids. Date press cake (DPC) is a by-product of date processing industries such as date honey industry, but it can cause environmental problems if not appropriately disposed. However, DPC contains a high percentage of carbohydrates and a reasonable proportion of protein and fiber, which qualifies it for use in ruminant nutrition[1]. On the other hand, corn is an important material in the animal feed industry. But corn in some areas is rarely found, leading to a higher price. Therefore, there must be alternatives to corn grains to reduce its consumption. Recently, date by-products could be used as an energy source to replace a part of the concentrates in the ration[2-5]. One of the advantages of DPC is that it contains a high percentage of carbohydrates and a relatively low fiber percentage, so it can be used as a good energy source and be an alternative to expensive cereals such as corn grain[6]. In previous research it was found that the percentage of fiber in DPC is estimated at 14.8%, while in corn grain was 2.3%[6]; therefore it is necessary to treat DPC with some additives such as fibrolytic enzymes to increase its nutritional value. Fibrolytic enzyme supplementation is one of the methods used to manipulate rumen fermentation and improve digestion coefficients[7-9]. The use of exogenous enzymes in animal feed as an additive improves the nutritional value of poor quality feeds due to the occurrence of solubilization in the dietary fiber[10]. Kholif *et al.*[10,11], Abdel-Aziz *et al.*[12] and Salem *et al.*[13] showed that the use of exogenous enzymes has the potential to improve the quality of feed used as natural additives for ruminant and non-ruminant feeding. Exogenous enzymes can stimulate increases in the total number of viable bacteria, increasing fiber digestion and improving the ability of rumen bacteria to ingest and degrade feed and secondary metabolites[14]. The exogenous fibrolytic enzymes can work synergistically with exogenous rumen microbial enzymes and thus could increase the digestion and nutritive value of fibrous diets[15]. Therefore, the hypothesis of this study was that different percentages of DPC only or with fibrolytic enzymes treatment can replace corn grains without negative effects on rumen activity.

MATERIALS AND METHODS

This study was conducted at the Dairy Science Department, National Research Center and Faculty of Agriculture, Ain Shams University Cairo Egypt, during September, 2018 to November, 2018.

Date press cake (DPC): Date press cake was collected from a factory to produce date honey. The Date press cake was then sun-dried, grinded and transported to the laboratory for use.

It is the exhausted date flesh with some residual sugar after the process of squeezing dates.

Enzyme products

ZAD: ZAD® is a liquid multi-enzyme commercially available feed additive produced from *Ruminococcus flavefaciens* and authorized by the Academy of Scientific Research and Technology in Egypt (Patent No.: 24551, Cairo, Egypt).

Experimental treatments: Ten experimental rations (Table 1) were used as following:

- Control: Clover hay 50%, corn grain 28. 8%, soybean meal 7.5%, wheat bran 12.5%, salt 0.40%, mineral mixture 0.15%, limestone 0.50%, calcium diphosphate 0.15%
- R1: Control+ZAD (2 mL/1 kg ration)
- R2: Replacement 25% from corn by DPC
- R3: Replacement 50% from corn by DPC
- R4: Replacement 75% from corn by DPC
- R5: Replacement 100% from corn by DPC
- R6: R2+ZAD (2 mL/1 kg ration)
- R7: R3+ZAD (2 mL/1 kg ration)
- R8: R4+ZAD (2 mL/1 kg ration)
- R9: R5+ZAD (2 mL/1 kg ration)

Replacement levels were recommended by Abd El-Rahman *et al.*[16].

***In vitro* gas production technique:** About 400 mg of each treatment was taken and placed in a 125 mL glass bottles. A buffer solution was prepared according to Szumacher-Strabel *et al.*[17] and then sprinkle continuously

Table 1: Chemical composition of the experimental rations (g kg^{-1} dry matter)

	Ration ingredients				
Items	Control	R2	R3	R4	R5
Dry matter	895.00	901.00	908.00	915.00	922.00
Organic matter	907.00	906.00	906.00	906.00	905.00
Neutral detergent fiber	313.00	334.00	355.00	376.00	398.00
Acid detergent fiber	161.00	185.00	209.00	233.00	257.00
Acid detergent lignin	36.66	54.33	71.99	89.65	97.32
Crude protein	158.00	160.00	162.00	163.00	165.00
Ether extract	43.49	40.75	38.00	35.25	32.51
Non-fibrous carbohydrate	391.00	371.00	350.00	330.00	309.00

R2: Replacement 25% from corn by DPC, R3: Replacement 50% from corn by DPC, R4: Replacement 75% from corn by DPC, R5: Replacement 100% from corn by DPC with carbon dioxide at 39°C during inoculation of the sample. Rumen fluid was obtained from slaughter house and it was collected from three sheep. The collected rumen fluid was mixed into a bottle (1 L) with an oxygen-free head space and immediately transported to laboratory at 39°C. Upon arrival at the laboratory, the rumen fluid was filtered through 4 layers of cheesecloth to eliminate large feed particles. The buffer solution was added to rumen liquid at proportion 4:1. Forty milliliter of this inoculum was added to each bottle and then was flushed with carbon dioxide and closed. The initial pH of the inoculum was from 6.8-6.9. Triplicates of each sample were used in two separate runs.

Degradability: Dry matter degradability (DMD %) was calculated as the (difference between the sample dry matter (DM) content and that in the residual after 48 h incubation/sample DM content×100). The residuals of acid detergent fiber (ADF) and neutral detergent fiber (NDF) after fermentation were analyzed with the same methods used for feed ingredient analysis[18]. Degradability of NDF, ADF, cellulose and hemicellulose were calculated as:

$$\text{Degradability} = \frac{\text{Nutrient before incubation} - \text{Nutrient after ncubation}}{\text{Nutrient before incubation}} \times 100$$

Total gas production: As described in Khattab *et al.*[19] and Elghandour *et al.*[20], at the end of incubation, after 24 h of samples incubation, the total gas production was estimated by the displacement of syringe piston, which was connected to the serum flasks. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank vessels (without substrate) from total gas produced in the vessels containing buffered rumen fluid and substrate.

Calculation: Metabolizable energy (ME), *in vitro* organic matter digestibility (OMD) were estimated according to Menke and Steingass[21], short chain fatty acid (SCFA) concentrations were calculated according to Getachew *et al.*[22], microbial biomass production (MCP) and efficiency of microbial biomass production (EMP) were calculated according to Blummel *et al.*[23] as followed:

$$\text{ME (MJ kg}^{-1}\text{ DM)} = 2.20+0.136\text{ GP}+0.057\text{ CP (\%)}$$

$$\text{OMD} = 14.88+0.889\text{ GP}+4.5\text{ CP (\%)}+0.0651\text{ ash (\%)}$$

$$\text{SCFA (mmol/200 mg DM)} = -0.00425+0.0222\times\text{GP}$$

$$\text{MCP (mg g}^{-1}\text{ DM)} = \text{DMD (mg)-GP}\times 2.2$$

$$\text{EMP} = \frac{\text{DMD (mg)-GP}\times 2.2}{\text{DMD (mg)}}$$

where, GP is net mL gas production from 200 mg of dry sample after 24 h of incubation, 2.2 mg mL^{-1} is a stoichiometric factor that empresses' mg of C, H and O required for the SCFA gas associated with production of 1 mL of gas. After 24 h of incubation, the filtrated rumen liquor for each sample was subjected for further investigation. The pH of rumen fluid was measured (pH meter) and quantitative analysis of ammonia concentration was carried out by Szumacher-Strabel *et al.*[17], total volatile fatty acids (TVFA) was determined according to Barnett and Reid[24].

Gas production calculation: After 24 h gas production was calculated according to Fahmy *et al.*[6] as followed:

$$\text{GP/DM} = \frac{\text{Total gas production (mL)}}{\text{Substrate DM (g)}}$$

$$\text{GP/OM} = \frac{\text{Total gas production (mL)}}{\text{Substrate OM (g)}}$$

$$\text{GP/NDF} = \frac{\text{Total gas production (mL)}}{\text{Substrate NDF (g)}}$$

$$\text{GP/ADF} = \frac{\text{Total gas production (mL)}}{\text{Substrate ADF (g)}}$$

Chemical analysis of feed ingredients: Ration ingredients were analyzed for DM and ash, crude fiber (CF), crude protein (CP) (Nitrogen×6. 25) and ether extract (EE) contents according to AOAC[25]. NDF, ADF acid detergent lignin (ADL) contents were analyzed sequentially[18] using the Ankom 200 Fiber Analyzer for NDF and ADF and thereafter soaking the residual with 72% sulfuric acid for 3 h. The NDF content was analyzed with 2 additions of heat-stable α-amylase and 1:1 g sodium sulfite per g sample in the neutral detergent solution. NDF and ADF are expressed inclusive of residual ash and hemicellulose and cellulose calculated from NDF, ADF and ADL values. Non-fiber carbohydrate (NFC) was calculated using the following equation as described in Kholif *et al.*[26]:

$$\text{NFC (\%)} = 100-(\text{NDF (\%)}+\text{CP (\%)}+\text{fat (\%)}+\text{ash (\%)})$$

Statistical analysis: The data of *in vitro* degradability and fermentation parameters were statistically analyzed as a completely randomized design according to statistical analysis system User's Guide of SAS 9.4 (SAS Inst, Inc, Cary, NC). Separation among means was carried out by using Duncan multiple test. The following model was used as defined by Steel and Torrie[27]:

$$Y_{ij} = \mu + S_i + E_{ij}$$

where, Y_{ij} is the observation of the model, μ is the general mean common element to all observation, S_i is the effect of the treatment (i = R1. . . R10) and E_{ij} is the effect of error.

RESULTS

Rumen basic parameters: There were no clear differences (p>0.05) for the pH values between the control and the other treatments. However, the mean values of ammonia nitrogen increased (p<0.05) with R1, R2, R6 and R9 than control group. There was an increase (p<0.05) in mean values of the total volatile fatty acids with R4, R5 and R6 compared with control (Table 2).

Rumen degradability: Regarding DMD values, there were no differences among the treatments. But, OMD values were decreased (p<0.05) with R2, R3, R4, R5 and R9 compared with the control diet. There were insignificant (p>0.05) differences among treatments R2, R3, R5, R7, R8, R9 and control in the overall mean of NDF degradation (Table 3).

Gas production: There were no significant differences in the total gas production between all groups except R3, R4 and R5 were decreased (p<0.05) than control (Table 4). GP/DM and GP/OM they were the same trend as the R1 group was the highest (p<0.05) value. While the R4 group was the lowest (p<0.05) compared to all treatments. The R2 was achieved a significant increase (p<0.05) in the value of GP/DM. GP/NDF, GP/NDFD and GP/ADF they were in the same trend with the R1 group was the highest (p<0.05) value.

Estimating ruminal microbial efficiencies: No significant differences were observed between treatments for MP and EMP (Table 5). Moreover, no differences were observed between R1 and R2 compared with the control for ME. The treatments R3, R4, R5, R6, R7 and R8 linearly decreased (p = 0.0001) Me compared with the control treatment. For SCFA, no significant differences were observed between control and R1, R2, R6, R7, R8 and R9; however, R3, R4 and R5 linearly decreased (p = 0.0001) SCFA compared with the control ration.

Table 2: Effect of replacing corn by treated date press cake on rumen pH, NH_3 (g L^{-1}) and TVFA (mmol L^{-1})

Items	pH	NH_3 (g L^{-1})	TVFA (mmol L^{-1})
Control	6.57	44.16^{def}	6.43^{bc}
ZAD (R1)	6.74	60.80^{a}	6.23^{c}
DPC			
25% (R2)	6.67	56.88^{ab}	6.13^{c}
50% (R3)	6.50	49.33^{cd}	6.03^{c}
75% (R4)	6.50	38.02^{f}	7.46^{a}
100% (R5)	6.56	46.40^{cde}	7.46^{a}
DPC+ZAD			
25% (R6)	6.56	50.97^{bc}	7.00^{ab}
50% (R7)	6.58	43.82^{def}	6.50^{bc}
75% (R8)	6.57	41.78^{ef}	6.33^{bc}
100% (R9)	6.66	51.26^{bc}	6.23^{c}
SEM	0.07	1.99	0.23
p-value			
Treatment	0.4297	0.0001	0.0007
Linear	0.3676	0.4347	0.0044
Quadratic	0.1596	0.0001	0.0183

DPC: Date press cake, SEM: Standard error of mean, NH_3: Ammonia (mmol L^{-1}) , TVFA: Total volatile fatty acids, $^{a-d}$Means in the same row with different superscripts significantly differ (p<0.05)

Table 3: Effect of replacing corn by treated date press cake on rumen degradability (%)

Items	DMD	OMD	NDFD
Control	60.54	54.81^{ab}	32.40^{ab}
ZAD (R1)	58.68	55.36^{a}	24.72^{d}
DPC			
25% (R2)	53.17	50.44^{cd}	27.40^{bcd}
50% (R3)	58.23	47.76^{d}	33.90^{a}
75% (R4)	55.14	47.11^{d}	31.68^{abc}
100% (R5)	52.15	48.08^{cd}	33.20^{a}
DPC+ZAD			
25% (R6)	56.09	52.06^{abc}	26.72^{cd}
50% (R7)	57.80	51.03^{bcd}	31.20^{abc}
75% (R8)	56.45	51.02^{bcd}	33.32^{a}
100% (R9)	54.02	50.03^{cd}	33.09^{a}
SEM	1.76	0.81	1.74
p-value			
Treatment	0.0615	0.0001	0.0094
Linear	0.0838	0.0001	0.7477
Quadratic	0.0040	0.3069	0.0012

DPC: Date press cake, SEM: Standard error of mean, DMD: Dry matter digestibility, NDFD: Neutral detergent fiber digestibility, OMD: Organic matter digestibility, $^{a-d}$Means in the same row with different superscripts significantly differ (p<0.05)

DISCUSSION

It is noticeable from the previous results that the use of DPC as an alternative to corn grain did not have a negative impact on the rumen activity[6]. The contents of total crude fiber and fiber fractions were high in DPC compared to that in corn grain, so it is possible to improve the nutritional value of

Table 4: Effect of replacing corn by treated date press cake on TGP (mL/200 mg DM), GP/DM (mL g^{-1}), GP/DMD (mL g^{-1}), GP/OM (mL g^{-1}), GP/NDF (mL g^{-1}), GP/NDFD (mL g^{-1}) and GP/ADF (mL g^{-1})

Items	TGP	GP/DM	GP/DMD	GP/OM	GP/NDF	GP/NDFD	GP/ADF
Control	80.33[a]	217[ab]	360	214[a]	621[ab]	1079[abc]	1202[a]
ZAD (R1)	82.0[a]	222[a]	380	219[a]	637[a]	1262[a]	1232[a]
DPC							
25% (R2)	76.33[abcd]	199[bcd]	376	198[abcd]	539[cd]	1040[bcd]	971[bc]
50% (R3)	70.33[cd]	184[cd]	318	185[cd]	472[efg]	838[d]	801[de]
75% (R4)	69.00[d]	181[d]	330	183[d]	440[fg]	852[d]	711[ef]
100% (R5)	72.00[bcd]	186[cd]	358	190[bcd]	432[g]	822[d]	669[f]
DPC+ZAD							
25% (R6)	79.33[ab]	209[ab]	373	207[ab]	563[bc]	1177[ab]	1015[d]
50% (R7)	77.67[abc]	203[abc]	351	203[abcd]	519[cde]	961[bcd]	881[cd]
75% (R8)	78.33[ab]	203[abc]	360	205[abc]	493[def]	882[cd]	797[de]
100% (R9)	74.67[abcd]	197[bcd]	366	201[abcd]	458[fg]	857[d]	709[ef]
SEM	1.49	4.24	13.86	4.21	11.57	44.1	21.43
p-value							
Treatment	0.0001	0.0001	0.0869	0.0001	0.0001	0.0001	0.0001
Linear	0.0001	0.0001	0.0574	0.0001	0.0001	0.0001	0.0001
Quadratic	0.8592	0.5943	0.0366	0.9586	0.0029	0.5079	0.0001

DPC: Date press cake, SEM: Standard error of mean, TGP: Total gas production, GP/DM: Gas production in dry matter, GP/DMD: Gas production in dry matter digestibility, GP/OM: Gas production in organic matter, GP/NDF: Gas production in acid detergent fiber, GP/NDFD: Gas production in neutral detergent fiber digestibility, GP/ADF: Gas production in acid detergent fiber, [a-e]Means in the same row with different superscripts significantly differ (p<0.05)

Table 5: Effect of replacing corn by treated date press cake on ME (MJ kg^{-1} DM), MP (g/100 g), EMP (mg g^{-1} DMD) and SCFA (mmol/200 mg DM)

Items	ME	MP	EMP	SCFA
Control	4.10[a]	46.84	0.21	0.97[ab]
ZAD (R1)	4.14[a]	35.53	0.17	0.99[a]
DPC				
25% (R2)	3.73[a]	35.04	0.17	0.89[bcd]
50% (R3)	3.19[b]	66.71	0.30	0.83[cd]
75% (R4)	3.20[b]	58.03	0.27	0.81[d]
100% (R5)	3.25[b]	42.66	0.21	0.83[cd]
DPC+ZAD				
25% (R6)	3.23[b]	38.2	0.18	0.93[ab]
50% (R7)	3.24[b]	49.92	0.22	0.91[abc]
75% (R8)	3.27[b]	45.19	0.21	0.91[abc]
100% (R9)	3.27[b]	39.86	0.19	0.88[bcd]
SEM	0.08	7.67	0.03	0.01
p-value				
Treatment	0.0001	0.1412	0.0902	0.0001
Linear	0.0001	0.1212	0.0561	0.0001
Quadratic	0.4628	0.0221	0.0408	0.4536

DPC: Date press cake, SEM: Standard error of mean, ME: Metabolizable energy, MP: Metabolizable protein, EMP: Efficiency of microbial biomass production, SCFA: Short chain fatty acid, [a-d]Means in the same row with different superscripts significantly differ (p<0.05)

DPC by adding fibrolytic enzymes to it[6]. Also using of exogenous fibrolytic enzyme (ZAD) as a feed additive was associated with an increase of nutrient digestibility in ruminant animals[28-30]. Therefore, the fibrolytic enzyme (ZAD) was added to DPC to improve its nutritional value in this study. The level of ammonia in rumen liquor is of particular nutritional importance because many species of rumen microorganisms use ammonia as a source of nitrogen[31]. Moreover, Ammonia content is considered an indicator for the process of protein digestion in the rumen[32]. The treatments R1, R2, R6 and R9 were significantly higher than the control. This is due to the fact that ZAD complex contains fibrolytic enzyme and thus provides the activity of microflora, which works to break the urea[10,33]. These results agreed with those obtained by Fahmy *et al.*[6] who used DPC in an *in vitro* experiment. Moreover, Abd El-Rahman *et al.*[16] and El-Shora and Abd El-Gawad[34] who found that inclusion ground dates in ruminant ration increased rumen ammonia nitrogen. Also, Alsersy *et al.*[35], found that ammonia nitrogen increased by added ZAD to sheep ration. In ruminant animals the rumen microflora fermented large amounts of indigestible carbohydrates to volatile fatty acids (VFA). Volatile fatty acids provide animal with more than 70% of the energy requirements. The total VFA molarities were significantly increased in some treatments and were not affected in some other treatments compared to the control group. Consequently, digestion and fermentation in rumen were good with different DPC treatments. These results agreed with those reported by Fahmy *et al.*[6], Abd El-Rahman *et al.*[16] and El-Shora and Abd El-Gawad[34].

The absence of significant differences for the values of DMD between different treatments was due to the possibility of a high percentage of carbohydrates easily degraded in DPC[6], that work to increase the microflora activity[36]. In contrast, it was also observed that the low fiber content of corn grains (control group) was the indirect cause of OMD increase with it compared to other treatments. Also the presence of the enzyme with R1, R6 and R7 and R8 worked to improve OMD values. In the case of NDFD has achieved the highest values with the control, R8 and R9 because the total fiber content in the corn grains (control) very few compared to DPC. Also, R8 and R9 contain ZAD which in turn helps break down the fiber. Fahmy *et al.*[6] reported increased the OM, NDF and ADF degradability for corn sample compared with DPC or ZAD treatments in *in vitro* trail. However, Taghinejad Roudbaneh *et al.*[37], found that addition date by-product in the rations of ruminants achieved increase in dry matter, organic matter and NDF degradation compared to control.

In vitro gas production technique is one of the best method to quickly evaluate the nutritive value of feeds[38-40] and feed additives[41-44]. Microbial fermentation, nutrient digestibility and rumen protein production can be expressed by measuring the gas production in the rumen[29]. The level of total gas production in *in vitro* fermentation depends on the composition of nutrient content such as (fiber, carbohydrates, protein), presence of inhibitor for gas production, the quality of ruminant diets and the fermentation activity of microorganism in the rumen liquor[45]. So, the gas measuring technique was used for evaluation of nutritive value to estimate some agro-industry by-products[46,47]. The slight improvement in gas production values with ZAD additives (R1, R6, R7, R8) is possible due to potentially improve fiber degradation through a hydrolytic action prior to feeding or *in vitro* incubation[30,48].

Microbial biomass provides animal with almost 70-85% of energy and 70-100% of the protein needs[14,49]. Therefore, MP and EMP values did not achieve significant differences between treatments due to the convergence of both NH_3 and GP values between treatments. SCFA values are the major nutrients produced by bacterial fermentation in ruminants. Thus, the results of SCFA values have taken almost the same trend of VFA values.

Finally, replacing corn with levels of treated date press cake has shown promising initial results for digestion coefficients, total gas production, ammonia nitrogen and total volatile fatty acids. Results of the present experiment may be applied in *in vivo* trials on goats and cows to reduce the cost of producing concentrated feed without affecting the efficiency of animals. Various studies of the effect of replacing corn with levels of treated date press cake on livestock production and performance are recommended.

CONCLUSION

The results of this study gave a little boost to explain the possibility of using DPC alone or with some enzymes as an alternative to corn grains in ruminants feed. However, further research is recommended that uses DPC as an alternative to corn grain and to study its impact on the productive performance of ruminants.

SIGNIFICANCE STATEMENT

This study discovers the using date press cake in the diet of ruminants alone or with fibrolytic enzyme that can be beneficial for enhancing rumen fermentation and feed utilization. This study will help the researcher to uncover the critical areas of including food by-product on the diet of ruminants that many negatively affect ruminal metabolism. Thus a new theory on binding dietary date press cake alone or with fibrolytic enzyme instead of corn grains on ruminal activity may be arrived at.

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