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DIGESTIBILITY AND SOME PERFORMANCE PARAMETERS FOR SHEEP FEEDING ON DATE SEED TREATED WITH BACTERIA

[38]

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ABSTRACT

This paper focuses on treated date seed with two cellulolytic bacteria (*Acetobacter xylinum* and *Thermonospora fusca*) isolated from sheep and evaluated these species by Invitro gas production and metabolism trail. We evaluated the influence of many rations contain several percentage from date seed untreated and treated on In vitro traild for DM,OM, NDF, ADF, cellulose and hemicellulose disappeara (samples incubated for 24 hrs). the best ration used in metabolism trail .

The experimental work was conducted in 2017, at the Department of Animal Production, faculty of agriculture, Ain Shams University, Cairo, Egypt and the experiment of the farm animals occurred in the Animal Production research institute.

Our results in this revealed that the ration content 25% untreated and treated with bacteria had significant values on NDF, ADF and hemicellulose degradability after 24 hours, especially treatment 2 (*Thermonospora fusca*) of date seed. In the experimental ration with ascending level of untreated and treated date seed had not significant effect on pH value while more effect on total gas production (GP),ammonia, TVFA's, MP, EMP and metabolizable energy ME (Mcal/ g). The differences were significant ($P<0.05$) between control ration and other experimental rations. Furthermore, ration content date seed treated (R3) had the highest values of DM, OM, CF and EE digestibility. It could be noticed that improving CP, CF and cell wall constituents (NDF, ADF, hemicellulose and cellulose) digestibility may be due to the increasing number of rumen cellulolytic bacteria.

In conclusion, the bacterial treatment (*Acetobacter xylinum* and *Thermonospora fusca*) with date seed successfully to improve chemical compostion of date seed and Invitro digestiability specially ration contain 25% from total dry matter. It showed that the strain (*Thermonospora fusca*) was the best in In vitro fermentation . Digestibility indicated that ration contain treated date seed (R3) was high DM, OM, ADF and nitrogen than other rations. It was concluded that treated date seed can replace concentrate in rations and improve In vitro degradability, digestibility trail and no effect on rumen and blood parameters without adversely affecting on helthy animals.

Key words: date seeds, Bacteria, sheep, In vitro gas production, Digestibility, Rumen parameters.

INTRODUCTION

Many research trends are to explore the use of waste from food industry and the waste utilization could provide economic gain to the farmers, industry, food security, environmental safety and sustainability. Date–pits are generally used as complementary feed materials for animals and poultry or as a conventional soil fertilizer . The world production of dates was 7.5 million tons **Guizani et al (2014),** meaning that approximately 750 thousand tons of date seeds were produced during that year. A large number of date seeds are being obtained from the date industries or the waste products annually. Date seeds contain high levels of valuable bioactive compounds and dietary fiber which makes them suitable for the preparation of fiber-based foods **Al–Farsi and Lee (2014).** Hence, utilization of this low cost agricultural by-product is important to date industry in the date producing countries.

Many studies have been carried out on date seeds, **(Basuni and AL-Marzooq, 2010)** reported that date seeds are commonly used as cheap source of energy in animal rations under desert condition, being available in abundance all over the year. Date seeds, also called pits, kernels, stones or pips, are a waste product of date processing and packing plants. Therefore, date seeds

have been a problem to the date industry, while they contain many valuable substances such as carbohydrates, oil, dietary fiber, protein, bioactive polyphenols and natural antioxidants. However, date seeds can be used for many applications like food products formulation, cosmetics and functional and medicinal supplements **Golshan et al (2017).** The seed forms about 10 to 30% of fruit weight,large amounts of date seeds are commonly used in desert areas as a source of feed energy . It is cheap and can be offered to animals in crushed or ground form. The chemical composition of date seeds showed , the contents of DM, CP, CF, EE, NFE, Ash, NDF, ADF, ADL, Hemicelluloses and cellulose were around (88.2; 6.1; 7.7; 2.9; 55.2; 1.0; 50.6; 40.6; 7.0; 6.4 and 25.9%), respectively.

El-Shaer et al (2004) found that using date stone with silage instead of berseem hay as a basal diets did not significantly affect the digestibility of DM, CP, EE, NFE and OM. Digestion coefficient of CF was affected by the type of fattening diets. The maximum digestibility coefficients of NDF, ADF and ADL were recorded in date stone diet. Also, indicated that the halophytic silage supplemented with ground date seeds can be recommended as a non-conventional fattening diet for sheep due to its nutrition and economic potentials. **Al-Ani and Farhan (2009)** fed Awassi lambs diets contained 62% date stone with different sources of nitrogen (soy bean meal, cotton seed meal and urea) and indicated that there was no significant difference in dry matter feed intake. **Al-Shanti et al (2013)** concluded that substitution of corn and barely by crushed date seeds up to 50% can be used to improve the growth performance of Assaf lambs.

The biological treatments of crop residues to improve the accessibility of cellulosic fractions, thus improving their digestibility and feeding value have been attracting the extensive interests among researchers **(Yu et al 2009)** although this process has a long history. The major obstruction in biological conversion of lignocelluloses is the physical protection of cellulose by lignin against cellulolytic enzymes. The potential of biological treatments has been explained by the ability of certain microbes (specifically bacteria or fungi) to disrupt plant cell wall by partial breakdown of the lignin-carbohydrate complex **Keller et al (2003)** thus improving their utilization in the rumen by increasing the availability of fermentable energy to ruminal microbes **Akin et al (1996). Gado and Abd El-Galil (2009)** reported that *Acetobacter xylinum* isolated from sheep recorded the highest value of DM disappearance (61.8%) then that isolated from camels (60.2%) or buffalo (59.4%) while the lowest value was recorded for cow (58.6%). In similar trend the highest value found for *Thermonospora fusca* isolated from sheep was 61.7% while the lowest value found in buffalo (51.3%).

The main goals of the present research are to compare effect of two strains of fibrolytic bacteria isolated from rumen of sheep (Acetobacter xylinum and *Thermonospora fusca*) on chemical composition, cell wall constituents of treated date seeds compared with untreated. It also compares effect of replacement of DM of concentrate feed mixture (CFM) in rations with treated and untreated date seeds on In *vitro* gas production parameters, digestibility, rumen parameters and some blood parameters .

MATERIAL AND METHODS

The present study was carried out in 2017, at the Department of Animal Production, faculty of agriculture, Ain Shams University, Cairo, Egypt and the experiment of the farm animals occurred in the Animal Production research institute, Cairo, Egypt .Where the *In vitro* digestibility consumed 24h but the incubation period was 1 month, while the *In vivo* digestibility was carried out through 21`day.

Small scale silo

Date seeds was sun dried to 90% DM and crushed to 2-4 cm and mixed with water, molasses, urea (2% w/w), formic acid and acetic acid according to **Abd El-Galil, (2006).** The two strains of bacteria were subjected to one of the following treatments using 1.5 liters (7.7×10^5 viable anaerobes/kg of wet silage) /ton:

UDS: untreated date seeds (control). T1DS: date seeds treated with *Acetobacter xylinum*. T2DS: date seeds treated with *Thermonospora fusca*. Treated samples were pressed in plastic bags for farm and used after incubated for 6 weeks

Bacterial cultures

Two strains of cellulolytic bacteria were isolated from rumen fluid of sheep and were grown as pure cultural. Rumen fluid of sheep was collected by a stomach tube. The separated strains were *Acetobacter xylinum and Thermonospora fusca*. The two isolated species were purified using the pour-plate technique according to **A.T.C.C. (1992).**

Chemical composition

The proximate analysis of concentrate feed mixture (CFM), control, treated date seeds and rice straw were determined according to **A.O.A.C. (1995)**. The chemical composition were used to determine dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash. The nitrogen free extract (NFE) was obtained by the difference. The chemical composition of feed in-

gredients and *Invitro* gas production rations are shown in **Table (1) and Table (2).**

Cell wall constituents analysis

CFM, control, treated date seeds and rice straw were analyzed according to **Van Soest and Breston (1979)** to determine neutral detergent fiber (NDF), Acid detergent fiber (ADF) and acid detergent lignin (ADL). Hemicellulose, cellulose and lignin were determined by difference.

In vitro Degradability

Dry matter degradability (% DMD) was calculated as the difference between the sample DM content before and after 48 h incubation / sample DM content * 100.

Also, degradability of NDF, ADF, cellulose and hemicellulose were calculated as difference between the content in the sample before and after incubation / content in the sample before incubation *100.

$$(\%\ \mathrm{DMD}) = \frac{DM\ before - DM\ after}{DM\ before} * 100$$

In vitro gas production

After 24 h of samples incubation, the total gas production (GP) 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 substrate.

Table 1. Chemical composition of feed ingredients.

Item	Rice Straw	Con-cen-trate	UDS Untreated Date Seed	T1DS T1(Aceto) Date Seed	T2DS T 2 (Thermo) Date Seed
DM	91.39	87.05	90.30	89.78	88.99
			On DM basis:		
OM	88.26	94.73	92.98	93.13	93.62
CP	2.66	16.03	7.50	12.48	14.91
EE	2.21	4.77	3.80	4.87	4.98
CF	42.13	20.67	16.78	13.53	12.91
NFE	36.24	54.10	64.90	62.25	60.82
Ash	11.74	5.27	7.02	6.87	6.38
			Cell wall constituents		
NDF	68.74	65.66	77.36	45.19	47.19
ADF	50.83	53.94	39.58	38.24	36.79
hemicel-lulose	17.91	15.24	37.78	6.95	10.40
cellulose	42.40	3.98	33.78	29.84	25.99

Where: Concentrate mixture contained Soya bean 17%, energy source 50 %, wheat bran 30%, Mineral and vitamins 1.5% , lime stone 1.5% . DM: dry matter OM: organic matter CP : crude protein CF: crude fiber EE :ether extract NFE : nitrogen free extract

Where : Control: 40% rice straw +60% concentrate. UDS: untreated date seed.T1DS: treated 1(*Acetobacter xylinum*) date seed

T2DS: treated 2 (*Thermonospora fusca*) date seed

Table 2. Chemical composition of rations used in *In vitro* gas production

item	Control	Untreated Date Seeds(UDS)				Treated(1) Date Seeds(T1DS)				Treated(2) Date Seeds (T2DS)			
	0%	25%	50%	75%	100%	25%	50%	75%	100%	25%	50%	75%	100%
DM	88.78	89.27	89.76	90.24	90.73	89.19	89.60	90.01	90.42	89.07	89.36	89.65	89.95
DM basis													
OM	92.14	91.87	91.61	91.35	91.09	91.90	91.66	91.42	91.18	91.97	91.80	91.64	91.47
CP	10.68	9.40	8.12	6.84	5.56	10.14	9.61	9.08	8.55	10.51	10.34	10.17	10.01
EE	3.74	3.60	3.45	3.30	3.16	3.76	3.77	3.79	3.80	3.77	3.80	3.84	3.87
CF	29.25	28.67	28.08	27.50	26.92	28.18	27.11	26.04	24.97	28.09	26.92	25.76	24.59
NFE	46.95	48.57	50.19	51.81	53.43	48.17	49.40	50.62	51.84	47.96	48.97	49.98	50.98
Ash	7.85	8.12	8.38	8.64	8.90	8.09	8.33	8.57	8.81	8.02	8.19	8.35	8.52
Cell wall constituents													
NDF	66.89	68.64	70.40	72.15	73.91	63.82	60.75	57.68	54.61	64.12	61.35	58.58	55.81
ADF	52.69	50.12	47.55	44.97	42.40	50.34	47.98	45.63	43.27	50.54	48.38	46.23	44.08
Hemi cell	16.30	20.10	23.90	27.70	31.50	15.06	13.82	12.57	11.33	15.16	14.01	12.87	11.73
	19.34	22.64	25.95	29.25	32.55	23.22	27.10	30.98	34.86	23.81	28.28	32.75	37.22

Where : Control: 40% rice straw +60% concentrate . UDS: untreated date seed

T1DS: treated 1(*Acetobacter xylinum*) date seed. T2DS: treated 2 (*Thermonospora fusca*) date seed

Calculation

Metabolizable energy (ME, Mcal/kg DM) , *In vitro* organic matter digestibility (OMD, g/kg OM) were estimated according to **(Menke and Steingass, 1988)**, short chain fatty acid (SCFA) concentrations were calculated according to **Getachew et al (2002),** microbial biomass production (MCP) and efficiency of microbial biomass production (EMP) were calculated according to **Blummel et al (1997)** as:

- ME (mJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP (%).
- OMD = 14.88 + 0.889 GP+ 4.5 CP (%) + 0.0651 ash (%).
- SCFA (mmol/200 mg DM) = -0.00425 + 0.0222 * GP
- MCP (mg/g DM) = mg dDM- GP*2.2.
- EMP = (mg DMD- GP*2.2))/ mg DMD.
- where : GP is net gas production in mL from 200 mg of dry sample after 24 h of incubation, 2.2 mg/ mL is a stoichiometric factor that expresses mg of C, H, and O required for the SCFA gas associated with production of 1 mL of gas.

After 24 hrs 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 Nesler method modified by **Szumacher-Strabel et al (2002)** and total volatile fatty acids (TVFA's) was analyzed according to **Barnett and Reid (1956).**

Animals and Metabolism trials

Nine Rahmany mature rams were randomly chosen and divide into three experimental groups, three animals in each group. All animals were reared under the same environmental condition. Each group of animals was fed the following rations :

R1 : 60%cocentrate :40% rice straw. R2: 25 %cocentrate: 25% date seed untreated :50% rice straw . R3: 25 %cocentrate : 25 % date seed treatment 2 (*Thermonospora fusca*):25% rice straw

Three digestion trials were carried out to determine nutrients digestibility, feeding values and nitrogen balance of tested ration by using metabolic cages. Three rams were used in each. At the end of the digestibility trial samples of rumen fluids were collected from each animal at zero, 3 and 6 hrs post feeding by stomach tube. Animals were fed at maintenance requirements using the allowances of **NRC (1985).** The chemical composition of experimental rations are shown in **Table (3).**

The rams were fed individually in metabolic cages. Water was available free. The trial extended for 21 days, adaptation period lasted for 14 days and collection period lasted for 7 days. Feces and urine were collected quantitatively daily during the collection period as described by **Mynard et al (1979).** Solution of 10% H_2So_4 was added to the representative feces samples before drying in oven at 60 °C for 24 hrs.

Table 3. Chemical composition of experimental Rations

Item	R1	R 2	R 3
DM	**87.78**	**89.76**	**89.36**
DM basis			
OM	**92.14**	**91.61**	**91.80**
CP	**10.68**	**8.12**	**10.34**
EE	**3.74**	**3.45**	**3.80**
CF	**29.25**	**28.08**	**26.92**
NFE	**46.95**	**50.19**	**48.97**
Ash	**7.85**	**8.38**	**8.19**
Cell wall constituents			
NDF	**66.89**	**70.40**	**61.35**
ADF	**52.69**	**47.55**	**48.38**
Hemicell	**16.30**	**23.90**	**14.01**
Cell	**19.34**	**25.95**	**28.28**

Where: Concentrate mixture contained Soya bean 17%, energy source 50 %, wheat bran 30%, Mineral and vitamins 1.5% , lime stone 1.5% .

R1 : 50%cocentrate :50% rice straw

R2: 25 %concentrate: 25% date seed untreated : 50% rice straw

R3: 25 %concentrate: 25 % date seed treatment 2 (*Thermonospora fusca*):25% or 50% rice straw.

Dried samples were ground and kept for chemical analysis. Add 50 ml of diluted Sulfuric acid (10%) was in urine collecting containers each day. A representative samples (10%) of urine volume was stored for nitrogen determination.

Analytical methods

Samples of feedstuffs and feces were taken and air dried at 55 C° for 48 hour in forced air oven up to about 10 -12% moisture, then kept to subsequent analysis. Dried samples were ground through a Wiley Mill fitted with a 1 mm screen and chemically analyzed according to **A.O.A.C (1995)** while NFE content was calculated by difference. Urine samples were subjected to N determination according to **A.O.A.C (1995)**. Ruminal pH was immediately determined with a digital pH meter (pH

ep®, pocket-sized pH meter Hana instruments, Italy) before rumen liquor was stored. Concentration of NH3-N was immediately determined using micro-diffusion method of **Conway (1963).** Frozen rumen liquor samples were analyzed for total volatile fatty acids (TVF's) by steam distillation according to **Warner (1964).** Blood serum was analyzed using special kits for urea **(Patton and Crouch, 1977),** total protein **(Henry, 1964)** and Creatinine **(Henry, 1974)**.

Statistical analysis

The data of *In vitro* gas production, dry matter, organic matter, hemicellulose, cellulose digestibility , and *In vitro* dry matter, organic matter, hemicellulose , cellulose degradability, digestion coefficient, rumen and blood parameters were statistically analyzed according to statistical analysis system User's Guide, **(SAS, 1998).** Separation among means was carried out by using Duncan Multiple test **(Duncan, 1955).** The following model was used:

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

Where: Y_{ij} = the observation of the model, μ = General mean common element to all observation, Si = the effect of the treatment (i = 1... 3), and α_{ij} = the effect of experimental error.

RESULTS AND DISCUSSION

In vitro DM, OM, Cellulose and Hemicellulose digestibility

A significant high (P<0.05) DM, OM and NDF degradability after 24 hours was recorded for ration contain untreated date seeds (25 %) while the highest value was recorded for ration control without date seed **(Table 4).** The present results pointed to no significant effect for rations contain treated date seed with bacteria (all different level of replacement) on DM and OM degradability which the high values were in control and untreated date seed (25%).

While the rations content 25% treated date seed with bacteria (T1 and T2) replacement were high values compared any different level replacement concentrate above 25 % decrease in OM degradability **(Table 4).** On the other hand the ration contain 25% treated untreated and treated with bacteria had significant values on NDF, ADF and hemicellulose degradability after 24 hours, especially treatment 2 (*Thermonospora fusca*) of date seed. Moreover rations contain untreated date seed 50% higher than control (without date seed) for cellulose degradation while rations treated date seed with bacteria T2 (*Thermonospora fusca*) 50 and 25 % then date seed treated (T1) with *Acetobacter xylinum* 25% replacement concentration . In addition, ration with ascending level of untreated and treated date seed had more effect on total gas production (GP) . This positive response for bacterial treatment (T1 or T2) on NDF, ADF, hemicellulose and cellulose degradability may be attributed to that effect of cellulolytic bacteria resulted in increase digestibility **(Table 4)** consequently increase cellulolytic enzyme activity.

Elghandour et al (2014) reported an improved fibers fractions degradability as a result of increased cellulolytic digester species : *Fibrobacter succinogenes, Ruminococcus flavifaciens* and *Selenomonas ruminantium* . Also **Colombatto et al (2007)** stated that fibrolytic enzymes enhanced the fermentation of cellulose and xylan by a combination of pre- and post- incubation effects. The gas production, form any substrate, depends mainly on nutrient availability for rumen microorganisms (**Elghandour et al 2014**).

Table 4. In vitro gas production (gas production 400 mg DM ml/) and invitro degradability of rations contained date seed untreated or treated with bacteria after 24 hours on DM basis

Item	control	Untreated Date Seeds(UDS)				Treated(1) Date Seeds(T1DS)				Treated(2) Date Seeds (T2DS)				SE
	0	25%	50%	75%	100%	25%	50%	75%	100%	25%	50%	75%	100%	±
Gas production GP24(Total GP)	44.00 ^{a}	45.00 ^{a}	33.33 ^{c}	35.33 ^{b}	30.00 ^{d}	42.66 ^{a}	35.66 ^{b}	32.00 ^{c}	20.33 ^{e}	38.33 ^{b}	32.33 ^{c}	28.66 ^{d}	20.66 ^{e}	0.85
DDM,%	47.00 ^{a}	41.12^{b}	32.91^{c}	34.55^{c}	29.51^{d}	35.48^{c}	34.22 ^{c}	25.11^{d}	20.80^{e}	36.21^{c}	27.18^{d}	26.03^{d}	16.43 ^{e}	0.70
DOM,%	37.80 ^{a}	38.08 ^{a}	32.16 ^{b}	32.97 ^{b}	30.32 ^{c}	37.01 ^{a}	33.35 ^{b}	31.53 ^{b}	25.79 ^{d}	34.85 ^{b}	31.70 ^{c}	30.00 ^{c}	26.06 ^{d}	0.27
DNDF,%	49.51^{a}	46.34^{a}	34.69 ^{b}	37.33^{b}	30.17^{c}	38.37^{b}	26.22^{d}	10.86 ^{e}	0	38.82^{b}	22.99^{d}	7.73^{e}	0	0.95
DADF,%	60.78^{a}	50.60^{b}	35.42^{d}	25. 80^{c}	10.31 ^{e}	43.96^{c}	33.54 ^{d}	16.12 ^{e}	0	46.62^{c}	30.93^{d}	16.77^{e}	0	1.65
DHemi,%	48.19^{b}	54.58^{a}	52.01^{a}	51.32^{a}	55.96^{a}	47.55^{b}	45.87 ^{b}	44.45^{b}	47.01^{b}	52.50^{a}	40.50^{c}	44.70^{c}	4.99 ^{d}	0.99
Dcellul,%	42.91^{c}	43.19^{c}	45.61^{c}	41.35^{c}	40.05^{c}	40.87^{c}	42.82 ^{c}	55.36^{b}	54.05^{b}	56.72^{b}	61.37^{a}	44.79^{c}	3.21 ^{d}	3.21

a,b,c,d and e : means in the same rows with different superscripts differed significantly at (p<0.05).
Where :
Control: 40% rice straw +60% concentrate.UDS: untreated date seed.
T1DS: treated 1(*Acetobacter xylinum*) date seed. T2DS: treated 2 (*Thermonospora fusca*) date seed .

Fermentation parameters

In the experimental ration with ascending level of untreated and treated date seed had not significant effect on pH value while more effect ammonia, TVFA's , MP, EMP and metabolizable energy ME (Mcal/ g) (Table 5). Ammonia concentration (mg/100 ml) and total volatile fatty acids (meq /100 ml) were recorded (P >0.05) increase as a result of supplemeating ration with date seed at 25% of untreated and treated compared to other levels. These results may be due to added treated date seed affect on bacteria activity which increased growth and activity ruminal bacteria and causes increase protein degradation **(Table 5).** Ruminant rations with untreated and treated date seed at 25% resulted in increase microbial mass protein (MP) and efficiency of microbial mass (EMP) compared to 100 % of replacement in all rations . Several studies have suggested that during fermentation due to increasing pH and lactate utilization making pH relatively more stable and meet the needs of rumen microbes to perform its activity **(Elghandour et al 2014).**

Fermentation of dietary carbohydrates to acetate, propionate and butyrate produces gases in the rumen. However, in the current study, standard ration had the same fiber fractions content in different additives. So, it is well clear that the increased gas production (GP) was a result of increased adding of nanocobalt to ration. It is well known that microorganisms has the ability to increase ammonia production in the rumen **(Hristov et al 2013)** by increasing protein degradation and increased the overall N excretion by the animal. In these study, the low level of date seed used (25 %) improved degradability, gas production and kinetics of fermentation (SCFA, NH_3 and MP) than the high level of date seed.

Table 5. Rumen parameters of rations contain date stone untreated or treated with bacteria

Item	control	Untreated Date Seeds(UDS)				Treated (1) Date Seeds (T1DS)				Treated (2) Date Seeds (T2DS)				SE
	0	25%	50%	75%	100%	25%	50%	75%	100%	25%	50%	75%	100%	±
Gas production														
GP24(Total GP)	44.00 ^{a}	45.00 ^{a}	33.33 ^{c}	35.33 ^{b}	30.00 ^{d}	42.66 ^{a}	35.66 ^{b}	32.00 ^{c}	20.33 ^{e}	38.33 ^{b}	32.33 ^{c}	28.66 ^{d}	20.66 ^{e}	0.85
Rumen parameters														
pH	6.64	6.54	6.54	6.50	6.53	6.56	6.61	6.60	6.53	6.53	6.64	6.60	6.01	0.01
NH3	12.87^{c}	8.81^{d}	7.88^{d}	9.22 ^{d}	10.37^{c}	23.32^{a}	18.61 ^{b}	17.95^{b}	11.99^{c}	16.10^{b}	10.87^{c}	11.46^{c}	2.69 ^{e}	0.69
TVFA's	7.09^{a}	6.89^{b}	6.80^{b}	6.16^{c}	6.94^{b}	6.83^{b}	6.14 ^{c}	7.11^{a}	7.89^{a}	7.23^{a}	6.34^{c}	6.39^{c}	4.15 ^{d}	0.15
MP	198.66^{a}	135.80^{b}	126.54^{c}	132.37^{b}	114.35^{d}	125.66 ^{c}	93.05^{e}	57.01^{f}	84.84^{e}	127.22^{c}	85.67^{e}	75.16^{e}	38.34 ^{f}	3.33
EMP	42.16^{a}	38.75^{a}	32.91^{b}	35.89^{b}	36.68^{b}	35.90 ^{b}	26.32 ^{c}	22.69^{d}	21.13^{d}	35.09^{b}	27.06^{c}	32.80 ^{b}	22.61 ^{d}	0.86
SCFA	2.73 ^{a}	2.77 ^{a}	2.04 ^{b}	2.14 ^{b}	1.82 ^{c}	2.63 ^{a}	2.18 ^{b}	1.95 ^{c}	1.23 ^{d}	2.36 ^{a}	1.98 ^{c}	1.75 ^{c}	1.26 ^{d}	0.04
M. E	2.78 ^{a}	2.61 ^{a}	2.23 ^{a}	2.08 ^{b}	1.81 ^{c}	2.68 ^{a}	2.47 ^{a}	2.33 ^{a}	2.05 ^{b}	2.65 ^{a}	2.51 ^{a}	2.42 ^{a}	2.26 ^{a}	0.01

a,b,c,d,e and f :means in the same rows with different superscripts differed significantly at (p<0.05). MP:microbial protein (mg/100 ml rumen liqour) - EMP: efficiency of microbial protein - SCFA: short chain fatty acid (µm).
M E : metabolic energy (Mcal/g DM). Where : Control: 40% rice straw +60% concentrate. UDS: untreated date seed. T1DS: treated 1 (Acetobacter xylinum) date seed. T2DS: treated 2 (Thermonospora fusca) date seed

Improving ME, MP and GP(24) were observed with the ration of low level than those other's . Rations with high protein content provide ruminal microflora with the essential nutrients for its activity. The highly activity reflected on higher GP, higher microbial protein synthesis, and higher degradability.

Digestability and degradability of In vitro gas production

Gas production per gram DM, OM, NDF, ADF, Hemicellulose and Cellulose after 24 hours incubation showed in **Table (6).** Potential gas production was significantly affected by replacement concentrate with treated date seed which significant decrease in gas production per gram DM, OM, NDF, ADF, cellulose and hemicellulose were observed for rations with all level of date seed untreated and treated 1 ecsept ration contain 25%. On the other hand, the ration contain treated date seed (2) resulted in numerically increase in gas production per gram DM, OM, NDF and ADF, ecsept hemicellulose and cellulose. **Siegel (1991)** suggested that gas production from cereal straws and from different classes of feeds incubated *In vitro* in buffered rumen fluid was closely related to the production of short chain fatty acid (SCFA) which was based on carbohydrate fermentation. **Bakker et al (1995)** reported a close association between SCFA and gas production *In vitro* studies, suggests a potential to make energy available to the ruminants.

After 24 hours incubation gas production degradability dry organic matter, NDF, ADF, cellulose and hemicellulose of 100% date seed untreated and treated bacteria (T1 and T2) were the lowest values compared any rations, but notes that the value of rations content treated (T1 and T2) date seed 25% were the highest values compared ration control without date seed . The results implying that increase growth of cellulolytic bacteria increase fermentation of cellulose and improve degradability of standard ration in the experiment. A higher potential gas production can contribute significantly to energy supply via short chain fatty acid production **(Remesy et al 1995).** Digestibility has been reported to be synonymous to *In vitro* gas production, with a high positive correlation obtained between gas production and dry matter digestibility **(Datt and Singh, 1995).** For gas volume and *In vitro* gas production characteristics, **Lina et al (2009)** suggested that gas volume at 24h after incubation is an indirect relationship with metabolisable energy in feedstuffs. Gas production can be considered as an indicator of carbohydrates degradation. **Lina et al (2009) and Rajendran (2013)** suggested that gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *In vitro* studies. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate **Sahoo (2014 a)** and substantial changes in carbohydrates fractions were reflected by total gas produced **(Te -Hsing et al 2007).**

Gas production from protein fermentation is relatively small as compared to carbohydrate fermentation, while contribution of fat to gas production is negligible, **Sahoo (2014 b).** Mathematical descriptions of gas production profiles allow analysis of data evaluation of substrates and media related differences and fermentability of soluble and slowly fermentable components of feeds **(Newman et al 2009).**

Table 6. *In vitro* gas production digestibility (ml / 1g DM) and degradability (g /kg DM) of rations contain date stone untreated or treated with bacteria

Item	Control	Untreated Date Seeds (UDS)				Treated (1) Date Seeds (T1DS)				Treated (2) Date Seeds (T2DS)				SE
	0	25%	50%	75%	100%	25%	50%	75%	100%	25%	50%	75%	100%	±
Gas production digestibility 24 hours (ml / 1g DM)														
GPDM	123.36^{a}	125.17^{a}	92.10^{c}	96.90^{c}	82.18^{d}	118.97^{b}	98.43^{c}	88.22^{c}	55.99^{e}	136.76^{a}	89.41^{c}	79.40^{d}	57.26^{e}	2.16
GPOM	133.88^{b}	120.24^{c}	100.53^{d}	106.07^{d}	61.41^{f}	136.46^{b}	107.37^{d}	100.49^{c}	90.22^{e}	146.08^{a}	116.85^{c}	102.38^{d}	96.65^{e}	2.29
GPNDF	163.74^{b}	162.77^{b}	117.39^{e}	121.24^{e}	100.88^{f}	166.39^{b}	145.10^{c}	137.58^{c}	0	184.38^{a}	130.13^{d}	121.51^{e}	0	6.12
GPADF	207.89^{a}	223.00^{a}	173.98^{c}	194.20^{b}	175.89^{c}	188.24^{b}	183.71^{b}	173.92^{c}	0	210.93^{a}	165.01^{d}	153.93^{e}	0	11.70
GPhemi	190.14^{a}	148.16^{b}	154.19^{b}	134.59^{c}	120.93^{d}	200.36^{a}	119.16^{d}	100.65^{d}	92.08^{e}	137.40^{c}	100.61^{d}	95.48^{e}	20.13^{f}	31.33
GPcell	195.11^{b}	177.88^{c}	100.00^{e}	81.97^{f}	72.23^{f}	235.64^{a}	113.79^{d}	104.39^{e}	81.37^{f}	160.40^{c}	122.75^{d}	81.50^{f}	14.40^{f}	17.69
Gas production degradability 24 hours (g /kg DM)														
GPdDM	374.72^{a}	587.41^{b}	518.41^{c}	500.02^{c}	477.10^{d}	652.30^{a}	575.02^{b}	551.93^{b}	494.54^{d}	690.54^{a}	650.03^{a}	513.14^{c}	477.91^{d}	2.41
GPdOM	943.76^{b}	960.88^{b}	940.37^{b}	905.94^{b}	408.63^{b}	969.89^{b}	986.98^{b}	951.18^{b}	416.17^{e}	1131.84^{a}	908.56^{b}	803.31^{c}	589.31^{d}	5.32
GPdNDF	331.42^{d}	351.31^{c}	338.03^{d}	334.39^{d}	338.92^{d}	433.83^{b}	556.71^{a}	409.03^{b}	0	576.41^{a}	384.41^{c}	338.78^{d}	0	14.04
GPdADF	341.93^{c}	440.87^{b}	492.31^{b}	480.27^{b}	160.24^{e}	479.36^{b}	548.88^{a}	237.85^{d}	0	564.47^{a}	403.32^{b}	139.39^{e}	0	15.53
GPdhemi	373.79^{b}	328.56^{b}	344.55^{b}	228.98^{d}	195.61^{e}	300.77^{c}	166.64^{f}	183.58^{e}	109.63^{f}	450.67^{a}	341.67^{b}	194.78^{e}	75.18^{f}	12.62
GPdcell	391.15^{c}	344.71^{c}	299.35^{d}	420.37^{b}	523.75^{a}	411.18^{b}	111.31^{e}	87.01^{f}	74.41^{f}	577.83^{a}	441.83^{b}	133.48^{e}	31.42^{f}	10.22

a,b,c,d, e and f :means in the same rows with different superscripts differed significantly at (p<0.05).

Gas production (ml / 1g DM) and degradability of DM OM, NDF,ADF, hemicellulose and cellulose (g /kg DM) after 24 hours incubation. Where: Control: 40% rice straw +60% concentrate. UDS: untreated date seed. T1DS: treated 1 (*Acetobacter xylinum*) date seed. T2DS: treated 2 (*Thermonospora fusca*) date seed.

Although gas production is a nutritionally wasteful products **(Ingale and Chaudhari, 2013),** but provides useful basis from which ME, OMD and SCFA may be predicted **(Yang and Sun, 2006).** There was a positive correlation between metabolisable energy calculated from *In vitro* gas production together with CP and fat content with metabolisable energy value of conventional feeds measured *In vivo* **(Kaiser et al 2014).** The OMD differed significantly with other agricultural wastes. **Iravani et al (2014)** found a high precision in prediction of *In vivo* OMD. This group further used a correlative approach to predict the ME content of

feed by *In vitro* gas production measurement and chemical constituents and concluded that the prediction of ME is more accurate when based on gas and chemical constituents only **(Lina et al 2009).** Other studies **(Lina et al 2009 and Rajendran et al 2013)** have also reported significant correlation between *In vitro* gas measurement and *In vivo* digestibility. **KinfAemi et al (2009)** showed that the *In vitro* gas production techniques can be used to assess the nutritive value of tropical agricultural wastes and to differentiate between their potential digestibility and metabolisable energy contents,also chemical composition and *In vitro* digestibility are very useful in estimation of OMD, SCFA and ME. Date–pits include high amount (14.4%) of crude fibre and sodium hydroxide treatment of date–pits increased the rate of *in–vitro* digestibility by solubilizing some of the cell wall components, such as neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose, and cellulose. However, lignin content was not affected by sodium hydroxide treatment (**Al–Yousef et al 1986**).

Digestion coefficient

Data in **(Table 7)** indicated that experimental rations in metabolic trial control ration without date seed (R1), ration content 25% untreated date seed (R2) and ration content 25% treated date seed (R3) there were significantly (P<0.05) increased in ration 3 (R3) for DM, OM, CP, EE, CF, ADF and cellulose digestibility as compared with control ration (R1) and ration 2 (R2). The highest values digestibility were recorded with R3 treated with date seed *Thermonospora fusca* than R1 and R2 but the lowest values in ration 1 without date seed recorded in OM and cellulose digestibility. On the other side, the lowest values (P<0.05) in ration 2 with untreated date seed recorded in CP,EE and CF digestibility. Data presented in **Table (7)** showed that no significant differences among animals fed rations R1 and R2 in DM and ADF digestibility.

The differences were significant (P<0.05) between control ration and other experimental rations. Furthermore, ration contain treated date seed (R3) had the highest values of DM, OM, CF and EE digestibility. It could be noticed that improving CP, CF and cell wall costatuents (NDF, ADF, hemicellulose and cellulose) digestibility may be due to the increasing number of rumen cellulolytic bacteria **(Gomez-Alarcon et al 1987)** and increase in population **(Newbold et al 1996)** and /or activity of rumen cellulolytic bacteria **(Dawson, 1993).** The results were harmony with **Beauchemin et al (2003)** reported that adding exogenous fibrolytic enzymes to dairy cow and feedlot cattle diets can potentially cell wall digestion and the efficiency of feed utilization by ruminants. The nutritive values as TDN % in **Table (7)** noticed that control ration without date seed had (P<0.05) the lowest value while, the ration of R2 and R3 were recorded significantly (P<0.05) the highest value of TDN.

Table 7. Effect of replacement concentrate with date seed untreated and treated on digestion coefficient in experimental ration

Item	R1	R 2	R 3	±SE
Digestibility coefficients, %				
DM	63.60^{b}	63.40^{b}	75.55^{a}	1.12
OM	69.61^{c}	72.52^{b}	79.67^{a}	1.14
CP	40.00^{b}	36.86^{c}	72.93^{a}	0.94
EE	78.71^{b}	67.34^{c}	80.77^{a}	1.27
CF	57.55^{b}	54.75^{c}	74.25^{a}	0.94
NFE	82.62^{b}	87.45^{a}	83.26^{b}	0.89
NDF	80.43^{b}	85.20^{a}	86.37^{a}	0.76
ADF	66.39^{b}	65.52^{b}	77.48^{a}	0.68
Hemicell	91.29^{a}	85.31^{b}	80.43^{c}	0.38
Cell	78.18^{c}	86.44^{b}	90.19^{a}	0.22
Nutritive values, %				
TDN	57.34^{c}	60.98^{b}	68.51^{a}	1.11
N balance	+2.16^{b}	+2.18^{b}	+3.76^{a}	0.43

a,b and c:means in the same rows with different superscripts differed significantly at (p<0.05).
Where: R1: 50%cocentrate: 50% rice straw. R2: 25% cocentrate: 25% date seed untreated : 50% rice straw.
R3: 25% cocentrate: 25% treaded date seed 2 (*Thermonospora fusca*): 25% rice straw.

The N-balance values were significantly (P<0.05) increased with rations of R3 than those in other rations. This result was reflected to increase values of CP digestibility. These results are agreement with report of **Abd El-Galil (2014)** who recorded that DCP had higher significantly values with fibrozyme and with mixed fibrozyme and yeast (8.15 and 8.01%) and with yeast (7.37%) than without supplement (6.59) These results means that biological additives or treatements effect on rations which may be increase the number of bacteria in the rumen and increase the digestibility and nutritive values in experimental diets.

Different schemes have been drawn up by different authors to draw together into a logical mode of action the various observations that have been made on microbial feed additives **(Wallace and Newbold, 1992).** These two observations are suggested to arise from a more active microbial population: the most effect of microbial feed additives is that they increase the viable count of anaerobic bacteria recovered from ruminal fluid. Increases of

50 to 100% are common **(Wallace and Newbold, 1993),** but increases of more than 10-fold compared with controls have been observed**.**

Cellulolytic bacterial numbers are increased **(Wallace and Newbold, 1993**) thus explaining in part the improvement in fiber breakdown and increased stability of the fermentation in animals receiving yeast and *A. oryzae* **(Williams et al 1991).** The estimated *In vivo* OM digestibility was in the 60-70% range calculated by **Shem et al (1995)** for bean straw, and are slightly higher than cereal straw OM digestibility **(**60%). Nitrogen balance and metabolism was found to be improved due to the inclusion of YC in the diet of sheep. This may be due to the increase in N digestibility as well as to a better utilization of the dietary N. Proteolytic bacteria count was increased and the flow of non-microbial non-ammonia N tended to be higher for cows fed YC **(Putnam et al 1997).** The nutritional value of date–pits is based on their dietary fibre content, which makes them suitable for the preparation of fibre–based foods, such as bread, biscuits, and cakes; and dietary supplements **(Larrauri et al 1995)** and Date–pits fibre could be used as an alternative to wheat bran, and it may provide a valuable contribution to dietary fibre intakes **(Larrauri et al 1995).** Finely , breads containing the fine date–pits fibre had higher dietary fibre contents than wheat bran controls, but lower desired colour, flavour, odour, chewiness, uniformity and overall acceptability of sensory scores **(Najafi, 2011).**

Golshan et al (2017) concluded that date seeds can be used as a functional food ingredient because they are a good source of dietary fiber, phenolic compounds and antioxidant activity. In addition, date seeds contain a considerable amount of food ingredients such as protein and minerals. Therefore, the potential uses of date seed in different industries are promising. Date seed presents a number of challenges. However, date seeds are available at low or no cost and the seed oil extraction may be feasible and worth consideration.

Rumen and blood parameters

Results obtained in **Table (8)** indicated that Effect of replacement concentrate with date seed untreated and treated on rumen and blood parameters**.**

The rumen parameters in metabolic trail indicated that PH and NH_3 recored no differences (P>0.05) in all rations experiement but TVFA's was the highest value in ration1 (R1) control without date seed.

Differences in serum total protein, urea and GOT (AST) concentration in R3 recorded the highest values while in R1 and R2 were aproxmatelly the same values might be attributed to synthesis liver function and higher digestibility of CP and OM of tested rations, which indicated better utilization of dietary protein owing to replecements concentrate by untreated and treated date seeds in rations. These results were found that serum albumin and creatinine concentration was no significantly differences in all rations while GPT (ALT) values were high significantly in ration 2 contain untreated date seed then R1 and R3 ,respectively.

Table 8. Effect of replacement concentrate with untreated date seed and treated on rumen and blood parameters

Item	R1	R 2	R 3	±SE
rumen parameters				
pH	6.96	6.76	6.81	0.06
TVFA's(meq/100ml)	9.16 [a]	8.10 [b]	8.06 [b]	0.15
NH3(mg/100ml)	14.95	14.86	14.91	0.21
blood parameters				
Total protein(g /dl)	2.96 [c]	3.08 [b]	4.85 [a]	0.15
Albumen (g/dl)	2.16	2.42	2.47	0.10
Urea	0.23 [c]	0.30 [b]	0.48 [a]	0.01
GPT(ALT)IU/L	0.41 [b]	0.51 [a]	0.39 [b]	0.02
GOT(AST)IU/L	0.47 [c]	0.63 [b]	0.70 [a]	0.04
Creatinine	1.36	1.33	1.42	0.17

a,b and c : means in the same rows with different superscripts differed significantly at (p<0.05).
Where:
R1 : 50%cocentrate +50% rice straw
R2: 25 %cocentrate+ 25% date seed untreated +50% rice straw
R3: 25 %cocentrate + 25 % date seed treatment 2 (*Thermonospora fusca*) +25% rice straw

However total protein in this study was lie within the normal range. In general from these results, it could be noticed that the ration 3 (R3) which cont treated date seed (25%) trended to significantly (P<0.05) affected in some blood parameters.

The demands of animal feed are increasing globally and date–pits can be used as an alternative feed. Date–pits could be a non–traditional carbohydrate sources in animal feed. Studies were conducted to use date–pits as a feed for sheep **(Aldosari et al 1995).** Date–pits were used in animal feed to enhance growth **(Elgasim et al 1995).**

These results came on line with those obtained by **Abd El-Galil (2008).** In most date producing

countries, date seeds are discarded or used on a small scale as animal feed **(Habib et al 2013).**

CONCLUSION

It can be concluded that, in this study, It's possible to replace% concentrate (R1) with 50% untreated date seeds (R2) or 50% treated date seeds (R3) as source of energy and protein in experimental rations. In addition that ration R3 containing using treated date seed was more effective on *Invitro* digestibility and digestion coefficient compared the other rations (R1 and R2). It could be use successfully untreated and treated date seeds by 50% replacement of concentrate to increase crude fiber, nutrients digestibility and nutritive value, while no effects on ruminal and blood parameters .

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تأثير تغذية الاغنام بنوى البلح المعامل بالبكتيريا على قابيلية الهضم وبعض معايير الاداء [38]

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الموجـــــز

يهدف هذا البحث الى دراسة تاثير نوى البلح بنوعين من البكتيريا الخلوية تسمى (Acetobacter Xylinum & Theromonospora Fusca) والتى تم عزلها من الاغنام وتقييم الاختلافات بين هاتين المعاملتين من خلال تجربة انتاج الغاز المعملية (Invitro Gas production)

وتجربة الهضم العملية حيث تم تقييم تأثير إضافة نسب مختلفة من نوى البلح غير المعامل والنوى المعامل بنوعية للعليقة الحيوانية على قيم NDF , ADF , Cellulose , Hemicellulose , OM , DM للتجربة المعملية (In vitro) بعد التحضين لمدة 24 ساعة حيث تم إختيار افضل نسبة مضافة للعليقة ليتم تطبيقها من خلال تجربة الهضم المعملية على الاغنام.

الجزء المعملى من الدراسة إجراء عام 2017م؛ فى قسم الإنتاج الحيوانى بكلية الزراعة جامعة عين شمس، القاهرة – مصر. أما عن تجربة حيوانات المزرعة فقد تمت فى معهد بحوث الإنتاج الحيوانى.

أوضحت نتائج الدراسة أن النسبة 25% من نوى البلح غير المعامل والمعامل بنوعية حققت افضل القيم من تكسير NDF , ADF , Hemicellulose , Cellulose بعد 24 ساعة وخاصة المعاملة الثانية اى نوى البلح المعامل ب(Theromonospora Fusca).

اظهرت التجربة المعملية أن تركيزات متزايدة من نوى البلح غير المعامل والمعامل لم يؤثر على قيم الأس الهيدروجينى PH بينما كانت مؤثرة فى حالة إنتاج الغاز الكلى– الامونيا، الأحماض الدهنية الطيارة (TVFA'S) وقيم MP, EMP وطاقة التكسير ME .

حيث كانت قيم الاختلاف بين العليقة المطابقة وباقى العلائق التجريبية $P< 0.05$ معنويا.

فضلا عن محتوى العليقة (R3) من نوى البلح المعامل بالمعاملة الثانية حقق اعلى قيم DM,OM,CF, EEفى تجربة الهضم، حيث يرجع هذا التحسين فى قيم CP, CF ومحتويات جدار الخلية Cellulose Hemicellulose , ADF , NDF لتجربة الهضم الى زيادة بكتيريا الكرش الخلوية.

وقد إتضح من النتائج السابقة، إن معاملة نوى البلح بالبكتيريا بنوعيها كان لها التأثير الايجابى الافضل حيث حسنت التركيب الكيميائى لنوى البلح وايضا لوحظ تحسن في نتائج تجربة الهضم المعملية (In vitro) خاصة فى حالة العليقة المحتوية على النسبة 25% من المادة الجافة الكلية، حيث اظهرت النتائج أن السلالة (Theromonospora Fusca) كانت الافضل على الاطلاق فى تجربة In vivo المعملية، حيث ارتفاع قيم ADF,OM, DM, النيتروجين قى المعاملة بالبكتريا الثانية (R3) بالمقارنة بالعلائق الاخرى، لذا توصي الدراسة بإمكانية إستبدال نوى البلح المعامل بالبكتيريا Theromonospora محل نسبة معينة من العلف المركز فى العليقة.

دون التأثير سلبا على سائل الكرش او معاملات الدم ودون التأثير السلبى على صحة الحيوان .

الكلمات الدالة: نوى البلح، بكتريا، أغنام، التجربة المعملية، الهضم، تجارب الهضم، مقاييس الكرش

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