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Research Article
Influence of Using Different Energy Sources on Growth Rate, Digestion Coefficients and Rumen Parameters in Sheep

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Abstract
Three rations were tested through two major trail metabolic and feeding trial to evaluate the replacement of different sources of energy in sheep rations. The rations contained: 30% berseem hay+70% CFM contain 50% yellow corn as control ration (R1), 30% berseem hay+70% CFM contain 50% date seeds (R2) and 30% berseem hay+70% CFM contain 50% sugar beet pulp (R3). No Significant differences ($p<0.05$) were observed in total dry matter intake and crude protein intake among the experimental rations. Ration 1 had the highest value of DM, OM, CP and NFE digestibility compared with R2 and R3. Total Digestible Nutrient (TDN) and N-balance had higher significantly values for R1 (74.1% and 6.57 g) and R2 (68.00% and 5.93 g) than R3 (63.3% and 4.46 g), respectively. The pH and total volatile fatty acids value had significant differences among all rations. Total cellulolytic bacteria had significant differences value in R3 than other experimental rations. Number of Cellulomonas, Bacillus, Acetobacter and Ruminococcus were high in R3 contain beet pulp, while Thermonospora was the highest number in R2. In feeding trail recorded that total gain, Average Daily Gain (ADG) and feed efficiency were significantly ($p<0.05$) higher for R1 followed by R2 and R3 orderly. It was concluded that using date seeds or sugar beet pulp as source of energy in rations of ruminants, have not any negative effect on digestibility, feed intake, ADG and feed efficiency compared with yellow corn ration.

Key words: Yellow corn, date palm, beet pulp, energy, digestibility, cellulolytic bacteria, feed efficiency, feed intake, ADG

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INTRODUCTION

Shortage of feedstuffs is one of the main problems facing animal production in Egypt. The gap between the available and requirements of animal feeds is wide and the feed shortage is nearly about 5.32 million tons of TDN (Ministry of Agriculture, 2006). Several efforts were carried out using the local by-products in animal feeds to reduce shortage problems.

This study hypothesized the possibility of using corn or date pits or sugar beet pulp in sheep diets, by substituting a part of the commercial concentrate feed due to its high energy content and palatability. Differences in source of energy, lead to differences in kind and quantity of microbial protein in rumen, where the source of energy is necessary to form an amino-acids microflora. The quantity of Sugar Beet Pulp (SBP) as source of energy in ration in Egypt as by-products approximately 161-491 t annually (MALR., 2003). Date seeds is another important agriculture by-products for its valuable nutrient. Annual production of date stone in Egypt is about 1.1 million tons representing 16% of total world production (FAO., 2004). The chemical composition of date pits ranged from 5.64-8.20% for CP, 1.60-9.35% for EE, 9.10-22.0% for CF, 58.5-75.4% for NFE, 38.5-73.1% for NDF and 17.2-35.3% for ADF (Aldhaheri *et al*., 2004). Previous studies reported that using of date seeds in animal feeding was successful (in sheep) (Abdel-Ghani, 1997). Grains are imported and globally jump in prices continuously, nutritionists aggressively work to replace part of starch grains by Date Seeds (DS) in ruminant rations aiming to reduce the nutritional gap between animal needs and local feed resource. Some previous studies (Kholif and Abo El-Nor, 1998; Kholif *et al*., 2001) proved that date seeds could be utilized as component in ruminant diets, but it is in need for supplementation with protein. 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. Sharifi *et al*. (2015) indicated that substitution part of dietary concentrate with date palm in the ration of dairy goats had no effect on DMI and nutrients digestibility. Milk yield and composition were not affected by treatments. In regard to, date palm is a cheap by-product and is considered as a natural antioxidant source.

Therefore, the objective of present study aimed to evaluate the effect of substituting partially three different sources of energy (corn, date seeds and sugar beet pulp) into traditional animal feed on sheep performance, which could be used as a corner stone for cheap rations in the future.

MATERIALS AND METHODS

Location: This study was carried out in Sakha station, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture.

Animals and feeding trial: Twenty one lambs (Finnish×Rahmani crossbred) with mean initial live body weight of 26.35 kg (±0.22) were randomly chosen and divide into three experimental groups, seven animals in each group. All animals were reared under the same environmental condition. Each group of animals was fed for 105 days on one of the following rations:

- **R1:** 30% berseem hay+70% concentrate mixture containing 50% corn (control ration)
- **R2:** 30% berseem hay+70% concentrate mixture containing 50% date seeds
- **R3:** 30% berseem hay+70% concentrate mixture containing 50% sugar beet pulp

Each group was kept in a separate shaded pen and fed as a group. Fresh water and salt blocks were available for each group all over the day. Animals were weighed biweekly, while feed consumption, total gain, average daily gain and feed efficiency were calculated. The chemical composition of feed ingredients and experimental rations are shown in Table 1 and 2.

Metabolism trials: 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, rumen fluids were collected from each animal at 0, 3 and 6 h post feeding by stomach tube. Animals were fed at maintenance requirements using the allowances of NRC (1985).

The rams were fed individually in metabolic cages. Water was available free. The trial extended for 21 days, adaptation

Table 1: Chemical composition of feed ingredients

	Nutrients (%), on DM basis						
Item	DM (%)	OM	CP	CF	EE	NFE	Ash
Concentrate 1	92.85	93.87	16.01	7.39	3.64	66.83	6.13
Concentrate 2	90.22	93.08	15.06	13.16	4.09	60.77	6.92
Concentrate 3	91.42	92.31	15.80	19.49	2.93	54.09	7.69
Berseem hay	86.00	92.00	12.50	33.00	2.30	44.20	8.00

Concentrate 1: Corn, Concentrate 2: Date seeds, Concentrate 3: Sugar beet pulp, DM: Dry matter, OM: Organic matter, CP: Crude protein, CF: Crude fiber, EE: Ether extract and NFE : Nitrogen free extract

Table 2: Chemical composition of experimental rations

	Nutrients (%), on DM basis						
Item	DM (%)	OM	CP	CF	EE	NFE	Ash
R1 (corn)	90.80	93.31	14.96	15.08	3.23	60.04	6.69
R2 (date palm)	88.95	92.75	14.29	19.11	3.55	55.80	7.25
R3 (sugar beet pulp)	89.79	92.22	14.81	23.54	2.75	51.12	7.78

Concentrate mixture contained soya bean 17%, energy source 50%, wheat bran 30%, mineral and vitamins 1.5%, lime stone 1.5%, R1: 30% berseem hay+70% concentrate mixture contain 50% corn (control ration), R2: 30% berseem hay+70% concentrate mixture contain 50% date seeds and R3: 30% berseem hay+70% concentrate mixture contain 50% sugar beet pulp

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 Maynard *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 h. 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.

Bacterial cultures: Five strains of cellulolytic bacteria were isolated from rumen fluid of sheep and were grown as pure culture. Rumen fluid was collected by stomach tube. The separated strains were *Cellulomonas cellulasea*, *Bacillus* sp., *Thermonospora fusca*, *Acetobacter xylinum* and *Ruminococcus albus*. The isolation of species used the pour-plate technique for pure preparation of cultures according to ATCC (1992). To determine viable counts of rumen cellulolytic bacteria, the rumen samples were immediately gassed with CO_2 and viable counts were determined according to the method described by Moir (1951) and Gall *et al*. (1949) and their classification were done according to Pounden and Hibbs (1948).

Blood samples: The blood samples were taken at zero, 3 and 6 h post morning feeding from 3 animals of each trial. The blood serum was obtained by centrifuging the blood samples soon after collection at 4000 rpm for 15 min. Blood serum was transferred into a clean dried glass vials and then stored in deep freezer at -20°C for subsequent specific chemical analysis.

Analytical methods: Samples of feedstuffs and feces were taken and air dried at 55°C for 48 h 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 AOAC (1995), while NFE content was calculated by difference. Urine samples were subjected to N determination according to AOAC (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 NH_3-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: Data were subject a one-way analysis with treatment effect by using General Linear Models (GLM), (SAS., 1998), separation among means was carried out by using Duncan (1955).

RESULTS AND DISCUSSION

Feed intake, digestion coefficients and nutritive values: Feed intake, digestibility and nutritive values for experimental rations are shown in Table 3. The results indicated that, no Significant differences ($p>0.05$) were observed in initial weight. Conversely, the final weights of sheep are significantly affected ($p<0.05$) with different rations. The group fed R3 diet (containing SBP) recorded the lowest final weight, while groups fed R1 and R2 (containing corn and date seeds, respectively) were the same final weights approximately but higher than R3. No significance differences among treated groups were observed for total Dry Matter Intake (DMI) and Crude Protein Intake (CPI).

Meanwhile, it agree with Al-Ani and Farhan (2009), who concluded that cotton seed meal with date stones in fattening diets of Awassi lamb achieved a better or similar response to soya been meal as a source of nitrogen and higher than that from urea.

Data of digestibility trials (Table 3) showed high values ($p<0.05$) of DM, OM, CP and NFE digestibility for R1 than the other two rations, while R3 had significantly higher digestibility of CF and EE than R2 or R1. Although chemical composition of rations approximately had equal values of protein, the digestibility of CP different significantly among rations. This is may be due to lower content of CF in R1 ration, which led to increasing rate of passage of digest in animals fed that ration, which the digestibility of nutrients negatively in addition of lack in rumen bacteria capable to digest protein content in the ration. While, increasing CF digestibility in R2 and R3, may be increasing the numbers of bacteria, especially cellulolytic bacteria and fungi in the rumen, which lead to crude fiber degradation (Ali, 2005).

High significantly value was recorded for R1 compared with R2 and R3 related to nutritive values as TDN (74.11, 68.00

Table 3: Total gain, ADG, feed efficiency, feed intake, digestibility and nutritive values for experimental rations

	Groups			
Item	R_1	R_2	R_3	±SE
Experimental period	105	105	105	-
No. of animals	7	7	7	-
Initial weight (kg)	26.14	26.29	26.57	0.38
Final weight (kg)	51.14[a]	50.71[a]	48.57[b]	0.42
Total gain (kg)	25.00[a]	24.42[a]	22.00[b]	0.45
ADG (kg h^{-1} day^{-1})	0.238[a]	0.233[a]	0.210[b]	4.23
Feed efficiency (killogram gain/killogram feed intake)				
DM	0.231[a]	0.224[ab]	0.203[b]	0.01
CP	1.329[a]	1.277[a]	1.214[b]	0.02
TDN	0.276[a]	0.305[a]	0.289[a]	0.02
Feed intake				
DMI (g h^{-1} day^{-1})	956.88	962.25	943.81	3.66
CPI (g h^{-1} day^{-1})	148.52[a]	141.53[b]	142.63[b]	1.08
Digestion coefficients				
DM	87.80[a]	74.50[b]	68.90[c]	0.18
OM	80.10[a]	73.00[b]	64.10[c]	0.19
CP	81.80[a]	75.90[b]	69.20[c]	0.18
CF	68.30[c]	70.50[b]	74.60[a]	0.21
EE	66.80[c]	68.20[b]	75.90[a]	0.16
NFE	86.70[a]	83.50[b]	75.40[c]	0.22
Nutritive value				
TDN*	74.11[a]	68.00[b]	63.30[c]	0.23
N-balance (g day^{-1})	+6.57[a]	+5.93[a]	+4.46[b]	0.12

[a,b,c]Means with different superscripts within each row for each parameter are significantly different ($p<0.05$). *Total digestible nutrient

Table 4: Rumen pH, TVFA's (m equ dL^{-1}) and NH_3-N (mg dL^{-1}) for tested rations

	Groups			
Items	R_1	R_2	R_3	±SE
pH				
0 h	6.92	6.89	6.94	0.05
3 h	6.02	6.15	6.27	0.03
6 h	6.11	6.32	6.36	0.06
Average	6.35	6.45	6.52	
TVFA's (m equ dL^{-1})				
0 h	8.28[a]	8.17[a]	7.90[b]	0.15
3 h	12.35[a]	11.40[b]	9.41[c]	0.19
6 h	11.13[a]	10.59[b]	8.22[c]	0.17
Average	10.59[a]	10.16[b]	8.51[c]	
NH_3-N (mg dL^{-1})				
0 h	28.50[a]	28.23[a]	27.70[b]	0.31
3 h	37.29[a]	35.38[b]	32.20[c]	0.37
6 h	35.76[a]	34.66[b]	30.90[c]	0.21
Average	33.85[a]	32.76[b]	30.27[c]	

[a,b,c]Means with different superscripts within each row for each parameter are significantly different ($p<0.05$)

and 63.30%, respectively) and N-balance (6.57, 5.93 and 4.46, respectively). These results were compatible with CF contents in the rations and nitrogen balance was positive for all experimental rations. Hoover and Stokes (1991) found that rate of digestion of carbohydrate (CF and NFE) is the major factor controlling energy and CP available for growth of rumen microbes. The high content of NFE in date pits has attracted the attention of number of researchers to evaluate its potential use in animal feed, with promising results. Dietary inclusion of date pits significantly improved the growth and feed utilization of sheep (Elgasim *et al*., 1995).

Rumen parameters: Rumen parameters are illustrated in Table 4. It is apparent that the pH values were within the normal range with no significant differences among the rations at all times post feeding. Meanwhile, the pH values tend to decrease by prolongation of time, reaching lowest at 3 h post-feeding then increased after 6 h feeding. Prasad *et al*. (1972) reported that the rumen pH values were between (6.39 and 7.57) at the different sampling time after feeding. Such range is suitable for growth and activity of cellulolytic bacteria. Previous studies reported that the pH value of Strained Rumen Liquor (SRL) ranged from 6.99-7.3 in healthy animals and 5.5-8.9 in digestive disorder cases. On the basis of pH value, the clinical cases were categorized into 4 kind of indigestion, viz., bloat (pH 5.52-5.72), acidic indigestion (pH 5.73-6.99), impaction (pH 7.00-7.49) and alkaline indigestion (pH 7.5-8.9). The above categorization on the basis of pH corroborates with the reports of Bhaskar (1971), Gupta *et al*. (1976) and Choudhuri *et al*. (1981).

There are significant differences among the experimental rations at different times were recorded in TVFA's value. In the present study, the different value of TVFA's may be due to different content of CF in rations, but the same value of CP in all rations (Table 2). Data indicated that different in CF in

rations, which effect on microbial fermentation of protein and fiber in rumen as microbial protein synthesis. Furthermore, rumen NH_3-N was recorded higher significant values for R1 than R2 and R3. Over all observation, TVFA's and NH_3-N values were higher after 3 h and tended to decrease after 6 h post feeding for all rations. These values were similar to that reported by Abd El-Galil (2006), who found the highest ammonia N concentration after 3 h. These differences in ammonia N concentration are referred to difference in treatments. However, it is well recognized that the ammonia N concentration found in the rumen at any given time presented the net concentration value of its production, utilization by rumen microbes and absorption across the rumen wall, the dilution by other factor and passage to the lower gut.

Cellulolytic bacterial count under the different feeding regimens and time intervals after feeding are given in Table 5. Total count in the experimental rations were the highest ($p<0.05$) values in R3 followed by R2 and R1. These result may be due to the higher content of CF% in R3 compared with R2 and R1 (17.65, 15.20 and 10.93%, respectively), which was the basic substance for activity of cellulolytic bacteria. The same trend was observed for number of cellulomonas, Bacillus, Acetobacter and Ruminococcus, which the highest value was in R3 then R2 but the lowest value was in R1. In another side, the result revealed highest value of Thermonospora count in R2 compared with other rations,while the highest value in control ration (R1) for number of Acetobacter. These results indicated that pH value, CP, CF, NFE and structure of fiber in rations effect on growth and species of ruminal bacteria especially cellulolytic bacteria. The rumen microbial population is very dense, containing 10^{10} bacteria mL^{-1}, 10^6 protozoa mL^{-1} and 10^3 fungi mL^{-1}, which the rumen is buffered over a range of 5.7-7.3 by phosphate and bicarbonate from saliva and bicarbonate from rumen fermentation.

The percentage of Thermonospora and Acetobacter from cellulolytic bacteria were maximum at 15.83 and 33.09%, in corn fed (R1), while the percentage of Cellulomonas, Bacillus and Ruminococcus were maximum at 30.25, 4.62 and 24.91% in date seeds fed (R2). Corn, date and sugar beet pulp were highly energy source and rapidly fermented by rumen microbes, leading to a rapid buildup in concentration of short-chain fatty acids.

This may depress rumen fluid pH and reduce cellulolytic activity in the rumen, unless the various processes in the rumen such as saliva from rumination (Wolin, 1981), ruminal NH_3 and absorption of the VFA's across the rumen epithelium or outflow to the lower parts of the gut (Sutherland, 1976).

Table 5: Ruminal cellulolytic bacteria count and percentage after 6 h of feeding on tested rations

	Groups			
Item	R1	R2	R3	±SE
Cellulolytic bacteria (nx10^5 mL^{-1})				
Total count	4.23[c]	5.62[b]	7.08[a]	
Cellulomonas	1.10[c]	1.70[b]	2.10[a]	0.06
%	26.00	30.25	29.66	
Bacillus	0.16[b]	0.26[a]	0.27[a]	0.09
%	3.78	4.62	3. 81	
Thermonospora	0.67[c]	0.76[a]	0.71[b]	0.26
%	15.83	13.52	10.02	
Acetobacter	1.40[b]	1.50[b]	2.30[a]	0.15
%	33.09	26.69	32.48	
Ruminococcus	0.90[c]	1.40[b]	1.70[a]	0.14
%	21.27	24.91	24.01	

[a,b,c]Means with different superscripts within each row for each parameter are significantly different ($p<0.05$)

Although the rumen pH of the animals on the three dietary rations was at times lower than 6.00, this was relatively short-term (<3 h) and this may explain why microbial degradation was not adversely affected. Hoover (1986) have suggested that the reduced pH decrease digestion of fibers and reducing feed intake.

Also, many of the bacteria in the rumen are free-floating in the liquid just before feeding and become attached to new feed particles after feeding. It is very difficult to remove and count the bacteria attached to feed particles and thus may explain the low numbers observed in the rumen after feeding, when fermentation rate is generally at its greatest. Bacterial numbers are generally assumed to be higher on high concentrate diets compared to high forage diets (Hespell *et al*., 1997). However, there are more fluid-associated bacteria with high concentrate diets and thus, easier to enumerate. The biggest differences due to diet are in the type of bacteria rather than the total number.

Among major rumen bacteria, *Fibrobacter succinogenes, Ruminococcus flavefaciens, Ruminococcus albus, Butyrivibrio fibrisolvens, Prevotella ruminicola, Eubacterium cellulosolvens* and *Eubacterium ruminantium* are recognized as fibrolytic bacterial species (Stewart *et al*., 1997). Varel and Dehority (1989) reported that the proportions of *F. succinogenes*, *R. flavefaciens* and *R. albus* in the total cellulolytic bacteria in cattle rumen were 33.0, 2.6 and 46.0%, respectively. In addition, the ability of these three species to digest cellulose is much higher than that of other cellulolytic Ruminal species. Therefore, *F. succinogenes, R. flavefaciens* and *R. albus* have been considered representative cellulolytic bacterial species in the rumen. Use of readily digestible carbohydrate supplements in low quality roughage poses a risk of lowering cellulolytic bacteria activity in the rumen that impacts negatively on digestibility of basal roughage and may reduce intake. Furthermore, as a source of glucogenic substrates

Table 6: Blood serum parameters for experimental rations on different sampling time

	Groups			
Item	R_1	R_2	R_3	±SE
Glucose (mg dL^{-1})	51.33[a]	52.00[a]	49.50[a]	0.80
Total protein (mg dL^{-1})	8.50[a]	8.49[a]	8.35[a]	0.15
Creatinine (mg dL^{-1})	1.31[a]	1.28[a]	1.18[a]	0.20

[a,b,c]Means with different superscripts within each row for each parameter are significantly different (p<0.05)

(or glucose) the supplement, when fermented in the rumen is also inefficiently utilized by the ruminant (Royes *et al*., 2001).

Blood parameters: Blood glucose, total protein and creatinine are shown in Table 6. Blood glucose value was higher in animals fed diet containing date seeds compared with other rations. This result was reflects on total body gain. However, the total blood protein value for all rations were not significantly differences (p>0.05) among the three experimental rations. Creatinine had no significant differences (p>0.05) among all treatments. It means that different rations not affected on kidney function as creatinine. The increase in blood studied constituents may be due to the role of Cellulomonas, Bacillus and Ruminococcus as Cellulolytic bacteria in improving all nutrient digestibility (Table 3) and rumen parameters (Table 4 and 5) of sheep fed date seeds and beet pulp and also may be probably led to an increase in the absorption rate from the digestive tract.

Thus, blood constituents of the supplemented animals reflected a corresponding increase of these values. These results are comparable with those obtained by Abd El-Galil (2006, 2008). Hematological data was used as an indication of the health status of the experimental animals. During the course of the experiment, all blood values recorded were within the normal range for animals of similar status. These results approve with Mahgoub and Lodge (1994). The result is in agreement with that reported by Kholif *et al*. (1996). Yagoub and Elemam (2012) recommended that using date pits by 10% give a good animal performance among trail diets as well as it had a lower cost. Mahmoud and El-Bana (2013) concluded that date stone and olive cake may be used to feed camels as substitution of barley grains without any adverse effect. Date stone showed better nutritive value than olive cake. Also, the mixture of barley grains plus olive cake or date stone gave the best results than other mixtures. Suliman and Mustafa (2014) reported that lambs should not be fed more than 20% of date pits replacing corn grain to maintain animal performance and productivity. Hossain *et al*. (2014) found that date pits showed effectives in animal and poultry feeds and it could be used as value added products, such as: Dietary fibers, functional polysaccharides, caffeine-free drinks (similar to coffee), oil (biofuel or cooking oil) and other functional or medicinal products. Al-Suwaiegh (2015) recommended that replacing the concentrate feed up to 20% by date pits without any adverse effect on goat health, productivity and its availability with low price as by-products in animal feeds.

CONCLUSION

It can be concluded that, in this study, the possible of replacement of corn (R1) by date seeds (R2) or sugar beet pulp (R3) as source of energy in experimental rations. These results indicated that ration R1 containing corn was more effective on total gain, average daily gain and feed efficiency compared the other rations but the count of cellulolytic bacteria had the highest value in R3. It could be use successfully date seeds or sugar beet pulp by the same proportion to replacement corn as source of energy due to improve the quality of rations with increased crude fiber, nutrients digestibility and nutritive value, while decreased cost of rations with no effects on ruminal and blood parameters.

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