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EFFECT OF ROUGHAGE TO CONCENTRATE RATIO ON DIGESTIBILITY IN SMALL RUMINANTS

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ABSTRACT

Three experimental rations differed in concentrate: roughage ratios {90:10 (R1), 80:20 (R2) or 70:30% (R3)} were designed to study chemical compositions and cell wall constituents analysis by *in vitro* at different incubation times, *in vitro* nutrient disappearance, *in vitro* fermentations, microbial protein, count and classification of ciliated protozoa, total and cellulolytic bacteria number. Also, utilization of energy and rumen protozoa in male sheep fed the experimental rations. Samples of rations were *in vitro* incubated with ruminal fluid from sheep fed concentrate feed mixture and good quality berseem hay for 2, 4, 6, 8, 24 and 48 h. *the* results indicated that R1 had the highest (P<0.05) DM, OM and CP contents, while R3 had the lowest (P<0.05) contents. Also, R3 had the highest (P<0.01) CF content and cell wall constituents, whereas, R1 showed the lowest (P<0.01) CF content and it's components. All contents and cell wall constituents showed gradual decrease (P<0.05) by progressed incubation time. Also, the obtained results revealed that R3 had the highest (P<0.05) nutrient disappearance, followed by R2 then R1. The best cell wall content disappearance also was for R3. All values of nutrients disappearance showed gradual increase (P<0.05) by progressed incubation time. *In vitro* fermentations, microbial protein and ruminal protozoa showed the highest values for R3. While, R2 had the best utilization of energy. It can be concluded that rations containing high roughage ratio enhanced nutrient degradability, *In vitro* fermentations, microbial protein and increased ruminal protozoa.

***Keywords**:* Roughage, *nutrients disappearance, in vitro fermentations, protozoa and bacteria.*

INTRODUCTION

Involving high roughage feeding in ruminant's diets increases the percentage of fiber and its contents in the diets, which decreases the digestibility coefficient of nutrients. Taiz and Zeiger (1991) stated that plant cell wall, the major reservoir of fixed carbon in nature, consists of three major polymers, involving cellulose, hemicelluloses and lignin. Many attempts had been made to increase the digestibility of these constituents which reversed on increasing the digestibility of nutrients. High proportion of concentrates into ruminant diets increased dietary energy, proteins, minerals, and vitamins and optimized the efficiency of feed utilization (Morand-Fehr and Sauvant 1987). Feeding rations containing high levels of roughage reduced endogenous fecal protein excretion (Webster *et al.*, 2000). Also, increasing the level of roughage in diets increases salivary flow rate (A.F.R.C. 1991), which enhances ruminal pH value and improves ruminal environment for rumen microbes growth, which reverses on rumen fermentations and improved animal performance.

In feed evaluation systems, the factors as rumen degradation characteristics of feed are particularly important. It would be of great advantage to be able to understand the degradation characteristics of feed as disappearance and fermentation to determine the optimal concentrate and roughage consumption.

The benefits of roughage inclusion in diets of ruminants seem to be caused by changes in ruminal fermentation, in particular by increased protozoal and bacterial activities, which in turn leads to increased degradability of forages and flow of microbial protein from the rumen (Wallace and Newbold, 1992).

The rumen has an important role and function in preparing fermentation end products as NH_3-N, volatile fatty acids (VFAs), microbial synthesis of VFAs and microbial synthesis of proteins for biosynthetic processes of ruminants. It was found that rumen was affected by type of feeds and roughage to concentrate ratio, which consequently influenced on rumen microorganisms and fermentation pattern (Khampa and Wanapat, 2007). Also, it is important to increase the ruminal microbial ecosystem to improve the efficiency of converting feed to produce consumable products by humans.

The main aims of the present research were to study the effects of rations varying in roughage to concentrate ratio on *in vitro* chemical compositions, *in vitro* cell wall constituents analysis at different sampling times and *in vitro* nutrient disappearance as a target for understanding the way of digestibility of these nutrients in the rumen. Also, study, *in vitro* fermentations, microbial protein, *in vitro* protozoa, bacteria count, utilization of energy and ruminal protozoa count in sheep as that rumen microorganisms are the main factor affected nutrients digestibility and rumen fermentation which reflects on animal performance.

MATERIALS AND METHODS

Three rations differed in concentrate: roughage ratios were designed and used in two trials. Firstly, *In vitro* trial was carried out to study *in vitro* chemical compositions and cell wall constituents analysis at different sampling times, also, to study *in vitro* nutrient disappearance of rations (as that *in vivo* digestibility coefficients of nutrients of the same rations were studied in pervious study, Badway *et al.*, 2013), *in vitro* fermentations, the identification and density of rumen ciliate protozoa and cellulolytic bacteria. Secondly, digestibility trial was carried out to investigate utilization of energy and ruminal protozoa in adult rams fed the three experimental rations.

Experimental rations:

The experimental rations contained 90: 10% (R1), 80:20% (R2) and 70:30% (R3), respectively. Ingredients, chemical composition and cell wall constituents of the experimental rations used in this work are presented in Table (1).

Table (1): Ingredients, chemical composition and cell wall constituents of the experimental rations.

Item	R1	R2	R3
Ingredient (%):			
Yellow corn	39	45	49
Soybean meal	12	14	15
Wheat bran	31	13	0
Hay	10	20	30
Molasses	5	5	3
Limestone	1.5	1.5	1.5
Sodium chloride	1	1	1
Minerals mixture and vitamins	0.5	0.5	0.5
Total	100	100	100
CP%	14.40	14.30	14.26
TDN (%)	67.23	66.99	66.47
Proximate chemical analysis, % (on DM basis):			
DM	90.59	91.17	91.02
OM	91.78	91.22	90.04
CP	14.00	13.98	13.98
CF	7.40	9.47	11.61
EE	3.44	2.82	2.10
ASH	8.22	8.77	9.96
NFE	66.94	64.96	62.35
Fiber fraction (%):			
NDF	37.44	34.04	39.68
ADF	11.50	13.85	15.68
ADL	2.48	3.35	4.94
Hemi-cellulose	14.82	10.72	32.7
Cellulose	7.33	8.45	9.89

CP and TDN were calculated. R1: 90% concentrate: 10% roughage. R2: 80% concentrate: 20% roughage. R3: 70% concentrate: 30% roughage.

In vitro incubation of the experimental rations:

Ruminal liquor (RL) was collected, two hours post-feeding from six adult rams fed concentrate feed mixture and good quality berseem hay at ratio of 70:30. Collected rumen liquor was kept warm in plastic Jug (35-37 ºC), strained through two layers of cheese cloth and mixed with urea-buffer under the lab conditions for *in vitro* study. A sample of this rumen liquor was taken to test before used in the *in vitro* trial. The ruminal liquor was incubated with the samples of the three rations, in two replicates for each sample at different incubation times (2, 4, 6, 8, 24, and 48 h) to estimated chemical compositions, cell wall constituents and nutrients disappearance at different incubation times.

Parameters of the ruminal liquor used for ration incubation are presented in Table (2).

Table (2): Examination of ruminal fluid used for ration incubation.

Item	Amount
pH value	6.8
TVFA's (ml equiv/100 ml)	6.30
Molar proportion of individual VFA's (%):	
Acetic acid (A)	29.98
Propionic acid (P)	14.22
Butyric acid	10.10
A/P ratio	2.11
Total nitrogen (mg/100 ml)	117.60
True protein nitrogen (mg/100 ml)	39.20
NPN (mg/100 ml)	78.40
Ammonia nitrogen (mg/100 ml)	37.33
Microbial protein (mg/100 ml)	54.75
Total protozoa count ($x10^4$ cell /ml)	1222.75
Entodinum spp.	998.50
Isotrchia spp.	48.10
Dasytrachia spp.	26.50
Epidinium spp.	45.85
Diplodinum spp.	53.85
Polyolastron spp.	24.10
Ophryoscolox spp.	25.85
Total bacterial number ($x10^8$ cell/ml)	2.35
Cellulolytic bacteria number ($x10^6$ cell/ml)	2.82

Proximate chemical analysis and analysis of cell wall constituents:

The proximate analysis of ration samples was carried out according to the A.O.A.C. (1990). The proximate analyses were used to determine dry matter (DM), crude protein (CP), crude fiber (CF), and ether extract (EE), while nitrogen free extract (NFE) was obtained by the difference. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the procedures of Van Soest *et al.* (1991). Cellulose and hemicelluloses were calculated by the difference between NDF and ADF for hemicelluloses, and ADF and ADL for cellulose.

***In vitro* nutrient *disappearance* at different times of incubation:**

The *in vitro* nutrient disappearance of dry matter (IVDM), organic matter (IVOM), curd protein (IVCP), crude fiber (IVCF) and either extract (IVEE) and cell wall constituents disappearance were determined according to the method described by Terry *et al.* (1969), modified by Norris (1976). The *in vitro* disappearance was estimated from the remaining in the tube after incubation times by drying at 105 ºC for 24 h.

In vitro fermentations:

In vitro rumen liquor samples were obtained at different incubation times (2, 4, 6, 8, 24, 48 h) and filtered through two layers of cheese cloth to remove feed particles. About 5 ml of the liquid that surround each tube of replicates at different sampling times were removed to be tested later. Value of pH in the rumen liquor was immediately determined using pH meter (model

the pHep), then 1 ml toluene and 1 ml paraffin oil were added to the strained ruminal fluid and stored in deep freeze at (-20ºC) until analyses. Ammonia nitrogen concentration (NH3-N) was determined according to A.O.A.C (1990), while concentration of total volatile fatty acids (TVFA's) was determined according to Warner (1964). Proportion of acetic, propionic and butyric was determined by gas liquid chromatography as described by Erwin *et al.* (1961).Concentration of total nitrogen (TN) and non-protein nitrogen (NPN) was determined by the modified semi-micro-kjeldahl digestion method according to A.O.A.C (1990), while concentration of true protein nitrogen (TP) was calculated by subtracting the non-protein nitrogen content from total nitrogen content. Yield of mmicrobial protein was estimated as described by Makkar *et al.* (1982).

In vitro ciliated protozoa count and classification:

Number and classification of *in vitro* rumen protozoa per 1 ml RL were determined at each sampling times (2, 4, 6, 8, 24, and 48 h). The collected contents were immediately flittered through one layer of gauze cloth, then fixed and stained with 4 times volume of methyl-green formalin saline solution as described by Ogimoto and Imai (1981) (100 ml formaldehyde 35% ,900 ml distilled water, methyl-green 0.6 g and sodium chloride 0.8 g), then stoked in dark place until examination.

After gentle mixing of fixed RL samples, one drop was poured on hemocytometer slide, covered with a cover slip and examined under a light microscope for identification of genera and species according to the description published by Dehority (1993).

The number of rumen protozoa per 1 ml RL was calculated as follows:

Number of protozoa/1 ml RL = N x 5 x 10 x 4

Where: N = Number of protozoa in one large corner square of white blood cells.

In vitro number of total bacteria and cellulolytic bacteria *was* determined by the anaerobic method of Bryant (1972) using the anaerobic diluents described by Mann (1968) to determine count of total bacteria and cellulolytic bacteria.

Digestibility trials:

Nine adult rams were used in digestibility trial to investigate utilization of energy, as well as rumen protozoa.

Animals were placed in metabolic cages (three in each group), weighed at the start and the end of the trial. The digestibility trial lasted for 17 days from which the first 10 days were considered as an adaptation and preliminary period, followed by 7 days as collection period. Over the collection period, daily amount of feed consumed and residuals were accurately weighed and recorded to determine the intake, and feces was quantitative collected from each animal to determined utilization of energy.

Rumen liquor (RL) samples were obtained at 0, 3, 6 and 8 hours post feeding to study ruminal ciliated protozoa count and classification.

Statistical analysis:

The data was statistically analyzed according to statistical analysis system of SAS (2000). The used design was two-way analysis, and the following model was used: $Y_{ij} = \mu + R_i + I_j + RI_{ij} + e_{ij}$

Where: Y_{ij} = experimental observation, μ = general mean, R_i = effect of ration, I_j = effect of sampling time, RI_{ij}=effect of interaction between ration and sampling time, and eij= random error.

The significant differences were carried out using Duncan's multiple tests (Duncan, 1955) at P<0.05.

RESULTS AND DISCUSSION

Chemical composition of the experimental rations at different incubation times:

Data presented in Table (3) showed that the average content of DM, OM and CP were significantly (P<0.05) affected by the experimental rations. The 1st ration (R1) had significantly (P<0.05) the highest DM, OM and CP contents, followed by R2, while R3 had significantly (P<0.05) the lowest contents. As affected by sampling time, the overall mean of DM, OM and CP contents showed significantly (P<0.05) gradual reduction by progressed incubation time, showing the highest (P<0.05) contents after 2 h of incubation, whereas, the lowest (P<0.05) ones were recorded after 48 h of incubation.

The observed reduction in CP content by progressed time of incubation may be due to rumen microorganisms (protozoa and bacteria), which consume ammonia of dietary CP digestion to convert it into microbial protein.

Data shown in Table (3) revealed that the average CF content showed significant (P<0.01) differences among the experimental rations. The R1 had significantly (P<0.05) the lowest CF content and the highest (P<0.05) EE and NFE contents, while, R1 showed an opposite trend. On the other hand, R2 had moderate contents of CF, EE and NFE. Contents of CF, EE and NFE was affected significantly (P<0.05) by sampling time with different rates. The overall means of CF content showed gradual decrease by progressed incubation time, while EE and NFE contents showed significantly (P<0.05) marked reduction after 6 h of incubation. The decrease of CF content by progressed time of incubation may be due to cellulolytic bacteria which secreted cellulase enzyme to degrade CF.

Table (3): Chemical composition of the experimental rations at different times of incubation.

Item	Time (h)	Treatment			Overall mean
		R1	R2	R3	
DM	2	85.62±0.089	84.96±0.089	83.40±0.089	84.66^{a}±0.051
	4	83.72±0.089	83.65±0.089	80.56±0.089	82.64^{b}±0.051
	6	81.77±0.089	80.10±0.089	76.80±0.089	79.55^{c}±0.051
	8	76.52±0.089	76.03±0.089	73.79±0.089	75.44^{d}±0.051
	24	55.39±0.089	54.02±0.089	51.18±0.089	53.53^{e}±0.051
	48	35.70±0.089	33.70±0.089	28.44±0.089	32.61^{f}±0.051
Overall mean		69.78^{a}±0.036	68.74^{b}±0.036	65.69^{c}±0.036	
OM	2	84.85±0.162	83.31±0.162	80.88±0.162	83.01^{a}±0.093
	4	82.42±0.162	80.81±0.162	78.04±0.162	80.42^{b}±0.093
	6	79.76±0.162	77.60±0.162	74.46±0.162	77.27^{c}±0.093
	8	50.33±0.162	47.23±0.162	43.50±0.162	47.02^{d}±0.093
	24	39.25±0.162	37.51±0.162	30.02±0.162	35.59^{e}±0.093
	48	33.26±0.162	30.54±0.162	22.87±0.162	28.89^{f}±0.093
Overall mean		61.64^{a}±0.066	59.50^{b}±0.066	54.96^{c}±0.066	
CP	2	11.79±0.13	10.60±0.13	9.78±0.13	10.72^{a}±0.074
	4	8.55±0.13	8.32±0.13	7.70±0.13	8.19^{b}±0.074
	6	6.68±0.13	6.09±0.13	4.94±0.13	5.90^{c}±0.074
	8	4.81±0.13	4.38±0.13	3.41±0.13	4.20^{d}±0.074
	24	2.97±0.13	2.36±0.13	1.38±0.13	2.24^{e}±0.074
	48	1.58±0.13	1.35±0.13	0.86±0.13	1.26^{f}±0.074
Overall mean		6.06^{a}±0.052	5.52^{b}±0.052	4.68^{c}±0.052	
CF	2	6.41±0.12	8.05±0.12	9.52±0.12	7.99^{a}±0.067
	4	5.71±0.12	6.85±0.12	7.75±0.12	6.77^{b}±0.067
	6	4.86±0.12	4.99±0.12	5.90±0.12	5.25^{c}±0.067
	8	4.08±0.12	4.42±0.12	4.2±0.12	4.23^{d}±0.067
	24	3.53±0.12	2.72±0.12	3.07±0.12	3.11^{e}±0.067
	48	2.82±0.12	1.41±0.12	1.22±0.12	1.82^{f}±0.067
Overall mean		4.57^{c}±0.047	4.74^{b}±0.047	5.27^{a}±0.047	
EE	2	3.11±0.53	2.38±0.53	1.70±0.53	2.40^{a}±0.30
	4	2.80±0.53	1.94±0.53	1.48±0.53	2.07^{ab}±0.30
	6	2.42±0.53	1.48±0.53	1.17±0.53	1.69^{ab}±0.30
	8	2.05±0.53	1.06±0.53	0.92±0.53	1.34^{bc}±0.30
	24	1.71±0.53	0.69±0.53	2.75±0.53	1.71^{ab}±0.30
	48	1.40±0.53	0.30±0.53	0.20±0.53	0.63^{c}±0.30
Overall mean		2.25^{a}±0.29	1.31^{b}±0.29	1.37^{b}±0.29	
NFE	2	63.52±2.9	62.27±2.9	59.86±2.9	61.88^{a}±1.67
	4	65.36±2.9	63.70±2.9	61.11±2.9	63.39^{a}±1.67
	6	65.79±2.9	65.03±2.9	62.44±2.9	64.42^{a}±1.67
	8	39.38±2.9	37.37±2.9	34.96±2.9	37.23^{b}±1.67
	24	31.04±2.9	31.73±2.9	25.56±2.9	29.44^{c}±1.67
	48	27.45±2.9	27.46±2.9	20.59±2.9	25.16^{c}±1.67
Overall mean		48.76^{a}±1.18	47.92^{a}±1.18	44.08^{b}±1.18	

Means with the different letters in the same row and column are significantly different at P<0.05.

Cell wall constituents analysis:

Data presented in Table (4) revealed that R1 showed significantly (P<0.05) the highest NDF and the lowest ADF and ADL contents as compared to other rations. Contents of ADF and ADL were the highest (P<0.05) in R3 and moderate in R2, while NDF content was nearly similar in R2 and R3. The overall means of NDF, ADF and ADL contents showed (P<0.05) gradual decrease by progressed incubation time. The highest value

(P<0.05) was after 2 h of incubation, while the lowest one was recorded after 48 h of incubation.

Results illustrated in Table (4) revealed that R3 showed significantly (P<0.05) the highest cellulose and hemi-cellulose contents. However, hemi-cellulose content was higher (P<0.05) and cellulose content was lower (P<0.05) in R1 than in R2. The overall means of cellulose and hemi-cellulose contents showed gradual decrease (P<0.05).

Cellulose, a long polymer of β-1, 4-glucose, is the major component of plant cell wall (Reiter, 2002). Rumen microbes (protozoa and cellulolytic bacteria) secrete many different types of cellulose enzymes for efficient degradation of this substrate.

The present results indicated that R3 had the highest contents of ADF, ADL, cellulose and hemicelluloses due to the increasing roughage to concentrate ratio (30:70%) as compared to other rations.

Table (4): Cell wall constituents of the experimental rations at different times of incubation.

Item	Time (h)	Treatment			Overall mean
		R1	R2	R3	
NDF	2	32.45±6.15	28.61±6.15	33.34^{a}±6.15	31.46^{a}±3.55
	4	27.61±6.15	22.96±6.15	26.92±6.15	25.83^{b}±3.55
	6	22.49±6.15	17.51±6.15	20.35±6.15	20.11^{c}±3.55
	8	17.30±6.15	12.30±6.15	13.82±6.15	14.47^{d}±3.55
	24	12.17±6.15	6.62±6.15	5.81±6.15	8.20^{e}±3.55
	48	7.07±6.15	2.06±6.15	1.62±6.15	3.58^{f}±3.55
Overall mean		19.84 ^{a}±2.51	15.01^{b}±2.51	16.97^{ab}±2.51	
ADF	2	10.02±0.57	11.51±0.57	13.85±0.57	11.79^{a}±0.33
	4	7.93±0.57	9.30±0.57	10.50±0.57	9.24^{b}±0.33
	6	6.11±0.57	7.29±0.57	7.65±0.57	7.01^{c}±0.33
	8	4.65±0.57	5.39±0.57	5.57±0.57	5.20^{d}±0.33
	24	3.02±0.57	3.46±0.57	5.56±0.57	4.01^{e}±0.33
	48	1.85±0.57	1.55±0.57	1.43±0.57	1.61^{f}±0.33
Overall mean		5.59^{c}±0.23	6.41^{b}±0.23	7.42^{a}±0.23	
ADL	2	2.30±0.24	2.97±0.24	3.87±0.24	3.04^{a}±0.14
	4	2.08±0.24	2.55±0.24	3.25±0.24	2.63^{b}±0.14
	6	1.90±0.24	2.16±0.24	2.62±0.24	2.22^{b}±0.14
	8	1.69±0.24	1.74±0.24	1.98±0.24	1.80^{c}±0.14
	24	1.48±0.24	1.35±0.24	2.43±0.24	1.75^{c}±0.14
	48	1.29±0.24	1.055±0.24	0.84±0.24	1.06^{d}±0.14
Overall mean		1.79^{b}±0.097	1.97^{b}±0.097	2.50^{a}±0.097	
Cellulose	2	7.72±0.081	8.54±0.081	9.98±0.081	8.75^{a}±0.047
	4	5.84±0.081	6.75±0.081	7.24±0.081	6.61^{b}±0.047
	6	4.20±0.081	5.13±0.081	5.03±0.081	4.79^{c}±0.047
	8	2.96±0.081	3.65±0.081	3.59±0.081	3.40^{d}±0.047
	24	1.54±0.081	2.11±0.081	1.62±0.081	1.76^{e}±0.047
	48	0.55±0.081	0.49±0.081	0.59±0.081	0.55^{f}±0.047
Overall mean		3.80^{c}±0.033	4.44^{b}±0.033	4.67^{a}±0.033	
Hemicellulose	2	22.43±8.58	17.10±8.5	19.49±8.5	19.67^{a}±4.95
	4	19.68±8.5	13.66±8.5	16.42±8.5	16.59^{ab}±4.95
	6	16.38±8.5	10.22±8.5	12.69±8.5	13.09^{ab}±4.95
	8	12.64±8.5	6.90±8.5	8.25±8.5	9.26^{ab}±4.95
	24	9.14±8.5	3.16±8.5	38.36±8.5	16.88^{ab}±4.95
	48	5.22±8.5	0.51±8.5	0.19±8.5	1.97^{b}±4.95
Overall mean		14.25^{b}±3.50	8.59^{c}±3.50	15.90^{a}±3.50	

Means with the different letters in the same row and column are significantly different at P<0.05.

All items of chemical composition and cell wall constituents of the experimental rations showed gradual decrease by progressed incubation time. This is due to the digestion of these nutrients by ruminal protozoa and

bacteria in the ruminal fluid used in incubation. Protozoa and cellulolytic bacteria secrete many different types of cellulose enzymes for efficient degradation of lingo-cellulosic substrates. The large number and diversity of enzymes secreted by these microorganisms reflect the complex chemical composition of polysaccharides surrounding the cellulose fibrils in the plant cell wall (Abd El-Galil and Khorshed, 2010). In this respect, Wallace and Newbold (1992) suggested that a more active bacterial population leads to an increase in fiber digestion.

In vitro disappearance at different incubation times:

Nutrients disappearance:

Data in Table (5) showed that overall mean of *in vitro* disappearance (IV) of DM, OM, CP and CF was the highest (P<0.05) in R3, followed by R2 and R1, respectively. However, *in vitro* disappearance of EE was higher (P<0.05) in R2 and R3 than in R2. <0.05<0.05<0.05

Overall mean of *in vitro* disappearance of DM, OM, CP, CF and EE showed gradual increase (P<0.05) by progressed incubation time, being the highest (P<0.05) at 48 h and the lowest (P<0.05) at 2 h after incubation.

Generally, the observed increase in disappearance of DM and OM was associated with decreasing of disappearance of CP, CF at different sampling times. These results indicated the highest disappearance of DM, OM, CP, CF and EE in R3 containing 70: 30 roughage to concentrate ratio as compared to R2 and R1 and may suggest that their digestibility was more efficient in R3 compared with R2 and R1. In this respect, **Sawe *et al.* (1998)** concluded that DM digestibility is positively correlated with CP content and negatively with CF, ADF and NDF. Also, Tiwari *et al.* (2007) mentioned that degradation of CF and cell wall constituents increased by increasing number of cellulolytic bacteria which secreted cellulase enzymes. The *in vitro* DM disappearance is in agreement with that obtained by Gado and Abd El-Galil (2009), who found that in *vitro* DM disappearance ranged from 51.3 to 64.8% by using five strains of cellulolytic bacteria.

***C*ell wall constituents disappearance:**

Results of cell wall constituents disappearance at different incubation times presented in Table (6) revealed that disappearance of NDF, ADF and ADL was significantly (P<0.05) the highest in R3, followed by R2 and R1, respectively. On the other hand, disappearance of cellulose was higher (P<0.05) in R2 and R3 than in R1, while hemicelluloses disappearance showed an opposite trend.

Overall mean of in vitro disappearance of NDF, ADF, ADL, cellulose and hemicelluloses showed gradual increase (P<0.05) by progressed incubation time. The highest (P<0.05) values were recorded at 48 h, while the lowest (P<0.05) values were recorded at 2 h after incubation.

According to the present results of the current study, rations contained high roughage to concentrate ratio tended to cause a significant increases in cellulose disappearance, which was in relation with increasing nutrients disappearance.

Table (5): *In vitro* disappearance (%) of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF) and ether extract (EE) of the experimental rations at different incubation times.

Item	Time (h)	Treatment			Overall mean
		R1	R2	R3	
DM %	2	5.49±0.098	6.80±0.098	8.37±0.098	6.89^{f}±0.057
	4	7.58±0.098	8.24±0.098	11.48±0.098	9.10^{e}±0.057
	6	9.73±0.098	12.14±0.098	15.61±0.098	12.49^{d}±0.057
	8	15.52±0.098	16.60±0.098	18.93±0.098	17.02^{c}±0.057
	24	38.85±0.098	40.74±0.098	43.77±0.098	41.12^{b}±0.057
	48	60.59±0.098	63.03±0.098	68.74±0.098	64.12^{a}±0.057
Overall mean		22.96^{c}±0.040	24.59^{b}±0.040	27.81^{a}±0.040	
OM %	2	7.56±0.178	8.67±0.178	10.17±0.178	8.80^{f}±0.102
	4	10.20±0.178	11.41±0.178	13.32±0.178	11.64^{e}±0.102
	6	13.10±0.178	14.94±0.178	17.30±0.178	15.11^{d}±0.102
	8	45.16±0.178	48.23±0.178	51.69±0.178	48.36^{c}±0.102
	24	57.23±0.178	58.88±0.178	66.65±0.178	60.92^{b}±0.102
	48	63.76±0.178	66.52±0.178	74.59±0.178	68.29^{a}±0.102
Overall mean		32.83^{c}±0.072	34.77^{b}±0.072	38.95^{a}±0.072	
CP %	2	15.75±0.920	24.17±0.920	30.01±0.920	23.31^{f}±0.531
	4	38.93±0.920	40.45±0.920	44.88±0.920	41.42^{e}±0.531
	6	52.25±0.920	56.40±0.920	64.62±0.920	57.75^{d}±0.531
	8	65.60±0.920	68.67±0.920	75.61±0.920	69.96^{c}±0.531
	24	78.78±0.920	83.08±0.920	90.09±0.920	83.98^{b}±0.531
	48	88.68±0.920	90.31±0.920	93.81±0.920	90.93^{a}±0.531
Overall mean		56.66^{c}±0.375	60.51^{b}±0.375	66.50^{a}±0.375	
CF %	2	13.31±1.24	14.94±1.24	17.96±1.24	15.40^{f}±0.716
	4	22.83±1.24	27.66±1.24	33.25±1.24	27.91^{e}±0.716
	6	34.25±1.24	47.31±1.24	49.18±1.24	43.58^{d}±0.716
	8	44.80±1.24	53.33±1.24	63.82±1.24	53.98^{c}±0.716
	24	52.30±1.24	71.22±1.24	73.04±1.24	65.52^{b}±0.716
	48	61.82±1.24	85.05±1.24	89.49±1.24	78.79^{a}±0.716
Overall mean		38.22^{c}±0.506	49.92^{b}±0.506	54.45^{a}±0.506	
EE %	2	9.44±0.689	15.42±0.689	18.81±0.689	14.56^{f}±0.398
	4	18.60±0.689	31.03±0.689	29.52±0.689	26.38^{e}±0.398
	6	29.50±0.689	47.34±0.689	44.28±0.689	40.37^{d}±0.398
	8	40.26±0.689	62.41±0.689	55.95±0.689	52.87^{c}±0.398
	24	50.29±0.689	75.53±0.689	77.38±0.689	67.73^{b}±0.398
	48	59.30±0.689	89.18±0.689	90.47±0.689	79.65^{a}±0.398
Overall mean		34.56^{b}±0.281	53.48^{a}±0.281	52.73^{a}±0.281	

Means with the different letter in the same row and column are significantly different at ($P<0.05$).

Table (6): *In vitro* cell wall constituents disappearance of the experimental rations at different incubation times.

Item	Time (h)	Treatment			Overall mean
		R1	R2	R3	
NDF (%)	2	13.32±0.215	15.93±0.215	15.97±0.215	15.07^{f}±0.124
	4	26.24±0.215	32.53±0.215	32.14±0.215	30.30^{e}±0.124
	6	39.93±0.215	48.56±0.215	48.71±0.215	45.73^{d}±0.124
	8	53.79±0.215	63.87±0.215	65.17±0.215	60.94^{c}±0.124
	24	67.49±0.215	80.55±0.215	80.28±0.215	76.11^{b}±0.124
	48	81.12±0.215	93.94±0.215	95.90±0.215	90.32^{a}±0.124
Overall mean		46.98^{c}±0.087	55.90^{b}±0.087	56.36^{a}±0.087	
ADF (%)	2	12.87±0.623	16.86±0.623	11.67±0.623	13.80^{f}±0.359
	4	31.04±0.623	32.81±0.623	33.03±0.623	32.29^{d}±0.359
	6	46.87±0.623	47.36±0.623	51.18±0.623	48.47^{e}±0.359
	8	59.52±0.623	61.05±0.623	64.47±0.623	61.68^{c}±0.359
	24	73.69±0.623	75.01±0.623	78.63±0.623	75.78^{b}±0.359
	48	83.91±0.623	88.80±0.623	90.85±0.623	87.8^{a}±0.359
Overall mean		51.31^{c}±0.254	53.65^{b}±0.254	54.97^{a}±0.254	
ADL (%)	2	7.25±0.577	11.19±0.577	21.66±0.577	13.37^{f}±0.333
	4	15.92±0.577	23.88±0.577	34.11±0.577	24.63^{e}±0.333
	6	23.18±0.577	35.52±0.577	46.96±0.577	35.22^{d}±0.333
	8	31.65±0.577	47.91±0.577	59.92±0.577	46.49^{c}±0.333
	24	40.32±0.577	59.70±0.577	70.95±0.577	56.99^{b}±0.333
	48	47.78±0.577	68.51±0.577	82.89±0.577	66.39^{a}±0.333
Overall mean		27.68^{c}±0.235	41.11^{b}±0.235	52.75^{a}±0.235	
Cellulose (%)	2	13.53±0.396	15.30±0.396	18.79±0.396	15.87^{f}±0.228
	4	24.11±0.396	32.34±0.396	31.56±0.396	29.34^{e}±0.228
	6	36.85±0.396	49.38±0.396	47.10±0.396	44.44^{d}±0.228
	8	51.25±0.396	65.80±0.396	65.62±0.396	60.89^{c}±0.228
	24	64.74±0.396	84.35±0.396	81.35±0.396	76.81^{b}±0.228
	48	79.87±0.396	97.47±0.396	99.21±0.396	92.18^{a}±0.228
Overall mean		45.06^{b}±0.161	57.44^{a}±0.161	57.27^{a}±0.161	
Hemi-cellulose (%)	2	14.41±0.860	18.66±0.860	7.07±0.860	13.38^{f}± 0.497
	4	35.20±0.860	35.67±0.860	32.54±0.860	34.47^{e}± 0.497
	6	53.38±0.860	51.14±0.860	53.12±0.860	52.54^{d}± 0.497
	8	67.18±0.860	65.24±0.860	66.57±0.860	66.33^{c}± 0.497
	24	82.87±0.860	79.90±0.860	82.17±0.860	81.65^{b}± 0.497
	48	93.85±0.860	95.28±0.860	94.50±0.860	94.54^{a}± 0.497
Overall mean		57.81^{a}±0.351	57.65^{a}±0.351	55.99^{b}±0.351	

Means with the different letter in the same row and column are significantly different at (P<0.05).

In the current study the data demonstrate that the rations contained high roughage to concentrate ratio (30:70 and 20:80 %) tended to increase dry matter, fiber and cell wall constituents degradation, this is may be due to cellulolytic bacteria which have a very important role fiber degradation as it secreted cellulase enzymes which degrade crude fiber and cell wall constituents to convert feedstuff to feed ruminant animals (Tiwari *et al.*, 2007).

Also, Wallace and Newbold (1992) suggested that a more active bacterial population leads to an increase in fiber digestion. Moreover, Gado

and Abd El-Galil (2009) stated that cellulolytic bacteria strains isolated from sheep was more effective in increased the *in vitro* DM disappearance than those isolated from cow, buffalo and camel, because these active strains were secreted cellulase enzymes most effective on roughage than other strains.

In vitro fermentation parameters and microbial protein:

pH value, total volatile fatty acids (TVFA's) concentration and molar proportion of individual VFA's (%):

Data presented in Table (7) showed that *in vitro* pH values were the lowest (P<0.05) in R3, followed by R2 and R1, respectively. However, concentration of VFA's, and molar proportion of acetic, propionic and butyric acids was higher (P<0.05) in R3, followed by R2, while R1 showed the lowest (P,0.05) values. Meanwhile, ratio of acetic to propionic acid was the highest in R2, moderate in R2 and the lowest in R3 (P<0.05).

As affected by sampling time, overall mean of pH showed a significant decrease (P<0.05) after 4 h, then gradually increased with progressed incubation time reaching the highest (P<0.05) values at 48 h after incubation. On the other hand, overall mean of VFA's concentration and proportion of acetic, propionic and butyric acids showed marked increase (P<0.05) by increasing sampling time from 2 to 4 h after incubation, then showed gradual reduction (P<0.05) up to 48 h after incubation. However, Acetic to propionic acid ratio showed the highest values 2 h after incubation and the lowest ones 4 h after incubation (Table 7).

This could be attributed to that *in vitro* fermentation process by rumen microorganisms which took place on the soluble carbohydrates is very soon producing more propionate, decreasing pH value. While fermentation of the structural carbohydrates needs more time producing more acetate dealing the decreased pH value.

The present results are in agreement with those obtained by El-Ashry *et al.*, (1997), who reported that the minmum pH values were observed at 3 h post- feeding (ranged between 6.29 and 6.83) and tended to increase at 6 h post-feeding. Also, similar results were obtained by Aziz (2004).

The present results of VFA's are in agreement with Richard and Allen (1967), who showed that the *in vitro* system could detect differences in VFAs concentration between feeds containing different levels of concentrate or soluble carbohydrate. Also, Khampa and Wanapat (2007) showed that increasing levels of concentrate feeding dramatically lowered pH and resulted in as a consequence a decrease in VFA's concentration. They also showed that at lower pH fiber digestion was inhibited, this result was in agreement with the results of fiber digestion in the present study whereas, pH was high and near the optimum for fiber digestion, as that R1 which was the highest level of concentrate had the lowest value of fiber digestion. Moreover, Slyer (1976) indicated that rumen pH was most affected by roughage to concentrate ratios in regards to saliva secretion, rumination, VFAs production and microbial population.

Table (7): Effect of experimental rations on *in vitro* pH value, volatile fatty acids (TVFA's) and molar proportion of individual VFA's (%):

Item	Time (h)	Treatment			Overall mean
		R1	R2	R3	
pH value	2	6.60±0.000	6.40±0.000	6.30±0.000	6.43^{f}±0.000
	4	6.45±0.000	6.20±0.000	6.10±0.000	6.25^{d}±0.000
	6	6.50±0.000	6.40±0.000	6.30±0.000	6.40^{e}±0.000
	8	6.55±0.000	6.50±0.000	6.50±0.000	6.51^{c}±0.000
	24	6.60±0.000	6.60±0.000	6.50±0.000	6.56^{b}±0.000
	48	6.80±0.000	6.70±0.000	6.60±0.000	6.70^{a}±0.000
Overall mean		6.58^{a}±0.00	6.46^{b}±0.00	6.38^{c}±0.00	
TVFA's (ml equiv/100 ml RL)	2	7.10±0.083	7.95±0.083	8.62±0.083	7.89^{c}±0.048
	4	8.57±0.083	9.26±0.083	10.10±0.083	9.31^{a}±0.048
	6	7.85±0.083	8.37±0.083	9.07±0.083	8.43^{b}±0.048
	8	7.44±0.083	7.77±0.083	8.17±0.083	7.79^{c}±0.048
	24	6.37±0.083	6.60±0.083	6.75±0.083	6.57^{d}±0.048
	48	4.67±0.083	5.12±0.083	5.87±0.083	5.22^{e}±0.048
Overall mean		7.00^{c}±0.034	7.51^{b}±0.034	8.10^{a}±0.034	
Molar proportion (%) of acetic acid (A)	2	35.44±0.021	39.15±0.021	42.09±0.021	38.89^{d}±0.012
	4	40.15±0.021	44.64±0.021	47.05±0.021	43.94^{a}±0.012
	6	38.75±0.021	42.27±0.021	44.95±0.021	41.99^{b}±0.012
	8	36.10±0.021	41.12±0.021	42.03±0.021	39.75^{c}±0.012
	24	17.20±0.021	18.91±0.021	20.06±0.021	18.72^{e}±0.012
	48	8.16±0.021	8.85±0.021	9.72±0.021	8.91^{f}±0.012
Overall mean		29.30^{c}±0.01	32.49^{b}±0.01	34.32^{a}±0.01	
Molar proportion (%) of propionic acid (P)	2	19.40±0.008	20.05±0.008	21.20±0.008	20.22^{d}±0.004
	4	25.49±0.008	28.82±0.008	29.27±0.008	27.86^{a}±0.004
	6	22.60±0.008	24.33±0.008	25.32±0.008	24.08^{b}±0.004
	8	20.16±0.008	21.92±0.008	22.55±0.008	21.54^{c}±0.004
	24	9.21±0.008	9.85±0.008	12.34±0.008	10.46^{e}±0.004
	48	4.45±0.008	4.71±0.008	5.85±0.008	5.00^{f}±0.004
Overall mean		16.88^{c}±0.003	18.28^{b}±0.003	19.42^{a}±0.003	
Molar proportion (%) of butyric acid	2	15.51±0.022	16.40±0.022	16.98±0.022	16.29^{d}±0.012
	4	17.31±0.022	18.21±0.022	18.86±0.022	18.13^{a}±0.012
	6	16.95±0.022	17.88±0.022	18.50±0.022	17.77^{b}±0.012
	8	16.49±0.022	17.43±0.022	18.00±0.022	17.30^{c}±0.012
	24	6.21±0.022	6.99±0.022	7.32±0.022	6.84^{e}±0.012
	48	3.05±0.022	3.37±0.022	3.65±0.022	3.35^{f}±0.012
Overall mean		12.58^{c}±0.009	13.38^{b}±0.009	13.88^{a}±0.009	
A/P ratio	2	1.82±0.002	1.95±0.002	1.98±0.002	1.92^{a}±0.001
	4	1.57±0.002	1.55±0.002	1.61±0.002	1.57^{f}±0.001
	6	1.71±0.002	1.74±0.002	1.77±0.002	1.74^{e}±0.001
	8	1.79±0.002	1.88±0.002	1.86±0.002	1.84^{b}±0.001
	24	1.87±0.002	1.92±0.002	1.62±0.002	1.80^{c}±0.001
	48	1.83±0.002	1.88±0.002	1.66±0.002	1.79^{d}±0.001
Overall mean		1.76^{b}±0.001	1.82^{a}±0.001	1.75^{c}±0.001	

Means with the different letter in the same row and column are significantly different at (P<0.05).

Concentration of ammonia nitrogen (NH_3-N), total nitrogen (TN), non-protein nitrogen (NPN), true protein nitrogen (TPN) and microbial protein:

Data in Table (8) revealed that TN, NPN, NH_3-N and microbial protein concentrations increased (P<0.05) in R3 more than R2 and R1, respectively. Overall mean of TN, NPN, NH_3-N and microbial protein showed marked

increase (P<0.05) after 4 h of incubation, then it showed gradual decrease to reach the lowest (P<0.05) value at 48 h after incubation.

It is of interest to note that TPN was higher 24 and 48 h after incubation more than after 2 h of incubation which means that the microorganisms used soluble carbohydrates, NPN and NH_3-N to convert it into microbial protein. As NH_3-N is essential source of nitrogen for microbial protein synthesis (Khampa and Wanapat, 2007). Also, microbial efficiency expressed as microbial protein (mg/100 ml RL) showed that R3 was the highest ration; this may de due to the high number of protozoa and bacteria in this treatment.

Table (8): Effect of experimental rations on *in vitro* total nitrogen, true protein nitrogen, non-protein nitrogen (NPN), ammonia nitrogen and microbial protein concentrations.

Item	Time (h)	Treatment			Overall mean
		R1	R2	R3	
Total nitrogen (mg/dl RL)	2	123.56±0.294	129.12±0.294	135.93±0.294	129.54^{c}±0.170
	4	136.49±0.294	148.75±0.294	156.18±0.294	147.14^{a}±0.170
	6	124.90±0.294	137.67±0.294	140.57±0.294	134.38^{b}±0.170
	8	112.85±0.294	124.35±0.294	129.99±0.294	122.39^{d}±0.170
	24	102.27±0.294	109.81±0.294	118.50±0.294	110.19^{e}±0.170
	48	85.05±0.294	86.75±0.294	87.80±0.294	86.53^{f}±0.170
Overall mean		114.18^{c}±0.120	122.74^{b}±0.120	128.16^{a}±0.120	
True protein nitrogen (mg/dl RL)	2	44.94±0.405	44.34±0.405	42.68±0.405	43.99^{f}±0.234
	4	54.58±0.405	60.57±0.405	59.42±0.405	58.19^{a}±0.234
	6	47.70±0.405	54.33±0.405	48.84±0.405	50.29^{d}±0.234
	8	53.90±0.405	58.90±0.405	56.18±0.405	56.33^{b}±0.234
	24	48.55±0.405	49.64±0.405	49.80±0.405	49.33^{e}±0.234
	48	58.71±0.405	54.24±0.405	46.67±0.405	53.21^{c}±0.234
Overall mean		51.39^{b}±0.165	53.67^{a}±0.165	50.60^{c}±0.165	
NPN (mg/dl RL)	2	78.62±0.189	84.78±0.189	93.25±0.189	85.55^{b}±0.109
	4	81.90±0.189	88.17±0.189	96.76±0.189	88.94^{a}±0.109
	6	77.19±0.189	83.34±0.189	91.73±0.189	84.08^{c}±0.109
	8	58.95±0.189	65.44±0.189	73.81±0.189	66.06^{d}±0.109
	24	53.72±0.189	60.17±0.189	68.70±0.189	60.86^{e}±0.109
	48	26.33±0.189	32.50±0.189	41.12±0.189	33.32^{f}±0.109
Overall mean		62.78^{c}±0.077	69.07^{b}±0.077	77.56^{a}±0.077	
Ammonia nitrogen (mg/dl RL)	2	37.68±1.03	40.39±1.03	44.39±1.03	40.82^{ab}±0.596
	4	39.19±1.03	42.05±1.03	46.08±1.03	42.44^{a}±0.596
	6	36.63±1.03	39.55±1.03	43.70±1.03	39.96^{b}±0.596
	8	28.46±1.03	31.17±1.03	35.24±1.03	31.62^{c}±0.596
	24	25.36±1.03	28.56±1.03	28.05±1.03	27.32^{d}±0.596
	48	12.58±1.03	15.79±1.03	19.70±1.03	16.02^{e}±0.596
Overall mean		29.98^{c}±0.421	32.92^{b}±0.421	36.19^{a}±0.421	
Microbial protein (mg/dl RL)	2	65.26±0.250	70.32±0.250	74.04±0.250	69.87^{e}±0.144
	4	112.75±0.250	125.43±0.250	138.44±0.250	125.54^{a}±0.144
	6	110.87±0.250	121.90±0.250	135.35±0.250	122.71^{b}±0.144
	8	105.24±0.250	115.03±0.250	127.98±0.250	116.08^{c}±0.144
	24	66.17±0.250	72.18±0.250	75.61±0.250	71.32^{d}±0.144
	48	62.26±0.250	67.51±0.250	69.92±0.250	66.56^{f}±0.144
Overall mean		87.09^{c}±0.102	95.39^{b}±0.102	103.56^{a}±0.102	

Means with the different letter in the same row and column are significantly different at (P<0.05).

Count and classification of ciliated protozoa:

Data in Table (9) represented the identification of ruminal protozoa species and their counts in the rumen liquor (x10^4 cell/ml RL) at different sampling times for the experimental rations. Seven genera with 13 species and 7 subspecies of ruminal protozoa were identified in ruminal fluid of sheep in this study. These generas (genus) are *Entodinum spp*. [*E. simplex, E. caudatum, E.bursa, E. minimum and E. triacum*], *Dasytrachia rummantium*, *Isotrchia spp.* [*I.intestinalis and I. prostoma*], *Epidinium ecaudatum,* Diplodinum *anisacanthum*, *Polyolastron multivesiculatum* and *Ophryoscolox spp.* [*O. caudatus and O. purkynjei*]. The highest presence among all species was for *Entodinum spps*.

Table (9): Effect of experimental rations on *in vitro* ciliated protozoa count and classification.

Item	Time (h)	Treatment			Overall mean
		R1	R2	R3	
Total protozoa count (x10^4 cell/m RL)	2	1561.17±31.96	1981.90±31.96	2318.68±31.96	1953.91^{d}±18.45
	4	2424.25±31.96	3026.89±31.96	3483.21±31.96	2978.12^{a}±18.45
	6	2221.31±31.96	2609.31±31.96	2896.03±31.96	2575.55^{b}±18.45
	8	2060.55±31.96	2372.49±31.96	2639.01±31.96	2357.35^{c}±18.45
	24	1044.42±31.96	1421.28±31.96	1645.45±31.96	1370.38^{e}±18.45
	48	724.17±31.96	906.59±31.96	993.36±31.96	874.70^{f}±18.45
Overall mean		1672.64^{c}±13.05	2053.07^{b}±13.05	2329.29^{a}±13.05	
Entodinum spp.	2	1273.36±31.45	1661.17±31.45	1950.18±31.45	1628.23^{d}±18.15
	4	1895.31±31.45	2390.18±31.45	2755.42±31.45	2346.97^{a}±18.15
	6	1752.84±31.45	2053.19±31.45	2229.32±31.45	2011.78^{b}±18.15
	8	1635.26±31.45	1873.20±31.45	2050.30±31.45	1852.92^{c}±18.15
	24	899.10±31.45	1254.29±31.45	1455.28±31.45	1202.89^{e}±18.15
	48	672.32±31.45	833.37±31.45	900.06±31.45	801.91^{f}±18.15
Overall mean		1354.69^{c}±12.84	1677.56^{b}±12.84	1890.09^{a}±12.84	
Isotrchia spp.	2	57.26±1.23	63.36±1.23	71.08±1.23	63.90^{d}±0.713
	4	98.32±1.23	123.20±1.23	141.18±1.23	120.90^{a}±0.713
	6	88.08±1.23	103.41±1.23	130.39±1.23	107.29^{b}±0.713
	8	79.77±1.23	91.12±1.23	108.00±1.23	92.96^{c}±0.713
	24	30.59±1.23	36.64±1.23	40.88±1.23	36.03^{e}±0.713
	48	11.32±1.23	17.32±1.23	25.68±1.23	18.10^{f}±0.713
Overall mean		60.89^{c}±0.504	72.50^{b}±0.504	86.20^{a}±0.504	
Dasytrachia spp	2	34.58±0.166	40.70±0.166	54.52±0.166	43.26^{d}±0.096
	4	67.47±0.166	82.16±0.166	90.62±0.166	80.08^{a}±0.096
	6	58.31±0.166	71.22±0.166	82.33±0.166	70.62^{b}±0.096
	8	50.60±0.166	60.50±0.166	79.00±0.166	63.37^{c}±0.096
	24	17.37±0.166	18.75±0.166	26.32±0.166	20.81^{e}±0.096
	48	6.94±0.166	9.35±0.166	11.15±0.166	9.14^{f}±0.096
Overall mean		39.212^{c}±0.067	47.11^{b}±0.067	57.32^{a}±0.067	

Means with the different letter in the same row and column are significantly different at (P<0.05).

Table (9): Continued

Item	Time (h)	Treatment			Overall mean
		R1	R2	R3	
Epidinium spp.	2	58.26±0.641	62.30±0.641	68.32±0.641	62.96^{d}±0.370
	4	98.33±0.641	123.65±0.641	139.10±0.641	120.36^{a}±0.370
	6	86.87±0.641	102.47±0.641	128.71±0.641	106.02^{b}±0.370
	8	79.25±0.641	88.03±0.641	104.54±0.641	90.60^{c}±0.370
	24	30.07±0.641	36.32±0.641	39.53±0.641	35.30^{e}±0.370
	48	10.34±0.641	19.85±0.641	24.78±0.641	18.32^{f}±0.370
Overall mean		60.52^{c}±0.261	72.10^{b}±0.261	84.16^{a}±0.261	
Diplodinum spp.	2	65.70±0.147	69.90±0.147	73.74±0.147	69.78^{d}±0.085
	4	96.32±0.147	106.60±0.147	116.10±0.147	106.34^{a}±0.085
	6	85.36±0.147	94.37±0.147	108.53±0.147	96.08^{b}±0.085
	8	72.72±0.147	82.74±0.147	96.78±0.147	84.08^{c}±0.085
	24	32.35±0.147	36.53±0.147	39.75±0.147	36.21^{e}±0.085
	48	8.02±0.147	9.22±0.147	12.56±0.147	9.93^{f}±0.085
Overall mean		60.08^{c}±0.060	66.56^{b}±0.060	74.57^{a}±0.060	
Polyolastron spp.	2	36.78±0.167	41.38±0.167	43.53±0.167	40.56^{d}±0.096
	4	70.11±0.167	78.40±0.167	96.47±0.167	81.66^{a}±0.096
	6	63.37±0.167	66.46±0.167	87.45±0.167	72.43^{b}±0.096
	8	57.61±0.167	64.34±0.167	79.07±0.167	67.01^{b}±0.096
	24	17.46±0.167	19.37±0.167	21.18±0.167	19.34^{e}±0.096
	48	7.07±0.167	8.50±0.167	9.28±0.167	8.28^{f}±0.096
Overall mean		42.07^{c}±0.068	46.41^{b}±0.068	56.16^{a}±0.068	
Ophryoscolex spp	2	35.23±0.166	43.09±0.166	57.30±0.166	45.20^{d}±0.096
	4	98.39±0.166	122.70±0.166	144.31±0.166	121.80^{a}±0.096
	6	86.47±0.166	118.17±0.166	129.30±0.166	111.31^{b}±0.096
	8	85.32±0.166	112.55±0.166	121.30±0.166	106.39^{c}±0.096
	24	17.47±0.166	19.37±0.166	22.50±0.166	19.78^{e}±0.096
	48	8.15±0.166	8.97±0.166	9.85±0.166	8.99^{f}±0.096
Overall mean		55.17^{c}±0.067	70.81^{b}±0.067	80.76^{a}±0.067	

Means with the different letter in the same row and column are significantly different at (P<0.05).

The data showed that R3 recorded the highest (P<0.05) count of total ruminal protozoa and the highest (P<0.05) values of all generas, followed by R2, while the lowest (P<0.05) value was for R1. It seems that *Entodinum spp*. appeared most frequently among all protozoa species, while, *Dasytrachia spp.* and *Polyolastron spp.* appeared less frequently among all species.

Overall means of total protozoa count of different species showed marked increase (P<0.05) after 4 h of incubation, then gradually decreased to reach the lowest (P<0.05) value at 48 h after incubation. The values obtained in this study considered as normal level in rumen (Hungate, 1966). These results of microbial population may be affected by values of pH, as it was shown that rumen pH value appeared to exert effect on type of rumen microorganisms (Khampa and Wanapat, 2007).

The present results are in agreement with Aziz (2004 and 2009). Also, Santra *et al.* (1998) reported that numerically the most important group of protozoa was the small spirotrichs (65.6-70.1% of the total population), whereas Isotricha and large spirotricha are numerically is fewer in number in

the rumen of sheep and goats. Hristove *et al.* (2001) showed that *Entodinum sp.* made up 89 and 91% of the ciliated protozoal population in cattle fed medium- or high- concentrate barley–based diets. It is worthy noting that x level of concentrate in the diet markedly influenced the rumen protozoa population in lambs (Christiansen *et al.*, 1964), and ruminal acids are altered by the presence or absence of protozoa in the rumen of lambs fed high or low concentrate diets (Luther *et al.*, 1966).

Number of total bacteria and cellulolytic bacteria:

Data in Table (10) clearly showed that R3 had the highest number of total bacteria and cellulolytic bacteria, followed by R2 and R1, respectively. Overall mean of total bacteria and cellulolytic bacteria increased ($P<0.05$) after 4 h of incubation, then it showed gradual decrease to reach the lowest ($P<0.05$) value at 48 h after incubation.

There are major cellulolytic bacteria within the rumen. One of the reasons for its predominance is its ability to readily degrade various forms of crystalline cellulose, hemicelluloses and lignocelluloses materials. In this way, Wallace and Newbold (1992) suggested that more active bacterial populations lead to an increase in fiber digestion and microbial protein supply. Colombatto *et al.* (2003) stated that fibrolytic enzymes secreted by cellulolytic bacteria enhanced the fermentation of cellulose and xylan by a combination of per and post incubation effects. It seems that total ruminal protozoa count and differential count of species, also total bacteria numbers and cellulolytic bacteria number had the same trend, thus R3 (70:30% concentrate: roughage) had the highest values, this is may be due to that the low concentrate to roughage ratio enhance ruminal microbial population (Aziz *et al.*, 2012).

Table (10): Effect of experimental rations on *in vitro* total numbers of bacteria and cellulolytic bacteria number.

Number	Time (h)	Treatment			Overall mean
		R1	R2	R3	
Total bacterial ($x10^8$ cell/ml RL)	2	2.70±0.048	2.93±0.048	3.73±0.048	3.12^{d}±0.028
	4	3.27±0.048	3.85±0.048	4.37±0.048	3.83^{a}±0.028
	6	3.07±0.048	3.64±0.048	4.20±0.048	3.64^{b}±0.028
	8	2.87±0.048	3.40±0.048	3.95±0.048	3.41^{c}±0.028
	24	1.37±0.048	1.59±0.048	1.90±0.048	1.62^{e}±0.028
	48	0.635±0.048	0.720±0.048	0.785±0.048	0.71^{f}±0.028
Overall mean		2.32^{c}±0.019	2.69^{b}±0.019	3.15^{a}±0.019	
Cellulolytic bacteria ($x10^6$ cell /ml RL)	2	3.35±0.058	4.01±0.058	4.75±0.058	4.03^{d}±0.033
	4	3.94±0.058	4.68±0.058	5.56±0.058	4.72^{a}±0.033
	6	3.72±0.058	4.37±0.058	5.28±0.058	4.46^{b}±0.033
	8	3.42±0.058	4.20±0.058	4.90±0.058	4.17^{c}±0.033
	24	1.76±0.058	2.00±0.058	2.28±0.058	2.01^{e}±0.033
	48	0.805±0.058	1.00±0.058	1.13±0.058	0.980^{f}±0.033
Overall mean		2.83^{c}±0.023	3.38^{b}±0.023	3.98^{a}±0.023	

Means with the different letter in the same row and column are significantly different at ($P<0.05$).

Digestibility trail:

Effect of experimental rations on utilization of energy:

Data in Table (11) revealed that R2 had the highest (P<0.05) values of gross, excreted, digested and metabolic energy (cal/h/day, cal/kg BW and cal/kg $BW^{0.75}$ and energy digestion coefficient %), followed by R1 then R3 which had the lowest values. It was clear that the differences between R2 and R1 were not significant, while the differences between R1 and R3 were not significant, except for energy digestion coefficient (%). The present data indicated that R2 (80:20% concentrate: roughage) was better in utilization of energy. The insignificant differences between R1 (90:10%) and R3 (70:30%) means that high roughage to concentrate diets doesn't affect utilization energy.

Table (11): Effect of experimental rations on utilization of energy.

Item	Treatment			±MSE
	R1	R2	R3	
Gross energy:				
cal/h/day	78703.87^{ab}	92449.34^{a}	71041.15^{b}	10042.11
cal/kg BW	2720.62^{a}	2445.72^{ab}	1712.01^{b}	373.76
cal/kg $BW^{0.75}$	4687.95^{ab}	5295.35^{a}	4230.16^{b}	498.97
Excreted energy:				
cal/h/day	7465.65^{ab}	8527.37^{a}	7150.87^{b}	410.67
cal/kg BW	257.57^{a}	226.99^{ab}	177.88^{b}	16.52
cal/kg $BW^{0.75}$	800.90^{ab}	885.84^{a}	772.62^{b}	33.70
Digested energy:				
cal/h/day	71238.22^{ab}	83921.97^{a}	63890.28^{b}	9883.63
cal/kg BW	2463.05^{a}	2218.73^{a}	1534.12^{b}	360.06
cal/kg $BW^{0.75}$	4350.49^{ab}	4924.32^{a}	3886.22^{b}	510.30
Metabolic energy:				
cal/h/day	58415.34^{ab}	68816.01^{a}	52390.03^{b}	8104.57
cal/kg BW	2019.70^{a}	1819.36^{ab}	1257.98^{b}	295.25
cal/kg $BW^{0.75}$	3748.85^{ab}	4243.33^{a}	3348.79^{b}	439.73
Energy digestion coefficient %	90.53^{a}	90.75^{a}	86.62^{b}	2.96

Means with the different letter in the same row and column are significantly different at (P<0.05).

In vivo ruminal protozoa count:

Data of *in vivo* ruminal protozoa count in Table (12) indicated presence of six genera with 16 species and 6 subspecies or formae of ruminal protozoa in RL of sheep in this study. These genuses were the same genus found in the *in vitro* trail, but there was no evidence of the presence of *Epidinum spp*. and this may be due to that the ruminal pH was not suitable for the growth of *Epidinum spp.* due to the composition of the rations which make ruminal pH higher than the suitable values.

Table (12): Effect of experimental rations on *in vivo* ciliated protozoa count and classification:

Count	Time (h)	Treatment			Overall mean
		R1	R2	R3	
Total protozoa ($x10^4$ cell /ml RL)	0	2307.95±24.02	2757.80±24.02	2755.92±24.02	2607.23^{b}±13.87
	3	1341.44±24.02	1233.83±24.02	1499.88±24.02	1358.38^{d}±13.87
	6	2089.36±24.02	2271.45±24.02	2333.83±24.02	2231.54^{c}±13.87
	8	2282.66±24.02	2897.42±24.02	2962.70±24.02	2714.26^{a}±13.87
Overall mean		2005.35^{c}±12.01	2290.12^{b}±12.01	2388.08^{a}±12.01	
Entodinum spp.	0	1972.83±96.11	2256.07±96.11	2483.98±96.11	2237.63^{a}±55.49
	3	1158.60±96.11	1206.73±96.11	1291.24±96.11	1218.85^{c}±55.49
	6	1750.46±96.11	1888.16±96.11	1974.67±96.11	1871.10^{b}±55.49
	8	1886.02±96.11	2119.35±96.11	2516.55±96.11	2173.97^{a}±55.49
Overall mean		1691.98^{c}±48.05	1867.57^{b}±48.05	2066.61^{a}±48.05	
Isotrchia spp.	0	52.53±2.80	92.95±2.80	96.71±2.80	80.73^{c}±1.62
	3	41.80±2.80	34.87±2.80	44.92±2.80	40.53^{d}±1.62
	6	55.49±2.80	96.52±2.80	101.54±2.80	84.52^{b}±1.62
	8	63.23±2.80	107.66±2.80	125.19±2.80	98.69^{a}±1.62
Overall mean		53.26^{c}±1.40	83.00^{b}±1.40	92.09^{a}±1.40	
Dasytrachia spp	0	31.86±0.187	32.62±0.187	69.90±0.187	44.79^{c}±0.108
	3	28.91±0.187	29.57±0.187	38.42±0.187	32.30^{d}±0.108
	6	37.67±0.187	40.87±0.187	57.87±0.187	45.47^{b}±0.108
	8	68.87±0.187	74.80±0.187	94.19±0.187	79.29^{a}±0.108
Overall mean		41.83^{c}±0.093	44.46^{b}±0.093	65.09^{a}±0.093	
Diplodinum spp.	0	130.51±0.884	118.22±0.884	195.07±0.884	147.93^{a}±0.510
	3	71.51±0.884	55.20±0.884	65.30±0.884	64.00^{d}±0.510
	6	90.48±0.884	72.55±0.884	90.76±0.884	84.59^{c}±0.510
	8	124.99±0.884	103.95±0.884	126.53±0.884	118.49^{b}±0.510
Overall mean		104.37^{b}±0.442	87.48^{c}±0.442	119.41^{a}±0.442	
Polyolastron spp.	0	83.58±0.601	70.34±0.601	100.90±0.601	84.94^{a}±0.347
	3	45.31±0.601	40.37±0.601	44.45±0.601	43.38^{c}±0.347
	6	67.18±0.601	60.52±0.601	81.80±0.601	69.83^{b}±0.347
	8	80.17±0.601	73.65±0.601	99.47±0.601	84.43^{a}±0.347
Overall mean		69.06^{b}±0.300	61.22^{c}±0.300	81.65^{a}±0.300	
Ophryoscolox spp	0	120.21±0.604	71.54±0.604	148.46±0.604	113.40^{b}±0.349
	3	59.24±0.604	38.22±0.604	45.50±0.604	47.65^{d}±0.349
	6	109.31±0.604	109.18±0.604	77.90±0.604	98.80^{c}±0.349
	8	148.34±0.604	119.41±0.604	99.58±0.604	122.44^{a}±0.349
Overall mean		109.28^{a}±0.302	84.59^{c}±0.302	92.86^{b}±0.302	

Means with the different letter in the same row and column are significantly different at (P<0.05).

It was cleared that *Entodinum spps.* was the most frequently among all species, whereas, *Isotrchia* and *Dasytrachia spps.* were the lowest frequently. Total protozoa count and count of *Entodinum, Isotrchia, Dasytrachia, Diplodinum* and *Polyolastron spps.* was the highest (P<0.05) for R3, followed by R2, while R1 showed the lowest count. Also, the highest (P<0.01) values at different sampling times for total protozoa count and these species were for R3 at 8 hrs post feeding, while the lowest (P<0.01) values were R1 at 3 hrs post feeding except for *Diplodinum, Polyolastron* and *Ophryoscolox spps.* the lowest (P<0.01) values were R2 at 3 hrs post

feeding. As for *Ophryoscolox spp.* the highest (P<0.01) value among rations was for R1, and the highest (P<0.01) value at different sampling times was for R1, which, suppose that high ruminal pH is suitable for *Ophryoscolox spp.* growth.

Overall mean of *in vivo* ruminal protozoa count and its species clearly showed a significant decrease (P<0.05) after 3 h of feeding, then gradually increased by progressed time of feeding.

In the present study, the decreasing number of protozoa after 3 h post-feeding may be related to the decreasing of ruminal pH after feeding as a result of higher TVFA's concentration. This finding is close with those reported by Aziz *et al.* (2012), who found the same species in the ruminal fluid of sheep lambs. They also found a significant decrease in protozoa count after 3 h of feeding, then it increased by increasing time of feeding. Similar results were reported by Franzolin and Dehorty (1996), who found that *Entodinium* constituted approximately 90% of the total protozoal numbers. Also, Ivan *et al.* (2000) found that *Entodinium* was the most detrimental of ciliated protozoa species.

The data of *in vitro* and *in vivo* rumen fermentations and microorganisms indicated that R3 (70:30% concentrate to roughage ratio) was more efficient in building the microbial society in the rumen more than other rations. Dehority and Orpin (1988) reported that diets containing between 40 to 60% concentrate will support maximal protozoa numbers with a diverse fauna containing species in most of the genera. Also, Mackie *et al.* (1978) reported that protozoal concentrations usually begin to decrease when the level of concentrate exceeds 60%. Also, R3 had the best nutrients disappearance which improve the digestibility of nutrients and enhance rumen fermentation and microbial protein.

It was of interest to show that the values of *in vivo* ruminal protozoa count was higher more than *in vitro* protozoa count which was under laboratory conditions, this is may be due to the individual differences among animals that reflects on the results.

CONCLUSION

The results of the present work indicated that ration containing high roughage to concentrate ratio (30: 70% roughage to concentrate ratio) enhanced *in vitro* nutrient degradability and improved *in vitro* rumen fermentations especially non protein nitrogen and microbial protein which are very important for rumen microbes to build their bodies and increase microbial growth as it is one of the most important parameters reveled on animal performance.

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تأثير نسبة المركز إلى الخشن على الهضم فى المجترات الصغيرة

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تم تصميم ثلاثة علائق مختلفة فى نسبة المركز: الخشن و تم استخدامها فى نوعين من التجارب:١- تجربة معملية لدراسة التحليل الكميائى ومكونات جدار الخلية على أوقات تحضين مختلفة و معدل اختفاء المادة الجافة والعضوية و المواد الغذائية الأخرى بالعلائق، ودراسة تخمرات الكرش معملياً و أعداد البروتوزوا والبكتريا. ٢- تجربة هضم لدراسة الاستفادة من الطاقة بالإضافة إلى أعداد بروتوزوا الكرش فى ذكور الأغنام المغذاه على هذه العلائق. وقد كانت العلائق تتكون من نسب مختلفة من المركز و المالئ : معاملة (١) بنسبة ٩٠ : ١٠ ، معاملة (٢) بنسبة ٨٠ : ٢٠ و معاملة (٣) بنسبة ٧٠ : ٣٠ %. تم تحضين العينات مع سائل كرش من أغنام تتغذى على مخلوط مركزات ودريس برسيم عالى الجودة فى أوقات تحضين مختلفة (٢، ٤، ٦، ٨، ٢٤، ٤٨). وتم أخذ عينات من سائل الكرش فى أوقات التحضين المختلفة.

و قد أظهرت النتائج أن العليقة الأولى كان لها أعلى قيم للمادة الجافة والمادة العضوية و البروتين الخام فى حين كان للعليقة الثالثة أعلى قيم للألياف الخام ومكونات جدار الخلية. كما أظهرت النتائج خلال أوقات التحضين المختلفة نقص تدريجى فى قيم كلا من المادة الجافة والمادة العضوية والبروتين الخام والألياف الخام ومكوناتها.

كما أوضحت النتائج وجود فرق معنوى بين العلائق حيث كان للعليقة الثالثة أعلى قيم لهضم أو اختفاء المادة الجافة و العضوية وباقى المواد الغذائية معملياً يليها العليقة الثانية ثم العليقة الأولى كما كان أيضاً للعليقة الثالثة أفضل معدل اختفاء معملي لمكونات جدار الخلية. وقد أظهرت جميع قيم الهضم المعملي للمواد الغذائية المختلفة زيادة معنوية مع زيادة مدة التحضين. وأظهرت التخمرات المعملية لسائل الكرش و البروتين الميكروبى وميكروبات الكرش اختلافاً معنوياً حيث أعطت العليقة الثالثة أعلى قيم لمعظم القياسات، بينما أظهرت العليقة الثانية أفضل استفادة من الطاقة. و يمكن استنتاج أن العلائق المحتوية على نسبة عالية من المادة المالئة تحفز هضم المواد الغذائية المختلفة و تخمرات الكرش و البروتين الميكروبى كما تؤدى إلى زيادة أعداد ميكروبات الكرش.