Effect of Inoculating New Born Lambs with Fresh or Lyophilized Rumen Fluid on Rumen Activity and Lamb Performance

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Abstract: Fresh or lyophilized digesta collected from mature Ossimi ewes fed berseem hay (*Triforum alxanderinum*) used to inoculate new born lambs. Nine ewes born twins (female and male) were divided into three equal groups. Lambs in the first group were left to suckle their mothers until 12 weeks of age (natural rearing) as a control group. Lambs in the 2nd group were inoculated with fresh rumen liquor (FRL) and the 3rd group was inoculated with lyophilized rumen liquor (LRL). Three male lambs of each group fed berseem hay (BH) were chosen to apply digestibility trials and to collect ruminal ingesta from each group separately to determine *in vitro* neutral detergent fiber (NDFD) and crud protein disappearance (CPD).

There weren't differences in total bacterial or protozoal number for either FRL or LRL groups. Daily milk consumption and feed conversion were significantly (P<0.05) decreased by male and female inoculated with FRL or LRL except females in group received LRL. Final body weight, total gain and average body gain increased with LRL or FRL inoculum.

Apparent digestibilities of DM, OM, ADF and hemicellulose for berseem hay were significantly (P<0.05) increased for lambs inoculated by either FRL or LRL compared to control. No significant (P>0.05) differences were found between either inoculated or control group for digestibilities of CP, EE, NFE, NDF and ADL. The values of TDN were significantly (P<0.05) increased with either FRL or LRL inoculum, while no significant (P>0.05) differences for DCP or nitrogen balance.

The values of pH lowered (P<0.05) significantly with inoculated lambs, while the concentration of short chain fatty acids (SCFA's), acetate (Ac), propionate (Pr) and butyrate (But) and NH_3-N were higher (P<0.05) significantly. The total count of bacteria and protozoa were higher (P<0.05) significantly with either FRL or LRL inoculum than control. Ruminal *Ruminococcus albus* and *Butyrivibrio fibrisolvens* (10^5) count were higher (P<0.05) with both inoculum than control. On the other hand, some species such as *Clostridium lochheadii* and *Clostridium longisporum* weren't detected in the rumen for all groups. *Enoploplastron triloricatum* and *Eudiplodinium maggii* count were higher (P<0.05) than another species of protozoa with either FRL or LRL inoculum. While, *Diploplastron afine* (10^3/ml) was not detected in the control group.

Positive improvement of NDF and CP disappearance for berseem hay was detected when in rumen liquor of new born lambs inoculated with FRL or LRL. Ruminal disappearance kinetics a, b, ED and PD for NDF and CP of BH for different groups showed similar trend. Concentration of SCFA's, Ac, pr and But significantly (P<0.05) increased except acetate at 24h incubation. The values of total nitrogen were significantly (P<0.05) increased at 48h of incubation than that control one. Microbial protein nitrogen and NH_3-N in incubated media was significantly (P<0.05) higher.

[Abo-Donia, F. M.; Ghada, S. Ibrahim; Safaa Nadi, and M. S. Sayah. Effect of Inoculating New Born Lambs with Fresh or Lyophilized Rumen Fluid on Rumen Activity and Lamb Performance. Journal of American Science 2011; 7(9): 409-422].(ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Rumens inoculate; feed intake, digestibility and rumen degradability.

1. Introduction:

At birth the rumen of the lamb is non-functional. Papillary growth, rumen wall uscularization and vascularization are minimal and the reticulorumen volume is small (Tamate, et. al., 1962). This transition phase (pre-ruminant) of the lamb is critical, where the development of the rumen is characterized by the increase in the size of the organ (mostly rumen wall development) and the development of the rumen mucosa (papillae development). Papillae and to a lesser extent rumen wall development can be influenced by feeding management. In ruminant nutrition, a smooth transition of the young lamb from the stage of pre-ruminant to ruminant, reducing its nutritional dependence on milk and the same time minimizing loss in growth, is always desired. Hence, to efficiently use concentrates and roughages, an adequate development of the rumen is necessary. Inoculation fresh or lyophilized digesta in the rumen

of small lambs at 2 weeks of age is expected to stimulate rumen development and consequently reduce abnormal oral behavior. Rumen development is triggered by the production of volatile fatty acids (VFA) resulting from fermentation of organic matter in the rumen (Flatt, et. al., 1958).

This study was carried out to investigate the effect of inoculation fresh or lyophilized digesta on rumen development, rumen fermentation and growth performance of new born lamb's performance.

2. Materials and Methods

In vivo and *in vitro* experiments were conducted at "Seds Experimental Station" in Middle Egypt, under supervision of the Animal Production Research Institute at Cairo.

Rumen inoculum

Four mature Ossimi ewes weighing approximately 56 kg were used as sources of rumen inoculum. The sheep were fed berseem hay (*Triforum elxanderinum*) *ad-libitum* with free access to water for 30 days prior to collection of rumen fluid. Rumen liquor was obtained via pass a stomach tube through Frick speculum (Fig. 1) used to the oral cavity; speculum prevents the animal from chewing the tube, also prevents mixing saliva with rumen fluid. Ruminal fluid was collected approximately 2 hrs post-feeding then strained through four layer of cheesecloth into an all-purpose thermos insulated drink container, pre-warmed 39°C. Total collections of the rumen liquor from the ewes were composited, flushed with CO_2 and sealed before being transported to the laboratory. The resulting fresh ruminal fluid was purged with deoxygenated CO_2 before use as the inoculum.

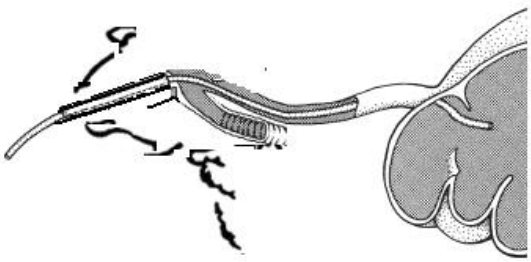

Figure 1: Position of stomach tube, used with a Frick speculum, through the oral passage into the rumen

The composited rumen fluid was lyophilized after prefreezing 300 ml aliquots with dry ice-acetone. About 24 hr were required for adequate drying. Dry matter yield from the liquor averaged 2.2%. Dried inocula were again composited in a sealed container and frozen until fed as a top dressing to recipient lambs.

Lyophilized and fresh rumen fluid in a complete roll tube medium used for enumeration of total rumen bacteria and protozoa. With one exception**,** no significant differences were found in total bacterial or protozoal numbers when lyophilized rumen fluid was substituted for fresh rumen fluid. Lyophilized of rumen fluid had to overcome the problems of handling of fresh rumen liquor. On the basis of these values, 50.68 mg of lyophilized rumen fluid is equivalent to 1 ml of fresh rumen fluid. Thirty grams of lyophilized rumen fluid were fed to each of the treated lambs in the third group as a top dressing on day 7 then repeated in the second week according recommendation of Waymack, (1976).

Feeding trials

Nine ewes of the herd were chosen (averaging 56 kg live weight) born twin in the same week, sex ratio (1:1), divided into three equal groups, three in each group. Lams in the first group were left to suckle their dams until 12 weeks of age (natural rearing) and served as a control group where they were received creep feeding *ad libitum*. Average daily milk consumed by lambs was determined once biweekly during the rearing period, by calf nursing technique according to Louca, et. al., (1974). Lambs from 2nd group were selected to drenched inoculum of fresh rumen liquor (FRL) and the 3rd group inoculated with lyophilized rumen liquor (LRL). Precautions taken to limit the transfer of microorganisms from other ruminants to these lambs until 12 wk. of age included the following: feed and equipments for maintaining the lambs and collecting weight data were stored in the isolation area about 1 mo. before the first lamb was brought in, and were kept in the area until 12 wk. of age. Mothers of both the second and third groups were excluded from their lambs in order to prevent infection and was being manually milking a day and record then pasteurized milk and submitted to the lambs (milk each mother for her lambs). The lambs were handled and fed by only three persons, who had little contact with the rest of the herd. Overalls, overshoes, and rubber gloves were kept in the area. The gloves were rinsed in 70% alcohol and a solution of disinfectant was walked through whenever the animals were approached.

Nursing periods were from 06:00 a.m. until 08:00 a.m. and from 12:00 noon until 04:00 p.m. daily. The lambs were received starter feeding *ad libitum*. Berseem hay long, chopped to be available free choice and water was access free. Feed consumed for lambs were recorded every week for both starter and berseem hay. Lambs were euthanized at 12 weeks of age. Mothers of the lambs were given two doses of CoVaccine-8™ during pregnant, a first

dose at the age of 3 months of pregnancy and the second 15 days before birth. The ingredients and chemical composition are presented in Table (2).

Animals were weighed biweekly intervals before morning meal, while daily feed intake, changes in live body weight, average daily gain, feed conversion (kg DMI/kg. gain) were determined. Starter was formulated and calculated according to recommendations of NRC, (1985) and offered to lambs *ad libitum*, water and salt blocks were freely available. Refusals was daily collected, while a composite sample was taken and dried for determination of dry matter intake and further proximate analysis.

Digestibility trials

Three digestibility trials were carried out after finished the feeding trials using three male lambs from each group to determine the nutrients digestibility coefficients and nutritive values of berseem hay (Trifolium alexandrinum). Feces and urine samples were collected daily during the collection period (last week of experiment). Samples of berseem hay (BH) and feces were dried in a forced air oven, then pooled for each animal, ground and chemically analyzed according to the methods of A.O.A.C. (1995).

Chemical analysis

Composite feed, fecal and urine samples were chemically analyzed according to A.O.A.C. (1995). Chemical compositions of tested ingredients are presented in Table (3). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Goering & Van Soest (1970). Hemi-cellulose and cellulose were calculated as the difference between NDF and ADF, ADL orderly.

At the end of the digestibility trials, rumen fluid samples were collected using a stomach tube at 0, 3 and 6hrs post feeding for two consecutive days. Rumen-pH was measured using an electronic pH-meter immediately (HANNA pH-meter model HI 8424) after the rumen fluid was obtained. Ammonia-N (NH_3-N) concentration as described by Conway, (1978), concentration of SCFA's (Eadie, et. al. (1967) and Molar proportions of SCFA's (Erwin, et. al. (1961).

Count and Identification of Bacteria and Protozoa

Samples of rumen liquor for count and identification of bacteria and protozoa were withdrawal by stomach tube just after 4 hr feeding in the morning at 45, 60 and 75 days from three lambs female of each group.

Rumen bacterial count in rumen content:

Fresh and lyophilized rumen fluid collected from donor animals and so that suction from an experimental lambs for the preparation of media. Total viable counts of rumen bacteria was determined by colony counts using the anaerobic roll tube procedure of Hungate, (1950) as modified by Bryant & Robinson (1961). A nonselective rumen fluid medium 98-5 with a 100% CO_2 gas phase (Bryant and Robinson, 1961) was one of the roll tube media used. The total number of colonies was determined from the average of counts from 4 replicate roll tubes prepared at each of 2 concentrations (0.2 and 1 ml of 10^8 dilution) from each rumen sample. A cultural bacterial count was also determined using the lactobacillus selective medium of Rogosa, et. al. (1951) and the anaerobic roll tube technique (Hungate 1950 and Bryant & Robinson 1961).The medium was modified to include a final concentration of 2% agar, 0.05% cysteine-HCl and a 100% CO2 gas phase. The total number of cultural bacteria was determined from the average of the colony counts from 2 roll tubes prepared at each of 2 concentrations (1 ml of 10^{-7} and 10^{-8} dilution) after 72 hours of incubation at 38°C.

Identification of Cellulolytic Bacteria Isolated from the Rumen:

The cellulolytic bacterial count was determined by the roll-tube method using cellulose agar after incubating the samples at 39°C for 4 weeks (Hobson, 1969). All the cellulolytic bacterial isolates showing zones of clearance on cellulose agar and recovered from a dilution were purified and identified according to procedures outlined by Dehority, (1963), Hobson, (1969) and Dehority, et. al. (1989)

Rumen protozoal count in rumen content:

Samples of rumen content (50 ml) taken before the morning feeding via stomach tube of the animals were filtered through a 4-mm^2 metal mesh to eliminate large plant particles. Two 5ml duplicate liquors of rumen fluid were separately taken and diluted five times by saline solution and lugol's iodine to fix and stain the protozoa cells. About 0.1ml of the diluted ruminal sample was poured on a dry clean slide which was then carefully covered by a dry clean cover slide. The stained samples were observed under microscope (400xs) to count protozoa cells and then calculated the protozoa number using the following equation.

$N \times D \times 4 \times 10^4$= (protozoa number/ mL) according to Wang, et. al. (2009),

Where,

N = Total number of protozoa in middle panes

D = Dilution times

Identification of Cellulolytic Protozoa Isolated from the Rumen:

All protozoa were identified from the descriptions given by Dogiel (1967), Kofoid & MacLennan (1933) and Hungate (1966) the latter's' nomenclature and classification being used where the authors differed.

Total bacteria populations were counted in a Neubauer chamber under 1200 x magnification after the preparation of rumen content samples following the procedure of Warner, (1962).

In-vitro procedure

At the end of digestibility trials about 250 ml ruminal ingesta was collected from lambs in each group separately by a stomach tube with a Frick speculum and strained through four layers of cheesecloth to provide inocula for NDFD and CPD determination of berseem hay. *In vitro* incubations were conducted as procedure described by the first stage of the Tilley & Terry (1963). Thus, 10 mL of ruminal inoculum and 40mL of buffer solution used by McDougall (1948) were added to 0.5 g of the sample of each by-product, in tubes. The tubes were flushed with CO2 and sealed. Treble bottles for each measurement were incubated in a 39 ºC shaking-water bath for each two replicates for 12, 24 and 48 h. At each time, crude protein (CP) was measured according to (A.O.A.C. 1995) and NDF disappearance was determined using a modified method (neutral-detergent fiber with heat-stable α-amylase) described by Van Soest, et. al. (1991).

To measurement of CP and NDF, the residues of each sample after incubation was filtered through Whatman® 541 paper then washed sequentially with water. The samples were dried and weighed to determine the *in vitro* component disappearance (CD). *In vitro* X disappearance was calculated as follows: (1 – ((CD residue – blank CD) / CD original)) × 100, where CD residue is the CD recovered after 12, 24 and 48 h of fermentation, $blank_{CD}$ is the CD recovered in the corresponding blank after the same fermentation time, and CD original is the CD of the substrate placed in the tube.

The disappearance rate was fitted to the following equation (Ørskov & McDonald, 1979): $\mathbf{P=a+b\ (1-e^{-ct})}$, where P = disappearance rate at time t, a = an intercept representing the portion of CP or NDF solubilized, b = the fraction of CP or NDF that will be degraded when given sufficient time for digestion in the rumen, c = a rate constant of disappearance of fraction b, and t = time of incubation. Effective degradability of NDF (EDNDF) and CP (EDCP) by the following equation (Ørskov & McDonald, 1979): **EDNDF or EDCP =a+bc/(c+k),** where k is the estimated rate of outflow from the rumen.

After each respective incubation interval, the fermentation medium for double bottle were decanted into plastic tubes, and then the tubes were centrifuged at 3000 ×g for 20 min. The values of pH were determined using HANNA pH-meter (model HI 8424), ammonia-N (Conway, 1978) and concentration of SCFA's (Eadie, et. al. (1967). Supernatants were decanted into glass bottles, 1 mL of 50% sulphuric acid was added and they were frozen at –20 ºC until analysis fractionation of SCFA's according to (Erwin, et. al. (1961).

Measurement of microbial protein in residue (apparent undigested residue) left after fermentation was analyzed using spectrophotometric method of Zinn & Owens (1986) after incorporating suggested modifications of Makkar & Becker (1999) and Obispo & Dehority (1999).

Statistical analysis

Analysis of variance (ANOVA) was carried out using the General Linear Model of SAS computer package (SAS, 2004). An F test of 5% probability level was used to test for significance differences between means, which were separated by Duncan's New Multiple Range Test (1955).

Prediction equation of voluntary feed intake from fiber fraction digestibility or kinetics of DM degradability and prediction of DM digestibility from kinetics of DM degradability were calculated using (SAS, 2004)

3. Results and Discussion

The data of microbiology, chemical and physical characters of fresh and lyophilized rumen liquor used in inoculation of lamb's rumen in Table (1) seemed to be comparable where no difference was found in total bacterial or protozoal numbers. The same results were obtained by (Waymack, 1976) when fed ewes on chopped wheat straw and (Abo-Donia, et. al. 2009) when fed rams reed and corn silage *ad libitum*. The data of total nitrogen, nitrogen fraction, SCFA's and fractionation of SCFA's were as same trend of data obtained by Abo-Donia, (2008) when fed sheep on egg plant bashes hay.

Table (1): Microbiology, chemical and physical characters of fresh and lyophilized rumen liquor inoculum used in inoculation of lambs rumen

Item	Rumen liquor inoculum	
	Fresh	Lyophilized
Bacterial count in rumen inoculum		
Total count (10^9/ml)	1,900	1,850
Viable count (10^9 /ml)	96.24	96.73
Ruminococcus albus (10^5 /ml)	75.19	75.09
Ruminococcus flaoefaciens (10^5/ml)	8.19	8.97
Bacteroides succinogenes ($10^{5/}$ ml)	19.27	19.42
Butyrivibrio fibrisolvens (10^5 /ml)	13.29	13.08
Clostridium lochheadii (10^5 /ml)	3.6	3.0
Clostridium longisporum (10^5/ml)	1.4	1.2
Other *Clostridium SPP* (10^5/ml)	35.21	35.06
Protozoal count in inoculum rumen digesta		
Total count (10^5 /ml))	8.59	8.00
Enoploplastron triloricatum (10^4/ml)	35.0	34
Eudiplodinium maggii (10^4 /ml)	25.0	25
Diploplastron afine (10^4/ml)	4.6	4.5
Diplodinium monacan thum (10^4/ml)	5.3	5.0
Diplodinium pentacanthum (10^4/ml)	1.78	1.8
Epidinium ecaudatum caudatum (10^4/ml)	3.2	3.0
Isotricha intestinalis (10^4/ml)	2.9	3.0
Dasytricha ruminantium (10^4/ml)	0.98	1.0
Polyplastron multivesiculatum (10^4/ml)	2.8	2.6
Entodinium caudatum (10^4/ml)	1.96	1.85
Nitrogen fractionation (mg/100ml)		
Total nitrogen (mg/100ml)	267.3	269
Microbial Protein Nitrogen (mg/100ml)	170.00	171.03
NH3-N (mg/100ml)	16.83	15.39
True PN (mg/100ml)	194.06	195.03
NPN (mg/100ml)	73.24	73.97
Total and Fractionation of SCFA's		
SCFA's (equl/ 100ml)	13.8	14
Acetate (mmol/l)	58.93	60
Propionate (mmol/l)	27	26
Butyrate (mmol/l)	12	13
Physical characteristics of rumen liquor		
Color	Green	Green
Sediment	1.80	1.79
pH	6.57	6.48

The data in Table (1) show that the lyophilization process for the rumen liquor inoculum did not affect sharply total bacterial count and viable count. The total count of bacteria in fresh inoculum was 1.900 X 10^9/ml. At the same time, it was 1.850 X 10^9 / ml in the lyophilized inoculum. This difference

appears clearly in the test of bacterial count and protozoa count. Many factors affect the counts obtained with rumen samples, including the time in relation to feeding and the position in the rumen which is sampled (Bryant & Robinson 1961and Hungate, 1969). The highest number of bacteria differentiation in both types of rumen liquor was for species *Ruminococcus albus* followed by *Bacteroides succinogenes* and *Butyrivibrio fibrisolvens* (10^5 /ml). In protozoa differentiation, the highest number was recorded for *Enoploplastron triloricatum* followed by *Eudiplodinium maggii* (10^4 /ml). The seamed values of total nitrogen and SCFA's and their fractionation were shown in both types of rumen liquor. Hungate (1950) demonstrated that *Ruminococcus albus*, *Ruminococcus flauefaciens*, and *Fibrobacter (Bacteroides)* succinogenes were the predominant cellulolytic bacteria in the rumen.

Table (2): Formulation of starter for lambs (on DM basis %)

Item	% of Ingredients
Yellow corn	55.00
Soybean meal	30.00
Wheat bran	7.00
Molasses	5.00
Lime stone	1.50
Sodium chloride	1.00
Mineral mix. And Vit. (Premix*)	0.50

*Primex, each kg contained 7,000,000 I.U. Vitamin A, 1,000,000 I.U. Vitamin D3, 30,000 mg Vitamin E, 50,000 mg Mn, 50,000 mg Zn, 50,000 mg Fe, 10,000 mg Cu, 8,000 mg I, 200 mg Co, 150 mg Se and 100 mg Mg.

Table (3): Chemical compositions of starter and berseem hay for lambs (on DM basis %)

Items	BH	Starter
Chemical composition (%)		
DM	88.74	90.90
OM	76.59	93.8
CP	12.40	19.10
EE	2.01	3.1
NDF	60.34	19.11
ADF	44.86	12.59
ADL	9.10	2.17
Cellulose	35.76	10.42
H. Cellulose	15.48	6.52
G. Energy (Mcal/kg)	3.624	4.842

Table (4): Effect of inoculation by lyophilized and fresh rumen liquor for lambs on daily milk consumption (g/h/d).

Item	Groups			±SE	P<					
	Control	FRL	LRL		G	Six	P	G*Six	G*P	Six*P
Male	127.86[a]	108.10[b]	113.33[b]	4.962						
Female	124.76[a]	104.05[b]	111.90[ab]	4.737	*	ns	*	ns	*	ns
Mean	126.3[a]	106.07[b]	112.61[b]	3.430						
±SE	5.361	4.595	4.554							

Means bearing different superscript in the same columns are significantly different P<* 0.05
G= group P= periods

Daily consumed milk significantly (P<0.05) decreased by male and female lambs inculcated with fresh (FRL) or lyophilized (LRL) rumen liquor except female group received LRL. According to Ørskov, et. al. (1970) the lack of ruminal development in milk-fed newborn animals may be due to the effective shunting of milk directly to abomasum by the reflexive closure of the reticular groove, thus preventing substrate for the establishment of ruminal fermentation from entering the rumen. The overall mean was shown the same trend. These results might be due to rumen

development and increase nitrogen retention, this suggestion is in agreement with Waymack, (1976). Abou Ward, et. al. (2008) mentioned to utilization of dietary N by early weaned lambs due to the higher DMI, which led in turn to higher N retention due to the well-developed rumination and fermentation in comparison with the later weaned lambs.

Table (5): Effect of inoculation by lyophilized and fresh rumen liquor for lambs on feed intake and growth performance.

Item	Six	Groups			±SE	P<
		Control	FRL	LRL		
Feed intake per group (on DM basis)					---	---
Starter (g/h/d)	---	399.18	444.72	447.78	---	---
Berseem hay (g/h/d)	---	346.38	387.35	390.28	---	---
Total intake (g/h/d)	---	745.55	832.10	838.07	---	---
Growth performance						
Initial body weight (kg/h)	Male	5.67 ±0.391	5.67 ±0.264	5.50 ±0.312	0.272	ns
	Female	5.17 ±0.391	5.33 ±0.264	5.33 ±0.312	0.373	ns
Overall mean		5.42	5.50	5.42	0.216	ns
Final body weight (kg/h)	Male	17.83^{b} ±0.782	20.17^{a} ±0.514	20.33^{a} ±0.565	0.527	*
	Female	16.33^{b} ±0.782	19.00^{a} ±0.514	19.33^{a} ±0.565	0.720	*
Overall mean		17.08^{b}	19.59^{a}	19.83^{a}	0.416	*
Gain (kg/h)	Male	12.33^{b} ±0.6667	14.67^{a} ±0.527	15.00^{a} ±0.471	0.430	*
	Female	11.33^{b} ±0.6667	13.67^{a} ±0.527	14.33^{a} ±0.471	0.667	*
Overall mean		11.83^{b}	14.17^{a}	14.67^{a}	0.408	*
Average daily gain (g/h/d)	Male	162^{b} ±10.410	193^{a} ±5.674	198^{a} ±4.972	5.292	*
	Female	149^{b} ±10.410	182^{a} ±5.674	187^{a} ±4.972	9.066	*
Overall mean		155^{b}	188^{a}	192^{a}	5.396	*
Feed conversion (kg DMI /kg gain)		4.82^{a}	4.44^{ab}	4.36^{b}	0.125	*

Means bearing different superscript in the same columns are significantly different (P<* 0.05)

As depicted in Table (5) there was an increase of final body weight, total gain and average daily gain of lambs inculcated with FRL and LRL than control. Feed conversion was significantly (P<0.05) decreased with lambs inoculated by LRL, while no significant difference was found with inoculate by FRL compared to control ones. Improved of feed intake and rumen fermentation for tested groups beginning of mid-suckling period to the end, may be reflected in the improvement in growth rates of lambs in the tested groups. Baldwant, et. al. (1992) reported that, rumen tissue weight was positively correlated with rumen capacity (r=0.92); DMI (r=0.74) and daily body weight gain (r=0.71). The length of rumen papillae increased (P<0.05) and was positively correlated with daily body weight gain (r = 0.5) and DM intake (r = 0.6). The work of Barth, et. al. (1961) did not support the concept of inoculates rumen of new lambs while Caffrey, et. al. (1967) and Ludwick, et al. (1971) obtained varying results in successive trials. Earlier work by Ewan, et. al. (1958) demonstrated an improvement in nitrogen utilization in lambs inoculated with rumen fluid.

The data in Table (6) showed that apparent digestibility of DM, OM, ADF and hemicellulose for berseem hay were significantly (P<0.05) increased with either FRL or LRL inoculum compared to control one. Digestibilities of CP, EE, NFE, NDF, ADL with inoculated group by either FRL or LRL showed no significant (P>0.05) differences among group. Waymack, (1976) found that inoculated lambs with lyophilized rumen liquor led to higher apparent nitrogen and dry matter digestibility. Inoculated lambs with of either FRL or LRL showed significantly (P<0.05) higher nutritive value as TDN than that of control, while no significant (P>0.05) difference was found between tested groups for DCP or nitrogen balance. These data are a good agreement

with Waymack, (1976), when lambs inoculated by lyophilized rumen liquor.

Table (6): Nutrient digestibility, cell wall constituent and nutritive value (%) of berseem hay by different experimental groups.

Item	Control	FRL	LRL	±SE	P<	
					G	F*L
Nutrient digestibility, (%)						
DM	43.65[b]	47.36[a]	47.35[a]	±0.855	*	ns
OM	46.97[b]	50.47[a]	50.45[a]	±0.758	*	ns
CP	43.29	47.05	47.05	±1.629	ns	ns
EE	58.58	61.33	61.34	±1.537	ns	ns
NDF	51.10	54.35	54.36	±1.720	ns	ns
ADF	45.73[b]	49.31[a]	49.29[a]	±0.578	*	ns
ADL	4.83	5.98	6.20	±0.842	ns	ns
Cellulose	66.67	68.95	69.05	±5.680	ns	ns
Hemicellulose	56.14[b]	60.34[a]	60.25[a]	±0.654	*	ns
Nutritive value, (%)						
TDN	42.19[b]	45.29[a]	45.28[a]	±0.690	*	ns
DCP	6.05	6.57	6.57	±0.227	ns	ns
Nitrogen balance (g/h/d)	1.59	1.87	1.84	±0.140	ns	ns

a, b and c Means in the same column for each category with different superscript are significantly different (P<0.05).
±SE: standard error of difference. G= between groups, T= time and F*L= fresh * lyophilized

Data in Table (7) showed lower (P<0.05) significant values of pH in the rumen for lambs inculcated with either type of RL compared to control one. While the concentration of SCFA's, Ac, Pr, But and NH_3-N were higher (P<0.05) significantly with added LRL or FRL. Abou Ward, et. al. (2008) concluded that the development of rumen papillae is aided by the fermentation of newborn starter to volatile fatty acids (VFA). Growth of rumen papillae aids in allowing the newborn to change from mono-gastric form to one characteristic of adult ruminant (Warner, 1961). There is significant difference among time for all groups and LRL was higher (P<0.05) significantly than that FRL for almost parameters. These data may be related to increase microorganisms according data obtained in Table (8 & 9) and increase their activities leading to increase SCFA's in the rumen (Table 7). Also data obtained from in vitro gas production from FRL and LRL in Tables (10, 11 and 12) supporting these results. The same results of NH_3-N are in a good agreement with Waymack, (1976), when inoculate lambs by lyophilized rumen liquor. End products of ruminal fermentation and digesta kinetics (i.e., rates of digestion and passage) affect production rate from a given diet (Singh & Gupta, 1993).

The results in Table (8) clearly show that, the total count of bacteria was higher (P<0.05) significantly in the lamb's rumen inoculated by either FRL or LRL. The species of *Ruminococcus albus* and *Butyrivibrio fibrisolvens* (10^5) was higher than the other bacteria species in the rumen at the same time was highly (P<0.05) significant with inoculation by both types of rumen liquor. The counts of bacteria species were in a good agreement with (Hungate, 1957). On the other hand, some species like *Clostridium lochheadii* and *Clostridium longisporum* (10^5/ml) were not detected in the rumen for all groups.

Data in Table (9) presenting the protozoal count in rumen liquor of lambs. Protozoal count was higher (P<0.05) significantly after different periods with addition of either FRL or LRL inoculum to the lambs than control. Total number of protozoa was significantly lower in rumen fluid than in whole rumen contents, depending on the time of sampling and the procedure used to separate the fluid and solid fractions (Abou Akkada, et. al. 1969 and Dehority, 1984). The species of *Enoploplastron triloricatum* and *Eudiplodinium maggii* (10^3/ml) were increased than another species of protozoa and higher (P<0.05) significantly with inoculate lamb's rumen with either FRL or LRL compared to control. While, *Diploplastron afine* (10^3/ml) was not detected at the control group compared with FRL and LRL. Coleman (1964) reported that protozoa have been grown *in vitro* for extended periods (2, 5, 9, 15, 16, and 19hr) in the presence of accompanying bacteria. The influence of inoculate on developing lamb's rumen leading to improve growth protozoa population, this confirmed well with the results of Afaf M. Fayed, (2009) which found that rams fed berseem hay had significant (p<0.05) higher of *Isotrachia, Epidinum, Eudiplodinum* and *Ophryoscolex* species and insignificant increase of *Entodinum* and *Polyplastron* species.

Table (7): Basic pattern of rumen fermentation for lambs received lyophilized or fresh rumen liquor.

Item	Time (hr)	Control	FRL	LRL	±SE	P<		
						G	T	F*L
pH	0	6.38	6.45	6.60	±0.096	ns	*	ns
	3	5.99[a]	5.48[b]	5.45[b]	±0.029	*	*	ns
	6	6.14[a]	5.74[b]	5.86[b]	±0.075	*	*	ns
Overall mean		6.17[a]	5.89[b]	5.97[b]	±0.042	*	*	*
SCFA's (meq/dl)	0	12.27[c]	14.81[b]	15.38[a]	±0.100	*	*	*
	3	13.83[b]	16.41[a]	16.21[a]	±0.103	*	*	ns
	6	12.98[c]	15.13[b]	15.76[a]	±0.056	*	*	*
Overall mean		13.03[c]	15.45[b]	15.78[a]	±0.051	*	*	*
Acetic (mM)	0	52.44[c]	54.67[b]	55.97[a]	±0.238	*	*	*
	3	54.06[b]	57.08[a]	56.97[a]	±0.143	*	*	ns
	6	54.60[b]	57.65[a]	57.54[a]	±0.144	*	*	ns
Overall mean		53.70[c]	56.47[b]	56.83[a]	±0.104	*	*	*
Propionic (mM)	0	32.89[c]	38.18[b]	39.46[a]	±0.173	*	*	*
	3	28.63[c]	33.31[b]	33.96[a]	±0.100	*	*	*
	6	28.92[c]	33.66[b]	34.30[a]	±0.103	*	*	*
Overall mean		30.15[c]	35.05[b]	35.91[a]	±0.075	*	*	*
(Ac/Pr)	0	1.59[a]	1.43[b]	1.42[b]	±0.004	*	*	ns
	3	1.89[a]	1.71[b]	1.68[c]	±0.008	*	*	*
	6	1.88[c]	1.71[b]	1.67[a]	±0.009	*	*	*
Overall mean		1.79[a]	1.62[b]	1.59[c]	±0.004	*	*	*
Butyric (mM)	0	12.16[c]	12.75[b]	13.22[a]	±0.060	*	*	*
	3	13.65[c]	14.23[b]	14.37[a]	±0.032	*	*	*
	6	13.78[c]	14.37[b]	14.51[a]	±0.030	*	*	*
Overall mean		13.20[c]	13.78[b]	14.03[a]	±0.025	*	*	*
NH_3-N (mg/100ml)	0	8.15[b]	12.64[a]	12.40[a]	±0.320	*	*	ns
	3	9.91[b]	14.40[a]	14.78[a]	±0.177	*	*	ns
	6	9.18[b]	13.36[a]	13.48[a]	±0.174	*	*	ns
Overall mean		9.08[b]	13.47[a]	13.55[a]	±0.135	*	*	ns

a, b and c Means in the same column for each category with different superscript are significantly different (P<0.05).
±SE: standard error of difference. G= between groups, T= time and F*L= fresh * lyophilized

In-vitro results in Table (10) show that, inoculated rumen of new bourn lambs by FRL or LRL positively improved NDF and CP disappearance of berseem hay when incubated in their rumen liquor than that incubated with rumen liquor collected from no inoculated lamb rumen. Berseem hay incubated with rumen liquor withdrawn from lambs rumen inoculated either FRL or LRL significantly (P<0.05) increased disappearance of NDF, while CP disappearance of berseem hay was not significantly (P>0.05) difference compared with either incubated with rumen liquor collected from rumen inoculated with FRL or rumen no inoculated. Ruminal disappearance kinetics especially a, b, ED and PD for NDF and CP of berseem hay were shown the same trend. These data are very logic with data in Tables (8&9) which include that rumen development could be enhanced by inoculated with either FRL or LRL. The corresponding value of NDF and CP disappearance and their kinetic for berseem hay (BH) when incubated with rumen liquor collected from inoculated lambs by either FRL or LRL was consistent with the view that (El-waziry & Kamel, 2001).

Incubation of berseem hay (BH) *in vitro* with rumen liquor withdrawal from lambs inoculated with either FRL or LRL at different times are show in Table (11). No significant (P>0.05) differences were found among different treatment at different incubation time between FRL and LRL. In contrary, concentration of SCFA's, acetate, propionate and butyrate significantly (P<0.05) increased when incubated BH with rumen liquor withdrawal from lambs inoculated by either FRL or LRL compared to that withdrawal from no inoculated lambs except acetate at 24h. The trends of basic pattern fermentation from liquor of *in vitro* after incubation BH are compatible with data finding in Table (7).

Similar trend was recorded by Elkholy, et. al. (2009) when Baladi rams fed on berseem hay or corn silages.

Data in Table (12) presenting total nitrogen, microbial nitrogen and NH_3-N in the liquor of rumen fermentation in-vitro at different incubations times of berseem and there kinetics. The values of total nitrogen in incubated media withdrawal from lambs inoculated by LRL were significantly ($P<0.05$) increased at 48h of incubation than that control one. On the other hand there is no significant ($P>0.05$) difference between LRL and FRL or between FRL and control. Microbial protein nitrogen and ammonia nitrogen in incubated media was significantly ($P<0.05$) higher with LRL and FRL compared control, while no significant ($P>0.05$) difference found between LRL and FRL. Moreover, the data show the bacterial count in rumen liquor of lambs after different periods from starting addition of lyophilized inoculum to the lambs. It appears clearly that the total count of bacteria for treated lambs compared with control (untreated lambs) increase sharply on throughout all the experimented periods. At the same time, all isolated celluolytic bacteria from the rumen which are identified had the same increasing compared with total count of bacteria.

Table (8): Bacterial count and identification of cellulolytic bacteria isolated from the rumen of tested groups at different periods.

Item	Periods (days)	Control	FRL	LRL	±SE	P<		
						G	D	F*L
	45	15.81 [b]	43.21 [a]	46.85 [a]	±2.327	*	*	ns
Total count (10^9/ml)	60	25.54 [b]	59.16 [a]	62.06 [a]	±1.076	*	*	ns
	75	23.23 [b]	54.03 [a]	54.21 [a]	±2.457	*	*	ns
Overall mean		21.53[b]	52.13[a]	54.37[a]	±1.184	*	*	ns
Identification of Cellulolytic Bacteria Isolated from the Rumen								
Ruminococcus albus (10^5/ml)	45	13.42 [b]	22.88	22.67	±0.582	*	*	ns
	60	24.56 [b]	40.92	43.44	±0.978	*	*	ns
	75	14.52 [b]	30.51	31.49	±1.436	*	*	ns
Overall mean		17.51[b]	31.43[a]	32.53[a]	±0.611	*	*	ns
Ruminococcus flaoefaciens (10^5/ml)	45	nd	nd	nd	±0.000	*	*	ns
	60	2.85	7.88	8.34	±0.533	*	*	ns
	75	4.84	7.50	7.83	±0.417	*	*	ns
Overall mean		2.56[b]	5.12[a]	5.39[a]	±0.226	*	*	ns
Bacteroides succinogenes (10^5/ml)	45	1.29[b]	5.34[a]	5.10[a]	±0.203	*	*	ns
	60	4.04[b]	7.80[a]	8.27[a]	±0.164	*	*	ns
	75	2.90[c]	6.61[a]	5.09[b]	±0.288	*	*	ns
Overall mean		2.74[b]	6.58[a]	6.15[a]	±0.129	*	*	ns
Butyrivibrio fibrisolvens (10^5/ml)	45	2.68[b]	7.63[a]	7.29[a]	±0.249	*	*	ns
	60	5.27[c]	14.62[b]	15.51[a]	±0.234	*	*	*
	75	2.90[c]	7.83[b]	10.17[a]	±0.430	*	*	*
Overall mean		3.62[b]	10.80[a]	10.21[a]	±0.183	*	*	ns
Clostridium lochheadii (10^5/ml)	45	nd	nd	nd	±0.000	*	*	ns
	60	nd	nd	nd	±0.000	*	*	ns
	75	nd	nd	nd	±0.000	*	*	ns
Overall mean		nd	nd	nd	±0.000	*	*	ns
Clostridium longisporum (10^5/ml)	45	nd	nd	nd	±0.000	*	*	ns
	60	nd	nd	nd	±0.000	*	*	ns
	75	nd	nd	nd	±0.000	*	*	ns
Overall mean		nd	nd	nd	±0.000	*	*	ns
Other *Clostridium SPP* (10^5/ml)	45	4.02[b]	7.80[a]	8.27[a]	±0.188	*	*	ns
	60	4.91[c]	11.35[b]	14.75[a]	±0.628	*	*	*
	75	11.27[b]	22.88[a]	21.87[a]	±0.763	*	*	ns
Overall mean		6.73[b]	15.14[a]	13.83[a]	±0.335	*	*	ns

a, b and c Means in the same row with different superscript are significantly different ($P<0.05$)
nd= Non- detectable, G= between groups, T= time and F*L= fresh * lyophilize

Table (9): Protozoal count and identification of cellulolytic protozoa isolated from the rumen of tested groups at different periods.

Item	Periods (days)	Control	FRL	LRL	±SE	P<		
						G	D	F*L
Total count (10^5/ml)	**45**	0.31^b	0.86^a	0.88^a	±0.019	*	*	ns
	60	0.44^b	1.51^a	1.50^a	±0.020	*	*	ns
	75	0.40^b	1.38^a	1.30^a	±0.059	*	*	ns
Overall mean		0.39^b	1.25^a	1.23^a	±0.022	*	*	ns
Identification of Cellulolytic Protozoa Isolated from the Rumen:								
Enoploplastron triloricatum (10^3/ml)	**45**	4.37^b	18.79^a	18.76^a	±1.264	*	*	ns
	60	4.95^b	22.07^a	22.42^a	±1.444	*	*	ns
	75	4.51^b	21.84^a	21.90^a	±1.306	*	*	ns
Overall mean		4.61^b	20.90^a	21.03^a	±0.774	*	*	ns
Eudiplodinium maggii (10^3/ml)	**45**	3.74^b	15.66^a	15.63^a	±1.084	*	*	ns
	60	6.77^b	18.34^a	18.69^a	±1.973	*	*	ns
	75	6.73^b	18.21^a	18.25^a	±1.960	*	*	ns
Overall mean		5.75^b	17.40^a	17.53^a	±0.995	*	*	ns
Diploplastron afine (10^3/ml)	**45**	nd	0.64	0.65	±0.022	*	*	ns
	60	nd	0.87	0.86	±0.007	*	*	ns
	75	nd	0.80	0.75	±0.034	*	*	ns
Overall mean		nd	0.77	0.76	±0.014	*	*	ns
Epidinium ecaudatum caudatum (10^{-3}/ml)	**45**	nd	1.80	1.90	±0.046	*	*	ns
	60	0.23^b	2.03^a	2.35^a	±0.154	*	*	ns
	75	0.38^b	1.96^a	2.34^a	±0.242	*	*	ns
Overall mean		0.23^b	1.93^a	2.20^a	±0.097	*	*	ns
Diplodinium monacan thum (10^3/ml)	**45**	nd	0.31	0.34	±0.011	*	*	ns
	60	nd	0.42	0.45	±0.004	*	*	ns
	75	0.08	0.37	0.39	±0.028	*	*	ns
Overall mean		0.08^b	0.37^a	0.39^a	±0.010	*	*	ns
Diplodinium pentacanthum (10^3/ml)	**45**	nd	0.58	0.59	±0.028	*	*	ns
	60	nd	0.80	0.78	±0.016	*	*	ns
	75	0.24	0.73	0.69	±0.041	*	*	ns
Overall mean		0.24^b	0.70^a	0.69^a	±0.017	*	*	ns

a, b and c Means in the same row with different superscript are significantly different (P<0.05)
nd= Non- detectable, G= between groups, T= time and F*L= fresh * lyophilize

Table (10): Degradation kinetics of neutral detergent fiber and CP at different incubations times of berseem hay in rumen liquor collected from experimented lamb's rumen.

Item	Disappearance			Ruminal degraded kinetics					
	12h (%)	24h (%)	48h (%)	a (%)	b (%)	c (%/h)	PD (%)	ED (5%h^{-1})	UND (%)
Neutral Detergent Fiber (%)									
Control	17.38^b	25.18^b	30.86^b	1.44^b	31.21^b	0.0.58	32.65^b	18.40^b	67.35^a
FRL	23.17^a	33.57^a	41.15^a	1.91^a	41.62^a	0.060	43.53^a	24.54^a	56.47^b
LRL	23.25^a	33.69^a	41.29^a	1.92^a	41.76^a	0.060	43.68^a	24.63^a	56.33^b
±SE	0.297	0.431	0.526	0.026	0.534	0.000	0.557	0.315	0.557
P<	*	*	*	*	*	ns	*	*	*
Crude protein (%)									
Control	15.64^b	22.65^b	27.77^b	1.29^b	28.08^b	0.0.49	29.37^b	16.56^b	70.62^a
FRL	20.85^{ab}	30.21^{ab}	37.03^{ab}	1.72^{ab}	37.44^{ab}	0.050	39.16^{ab}	22.08^{ab}	60.84^{ab}
LRL	22.50^a	32.60^a	39.95^a	1.86^a	40.41^a	0.050	42.26^a	23.83^a	57.74^b
±SE	1.627	2.886	2.889	0.135	2.923	0.000	3.057	1.724	3.056
P<	*	*	*	*	*	ns	*	*	*

a, b and c Means in the same column for each category with different superscript are significantly different (P<0.05).
±SE: standard error of difference. **ED**: effective degradability at (5%h-1). **PD**: potential degradability = a + b

Table (11): Changing in pH values, SCFA's and fractionation at different incubations times of berseem hay from liquor of *in vitro*.

Item	Disappearance			P<		
	12h (%)	24h (%)	48h (%)	G	T	F*L
pH						
Control	6.20	6.00	6.00	ns	ns	ns
FRL	6.37	6.08	5.76	ns	ns	ns
LRL	6.40	6.09	5.82	ns	ns	ns
±SE	±0.077	±0.068	±0.076			
Total SCFA's (mequel/100ml)						
Control	5.60^{c}	7.88^{b}	8.95^{b}	*	*	ns
FRL	7.03^{b}	9.16^{ab}	11.29^{a}	*	*	ns
LRL	7.79^{a}	9.75^{a}	11.77^{a}	*	*	ns
±SE	±0.154	±0.373	±0.439			
Acetate (mM/100ml)						
Control	50.40^{b}	58.86	53.68^{b}	*	*	ns
FRL	57.00^{a}	59.82	60.53^{a}	*	*	ns
LRL	57.59^{a}	59.67	61.06^{a}	*	*	ns
±SE	±1.266	±1.403	±1.150			
Propionate (mM/100ml)						
Control	22.34^{b}	24.11^{b}	24.51^{b}	*	*	ns
FRL	24.87^{a}	25.48^{a}	25.66^{ab}	*	*	ns
LRL	24.91^{a}	25.81^{a}	26.43^{a}	*	*	ns
±SE	±0.454	±0.394	±0.389			
Butyrate (mM/100ml)						
Control	8.93^{b}	9.64^{b}	9.80^{b}	*	*	ns
FRL	10.19^{a}	10.45^{a}	10.52^{a}	*	*	ns
LRL	10.21^{a}	10.58^{a}	10.83^{a}	*	*	ns
±SE	±0.184	±0.161	±0.160			

G= between groups, **T**= time and **F*L**= fresh * lyophilized

Table (12): Total nitrogen, microbial nitrogen and NH_3-N in the liquor of rumen fermentation *In vitro* at different incubations times of berseem and their kinetics

Item	Disappearance			Disappearance kinetics		
	12h (%)	24h (%)	48h (%)	a (%)	b (%)	c (%/h)
Total nitrogen(mg/100ml)						
Control	72.70	83.60	90.63^{b}	47.64^{b}	44.85	0.070
FRL	80.88	93.10	102.17^{ab}	50.26^{ab}	61.42	0.063
LRL	84.33	94.07	104.53^{a}	68.28^{a}	65.18	0.036
±SE	±3.745	±4.568	±3.788	±5.142	±12.635	±0.018
P<	ns	ns	*	*	ns	ns
Microbial protein nitrogen (mg/100ml)						
Control	23.03^{b}	27.46^{b}	31.83^{b}	15.76	19.00	0.0413
FRL	25.90^{a}	31.30^{a}	35.67^{a}	15.26	22.57	0.052
LRL	26.76^{a}	32.20^{a}	36.00^{a}	14.59	22.97	0.064
±SE	±0.385	±0.806	±0.832	±2.328	±2.608	±0.011
P<	*	*	*	ns	ns	ns
Ammonia nitrogen (mg/100ml)						
Control	4.20^{b}	4.92^{b}	5.60^{b}	2.63	3.22^{b}	0.056
FRL	5.03^{a}	6.44^{a}	7.78^{a}	2.40	6.19a	0.050
LRL	5.44^{a}	7.12^{a}	8.00^{a}	2.97	6.86a	0.040
±SE	±0.174	±0.122	±0.168	±0.766	±0.624	±0.013
P<	*	*	*	ns	*	ns

a, b and c Means in the same column for each category with different superscript are significantly different (P<0.05).
±SE: standard error of difference

Conclusion

The data obtained pointed to improve rumen activity of lambs inoculated with either FRL or LRL. We suggest inoculate thirty grams of lyophilized or equivalent of fresh rumen fluid to inoculate lambs as a top dressing on day 7 then repeated in the second week.

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