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EFFECT OF RUMINAL DEGRADABILITY OF CRUDE PROTEIN AND NON STRUCTURAL CARBOHYDRATES ON THE PERFORMANCE OF LACTATING GOATS:

1. SOME RUMEN FLUID PARAMETERS AND MICROBIAL ACTIVITIES

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ABSTRACT

Twenty four lactating Zaraibi goats in mid lactation were used in a 2 x 2 factorial experimental design to evaluate two different sources of ruminally degradable non structural carbohydrates (high and low RDNSC) and two different sources of ruminally degradable protein (high and low RDP). Four experimental diets were formulated to study their effects on some rumen fluid parameters and microbial activities.

The obtained results showed that high RDP as well as RDNSC gave significant ($P<0.05$) higher values of pH. A significant ($P<0.05$) reduction in NH_3-N concentrations recorded with diets contained low RDP without significant differences due to RDNSC sources. No significant differences were noticed between VFA concentrations among the tested diets, except that contained high RDP with low RDNSC which gave the highest significant ($P<0.05$) value.

It seems that the conditions in rumen environment from pH, NH_3-N and VFA concentrations was favorable for coccid form bacteria to dominate the bacterial population and the bacterial growth (TBC or OD) which was clearly reflected on the enzymatic activities, since most enzymatic activities recorded for cellulolytic group. On the other hand, the gas volume measured was higher with amylolytic and proteolytic bacterial groups than cellulolytic ones indicating for the utilization of high RDNSC and low RDP in the rumen of experimental animals. This means that bacterial groups in the rumen utilized the tested sources of RDP and RDNSC in the manner that kept most nutrients to be utilized in the lower part of digestive system. This explanation could be reasonable since the results support the energy values of tested diets which showed the superiority of low RDP with high RDNSC containing diet. Microbial protein synthesis in the rumen fluid was significantly ($P<0.05$) affected by RDNSC more than RDP. The highest DNA, RNA and ON values were obtained when goats fed low RDNSC with high RDP.

From the above mentioned results the present study came to the conclusion that feeding low RDP with high RDNSC would be of beneficial effect on rumen environment and its bacterial activities. Since the diet contained high RDP with low RDNSC tended to give better values of rumen parameters and bacterial activities as well as microbial protein synthesis it is recommended under similar experimental conditions to use soybean meal with corn to improve the nutritive value of diets for lactating goats.

Keywords: Goats, rumen parameters, rumen bacteria, degradability, NSC, microbial protein, DNA, RNA, enzymatic activity.

INTRODUCTION

Synchronization of the ruminal degradability of NSC and RDP should maximize microbial protein synthesis to support animal growth and milk production. For lactating cows, milk production, flow of bacterial N and synthesis of microbial protein have been altered by varying NSC sources and NSC to RDP ratios (Nocek and Russell, 1988; Hoover and Stokes, 1991; Nocek and Tamminga, 1991 and Clark, *et al.,* 1992).

Greater dietary concentrations of NSC have increased the utilization of ruminal NH_3-N for synthesis of microbial protein (Hoover and Stokes, 1991; Nocek and Russell, 1988 and Nocek and Tamminga, 1991). Hoover and Stokes (1991) also reported that decreasing the NSC to RDP ratio increased the quantity of microbial protein synthesized *in vitro* and *in vivo*. Casper *et al.* (1990) suggested that differences in NSC solubility (more than degradability) may result in differences in animal responses. Perhaps the synchronization of NSC and RDP, by reducing instead of increasing CP degradability, improves performance of lactating dairy cows fed barley-based diets.

High concentrate diets are rapidly fermented in the rumen, leading to high concentrations of VFA in ruminal fluid and relatively low ruminal pH (Beauchemin *et al.*, 2001). Low ruminal pH may affect fiber and protein degradation (Hoover, 1986 and Shriver *et al*., 1986) and the efficiency of microbial protein synthesis (Strobel and Russell, 1986). Heldt *et al.* (1999) studied the effects of level and source of carbohydrate as well as level of degradable protein on intake and digestion of low-quality Tall grass-Prairie hay by beef steers. They recorded that supplementation treatments had significant effect on ruminal NH_3-N concentration depending on source and level of carbohydrate offered, as well as level of degradable protein provided. Most research studies with dairy cattle reported a greater VFA concentration in barley-based diets, due to the greater rate of NSC degradability of barley (McCarthy *et al*., 1989 and Khorasani *et al*., 2001). Other studies reported no differences between barley and corn-based diets (Casper and Schingoethe, 1989), or greater VFA concentration for corn-based diets (Casper *et al*., 1999). Surber and Bowman (1998) reported a greater VFA concentration in barley-based diets compared with corn-based diets with beef cattle.

There was no effect of the level of silage on microbial N flow to the intestine ($P>0.10$), but there was a tendency for improved efficiency of microbial protein synthesis (g of microbial N/kg of OM truly fermented in the rumen) for the 20% barley silage diets ($P = 0.072$). The efficiency of microbial protein synthesis is affected by several factors, including the maintenance energy requirements of the bacterial populations (Russell *et al*., 1992) and the turnover rate of the solid and liquid pools (Rode and Satter, 1988). Maintenance requirements are higher for bacteria that ferment nonstructural carbohydrates (starch, pectin, and sugars; Russell *et al*., 1992), and thus would be expected to be higher for the bacterial populations in steers fed the 5% silage diets with a greater amount of ruminal starch digestion.

The amount of microbial protein synthesized in the rumen is largely driven by the amount of energy derived from ruminal fermentation of

carbohydrate, but is also influenced by ruminal pH and the bacterial requirements for N (Russell, 1998). The energy derived from carbohydrate digestion in the rumen drives microbial protein synthesis, and in turn, the supply of protein to the growing animal. However, feeding extensively processed grain with rapid and complete digestion can create unfavorable conditions within the rumen that limit microbial protein synthesis and fiber digestion.

So, the aim of the present part of this investigation was to follow the degree that different rumen degradable sources of proteins and carbohydrates degrade in the rumen and the extent of the synchrony of their fermentation in order to maximize the animal benefit from feed throughout microbial protein synthesis and maximizing bypass nutrients.

MATERIALS AND METHODS

The current investigation was carried out at El-Serw Experimental Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt, using twenty four Zaraibi goat does during lactation period. These does were assigned to four similar experimental groups (6 does each) taking into account its parity, milk yield and their body weights. Animals of each group were housed in stalls (6 x 4 m) and fed in groups. Fresh and clean drinking water was available all times. The experiment started after weaning and began after 10 days of adaptation with the tested diets and lasted for 12 weeks. The experimental design was a 2 x 2 factorial arrangement of the treatments. The four animal groups were assigned at random to receive the four experimental diets.

Four experimental diets were formulated using local resources of ingredients (corn silage, CS) was used as roughage for all groups. Yellow corn grains (low RDNSC) or barley grains (high RDNSC) as tested carbohydrate sources and the tested protein sources were soybean meal (high RDP) and corn gluten (low RDP). The diets (Table 1) consisted of:

1. Low RDNSC and high RDP.
2. High RDNSC and high RDP.
3. Low RDNSC and low RDP.
4. High RDNSC and low RDP.

The tested materials were ground together and mixed with other ingredients to make pellets (6 mm) of 4 concentrate feed mixtures (CFM). The experimental diets were formulated to exceed or equal the NRC (1989) recommendations based on their feeding values determined in the second part of this series of experimental (El-Shabrawy *et al*, 2010). All animals were fed on the tested diets containing 60% concentrate: 40% roughage ratio which were hand-mixed just before feeding once daily.

Samples of the four tested diets (Table 2) were analyzed for dry matter, ash, crude fiber, crude protein and ether extract according to A.O.A.C (1990). The NDF % was determined according to Robertson and Van Soest (1981) and the NSC % was calculated according to Calsemiglia *et al.* (1995).

For this part of the present study, during the last week of the experimental period, rumen fluid samples were taken from three animals each group just before offering morning meal and 2, 4, 6 and 8 hours post-

feeding using stomach tube. One part of rumen fluid used for measuring pH, NH_3-N and total volatile fatty acids (VFA) concentrations. Rumen-fluid pH was measured immediately on a fresh aliquot using battery operated pH meter. All samples were filtered through two layers of surgical gauze. One part of each sample was acidified with 1.0 ml of diluted H_2SO_4 (50% v/v) to retard ammonia and kept frozen at -20°C until analysis of NH_3-N (Conway and O'Mally, 1957) and total VFAs (Warner, 1964).

Table 1. Formulation of the experimental diets (% of DM).

Ingredients	High RDP		Low RDP	
	Low RDNSC (Diet 1)	High RDNSC (Diet 2)	Low RDNSC (Diet 3)	High RDNSC (Diet 4)
Whole corn silage, WCS	60	60	60	60
Corn gluten meal, CGM	-	-	14	14
Barley grain, BG	-	18	-	24
Corn grain, CG	18	-	24	-
Soybean meal, SBM	20	20	-	-
Limestone	1.1	1.1	1.1	1.1
Salt	0.5	0.5	0.5	0.5
Di-calcium phosphate	0.2	0.2	0.2	0.2
Minerals and vitamins mixture*	0.2	0.2	0.2	0.2

*** Each Kg contained P, 40 g, Ca, 50 g, Mg. 50 g, Mn, 4.5 g, S, 12 g, Fe, 7 g, Cu, 2 g, Se, 12 mg, Co, 50 mg, vitamin A, 2000000 IU, vitamin D, 20000 IU and vitamin E, 20 mg (Biomix 33, Produced by Biochema, A.R.E., Cairo).**

The second part of the rumen liquor samples was used for determination of gas length measured by a ruler after incubating the samples at 39°C according to the Vaspar broth method (West and Wilkins, 1980). Bacterial cell density measured at 600 nm in 1 cm cell using Spekol spectrophotometer. Direct bacterial count was carried out using breed slide techniques as described by Collins and Lyne (1985). Cellulase activity using 3 x 1 cm diameter pieces (25 mg) of filter paper No. 1 according to the method outlined by Gadgil *et al.* (1995); Amylase activity using 1% soluble starch solution according to Kochhar and Dua (1990) and quantitative assay of proteinase activity was carried out according to the modified casein digestion method described by Lupin *et al*. (1982). Moreover, microbial protein synthesize was measured throughout the estimation of DNA, RNA and oligoneucleotide concentrations in the rumen liquor using spectrophotometric quantitative technique and equations according to Maniatis *et al.* (1982). The absorbency was measured at 260 and 280 nm using Milton Roy Spectronic 1201 spectrophotometer using distilled water as blank and the DNA and RNA calibration curve were setup and the following equations were adopted:

For DNA: $Y = a x + b$

Where: a = 0.021 and b = 0.007 and r=1.0

For RNA: $Y = a x + b$

Where: a = 0.018 and b = 0.033 and r=0.999

Data were subjected to 2x2x5 factorial statistical analysis by the computer program of SAS (1996) using the General Linear Model (GLM). The data of rumen liquor, shapes of rumen liquor's bacteria and its activities and

single cell protein concentration were subjected to analysis of variance for examining the effects of treatments ($diet_1$, $diet_2$, $diet_3$ and $diet_4$) and high and low (rumen degradable protein, RDP and rumen degradable non structural carbohydrates, RDNSC) and time of sampling (0,2,4,6 and 8 hours) and their interaction according to the following model:

$Y_{ijkl}= U+ P_i+ D_j+T_k+PD_{ij}+PT_{ik}+DT_{jk}+ PDT_{ijk}+e_{ijkl}$

where: Y_{ijkl} = observed traits, U= overall mean, P_i= effect of RDP and RDNSC 1-4 (1= high RDP, 2= low RDP, 3= high RDNSC and 4= low RDNSC),D_j= effect of experimental diets 1-4, T_k= time of sampling, PD_{ij}= interaction RDP and RDNSC X experimental diets, PT_{ik}= interaction RDP and RDNSC X time of sampling, DT_{jk}= interaction experimental diets X time of sampling, PDT_{ijk}= interaction RDP and RDNSC X experimental diets X time of sampling, e_{ijkl}= Random error. Means were compared according to Duncan's Multiple Range Test at 0.05 level (Duncan, 1955).

RESULTS AND DISCUSSION

The results presented in Table 2 show that the chemical analysis of the different ingredients used in this study were within the normal range of similar materials as discussed and reviewed previously by (Mehrez, 1992; Mabjeesh *et al,* 1997; El-Badawi *et al.* 2001 and El-Shabrawy and El-Fadaly, 2006). The calculated chemical composition of the tested formulated diets using these ingredients seemed similar in all nutrients, except for NSC which was higher in corn-gluten diet and was lower in barley-SBM diet. This could be attributed to their variable contents in both corn grains (74.63) and barley grains (66.37).

Table 2. Chemical composition of the tested ingredients and the experimental diets

Ingredients	DM (%)	Chemical composition on DM basis (%)							
		OM	CP	EE	CF	NFE	Ash	NDF	NSC*
WCS	26.04	92.92	8.23	2.30	25.70	56.69	7.08	45.40	36.99
CGM	91.21	97.98	53.36	1.90	2.80	39.92	2.02	14.15	28.57
BG	89.43	97.52	9.30	1.85	7.10	79.27	2.48	20.00	66.37
CG	87.50	98.10	9.11	2.35	2.70	83.94	1.90	12.01	74.63
SBM	89.92	93.20	42.48	3.40	6.21	41.11	6.80	14.25	33.07
Calculated chemical composition of the tested diets									
1	49.35	92.05	15.07	2.48	17.15	57.35	7.95	32.25	42.23
2	49.69	91.94	15.10	2.39	17.94	56.51	8.06	33.69	40.75
3	49.39	93.01	14.60	2.21	16.46	59.74	6.99	32.10	44.10
4	50.85	92.87	14.64	2.29	17.51	58.43	7.13	34.02	42.12

*** NSC % = 100 – (NDF% + CP% + Fat% + Ash%). Calsemiglia *et al.* (1995)**

Regarding the dry matter intake (DMI), organic matter intake (OMI) and crude protein intake (CPI), data in Table 3 show that they were similar in the four tested groups with averages of 1567, 1450 and 233 g/h/d, respectively. However, the RDP as % of CPI decreased when gluten replaced SBM in diets 3 and 4 since the degradability of corn gluten meal CP was lower than that of SBM (Mabjeesh *et al.*, 1997 and Casper *et al.*, 1999).

Moreover, the calculated RDNSC was higher in barley based diets, while recorded lower values in corn based diets. This could be referred back to the higher content of barley grains which its starch may be more rapidly fermented in the rumen than corn starch (McCarthy *et al.,* 1989; Herrera-Saldana *et al.*, 1990 and Mabjeesh *et al.*, 1997).

Although GE values showed no differences among the four tested diets, the values of eNDF were high in barley based diets than that based on corn grains and it was higher in diets with high RDP than those contained low RDP. On the other hand, RFV, DE, ME and NE_L values were higher in diets contained high RDNSC (barley) than those with low RDNSC (corn) and with low RDP (corn gluten meal) than those with high RDP (SBM).

Table 3. Intake, rumen degradability, energy and relative feeding value of the experimental diets

Items	Experimental diets			
	1	2	3	4
Total DM intake, g/head/day	1550	1570	1560	1590
Total OM intake, g/head/day	1427	1443	1451	1477
Total CP intake, g/head/day	234	237	228	233
RDP intake, g/head/day	150	158	101	110
RDP% from DM intake	9.68	10.06	6.47	6.92
RDP* % from CP intake	64.10	66.67	44.29	47.21
Total NSC intake, g/head/day	655	640	688	670
RDNSC intake, g/head/day	473	558	457	572
RDNSC% from DM intake	30.52	35.54	29.29	35.97
RDNSC* % from NSC intake	72.21	87.19	66.42	85.37
eNDF	15.49	18.09	14.07	17.85
RFV	119.4	127.1	130.3	136.1
GE (MJ/Kg DM)	1.79	1.78	1.79	1.79
DE (Mcal/Kg DM)	2.89	2.93	3.00	3.05
ME (Mcal/Kg DM)	2.46	2.51	2.58	2.63
NE_L (Mcal/Kg DM)	1.45	1.51	1.57	1.58

*** Rumen degradable protein (RDP) and non-structural carbohydrate (RDNSC) calculated according to Mabjeesh *et al.* (1997).**

eNDF (Effective neutral detergent fiber)=(pH-5.425)/0.04229 Fox *et al.* (2000)
RFV (Relative feeding value) = DMI x DDM / 1.29 Moore and Coleman (2001)
GE (MJ/Kg DM)=0.0226 CP+0.0407 EE+0.0192 CF+0.0177 NFE. MAFF (1975)
DE (Mcal/Kg DM) = % TDN x 0.04409 (NRC, 1978).
ME (Mcal/Kg DM) = -0.45 + 1.01 DE (Mcal/Kg DM) (NRC, 1978).
NE_L (Mcal/Kg DM) = -0.12 + 0.0245 TDN (% of DM) (NRC, 1978).

The obtained trends are in harmony with the results of El-Deeb *et al.* (2005) who stated that RFV and both of DE and ME of ensiled alfalfa increased than those of green and hay of alfalfa which were accompanied with a reduction in eNDF values, specially with increased NSC content of formaldehyde-treated silages (as rumen undegradable protein source).

Regarding the effect of RDP on pH values of rumen liquor during different sampling times (Table 4), it is clear that high RDP tended to significantly ($P<0.05$) increased pH values compared to low RDP diet.

4

Data showed also that high RDNSC in diets of the experimental animals significantly (P<0.05) increased pH values than that obtained when diets contained low RDNSC were fed. With sampling time order advancement pH values followed its known curve, since it significantly (P<0.05) decreased after 2 hrs of feeding and start to slightly increase after 4 hrs post feeding. In general, the range of pH values was between 5.57 and 6.76 and remained above 5.8 during all sampling times. Khorasani *et al.* (2001) observed that the average pH values was not affected by the grains source, but the rate of decrease of pH values after feeding was faster for cows fed barley than for cows fed corn. In barley-based diets, the highest pH values was greater (P = 0.041) when combined with SBM, and the pH change tended to be greater for the desynchronized diets (P = 0.10).

The concentration of NH_3-N gave the highest values with diets contained high RDP compared to those contained low RDP with significant (P<0.05) differences (29.95 *Vs.* 13.15 mg/100 ml). Moreover, RDNSC positively (P<0.05) affected NH_3-N concentration, since it increased with diets contained high RDNSC than with those contained low RDNSC (22.12 *Vs.* 20.98 mg/100 ml). With sampling times order advancement NH_3-N concentration gradually (P<0.05) decreased from 28.12 before feeding to 17.10 mg/100 ml after 8 hrs from feeding. The range of NH_3-N values of tested diets along different sampling times was between 40.23 at 0 time and 8.77 mg/100 ml at 8 hrs post feeding. This range is covered the required amounts for microbial protein synthesis, since the minimum value reported in this concern was 3.3 to 8.5 mg/100 ml (Kang-Meznarich and Broderick, 1981).

Ruminal NH_3-N concentrations throughout the day serve as a good indicator of both CP degradability and energy availability. Within this domain, most studies in dairy cattle reported lower NH_3-N concentration in barley than corn-based diets, mainly due to the greater rate of NSC degradability of barley and the greater incorporation of NH_3-N into microbial protein synthesis (McCarthy *et al*., 1989 and Casper *et al*., 1990 and 1999). Moreover, Sindt *et al.* (1993) reported that ruminal NH_3-N was higher in steers fed dry-rolled corn when urea-blood meal / feather meal was the protein supplement source. However, steers fed dry-rolled grains had similar ruminal NH_3-N regardless of the source of supplemental protein.

On contrast, the results obtained by Overton *et al.* (1995) revealed that there was a linear decrease in NH_3-N concentrations when barley gradually replaced corn in diets of dairy cows, but this decrease was not accompanied by an increase in the passage of microbial N to the duodenum. Also, Surber and Bowman (1998) reported a greater ruminal NH_3-N concentration for steers fed barley than for steers fed corn.

The concentration of total volatile fatty acids (VFAs) ranged between 4.78 and 9.00 meq./100 ml RL. The highest value was recorded with goats fed high RDP compared to those fed low RDP (7.35 *Vs.* 6.39 meq./100ml RL on average). In the meantime, diets contained high RDNSC gave low VFA values than those contained low RDNSC (7.07 *Vs.* 6.67) without significant differences. With sampling time order advancement total VFAs values significantly (P<0.05) increased after 2 hrs of feeding and started to

significantly (P<0.05) decrease at 8 hrs post feeding. In general, the decreased values of VFAs obtained in the present study with corn based diets (low RDNSC) are in agreement with those obtained by Casper *et al*. (1999) who found a greater VFA concentration for corn based diets.

In this concern, Owens and Goetsch (1988) stated that the concentration of VFA in the ruminal fluid was higher for high and medium crude protein (HCP & MCP) than for low crude protein (LCP), which is in contrast with changes in ruminal pH. However, the ruminal concentration of VFA is the net result of production; absorption and the rate of absorption which increases as ruminal pH decreases (Merchen, 1988). Therefore, Ipharraguerre *et al.* (2005) documented that it is possible that differences detected for the concentration of VFA arose from a faster rate of removal of VFA from the rumen of cows fed LCP. More importantly, these differences were small and probably were not biologically relevant. On the other hand, Sindt *et al.* (1993) stated that the reasons for the reduced total VFA and higher ruminal NH_3-N concentrations in steers fed dry-rolled corn supplemented with escape protein are unknown.

Data in Table 5 show the effect of feeding the tested diets on some bacterial forms in rumen liquor. It is clear that the highest values for coccoid shaped bacteria was recorded for diet 4 (high RDNSC and low RDP) being 7.03 x 10^7 CFU/ml. Data showed no significant differences between the mean values for high RDP and low RDP sources, while the mean value (6.96) of coccoid shaped bacteria (6.96x10^7) obtained from barley based diets (high RDNSC) was significantly (P<0.05) higher than that (6.88x10^7) obtained from corn based diets (low RDNSC). With sampling time order advancement, the highest value (8.05 x 10^7 CFU/ml) was recorded for coccoid shaped bacteria after 2 hrs post feeding and then values gradually decreased.

The second population of bacteria found was short rod shaped ones. This form of bacteria seems to be activated and gave the highest value (6.15 x 10^7 CFU/ml) when diets contained high RDP with high RDNSC (diet 2) was fed. This group of bacteria showed a significant (P<0.05) difference between the mean value of high RDP and low RDP (6.06 x 10^7 *Vs.* 5.80 x 10^7 CFU/ml) and the same trend was recorded for the two RDNSC sources (being 5.99 x 10^7 *Vs.* 5.88 x 10^7 CFU/ml with high and low RDNSC, respectively). As for sampling time effect on short rod shaped bacteria, data showed that this form significantly (P<0.05) decreased after 4 hrs of feeding and significantly (P<0.05) fluctuated thereafter.

The third bacterial group found in the rumen liquor investigated in the present study was long rod shaped bacteria. The mean values of this long rod bacterial form recorded for high and low RDP were significantly (P<0.05) different (being 2.83 x 10^7 CFU/ml and 2.74 x 10^7 CFU/ml, respectively). Low RDNSC diets (corn based diets) recorded the highest significant (P<0.05) values of long rod shaped bacteria compared to that recorded with high RDNSC (barley based diets).

5

The effect of sampling times on long rod bacteria showed that at 0 time was 2.81 x 10^7 CFU/ml then decreased to reach its minimum (2.61 x 10^7 CFU/ml) at 4 hrs post feeding and start to increase again to reach its maximum (3.05 x 10^7 CFU/ml) after 8 hrs from feeding. All interaction effects among feed sources and sampling times significantly (P<0.05) affected the studied of rumen liquor's bacterial shapes.

In this connection, Tarakanov and Lavlinskii (1998) worked on cellulolytic cocci, from the rumen of cows bred at different farms and found that ruminococci were heterogeneous with respect to cellulose hydrolysis and could be classified into high, medium and low activity strains. The rate of cellulose hydrolysis showed to be directly related to the capacity of ruminococci to adhere to the substrate.

Regarding the effect of tested diets on total bacterial counts (TBC, CFU x 10^7/ml) in the rumen fluid, Figure 1 show that the highest values of bacterial count was recorded with diet low in RDNSC and high RDP after 2 hrs post feeding. The lowest value was recorded also with diet contained low RDNSC, but with low RDP after 8 hrs post feeding. In general, rations based on barley (high RDNSC) showed no significant differences in TBC being 5.20 x 10^7 *Vs.* 5.22 CFU/ml with rations high and low RDNSC, respectively, but this parameter showed to be influenced significantly (P<0.05) with RDP being 5.27 x 10^7 *Vs.* 5.16 CFU/ml with rations high and low RDP, respectively. These results could be explained by the availability of N for bacterial growth in the rumen. These results are in harmony with Hoover and Stokes (1991) who found that total population achieved the highest growth rate on mixture of peptides, amino acids and ammonia (fractions of protein digestion). They added that carbohydrates are digested by exoenzymes to oligosaccharides that are available for cross feeding by the mixed microbial population. So, the rate of carbohydrates digestion is the major factor controlling the energy available for microbial growth. Moreover, Hung *et al.* (1995) studied the ruminal microbial density in Taiwan native goats. They found that high total bacterial and protozoal numbers were obtained when goats received rapid starch and protein ruminal degradation rates.

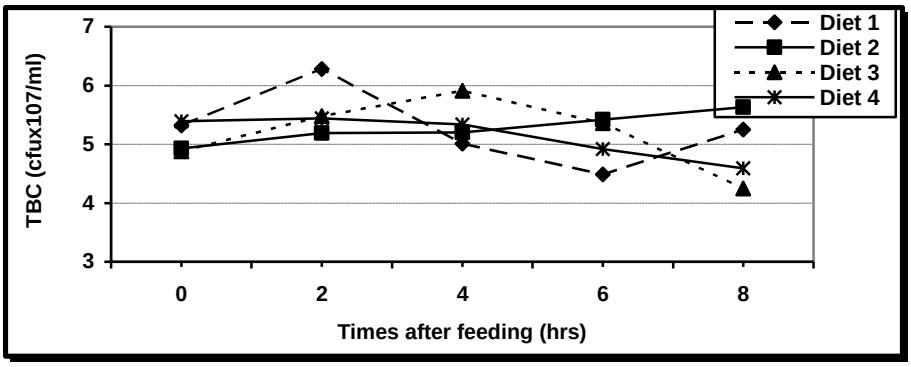

Figure 1. Effect of feeding the tested diets to Zaraibi goats on total bacterial counts (TBC, CFU x 10^7/ml) in their rumen fluid.

Moreover, Russell *et al.* (1992) stated that bacterial yield is decreased when forage NDF is <20%. In case of structural carbohydrate (SC), bacteria can utilize only ammonia as a N source, but in case of non-structural carbohydrate (NSC), bacteria can utilize either ammonia or peptides. The yield of NSC bacteria is enhanced by as much as 18.7% when proteins or peptides are available.

Data obtained in Figure 2 showed clearly that bacterial growth assessed in terms of optical density (OD) was significantly (P<0.05) higher in high RDP diets than those with low RDP ones (being 0.165 *Vs.* 0.158 on average), but took the reverse picture with RDNSC, since it was higher with diets contained low RDNSC compared to that contained high RDNSC (being 0.186 *Vs.* 0.137 on average). With sampling time order advancement, diets (1, 2 and 3) showed to follow similar pattern, while they showed the reverse before feeding and at 2 hrs post feeding since it gave obvious decrease in OD values till 6 hrs post feeding and start to increase again.

These results could be explained by the findings of Russell *et al.* (1992) who found that the NSC bacteria produced less ammonia when the carbohydrate fermentation rate (growth) is rapid, but 34% of the ammonia production is insensitive to the rate of carbohydrate fermentation. Ammonia production rates are also moderated by the rate of peptide, and amino acid uptake. Peptides and amino acids can pass out of the rumen if the rate of proteolysis is faster than in rate of peptide utilization.

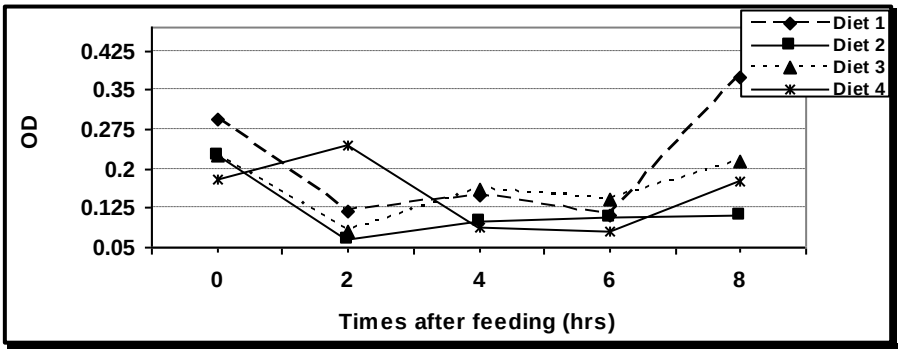

Figure 2. Effect of feeding the tested diets to Zaraibi goats on optical density (OD, 600 nm) of their rumen fluid.

However, Leedle *et al.* (1982) documented that soluble carbohydrate-utilizing bacteria predominated at all times throughout the day. The cellulolytic subgroup followed a diurnal pattern with few variations in bacterial cell composition and ruminal fluid parameters between diets. Moreover, Leedle *et al.* (1986) found that the variation of postprandial within the bacterial population carbohydrate-fermenting groups was small.

Data in Table 6 present the effect of formulated diets on some enzymatic activities and gas column produced as one of the fermentation process indicators and result of enzymatic activities.

6

Although, data clearly indicated that the most dominant enzymatic activity was recorded for cellulolytic bacteria followed by proteolytic and amylolytic, the lowest values of resultant gas column was recorded with cellulolytic bacteria. Feeding diet contained high RDP with low RDNSC recorded the highest values of bacterial enzymatic activities in forms of cellulolytic (being 103.40 mg glucose/ml RL), proteolytic (being 2.48 mg tyrosine/ml RL) and amylolytic (being 1.69 mg glucose/ml RL). In the same time, the produced gas length (cm) by different types of bacteria showed that the highest ($P<0.05$) value (2.11 cm) obtained with amylolytic bacteria when goats were fed diet contained high RDNSC with high RDP; and the lowest ($P<0.05$) value (0.83 cm) was obtained with cellulolytc bacteria when goats fed diet contained low RDNSC with high RDP.

In general, high RDP as well as high RDNSC in diets of goats recorded significantly ($P<0.05$) higher values of rumen bacterial enzymatic activities than those fed diets contained low RDP and RDNSC. Gas column length took the same trend in case of RDP influence, while the reverse picture took place with RDNSC since higher ($P<0.05$) gas column length was recorded with diets contained low RDNSC. These results are on line with those obtained by Cecava *et al.* (1988).

With sampling time order advancement, data in Table 6 revealed that enzymatic activities decreased gradually and significantly ($P<0.05$) compared to that recorded before feeding (0 time) and showed to be significantly ($P<0.05$) increased after 8 hrs from feeding. Gas column length recorded with proteolytic and amylolytic activities significantly ($P<0.05$) increased till 4 hrs post feeding and that gas column length for amylolytic bacteria reached its maximum after 6 hrs post feeding and declined thereafter. On the other hand, gas column length recorded for cellulolytic bacteria significantly ($P<0.05$) fluctuated with time order advancement recording its highest value before feeding followed by that recorded at 4 hrs post feeding and the other sampling times values remained low.

All interaction effects among feed sources and sampling times significantly ($P<0.05$) affected gas column length and enzymatic activities of the studied rumen liquor's bacterial types.

Regarding microbial growth and efficiency, Nocek and Russell (1988) stated that nitrogen sources other than NH_3-N are required to maximize microbial growth and efficiency. Moreover, Sniffen and Robinson (1987) found similar effect especially for diets that contain high amounts of concentrates, causing the noncellulolytic bacterial population to dominate the ruminal microflora.

Martin *et al.* (1999) found that most fibrolytic activities of the solid-associated microorganisms were lower in animals fed wheat than those fed corn. Differences in fibrolytic activities of solid-associated microorganisms between two corn genotypes were not statistically significant. They also concluded that the faster the starch is degraded limited digestion in the rumen and this may be an alternative to minimizing digestive interactions in the rumen by avoiding large microbial changes.

Demeyer and Van Nevel (1986) hypothesized that microbial N produced in the rumen and flowing to the duodenum is related to the total

amount of OM fermented or apparently digested in the rumen. This relationship is mainly affected by the physical and chemical properties of feeding carbohydrates and the amounts ingested. Since, high fermentation rates are associated with lactate production, low methane production and transient polysaccharide synthesis were observed.

Data in Table 7 represent the effect of formulated diets on microbial protein synthesis measured in the present study as DNA, RNA and oligonucleotide (single cell protein indicators). The results showed that the three measured parameters significantly ($P<0.05$) increased when diets contained low RDNSC compared to those contained high RDNSC when both fed with low RDP, while such effect was not significant when they were fed with high RDP source. The tested parameters did not show any appreciable change with sampling time order advancement.

The obtained results are supported with those obtained by Mabjeesh *et al.* (1997) who stated that, despite the quantitative matching between RDP and RDNSC concentrations in both diets with high RDP and high RDNSC concentrations and low RDP and low RDNSC concentrations, better efficiency and higher microbial yield were observed in the latter diet. This result suggests that a deficient supply of AA and peptides in the rumen is responsible for the lower bacterial crude protein flow in both diets with high concentrations of RDP, consequently influencing the efficiency of bacterial crude protein synthesis in those diets, which include a high ratio of concentrates.

Table 7. Effect of feeding the tested diets to Zaraibi goats on single cell protein concentration (µg/ml) in their rumen liquor

Items	Time from feeding (hrs)	Experimental diets				SEM	RDP		RDNSC		SEM	Times' means
		1	2	3	4		High	Low	High	Low		
DNA	0	52.64	52.56	52.43	51.77	0.59	52.60	52.10	52.17	52.54	0.42	52.35
	2	52.41	52.43	52.00	51.98		52.42	51.99	52.21	52.21		52.32
	4	52.08	51.74	52.72	52.75		51.91	52.73	52.24	52.39		52.32
	6	52.64	52.28	52.41	51.95		52.46	52.18	52.12	52.53		52.21
	8	52.72	52.84	52.92	48.29		52.78	50.61	50.57	52.82		51.69
Rations' means		52.49	52.37	52.49[a]	51.35[b]	0.26	52.43	51.92	51.86[b]	52.49[a]	0.18	
RNA	0	42.12	42.05	41.94	41.42	0.47	42.08	41.68	41.73	42.03	0.33	41.88
	2	41.93	41.94	41.60	41.59		41.94	41.59	41.76	41.76		41.86
	4	41.66	41.39	42.17	42.19		41.52	42.18	41.79	41.92		41.85
	6	42.11	41.83	41.93	41.56		41.97	41.74	41.69	42.02		41.76
	8	42.17	42.27	42.34	38.64		42.22	40.49	40.45	42.26		41.36
Rations' means		41.99	41.89	41.99[a]	41.08[b]	0.21	41.95	41.54	41.48[b]	41.99[a]	0.15	
ON	0	21.06	21.03	20.97	20.71	0.24	21.04	20.84	20.87	21.01	0.17	20.94
	2	20.96	20.97	20.80	20.79		20.97	20.79	20.88	20.88		20.93
	4	20.83	20.69	21.08	21.09		20.76	21.09	20.89	20.96		20.93
	6	21.06	20.91	20.96	20.78		20.98	20.87	20.85	21.01		20.88
	8	21.08	21.14	21.17	19.32		21.11	20.24	20.23	21.13		20.68
Rations' means		20.99	20.94	20.99[a]	20.54[b]	0.11	20.97	20.77	20.74[b]	20.99[a]	0.07	

SEM: standard error of means.

a, b means within the same row having different superscripts are significantly different at P<0.05.

The interaction between protein sources and sampling times as well as the interaction among protein sources, carbohydrate sources and sampling times significantly (P<0.05) affected DNA, RNA and ON values. In the mean time, protein sources as well as its interaction with carbohydrate sources had no significant effect on these parameters, while carbohydrate sources significantly (P<0.05) affected the obtained values.

In this concern, the amino acids and peptides derived from dietary protein degradation are important sources of N for the growth of ruminal bacteria (Argyle and Baldwin, 1989), and, if they are deficient, ruminal fermentation may become uncoupled. Consequently, optimal bacterial crude protein synthesis may not be realized because the availabilities of energy and N are not synchronized (Polan, 1988 and Clark, *et al.*, 1992).

The obtained trends of studied parameters showed clearly that bacterial groups in the rumen utilized the tested sources of RDP and RDNSC in the manner that kept most nutrients to be utilized in the lower part of digestive system. This explanation could be reasonable since the results support the energy values of tested diets (Table 3) which showed the superiority of low RDP with high RDNSC containing diet.

On the basis of the results of the present study, it could be recommended to formulate diets containing low degradable protein (corn gluten meal) along with high fermentable carbohydrate (barley grains) sources in the diets of lactating Zaraibi goats in order to optimize rumen environment for microbial activity.

The second part of this research work (El-Shabrawy et al,2010) will be directed to study the effect of these recommendations on feed utilization and milk production and its composition by Zaraibi goats.

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تأثير البروتين والكربوهيدرات المتكسرين في الكرش على الأداء الإنتاجي للماعز الحلاب:

١- بعض قياسات سائل الكرش والنشاط الميكروبي

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أجريت هذه الدراسة في محطة بحوث الإنتاج الحيواني بالسرو على ٢٤ عنزة زرايبي في مرحلة الحليب المتوسط ، وقسمت عشوائياً إلى ٤ مجموعات (٦ حيوانات بكل مجموعة) في تصميم عاملي (٢ × ٢) إشتملت على مصدرين من الكربوهيدرات (أذرة صفراء "منخفض التكسر بالكرش" و شعير "عالي التكسر بالكرش") ومصدرين من البروتين (كسب فول صويا "عالي التكسر بالكرش" و جلوتين الأذرة "منخفض التكسر بالكرش"). وقد تم تكوين أربعة علائق تجريبية من هذه المصادر بهدف معرفة تأثير التغذية على تلك العلائق على بعض قياسات سائل الكرش والنشاط الميكروبي لبكتريا الكرش في الحيوانات التجريبية.

- سجلت العليقة المحتوية على بروتين عالي التكمسير مع كربوهيدات عالية التكسير في الكرش أعلى قيمة لتركيز الأس الهيدروجيني. بينما حدث إنخفاض في تركيزات أمونيا الكرش مع العلائق المحتوية على بروتين منخفض التكسير في الكرش دون فروق معنوية بين مصدري الكربوهيدات في العلائق. ولم تكن هناك فروق معنوية في تركيزات الأحماض الدهنية الطيارة الكلية في سائل الكرش ، باستثناء العليقة المحتوية على بروتين عالي التكسير في الكرش مع كربوهيدرات منخفضة التكسير في الكرش والتي أعطت أعلي (معنوياً) تركيز في هذا المعيار.
- اتضح أن بيئة الكرش كانت مناسبة من حيث تركيز الأس الهيدروجيني ونتروجين الأمونيا والأحماض الدهنية الطيارة الكلية لنمو ونشاط البكتريا الكروية الشكل والذي ظهر جلياً في

النشاط الإنزيمي السائد والذي ينتمي لصورة البكتريا السائدة (كرويات محللة للسليولوز). بالرغم من ذلك فإن حجم الغاز الناتج قد تفوق في البكتريا المحللة للنشا والبروتين مقارنة بذلك المسجل مع المجموعة المحللة للسليولوز والذي أوضح أن هناك إستفادة أفضل من العليقة عندما احتوت على كربوهيدات عالية التكسير في وجود مصدر بروتين منخفض التكسير. وهذا يعني أن النمو البكتيري في الكرش قد إستفاد من العلائق المحتوية على مصادر البروتين المتكسر والكربوهيدرات المتكسرة في الكرش بطريقة ضمنت الحفاظ على غالبية العناصر المغذية للإستفادة بها في مرحلة الهضم فيما بعد التخمر في الكرش.

- أظهرت معايير قياس البروتين الميكروبي المتكون في الكرش تأثراً معنوياً بمصدر الكربوهيدرات في العليقة ، بينما لم يكن مصدر البروتين ذو تأير معنوي. وقد أعطت العليقة المحتوية على مصدر كربوهيدرات منخفض التكسير في الكرش مع مصدر بروتين عالي التكسير في الكرش أعلى قيم في كل من الحمض النووي الريبوزي المفرد والمزدوج السلسلة (DNA , RNA) وعديدات النيوكليوتيد (ON) كدلائل على البروتين الميكروبي المخلق في سائل الكرش.

من النتائج المتحصل عليها توصي هذه الدراسة باستخدام مصدر بروتين منخفض التكسير (حبوب جلوتين الأذرة) مع مصدر كربوهيدرات عالي التكسير (حبوب الشعير) في الكرش للحصول على بيئة كرش مناسبة للنمو والنشاط البكتيري. وحيث أن القيم العالية لقياسات الكرش والنمو البكتيري المدروسة والتي تم الحصول عليها تحت ظروف التجربة الحالية، فإنه يوصى تحت الظروف المماثلة أن يستخدم كسب فول الصويا عند توفرة مع حبوب الأذرة لتحسين القيمة الغذائية في علائق الماعز الحلاب.

قام بتحكيم البحث

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Table 4. Effect of feeding the tested diets to Zaraibi goats on some rumen liquor parameters

Items	Time from feeding (hrs)	Experimental diets				SEM	RDP		RDNSC		SEM	Times' means
		1	2	3	4		High	Low	High	Low		
pH	0	6.41	6.53	6.32	6.76	0.10	6.47	6.45	6.65	6.36	0.07	6.51^{a}
	2	5.84	6.09	5.57	5.78		5.96	5.67	5.94	5.70		5.82^{c}
	4	6.08	5.99	5.78	5.92		6.04	5.85	5.95	5.93		5.94^{c}
	6	5.98	6.23	6.11	6.35		6.10	6.23	6.29	6.04		6.16^{b}
	8	6.12	6.13	6.30	6.14		6.13	6.22	6.14	6.21		6.17^{b}
Rations' means		6.08^{ab}	6.19^{a}	6.02^{b}	6.18^{a}	0.05	6.14	6.10	6.19^{a}	6.05^{b}	0.03	
NH_3-N (mg/100 RL)	0	40.23	33.23	22.40	16.61	3.12	36.73	19.51	24.92	31.31	2.20	28.12^{a}
	2	31.73	35.75	13.25	15.96		33.74	14.61	25.85	22.49		24.17^{a}
	4	28.09	27.16	6.53	15.87		27.63	11.20	21.51	17.31		19.41^{b}
	6	22.31	30.71	9.15	13.63		26.51	11.38	22.17	15.73		18.95^{b}
	8	26.79	23.52	9.33	8.77		25.15	9.05	16.15	18.06		17.10^{b}
Rations' means		29.83^{a}	30.07^{a}	12.13^{b}	14.17^{b}	1.39	29.95^{a}	13.15^{b}	22.12	20.98	0.98	
Total VFAs (meq./100 ml RL)	0	6.82	6.45	5.68	4.78	0.47	6.63	5.23	5.62	6.25	0.33	5.93^{c}
	2	9.00	7.87	7.87	7.08		8.43	7.47	7.47	8.43		7.95^{a}
	4	7.83	6.28	5.95	6.52		7.06	6.23	6.40	6.89		6.65^{b}
	6	7.85	7.68	7.25	7.28		7.77	7.27	7.48	7.55		7.52^{a}
	8	7.33	6.38	5.15	6.33		6.86	5.74	6.36	6.24		6.30^{bc}
Rations' means		7.76^{a}	6.93^{b}	6.38^{b}	6.40^{b}	0.21	7.35^{a}	6.39^{b}	6.67	7.07	0.15	

SEM: standard error of means.

a, b,c means within the same row or column having different superscripts are significantly different at P<0.05.

All interaction effects on NH_3-N and VFA values was not significant, except the interaction between carbohydrate sources and sampling times which was significant at P<0.05.

Table 5. Effect of feeding the tested diets to Zaraibi goats on some shapes of rumen liquor's bacteria (CFU x 10^7/ml).

Items	Time from feeding (hrs)	Experimental diets				SEM	RDP		RDNSC		SEM	Times' means
		1	2	3	4		High	Low	High	Low		
Coccoid shaped	0	5.84	6.15	6.23	7.95	0.08	5.99	7.09	7.05	6.04	0.06	6.54^{c}
	2	8.61	7.94	7.63	8.00		8.27	7.82	7.97	8.12		8.05^{a}
	4	7.40	8.11	8.04	7.58		7.76	7.81	7.84	7.72		7.78^{b}
	6	5.78	6.27	6.95	6.42		6.02	6.68	6.34	6.36		6.35^{d}
	8	7.05	5.97	5.26	5.22		6.51	5.24	5.59	6.15		5.87^{e}
Rations' means		6.94^{ab}	6.89^{bc}	6.82^{c}	7.03^{a}	0.04	6.91	6.93	6.96^{a}	6.88^{b}	0.03	
Short-rod shaped	0	6.52	5.90	6.21	5.52	0.07	6.21	5.86	5.71	6.36	0.05	6.04^{a}
	2	6.94	5.58	6.19	5.63		6.26	5.91	5.60	6.56		6.08^{a}
	4	5.34	4.95	5.95	6.52		5.15	6.24	5.74	5.65		5.69^{c}
	6	5.57	6.88	5.90	5.63		6.22	5.77	6.25	5.73		5.99^{a}
	8	5.54	7.46	4.67	5.77		6.50	5.22	6.62	5.10		5.86^{b}
Rations' means		5.98^{b}	6.15^{a}	5.78^{c}	5.82^{c}	0.03	6.07^{a}	5.79^{b}	5.98^{a}	5.88^{b}	0.02	
Long-rod shaped	0	3.59	2.73	2.21	2.72	0.03	3.16	2.46	2.72	2.90	0.02	2.81^{b}
	2	3.28	2.05	2.63	2.68		2.66	2.66	2.37	2.95		2.66^{c}
	4	2.27	2.53	3.73	1.92		2.40	2.83	2.22	3.00		2.61^{d}
	6	2.11	3.10	3.21	2.72		2.61	2.96	2.91	2.66		2.78^{b}
	8	3.16	3.46	2.83	2.76		3.31	2.79	3.12	2.99		3.05^{a}
Rations' means		2.88^{a}	2.77^{b}	2.92^{a}	2.56^{c}	0.01	2.83^{a}	2.74^{b}	2.67^{a}	2.90^{b}	0.01	

SEM: standard error of means.

a, b,c means within the same row or column having different superscripts are significantly different at P<0.05.

Table 6. Effect of feeding the tested diets to Zaraibi goats on some bacterial activities in their rumen liquor

Items	Time from feeding (hrs)	Experimental diets				SEM	RDP		RDNSC		SEM	Times' means
		1	2	3	4		High	Low	High	Low		
Proteolytic activity (mg tyrosine/ ml RL)	**0**	3.54	2.67	2.71	2.13	0.01	3.11	2.42	2.40	3.13	0.01	2.76^{a}
	2	1.36	0.69	0.87	2.91		1.03	1.89	1.80	1.11		1.46^{c}
	4	1.73	1.10	1.90	0.98		1.42	1.44	1.04	1.81		1.43^{d}
	6	1.27	1.24	1.66	0.87		1.25	1.26	1.05	1.46		1.26^{e}
	8	4.52	1.29	2.54	2.06		2.91	2.29	1.67	3.53		2.60^{b}
Rations' means		2.48^{a}	1.40^{d}	1.93^{b}	1.79^{c}	0.006	1.94^{a}	1.86^{b}	1.59^{b}	2.21^{a}	0.005	
Gas column length (cm)	**0**	0.91	1.11	1.37	0.20	0.02	1.01	0.78	0.66	1.14	0.01	0.90^{d}
	2	2.05	1.32	1.29	0.20		1.68	0.75	0.76	1.67		1.22^{b}
	4	1.34	1.90	0.66	1.60		1.62	1.13	1.75	1.00		1.37^{a}
	6	0.00	1.47	1.37	1.37		0.74	1.37	1.42	0.68		1.05^{c}
	8	1.90	0.81	0.10	1.37		1.36	0.73	1.09	1.00		1.04^{c}
Rations' means		1.24^{b}	1.32^{a}	0.96^{c}	0.95^{c}	0.007	1.28^{a}	0.95^{b}	1.13^{a}	1.09^{b}	0.005	
Cellulolytic activity (mg glucose / ml RL)	**0**	150.59	111.92	113.60	87.82	0.59	131.25	100.71	99.87	132.09	0.42	115.98^{a}
	2	53.07	23.37	31.21	122.57		38.22	76.89	72.97	42.14		57.56^{c}
	4	69.88	41.86	77.17	36.26		55.87	56.71	39.06	73.53		56.29^{d}
	6	49.15	48.03	66.52	31.21		48.59	48.87	39.62	57.84		48.73^{e}
	8	194.30	50.27	105.75	84.46		122.28	95.11	67.36	150.03		108.70^{b}
Rations' means		103.40^{a}	55.09^{d}	78.85^{b}	72.46^{c}	0. 26	79.24^{a}	75.66^{b}	63.78^{b}	91.13^{a}	0.18	
Gas column length (cm)	**0**	1.29	1.87	1.24	1.39	0.01	1.58	1.31	1.63	1.26	0.008	1.45^{a}
	2	0.10	1.26	0.71	1.04		0.68	0.87	1.15	0.40		0.78^{d}
	4	1.46	1.31	0.96	0.81		1.39	0.88	1.06	1.21		1.14^{b}
	6	0.20	1.62	0.10	0.51		0.91	0.30	1.06	0.15		0.61^{e}
	8	1.11	0.25	1.26	0.91		0.68	1.08	0.58	1.19		0.88^{c}
Rations' means		0.83^{d}	1.26^{a}	0.85^{c}	0.93^{b}	0.005	1.05^{a}	0.89^{b}	1.09^{a}	0.84^{b}	0.004	
Amylolytic activity (mg glucose / ml RL)	**0**	2.40	1.82	1.84	1.45	0.02	2.11	1.65	1.64	2.12	0.01	1.88^{a}
	2	0.94	0.49	0.61	1.96		0.72	1.28	1.23	0.77		1.00^{c}
	4	1.19	0.77	1.29	0.69		0.97	0.99	0.73	1.24		0.98^{c}
	6	0.88	0.86	1.14	0.61		0.87	0.87	0.74	1.01		0.87^{d}
	8	3.05	0.90	1.71	1.41		1.97	1.56	1.15	2.38		1.77^{b}
Rations' means		1.69^{a}	0.97^{d}	1.32^{b}	1.22^{c}	0.007	1.33^{a}	1.27^{b}	1.09^{b}	1.50^{a}	0.005	
Gas column length (cm)	**0**	0.71	1.44	1.46	1.06	0.02	1.08	1.26	1.25	1.08	0.01	1.17^{d}
	2	1.17	2.25	1.26	1.06		1.71	1.16	1.65	1.22		1.44^{c}
	4	1.74	2.49	1.18	2.34		2.12	1.76	2.41	1.46		1.94^{b}
	6	1.91	2.43	2.43	2.25		2.17	2.34	2.34	2.17		2.26^{a}
	8	0.10	1.95	1.18	0.81		1.03	0.99	1.38	0.64		1.01^{e}
Rations' means		1.13^{b}	2.11^{a}	1.50^{c}	1.50^{c}	0.008	1.62^{a}	1.50^{b}	1.81^{a}	1.32^{b}	0.006	

SEM: standard error of means.

a, b,c,d,e means within the same row or column having different superscripts are significantly different at P<0.05.