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Azzawi Dates (*Phoenix dactylifera*) as a Substitute for Corn as an Energy Source in Sheep Diet: *In vitro* Gas Production and Fermentation

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ABSTRACT

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In vitro gas production technique was used in the current study to evaluate Azzawi date (AD) as a substitute for corn grain (CG). The AD was used to replace corn grain at graded levels in the proportion: 0:100 (CG), 25:75 (AD25), 50:50 (AD50), 75:25 (AD75), and 100:0 (AD100). Gas production (GP) was continuously measured by incubating samples in buffered rumen fluid from cannulated sheep and it was recorded at 3, 6, 9, 12, 24, 48 and 72h of incubation. Cumulative GP, kinetics of GP (a, b and c), ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA) concentrations were determined, while metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD), microbial protein (MP) were estimated. The gas volume was increased ($P<0.05$) at 12h with increasing the substitution levels of AD in the feed, the values ranged between 31.3 and 44.7 ml/200 mg DM. However, it was found to be decreased ($P<0.05$) at 48 and 72h with increasing substitution levels of AD. There were no differences in the gas production found at 24h. Data of VFA, NE, OMD and MP were similar ($P>0.05$) among the feeds. The energy (ME; MJ/kg DM) value of AD25 (10.4) and AD50 (10.3) were comparable to that of CG (10.5); however it was reduced ($P<0.05$) with AD75 and AD100. Data demonstrated that, Azzawi date at the level of 50 g/100g of substrate (*i.e.,* AD50) may have similar energy contents as of corn grain, and it can be used as a source of energy in ruminant diets either alone or in combination with corn grain.

Key words: Azzawi date, Corn grain, Gas production, *In vitro*.

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INTRODUCTION

The price of energy supplements has been increased dramatically in the recent years with the increase of demand of feeds for animals. The increase in feed price encouraged nutritionists to search for cheaper high-energy feed ingredients. In Egypt, production of dates (*Phoenix dactylifera*) leaves behind a sizable amount of by-products in the form of discarded dates and date pits, which are not suitable for packing. Azzawi date which produced in Siwa Oasis can be classified by: poorer quality varieties; discarded date; small shriveled fruit; unripe, seedless; un-pollinated fruits of the date and insect or worms infected date. Farmers in this region use Azzawi date to feed their animals intact or after grinding. Azzawi dates can be used as a source of energy to replace part of the corn grain used in the diets of ruminants as main source of energy.

Al-Dabeeb (2005) found that, diets supplemented with up to 20% dates could be used efficiently in feeding Najdi lambs without adverse effects on growth performance and digestion of nutrients. An earlier report by El-Hag *et al*. (1993), indicated that the inclusion of dates at 15% of the whole DM of ration was associated with an increase in growth rate of Awassi lambs.

The *in vitro* gas production (GP) technique may be a good tool in feed evaluation, to determine the rate and extent of DM degradation, and allows for more control of the experimental conditions than *in vivo* experiments. Fermentation of feeds by rumen micro-organisms produce VFA and Microbial protein (MP) and gases (Blümmel *et al*., 1997). Estimation of fermentable energy by the *in vitro* GP technique for a range of feedstuffs incubated alone or in combination has been investigated by several authors (Fakhri *et al*., 1998; Ben Salem *et al*., 2002). Results of GP obtained by simulated diets in calibrated syringes were parallel and matched the results obtained *in vivo* for digestible OM intake and live weight gain (Blümmel and Ørskov, 1993).

The information available about the inclusion of discarded dates in diets of ruminant in Egypt is very scarce. Therefore, the main objective of the present study was to evaluate the effects of substitution the discarded Azzawi date (AD) for corn grains (CG) as a source of energy in sheep diets using *in vitro* GP technique.

MATERIALS AND METHODS

Feed samples

Samples of corn grain and Azzawi date were collected in triplicate from different areas. Samples of Azzawi date, collected from Siwa Oasis, which lies in the western desert of Egypt 65 km of Libyan borders, were mixed with corn grain (in triplicate) by the following proportion : 0:100 (CG), 25:75 (AD25), 50:50 (AD50), 75:25 (AD75), and 100:0 (AD100). Feed samples were ground in mills to pass a 1 mm sieve prior to chemical analysis, *in vitro* GP measurements and rumen fermentation. The chemical composition of the feeds used for *in vitro* GP is presented in Table 1.

Table 1. Chemical composition (g/kg DM) of corn grain (CG) and Azzawi date (AD)

Parameter	Feedstuff		SEM	P *value*
	CG	AD		
Ash	15.8[b]	43.1[a]	4.53	<0.001
Crude protein	85.3[a]	74.2[b]	8.56	0.048
Ether extract	41.6	38.3	5.12	0.082
Nitrogen free extract	831.9[a]	769.2[b]	23.60	<0.001
Neutral detergent fibre	114.1[b]	188.3[a]	11.63	<0.001
Acid detergent fibre	53.6[b]	182.8[a]	10.34	<0.001
Total sugars	82.8[b]	469.5[a]	13.49	<0.001

[a,b]Different superscripts following means in the row indicate differences at P<0.05.

In vitro gas production

In vitro gas production was carried out using the method described by Menke and Steingass (1988). Buffer and mineral solution were prepared and placed in a water bath at 39°C under continuous flushing with CO_2. Both solid and liquid rumen fractions were collected before the morning feeding from three fistulated Barki sheep fed twice daily with diet containing berseem hay (600 g) and concentrate mixture (400 g) ration divided into two equal meals at 8:00 and 16:00 hrs daily. Sheep were supplemented with minerals and had free access to water throughout the experiment. Rumen contents were collected into pre-warmed insulted bottles, pooled among sheep, homogenized in a laboratory blender, filtered through two layers of cheese-cloth and purged with CO_2. The well mixed and CO_2 flushed rumen fluid was added to the buffered rumen fluid solution (1:2 v/v), which was maintained in a water bath at 39°C, and mixed.

Approximately 200 mg DM of finely ground samples were accurately weighed into calibrated glass syringes (100 ml). Buffered rumen fluid (30 ml) was pipetted into each syringe, containing the feed samples, and the syringes were immediately placed into the water bath at 39°C. Three syringes with only buffered rumen fluid were incubated and considered as the blanks. A total of 135 syringes (three syringes of each triplicate sample within each of the five feeds in three runs on different weeks with three syringes as blanks at each run) were incubated for a period of 72h.

The gas production was recorded after 3, 6, 9, 12, 24, 48 and 72h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 200 mg of DM. After 72h of incubation, the contents of the incubation syringes were transferred into pre-weighed 50ml centrifuge tubes, rinsed into the tubes and centrifuged. The supernatant was taken for determination of NH_3-N and volatile fatty acids (VFA). Blank 0h samples were also analyzed for NH_3-N and VFA, and these estimates were used to calculate net NH_3-N and net total VFA concentrations.

The gas production fractions were estimated by fitting the mean gas volumes to the exponential equation of Ørskov and McDonald (1979):

$$\text{Gas (GP)} = a + b\,[1 - e^{-(c \times t)}],$$

where, GP is the gas production (ml/200 mg DM) at time *t*, *a* is the gas production from the immediately soluble fraction, *b* is the gas production from the insoluble fraction, *c* is the gas production rate constant for the insoluble fraction (*b*).

Chemical analysis

Feed samples were analyzed for DM (#934.01), ash (#942.05), N (#954.01), CP (N×6.25, #954.01) and ether extract (#920.39) according to AOAC (1997). The neutral detergent fibre (NDF; Van Soest *et al.*, 1991), acid detergent fibre (ADF; #973.18 of AOAC, 1997) analyses used an ANKOM200 Fibre Analyzer unit (ANKOM Technology Corporation, Macedon, NY, USA). The NDF was assayed without use of α-amylase but with sodium sulfite. Both NDF and ADF are expressed without residual ash. The concentration of NH_3-N in rumen fluid was determined by a Kjeldahl distillation method according to AOAC (1997; #954.01), and ruminal total VFA were determined by steam distillation as described by Warner (1964). Total sugars were measured a colorimetric method of Dubois *et al*. (1959).

Calculations and statistical analysis

The energy values were calculated from the amount of gas produced at 24h of incubation with supplementary analysis of CP, ash and ether extract. This approach was developed by the research group in Hohenheim (Germany) and the ME, OMD and NE values in feeds were calculated using equations of Menke *et al*. (1979) and Menke and Steingass (1988).

$$\text{ME (MJ/kg DM)} = 1.06 + 0.157 \times \text{GP} + 0.084 \times \text{CP} + 0.22 \times \text{EE} - 0.081 \times \text{ash}$$

$$\text{OMD (\%)} = 14.88 + 0.889 \times \text{GP} + 0.45 \times \text{CP} + 0.0651 \times \text{CA}$$

$$\text{NE (MJ/kg DM)} = 0.096 \times \text{GP} + 0.0038 \times \text{CP} + 0.000173 \times \text{EE} + 0.54$$

where, GP is 24-h net gas production (ml/200 mg DM), and CP, EE, ash, ME, OMD, NE are crude protein, ether extract, metabolizable energy, organic matter digestibility and net energy, respectively.

Microbial protein was calculated as 19.3g microbial nitrogen per kg OMD according to Czerkawaski (1986).

Data of *in vitro* ruminal GP, fermentation parameters, and microbial protein syntheses were analyzed as a complete random design using the GLM option of SAS (2002). Data of each one of the three runs within the same feeds sample were averaged. Mean values of each individual sample within each feed sample (three samples of each) were used as the experimental unit. Turkey's test was used for the multiple comparisons among mean values for the feeds samples used.

RESULTS

The ash, NDF, ADF and total sugars contents were higher ($P<0.05$) in Azzawi date as compared to corn grain, while the CP and NFE contents were lower ($P<0.05$) in AD; there was no difference in the fat content between the two feedstuffs (Table 1).

Gas volume was increased ($P<0.05$) at 12, 48 and 72h with increasing substitution levels of AD in the diets (Table 2). Corn grain (CG) increased ($P<0.05$) *b* fraction, while it was lowest ($P<0.001$) with AD100 and their substitutions. In contrast, the intercept *a* fraction (ml/g OM) and rate of fermentation *c* (h^{-1}) were decreased ($P<0.05$) in CG and increased with increased proportion of AD in the diet.

Table 2. *In vitro* gas production at different time of incubation and gas production kinetics of corn grain, and Azzawi date at different substitution levels

Item	Feeds†					SEM	P *value*
	CG	AD25	AD50	AD75	AD100		
Gas production, ml/200 mg DM							
GP_3	5.3^{d}	14.3^{c}	15.0^{c}	25.7^{b}	29.3^{a}	0.56	<0.001
GP_6	15.0^{d}	22.0^{c}	28.0^{b}	38.3^{a}	40.0^{a}	0.53	<0.001
GP_9	21.0^{d}	29.3^{c}	32.3^{b}	41.0^{a}	42.3^{a}	0.54	<0.001
GP_{12}	31.3^{d}	34.3^{c}	40.0^{b}	43.0^{a}	44.7^{a}	0.58	<0.001
GP_{24}	50.3	50.3	50.7	50.0	49.7	0.39	0.485
GP_{48}	68.3^{a}	64.3^{b}	60.7^{bc}	57.0^{cd}	54.3^{d}	0.79	<0.001
GP_{72}	72.0^{a}	68.3^{ab}	65.7^{b}	60.0^{c}	57.7^{c}	0.89	<0.001
Gas production kinetics‡							
a	1.6^{c}	5.5^{b}	6.5^{b}	20.7^{a}	23.8^{a}	7.36	<0.001
b	69.3^{a}	64.7^{b}	57.8^{c}	37.8^{d}	31.9^{e}	1.01	<0.001
c	0.040^{e}	0.060^{d}	0.067^{c}	0.078^{b}	0.091^{a}	0.0002	<0.001

†Contained g/100 g: CG, 100g corn grain; AD25, 25g Azzawi date plus 75g corn grain; AD50, 50g Azzawi date plus 50g corn grain; AD75, 75g Azzawi date plus 25g corn grain; AD, 100g Azzawi date.

‡*a*; soluble fraction (ml/g OM), *b*; insoluble fraction (ml/g OM), *c*; production rate (h^{-1}).

^{abcd}Different superscripts following means in the row indicate differences at $P<0.05$.

Table 3. *In vitro* ruminal fermentation activities, energy contents, organic matter digestibility (OMD) and microbial protein synthesis (MP) of corn grain and Azzawi date at different substitution levels

Item	Feeds†					SEM	P *value*
	CG	AD25	AD50	AD75	AD100		
NH_3-N (mg/dL)	16.38^{a}	12.09^{ab}	9.57^{abc}	7.05 ^{b}	3.76^{c}	0.818	<0.001
VFA (M.eq/dL)	27.0	26.5	26.1	26.6	26.4	1.06	0.982
ME (MJ/kg DM)	10.5^{a}	10.4^{ab}	10.3^{ab}	10.1^{bc}	9.9^{c}	0.06	0.002
NE (MJ/kg DM)	5.41	5.40	5.44	5.37	5.34	0.039	0.485
OMD (%)	63.55	63.47	63.69	63.03	62.66	0.351	0.276
MP (g/kg DM)	76.7	76.6	76.8	76.0	75.6	0.31	0.073

†Contained g/100 g: CG, 100g corn grain; AD25, 25g Azzawi date plus 75g corn grain; AD50, 50g Azzawi date plus 50g corn grain; AD75, 75g Azzawi date plus 25g corn grain; AD, 100g Azzawi date.

^{abc}Different superscripts following means in the row indicate differences at $P<0.05$.

The ruminal NH_3-N concentration was decreased ($P<0.05$) with increasing the substitution levels of AD with CG. Data on VFA, NE, OMD and MP did not differ among the feeds tested. The energy contents (ME and NE) of CG and AD100 feeds and their substitutions levels, were similar with AD50 although the energy contents were decreased with the other AD levels *versus* GC.

DISCUSSION

Compared to the CG control, inclusion of Azzawi date induced higher gas production during the period from 3 to 12h of incubation. However, during the periods from 48 to 72h, CG showed higher GP than AD100. The differences in the GP are related to the proportion of fermentable substrate of feeds and could be correlated to the observed higher sugars contents in AD (Table 1). Higher fermentation rate and GP in AD containing feeds *vs*. CG was probably due to higher sucrose contents in AD; conversely CG which had a higher starch contents that has lower fermentation rate than the sucrose in the rumen (Sniffen *et al*., 1992; Adesogan *et al*., 2005). However, gas is produced mainly when carbohydrates of the feedstuffs are fermented to short chain fatty acids. The rate of GP is associated with the rapid growth phase of microorganisms and in a mixed culture system, the rate of fermentation will be a result of the interactions between the microorganisms present and the manner in which they digest the particular feed within the system (Mauricio *et al*., 2001).

The total GP of CG and AD100 was found to be 50.3 and 49.7 ml/200 mg DM, respectively, at 24h of incubation. These values are apparently lower than those reported for barley (64-71 ml/200 mg DM), wheat (60-73 ml/200mg DM) and corn grains (60-82 ml/200 mg DM) by Getachew *et al*. (2002) and Umacalilar *et al*. (2002). Gas production reflects all nutrients fermented, soluble as well as insoluble; and fractions that are not fermentable do not contribute to GP. The GP is the result of the fermentation of carbohydrates and the amount of gas produced is affected by the rate of carbohydrate fermentation, the molar proportions of the VFA and amount of VFA produced (Dijkstra *et al*., 2005). Differences in parameters '*a*' and '*c*' indicate different fermentation patterns, suggesting that AD is fermented faster, and to a greater extent.

The CP content of CG was higher than AD and our results showed that CG produce higher ($P<0.05$) in NH_3-N *vs*. AD100. This may be explained by the possible difference in the rumen degradability in CP between CG and AD. The increase in NH_3-N concentration suggests that CG had high ruminal-degradable protein and thereby the potential to produce higher levels of microbial protein production. Production of NH_3-N from *in vitro* GP technique reflects a balance between protein degradation and the uptake of NH_3-N for microbial protein synthesis. The principal energy source for the uptake of NH_3-N by microorganisms is carbohydrate; therefore the rate of carbohydrate fermentation had a strong relationship with the rate of rumen protein degradation to NH_3-N and for microbial protein production (Van Soest, 1982).

Thus it is important that the rate of release of NH_3-N matches as closely as possible the release of energy (NRC, 2007). In diets that are rich in protein but low in digestibility, protein degradation rate exceeds carbohydrate fermentation rate and, as energy is the limiting factor for microbial protein synthesis, excess NH_3-N is produced (Andrews *et al*., 1992). The present data of microbial protein was not affected by feeds, being ranged from 75.58 to 76.66 g/kg OMD. This range is higher than that reported in the *in vitro* studies by Van Nevel and Demeyer (1977) which was 18.2-44.6g N/kg OM digested.

The higher GP as well as OMD value of AD feeds could be attributed to relatively low cell wall content as reported by Khan *et al*. (2002). Moreover, the slightly higher GP from fermentation of intact AD feeds compared to CG can be attributed to the presence of higher readily digestible sugars and other short-chain oligosaccharides not present in the extracted fiber. Gas produced is a direct measure of microbial activity and can be a better index of feed ME than an indirect *in vitro* measure based on nutrients fermented. Our values of ME and NE values were lower than that reported by Ismail *et al*. (2005), they calculated ME and net energy for lactation (NE_L) values of corn, wheat, and barley by *in vitro* GP, the values were 13.08, 12.99, and 12.45 MJ/kg DM and 8.30, 8.25, and 7.84 MJ/kg DM, respectively. The ME and NE contents of these feeds were similar to that reported from *in vivo* work of Seker (2002), while NE values were lower than that reported in NRC (2001) tables. Energy values in grains are related to their grain formation, therefore, lower predicted energy value for grains might be attributable to an early stage of harvest (Krishnamoorthy *et al*., 1995).

CONCLUSION

Supplementation of Azzawi date at the level of 50g per 100g of substrate apparently has similar energy content as that of corn grain and it can be used as a source of energy in concentrate diets for ruminants either alone or in combination with corn grain. However, this readily available cheaper feed should perferably be used in arid tropics for reducing feed cost or for feeding during of the season of nutritional shortage or for covering the maintenance requirements for animals.

REFERENCES

Adesogan, A.T., Krueger, N.K. and Kim, S.C. 2005. A novel, wireless, automated system for measuring fermentation gas production kinetics of feeds and its application to feed characterization. *Animal Feed Science and Technology*, 123: 211-223.

Al-Dabeeb, S.N. 2005. Effect of feeding low quality date palm on growth performance and apparent digestion coefficients in fattening Najdi sheep. *Small Ruminant Research*, 57: 37-42.

Andrews, A.H., Blowey, R.W., Boyd, H. and Eddy, R.G. 1992. *Bovine Medicine: Diseases and Husbandry of Cattle*. Blackwell Science, Oxford, pp. 77-78.

AOAC. 1997. *Official Methods of Analysis*, 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Ben Salem, H., Nefzaoui, A. and Ben Salem, L. 2002. Supplementation of *Acacia cyanophylla* Lindl. foliage-based diets with barley or shrubs from arid areas (*Opunita ficus-indica* f. *inermis* and *Atriplex nummularia* L.) on growth and digestibility in lambs. *Animal Feed Science and Technology*, 96: 15-30.

Blümmel, M., Makkar, H.P.S. and Becker, K. 1997. *In vitro* gas production a technique revisited. *Journal of Animal Physiology and Animal Nutrition*, 77: 24-34.

Blümmel, M. and Ørskov, E.R. 1993. Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. *Animal Feed Science and Technology*, 40: 109-119.

Czerkawaski, J.W., 1986. *An Introduction* to *Rumen Studies*. Pergamon Press, Oxford, New York.

Dijkstra, J., Forbes, J.M. and France, J. 2005. *Quantitative Aspects of Ruminant Digestion and Metabolism*. CABI Publishing, Cambridge, MA.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1959. Colorimetric method for the determination of sugars and related substances. *Analytical Chemistry*, 28: 350-356.

El-Hag, G.A., Al-Yousef, Y.M. and Al-Mulhim, F.N. 1993. A study of different proportions of dates in the ration of sheep. In: *Proceedings on the III Symposium on the Date Palm in Saudi Arabia*. Al-Hassa, Kingdom of Saudi Arabia, pp. 343-350.

Fakhri, S., Moss, A. R., Givens, D.I. and Owen, E. 1998. Estimation of fermentable energy by the automatic *in vitro* gas production technique for a selection of feedstuffs incubated alone and in combination. In: *Proceedings of the British Society of Animal Science*. *BASAS Annual Meeting*, March, 1998, Scarborough, p. 69.

Getachew, G., Crovetto, G.M., Fondevila, M., Krishnamoorty, U., Singh, B., Spanghero, M., Steingass, H., Robinson, P.H. and Kailas, M.M. 2002. Laboratory variation of 24h *in vitro* gas production and estimated metabolizable energy values of ruminant feeds. *Animal Feed Science and Technology*, 102: 169-180.

Ha, J.K. and Kennelly, J.J. 1984. Effect of protein on nutrient digestion and milk production by Holstein cows. *Journal of Dairy Science*, 67: 2302.

Ismail, A., Haydar, O., Can, K. and Recep, K.: 2005. Determination of the metabolizable energy (ME) and net energy lactation (NE_L) contents of some feeds in the Marmara region by *in vitro* gas technique. *Turkish Journal of Veterinary and Animal Sciences*, 29: 751-757.

Khan, M.J., Steingass, H. and Drochner, W. 2002. Evaluation of some aquatic plants from Bangladesh through mineral composition, *in vitro* gas production and in situ degradation measurements. *Asian-Australasian Journal of Animal Sciences*, 15: 537-542.

Krishnamoorthy, U., Soller, H., Steingass, H. and Menke, K.H. 1995. Energy and protein evaluation of tropical feedstuffs for whole tract and ruminal digestion by chemical analyses and rumen inoculum studies *in vitro*. *Animal Feed Science and Technology*, 52: 177-188.

Mauricio, R., Owen, E., Mould, F.L., Givens, I., Teodorou, M.K., France, J., Davies, D.R. and Dhanoa, M.S. 2001. Comparison of bovine rumen liquor and bovine faeces as inoculum for an *in vitro* gas production technique for evaluating forages. *Animal Feed Science and Technology*, 89: 33-48.

Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analyses and gas production using rumen fluid. *Animal Research and Development*, 28: 7-12.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz D. and Schneider, W. 1979. The estimation of the digestibility and metabolisable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor. *Journal of Agriculture Science*, Cambridge, 93: 217.

NRC. 2007. *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*. Committee on Nutrient Requirements of Small Ruminants. National Research Council, The National Academies Press, Washington, DC.

NRC. 2001. *Nutrient Requirements of Dairy Cattle*, 7th rev. ed. National Research Council, National Academy Press Washington, D.C.

Newbold, J.R., Baughan, J., Davies, D.R., Theodorou, M.K., 1996. Use of automated pressure evaluation system to measure the fermentable energy content of ruminant feedstuffs: additively of estimates. *Proceedings of the British Society of Animal Science*, p. 221.

Ørskov, E.R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agriculture Science*, Cambridge 92: 499.

Pirt, S.J. 1975. *Principles of Microbe and Cell Cultivation*. Blackwell Scientific Publications, Oxford, UK.

SAS. 2002. *SAS User's Guide: Statistics*. Ver 9.0. SAS Institute. Cary, N.C. USA, 956 pp.

Seker, E. 2002. The determination of the energy values of some ruminant feeds by using digestibility trial and gas test. *Revue de Médecine Vétérinaire*, 153: 323-328.

Sniffen, C.J., O'Connor, J.D., Van Soest, P.J., Fox, D.G. and Russell, J.B. 1992. A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. *Journal of Animal Science*, 70: 3562-3577.

Umacalilar, H.D., Coskun, B. and Gulsen, N. 2002. *In situ* rumen degradation and *in vitro* gas production of some selected grains from Turkey. *Journal of Animal Physiology and Animal Nutrition*, 86: 288-297.

Van Nevel, C.J. and Demeyer, D.I. 1977. Determination of rumen microbial growth *in vitro* from ^{32}P-labelled phosphate incorporation. *British Journal of Nutrition*, 38: 101-114.

Van Soest, P.J., 1982. *Nutritional Ecology of the Ruminant*, 2nd ed. Cornell University Press, New York, pp. 266, 301.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods of dietary fiber, neutral detergent fiber and non-polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74: 3583-3597.

Warner, A.C.I. 1964. Production of volatile fatty acids in the rumen. Methods of measurements. *Nutrition Abstracts and Reviews*, 34: 339-352.