Iranian Journal of Applied Animal Science

Effect of Supplementing Common Reed (*Phragmites australis*) with Urea on Intake, Apparent Digestibility and Blood Metabolites of Baluchi Sheep

Research Article

A. Mokhtarpour[1*] and M. Jahantigh[2]

[1] Research Center for Special Domestic Animals, Research Institute at University of Zabol, Zabol, Iran
[2] Department of Clinical Science, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran

Received on: 7 Mar 2017
Revised on: 21 May 2017
Accepted on: 31 May 2017
Online Published on: Dec 2018

*Correspondence E-mail: am.mokhtarpour@uoz.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

To evaluate the effects of treating or supplementing common reed (*Phragmites australis*) with 2% urea on intake, nutrient apparent digestibility, and blood metabolites, fifteen Baluchi rams (35.4±2.3 kg body weight) were used in a completely randomized design. Treatments were as follows: 1) common reed, 2) common reed supplemented with 2% urea solution at feeding, and 3) common reed treated with 2% urea solution and stored for 21 d. Results showed that crude protein (CP) content increased with addition or treatment of urea (P<0.05). The contents of dry matter (DM) and nutrients were not affected by urea. The CP intake significantly increased in sheep fed urea whereas intake of DM, organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF) and estimated energy intake (MJ/d) were not affected by supplementing with urea. Apparent digestibility of CP increased (P<0.05) in urea fed sheep and also a trend (P<0.10) for more DM and NDF digestibilities was observed in sheep fed urea treated common reed compared to control group. Blood serum urea nitrogen (BUN) concentration was higher in sheep fed urea compared with control (P<0.05), however, the other blood metabolites were not affected by supplementing or treating with urea. It can be concluded that with the exception of slight increase in DM and NDF digestibilities, no significant advantage of treating common reed with urea compared with supplementing with urea was observed. However, compared to untreated and un-supplemented common reed, urea at 2% of DM increased feed efficiency and hence can be used in Baluchi sheep diet.

KEY WORDS common reed, sheep, urea.

INTRODUCTION

Inadequate feeds and improper feeding are two major limiting factors of domestic animals (local breeds) productivity in arid and semi-arid areas. Ruminant livestock in smallholders are mainly dependent on forage crops, crop residues and agro-industrial by-products for survival, growth, meat or milk production and reproduction (Makkar, 2002). Furthermore, quantity and quality of the forages are mostly characterized by the season. As these feeds are usually rich in structural carbohydrates and low in protein content, animal productivity will severely diminish in harsh climates with prolonged summer period and scarce amount of forage resources. Common reed (*Phragmites australis*) is a plant largely distributed in wetlands and drained water found in Sistan region (north part of Sistan and Baluchestan province of Iran). It is a tall (2-4 m) perennial grass with an annual production of about 3 to 30 tonnes per hectare (Köbbing *et al*. 2013). Common reed is widely used as a main constituent of ruminant rations in Sistan. However, consumption of common reed may be restricted by high content of NDF (~70% of DM) (Mashayekhi and Ghorbani, 2004). Similar to other crop residues which are high in fiber and low in protein content, the nutritive value of common reed may be improved by different treating methods including chemical, mechanical and biological treatments (Abebe *et al.* 2004). Among chemical treatments (sodium hydroxide, ammonia, urea), urea is seemed to be the best method for treating low quality forages in smallholders because urea is the most commonly available, easily transportable and is relatively safe to use (Smith, 2002; Abebe *et al.* 2004). Urea is non-protein nitrogen (NPN) compounds which are widely used in ruminant nutrition either as a supplement in concentrate or as liquid for addition to the diets. In ruminants, NPN is utilized by rumen bacteria to produce amino acids and proteins hence provide high-quality animal protein (meat and milk; Bach *et al.* 2005). Therefore, treating reed not only may promote digestion, but also add nitrogen to the residue.

Baluchi sheep are a native meat and milk type breed in Iran, which is well adapted to the arid zone of Sistan. Sheep and goats reared in this region are facing serious nutritional shortages and they depend on low-quality crop residues such as common reed. The aim of this study, therefore, was to evaluate whether common reed can be used as sole roughage in sheep ration and adding or supplementing urea can affect intake, digestion and blood metabolites.

MATERIALS AND METHODS

Animals, experimental diets and management

Fifteen male Baluchi sheep with the average body weight of 35.4 ± 2.3 kg were used in a completely randomized design with three treatments. The experimental period lasted 28 d including 21 d for adaptation and 7 d for measurements and data collection. Animals were kept in individual stalls in a barn protected from rain and wind.

Common reeds are usually harvested by hands or small movers and after feeding to animals, the crop residue containing tall stems and small amount of leaves are collected, stored and then chopped for using in winter. Common reed was chopped in a theoretical length of 3 cm. The diets consisted of untreated and un-supplemented common reed (control), common reed supplemented with urea (USCR), and common reed treated with urea (UTCR). Urea supplementation was achieved by dissolving 20 g urea in 500 mL water per kg common reed DM. Common reed was treated by addition of 20 g urea in 500 mL water per kg DM and stored in plastic bags for 21 d to allow for hydrolysis of urea to ammonia. Feeds were offered daily *ad libitum* and all animals had free access to water.

Measurements

Samples of feeds given to the animals were collected daily, dried in an oven at 60 ˚C to a constant weight and then composited for each treatment. Feed refusals were collected before the morning feeding and weighed daily during the measurement period. Dry matter intake was calculated by difference between total amount of DM offered and refused. Apparent digestibility of OM, CP, NDF and ADF were determined during a measurement period by collecting fecal samples of each animal through the 5 d. Feeds and orts were sampled daily during the collection period. Composite samples of the feeds, feed refusal and feces were dried in an oven, then ground to pass through a 2 mm screen and stored for later analysis. Blood samples were taken from the jugular vein (10 mL) on day 27, just 2 h after the morning feeding, centrifuged, and the serum was recovered and stored at −20 ˚C.

Laboratory analysis

Dry matter content of feeds, orts and feces was determined by drying in an oven at 100 ˚C to a constant weight (AOAC, 2005). Ash (method 942.05) and acid detergent fiber (ADF; method 973.18) were measured according to AOAC (2005). Crude protein (Kjeldahl N×6.25) was determined by the block digestion method using copper catalyst and steam distillation into boric acid (method 2001.11) on 2100 Kjeltec distillation unit as described in AOAC (2005). Neutral detergent fiber (NDF) was determined by Van Soest *et al.* (1991). Both NDF and ADF were expressed exclusive of residual ash. Apparent digestibility of OM, CP, NDF and ADF were determined by measuring their concentrations and the concentrations of acid insoluble ash (AIA) as an internal marker in the feed and fecal samples (Van Kuelen and Young, 1977).

Blood samples were centrifuged at 3000 × g for 10 min, then serum was separated and frozen at −20 ˚C. Serum urea nitrogen, glucose, protein, albumin, creatinine, AST, ALT and ALP were determined using an autoanalyzer (Biosystems A 15; 08030 Barcelona, Spain). Globulin was determined by subtracting albumin from total protein.

Statistical analysis

Data were analyzed using GLM procedure of SAS (2003) as the following model for a complete randomized design:

$$Y_{ij}= \mu + T_i + \varepsilon_{ij}$$

Where:
Y_{ij}: dependent variable.
μ: overall mean.
T_i: effect of treatment (i=1, 2, 3).
ε_{ij}: random residual error.

Least squares means procedure (LSMEANS) was used to test the differences among means if a value of $P < 0.05$ was detected. Trends were discussed at $P < 0.10$.

RESULTS AND DISCUSSION

The chemical composition of untreated, treated and supplemented common reed with urea is presented in Table 1. It has been well documented that treatment of straws and low quality forages with urea can improve their nutritional value (Vadiveloo and Fadel, 2009; Abo-Donia *et al.* 2014). Common reed used in our experiment contained high fiber (74.3% NDF, 50.3% ADF) and low CP (3.8%) which was consistent with Nakhaei-Moghadam and Dehghani (2014) who reported that concentrations of ash, CP, NDF and ADF of common reed collected from Sistan wetlands were 8.7, 2.1, 74.3 and 55.0%, respectively. The content of OM, CP and ADF of common reed harvested from Khuzestan wetlands ranged from 86.0 to 87.5%, 8.5 to 11.6% and 39.0 to 42.7%, respectively (Kardooni and Alemzadeh, 2005). The lower CP and higher ADF contents in our study could be due to higher stem to leaf ratio as we used mostly stalks of common reed. Supplementation with urea had no effect on chemical composition of common reed except CP concentration which was significantly increased compared to control.

Dry matter intake (DMI) as g per day, % body weight (BW) and g per kg of metabolic BW was not significantly affected by treatments, however, DMI (g/d) was increased by 7.7% in sheep fed UTCR compared to those fed control (Table 2). Intake of CP was higher in USCR and UTCR than that of the Control. The amounts of DM, metabolizable energy (ME) and CP intake to meet maintenance requirements of sheep are estimated to be about 2% of BW, 7.11 MJ per d and 66 g per day, respectively (NRC, 1985). However, as it is shown in table 2, DMI, ME and CP intake of sheep ranged from 0.96 to 1.03% BW, 2.52 to 2.85 MJ per d and 13.0 to 32.0 g per day, respectively, which indicated that our treatments could not meet maintenance requirements of sheep. Conversely, Kirby *et al.* (1989) stated that common reed could provide maintenance requirements of local breed animals. In order to estimate DMI, it is assumed that protein requirements are met by the forage or supplementation of protein sources, however when protein requirements are not met, forage intake will be lower than the estimated values (Hibbard and Thrift, 1992). Furthermore, common reed is rich in fiber and low in nitrogen, both of which restrict DMI and there is always negative correlation between DMI and NDF content. In spite of supplementing and treating with urea, it could not compensate nutrients deficiencies. Our results are accordance with Thanh (2012) who found no difference in DMI of cattle and buffalo fed rice straw with addition of 0.5, 1.0 and 2% urea. Compared to control group, an increase in CP intake of sheep fed USCR and UTCR could be simply due to higher CP concentration.

Apparent digestibility of DM and nutrients is given in Table 3. Dry matter and NDF digestibilities had a tendency to increase in sheep fed UTCR compared to other treatments ($P<0.10$). Compared with the control, increases in CP digestibility were 56% and 54% on USCR and UTCR, respectively ($P<0.01$). Several authors declared the priority of urea treatment rather than urea supplementation of crop residues (Tuen *et al.* 1991; Manyuchi *et al.* 1994). The goals of urea treatment are to break down the lignocellulose bonds by ammonia generated from hydrolysis of urea, hence increasing degradability and also to add nitrogen to the residues (Ben Salem and Smith, 2008). Thus, a trend for higher DM and NDF digestibilities of UTCR could be attributed to the combination effect of urea on cell wall structure and also the effect of added nitrogen on rumen bacterial activity as Ben Salem and Smith (2008) stated that fiber ruminal digestion dependent on adequate number of cellulolytic bacteria which need a constant supply of degradable protein.

Therefore, improvement in nutritive value of low quality forages will increase N availability for bacterial growth and consequently increase ruminal digestion. Our finding are consistent with Malekkhahi *et al.* (2014) who reported that treating sesame stover with 3% urea increased DM digestibility in sheep. However, Manyuchi *et al.* (1994) found no effect on DM and OM digestibilities of lambs fed maize stover treated or supplemented with urea.

Serum urea N significantly increased in sheep fed common reed supplemented or treated with urea. Blood metabolites are indicators of animal health status. Blood glucose and BUN levels indicate ruminant nutritional status (Hammond *et al.* 1994). Glucose concentration is affected by energy and protein intakes (Smith *et al.* 2002) and in spite of low energy and protein intakes of sheep, glucose levels were within the normal range (44-81 mg/dL) for sheep (Merck Veterinary Manual, 2009). Tanaka *et al.* (2008) reported that restricted feeding in ewes significantly reduced body weight compared to control feeding without minor or no changes in the concentrations of plasma glucose and total proteins.

The concentrations of BUN were lower than normal values (10-26 mg/dL) reported by Frandson *et al.* (2009) and Merck Veterinary Manual (2009) which revealed CP deficiency of feeds. Urea N concentration is influenced by the nutritional status of the animals and affected by various factors including chemical composition of the diet, dietary CP intake and rumen degradability, CP to rumen fermentable OM ratio, post-ruminal metabolism of protein, endogenous secretion of urea N and function of kidney and liver (Rosler *et al.* 1993; Ndlovu *et al.* 2009). The concentrations of total protein, albumin and globulin were within the normal range.

Table 1 Chemical composition of common reed supplemented or treated with urea (% of DM)

Item	Treatment			SEM	P-value
	CR	USCR	UTCR		
DM	43.80	44.10	45.01	0.317	0.30
OM	91.70	88.50	88.90	0.631	0.10
CP	3.83[c]	9.08[a]	7.36[b]	0.802	< 0.01
NDF	74.25	74.20	71.31	0.751	0.19
ADF	50.26	50.40	49.37	0.524	0.73

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; CR: common reed without supplemented or treated with urea; USCR: common reed supplemented with urea and UTCR: common reed treated with urea.
SEM: standard error of the means.

Table 2 Dry matter and nutrients intake of sheep fed common reed supplemented or treated with urea

Item	Treatment			SEM	P-value
	CR	USCR	UTCR		
DM intake					
g/d	339	352	365	6.48	0.29
% BW	0.96	0.99	1.03	0.019	0.20
g/kg $BW^{0.75}$	23.4	24.3	25.2	0.406	0.12
OM intake (g/d)	311	312	364	5.05	0.57
CP intake (g/d)	13.0[c]	32.0[a]	26.9[b]	2.85	< 0.01
NDF intake (g/d)	252	261	260	3.60	0.59
Estimated energy intake*					
ME (MJ/kg DM)	8.07	8.30	8.78	0.155	0.18
ME (MJ/d)	2.52	2.58	2.85	0.072	0.14

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; CR: common reed without supplemented or treated with urea; USCR: common reed supplemented with urea and UTCR: common reed treated with urea.
* Estimated based on the equation derived from Kearl (1982): 1 kg of digestible organic matter (DOM)= 15.9 MJ ME/kg
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Nutrients apparent digestibility of sheep fed common reed supplemented or treated with urea (%)

Item	Treatment			SEM	P-value
	CR	USCR	UTCR		
DM	52.9	54.3	57.2	1.03	0.09
OM	50.8	52.2	55.2	1.29	0.19
CP	35.2[b]	54.9[a]	54.2[a]	3.27	< 0.01
NDF	48.2	49.4	52.3	0.79	0.07
ADF	43.4	44.9	47.6	0.92	0.18

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; CR: common reed without supplemented or treated with urea; USCR: common reed supplemented with urea and UTCR: common reed treated with urea.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Blood metabolites of sheep fed common reed supplemented or treated with urea

Item	Treatment			SEM	P-value
	CR	USCR	UTCR		
Glucose (mg/dL)	51.9	53.6	54.4	1.04	0.67
BUN (mg/dL)	7.13[b]	9.95[a]	9.61[a]	0.507	0.04
Total protein (g/dL)	6.20	6.42	6.38	0.122	0.85
Albumin (g/dL)	2.69	2.80	2.82	0.037	0.34
Globulin (g/dL)	3.51	3.62	3.56	0.133	0.97
Creatinine (mg/dL)	1.60	1.66	1.65	0.042	0.87
ALT (u/L)	35.8	32.9	32.4	1.14	0.53
AST (u/L)	66.7	62.3	63.6	1.26	0.32
ALP (u/L)	89.0	98.1	96.8	2.98	0.46

BUN: blood serum urea nitrogen; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; CR: common reed without supplemented or treated with urea; USCR: common reed supplemented with urea and UTCR: common reed treated with urea.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Albumin and globulin values are measured to assess long term protein status, as well as the presence of chronic inflammatory disease (Whitaker *et al.* 1999).

Low albumin to globulin ratio in our study may be associated with low protein uptake (Thrall *et al.* 2012). The concentration of creatinine was also within the normal range (0.9-2.0 mg/dL) (Merck Veterinary Manual, 2009) and it can be considered as an indicator of total muscle mass (Myer *et al*. 1996). Aspartate aminotransferase (AST) alanine aminotransferase (ALT) and alkaline phosphatase (ALP) have been used for evaluating bile obstruction and liver damages (Silanikove and Tiomkin, 1992). However, Braun *et al*. (2010) stated that AST, ALT and ALP are poorly specific to the liver whereas glutamate dehydrogenase (GLD) is considered to be liver specific and AST (but not ALT) maybe used when GLD measurement is not available.

CONCLUSION

Our results revealed that treating or supplementing common reed with urea enhanced CP intake and its digestibility. In spite of numerically higher digestion of DM and NDF in urea treated compared to urea supplemented common reed, there was no significant advantage of urea treatment over urea supplementation. Feeding common reed without or with addition or treatment of urea could not met animal requirements and therefore, it is suggested not be used as a sole feed in Baluchi sheep nutrition.

ACKNOWLEDGEMENT

The authors would like to acknowledge Research Institute of University of Zabol for its financial support.

REFERENCES

Abebe G., Merkel R.C., Animut G., Sahlu T. and Goetsch A.L. (2004). Effects of ammoniation of wheat straw and supplementation with soybean meal or broiler litter on feed intake and digestion in yearling Spanish goat wethers. *Small Rumin. Res*. **51,** 37-46.

Abo-Donia F.M., Abdel-Azim S.N., Elghandour M.M.Y., Salem A.Z.M., Buendía G. and Soliman N.A.M. (2014). Feed intake, nutrient digestibility and ruminal fermentation activities in sheep-fed peanut hulls treated with *Trichoderma viride* or urea. *Trop. Anim Health. Prod*. **46,** 221-228.

AOAC. (2005). Official Methods of Analysis. 18th Ed. Association of Official Analytical Chemists, Arlington, Washington, D.C.

Bach A., Calsamiglia S. and Stern M.D. (2005). Nitrogen metabolism in the rumen. *J. Dairy Sci*. **88(1),** 9-21.

Ben Salem H. and Smith T. (2008). Feeding strategies to increase small ruminant production in dry environments. *Small Rumin. Res*. **77,** 174-194.

Braun J.P., Trumel C. and Bézille P. (2010). Clinical biochemistry in sheep: A selected review. *Small Rumin. Res.* **92,** 10-18.

Frandson R.D., Wilke W.L. and Fails A.D. (2009). Anatomy and Physiology of Farm Animals. A John Wiley and Sons, Inc., Hoboken, New Jersey.

Hammond A.C., Kunkle W.E., Genho P.C., Moore S.A., Crosby C.E. and Ramsay K.H. (1994). Use of blood urea nitrogen concentration to determine time and level of protein supplementation in wintering cows. *Prof. Anim. Sci*. **10,** 24-31.

Hibbard C.A. and Thrift T.A. (1992). Supplementation of forage based diets: Are results predictable*? J. Anim. Sci*. **70,** 181-187.

Kardooni A. and Alemzadeh B. (2005). Determination of chemical composition and digestibility coefficients of *Phragmatis australis* with *in vivo* and *in vitro* in Khuzestan province. *Anim. Sci. J. (Pajouhesh and Sazandegi)*. **68,** 24-30.

Kearl L.C. (1982). Nutrient requirements of ruminants in developing countries. MS Thesis. Utah State Univ., Lake, Utah.

Kirby D.R., Green D.M. and Mings T.S. (1989). Nutrient composition of selected emergent macrophytes in northern prairie wetlands. *J. Range. Manage*. **42,** 323-326.

Köbbing J.F., Thevs N. and Zerbe S. (2013). The utilisation of reed (*Phragmites australis*): A review. *Mires and Peat*. **13,** 1-14.

Makkar H.P.S. (2002). Development and field evaluation of animal feed supplementation packages. Proceedings of the final review meeting of an IAEA Technical Co-operation Regional AFRA Project organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

Malekkhahi M. and Mesgaran M.D. (2014). Effect of chemical treatment of sesame stover with NaOH and urea on chemical composition and *in vivo* rumen digestion in sheep. *Not. Sci. Biol*. **6,** 36-40.

Manyuchi B., Mikayiri S. and Smith T. (1994). Effect of treating or supplementing maize stover with urea on its utilization as feed for sheep and cattle. *Anim. Feed Sci. Technol*. **49,** 11-23.

Mashayekhi M.R. and Ghorbani G.R. (2004). Variation of chemical composition and digestibility of common reed forage during growth stage and characteristics of reed forage ensilage. *Anim. Sci. J. (Pajouhesh and Sazandegi)*. **68,** 93-98.

Merck Veterinary Manual. (2009). Serum biochemical reference ranges. WebMD. www.merckvetmanual.com Accessed Mar. 2009.

Myer H.H., Abdulkhaliq A., Davis S.L., Thompson J., Nabioullin R., Wu P. and Forsberg N.E. (1996). Effects of the callipyge phenotype on serum creatinine, total cholesterol, low-density lipoproteins, very-low-density lipoproteins, high-density lipoproteins, and triacylglycerol in growing lambs. *J. Anim. Sci*. **74,** 1548-1552.

Nakhaei Moghadam S.H. and Dehghani M.R. (1392). Nutritional value of Commom reed forage treated with Oyster fungus Pleurotus ostreatus. Pp. in Proc. 1st Nat. Conf. Stable Agric. Nat. Res., Tehran, Iran.

Ndlovu T., Chimonyo M., Okoh A.I., Muchenje V., Dzama K., Dube S. and Raats J.G. (2009). A comparison of nutritionally-related blood metabolites among Nguni, Bonsmara and Angus steers raised on sweetveld. *Vet. J*. **179,** 273-281.

NRC. (1985). Nutrient Requirements of Sheep. 6th Ed. National Academy Press, Washington, DC, USA.

Rosler D.K., Ferguson J.D., Sniffen C.J. and Letrrema J. (1993). Dietary protein degradability effects on plasma and milk urea nitrogen and milk non protein nitrogen in Holstein cows. *J. Dairy Sci*. **16,** 525- 534.

SAS Institute. (2003). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Silanikove N. and Tiomkin D. (1992). Toxicity induced by poultry litter consumption: effect on parameters reflecting liver function in beef cows. *Anim. Prod.* **54,** 203-209.

Smith J.M., Van Amburgh M.E., Diaz M.C., Lucy M.C. and Bauman D.E. (2002). Effect of nutrient intake on the development of the somatotropic axis and its responsiveness to GH in Holstein bull calves. *J. Anim. Sci*. **80,** 28-37.

Smith T. (2002). On-farm treatment of straws and stovers with urea. MS Thesis. University of Reading, Reading, United Kingdom.

Tanaka Y., Mori A., Tazaki H., Imai S., Shiina J., Kusaba A., Ozawa T., Yoshida T., Kimura N., Hayashi T., Kenyon P.R., Blair H. and Arai T. (2008). Plasma metabolite concentrations and hepatic enzyme activities in pregnant Romney ewes with restricted feeding. *Res. Vet. Sci.* **85,** 17-21.

Thrall M.A., Baker C., Campbell T.W., DeNicola D., Martin J.F., Lassen E.D., Rebar A. and Weiser G. (2012). Veterinary Haematology and Clinical Chemistry. Lippincott Williams and Wilkins, Philadelphia, Pennsylvania.

Thanh V.T.K. (2012). The effect on intake digestibility and microbial protein production of adding urea to rice straw for cattle and buffalo calves. *Livest. Sci.* **150,** 111-113.

Tuen A.A., Daham M.M., Young B.A. and Vijchulata P. (1991). Intake and digestibility of ureatreated, urea-supplemented and untreated rice straw by goats. *Anim. Feed Sci. Technol*. **32,** 333-340.

Vadiveloo J. and Fadel J.G. (2009). The response of rice straw varieties to urea treatment. *Anim. Feed Sci. Technol*. **151,** 291-298.

Van Kuelen J. and Young B.A. (1977). Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* **44,** 282-287.

Van Soest P.J., Robertson J.B. and Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci*. **74,** 3583-3597.

Whitaker D.A., Goodger W.J., Garcia M., Perera B.M.A.O. and Wittwer F. (1999). Use of metabolic profiles in dairy cattle in tropical and subtropical countries on smallholder dairy farms. Pp. 87-98 in Proc. Final Res. Co-ordination Meet. Coordinated Res. Project Organ., Vienna, Austria.