Proceedings 5th World Rabbit Congress, 25-30 July 1992, Corvallis – USA, 914-921.

RATE OF PASSAGE OF BLACK LOCUST LEAF AND ALFALFA MEAL IN THE RABBIT GUT

B. R. Luick, A. Ayers and P.R. Cheeke.

Department of Animal Sciences, Oregon State University, Corvallis, OR 97331-6702

Abstract

Total mean retention times (TMRT) of particulate (Cr) and soluble (Co)markers were estimated in twelve New Zealand White rabbits of 1.94 ± 0.091 kg body weight, fed either a 50% alfalfa or 50% black locust (*Robinia psuedoacacia*) based diet. Daily gains were significantly higher in the alfalfa diet relative to black locust (39.8 ± 16.8 vs. 20.6 ± 11.9, respectively). Daily gains were negative when cecotrophy was prevented. However, DM intake and fecal output were not significantly different between diets regardless of whether cecotrophy was prevented. When cecotrophy was prevented, dry matter digestibility was significantly less among black locust fed animals. The time profile of the concentration of markers in the feces had recurrent peaks, the peaks corresponded to cecotropes in animals prevented from cecotrophy. Therefore, the majority of the marker dose within the animals likely resided within the cecum which allowed for extensive recirculation of the marker via cecotrophy. Cecotrophy caused an approximate doubling of TMRT and the TMRT of the soluble marker was approximately double that of the particulate marker. Little differences were noted in TMRT between alfalfa and black locust fed rabbits. Differences in growth performance between alfalfa and black locust fed rabbits were not due to differences in rates of passage indicating causes due to anti-nutritive factors of black locust affecting digestive and/or systemic activities.

Introduction

The use of Black Locust (*Robinia psuedoacacia*) as a forage material is limited by its well documented inhibition of growth in rabbits, sheep and cattle (Kumar and Vaithiyanathan 1990). However, interest in this leguminous tree remains high because of its high nutrient content and ability to thrive in poor soils. Poor performance of livestock fed black locust is thought to result from the high levels of tannin and robin, a lectin, occurring throughout the plant (Cheeke and Shull 1985). Tannins are known to bind with salivary (Robbins et al. 1987) and intestinal proteins (Horigome et al. 1988) resulting in reduction of nitrogen retention. There is additional evidence that tannins are labile within the gut resulting in their absorption with consequent systemic effects (Clausen et al. 1990).

Rates of digestion and passage are interrelated and are influenced by level of intake (Mertens 1987). Reduced dry matter (DM) intake and digestibility have been observed in rabbits fed black locust leaf relative to alfalfa (Raharjo et al. 1990). In animals normally having high gut fill, rate of passage correlates with the level of intake (Warner 1981). This experiment was therefore undertaken to determine relative rates of passage of digesta in rabbits fed black locust or alfalfa based diets. The design included the prevention of cecotrophy to determine if an interrelationship existed between that behavior, the black locust diet and the mean retention time of digesta.

Materials and Methods

Twelve New Zealand White rabbits of 1.94 ± 0.09 kg (mean, SD) body weight were randomly assigned to either an alfalfa or black locust based diet (Table 1). Animals were housed individually in hanging wire cages in a forced draft ventilated structure. Black locust leaf was collected in August in the vicinity of Corvallis, Oregon. Both the black locust and the alfalfa were dried at 60 C for 48 h prior to diet formulation. The black locust was ground in a #4 Wiley mill fitted with a 2 mm screen. Mean particle size for both alfalfa and black locust was 0.4 mm (Lapple 1968). Feed intake was recorded daily and bodyweights were taken at three times in the course of the experiment.

Markers were prepared from destarched (Pond 1986) alfalfa meal and black locust leaf, using the methods of Uden et al. (1980) as described in Luick and Penner (1991). After nine days on the diet, animals were removed from their cages and orally administered a prepared dose of approximately 0.3 g cobalt EDTA (Co EDTA) and 0.6 g chromium mordanted alfalfa or black locust (Cr mordant). Fecal collections were made every two hours for two days, every six hours for an additional day and daily for three more days.

Fecal collections were immediately frozen at -10 C and transferred to -35 C within a week awaiting analysis. The thawed collections were dried at 80 C for at least 12 h, and ground in a Krups model 506 coffee mill. The chromium and cobalt content of the fecal collections were determined on a Perkin-Elmer model 303 atomic absorption spectrometer (Norwalk, CT) using an oxidizing flame. Dry matter oxidation of the 0.5 to 1.0 g analytical subsamples were done as follows. A sample was placed in a heavy wall 100 ml volumetric flask and heated with 10 ml concentrated nitric acid. To the cooled flask was added 5 ml 30% H_2O_2 and heated briefly. An additional 5 ml of H_2O_2 was added and the flask heated until the reaction subsided. The cooled flask was diluted close to the mark, one ml of hexane added, and finally diluted to the mark and mixed.

Table 1. Composition of Diets (%)

Ingredients	Alfalfa	Black Locust
Alfalfa meal	50	--
Black locust leaf	--	50
Ground barley	41.5	41.5
Molasses	5	5
Dicalcium Phosphate	1	1
Trace mineral salt	0.5	0.5
Vegetable oil	2	2
Chemical composition[1]		
Crude protein	17.7	17.0
ADF	19.9	15.3
NDF	36.2	34.2

[1] From Raharjo et al. 1990. J. Appl. Rabbit Res. 13:56-61.

Total mean retention time (TMRT) was calculated as $\Sigma m_i t_i / \Sigma m_i$, where m was mass of marker metal recovered in the ith sample, t hours after administering the dose. Statistical analysis of TMRT was performed by 2x2x2 factorial analysis of variance (diets, markers, collars). The significance of difference between the means of planned comparisons was evaluated by t-tests based on the pooled variance estimate. Comparisons of growth performance was performed by independent t-

tests. Statistical comparisons were not made of growth performance between collared and uncollared animals. Statistical significance was accepted at the 95% confidence level.

Results and Discussion

As shown in Table 2, growth performance was compromised among rabbits fed the black locust diet. Feed intakes and fecal output were not significantly different regardless of the use of the collars. Dry matter digestibility (DMD) did not differ greatly between dietary groups when cecotrophy was not prevented. However, the black locust fed animals fell significantly short of the DMD achieved by the alfalfa fed animals when cecotrophy preventing collars were fitted, which indicated the importance of cecotrophy to the growth performance of these animals. Raharjo et al. (1990) found that DMD was less compromised in alfalfa than black locust fed rabbits regardless of collars.

Table 2. Performance of rabbits fed alfalfa or black locust leaf based diets[1].

	Normal		Collared	
	Alfalfa	Black locust	Alfalfa	Black locust
n	6	6	6	6
Body weight (g)				
Day 0[2]	1938[a] ± 97	1943[a] ± 94	--	--
Day 7	2131 ± 172	1992 ± 70		
Day 13	2370[a] ± 222	2116[b] ± 71	--	--
Day 17	--	--	2330[a] ± 210	2075[b] ± 53
DM[3] intake (g/d)	164 ± 38	154 ± 11	186 ± 39	166 ± 14
DM output (g/d)	76.9 ± 29.9	62.6 ± 22.1	65.8 ± 16	71.9 ± 14
DMD (%)	54.1 ± 9.2	59.3 ± 14.5	64.8[a] ± 3.1	56.7[b] ± 7.3
ADG (g/d)	39.8[a] ± 16.8	20.6[b] ± 11.9	-10.1 ± 33	-10.3 ± 8.2
FER	4.9 ± 2.4	12.9 ± 12.3	--[4]	--[4]

1 Row means bearing different superscripts are significantly different ($p \leq 0.05$) within a treatment (collars). Statistical comparisons were not made between collared and uncollared animals.

2 Animals (day 0) were distributed for equal initial mean body weight. First dosing was day 7 of experiment, animals were fitted with collars and dosed a second time on day 13.

3 DM, dry matter intake and fecal output; DMD, DM digestibility; ADG, average daily gain; FER, feed efficiency ratio, g gain/ g intake.

4 Negative values.

Figure 1 shows a representative plot of the time profile of the natural logarithm of concentration (ug cr/g DM) of the particulate and soluble markers in the feces. Marker rapidly appeared (< 4 h) in the feces indicating a brief transit time for particles in the rabbit. This agrees with the results of Brandt and Thacker (1958) and Sakaguchi et al. (1987). Compared to the soluble marker, the particulate marker had a nonlinear pattern of excretion. As shown in Figure 2, the disturbances were greatly emphasized when cecotrophy was prevented. This indicated that the nonlinearity stemmed from changes in fecal composition associated with the excretion of cecotropes. The peaks

in figure 2 were observations from feces identified as cecotropes or mixed hard and soft feces and were seen to correspond to peak marker concentration levels. Since cecotropes originate from cecal material (Pickard and Stevens 1972) it was hypothesized that the marker dose resided primarily in the cecum and that the rate of marker loss from the animals was determined primarily by loss from the cecum. This point is supported by the clear similarity in slopes between consecutive peaks and consecutive valleys of the time course of the soluble marker excretion. Marker apparently moved continuously from the cecum into materials not associated with cecotropes. Marker loss was therefore primarily a function of hard feces production in normal animals and included cecotropes in the collared animals. Chromium mordants have been found stabile to microbial digestion (Uden et al. 1980). The fecal matter collected either side of the cecotropes was generally of a mixed hard and soft feces. Cecotrope formation appeared to occur about every 12 h. Since the concentration peaks were characteristic of both markers it appeared that considerable particulate marker remained in the cecum. The mean particle size of the diet and marker was 0.4 mm with an upper limit of about 1.2 mm and an undetermined lower limit. The distribution of particle sizes complicated interpretation of the particulate marker excretion although it can be expected that the larger particles would have been lost relatively more rapidly.

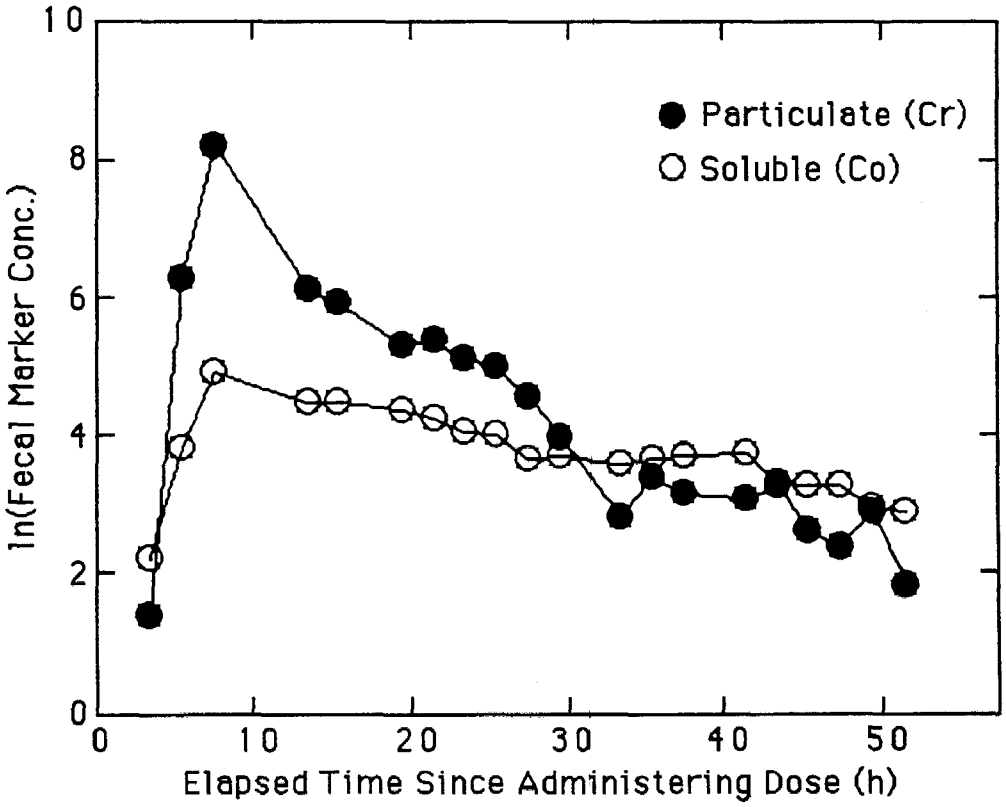

Figure 1. Representative plots of the time profile of the fecal concentration (ug/g DM) of the particulate and soluble markers from one rabbit allowed to practice cecotrophy.

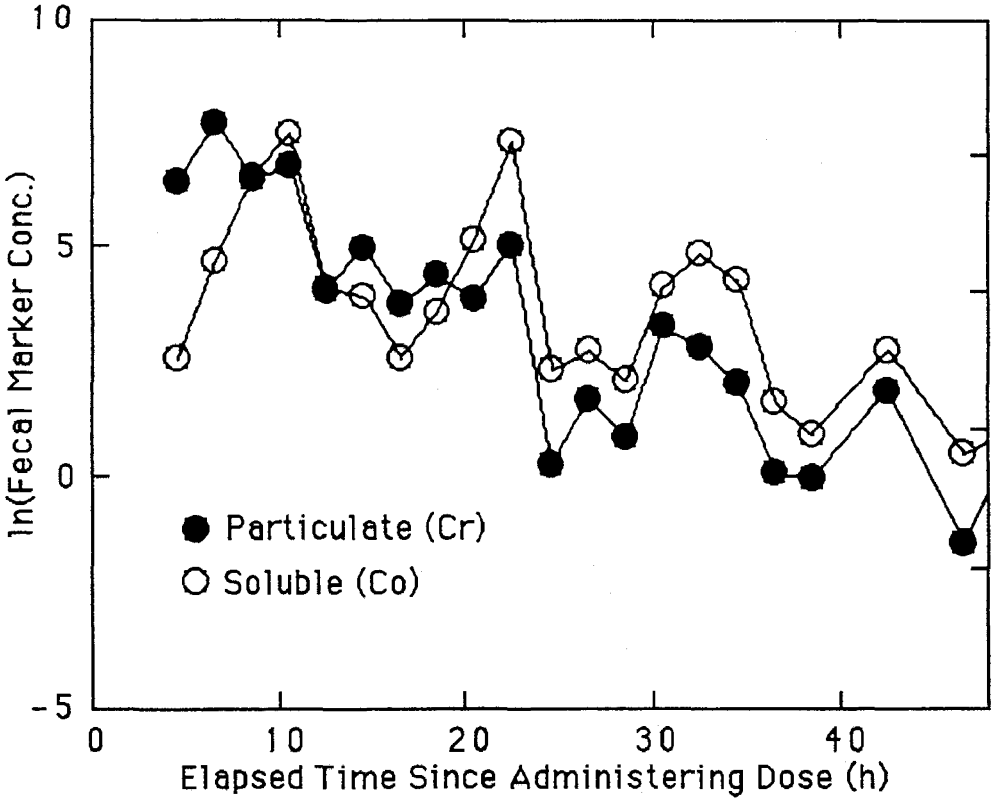

Figure 2. Representative plots of the time profile of the fecal concentration (ug/g DM) of the particulate and soluble markers from one rabbit prevented from cecotrophy.

Table 3. Total mean retention time (TMRT, h) of particulate (Cr mordant) and soluble (Co EDTA) markers in the gastrointestinal tract of rabbits allowed or prevented from cecotrophy (mean ± SD).

Group	Diet[1]	Marker	+/-[2]	TMRT	(h)	N
1	Alfalfa	Cr mordant	+	19.5 ± 6.1	6	
2	Alfalfa	Cr mordant	-	10.0 ± 1.6	6	
3	Alfalfa	Co EDTA	+	44.0 ± 13.0	6	
4	Alfalfa	Co EDTA	-	19.1 ± 3.2	6	
5	Blk Locust	Cr mordant	+	21.3 ± 3.3	6	
6	Blk Locust	Cr mordant	-	10.8 ± 2.9	6	
7	Blk Locust	Co EDTA	+	46.3 ± 11.3	6	
8	Blk Locust	Co EDTA	-	15.9 ± 1.8	6	

Tests of significance[3]

1x5	1x3*	1x2*
2x6	2x4*	3x4*
3x7	5x7*	5x6*
4x8	6x8	7x8

1 Primary ingredient.
2 Cecotrophy allowed (+) or not allowed (-).
3 Significantly different means ($p \leq 0.05$, df = 40, MSE = 45.94)) are indicated with an asterisk.

Table 3 shows the estimated TMRT and the results of t-tests among group means based on the experiment wide variance estimate (MSE). Little difference was detected between alfalfa and black locust retention times either for the particulate (Cr mordant) or soluble (Co EDTA) marker. With one exception, the soluble marker was retained for a significantly longer time than the particulate marker, and prevention of cecotrophy significantly shortened the TMRT of the markers. The exception followed the same trend.

Figure 3 presents the retention times graphically. Within a marker type, the difference in height of like columns is an indication of the increase in TMRT due to cecotrophy. In all cases it is seen that recirculation of the marker approximately doubles the residence time. Between marker types, it is readily seen that the soluble fraction of the diet had approximately twice the residence time of the particulate fraction. This difference is dependent on the mean size of the Cr mordant marker particle.

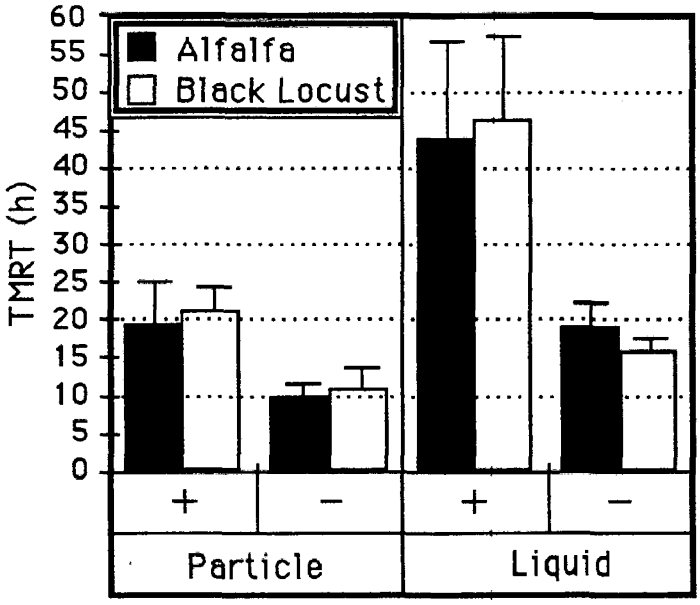

Figure 3. Total mean retention time (TMRT (h), mean + SD) of particulate and soluble digestion markers in the gut of rabbits fed a 54% alfalfa or black locust based diet. TMRT estimated with cecotrophy allowed (+) or prevented (-).

Table 4 shows a comparison of published values for the TMRT of various markers in the gut of rabbits fed several diets. The particular values are dependent on diet and marker particle size, age of the animal, fiber content of the diet, time of dosing relative to cecotrope formation, choice of marker and mathematical calculation of TMRT. Nevertheless, TMRT of particles are on the order of 20 h and approximately half that when cecotrophy is prevented. Our data indicated a TMRT for the liquid marker of about 45 h. The maximum individual observation was 64 h, and the maximum individual observation of TMRT when calculated by compartmental analysis, as done by Sakaguchi et al. (1991), was 83.3 h. However, the average value by compartmental analysis was 41 h, which was close to the present calculations. Their conclusion that agar influenced the soluble fraction of the digesta seems reasonable based on the similarity to published results of TMRT of the particulate but not soluble marker.

Table 4. A comparison of published values of total meant retention time (TMRT) of digesta in rabbits fed various diets (mean ± SD).

Diet[1]	Marker[2]	+/-[3]	TMRT	(h)	n
(Roughage)	Cr_2O_3	+	21.6 ± 1.7	--	Brandt 1958
(Roughage)	Cr_2O_3	-	8.4 ± 0.6	--	Brandt 1958
Barley/Alfalfa	S.P.	+	16.5	5	Fraga 1991
Barley/Alfalfa	S.P.	-	9.6	5	Fraga 1991
Hay/Alfalfa	Yb	+	14.8 ± 2.9	4	Gidenne 1987
Alfalfa	Cr mordant	+	19.7 ± 1.8	3	Gidenne 1989
Alfalfa	Yb	+	22.4 ± 1.8	3	Gidenne 1989
Alfalfa	Yb	+	19.8 ± 1.8	7	Gidenne 1991
Alfalfa	Cr mordant	+	15.7	7	Sakaguchi 1987
Alfalfa/Agar	Cr mordant	+	16.9	4	Sakaguchi 1991
Alfalfa/Agar	Cr mordant	-	13.2	4	Sakaguchi 1991
Alfalfa/Agar	Co EDTA	+	80.0	4	Sakaguchi 1991
Alfalfa/Agar	Co EDTA	-	46.1	4	Sakaguchi 1991

1 Primary ingredient.
2 Cr mordant and Yb are markers of particulate organic matter, Cr_2O_3 is insoluble and Co EDTA is soluble.
3 Cecotrophy allowed (+) or not allowed (-).

It is unlikely that the effects of black locust on digestibility and growth performance can be attributed to differences in TMRT. Consequently, the anti-nutritive properties of black locust seem not to act through changes in rates of passage but rather through inhibition of digestion and possible systemic effects.

Summary

Black locust caused a significant decrease in rate of gain relative to alfalfa. However, DM intake and fecal output was not significantly different between diets regardless of cecotrophy. When cecotrophy was prevented, dry matter digestibility was significantly less among black locust fed animals. The time profile of the concentration of markers in the feces had recurrent peaks, the peaks corresponded to cecotropes in animals prevented from cecotrophy. Therefore, the majority of the marker dose within the animal likely resided within the cecum which allowed for extensive recirculation of the marker via cecotrophy. Cecotrophy caused an approximate doubling of TMRT and the TMRT of the soluble marker was approximately double that of the particulate marker. Little differences were noted in TMRT between alfalfa and black locust fed rabbits.

References

Brandt, C. S. & Thacker, E. J. (1958) A concept of rate of food passage through the gastro-intestinal tract. J. Anim. Sci. 17: 218-223.

Clausen, T.P., Provenza, F.D., Burrit, E.A., Reichardt, P.B. and Bryant, J.P. 1990 Ecological implications of condensed tannin structure: a case study. J. Chem. Ecol. 16: 2381-2392.

Gidenne, T., Carre, B., Segura, M., Lapanouse, A., and Gomez, J. 1991 Fibre digestion and rate of passage in the rabbit: effect of particle size and level of lucerne meal. Anim. Feed Sci. Technol. 32: 215-221.

Gidenne, T., Poncet, C. and Gomez, L. 1987. Addition of a fibre-rich concentrate to a hay-based diet at two levels of feeding to adult rabbits. 1. Mean retention time of digesta. Reprod. Nutr. Dev. 27 (3): 733-743.

Gidenne, T. and Ruckebusch 1989. Flow and passage rate studies at the ileal level in the rabbit. Reprod. Nutr. Dev. 29: 403-412.

Horigome, T., Kumar, R. and Okamoto, K. 1988 Effects of condensed tannins prepared from leaves of fodder plants on digestive enzymes in vitro and in the intestines of rats. Br. J. Nutr. 60: 275-285.

Kumar, R. and Vaithiyanathan, S. 1990 Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Anim. Feed Sci. Technol. 30: 21-38.

Lapple, C.E. 1968 Particle-size analysis and analyzers. Chem. Eng. 75:149-156.

Luick, B. and Penner, M.H. 1991 Nominal response of passage rates to fiber particle size in rats. J. Nutr. 121:1940-1947.

Mertens, D.R. 1987 Predicting intake and digestibility using mathematical models of ruminal function. J. Anim. Sci. 64:1548-1558.

Pickard, D.W. and Stevens, C.E. 1972 Digesta flow through the rabbit large intestine. Am J. Physiol. 222: 1161-1166. Check to see if this talks about bran.

Pond, W. G., Pond, K. R., Ellis, W. C. & Matis, J. H. (1986) Markers for estimating digesta flow in pigs and the effects of dietary fiber. *J. Anim. Sci.* 63: 1140-1149.

Raharjo, Y.C., Cheeke, P.R. and Patton, N.M. 1990 Effect of cecotrophy on the nutrient digestibility of alfalfa and black locust leaves. J. Appl. Rabbit. Res. 13:56-61.

Robbins, C.T., Mole, S., Hagerman, A.E. and Hanley, T.A. 1987 Role of tannins in defending plants against ruminants: reduction in dry matter digestion? Ecology 68(6): 1606-1615.

Sakaguchi, E., Itoh, I., Uchida, S. & Horigome, T. (1987) Comparison of fibre digestion and digesta retention time between rabbits, guinea-pigs, rats and hamsters. Br. J. Nutr . 58: 149-158.

Sakaguchi, E., Kaizu, K. and Nakamichi, M. 1991 Fibre digestion and digesta retention on different physical forms of the feed in the rabbit. Proc. 3rd Internat. Symp. of Herbivores. 43400 UPM, Serdang, Selangor, Malaysia:102.

Uden, P., Colucci, P.E., & Van Soest, P.J. (1980) Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food Agric . 31: 625-632.