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The chemical composition and ruminal degradation of the protein and fibre of tetraploid *Robinia pseudoacacia* harvested at different growth stages*

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ABSTRACT

Samples of leaves, stems and whole plant of tetraploid *Robinia pseudoacacia* harvested at four different growth stages (first rapid growth, slow growth, second rapid growth, and leaf-colour changing) were analysed for chemical composition and *in situ* disappearance of protein and fibre using the nylon bag technique. The crude protein content was the highest in leaves, followed by whole plant, and the lowest in stems, while the opposite trend was found for dry matter, NDF, and ADF. Moreover, the crude protein content of the three plant parts decreased during maturation. Effective degradability of crude protein was higher for stems (519.0 g kg^{-1}) than for whole plant (353.6 g kg^{-1}) and leaves (270.4 g kg^{-1}). Effective degradability of ADF was significantly higher in leaves than in the whole plant and stems. Ruminal disappearance of nutrients in the three plant parts was higher during the first rapid growth stage than at later stages.

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INTRODUCTION

Black locust (*Robinia pseudoacacia* L.) is a multi-purpose tree species suitable for production of timber, fuel wood, land reclamation, beekeeping, and forage (Barrett et al., 1990). Its use for forage is becoming a common practice in China and other parts of the world with a temperate climate due to the high leaf biomass and high nutritive value for livestock when compared with other woody fodder species (Baertsche et al., 1986; Papachristou and Papanastasis, 1994; Papanastasis et al., 1997; Ainalis and Tsiouvaras, 1998; Burner et al., 2005). Moreover, no significant differences have been observed between goats fed *R. pseudoacacia* and at 300-600 g kg^{-1} in terms of average daily gain. Neither were there any significant differences in milk yield and milk quality between dairy cows fed *R. pseudoacacia* and lucerne at 100 g kg^{-1} (unpublished report).

Tetraploid *R. pseudoacacia*, which was colchicine-induced from black locust in South Korea (Kim and Lee, 1973), is considered to be a more promising forage tree than the diploid form (Zhang et al., 2007; Li et al., 2009). Some traits of the tetraploid are twice of those of the diploid (unpublished), such as dry weight of one hundred leaves, leaf area, and leaf thickness, while the dry matter (DM), crude protein (CP), NDF and ADF, Ca, P, and tannin contents in the leaves of 2-year-old trees are similar. In addition, the chemical composition of the tetraploid leaves has been evaluated at three different growth stages, excluding the first rapid growth stage (Li et al., 2006).

Comprehensive research is not available, however, on the chemical composition and ruminal degradation of protein and fibre from different plant parts at different growth stages of tetraploid *R. pseudoacacia*. The objectives of this study were, therefore, to: 1. analyse the chemical composition of different plant parts at different growth stages, 2. evaluate ruminal degradability (disappearance) of protein and fibre of different plant parts of tetraploid *R. pseudoacacia* harvested at different growth stages.

MATERIAL AND METHODS

Three-year-old tetraploid *R. pseudoacacia* clones coppiced at a height of 0.2 m above ground level in March each year were used. The experimental design was a randomized complete block with four growth stages (first rapid growth

stage - June, slow growth stage - July, second rapid growth stage-August, and leaf - colour changing stage - September) as 4 plots in 4 replications of 16-tree plots with buffer rows. Each tree within one plot was cut, then samples of leaves and stems from the same randomized eight trees within one plot, and samples of whole plant from the other randomized eight trees were taken. Samples of stems and whole plant were shredded. Samples collected from the 4 replications within the same stage of growth were mixed together before chemical composition and ruminal disappearance were determined.

In situ procedures

In situ rumen degradation was determined by the nylon bag technique using three mature steers (575±25 kg body weight) as three replications, each with a permanent fistula in the rumen. Steers housed in individual pens with fresh water always available were fed equal amounts of a standardized diet consisting of rice straw (5 kg per day), maize (2 kg per day), wheat bran (0.5 kg per day), soyabean meal (0.5 kg per day), and salt (0.09 kg per day) divided into two parts and fed at 08.00 and 17.00 daily.

Five grams (DM basis) of each plant part (leaves, stems, and whole plant) harvested at leaf-colour changing stage that had been ground after drying (60°C for 48 h) to pass through a 2-mm screen were placed separately in 126 bags (3 plant parts × 3 animals × 2 duplicates × 7 incubation periods), which were anchored with a 50 cm length of braided fishing line. The bags, 17 cm × 13 cm, were made from Dacron cloth with a pore size of 50 μm (Vanzant et al., 1998). All bags for each steer and time period were soaked in warm (39°C) water for 20 min prior to ruminal incubation. Subsequently, all bags (except those at 0 h) were inserted into the ventral rumen of all three steers simultaneously before the morning feeding (08.00) and incubated for 6, 16, 24, 48, 72 and 96 h. The bags were removed from the rumen of steers after incubation and washed in cold tap water until the wash water was clear and colourless.

Ruminal DM, OM, CP, NDF and ADF disappearance data were used to estimate ruminal kinetic parameters (a, b, c) as described by Ørskov and McDonald (1979). Ruminal effective degradability (ED) of nutrients and the rumen bag incubation time T* that would yield the same degradation as ED were also calculated using the equations described by Ørskov and McDonald (1979).

Ruminal disappearance of DM and nutrients of the three plant parts harvested at four growth stages were determined by incubating each 3.5 g ground (2-mm screen) feed (DM basis) in 72 nylon bags (4 dates × 3 plant parts × 3 animals × 2 duplicates), in the rumen of three fistulated steers for 24 h. Another 72 nylon bags were soaked in warm water and washed in cold tap water directly (incubation 0 h). Other procedures and protocol were as described above.

Chemical analysis

Dry matter content was determined by drying duplicate samples from each treatment in a 60°C forced-air oven for 48 h and analysis for OM was carried out on samples according to AOAC (1990). To measure nitrogen content, samples were ground to pass a 1-mm screen and subjected to Kjeldahl N analysis (AOAC, 1990). Samples were also analysed for NDF and ADF by the methods of Van Soest et al. (1991).

Statistical analysis

The ruminal parameters a, b, and c were estimated according to the equation described by Ørskov and McDonald (1979) by means of an interactive least square method applying the NLR (nonlinear regression) procedure in SPSS (version 16.0 for Windows, SPSS Inc., Chicago, IL, USA). In fitting the equation the estimates of a and b were constrained to ensure that their total does not exceed 1000 g kg^{-1}. The parameters a, b, c, potential degradable fraction a + b and ED of different plant parts at leaf-colour changing stage were analysed for a fixed effect of plant part and random effects of each steer and replication with the following model using the GLM procedure in version 16.0 of SPSS as:

$$Y_{ij} = \mu + P_i + A_j + \varepsilon_{j(i)}$$

where: Y_{ij} - the dependent variable under examination, μ - the general mean, P_i - the effect of plant part i (i =1, 2, 3), A_j - the effect of steer (replication) j (j =1, 2, 3) and $\varepsilon_{j(i)}$ - the residual.

Ruminal disappearance after 24 h for nutrients of different plant parts harvested at different growth stages were analysed for fixed effects of date and random effects of steer (replication) using the GLM procedure of SPSS as:

$$Y_{ij} = \mu + D_i + A_j + \varepsilon_{j(i)}$$

where: Y_{ij} - the dependent variable under examination, μ - the general mean, D_i - the effect of date i (i=1, 2, 3, 4), A_j - the effect of steer (replication) j (j=1, 2, 3), $\varepsilon_{j(i)}$ - the residual. Results are as estimated marginal means with their SEM (standard error of means). If differences among treatment means were detected, the Duncan multiple range test was applied to separate means. A significance level of $P<0.05$ was used during analysis.

RESULTS

Chemical composition

Figure 1 shows the dynamics of changes in chemical composition of three plant parts of tetraploid *R. pseudoacacia* harvested at different growth stages. The CP content of these parts decreased rapidly from first rapid growth stage to slow growth stage, after which it declined gradually to leaf-colour changing stage. The CP content of leaves ranged from 242 g kg^{-1} (first rapid growth stage) to 160 g kg^{-1} (leaf-colour changing stage), while the CP content of the whole plant ranged from 212 to 84 g kg^{-1}, and that of stems, from 126 to 69 g kg^{-1}. As expected, the DM, NDF and ADF contents of all three plant parts increased slowly during growth and their concentrations in leaves were the lowest, while those in the stems were the highest. The OM content of the three plant parts did not fluctuate greatly.

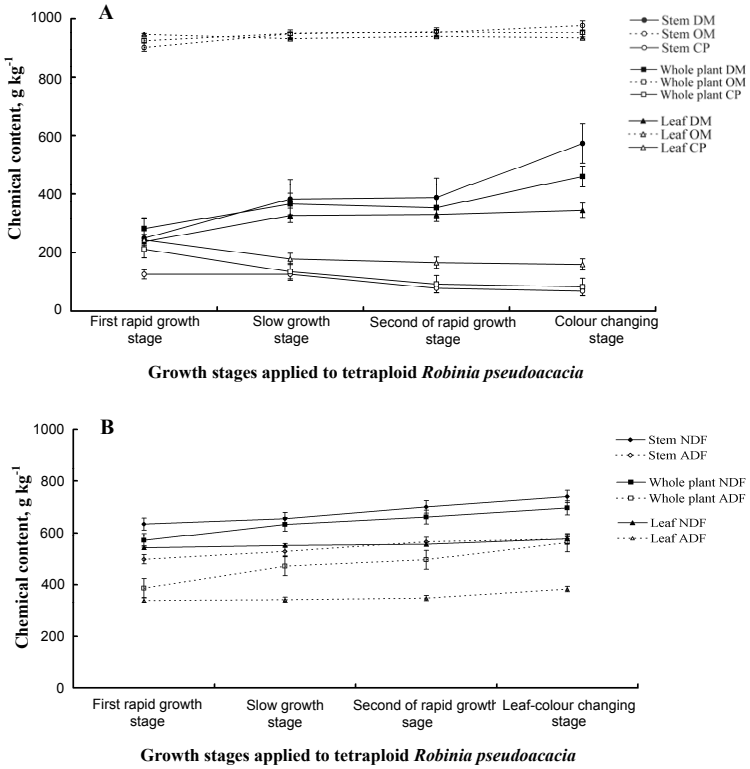

Figure 1. (A) DM, OM and CP contents, (B) NDF and ADF contents in stems, whole plant and leaves of tetraploid *Robinia pseudoacacia* harvested at different growth stages

Ruminal degradation parameters of nutrients of three plant parts

The ruminal degradation of nutrients of three plant parts of tetraploid *R. pseudoacacia* harvested at leaf-colour changing stage is given in Table 1. The soluble fraction (a) of DM and OM differed significantly ($P<0.05$) among the three plant parts, and was the highest in leaves, followed by whole plant, and the lowest in stems. The degradable fraction (b) of DM and OM for leaves was significantly lower, however, compared with whole plant and stems ($P<0.05$), and the degradable fraction (b) of DM and OM for whole plant was also significantly lower than for stems ($P<0.05$). The same result was also found in the degradation

Table 1. Soluble (a) and degradable fraction (b), degradation rate (c), potential degradability (a + b) and *in situ* effective degradability (ED) of dry matter, organic matter, crude protein, neutral detergent fibre and acid detergent fibre in leaves, whole plant and stems of tetraploid *R. pseudoacacia* harvested at leaf-colour changing stage

Nutrient	Material	a	b	c	a + b	ED	T*
		g kg^{-1}		g kg^{-1} h^{-1}	g kg^{-1}		h
Dry matter	Leaves	240.7^{a}	189.7^{c}	58	430.4	373.4	20.6
	Whole plant	188.4^{b}	234.8^{b}	57	423.2	351.9	20.8
	Stems	154.4^{c}	285.2^{a}	67	439.5	362.1	19.4
	SEM	4.1	3.9	2	8.0	8.3	
Organic matter	Leaves	262.0^{a}	182.2^{c}	27^{c}	444.3	357.0	27.0
	Whole plant	159.8^{b}	247.3^{b}	56^{b}	407.2	331.1	20.9
	Stems	111.1^{c}	314.7^{a}	80^{a}	425.7	350.4	18.0
	SEM	3.4	3.2	1	6.6	7.2	
Crude protein	Leaves	211.6	84.5^{c}	58^{b}	296.1^{c}	270.4^{c}	20.7
	Whole plant	235.6	151.2^{b}	89^{a}	386.8^{b}	353.6^{b}	17.0
	Stems	221.9	373.0^{a}	98^{a}	594.8^{a}	519.0^{a}	16.2
	SEM	6.2	5.9	4	12.1	12.3	
NDF	Leaves	131.2^{a}	342.3^{a}	7^{b}	473.5^{a}	204.9	35.0
	Whole plant	0.0^{b}	274.3^{b}	57^{a}	274.3^{b}	191.0	20.8
	Stems	0.0^{b}	276.4^{b}	62^{a}	276.4^{b}	196.8	20.1
	SEM	1.2	4.8	2	5.5	9.4	
ADF	Leaves	233.1^{a}	472.3^{a}	3^{c}	705.3^{a}	285.5^{a}	37.8
	Whole plant	99.4^{b}	144.0^{c}	61^{b}	243.4^{b}	201.7^{b}	20.0
	Stems	0.0^{c}	201.7^{b}	102^{a}	201.7^{c}	162.0^{c}	15.9
	SEM	2.0	8.4	2	9.5	7.2	

means in the same column with different superscripts are significantly different ($P<0.05$); Ruminal parameters a, b and c according to the equation $P = a + b\ (1-\exp^{-ct})$; ED, calculated as $ED=a + bc/(k + c)$, in which ruminal particulate passage rate (k) was 25 g kg^{-1} h^{-1} (Singh et al., 1989); the rumen bag incubation time T* which yields the same degradability as ED, calculated by equation $T^*=1/c\times\ln[(k + c)/k]$

rate (c) of OM. No differences between leaves, whole plant and stems of tetraploid *R. pseudoacacia* were observed in degradation rate (c) of DM, potential degradability (a + b) of DM, and OM, ED of DM and OM.

The degradable fraction (b) of CP for stems (373.0 g kg^{-1}) was significantly higher compared with whole plant (151.2 g kg^{-1}) and leaves (84.5 g kg^{-1}) ($P<0.05$), and the degradable fraction (b) of CP for whole plant was also significantly higher than that for leaves ($P<0.05$). Similar results were observed in the potential degradability (a + b) of CP and ED of CP. No significant difference was observed, however, in the soluble fraction (a) of CP among three plant parts. The rate of degradation (c) of CP was not different between stems and whole plant, but they were both significantly higher than that for leaves ($P<0.05$).

ED of NDF was not different between three plant parts. The soluble fraction (a), degradable fraction (b), and potential degradability (a + b) of NDF were significantly higher ($P<0.05$) for leaves than for both whole plant and stems, and there were no significant differences between these parts. The degradation rate (c) of NDF for leaves was significantly lower, however, compared with both whole plant and stems ($P<0.05$), and no significant difference was found between the last two.

Soluble fraction (a), potential degradability (a + b) and ED of ADF differed significantly ($P<0.05$) among the three plant parts, with the highest in leaves, followed by whole plant, and the lowest in stems. The degradation (c) of ADF differed significantly ($P<0.05$) among three plant parts, with the highest in stems, followed by whole plant, and the lowest in leaves. The degradable fraction (b) of ADF for leaves was significantly higher compared with whole plant and stems ($P<0.05$), and the degradable fraction (b) of ADF for stems was also significantly higher than for the whole plant.

Ruminal disappearance of nutrients of tetraploid R. pseudocacia harvested at four growth stages

Ruminal disappearance of nutrients of tetraploid *R. pseudocacia* harvested at different growth stages at an incubation time of 24 h is presented in Table 2. The choice of 24 h for this experimental incubation time is justified by the finding that rumen incubation for up to 24 h is enough for degradability of tetraploid *R. pseudoacacia* DM and nutrients (almost all of the rumen bag incubation times T* yielding the same degradability as ED in Table 1 were less than 24 h).

Harvest date had an effect on the ruminal disappearance of nutrients in all the three plant parts, with higher values found at the first rapid growth stage than at other stages.

Table 2. Ruminal disappearances at an incubation time of 24 h of DM, OM, CP, NDF and ADF in leaves, whole plant and stems of tetraploid *R. pseudoacacia* harvested at different growth stages

Material	Growth stage	DM	OM	CP	NDF	ADF
		g kg^{-1}	g kg^{-1} DM			
Leaves	First rapid growth	506.1^{a}	518.5^{a}	405.4^{a}	268.5^{a}	298.2^{a}
	Slow growth	418.3^{b}	399.2^{c}	285.9^{b}	188.5^{b}	270.1^{b}
	Second rapid growth	431.2^{b}	426.0^{b}	252.5^{c}	155.7^{c}	253.6^{c}
	Leaf-colour changing	393.4^{c}	368.8^{d}	266.2^{bc}	179.1^{b}	249.1^{c}
	SEM	4.2	6.2	5.4	7.2	6.6
Whole plant	First rapid growth	456.6^{a}	447.7^{a}	423.6^{a}	247.5^{a}	265.3^{a}
	Slow growth	425.4^{b}	415.3^{b}	391.4^{b}	223.4^{b}	248.2^{b}
	Second rapid growth	336.0^{c}	340.2^{c}	352.8^{c}	162.1^{d}	246.6^{b}
	Leaf-colour changing	339.8^{c}	321.2^{d}	340.1^{c}	190.6^{c}	191.0^{c}
	SEM	6.4	7.8	8.0	4.6	5.0
Stems	First rapid growth	410.4^{a}	393.5^{a}	657.8^{a}	237.1^{a}	286.3^{a}
	Slow growth	332.4^{bc}	320.1^{c}	559.9^{b}	199.6^{b}	208.4^{b}
	Second rapid growth	322.1^{c}	289.1^{d}	373.3^{d}	180.1^{c}	197.3^{b}
	Leaf-colour changing	345.8^{b}	344.8^{b}	483.2^{c}	191.9^{bc}	172.3^{c}
	SEM	6.0	5.2	12.7	6.0	2.4

means in same column with different superscripts are significantly different (P<0.05)

In situ 24 h CP disappearance of leaves was significantly higher in the first rapid growth stage compared with the other three stages (P<0.05). *In situ* NDF disappearance of stems and leaves and DM disappearance of stems were also significantly higher in the first rapid growth stage compared with the other three stages (P<0.05). *In situ* whole plant CP disappearance was significantly higher in the first rapid growth stage than in the slow growth stage (P<0.05), and whole plant CP disappearance in these two stages was significantly higher than in both the second rapid growth stage and leaf-colour changing stage (P<0.05). Similar results were also found in *in situ* whole plant DM and leaf NDF disappearance among the four growth stages. *In situ* stem CP disappearance differed significantly (P<0.05) among the four growth stages, with the highest in the first rapid growth stage and the lowest in the second rapid growth stage. Similar results were observed for *in situ* stem OM and whole plant NDF disappearance among the four growth stages. *In situ* disappearance of whole plant and stem ADF was significantly higher in the first rapid growth stage than in the slow growth and the second rapid growth stages (P<0.05), and whole plant and stem ADF disappearance was significantly higher in the slow growth and second rapid growth stages than in the leaf-colour changing stage (P<0.05).

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DISCUSSION

As expected, young leaves and young stems of tetraploid *R. pseudocacia* contained more CP than older parts. A similar pattern of seasonal variation in CP

content was also reported by other researchers (Karachi, 1998; Peiretti and Gai, 2006). DM, NDF and ADF contents increased as plants matured (Papachristou and Papanastasis, 1994; Karachi, 1998; Peiretti and Gai, 2006). These results are both in agreement with the report of Kamalak et al. (2005), who found that NDF and ADF contents increased and CP content decreased with increased maturity. CP in leaves declines with maturity, but leaves always contain a higher protein concentration and less NDF than stems and whole plant.

Nutrients appeared to be equally digested from stems as from leaves, except for ADF and CP. Moreover, CP was easier to digest from stems than from leaves of tetraploid *R. pseudocacia*. These two results confirm the slow degradation of leaf, but also suggest a relatively high nutritive value of the stems, despite their moderate concentration of ADF. In addition, comparison of ED of NDF with ED of OM indicates that ED for cell contents (OM minus NDF) was also greater for stems than for leaves (i.e. ED for stems = 820 g kg^{-1}; ED for leaves = 620 g kg^{-1}). This may be due to the tannin content in the leaves (1.2 g kg^{-1}, unpublished report) being greater than that in the stems (0.7 g kg^{-1}, unpublished report). Some previous studies with black locust indicate that tannins interfere with digestion particularly of leaves (Cheeke, 1992; Ayers et al., 1996), as removal of tannins can increase *in situ* disappearance and supplemental PEG can improve digestibility (indicative of a tannin effect).

In situ nutrient disappearance from leaves and stems were both higher in the first rapid growth stage compared with the three later stages. The decline in the ruminal degradability of DM, CP, NDF and ADF with increasing stage of maturity could be explained by increases in the indigestible fraction of forages. Blade et al. (1993) reported that the stages of maturity affect both degradability and digestion and are associated with an increase in the indigestible fraction of forage, NDF, and an increase in the lignification of NDF. Karachi (1998) observed that the DM of young leaves and young stems was more digestible than in older parts. Burner et al. (2005) also reported that black locust foliar CP and DM degradation decreased as leaves aged. Therefore, this shift in CP degradability with stage of maturity should be taken into consideration when formulating ruminant diets for meeting their requirements for rumen degradable and undegradable protein (Hadjipanayiotou et al., 1996).

CONCLUSIONS

The crude protein of tetraploid *R. pseudocacia* was shown to be easier to digest from stems than from leaves, though its content was the highest in leaves. Except for ADF, nutrients appeared equally digested from stems as from leaves.

Accordingly, tetraploid *R. pseudocacia* stems appear useful as ruminant fodder. Moreover, older leaves and stems of tetraploid *R. pseudocacia* were less digestible than young fractions. Therefore, if economics are not considered, depending on the rate of re-growth, biomass, and nutrition, perhaps multiple harvests at younger stages of growth would be superior to a single harvest late in the year.

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