Journal of Animal and Feed Sciences, 22, 2013, 354–359
The Kielanowski Institute of Animal Physiology and Nutrition, PAS, Jabłonna

The effect of tetraploid *Robinia pseudoacacia* leaf meal on performance, egg quality, and nutrient digestibility in laying hens

G.J. Zhang[1,2], Y. Li[1,4], Y.H. Sun[1], C.Q. Yuan[1] and Z.H. Xu[1,3]

[1] Beijing Forestry University, National Engineering Laboratory for Tree Breeding, College of Biological Sciences and Biotechnology Beijing 100083, P.R. China
[2] Hebei Normal University of Science and Technology, College of Horticulture Science and Technology Qinhuangdao 066600, P.R. China
[3] Beijing Forestry University, College of Forestry, Beijing 100083, P.R. China

KEY WORDS: tetraploid *Robinia pseudoacacia*, laying hen, egg quality, performance, digestibility

Received: 07 November 2012
Revised: 21 August 2013
Accepted: 12 November 2013

[4] Corresponding author: e-mail: yunli@bjfu.edu.cn

ABSTRACT. A 60-day feeding experiment was conducted to study the effects of including 0, 20, 40, or 60 g · kg^{-1} leaf meal of tetraploid *Robinia pseudoacacia* (TRLM) in diets on performance, egg quality, and apparent nutrient and amino acid digestibility of hens. Egg mass and egg weight were both higher when feeding 40 g · kg^{-1} TRLM than the other three diets, the egg shape index linearly increased ($P < 0.05$). No significant effects of diet were seen on the other hen performance or egg quality parameters. Apparent nutrient and amino acid digestibilities increased at first and subsequently decreased with increasing inclusion of the foliage, but no significant differences were observed in dry matter or glycine. Digestibility of crude protein, ether extract, gross energy and acid detergent fibre increased up to 20 g · kg^{-1} TRLM and subsequently decreased, and similar trends were observed for 13 amino acids, but the digestibility of neutral detergent fibre decreased up to 40 g · kg^{-1} TRLM and subsequently increased. A significant linear decrease ($P < 0.05$) in the digestibility of proline was observed with increasing inclusion of TRLM into the diets. Tetraploid *Robinia pseudoacacia* leaf meal could be a potential supplementary protein source in laying hen diets at inclusion levels not exceeding 60 g · kg^{-1}.

Introduction

Black locust (*Robinia pseudoacacia* L.) is a leguminous, deciduous, fast growing tree species native to the southeastern USA. It 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 prominent practice in China and other parts of the world with a temperate climate owing to the superiority of biomass production and high protein content compared with other woody fodder species (Papanastasis et al., 1997; Ainalis and Tsiouvaras, 1998; Burner et al., 2005).

In addition, Takada et al. (1980) compared lucerne meal and black locust meal at the 50 g · kg^{-1} level in poultry diets. There were no differences in body weight or mortality between the two treatments.

When Cheeke et al. (1983) compared lucerne and black locust at a high (200 g · kg^{-1}) level in broiler chicken diets, however, they found a pronounced growth depressing effect of black locust, which was partially overcome by autoclaving (indicative of a tannin effect). Therefore, development of low-tannin cultivars, selected for superior feeding responses, would enhance the prospects of black locust as forage.

Tetraploid *Robinia pseudoacacia*, which was colchicine-induced from black locust (Kim and Lee, 1973), is considered to be a more promising forage tree than diploid *R. pseudoacacia* (Li et al., 2009). The tannin content in leaves ranged from 10 to 20 g · kg^{-1} in tetraploid *R. pseudoacacia* (Zhang et al., 2009) as compared with 65.9 to 104 g · kg^{-1} in diploid *R. pseudoacacia* (Negi et al., 1989; Unruh Snyder et al., 2007). Moreover, the content of crude protein (CP) and 18 amino acids (AA) in tetraploid *R. pseudoacacia* leaves can reach 270 g · kg^{-1} and 180 g · kg^{-1}, respectively, making the tree an excellent fodder for animals (Zhang et al., 2007, 2012). Moreover, no significant differences have been observed among goats fed *R. pseudoacacia* at 300–600 g · kg^{-1} in terms of average daily gain. Neither were there any significant differences in milk yield or milk quality between dairy cows fed *R. pseudoacacia* and lucerne at 100 g · kg^{-1} (unpublished report).

There is no information, however, regarding the effect of tetraploid *Robinia pseudoacacia* leaf meal (TRLM) on monogastric animals. The objective of the present study was to examine the effects of increasing proportions of TRLM in laying hen diets on performance, egg quality, and nutrient and AA digestibility.

Material and methods

Husbandry and diets

A total of two thousand 240-day-old Hy-Line Brown laying hens were housed in cages. Sixteen uniform groups of 125 hens each constituted the experimental units. The trial lasted for 60 d and started after a pre-experimental period of 7 d, during which the birds received the same diet. Diets were fed in mash form. Feed and water were supplied *ad libitum*. The hens received 15 h light per day throughout the experiment. Room temperature was also controlled at about 25°C.

Four replicates were randomly assigned to each of the four dietary treatments, which consisted of 0, 20, 40, or 60 g · kg^{-1} TRLM. The ingredient and chemical compositions of the TRLM and basal diets are shown in Tables 1 and 2.

Table 1. Chemical composition of tetraploid *Robinia pseudocacia* leaf meal in early August, g · kg^{-1}

Nutrient	Content	Nutrient	Content	Nutrient	Content
DM	339.8	Tannin	15.2	Valine	8.7
CP	195.9	Aspartic acid	24.3	Isoleucine	6.5
EE	49.5	Threonine	6.8	Leucine	11.8
CF	164.4	Serine	8.0	Tyrosine	5.3
NDF	471.9	Glutamic acid	13.5	Phenylalanine	8.5
ADF	315.2	Proline	12.6	Histidine	7.7
Calcium	10.7	Glycine	7.5	Lysine	8.4
Phosphorus	1.6	Alanine	8.1	Arginine	9.0

DM – dry matter; CP – crude protein; EE – ether extract; CF – crude fibre; NDF – neutral detergent fibre; ADF – acid detergent fibre

Table 2. Ingredients and nutrient composition of the experimental diets, g · kg^{-1} as fed basis

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
Tetraploid *Robinia pseudocacia* leaf meal	0	20	40	60
Maize	600.0	592.5	583.9	577.9
Wheat bran	30	27	25	19
Soyabean meal	200	192	184	177
Cotton seed meal	50	50	50	50
Limestone	89	87	85	84
Calcium phosphate dibasic	14.0	14.5	15.0	15.0
Salt	3	3	3	3
Vitamin–mineral premix	10	10	10	10
Choline (VB_4 500, g · kg^{-1})	1	1	1	1
Lysine	2	2	2	2
DL–Methionine	1.0	1.0	1.1	1.1
Calculated analysis				
crude protein	166.4	166.4	166.4	166.4
crude fibre	27.9	29.6	31.4	33.0
ether extract	26.8	26.7	26.6	26.5
calcium	35.0	35.0	35.0	35.2
phosphorus	5.9	5.9	6.0	5.9
available phosphorus	3.6	3.6	3.7	3.6
lysine	9.50	9.50	9.51	9.52
methionine	3.63	3.63	3.65	3.61
methionine + cystine	6.61	6.55	6.42	6.33
metabolizable energy, MJ, · kg^{-1}	10.78	10.78	10.78	10.78

composition of vitamin-mineral premix provided per kg: IU: vit. A 2,000,000, vit. D 400,000; vit. E, 4,000; mg: vit. K_3 200, vit. B_1 200, vit. B_2 800, vit. B_6 200, vit. B_{12} 2, D-calcium pantothenic acid 2000, niacin 6000, folic acid 2, biotin 6, iodine 160, selenium 80; g: iron 12, copper 3, manganese 15, zinc 16

Performance and egg quality

Egg production, egg weight, feed consumption and mortality were recorded daily throughout the trial. Twelve eggs per replicate were collected during the last 3 days to determine egg quality traits. Haugh units, yolk colour and albumen height were measured by an egg multitester EMT-5200 (Robotmation, Co., Ltd., Tokyo, Japan). Shell colour and shell thickness were measured by taking the mean of three pieces – from the two ends and from the middle – using a QCR colour reflectometer (Technical Services and Supplies, UK) and a micrometer.

Egg length and width were measured using a calliper and were recorded in centimetres. Egg shape index was defined as the length-to-width ratio. Shell strength was assessed using an robotmation machine (Model-II, Robotmation, Co., Ltd., Tokyo, Japan) and was recorded in kilograms of force required to crack the shell surface (Hammerle, 1969). The feed conversion ratio was calculated as grams feed consumption per day per hen divided by gram egg mass per day per hen.

Nutrient and AA apparent digestibility

During the last 3 days, feed intake was monitored, and the excreta were collected daily at 09.00 h. Care was taken to avoid contamination from feathers, scales and debris. Each day, the excreta were frozen with 10% H_2SO_4 10 ml per 100 g. For each treatment per replicate, the 3 excreta samples from the 3 days were pooled and dried for 24 h at 80°C in a forced-air oven for analysis. There were altogether 16 excreta samples for the 4 replicates, each of which weighed about 300 g. The dried excreta were allowed to equilibrate to atmospheric conditions before being weighed. The apparent digestibility ($g \cdot g^{-1}$) of nutrients and AA was calculated according to the equation:

$$\text{apparent digestibility} = \frac{\text{INT} - \text{FME}}{\text{INT}}$$

where: INT (g per day) – the intake, FME (g per day) – faecal matter excreted.

Chemical analyses

Prior to chemical analysis, samples of feed and dried faecal material were ground in a laboratory sample mill and passed through a 0.25 mm screen. Dry matter (DM ID 934.01), CP (ID 984.13) and ether extract (EE ID 920.39) were subsequently analysed by the procedures of AOAC (2000). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest et al. (1991) and expressed inclusive of residual ash. The gross energy of diet and the excreta samples was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, UK) standardized with benzoic acid. AA concentrations of samples were analysed by an AA analyser (L-8500A, Hitachi, Tokyo, Japan) after acid hydrolysis (6 N HCl for 24 h at 110°C).

Statistical analysis

All data were analysed as a randomized complete block design for a fixed effect of diet and random effect of replicate with the following model using the GLM procedure in version 16.0 of SPSS (SPSS Inc., Chicago, IL, USA) as:

$$Y_{ij} = \mu + R_i + D_j + \varepsilon_{ij}$$

where: Y_{ij} – the dependent variable under examination, μ – the general mean, R_i – the effect of replicate i (i = 1, 2, 3, 4), D_j – the effect of diet j (j = 1, 2, 3, 4), ε_{ij} – the residual.

Results are presented as means with SEM (standard error of means). If differences among treatment means were detected by ANOVA, the Duncan multiple range test was applied to separate means. Contrast statements were utilized to test for linear or quadratic dietary effects. A significance level of $P < 0.05$ was used during analysis.

Results

Egg mass was higher ($P < 0.05$) when feeding 40 $g \cdot kg^{-1}$ TRLM to laying hens than 0 $g \cdot kg^{-1}$ TRLM (Table 3). There were no significant effects of diet on laying rate, feed intake, feed conversion, or mortality rate throughout the experimental period. Neither feed intake nor feed conversion was influenced by TRLM supplementation.

Egg weight was higher ($P \leq 0.05$) in the diet containing 40 $g \cdot kg^{-1}$ TRLM compared with the diets containing 20 $g \cdot kg^{-1}$ and 60 $g \cdot kg^{-1}$ TRLM (Table 4). The egg shape index increased linearly ($P < 0.05$) from 1.28 to 1.32 with increasing TRLM levels. Egg quality parameters such as eggshell colour, eggshell strength, eggshell thickness, albumen height, Haugh unit and yolk colour were not affected, however, by diet.

The apparent digestibility of CP, GE, NDF, and ADF was significantly different ($P < 0.05$) among diets, whereas DM and EE were not affected (Table 5). The apparent digestibility of EE first increased with up to 20 $g \cdot kg^{-1}$ TRLM and then decreased. Similar trends were found in the apparent digestibility of CP, GE and ADF, while the trend for the apparent digestibility of NDF was the opposite, as it decreased up to 40 $g \cdot kg^{-1}$ TRLM and subsequently increased. Feeding 20 g kg^{-1} and 40 $g \cdot kg^{-1}$ TRLM to laying hens resulted in higher ($P < 0.05$) apparent digestibility of CP and ADF as compared with diets containing 0 $g \cdot kg^{-1}$ and 60 $g \cdot kg^{-1}$ TRLM, while the apparent digestibility of GE exhibited a significantly higher value ($P < 0.05$) in the diet containing 20 $g \cdot kg^{-1}$ TRLM than the other three diets.

Except for glycine, the apparent digestibility of all of the other 14 AA was significantly influenced ($P < 0.05$) by the dietary treatments (Table 6). A significant linear decrease ($P < 0.05$) in the apparent digestibility of proline was observed with increasing inclusion

Table 3. Effect of tetraploid *Robinia pseudocacia* leaf meal on performance of laying hens at 240 d of age

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	SEM	Probability		
						main effect	contrasts	
							linear	quadratic
Laying rate, %	85.80	85.74	83.83	84.32	0.76	0.229	0.094	0.727
Egg mass, g · hen^{-1} d^{-1}	57.2^{b}	58.0^{ab}	59.0^{a}	58.2^{ab}	0.42	0.043	0.061	0.096
Feed intake, g · hen^{-1} d^{-1}	128.7	131.5	130.1	129.7	1.90	0.778	0.876	0.429
Feed conversion, g · g^{-1} eggs	2.63	2.65	2.64	2.64	0.02	0.935	0.703	0.802

means within a row with different superscript (a, b) differ significantly, $P < 0.05$

Table 4. Effect of tetraploid *Robinia pseudocacia* leaf meal on egg quality of laying hens at 240 d of age

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	SEM	Probability		
						main effect	contrasts	
							linear	quadratic
Egg weight , g	60.42^{ab}	58.35^{b}	61.50^{a}	58.85^{b}	0.82	0.050	0.681	0.731
Shell colour	30.20	34.06	33.85	33.79	1.38	0.213	0.119	0.187
Shell strength, kg · cm^{-2}	3.933	3.984	4.338	4.102	0.184	0.453	0.323	0.455
Shell thickness, mm	0.352	0.347	0.354	0.357	0.006	0.706	0.455	0.470
Albumen height, mm	7.36	7.20	6.76	7.10	0.18	0.180	0.157	0.197
Haugh units	85.50	85.18	81.95	84.27	1.05	0.143	0.175	0.241
Yolk colour	7.92	7.42	7.92	7.63	0.24	0.436	0.739	0.679
Egg shape index	1.28	1.30	1.31	1.32	0.01	0.194	0.045	0.716

means within a row with different superscript (a, b) differ significantly, $P < 0.05$

Table 5. Effect of tetraploid *Robinia pseudocacia* leaf meal on apparent nutrient digestibility of laying hens at 240 d of age, g · g^{-1}

Nutrient	Diet 1	Diet 2	Diet 3	Diet 4	SEM	Probability		
						main effect	contrasts	
							linear	quadratic
DM	0.697	0.720	0.687	0.681	0.010	0.104	0.118	0.192
CP	0.431^{b}	0.626^{a}	0.565^{a}	0.449^{b}	0.028	0.002	0.956	< 0.001
EE	0.619	0.713	0.656	0.579	0.037	0.141	0.310	0.048
GE	0.736^{b}	0.768^{a}	0.732^{b}	0.716^{b}	0.008	0.005	0.017	0.010
NDF	0.668^{a}	0.625^{a}	0.529^{b}	0.620^{a}	0.018	0.003	0.015	0.005
ADF	0.446^{b}	0.661^{a}	0.652^{a}	0.449^{b}	0.023	< 0.001	0.997	< 0.001

means within a row with different superscript (a, b) differ significantly, $P < 0.05$; DM – dry matter; CP – crude protein; EE – ether extract; GE – gross energy; NDF – neutral detergent fibre; ADF – acid detergent fibre

Table 6. Effect of tetraploid *Robinia pseudocacia* leaf on apparent amino acid digestibility of laying hens at 240 d of age, g · g^{-1}

Amino acid	Diet 1	Diet 2	Diet 3	Diet 4	SEM	Probability		
						main effect	contrasts	
							linear	quadratic
Lysine	0.786^{bc}	0.822^{a}	0.814^{ab}	0.756^{c}	0.010	0.004	0.052	0.001
Threonine	0.805^{ab}	0.826^{a}	0.796^{b}	0.745^{c}	0.008	< 0.001	< 0.001	0.002
Histidine	0.742^{a}	0.764^{a}	0.731^{a}	0.675^{b}	0.011	0.002	0.001	0.007
Leucine	0.863^{a}	0.877^{a}	0.862^{a}	0.821^{b}	0.007	0.001	0.001	0.003
Isoleucine	0.830^{a}	0.851^{a}	0.825^{a}	0.762^{b}	0.010	0.001	0.001	0.002
Phenylalanine	0.873^{a}	0.885^{a}	0.869^{a}	0.835^{b}	0.005	< 0.001	< 0.001	0.002
Arginine	0.916^{a}	0.919^{a}	0.898^{a}	0.861^{b}	0.007	0.001	< 0.001	0.016
Valine	0.814^{a}	0.835^{a}	0.809^{a}	0.761^{b}	0.011	0.007	0.005	0.013
Glycine	0.461	0.418	0.489	0.515	0.027	0.143	0.089	0.235
Aspartic acid	0.845^{b}	0.867^{a}	0.843^{b}	0.804^{c}	0.007	0.001	0.001	0.002
Serine	0.848^{ab}	0.859^{a}	0.836^{b}	0.804^{c}	0.006	0.001	< 0.001	0.008
Glutamic acid	0.898^{ab}	0.908^{a}	0.890^{b}	0.855^{c}	0.005	< 0.001	< 0.001	0.001
Alanine	0.793^{bc}	0.827^{a}	0.813^{ab}	0.770^{c}	0.010	0.015	0.097	0.004
Tyrosine	0.840^{a}	0.849^{a}	0.839^{a}	0.808^{b}	0.008	0.028	0.018	0.034
Proline	0.858^{a}	0.840^{a}	0.841^{a}	0.778^{b}	0.011	0.003	0.001	0.075

means within a row with different superscript (a–c) differ significantly, $P < 0.05$

of TRLM into the diets, whereas the apparent digestibility of the remaining 13 AA first increased up to 20 g · kg^{-1} TRLM, and subsequently decreased. Moreover, there were no significant differences between the diets containing 0 g · kg^{-1}, 20 g · kg^{-1}, or 40 g · kg^{-1} TRLM in the apparent digestibilities of histidine, leucine, isoleucine, phenylalanine, arginine, valine, tyrosine, and proline, but they were significantly higher ($P < 0.05$) than in the diet containing 60 g · kg^{-1} TRLM. In addition, the apparent digestibility of aspartic acid was higher ($P < 0.05$) in the diet containing 20 g · kg^{-1} TRLM compared with the diets containing 0 g · kg^{-1} and 40 g · kg^{-1} TRLM; in both of these diets, the apparent digestibility of this amino acid was higher ($P < 0.05$) than in the diet containing 60 g · kg^{-1} TRLM.

Discussion

Egg mass and egg weight were both higher when feeding 40 g · kg^{-1} TRLM to laying hens than the other three diets. Yang et al. (1992) found that substituting from 30 g · kg^{-1} to 50 g · kg^{-1} black locust leaf meal for wheat bran would enhance egg mass and egg weight. Dancea et al. (2005) also found that combined feed containing 63 g · kg^{-1} black locust leaf meal positively influenced egg production and egg weight in layers. Furthermore, there were no significant differences between the diets containing 40 to 50 g · kg^{-1} black locust leaf meal and lucerne in egg weight, body weight and mortality (Takada et al., 1980; Wu and Han, 1985).

No significant effect of TRLM supplementation on laying rate, feed intake, feed conversion, or mortality rate was found throughout the experimental period. Moreover, egg quality parameters such as eggshell colour, eggshell strength, eggshell thickness, albumen height, Haugh units and yolk colour were not affected by the diet. These findings showed that the inclusion of TRLM did not constitute any physically visible health hazards. Ran et al. (1996) reported, however, that feeding 30 g · kg^{-1} to 50 g · kg^{-1} black locust leaf meal to 40 weeks age of Roman hens increased laying rate, feed conversion rate, and economic benefits. Wang and Yin (1992) found that substituting 50 g · kg^{-1} black locust leaf meal for wheat bran could enhance the laying rate by 9.76%. Dancea et al. (2005) also reported that combined feed containing 63 g · kg^{-1} black locust leaf meal positively influenced health and egg yolk colour in layers, and their metabolism was also much more uniform. Paterson et al. (2000) and Yang et al. (1992) both suggested that the use of high-protein tree fodder in poultry rations might reduce feeding costs and provide sufficient pigment to make the eggs more attractive and nutritious.

The apparent digestibility of CP, EE, GE, ADF and 13 AA (except glycine and proline) first increased with up to 20 g · kg^{-1} TRLM and then decreased with increasing TRLM. To the authors' knowledge, no comparable published data on the influence of black locust leaf meal on nutrient and AA digestibility of a laying hen diet are available. Cheeke et al. (1983) did, however, find pronounced growth depressing effect of black locust at a high (200 g · kg^{-1}) level in broiler chicken diets. Tannins and other phenolics are the likely cause of the low nutrient availability (Cheeke, 1992). Apart from tannins, D'Mello (1992) postulated that the poor digestibility and availability of nutrients resulting from the presence of a component with a high fibre content could explain the poor performance of poultry fed on leguminous leaf meals. These results suggest that TRLM might depress the digestibility of both protein and organic matter in poultry, and may only be useful as a feed supplement in laying hens at low levels of supplementation, particularly at inclusions not exceeding 60 g · kg^{-1}. Although tetraploid *Robinia pseudoacacia* has tannins and fibre that reduce nutrient digestibility, wildlife and livestock will consume it, especially as a source of nutrients for a ruminant species well adapted to highly fibrous ingredients.

Conclusions

Tetraploid *Robinia pseudoacacia* leaf meal (TRLM) shows promise as an alternative diet for laying hens at 20 g · kg^{-1} – 40 g · kg^{-1} without any adverse effects on performance, egg quality, apparent nutrient and amino acid (AA) digestibilities. Although 20 g · kg^{-1} TRLM addition to diets had no significant effect on hen performance or egg quality, apparent nutrient and AA digestibilities were improved.

Acknowledgements

The authors thank Harry Wu and Miloš Ivković of CSIRO Plant Industry in Australia for revising this manuscript. We also acknowledge financial support from the following programmes: Forestry Public Benefit Research Programme (No.201004008), National Key Project of Scientific and Technical Supporting Project (No. 2012BAD01B0601), National High-tech R&D Programme (863 No. 2007AA100105).

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