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EFFECT ON WEANLING RABBITS OF THE ADDITION OF POLYETHYLENE GLYCOL, PHYTASE, METHIONINE AND CHOLINE CHLORIDE TO DIETS CONTAINING BLACK LOCUST (*ROBINIA PSEUDOACACIA*) LEAF MEAL

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Abstract

Forty New Zealand White weanling rabbits were fed diets containing 50% alfalfa (control), 25% alfalfa and 25% black locust leaf meal (BLM) (BLM control), BLM + 1% polyethylene glycol (PEG), BLM + 1% phytase, and BLM + 0.3% L-methionine and 0.3% choline chloride. The digestibilities of dry matter (DM), crude protein (CP), ADF, NDF, ash, calcium and phosphorous were significantly higher for the 50% alfalfa control than for the BLM treatments (respective p values < .01, .01, .01, .01, .01, .01, .01). The average daily gain was higher for the control (p < .01) and feed/gain was lower (p < .01) than for the BLM treatments. The lower utilization of nutrients in the BLM diets is probably due to the presence of condensed tannins. The addition of PEG increased CP (p < .01) and ADF (p < .03) digestibilities compared to the BLM control. The addition of L-methionine and choline chloride increased ADF digestibility (p < .02) compared to the BLM control. It was concluded that PEG is partially effective in alleviating the reduced nutrient availability due to tannins.

Introduction

Black locust (*Robinia pseudoacacia*) is a fast-growing, leguminous tree; its leaves can be harvested for animal feed. In China, the trees are grown to provide leaves as a principle source of rabbit feed (Cheeke and Patton, 1987) and as a source of pigmenting agents for poultry feed (Takada et al., 1980). Black locust (BL) is intensively cultivated in Hungary for sawlogs (particularly a variety called "shipmast locust"), poles and posts, beekeeping and decorative planting (Keresztesi, 1983).

Black locust is indigenous to the eastern U.S. It has a rapid growth rate which can average 2 feet per year, and the tree can reach a height of 70 feet. It is very adaptable to soils and climate, having the ability to grow in any type of soil except permanently wet soil and to fix atmospheric nitrogen (Dirr, 1990).

Although BL leaves are similiar to alfalfa meal in nutrient composition (Cheeke, 1992), feeding trials with BL leaf meal have generally given poor animal performance (Horton and Christensen, 1981, Harris et al., 1984, Raharjo et al., 1990, Takada et al., 1980).

Black locust leaves contain the anti-nutritive factors robin and tannins. Robin is a lectin that may cause adverse effects on animals by binding to the intestinal wall and is suspected of poisoning horses when they eat the bark (Cheeke and Shull, 1985). Tannins are phenolic substances which bind dietary proteins and digestive enzymes and reduce nutrient digestibility (Cheeke and Shull, 1985). Tannins are responsible for the low protein digestibility of BL leaves (Horigome et al., 1984).

Various methods of detoxifying tannins have been demonstrated. Dietary supplementation with polyethylene glycol (PEG) reduces adverse effects because tannins bind more strongly to PEG than to proteins (Oh et al., 1980). Methionine and choline chloride may act as methyl donors to facilitate the excretion of absorbed phenolic acids (Cheeke and Shull, 1985). Sulfur-containing amino acids counteract adverse effects of tannic acid in chickens (Fuller et al., 1967).

Tree leaves are often low in phosphorous (Kumar and Vaithiyanathan, 1990). Phosphorous was found to be poorly utilized by lambs fed BL leaves as a sole feed (Horton and Christensen, 1981). A phytase-rich diet has been shown to increase P availability in swine (Pointillart, 1991).

The objectives of this study were to determine the effects of adding PEG, phytase, and methionine and choline chloride to diets containing BL leaves on nutrient digestibility and growth performance in weanling rabbits. A diet with an equal amount of alfalfa meal in place of BL served as the control.

Materials and Methods

Forty New Zealand White weanling rabbits aged 5 to 6 weeks old were randomly assigned to 5 treatment groups:
1) 50% alfalfa (control)
2) 25% alfalfa and 25% black locust leaf meal (BLM) (BLM control)
3) 25% alfalfa and 25% BLM + 1% PEG[1]
4) 25% alfalfa and 25% BLM + 1% phytase[2]
5) 25% alfalfa and 25% BLM + 0.3% L-methionine[3] and 0.3% choline chloride[4].

The trial consisted of a 10-day adaptation period followed by a 10-day digestibility period. Rabbits were housed in individual cages with automatic water valves. Feed intake was measured throughout the trial and during the digestibility period. Feed intake was adjusted by collecting and measuring wasted feed which fell from the feeder. Initial, weekly and

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[1]Sigma Chemical Co., St. Louis, MO. Average molecular weight:3350.

[2]Alltech Biotechnology, Inc., Nicholasville, KY

[3]U.S. Biochem. Corp., Cleveland, OH

[4]U.S. Biochem. Corp., Cleveland, OH

final weights were taken, along with initial and final weights for the digestibility period. Total fecal output for the digestibility period was collected with screens suspended underneath each cage.

Animals were fed the diets *ad libitum* (Table 1). The BL leaves were hand-harvested from mature trees felled on a local farm in July 1990. The branches and leaves were air-dried on screens. The leaves were stripped from branches and put through a shredder. The leaves were dried in an oven at 60° C for 48 hours and then ground through a 2mm screen.

After the end of the digestibility period, the total wet feces were weighed and a subsample taken which was dried in an oven at 60° c for 72 hours. The dry subsample was weighed and ground through a 1mm screen. Feed and feces samples were analyzed for dry matter and ash by standard procedures (AOAC, 1984). Samples were analyzed for ADF and NDF as described by Goering and Van Soest (1970), modified by a micro method (Waldern, 1971). Crude protein was determined by the Macro Kjeldahl method (AOAC 1984). Ca was measured by atomic absorption spectrophotometry (AOAC 1984) and P was measured on a spectrophotometer by the vanadomolybdate method (AOAC 1984).

The data were analyzed following the general linear model procedure (SAS, 1985). Initial F-screening was done at the .05 level. Treatment means were compared using a series of orthogonal contrasts from 4 pre-planned comparisons.

Results and Discussion

The digestibility and growth performance data are given in Table 2 and statistical comparisons in Table 3.

In Comparison 1, the digestibilities of dry matter (DM), crude protein (CP), ADF, NDF, ash, Ca and P were significantly higher for the alfalfa control than for the treatments with the BLM (respective p values < .01, .01, .01, .01, .01, .01, .01). The overall model for average daily gain (ADG) approaches significance with the F-screening ($p < .09$), but when examining Comparison 1, it is evident that the ADG was significantly higher ($p < .01$) for the alfalfa control than for the BLM treatments. Feed efficiency was lower for the alfalfa control than for the BLM treatments ($p < .01$).

Comparison 2 (BLM control vs. BLM + PEG) showed significantly higher crude protein ($p < .01$) and ADF digestibilities when PEG was added to the diet ($p < .03$).

Comparison 3 (BLM control vs. BLM + phytase) showed a slight, although not significant, increase in digestibility of dry matter when phytase was added to the diet ($p < .07$).

In Comparison 4 (BLM control vs. BLM + L-methionine and choline chloride), the addition of L-methionine and choline chloride statistically increased ADF digestibility ($p < .02$) and gave a slight, although not significant, increase of P digestibility ($p < .07$).

The diets containing BLM were more poorly digested than the alfalfa control. This is most likely due to the presence of

TABLE 1. DIET COMPOSITION AND CHEMICAL ANALYSIS (%).

Diet #	1	2	3	4	5
Alfalfa meal, sun-cured	50	25	25	25	25
Black locust leaves	-	25	25	25	25
Ground barley	42.5	42.5	41.5	41.5	41.5
Molasses	5	5	5	5	5
Trace mineral salt*	0.25	0.25	0.25	0.25	0.25
Vegetable oil	2	2	2	2	2
Vitamin mix**	0.25	0.25	0.25	0.25	0.25
Choline chloride	-	-	-	-	0.3
L-Methionine	-	-	-	-	0.3
Phytase	-	-	-	1	-
Polyethylene glycol	-	-	1	-	-
Chemical Analysis:					
Crude protein	15.53	15.7	15.6	15.9	16.31
ADF	18.53	18.34	18.85	18.49	18.86
NDF	30.32	30.27	30.11	29.55	29.80
Ash	7.02	7.44	7.68	7.84	7.67
Ca	0.98	1.49	1.61	1.43	1.64
P	0.33	0.27	0.25	0.28	0.30

*Ingredients: sodium chloride, zinc oxide, manganous oxide, iron carbonate, copper oxide

**Contains:		
	Vitamin A	3,000,000 IU
	D-3	1,000,000 IU
	E	1000 IU
	K	0.5 gm
	B-12	5 mg
	Riboflavin	3 gm
	Pantothenic acid	5 gm
	Niacin	20 gm
	Choline chloride	200 gm
	Folic acid	200 gm
	Ethoxyquin	56.75 gm

* and ** manufactured by Inman & Co., Inc.; 12530 SE Jennifer St., Clackamas, OR 97015

tannins in the BL leaves. Tannins may be classified as condensed or hydrolyzable tannins (Salunkhe et al., 1990). Black locust leaves contain primarily condensed tannins (Kumar and Horigome, 1986). Tannins precipitate proteins by hydrogen-bonding and hydrophobic interactions (Haslam, 1974). Kumar and Horigome

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TABLE 2. MEANS OF DIGESTIBILITIES (%) OF NUTRIENTS AND GROWTH INDICATORS OF WEANLING RABBITS FED DIETS CONTAINING ALFALFA AND BLACK LOCUST LEAF MEAL (BLM) MODIFIED BY VARIOUS TREATMENTS.

Trt	CP	ADF	NDF	DM	Ash	Ca	P	ADG	DM Intake	Feed/ Gain
1. 50% alfalfa (Control)	77.07 ±1.34	15.81 ±1.79	25.78 ±1.20	68.80 ±0.85	75.26 ±1.34	84.51 ±1.58	70.33 ±2.56	37.0 ±1.7	95.9 ±3.2	2.9 ±0.1
2. 25% alfalfa & 25% BLM (BLM Control)	58.80 ±1.34	2.63 ±1.79	19.79 ±1.20	62.32 ±0.85	66.32 ±1.34	75.62 ±1.58	58.48 ±2.39	30.4 ±1.7	93.0 ±3.2	3.4 ±0.1
3. BLM Control + PEG	66.30 ±1.34	8.55 ±1.79	18.38 ±1.20	63.64 ±0.85	68.13 ±1.34	75.33 ±1.58	61.60 ±2.39	32.4 ±1.7	100.1 ±3.2	3.5 ±0.1
4. BLM Control + phytase	60.06 ±1.34	6.91 ±1.79	18.94 ±1.20	64.50 ±0.85	68.67 ±1.34	73.72 ±1.58	63.65 ±2.39	31.4 ±1.7	91.2 ±3.2	3.2 ±0.1
5. BLM Control + L-methio-nine & choline chloride	57.77 ±1.34	8.81 ±1.79	17.14 ±1.20	62.43 ±0.85	67.90 ±1.34	74.58 ±1.58	64.83 ±2.39	33.3 ±1.7	94.4 ±3.2	3.2 ±0.1
Average of 2, 3, 4 and 5	60.73 ±1.34	6.73 ±1.79	18.56 ±1.20	63.22 ±0.85	67.76 ±1.34	74.81 ±1.58	62.14 ±2.39	31.9 ±1.7	94.7 ±3.2	3.3 ±0.1

TABLE 3. STATISTICAL COMPARISONS BETWEEN NUTRIENT DIGESTIBILITY MEANS (%) AND GROWTH INDICATOR MEANS OF WEANLING RABBITS FED DIETS CONTAINING ALFALFA AND BLACK LOCUST LEAF MEAL (BLM) MODIFIED BY VARIOUS TREATMENTS.

Contrast	Crude Protein	ADF	NDF	Dry Matter	Ash	Ca	P	ADG (g)	DM Intake g/day	Feed / Gain
1	77.07 vs 60.73*	15.81 vs 6.73*	25.78 vs 18.56*	68.80 vs 63.22*	75.26 vs 67.76*	84.51 vs 74.81*	70.33 vs 62.14*	37.0 vs 31.9*	95.9 vs 94.7	2.9 vs 3.3*
2	58.80 vs 66.30*	2.63 vs 8.55*	19.79 vs 18.38	62.32 vs 63.64	66.32 vs 68.13	75.62 vs 75.33	58.48 vs 61.60	30.4 vs 32.4	93.0 vs 100.1	3.4 vs 3.5
3	58.80 vs 60.06	2.63 vs 6.91	19.79 vs 18.94	62.32 vs 64.50	66.32 vs 68.67	75.62 vs 73.72	58.48 vs 63.65	30.4 vs 31.4	93.0 vs 91.2	3.4 vs 3.2
4	58.80 vs 57.77	2.63 vs 8.81*	19.79 vs 17.14	62.32 vs 62.43	66.32 vs 67.90	75.62 vs 74.59	58.48 vs 64.83	30.4 vs 33.3	93.0 vs 94.4	3.4 vs 3.2

Contrasts are:
1. Control vs. BLM Treatments (Groups 1 vs. 2, 3, 4 and 5)
2. BL Control vs. BLM + PEG (Groups 2 vs. 3)
3. BL Control vs. BLM + phytase (Groups 2 vs. 4)
4. BL Control vs. BLM + L-methionine and choline chloride (Groups 2 vs. 5)

* indicates statistical significance at .05 level

(1986) examined the nature of BL tannins. The proanthocyanidins (condensed tannins) in BL may exist in 5 molecular sizes. Their protein-precipitating capacity increases with an increasing degree of polymerization.

Horton and Christensen (1981) found that when lambs were fed BL leaves as a sole feed, the digestibilities of organic matter, CP, ADF, NDF and P were less than half those of alfalfa. Although no significant differences were seen between lambs fed BL leaves and alfalfa in terms of ADG or DM intake, it was concluded that BLM was not a satisfactory replacement for alfalfa. Harris et al. (1984) found ADG for weanling rabbits fed a 40% BL diet to be lower than the alfalfa control even though DM intake was higher for the BL diet. Raharjo et al. (1990) also found ADG to be lower for rabbits fed a 50% BL diet when compared to an alfalfa control, and reported twice as much N bound to the ADF fraction in the diets containing BL leaves as compared to the alfalfa control diet.

In our experiment, the addition of 1% PEG improved crude protein digestibility from 58.8% to 66.3%. This is in agreement with the findings of Horigome et al. (1984), who found that adding 1.6% PEG to rat diets containing BL leaves increased CP digestibility from 49.1% to 70.7%. Only 46% of the PEG was recovered in the feces compared to 91% from a diet containing brewers' grains, thus showing that a water-insoluble complex was formed in the gut. Pritchard et al. (1988) fed sheep *Acacia aneura* (which contained 6.1% tannins) and found that drenching the sheep every day with PEG increased body weight and wool growth. These findings contrast with those of Nuñez-Hernandez et al. (1991), who determined that adding PEG to diets containing mountain mahogany leaves, which have condensed tannins, did not improve nutrient digestion or affect N balance in goats and lambs. Garrido et al., (1991) found that the addition of PEG to faba bean seed coat, at a level of 200 mg of PEG per gram of seed coat, increased CP digestibility in vitro from 52.4% to 75.5%. A reduction of 97% of the tannins was observed by the addition of increasing levels of polyvinylpyrrolidine.

Tannins may affect not only CP digestibility but the digestibilities of other nutrients as well. A lower digestibility of DM and energy was observed in lambs fed BLM as compared to alfalfa (Horton and Christensen, 1981). In our study, ADF digestibility was also improved by addition of PEG to the BLM diet. The ADF was poorly digested for the control BLM diet (2.63%), but increased slightly to 8.55% with addition of PEG. Insoluble tannin-fiber complexes may be measured as ADF in the feces to give very low ADF digestibilities (Cheeke, 1992). It is evident from the NDF values in this study that the hemicellulose portion is better utilized for the alfalfa control than the BLM treatments.

The effect that tannins may have on mineral availability is not entirely clear, but tannins appear to form insoluble complexes with divalent metal ions which make them less absorbable (Salunke et al., 1990). Horton and Christensen (1981) found P to be poorly digested by lambs fed BLM. In our study, Ca and P were less available in the BLM control diet as compared to the alfalfa control and the addition of phytase did not improve P or Ca utilization; however, there was a trend towards improved DM digestibility with addition of phytase. Pointillart (1991) found that feeding a 20% rye bran diet high in phytase (1200 IU/kg) to pigs increased P absorption (55 vs 36%) and retention (50 vs 36%) when compared to a control without rye bran.

The addition of methionine and choline chloride to the BLM diet did not improve CP digestibility, but it did improve ADF digestibility, giving an increase from 2.63% to 8.81%. This may perhaps be explained by the tannins binding more strongly to protein than to fiber, allowing a better use of ADF. There was also a trend towards improved P utilization with addition of methionine and choline chloride. In a study by Myer et al. (1986), 0.1% DL-methionine was added to diets containing bird-resistant (high tannin) and non-bird-resistant (low tannin) grain sorghum. Dietary methionine was not effective in alleviating the detrimental effects that tannins have on swine. No improvement was seen in ADG or feed efficiency. Fuller et al. (1967) found that adding MHA (methionine hydroxy analogue) or choline to diets containing 0, 0.5, 1% tannic acid partially alleviated the growth depression due to the tannic acid in chicks. They proposed that the methionine probably provides labile methyl groups for the production of 4-O-methyl gallate, found by Booth et al. (1959) to be the major metabolite in the urine of rats and rabbits fed tannic acid and gallic acid.

Black locust leaf meal is not a satisfactory replacement of alfalfa in the diets of weanling rabbits. The nutrient digestibilities and ADG were lower for the BLM diets than for the alfalfa control. Since the DM intake did not increase to compensate for this, palatability may be a problem. The addition of PEG to diets containing BLM leaves improved nutrient utilization and warrants further investigation into the use of black locust as animal feed when treated with PEG. At the levels used in this study, addition of phytase and L-methionine and choline chloride had little effect on nutrient utilization. Further trials could be conducted using higher levels.

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