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EFFECTS OF REPLACEMENT OF ALFALFA BY BIG-LEAF MULBERRY ON GROWTH PERFORMANCE, DIGESTION AND MEAT QUALITY IN GROWING RABBITS

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Abstract: This study aimed to investigate the effect of sundried big-leaf mulberry (BLM) as a replacement for alfalfa on the growth performance, digestibility of nutrients, nitrogen (N) utilisation and meat quality in New Zealand White rabbits. One hundred and sixty weaned rabbits, aged 35±1 d and with a body weight of 755±26 g, were randomly assigned to the 4 treatments with 20 replicates of 2 rabbits (1 male and 1 female) each. Animal performance was evaluated between the 35th and 77th d of age in 40 animals per treatment. The coefficients of total tract apparent digestibility (CTTAD) of nutrients and N utilisation were measured between 77 to 83 d of age in 30 animals per treatment. The control rabbits were fed a corn-soybean meal-alfalfa meal based diet. The three experimental groups were fed a similar diet in which the alfalfa meal was replaced by 10% (BLM10), 20% (BLM20), or 30% (BLM30) BLM. The final body weights and average daily gain were higher ($P<0.05$) in BLM20 rabbits compared to control and BLM30 rabbits. Although BLM inclusion had no effects on the CTTAD of dry matter, organic matter and crude protein ($P>0.10$), the CTTAD of ether extract was higher in rabbits fed the diets based on BLM ($P<0.05$). No effect of treatment was noted on ingested and faecal N. Urinary ($P<0.05$) and retained N ($P<0.10$) were higher in animals fed with BLM30 and BLM20 diets, respectively. Dietary BLM supplementation increased a* ($P<0.05$) and tended to reduce L* ($P<0.10$) in rabbit meat samples. It was concluded that replacing alfalfa meal with BLM (20%) can promote growth and increase ether extract digestibility. Additionally, dietary BLM supplementation can increase the redness value of meat.

Key Words: big-leaf mulberry, digestibility, growth performance, meat quality, rabbit.

INTRODUCTION

In China, rabbit production is seriously affected by dependence on imports for the majority of feed ingredients, such as soybean meal and alfalfa. In recent years, China's soybean imports have risen steadily, and its dependence on foreign trade has reached 87%. The planting of alfalfa in China lagged behind, meeting only 50% of the demand, and depended on imported alfalfa. Therefore, rabbit production needs to find local feed crop resources instead of alfalfa and soybean meal.

In Africa and China, the use of dried foliage and pea grains in rabbit feeding has been reported in several studies (Lounaouci-Ouyed *et al.,* 2014; Safwat *et al.,* 2015; Abu Hafsa *et al.,* 2016; Shang *et al.,* 2017), while in China, data on the use of these local raw materials in concentrate pelleted diets are inexistent for mulberry. All cited studies indicated that rabbits can use grass and leaves efficiently. Nakkitset *et al.* (2008) found that feeding head lettuce residue, *Mimosa pigra* and water spinach resulted in higher growth rate and digestibility than feeding *Brachiaria ruziziensis*, and can be recommended as alternative feeds.

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Alfalfa is an ideal feed for rabbits, but the low yield and high price of Chinese alfalfa have seriously affected the economic benefits of rabbits. Therefore, the development of new roughage is of great significance. Hybrid *Broussonetia papyrifera*, commonly known as the big-leaf mulberry (BLM), is a species of mulberry native to China. It is highly adaptable, with a fast growth rate and strong regeneration ability. According to statistics, yearly output yield of BLM is something around 207 tons (fresh weight) per hectare (Ma *et al.,* 2012). The leaves of big-leaf mulberry contain 26.1% crude protein (CP), 5.2% ether extract (EE), and 3.4% calcium (Tu *et al.,* 2009). Big-leaf mulberry has recently been used in diets for growth-finishing pigs (17% dry matter [DM], BLM) (Zhang *et al.,* 2015), and growing lambs (10% DM) (Mei-Rong *et al.,* 2014).

Information on the nutritive value of big-leaf mulberry is limited, especially in rabbits (Deshmukh *et al.,* 1993). The study aimed to determine the effect of the dietary inclusion of BLM on the growth performance, coefficients of total tract apparent digestibility of the diets (CTTAD), nitrogen (N) utilisation and meat quality of growing rabbits.

MATERIAL AND METHODS

The experiment was carried out at the Animal Science and Technology Park (Guangxian, China). The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Anhui Science and Technology University (Bengbu, China).

Animals, experimental diets and management

One hundred and sixty weaned New Zealand White rabbits, aged 35±1 d and with a body weight of 755±26 g, were randomly assigned to the 4 treatments with 20 replicates of 2 rabbits (1 male and 1 female) each. The animals were housed in wire cages (60×50×40 cm; 2 rabbits per cage) and had free access to clean drinking water. The temperature and relative humidity in the rabbitry were 18±5°C and 55±10%, respectively. A cycle of 14 h of light and 10 h of dark was used throughout this trial.

The big-leaf mulberry was harvested around the experimental site. Nutrient composition of BLM and alfalfa is shown in Table 1. Values of CP, EE, ash, neutral detergent fibre (NDF) and calcium in BLM than in alfalfa meal, while the content of phosphorus in BLM was lower than that of alfalfa meal. The control rabbits were fed a corn-soybean meal-alfalfa meal based diet (Table 2). The three treatment groups were fed a similar diet in which the alfalfa meal was replaced by 10% (BLM10), 20% (BLM20), or 30% (BLM30) BLM. All groups received the diets in the form of pellets, and had *ad libitum* access to feed throughout the study.

Experimental protocol and sample collection

All rabbits were weighed at the beginning (d 35) and end (d 77) of the feeding trial. Offered and refused feeds were recorded daily during the entire experiment period. The average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio were calculated for each experimental unit (1 male and 1 female).

At the end of the feeding trial, 40 rabbits (10 rabbits per group, male and female had half each) were euthanised in accordance with normal farming practice. The whole *longissimus lumborum* (LL) muscle (between the 1st and 7th lumbar vertebra) was excised and used for the meat quality analyses. The LL was then divided into 3 sub-samples.

Table 1: Nutrients concentration (% dry matter) of big-leaf mulberry (BLM) and alfalfa.

Item	Alfalfa	BLM
Dry matter	88.83±1.56	90.05±1.33
Crude protein	19.75±0.78	21.59±0.50
Crude fibre	33.42±0.87	34.08±0.96
Ether extract	2.29±0.12	4.29±0.21
Ash	7.62±0.24	11.66±0.33
Acid detergent fibre	25.45±0.39	24.93±0.42
Neutral detergent fibre	36.67±0.93	39.10±0.71
Calcium	1.41±0.04	1.92±0.06
Phosphorus	0.50±0.02	0.44±0.02

Table 2: Ingredients and chemical composition of experimental diets.

Items	Control	BLM10	BLM20	BLM30
Ingredients (%)				
Big-leaf mulberry	0	10	20	30
Alfalfa meal	30	20	10	0
Wheat bran	39	39	39	39
Corn grain	20	20	20	20
Soybean meal	5	5	5	5
Molasses	2.5	2.5	2.5	2.5
Di-calcium phosphate	1.2	1.2	1.2	1.2
Limestone	0.5	0.5	0.5	0.5
Lysine	0.2	0.2	0.2	0.2
Methionine	0.2	0.2	0.2	0.2
NaCl	0.4	0.4	0.4	0.4
Premix[1]	1	1	1	1
Chemical analysis (% on dry matter basis)				
Dry matter	89.42	89.62	89.82	90.02
Crude protein	16.66	16.87	17.08	17.29
Ether extract	2.93	3.08	3.22	3.36
Crude fibre	16.14	16.04	15.94	15.84
Ash	5.68	5.93	6.17	6.42
Neutral detergent fibre	34.89	34.64	34.38	34.13
Acid detergent fibre	16.36	15.68	14.99	14.30
Gross energy (MJ/kg)	15.26	15.34	15.43	15.52

BLM10=10% supplemental BLM; BLM20=20% supplemental BLM; BLM30=30% supplemental BLM.

[1]Each 1 kilogram of premix contains: vitamin A, 1050000 IU; vitamin D3, 150000 IU; vitamin E 9000, vitamin K3, 0.15 g; thiamin, 0.25 g; calcium d-pantothenate, 0.8 g; riboflavin, 0.25 g; pyridoxine, 0.12 g; biotin, 0.005 g; nicotinic acid, 2.5 g; choline chloride, 15 g; folic acid, 0.25 g.

One sub-sample was used for pH measurements at 45 min and 24 h. The muscle colour (L*, a* and b*) and Warner-Bratzler (WB) shear force values were determined at the end of the chilling period (24 h) on the second and third sub-samples, respectively. Drip loss were measured according to Blasco and Ouhayoun (1996). Each sample were cores (Ø=1.25 cm, thickness=2 cm) obtained from LL samples, cut perpendicularly to the fibre direction and previously cooked in a water bath (80°C, 10 min).

Digestion of nutrients and N utilisation trial

At 77 d of age, remaining animals (30 rabbits per group; 15 replicates of 2 rabbits) were housed in metabolic crates (60×50×40 cm; 1 male and 1 female) that allowed separation of urine and faeces and were used to determine the CTTAD for DM, organic matter (OM), CP, EE, acid detergent fibre (ADF) and NDF in accordance with the European Group on Rabbit Nutrition (Pérez *et al.,* 1995), and to determine N balance and N retention. Faeces and urine samples were collected between 77 to 83 d of age. During the collection period, excreta were collected over 24 h and weighed at 06:00, 14:00 and 21:00. A portion of around 250 g of faeces was sampled for chemical analysis. Urine excreted in 24 h was collected into plastic bottles and acidified with 20 mL/L (v/v) H_2SO_4 (95%), measured daily at 09:00 h (Calvet *et al.,* 2008; Gidenne *et al.,* 2013). Sufficient aliquots of urine (10 mL/100 mL excreted) were collected, pooled and frozen (–20°C) for later chemical analysis.

Meat quality parameters

The pH value was determined from each rabbit 45 min post-mortem and d 1 with a pH meter equipped with a pH probe by penetrating 3 mm into the LL. Warner-Bratzler shear force values (kg·f) of the LL was evaluated on cores (1.25×2 cm) obtained from the mid-portions of the cooked samples up to 80°C internal temperature of the LL by cutting them perpendicular to fibre direction, using the WB meat shear apparatus (C-LM, USA) (Ouhayoun and Dalle Zotte, 1996).

Three samples of per LL were used for shear force measurement and the values were averaged. The colour, including L* (lightness or brightness), a* and b* (redness and yellowness) parameters (CIE, 1976), was measured at the muscle surface of the LL using Chromameter type CR-100 (Minolta, Tokyo, Japan).

Chemical analyses

Laboratory analyses were carried out on feed, leaf meals and faecal and urine samples using the standard AOAC (2005) procedures to determine DM (930.15), EE (920.39; the EE determination on faeces was not preceded by acid hydrolysis), CP (984.13; N converted to CP, N×6.25), ash (942.05), crude fibre (CF, 978.10), phosphorus (995.11) and calcium (927.02) content. Acid detergent fibre and NDF were determined using methods described by Van Soest *et al.,* (1991). A bomb calorimeter (Adiabatic Oxygen Bomb Calorimeter, Par Instrument Co., Moline, IL, USA) was used to determine gross energy (GE).

Statistical analysis

Nutrient concentrations of BLM and alfalfa were analysed statistically using *t* test procedure of SAS (2009) with a model that included the effect of forage (n=6). Data were analysed as a completely randomised design using the Proc MIXED procedures of SAS (2009) with a model that included the dietary effect of BLM levels as main effect. Cage was the experimental unit. Differences among treatments were examined using Duncan's multiple range test and were considered significant at $P<0.05$, and *P*-values between 0.05 and 0.1 were considered a trend.

RESULTS

Digestion of nutrients and N utilisation

Data describing the CTTAD of the diets and N utilisation are presented in Table 3. The different treatments had no effects on CTTAD of DM, OM, CP, NDF and ADF. The CTTAD of EE was increased with the dietary BLM inclusion ($P<0.05$). Although ingested nitrogen and faecal nitrogen were not affected by dietary BLM concentrations, urinary nitrogen ($P<0.05$) and retained nitrogen ($P<0.10$) were increased in rabbits fed the BLM30 and BLM20 diets, respectively.

Table 3: Influence of dietary inclusion of big-leaf mulberry (BLM) on coefficients of total tract apparent digestibility of the diets (CTTAD) and N utilisation in rabbits[1] between 77 and 83 d of age.

Item	Control	BLM10	BLM20	BLM30	Pooled SEM	*P*-value
Average daily feed intake (g/d)	121	123	122	126	4	0.9063
CTTAD						
Dry matter	0.67	0.68	0.68	0.68	0.01	0.2779
Organic matter	0.68	0.71	0.70	0.71	0.01	0.1611
Crude protein	0.73	0.72	0.75	0.76	0.01	0.1158
Ether extract	0.79[a]	0.83[b]	0.84[b]	0.84[b]	0.01	0.0025
Acid detergent fibre	0.40	0.40	0.43	0.40	0.01	0.3715
Neutral detergent fibre	0.50	0.52	0.53	0.53	0.01	0.1245
Nitrogen utilisation (g/d)						
N intake	3.24	3.31	3.33	3.48	0.12	0.5856
N excretion in faeces	0.89	0.92	0.83	0.85	0.05	0.7254
N excretion in urine	1.16[a]	1.14[a]	1.12[a]	1.40[b]	0.07	0.0337
Retained N[2]	1.18	1.26	1.38	1.22	0.05	0.0857

[1]n = 30 rabbits (15 cage) per experimental diet.

[2]as nitrogen intake–total nitrogen excreted (faeces+urine).

Means within a row with different superscripts differ ($P<0.05$).

BLM10=10% supplemental BLM; BLM20=20% supplemental BLM; BLM30=30% supplemental BLM; SEM, standard error of mean.

Table 4: Influence of dietary inclusion of big-leaf mulberry (BLM) on growth performance in rabbits[1] between 35 and 77 d of age.

Item	Control	BLM10	BLM20	BLM30	Pooled SEM	*P*-value
Initial body weight (g, at weaning)	755	752	755	757	8	0.9843
Final body weight (g)	2115[a]	2180[ab]	2212[b]	2104[a]	25	0.0160
Average daily gain, (g)	32[a]	34[ab]	35[b]	32[a]	0.61	0.0171
Average daily feed intake, (g)	95	96	97	96	2	0.8984
Feed conversion ratio	2.94[ab]	2.82[a]	2.81[a]	2.99[b]	0.05	0.0498

[1]n = 40 rabbits (20 cage) per experimental diet.
Means within a row with different superscripts differ ($P<0.05$).
BLM10=10% supplemental BLM; BLM20=20% supplemental BLM; BLM30=30% supplemental BLM; SEM, standard error of mean.

Growth performance

The results concerning the growth performance of rabbits are shown in Table 4. No effect of treatment was noted on ADFI. A higher ADG (by 3 g; $P<0.05$), resulting in higher final live weight of rabbits at d 77 of age ($P<0.05$), was detected in rabbits fed the BLM20 diet than in those fed the control or BLM30 diets. The feed conversion ratio was better in rabbits fed the BLM10 and BLM20 diet than in rabbits fed the BLM30 diet ($P=0.050$).

Meat quality

The results concerning pH values, meat colour, drip loss or WB shear force values measured in LL muscles are shown in Table 5. Dietary inclusion of BLM increased a* ($P<0.05$) and tended to reduce L* ($P<0.05$) of LL muscles compared to rabbits fed the control diet. Drip loss percentage, pH, b*, and WB shear force values were not affected by the dietary BLM addition.

DISCUSSION

The big-leaf mulberry could be a kind of feed resource to provide more crude protein, but the proportion of calcium and phosphorus should be adjusted in application. As a non-ruminant herbivore, rabbits have the ability to digest a variety of natural pastures and leaves.

In the present study, the CTTAD of EE was significantly higher in rabbits fed the diets based on BLM than in rabbits fed the control diet. These results are probably associated with the slightly higher dietary EE contents in the diets based on BLM compared to the control diet (Xiccato, 2010). The crude protein ingested by rabbits is used for protein synthesis in the body, and the other part is discharged in faeces and urine (Calvet *et al.,* 2008). The results of the present study showed that there was no significant difference in ingested nitrogen and faecal nitrogen, whereas urinary nitrogen was significantly increased when alfalfa was completely replaced by BLM. This finding may be explained by the different dietary compositions used in this study (Gidenne *et al.,* 2013).

Table 5: Influence of dietary inclusion of big-leaf mulberry (BLM) on meat quality in rabbits[1] at 77 d of age.

Item	Control	BLM10	BLM20	BLM30	Pooled SEM	*P*-value
pH (45 min)	6.78	6.94	6.89	7.01	0.08	0.2234
pH (24 h)	5.83	5.94	5.96	5.98	0.06	0.4163
L*	35.33	34.71	33.61	33.19	0.58	0.0682
a*	3.58[a]	3.73[b]	3.89[bc]	3.88[c]	0.05	0.0004
b*	2.08	2.13	2.13	2.11	0.04	0.8630
Drip loss (%)	3.62	3.52	3.62	3.48	0.08	0.5307
WB shear force[4] (kg.f)	11.81	11.96	11.70	12.94	0.75	0.6711

[1]n = 10 rabbits (5 male and 5 female) per experimental diet.
Means within a row with different superscripts differ ($P<0.05$); SEM, standard error of mean; BLM10=10% supplemental BLM; BLM20=20% supplemental BLM; BLM30=30% supplemental BLM; L*, lightness; a*; redness; b*, yellowness; WB, Warner-Bratzler.

There are few data available on the effect of dietary inclusion of BLM on the growth performance of rabbits to compare and explain our results (Martinez *et al.,* 2005; Mora-Valverde, 2010). The results of this study showed that the addition of 20% BLM to rabbit diets significantly increased daily gain, and there was no significant difference in feed intake between groups.

Shear force and drip loss can reflect the tenderness of rabbit meat (Rodriguez *et al.,* 2017). The flesh colour reflects the freshness of the meat and is one of the main sensory indicators of meat products, mainly depending on the pigment substances myoglobin and haemoglobin in the muscle (Dalle Zotte *et al.,* 2016; Wang *et al.,* 2018). The results of this experiment indicated that the addition of BLM to the diet had no significant effect on shearing force and drip loss, but significantly increased the redness value of the meat. Tu *et al.* (2009) showed that the hybrid leaves had higher iron content than the alfalfa powder, which may be the reason for the improvement in meat colour. In addition, a study in lambs also indicated that BLM supplementation had no effect on shear stress, drip loss and cooking loss (Mei-Rong *et al.,* 2014).

CONCLUSION

Big-leaf mulberry is good feed material, rich in protein and calcium, which may replace some alfalfa meal and soybean meal in the diet of herbivores. Feeding BLM as a supplemental forage source could be a potentially feasible strategy in China. Replacing alfalfa with BLM can promote growth and increase fat digestibility. However, total replacement of alfalfa by BLM reduced ADG. Thus, it seems that up to 66% alfalfa hay can be replaced with BLM in diets of growing rabbits without adverse effects on their growth performance. Additionally, dietary BLM supplementation can increase the redness value of meat.

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