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Evaluation of mulberry (*Morus alba*) leaves as a concentrate substitute in rabbit diet: effect on growth performance and meat quality

Kamran KHAN[1,*], Inam ULLAH[1], Nazir Ahmad KHAN[2], Sarzamin KHAN[2]

[1]Department of Animal Sciences, Shaheed Benazir Bhutto University, Sheringal, Dir (U), Khyber Pakhtunkhwa, Pakistan

[2]Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar, Peshawar, Khyber Pakhtunkhwa, Pakistan

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Abstract: Mulberry (*Morus alba*) leaves contain a high amount of digestible carbohydrates, protein and minerals, making it an attractive, natural, low-cost feed ingredient in rabbit rations. The present study evaluated the effect of stepwise replacement of a concentrate diet with wilted *M. alba* leaves on growth performance and meat quality of rabbits. Sixty indigenous rabbits (age, 36 ± 2 days) were assigned to 5 diets (12 rabbits per diet). The 5 diets consisted of a commercial concentrate diet (control; WML0), and stepwise replacement of the concentrate with 25% (WML25), 50% (WML50), 75% (WML75), and 100% (WML100) wilted *M. alba* leaves. Final body weight (1238 g/rabbit), and total (904.1 g/rabbit) and daily (18.8 g/day/rabbit) weight gains were higher ($P < 0.05$) in rabbits fed with WML50. The WML50 also supported the best ($P < 0.05$) feed to gain ratio (4.50). Moreover, higher ($P < 0.05$) reference carcass weight (571.7 g) and a higher dressing percentage (55.8%) were recorded for WML50. Composition of the diets did not alter ($P > 0.05$) meat pH, water release and cooking loss. In conclusion, *M. alba* successfully replaced 50% of concentrate in rabbit diet and supported a higher growth performance and carcass yield without negative effects on meat quality.

Key words: Mulberry leaves, rabbit, growth performance, meat quality

1. Introduction

Northern regions of Pakistan are home to the largest rabbit population of the country because of the favourable agroclimatic conditions and suitable feed resources for rabbit farming [1,2]. Traditionally, rabbit farming serves as an important family enterprise for low-income farmers [3]. Within the last decade, however, rabbit production has been rapidly changing in the area from household enterprise to medium-subsistence and larger-scale (semi-) commercial production. As a result of this change in production systems, the farmers are often feeding high amounts of concentrates to the rabbits to obtain better growth rates. Feeding large amounts of concentrates are reducing the profit margins for progressive farmers because of their high costs. Therefore, the current study focused on the exploration of low-cost, natural feed resources that can partly fulfil the digestible crude protein (CP), energy and minerals requirements of rabbits. In this regard, trees foliage has received increasing attention as a strategic feed supplement in many developing countries in the tropics due to its higher contents of metabolizable protein, energy, and minerals [4].

Research has established that tree foliage is a rich source of fermentable organic matter, CP, and minerals, and that it can be used as a forage ingredient in ruminant rations [5]. Mulberry (*Morus alba L.*) is an economically important tree, providing many valuable inputs for food, feed, and silk industries. This woody fodder species produces a remarkable forage yield (leaves) on a yearly basis, ranging from 25 to 30 tons ha^{-1} [6,7]. Furthermore, it also yields higher digestible nutrients per unit area than other traditional forage trees [8,9]. Moreover, it is a highly nutritious fodder bank of Pakistan (4,10]; and like other promising fodder trees, it provides good-quality green fodder throughout scarcity periods [11]. Several authors have reported positive effects of feeding mulberry foliage to ruminants [7,12,13]. The application of herbal plants in rabbit diets improves nutrient utilization, growth performance [14], and meat quality [15]. In our previous study, *M. alba* leaves showed better nutrient composition and dry matter digestibility (in vitro) among 5 promising tree species in Northern Pakistan [10]. Consequently, the current study was conducted to further investigate the effects of stepwise replacement of commercial concentrate with wilted *M. alba* leaves on growth traits and meat quality of rabbits.

* Correspondence: dr.kamran@sbbu.edu.pk

2. Materials and methods

2.1. Experimental design and rabbit selection

Sixty indigenous rabbits (36 ± 2 days; weight: 337 ± 4.3 g) were chosen and distributed randomly into 5 dietary groups according to randomized complete block arrangement. Each group was subdivided further into 3 replicates, having 4 rabbits per replicate. The blocks were balanced in terms of age, weight, and sex. The control group rabbits were fed a commercial concentrate-based mixed diet (control; WML0). The other 4 diets were formulated with stepwise replacement of the control diet with 25% (WML25), 50% (WML50), 75% (WML75) and 100% (WML100) of wilted *M. alba* leaves. The *M. alba* leaves were cut daily in the morning, withered in shade, and offered the next day. The ingredients of the control diet were mixed and converted to pellet form (2 mm). The nutrient profile of control diet and mulberry leaves is presented in Table 1. Each rabbit had free access to clean drinking water, with provision of 16 h light. The experimental period of 55 days was established, 7 of which were intended for adaptation of the animals to the diets.

Table 1. Chemical composition (% dry matter, DM) of concentrate-based diet and *Morus alba* leaves.

Chemical composition	Concentrate[1]	*Morus alba* leaves[2]
Crude protein	18.57	21.84
Neutral detergent fibre	37. 73	35.53
Acid detergent fibre	23.64	23.83
Ether extract	4.50	3.313
Total ash	9.30	15.32

[1]Standard concentrate-based mixed diet contained wheat bran (25%), maize grain (15%), cotton seed cake (15%), soybean meal (8%), palm oil cake (15%), maize gluten (15%), molasses (5%), Di-Ca-phosphate (1%), DL-methionine (0.1%), sodium chloride (0.5%), vitamin and mineral premix (0.5%)

[2]*Morus alba* leaves are in green wilted form (daily cut in the morning and offered the next day)

2.2. Growth performance and carcass traits

Before the commencement of the study, the body weight (BW) of an individual rabbit was recorded for 2 consecutive days before morning feeding. Then, weekly weight gain was recorded using a similar procedure, and the average daily weight gain was calculated (g/d). A predetermined amount of feed was offered to each replicate group 2 times a day, and the refusals were collected and weighed the next morning (at 8:00 am). Similarly, the feed intake (g/day) and feed to gain ratio (F/G) were computed for each replicate group. Finally, the rabbits were fasted for 15 h with only access to clean drinking water for measurements of carcass quality. Six randomly selected rabbits from each treatment group (2 from each replicate) were sacrificed to record carcass traits. The slaughtering process and the measurement of carcass characteristics were carried out by using the Procedures of World Rabbit Science Association as previously described by Khan et al. [16].

2.3. Meat quality

From all slaughtered animals, muscle of *longissimus lumborum* (LL) was taken for measurement of cooking loss and water release. Then, immediately the collected samples were packed (vacuum) and frozen at –20 °C. For pH determination, another sample (10 g) was taken 24 h postmortem from LL muscles. Before pH measurement, the samples were homogenised with 50 mL of water. The pH of the homogenized samples was measured using a digital pH meter (JENWAY 3505, UK). Prior to cooking, the frozen samples were thawed at 4 °C for 24 h, and then cut into 2.5 cm and 1 cm pieces. The thawed samples (2.5 cm; 150 g) were cooked individually in water bath (at 80 °C for 90 min), and then cooled in tap water for 20 min, dried from fluids and weighed. Cooking loss was calculated as follows:

% cooking loss = [(raw weight - cooked weight)/(raw weight)] × 100

For determination of water release, the thawed samples (1 cm thick; 300 mg) were enclosed in previously desiccated, weighed, and labelled Whatman filter-papers, and pressed by 2.25 kg load for 5 min. The weight of damp filter paper was recorded immediately after removing the load. Water release was determined as a percentage of weight loss before and after pressing [17]. The moisture, CP (Nitrogen × 6.25), crude fat and ash contents of LL muscles were calculated according to the standard method of the AOAC [18]. Moisture content was calculated by drying meat (1 g) in an oven (100 °C) till a constant weight. Crude protein (N% × 6.25) content was determined via Kjeldahl method. Soxhlet extraction method was used to determine the fat content using petroleum ether. The content of ash was determined by the complete ignition of the meat in muffle furnace (550 °C for 3 h).

2.4. Statistical analysis

Data on the effect of stepwise replacement of commercial concentrate with wilted *M. alba* leaves on rabbit growth performance, carcass traits, meat physical (pH, water release, cooking loss) and chemical (moisture, protein, fat, ash) properties were analysed by PROC MIXED procedure of the Statistical Analysis System (SAS Institute, 2009). Diet was fixed effect, and rabbit was considered as random effect. Post hoc analyses were performed using the Tukey–Kramer test to determine if the results were significant ($P < 0.05$). The results are presented as least square mean along with model estimated standard error of the mean.

3. Results

Data on the effects of stepwise replacement of commercial concentrate diet with wilted *M. alba* leaves on growth performance of rabbits are shown in Table 2. Results revealed that final body weight (1238 g/rabbit), and total (904.1 g/rabbit) and daily (18.8 g/rabbit/day) weight gains were higher (P < 0.05) in rabbits fed with WML50 diet. The lowest (P < 0.05) feed intake was recorded for WML100 group, while the other dietary groups did not differ (P > 0.05) in feed intake. The best (P < 0.05; 4.50) feed to gain ratio was recorded for the rabbits fed with WML50. No mortality was recorded in the rabbits during the experimental period.

Data on the effect of the stepwise replacement of commercial concentrate with wilted *M. alba* leaves on carcass characteristics of the rabbits are summarized in Table 3. Reference, chill and hot carcass weight, and dressing percentage were higher (P < 0.05) in the rabbits fed with WML50 diet. However, the composition of the diets did not alter (P > 0.05) the weight of heart, kidney, liver, spleen, and lungs.

The pH, water release, cooking loss, contents of moisture, CP, fat, and ash of *longissimus lumborum* muscle did not vary (P > 0.05) due to the substitution of commercial concentrate with wilted *M. alba* leaves in the rabbit diets (Table 4).

Table 2. Effect of stepwise inclusion of *Morus alba* leaves in concentrate-based diet on growth performance of local rabbits.

Traits	Diets[1]					SEM	Significance
	WML0	WML25	WML50	WML75	WML100		
Final body weight, g	1196^{c}	1203^{b}	1238^{a}	1207^{b}	1154^{d}	1.82	***
Total weight gain, g	860^{d}	866^{c}	904^{a}	872^{b}	817^{e}	2.50	***
Weight gain, g/day	17.9^{b}	18.0^{b}	18.8^{a}	18.2^{b}	17.0^{c}	0.05	***
Dry matter intake, g/day	85.6^{a}	86.2^{a}	84.8^{a}	83.7^{ab}	81.8^{b}	1.11	**
F/G	4.78^{a}	4.78^{a}	4.50^{c}	4.61^{b}	4.80^{a}	0.06	***

Values are shown as mean; in the same row, carrying different superscript letter a, b, c, d means significant difference at P < 0.05
SEM, standard error of the mean; F/G, feed to gain ratio
**P < 0.01; ***P < 0.001
[1]WML0, control diet (standard concentrate-based mixed diet with 18.57% CP), and diets based on stepwise replacement of WML0 with 25% (WML25), 50% (WML50), 75% (WML75) and 100% (WML100) with wilted *M. alba* leaves

Table 3. Effects of stepwise inclusion of *Morus alba* leaves in concentrate-based diet on carcass traits of rabbits.

Traits	Diets[1]					SEM	Significance
	WML0	WML25	WML50	WML75	WML100		
Hot carcass weight (g)	693^{c}	703^{b}	714^{a}	704^{b}	664^{d}	2.21	***
Chill carcass weight (g)	644^{c}	655^{b}	674^{a}	655^{b}	617^{d}	2.34	***
Reference carcass weight (g)	544^{c}	554^{b}	572^{a}	555^{b}	522^{d}	2.22	***
Dressing (%)	54.1^{b}	53.7^{b}	55.8^{a}	53.3^{b}	53.5^{b}	0.21	**
Liver (g)	56.3	55.9	56.8	56.6	55.6	2.17	ns
Kidney (g)	8.98	9.69	8.58	8.39	8.65	0.18	ns
Heart (g)	4.12	4.01	3.99	4.03	4.15	0.05	ns
Lungs (g)	8.98	8.86	8.82	8.78	9.04	0.21	ns
Spleen (g)	0.69	0.67	0.67	0.68	0.69	0.01	ns

Values are shown as mean; in the same row, carrying different superscript letter a, b, c, d means significant difference at P < 0.05
SEM, standard error of the mean
ns, nonsignificant; **P < 0.01; ***P < 0.001
[1]WML0, control diet (standard concentrate-based diet with 18.57% CP), and diets based on stepwise replacement of WML0 with 25% (WML25), 50% (WML50), 75% (WML75) and 100% (WML100) with wilted *M. alba* leaves

Table 4. Effects of stepwise inclusion of *Morus alba* leaves in concentrate-based diet on physicochemical quality of *longissimus lumborum* muscle of rabbits.

	Diets[1]						
	WML0	WML25	WML50	WML75	WML100	SEM	Significance
Chemical quality (%)							
Moisture	71.5	71.6	71.8	72.1	71.4	0.25	ns
Protein	21.4	21.6	21.8	21.6	21.4	0.23	ns
Fat	1.85	1.87	1.83	1.79	1.81	0.03	ns
Ash	1.25	1.23	1.32	1.29	1.24	0.03	ns
Physical quality							
pH24	5.36	5.46	5.34	5.41	5.37	0.02	ns
Water release (%)	15.5	15.8	16.1	15.6	15.9	0.18	ns
Cooking losses (%)	35.8	35.7	35.6	36.1	35.5	0.23	ns

Values are shown as mean; in the same row, carrying no superscript letter means no significant difference at $P > 0.05$
SEM, standard error of the mean
ns, nonsignificant
[1]WML0, control diet (standard concentrate-based diet with 18.57% CP), and diets based on stepwise replacement of WML0 with 25% (WML25), 50% (WML50), 75% (WML75) and 100% (WML100) with wilted *M. alba* leaves

4. Discussion

Research has established that tree foliage is a low-cost CP and mineral-rich supplement to low-quality fibrous diets under subsistence farming systems [19], making it an attractive feed ingredient for small ruminants, particularly, in the arid and/or semiarid areas of the world. Our previous study revealed that *M. alba* leaves contain high amounts of CP (21.8%) and minerals, and the leaf biomass was highly digestible in rabbits (62.5%) [10], highlighting their potential as a low-cost alternate feed ingredient for fattening rabbits. Therefore, the present study was conducted to examine the stepwise replacement of the concentrate with *M. alba* leaves on performance, carcass characteristics, and meat physicochemical quality of the rabbit.

The results revealed that the replacement of 50% commercial concentrate diet with wilted *M. alba* leaves supported the highest improvement in body weight gain, feed to gain ratio, and carcass yield. This could be related to better fermentation ability and higher digestibility of nutrients in the browse in a well-developed caecum of rabbits [20]. Thus, the inclusion of wilted *M. alba* leaves (50%) in the rabbit diet can not only increase the economic profitability but can also provide long-term sustainability to the rabbit industry. The rabbits readily consumed *M. alba* leaves, and the leftovers were very little, indicating that the leaves were highly palatable. However, increasing the inclusion levels up to 75% and 100% resulted in higher feed consumption per unit weight gain. Moreover, the total weight gain of the rabbit reduced with 100% replacement of concentrate-based diet with *M. alba* leaves, which could be related to the relatively lower digestibility of the leaves [10]. Our findings were consistent with the previous research, which reported significant improvement in the growth traits of rabbit consuming concentrate and forage mixed diet [16,21,22].

In agreement with earlier findings, the carcass weight was mostly influenced by the final body weight of the rabbit [16]. Moreover, the dressing percentage is a vital factor determining the proportion of lean meat. In general, the higher the dressing percentage, the greater the proportion of lean meat, and vice versa. In the present study, a higher reference carcass weight and dressing percentage were reported in the rabbit fed with WML50 diet. Our results are consistent with those of Martinez et al. [23], who recorded no significant dietary influence on the relative weight of kidney, liver, heart, and spleen.

Postmortem pH decline is a vital indicator of water holding capacity of meat, which in turn largely influences meat quality characteristics, such as juiciness of raw and cooked meat [24,25]. In the present study, pH of *longissimus lumborum* was the same in all groups, which suggests that acidification process in meat was not altered by the inclusion of wilted *M. alba* leaves in the diet. Similarly, cooking loss and water release of meat were not affected by the diets. Moreover, the substitution of concentrate with *M. alba* leaves did not cause any significant changes in the protein, ash, fat, and moisture contents of the meat. However, the values of chemical components reported in the present study were in line with the literature ranges [16]. Earlier studies have shown that rabbit meat is prone to lipid peroxidation process [26] due to higher amounts of polyunsaturated fatty acids [27]. Inclusion of *M. alba* leaves can prevent meat lipid peroxidation due to their powerful antioxidant property without any adverse effects on organoleptic attributes. This study indicated that the inclusion of 50% *M. alba* leaves in the rabbit ration supports optimum growth rate of rabbits. However, long-term feeding trials are needed for concrete recommendations with respect to overall performance of rabbits. The mulberry leaves have potential to replace 50% of the concentrate-based diet for fattening rabbits, without negative effects on meat quality and can be helpful to enhance the economic profitability and long-term sustainability of the rabbit industry.

In conclusion, this study investigated the effect of stepwise replacement of commercial concentrate-based diet with wilted *M. alba* leaves on the growth performance and carcass quality of rabbits. The results revealed that weight gain, feed to gain ratio, and carcass yield of fattening rabbits were significantly improved with 50% replacement of a commercial concentrate-based diet with wilted *M. alba* leaves, without any adverse effect on meat physicochemical characteristics. However, a further increase in the inclusion levels of *M. alba* leaves (75% or 100%) did not cause further improvement in the rabbit growth performance and meat quality. These findings show that wilted *M. alba* leaves are a valuable feed resource for rabbits, particularly in the small holding and semicommercial production systems.

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Conflict of interest
We state that there are no conflicts of interest.

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