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## **Dietary mulberry (*Morus alba*) leaf affects on apparent nutrient digestibility and growth performance of crossbred rabbits (New Zealand x Local)**

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### **Abstract**

Two experiments were performed to determine the effect of mulberry (*Morus alba*) leaf meal (MLM) in diets on apparent nutrient digestibility and growth performance of crossbred rabbits (New Zealand x Local). Feed protein source of fish meal were replaced by 5 levels of MLM in the diet (0%, 25%, 50%, 75% and 100%) in the 1<sup>st</sup> experiment on nutrient digestibility and in the 2<sup>nd</sup> experiment on growth performance. The results showed that the digestibility of DM, OM, CP and CF decreased according to the increasing level of mulberry leaves in the rabbit's diet. There were no different significances in DM intake, CP intake and FCR among 5 treatments ( $P > 0.05$ ). In conclusion, rabbits fed diets containing fishmeal protein replacement by mulberry leaves were optimized growth and feed conversion rates at 50% replacement level.

**Keywords:** *digestibility, feed conversion, feed intake, mulberry, weight gain*

### **Introduction**

Rabbits in Vietnam are increasingly interested by farmers and government agencies. Rabbits are seen as a means to raise the incomes of the rural poor mainly because of its low investment capital, variety, availability and non-competitiveness with other livestock for feed. Rabbit's feed is abundant, easy to find and does not compete for food with other livestock (Nguyen Van Thu 2019).

Industrial feed pellets used for rabbits are gradually becoming popular. But due to the high cost, farmers cannot use industrial pellets completely. Therefore, the effective use of some available forage crops and agricultural by-products to mix feed pellets on the spot is essential both to reduce costs and to create initiative for farmers (Le Duc Ngoan and Le Thi Lan Phuong 2010; Nguyen Van Dat et al 2015).

Mulberry trees are grown in many parts of Vietnam and the leaves can be harvested all year round and have a high yield of 15 - 16.7 tons/ha (Le Hong Van et al 2017). Mulberry leaves are considered as a potential source of protein with crude protein content of mulberry leaves ranging from 18-25% (Le Thi Lan Phuong et al 2012).

Our previous study showed that using mulberry leaves in the rabbit's diet increased the weight of rabbits (Le Thi Lan Phuong et al 2012). Kamran Khan et al (2020) reported that mulberry leaves are a valuable feed resource for rabbits, particularly in the small holding and semicommercial production systems. The current study aimed to determine the effect of mulberry (*Morus alba*) leaf pellets in diets on apparent nutrient digestibility and growth performance of crossbred rabbits (New Zealand x Local).

## Materials and methods

### Location

The experiment was carried out at the experimental farm of Institute of Biotechnology, Hue University, Thua Thien Hue province.

### Animals

The hybrid rabbits (New Zealand x Local) of about 3 months of age were used in this study. Before starting the experiments, the rabbits were adaptably kept and controlled against external parasites with injections of Ivermectin solution (1 ml per 3 kg body weight) and vaccinated against VHD (virus haematologist disease).

The cages of rabbits was constructed from wood and wire mesh. The dimensions width, length, height (m) of the growth cages were 0.5, 0.6 m and 0.4 m and the digestibility cages were 0.15, 0.5 and 0.3 m.

### Experimental feed

Mulberry was cut with 30-35 cm in length. Then, the leaves and top of the mulberry tree are dried in the sun and finely ground to make mulberry leaf powder. After that, mulberry leaf powder was mixed with other ingredients in different proportions to make experimental pellets. The feed used in the experiments consisted of 05 homemade pellets and Para grass. Para grass was cut daily with 20-25 cm in length.

**Photo 1.** Leaves and top of mulberry were used to make mulberry leaf meal in this study

**Photo 2.** The hybrid rabbit (New Zealand x Local)

**Photo 3.**

**Photo 5.** Processing process of experimental feed pellets

The recommendations of NRC (1977), INRA (1984), De Blas and Mateos (1998) were applied to combine basal pellets with 17% protein (DM). This basal feed did not contain mulberry leaf meal (MLF) and contained fish meal (32.7% of the total protein of pellets). Then, the protein of fish meal were replaced by protein of MLF with levels 0%, 25%, 50%, 75% and 100% in the pellets (corresponding to MLF protein in pellets of 0.00%, 7.49%, 16.23%; 26.58% and 44.03%, respectively). The mixing ratios of the material (% DM) of pellets and chemical composition of experimental feeds were shown in Table 1 and Table 2.

**Table 1.** Ingredients in the experimental diets

	Experimental feed pellets				
	ML0	ML25	ML50	ML75	ML100
<i>Composition of diets, % DM basis</i>					
Vitamin-mineral amino acid	0.50	0.50	0.50	0.50	0.50
Corn flour	49.8	43.9	41.1	28.8	27.5
Peanut oil cake	5.00	5.50	5.50	6.00	6.00
Molasses	8.00	6.00	4.00	4.00	5.00
Rice bran	7.46	10.8	11.9	14.4	7.24
Salt	0.50	0.50	0.50	0.50	0.50
Beer residue	5.00	5.50	5.52	6.00	5.50
Broken rice	8.00	8.00	6.00	6.00	6.00
Soya residue	5.00	5.00	6.00	5.00	5.00
MLF	0.00	6.26	13.6	27.4	36.8
Fish meal	10.8	8.07	5.43	1.50	0.00

*ML0, ML25, ML50, ML75, ML100 corresponding to replaced protein of fish meal by protein of mulberry leaf meal with levels 0%, 25%, 50%, 75% and 100%*

## Experiment 1

### *Animals and design*

The digestibility trial was conducted according to the European method (Perez et al 1995). Forty crossbred rabbits (New Zealand x Local) with an average weight of  $1626.9 \pm 60.7$ g were kept in individual cages on this experiment. Rabbits were arranged in a completely randomized design with 5 treatments, corresponding to 5 replacing protein levels of fish meal by MLF in pellets levels: 0, 25, 50, 75 and 100%. There were 8 replications (digestive cages) with 8 rabbits (balanced for sex) in each experimental unit. The experimental diet was experimental pellets *ad libitum* and fresh Para grass (8% live weight).

The digestibility experiment was done during 12 days, comprising 7 days of adaptation to each diet followed by 5 days for collection of refused feed and faeces. The rabbits were fed 4 times a day at 7:00, 11:00, 14:00 and 22:00. The refused feed and faeces of each experimental rabbits were collected and recorded daily.

### *Sample collection and calculations*

Faeces were stored at  $-18^{\circ}\text{C}$  and at the end of each experimental rabbits samples will be pooled and mixed. Sub-samples were taken and dried at  $60^{\circ}\text{C}$  prior to chemical analysis. The apparent digestibility ratios were calculated from the

individual ratios of components to marker in the diet at the respective site of sampling. DM, OM, CP and NDF digestibility were calculated according to McDonald (2002). The apparent digestibility ratios were calculated according to the following formula.

$$\text{The apparent digestibility ratios (\%)} = (A-B)/A * 100$$

Where, A and B are the nutrient in feed and faeces.

## Experiment 2

The design and data collection of the rabbit nutrition study was performed according to the recommendations of the European Association for the study of rabbit nutrition (EGRAN) described by Fernández-Carmona et al (2005).

Fifty crossbred rabbits (New Zealand x Local) (2.5-3.0 months of age, body weight of 1216.4±105.8 g) were used in a completely randomized design with 5 treatments and 5 replications. There are 5 treatments corresponding to 5 replacing protein levels of fish meal by MLF in pellets: 0, 25, 50, 75 and 100%. The animals were fed four times a day at 7:00, 11:00, 14:00 and 22:00. The experimental diet was experimental pellets *ad libitum* and fresh Para grass (8% live weight).

### Chemical analysis

Feeds, refusals feed and faeces were taken for analyses of DM, OM, CP, CF and Ash following the procedures of AOAC (2000), Acid detergent fiber (ADF) and neutral detergent fiber (NDF) was determined according to Van Soest et al (1991).

### Statistical analysis

Data from the experiment were analyzed by ANOVA using the General Linear Model (GLM) procedure (Minitab Version 16.1, 2010). Pair-wise comparisons with a confidence level of 95 will be used to determine the effects of dietary treatment between groups. Results will be presented as Least Squares Means with their pooled standard errors.

## Results and discussion

### Feed characteristics

The chemical composition of experimental feeds were shown in table 2. Protein levels of the experimental pellets did not different, ranging from 17.0 to 17.1% in DM. Crude fiber, ADF and NDF in pellets increased corresponded to the increase in substitution of fishmeal protein by mulberry leaf protein in pellets.

**Table 2..** Chemical composition of experimental feeds (% DM)

	DM (%)	% in DM				
		OM	CP	ADF	NDF	Ash
ML0	92.9	94.3	17.0	7.56	13.0	5.75

ML25	93.1	94.1	17.1	9.21	15.0	5.87
ML50	92.1	93.0	17.1	12.2	15.6	6.89
ML75	93.1	92.8	17.0	14.8	18.7	7.24
ML100	92.2	92.3	17.1	15.6	19.5	7.75
Para grass	15.3	87.8	16.6	33.1	62.4	12.2

*DM: dry matter, OM: organic matter, CP: crude protein, CF: crude fiber; ADF: acid detergent fiber; NDF: neutral detergent fiber*

### Digestibility and nitrogen balance of rabbits

The digestibility of DM, OM, CP and CF was affected by replacing protein levels of fish meal by mulberry leaf meal, except for ADF and NDF (Table 3). The digestibility of DM, OM, CP and CF decreased corresponding to the increase of MLF in the diet.

**Table 3.** Apparent nutrient digestibility in different treatments

Item	Treatments					SEM	<i>p</i>
	ML0	ML25	ML50	ML75	ML100		
Digestibility, %							
DM	74.2 <sup>a</sup>	67.6 <sup>b</sup>	63.5 <sup>bc</sup>	61.9 <sup>bc</sup>	61.0 <sup>c</sup>	1.42	<0.001
OM	75.3 <sup>a</sup>	68.9 <sup>b</sup>	64.5 <sup>bc</sup>	62.7 <sup>c</sup>	61.3 <sup>c</sup>	1.40	<0.001
CP	71.7 <sup>a</sup>	60.7 <sup>b</sup>	49.1 <sup>c</sup>	49.1 <sup>c</sup>	48.2 <sup>c</sup>	2.20	<0.001
CF	36.9 <sup>a</sup>	33.2 <sup>ab</sup>	30.7 <sup>ab</sup>	29.8 <sup>ab</sup>	26.9 <sup>b</sup>	2.29	0.043
ADF	32.3	24.9	28.9	36.4	35.3	3.02	0.062
NDF	40.3	32.0	32.8	38.6	38.3	2.65	0.117

*DM: dry matter, OM: organic matter, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber ML0, ML25, ML50, ML75, ML100 corresponding to replaced protein of fish meal by protein of mulberry leaf meal with levels 0%, 25%, 50%, 75% and 100%. <sup>a, b, c</sup> values with different superscript letters within one row are significantly different at the level of 5%*

### Feed intake, growth rate and FCR

Results of Table showed that the increase in substitution of fish meal protein with MLF weren't affected on DM and CP intake, except for ADF and NDF intake, however, ADF and NDF intake increased significantly ( $P < 0.05$ ) with the increase of MLF in the diet.

**Table 4.** Mean values for DM intake of rabbits

Item	Treatments					SEM	<i>p</i>
	ML0	ML25	ML50	ML75	ML100		

DM intake, g/d							
Para grass	21.2	20.8	20.6	20.0	19.5	0.74	0.540
Pellets	56.8	60.9	60.8	61.0	61.0	2.32	0.653
Total	78.0	81.7	81.4	80.9	80.5	2.33	0.804
CP intake, g/d	13.2	13.9	13.8	13.7	13.7	0.44	0.823
ADF intake, g/d	11.4 <sup>a</sup>	12.5 <sup>a</sup>	14.2 <sup>b</sup>	15.6 <sup>bc</sup>	16.0 <sup>c</sup>	0.40	<0.001
NDF intake, g/d	20.7 <sup>a</sup>	22.1 <sup>ab</sup>	23.5 <sup>ab</sup>	23.7 <sup>b</sup>	24.1 <sup>b</sup>	0.66	0.010

DM: dry matter, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber ML0, ML25, ML50, ML75, ML100 corresponding to replaced protein of fish meal by protein of mulberry leaf meal with levels 0%, 25%, 50%, 75% and 100%. <sup>a, b, c</sup> values with different superscript letters within one row are significantly different at the level of 5%

DM and CP intake were not affected by the increasing MLF level in the diet (table 4, figures 1).

Live weight gain and FCR of rabbits tended to curve. Daily gain and FCR showed positive trends only at the replacement of 50% protein of fish meal by MLF in the pellets, then showed negative trends with further increases in MLF protein in diet (Table 5, Figures 2 and 3).

**Table 5.** Mean values for feed intake, live weight changes and FCR

Item	Treatments					SEM	p
	ML0	ML25	ML50	ML75	ML100		
Live weight, g							
Initial	1266	1226	1226	1177	1187	49.4	0.722
Final	2151	2142	2223	2117	2039	61.1	0.351
Daily gain, g/d	15.8 <sup>ab</sup>	16.4 <sup>ab</sup>	18.5 <sup>a</sup>	17.0 <sup>ab</sup>	15.4 <sup>b</sup>	0.86	0.010
DM intake, g/d	78.0	81.7	81.4	80.9	80.5	2.33	0.804
FCR	4.97	5.01	4.40	4.81	5.29	0.24	0.166

DM: dry matter ML0, ML25, ML50, ML75, ML100 corresponding to replaced protein of fish meal by protein of mulberry leaf meal with levels 0%, 25%, 50%, 75% and 100%. <sup>a, bc</sup> values with different superscript letters within one row are significantly different at the level of 5%

**Figure 1.** Effect of replacing protein levels of fish meal by MLF in pellets levels on DM feed intake of rabbits

**Figure 2.** Effect of replacing protein levels of fish meal by MLF in pellets levels on live weight gain of rabbits

**Figure 3.** Effect of replacing protein levels of fish meal by MLF in pellets levels on FCR of rabbits

## Conclusions

- The protein substitution of fishmeal by MLF affected the digestibility of DM, OM, CP, CF and live weight gain, but didn't affect the DM intake, the digestibility of ADF, NDF and FCR.
- Rabbits fed diets containing fishmeal protein replacement by MLF were optimized growth and FCR rates at 50% replacement level.

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