Inclusion of *Morus alba* leaf meal: its effect on apparent retention of nutrient, productive performance and quality of the carcass of naked neck fowls

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In order to evaluate the effect of mulberry (*Morus alba*) meal on apparent retention of nutrients, productive performance, yield and quality of the carcass of heterozygous naked neck fowls, two experiments were carried out. In the first experiment, a total of 24 male fowls of seven weeks old and 1.8 kg of liveweight were analyzed (six/treatment), using a completely randomized design. In the second experiment, an amount of 168 fowls, without being sexed and 28 d old, were analyzed (42 fowls/treatment), using a random block design. In both experiments, treatments were 0, 3, 6 and 9 % of inclusion of mulberry leaf meal in diets. In experiment 1, the apparent retention of DM, CP and NDF were measured. In experiment 2, the study determined food intake, weight gain, food conversion and sensorial quality of carcass. Apparent retention of neutral detergent fiber was reduced with 6 and 9 % of mulberry leaf meal ($P<0.001$), regarding apparent retention of DM when including 0, 3 and 9 % ($P<0.05$). Food conversion was more efficient ($P < 0.001$) (2.94) in fowls that consumed a diet with 3 % of this meal. Weights of the carcass, drumsticks + tighs, breast and abdominal fat were similar in the control and with 3% of the meal, and differed ($P < 0.001$) with the inclusion of 6 and 9 % of the meal. Indicators of taste, tenderness and juiciness did not show differences among treatments. Fowls consuming mulberry meal presented a higher pigmentation regarding control (0 %). Results suggest that it is possible to include up to 3% of mulberry leaf meal, without affecting apparent retention of nutrients, productive performance, yield and quality of carcass in heterozygous naked neck fowls.

Key words: *heterozygous naked neck fowls, mulberry leaf meal, productive performance*

Currently, maize and soybean are used for producing ethanol and agro-diesel (biocofuel), therefore, the prices of input used in diets for feeding animals has increased. This affects the production of animal origin protein, which is essential for human feeding (Simol *et al*. 2012).

The use of tree sources, in monogastric species, has become a valid strategy (Dale 2007), mainly in tropical countries, where there is a high production of plant biomass that could allow to reduce the use of food sources from other regions (Roa 2011).

Mulberry is one of the shrub forage plant that better adapts to tropical and subtropical areas. It shows high content of proteins (15 - 35 %), minerals (2.42 - 4.71 %), Ca (2.96 %), P (0.23-0.97) and metabolizable energy (ME) (9.4 MJ/kg) (Srivastava *et al*. 2006). However, according to Savón (2005), the use of these fibrous tropical feed has some limitations like incomplete characterization of its content of nutrients, presence of secondary metabolites and high content of cell wall. These characteristics could influence on digestive processes and on zoo-technique behavior of fowls, pigs, rabbits and Guinea pigs that receive these food sources.

The objective of this study was to evaluate the effect of mulberry leaf meal on apparent retention of nutrients, productive performance, yield and quality of carcass in heterozygous naked neck chickens.

Materials and Methods

Location, soil and sowing of plants. This research was carried out in the Universidad Técnica Estatal de Quevedo, Ecuador, located at 01°06' SL and 79°29' WL, at 75 m o.s.l., with a mean annual temperature of 24.7 °C, 87 % of relative humidity, 2613 mm of precipitations, 886 h of annual heliophany and soil of clay, sand and lime.

A total of 5,000 m^2 of mulberry were sowed, with sticks of 40 cm, at a rate of 40 cm between plants and a meter between furrows. Plants had a year of establishment and they were fertilized with natural fertilizers. For the chemical analysis, six samples were taken per age (30, 45 and 60 d of re-growth) (1 kg/age/sample), which were sent to the laboratory. Those of 45 d were the ones used due to their high content of protein.

Mulberry leaf meal obtaining. The mulberry leaf drying process was carried out under a roof, over a cement floor for three days. They were turned over three times every day for achieving a uniform drying. Later, they were grounded in hammer mill, branded Henkel 2040, with a capacity of 30 kg and a sieve of 1 mm. The mean chemical composition of mulberry leaf of 45 d of re-growth was (%): 92.81 DM, 24.78 CP, 2.96 Ca, 0.38 P, 39.54 NDF, 27.0 ADF, 17.5 hemicellulose, 12.4 cellulose, 6.10 lignin and 8.74 MJ ME/kg. For this study, the animals consumed four diets (treatments) during final and growth stages, with three levels of mulberry leaf meal (3, 6 and 9 %) and a control (0 % of mulberry leaf meal), formulated according to the requirements of this lineage (SASSO 2010) (table1).

Experimental procedure. In experiment 1, a total of

Table 1. Inclusion of mulberry leaf meal in diets of growth and ending for naked neck chickens

Raw materials, %	Mulberry meal, %							
	Growth stage (28-63 d)				Final stage (63-91 d)			
	0	3	6	9	0	3	6	9
Maize	56.33	54.22	52.10	49.88	52.33	50.38	48.31	46.09
Rice powder	15.00	15.00	15.00	15.00	20.00	20.00	20.00	20.00
Mulberry	0.00	3.00	6.00	9.00	0.00	3.00	6.00	9.00
Fish meal	4.00	3.70	3.50	3.30	3.20	3.00	2.70	2.50
Soybean cake	20.00	19.00	18.00	17.00	18.00	17.00	16.00	15.00
Palm tree oil	0.90	1.50	2.00	2.60	2.80	3.25	3.80	4.40
Calcium carbonate	1.40	1.20	1.00	0.80	1.30	1.00	0.80	0.60
Biofos	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Salt	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Premix Broiler[1]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DL-Metionine	0.20	0.20	0.20	0.20	0.22	0.22	0.22	0.22
L-lisine	0.12	0.13	0.15	0.17	0.10	0.10	0.12	0.14
Chemical Analysis[2]								
Crude Protein, %	17.68	17.01	17.05	17.08	16.74	16.78	16.70	16.80
ME, MJ/kg	12.34	12.00	12.13	12.33	12.30	12.34	12.32	12.40
Calcium, %	0.96	0.96	0.97	0.97	0.86	0.90	0.87	0.88
Total Phosphorous , %	0.63	0.64	0.62	0.64	0.62	0.65	0.68	0.67

[1]A kg of food contains: vitamin supplement: vitamin A (10000 UI), vitamin D3 (2000 UI), vitamin E (10 mg), vitamin K 3 (2 mg), Thiamine (1 mg)-B1, Riboflavin (5 mg), Pyridoxine (2 mg)- B 6 ,Vitamin B12 (15 mg), nicotinic acid (125 mg), calcium Panthetonate (10 mg), pholic acid (0.25 mg), and Biotin (0.02 mg). Mineral supplement: mineral selenium: Selenium (0.1 mg), Iron (40 mg), Copper (12 mg), Zinc (120 mg), Mg (100 mg), Iodine (2.5 mg) and Cobalt (0.75 mg).

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24 male heterozygous naked neck chickens were used for testing apparent retention of nutrients, which had a mean weight of 1.8 kg, seven weeks old, belonging to T451N breed, from a crossing between roosters T44N and recessive hens SA51(Sasso 2010). They were distributed in four treatments (0, 3, 6, and 9 % of mulberry leaf meal) and six repetitions per treatment.

The test of apparent retention of nutrients lasted 15 d: five for adapting (cages and diets) and ten for collecting feces. A mixture was made with 10 % of the feces, collected during 10 d and put into a freezer. After the collecting period, feces were mixed and 10% of them were taken for being dried at 60 °C, for 72 h at a forced air oven. Later, they were grounded up to 1 mm for chemical analysis.

Apparent retention was calculated through these formulas:

$$\text{Apparent retention DM} = \frac{\text{Consumed dry matter} - \text{Excreted dry matter}}{\text{Consumed dry matter}} \times 100$$

$$\text{Apparent retention of nutrients} = \frac{\text{DM x \% nutrient} - \text{DM in feces x \% nutrient}}{\text{Consumed DM x \% consumed nutrien}} \times 100$$

In experiment 2, a total of 168 heterozygous naked neck fowls without being sexed, from 28 to 91 d old, were used for measuring productive performance, yield and quality of carcass. They were distributed in four treatments, with six repetitions (7 fowls/ repetition) per treatment. The treatments conducted were the same as in the previous experiment (table 1). Water and food were offered *ad libitum*. Daily lightning was of 12 h. The fowls were vaccinated against Newcastle.

Intake (kg) (offered-rejected food), weight gain (kg) and food conversion were determined. The fowls of 91 d old were weighed and 12 of them were sacrificed per treatment, using the jugular vein bleeding method (Sánchez 1990), in order to determine the weights of carcass (kg), heart, liver, gizzard, drumsticks + thighs (g), breast (g), edible viscera (g) and abdominal fat (g).

For sensorial analysis of meat, samples of breast were kept at 25 °C. In order to determine organoleptic characteristics (taste, texture and juiciness) of breast meat of heterozygous naked neck fowls, the methodology of Anzaldúa (2005) was used. The sensorial quality of breast meat was determined by a panel of 12 tasters, selected from the Facultad de Ingeniería en Alimentos, from Universidad Técnica Estatal de Quevedo, Ecuador. Samples were defrosted

and cooked without salt for 30 min., at 100 °C (Anzaldúa 2005).

The members of the panel were selected according to the following criteria: healthy, non smokers, with no habits of drinking coffee or alcoholic drinks and aged between 35 and 50 years. A total of 12 trained panel members participated in this test, who received the samples (twelve portions of 10 g of cooked breast meat, without flavorings) previously codified. The quality sensorial parameters were: taste (herbal or normal flavor), tenderness (tender or hard), juiciness (juicy or dry) and pigmentation (white, creamy, pale yellow or intense pale yellow).

Chemical analysis. Samples were analyzed in Laboratorio del Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP), Ecuador. The content of DM and protein were determined according to AOAC (2006), and the contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose and lignin according to Goering and van Soest (1970).

Statistical methods. In experiment 1, in order to determined sensorial quality of carcass, a completely random design was used, with four treatments and six repetitions. In experiment 2, a random blocks design was applied with four treatments and six repetitions per treatment (7 fowls/repetition). The computer statistical package DiRienzo *et al.* (2012) was used for the statistical analysis of results. Mean values were compared using the test of Duncan (1955).

Results and Discussion

Experiment 1. Table 2 shows the values of apparent retention of DM, CP and NDF, with different levels of mulberry leaf meal.

The apparent retention of neutral detergent fiber (ARNDF) was reduced with the inclusion of 6 and 9 % of mulberry leaf meal ($P < 0.001$) compared to control, and 3 % of mulberry leaf meal and 9 % on the apparent retention of DM ($P < 0.05$). With 6 and 9 %, the concentration of insoluble fibrous material within the ration increased, which could provoke a higher speed of intestinal flow. With that, the time of food stay for the development of fermentative processes in the caecum decreases. With 3%, the animal has more digestible nutrients, given by the remaining ingredients of the diet (maize-soybean) (Martínez 2011).

Studies carried out with other fibrous sources have demonstrated that apparent retention of dry matter, crude protein and energy decreases with the increase of fiber level (Itzá *et al*. 2010 and Roa 2011). On this matter, Cáceres *et al*. (2006) informed that apparent retention of NDF of forage plants is affected due to the high content of mulberry cell wall (28.06- 38.3 %), which is confirmed in this study.

Experiment 2. Table 3 shows productive indicators of fowls.

Liveweight and weight gain, of 6 and 9 %, differed ($P < 0.001$) between them, and with the rest of treatments. This could be owed to the decrease of intake of meal treatments. However, despite that there was lower intake with 3 % of mulberry leaf meal regarding control, a higher food usage was demonstrated, which was represented by a better efficiency in conversion (2.94). These results are related to those informed in the test of apparent retention of DM and NDF (table 2). Control and 3 % of mulberry leaf meal presented similar performances and differed when 6 and 9 % of mulberry leaf meal was included. Apparently, the content of fibrous material in 3% did not affect these indicators.

Simol *et al*. (2012) used high levels of mulberry meal (20, 30, 40 and 50 %), and a control (0 %) in fattening fowls. These authors reduced cost/food in 26.09 %. Besides, intake decreased, while the level increased. Weights, except control, were neither significative (2047, 1992, 1994, 1758 and 1265 g) nor substantial in the market.

Olmo *et al*. (2012) worked with country fowls (70 d), and, when including levels of mulberry meal (leaves and stems), they confirmed that productive indicators decreased while the mulberry leaf meal levels increased. Weights were inferior (2033, 1680, 1539 and 1374 g) to those informed by Simol *et al*. (2012). Casamachín *et al*. (2007) studied the effect of mulberry leaf meal on captivity fowls. These authors

Table 2. Apparent retention of DM, CP and NDF in heterozygous naked neck chickens, with three levels of mulberry meal

Indicators	Levels of inclusion of mulberry meal, %				Sign.SE (±)
	0	3	6	9	
ARDM [1]	73.8[a]	73.4[a]	63.8[ab]	61.6[b]	3.1*
ARCP [2]	81.4	80.8	73.8	74.2	2.3
ARNDF [3]	80.3[a]	82.4[a]	61.5[b]	49.8[c]	3.6***

[abc] Different letters within the same line differ significantly at $P < 0.05$ (Duncan 1955).

[1] ARDM = Apparent retention of dry matter

[2] ARCP = Apparent retention of crude protein

[3] ARNDF= Apparent retention of neutral detergent fiber

* $P<0.05$ ***$P<0.001$

Table 3. Behavior of fowls from 28 to 91 days old

Indicators	Inclusion of mulberry leaf meal, %				Sign.SE (±)
	0	3	6	9	
Liveweight , kg/fowl	3.32[a]	3.33[a]	3.05[b]	2.80[c]	0.02***
Total weight gain, kg/chicken	2.61[a]	2.67[a]	2.45[b]	2.19[c]	0.03***
Total intake, kg/fowl	8.06[a]	7.84[b]	7.42[c]	7.35[c]	0.05***
Total food conversion	3.09[b]	2.94[c]	3.07[b]	3.35[a]	0.03***

[abc] Different letters within the same line differ significantly at P<0.05 (Duncan 1955).
*** P<0.001

used levels (0, 5, 10 and 15 %) of mulberry leaf meal and demonstrated that up to 5 % of mulberry leaf meal could be used without affecting productive indicators. However, Itzá *et al*. (2010) recommend the use of up to 4 % of mulberry leaf meal in meat fowls. Fowls do not use great amounts of fiber due to their digestive condition, which does not allow to degrade high amounts of food (Gonzalvo *et al*. 2001).

Diets with high content of fiber have low energy. It is known that fowls consume food until they cover their energy needs, and because of the great volume of fiber diets, a distention of the crop and gizzard is produced, leading to a decrease of intake. According to Gernat (2007), there are, in these organs, receptors sensitive to the pressure exerted to them and they send signals to the brain, where the satiation effect is produced and the intake stops.

Table 4 shows the relative weight (%) of the carcass of naked neck fowls. The weight of carcass, drumsticks + thighs, breast and abdominal fat from the control was similar to that with 3 % of mulberry leaf meal. These values differed (P < 0.001) when 6 and 9 % of mulberry leaf meal were included. These results demonstrate that fowls have higher food efficiency and response up to this level (3 % of mulberry leaf meal), which confirms the answers presented in the test of apparent retention of nutrients.

Fowls consuming diets with mulberry meal (3, 6 and 9 %) had higher weight of edible viscera and neck development. This is probably owed to the size or weight of an organ, which corresponds to the increase of its specific functions (Rodríguez *et al*. 2006). In this case, the highest mechanical function of the gizzard is physically reflected on the increase of its weight.

Fowls consuming 9 % of mulberry leaf meal showed higher yield of the carcass (79 %) and lower abdominal fat deposition (1.56 %), which represented a 53 % regarding the control (2.93 %).

It has been demonstrated that diets with high amounts of soluble fiber in fowls decrease the absorption of lipid and cholesterol at intestinal level. This physiological effect may be caused by soluble fiber (peptine) and lignin (Ayerza *et al*. 2002). The crude fiber and lignin of mulberry meal were of 14, 28 and 6, 10 %, respectively.

Table 5 shows results of the sensorial analysis performed to the quality of breast meat.

Indicators of taste, tenderness and juiciness did not show differences between treatments. Fowls consuming mulberry leaf meal had higher pigmentation (P <0.001) (visual appreciation) regarding fowls with 0 % of mulberry leaf meal. Machii (2000) and Moller *et al*. (2000) found the same effect on the egg yolk of grazing hens. Casamachín *et al*. (2007), Itzá *et al*. (2010) and Olmo *et al*. (2012) also confirmed this in fowls fed with mulberry meal.

Results suggest that it is possible to include up to 3% of mulberry leaf meal without affecting productive

Table 4. Effect of the inclusion of mulberry meal on the relative weight (%) of the carcass of naked neck fowls, at 91 d old

Indicators	Inclusion of mulberry leaf meal, %				Sign.SE (±)
	0	3	6	9	
Carcass yield	77.00[b]	74.00[c]	75.00[c]	79.00[a]	0.48***
Fat	2.93[a]	2.79[a]	2.01[b]	1.56[c]	0.05***
Drumsticks + thighs	22.30[a]	22.96[a]	20.62[b]	19.22[b]	0.52***
Breast	25.21[a]	25.96[a]	23.31[b]	21.73[b]	0.59***
Neck	3.85[c]	3.94[bc]	4.21[ab]	4.34[a]	0.11**
Edible viscera	3.42[b]	3.81[a]	4.11[a]	4.07[a]	0.10***
Carcass weight, kg	2.50[a]	2.40[ab]	2.30[bc]	2.20[c]	0.05***

[abc] Different letters within the same line differ significantly P<0.05 (Duncan 1955).
[1] Edible viscera (heart, gizzard and liver).
*** P<0.001 ** P<0.01

Table 5. Taste, texture and juiciness of breast meat and pigmentation of the carcass of heterozygous naked neck chickens, with different levels of inclusion of mulberry leaf meal

Treatments Indicators	Inclusion of mulberry leaf meal, %				Sign.SE (±)
	0	3	6	9	
Taste	1.67	1.50	1.42	1.08	0.15
Texture	1.75	1.58	1.42	1.33	0.14
Juiciness	1.83	1.58	1.50	1.92	0.14
Pigmentation	2.17^{c}	2.83^{b}	3.33^{ab}	3.67^{a}	0.16***

^{abc} Different letters within the same line differ significantly at P < 0,05 (Duncan 1955).
Taste: 1-2 normal, 2.3 others
Texture: 1-2 tender, 2-3 hard
Juiciness: 1-2 juicy, 2-3 dry
Pigmentation: 1 white, 2 creamy, 3 light yellow, 4 intense yellow
*** P < 0.001

performance, apparent retention of nutrients and carcass quality of heterozygous fowls.

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