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APPARENT DIGESTIBILITY OF RHODE ISLAND RED HENS' DIETS CONTAINING *Leucaena leucocephala* AND *Moringa oleifera* LEAF MEALS

[DIGESTIBILIDAD APARENTE DE LA DIETA CON HARINA DE HOJAS DE *Leucaena leucocephala* y *Moringa oleífera* EN GALLINAS RHODE ISLAND RED]

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SUMMARY

This study consisted of two experiments aimed to evaluate the dietary digestibility by Rhode Island Red (RIR) hens fed different levels of *Leucaena leucocephala* (LLM) or *Moringa oleifera* (MOLM). In each experiment, four groups each of nine birds were assigned to four dietary treatments containing 0, 5, 10 and 15 % of LLM (Exp 1) or MOLM (Exp 2). All groups received smashed diets, containing similar metabolizable energy and crude protein for six weeks and during the last four days, feed intake was recorded daily and excreta was totally collected twice a day and weighed individually. The dietary treatments had no significant effects on the intake of dry matter (DM), organic matter (OM), gross energy (GE), crude protein (CP) or neutral detergent fiber (NDF) in both experiments, while the acid detergent fiber (ADF) intake increased linearly with the increase of LLM and MOLM levels. The apparent digestibility of DM, CP, ADF, and OM decreased linearly with the increase of LLM and MOLM levels. The apparent digestibility of gross energy decreased linearly in LLM experiment, while it was not affected in MOLM experiment. LLM and MOLM could be recommended for RIR hens between 5 and 10% of the diet.

Keywords: Digestibility; nutrients intake; forages; hens; *Leucaena leucocephala; Mo*ringa oleifera; Rhode island hens.

RESUMEN

Este estudio consistió en dos experimentos destinados a evaluar la digestibilidad de la dieta en gallinas Rhode Island Red (RIR) alimentadas con diferentes niveles de Leucaena leucocephala (LLM) o Moringa oleífera (MOLM). En cada experimento, cuatro grupos, de nueve aves fueron asignados a cuatro tratamientos dietéticos que contenían 0, 5, 10 y 15% de LLM (Exp. 1) o MOLM (Exp. 2). Todos los grupos recibieron dietas con la misma energía metabolizable y proteína cruda, durante seis semanas. Durante los últimos cuatro días, el consumo de alimento se registró diariamente y la excreta se recogió dos veces al día y el peso se registró individualmente. Los tratamientos dietéticos no tuvieron efectos significativos en la ingesta de materia seca (MS), materia orgánica (MO), energía bruta (EB), proteína cruda (PC) o fibra neutro detergente (FND) en ambos experimentos, mientras que el consumo de fibra detergente ácida (ADF) aumentó linealmente con el aumento de los niveles de LLM o MOLM. La digestibilidad aparente de MS, PC, ADF, y OM disminuyó linealmente con el aumento de los niveles de LLM y MOLM. El coeficiente de digestibilidad de la energía bruta se redujo linealmente en el experimento de LLM, mientras que no afectó en el experimento MOLM. La inclusión en la dieta de LLM y MOLM podría ser recomendada para las gallinas RIR entre 5 y 10%.

Palabras clave: Digestibilidad; *Luecaena leucocephala; Moringa oleífera;* gallinas Rhode Island.

INTRODUCTION

The current acute short supply of animal protein for humans in most developing countries along with the expensive prices of the conventional feedstuffs have necessitated several investigations on the potentials of some novel feed resources for livestock; such as leaf meals which are usually cheap and easily available (Sarmiento, 2001; Odunsi *et al*., 2002; Ige *et al.*, 2006; Nworgu and Fasogbon, 2007; Atawodi *et al*., 2008). The feed cost represents 60 to 80% of the total costs involved in poultry enterprises. Therefore, any reduction in feed cost will reduce remarkably the total production costs. In addition, using leaf meals could help in alleviating the competition between humans and animals for some conventional feedstuffs, such as soybean meal and maize (Ige *et al*., 2006; Horsted, 2006).

Leucaena leucocephala and *Moringa oleifera* leaf meals (LLM and MOLM) are seemingly good feed alternatives to commercial livestock and poultry in the tropics (Makkar and Becker, 1997; Agbede, 2003). *Leucaena leucocephala* and *Moringa oleifera* leaves are available and cheap in vast areas worldwide (Brewbaker and Sorensson, 1990; Morton, 1991; Price, 2000). The LLM and MOLM contain considerable amounts of crude protein, essential amino acids, metabolizable energy, vitamins and minerals. Therefore, the LLM and MOLM are expected to be sustainable resources for commercial poultry production in the tropics (Makkar and Becker, 1997; Price, 2000; Elkhalifa *et al*., 2007; Kakengi *et al*., 2007; Atawodi *et al*., 2008). In comparison with ruminants and other monogastric animals like rabbits and pigs, the digestive system of chickens is very simple and has limited capacity to digest high fibrous materials (Esonu *et al*., 2006; Ige *et al*., 2006). Poor laying and growing performances of chickens which fed diets with high inclusion levels of leaf meals were reported. Therefore, including the leaf meals in the chicken diets might go through good knowledge of their nutritional value and its impact on nutrients digestibility (Angkanaporn *et al*., 1994; Esonu *et al*., 2006; Kakengi *et al.*, 2007; Iheukwumere *et al*. 2008; Ayssiwede *et al*., 2010).

Measuring digestibility is a way to evaluate the availability of nutrients. In addition, an accurate prediction of overall nutritive value can be made when digestibility is combined with nutrients intake data (Khan *et al*., 2003). The objective of this study was to evaluate the effect of dietary inclusion of either *Leucaena leucocephala* or *Moringa oleifera* leaf meal on nutrients digestibility by Rhode Island Red hens, which is expected to present a recommendation about the possible inclusion level of LLM and MOLM in the laying hen diets.

MATERIALS AND METHODS

The experimental site

The experimental work was carried out using the poultry facilities of the Faculty of Veterinary Medicine and Animal Science (FMVZ), University of Yucatan (UADY), Yucatán, Mexico. The climate is sub-humid, with an average annual rainfall (highly variable) of 960 mm, and 6-7 months of dry period; the annual average temperature is 26 °C. The soils, are calcareous and mainly shallow (<10 cm in depth), are classified as Rendzinas and Lithosols and are of moderate fertility, with 1.0 to 1.5 % organic carbon content and a pH range of 7.5 to 7.8.

Birds, treatments and experimental design

The study consisted of two feeding experiments, which were carried out simultaneously following similar design. In each experiment, thirty six Rhode Island Red hens at 36 weeks of age with 60% egg laying rate on average and 2 kg body weight, were randomly divided into four groups each of nine birds which were allocated in individual cages (40 x 40 x 40 cm). The four groups were corresponded to four dietary treatments containing 0 (control), 5, 10 and 15 % of *LLM* (Exp1) or MOLM (Exp 2), respectively. All groups received diets in the form of meal, containing similar metabolizable energy and CP levels (16% and 2900 kcal ME/kg diet, as fed basis).

Preparing the leaf meals

L. leucocephala and *M. oleifera* fresh leaves were harvested from trees (two- year old, last harvest was 4 months before) growing at the FMVZ farm, under the tropical conditions of the Yucatán Peninsula, southern Mexico. As described by Morton (1991) and Shelton and Brewbaker (1994), the young branches were cut from trees, leaves were separated from branches, spread out and dried under shade for a period of 1 day. Thereafter, they were dried in ovens (60ºC) for two days. The dried leaves were grounded with a hammer mill (3.0 mm sieve) to make the LLM or MOLM, which were incorporated to the experimental diets.

Digestibility study

The hens were fed on the experimental diets for six weeks, and during the last four days (Ige *et al*., 2006), feed intake was recorded daily for the individual birds. Fresh droppings (feces + urine) were collected and weighed two times a day for the individual birds, kept individually, oven dried at 60˚C and weighed. At the end, the collected excreta per bird were pooled, mixed, grounded and stored respectively for chemical analyses.

Laboratory analyses

Chemical composition of experimental diets (Table 1), leaf meals (Table 2) and excreta samples including; dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), gross energy (GE), ash, calcium and phosphorus were determined at FMVZ nutrition lab, following the standard methods of AOAC (1990). A graded volumetric flask was used to measure the volume of each diet, in which five replicates (each of 100 g) of each diet were included (Table 3).

Determination of apparent nutrients digestibility

The apparent coefficients of nutrients digestibility were determined according to the following formula: Apparent nutrient digestibility= [(NI-NE) / NI] x100 Where, NI represented the nutrient intake and NE expressed the nutrient excreted (Ayssiwede *et al.*, 2010). In case of energy, this estimate was called apparent metabolizability of energy (AME), the average daily ME intake was calculated by multiplying the average GE intake by its AME coefficient (Kendeigh *et al*., 1977; Karasov, 1990). Similarly, the ME for the experimental diets was calculated by multiplying its GE (Kcal/kg diet) content by its AME coefficient.

Statistical analyses

The complete randomized design was used and data in each experiment were analyzed by response surfaces using orthogonal contrasts to evaluate the linear, quadratic and cubic effects of the LLM or MOLM levels, using the general linear model (GLM) procedure of SAS (2002).

Table 1. Composition of experimental diets.

Items	Exp. 1 diets (LLM[1] treatments)				Exp. 2 diets (MOLM[2] treatments)			
	0% Control	5%	10%	15%	0% Control	5%	10%	15%
Ingredients (% diet)								
Leaf meal	0.00	5.00	10.00	15.00	0.00	5.00	10.00	15.00
Sorghum	63.94	60.32	56.22	52.13	61.23	59.34	57.43	55.52
Soya bean meal	18.32	16.22	14.21	12.21	19.30	15.96	12.63	9.30
$CaCO_3$ (38%)	9.10	8.75	8.48	8.21	9.00	8.67	8.34	8.00
Canola meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soya oil	1.00	1.92	3.13	4.33	2.83	3.23	3.62	4.02
Di-Cal-phosphate	1.53	1.56	1.59	1.62	1.52	1.50	1.50	1.50
Lysine	0.10	0.18	0.27	0.35	0.11	0.23	0.35	0.47
Methionine	0.17	0.21	0.26	0.31	0.17	0.23	0.29	0.35
NaCl	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Flavomicine 4%	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mycosorb	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Funginat 42	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Minerals premix [a]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamins premix [b]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Anti-oxidant	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Chemical composition (on DM basis)								
DM (%)	89.55	89.82	90.68	90.68	89.96	89.8	89.9	90.24
GE (Kcal/kg)	3886.3	4010.9	3889.1	4010.8	3999.4	4086.1	4123.6	4128.2
CP (%)	18.06	18.24	18.58	18.83	18.39	17.66	17.88	17.45
ADF (%)	7.87	9.39	10.32	10.49	7.87	9.5	10.16	10.16
NDF (%)	54.35	58.19	59.14	66.85	52.34	59.74	61.04	60.29
Calcium (%)	3.47	3.69	3.42	3.64	3.1	3.45	3.38	3.3
Phosphorus (%)	0.64	0.65	0.62	0.68	0.64	0.66	0.67	0.51
Ash (%)	13.49	12.5	12.09	12.88	13.04	12.47	12.4	12.78

[a]: Content kg^{-1} of diet: Manganese, 65 mg; iodine, 1 mg; iron, 55 mg, copper, 6 mg; zinc, 55 mg; selenium, 0.3 mg; [b]: Content kg^{-1}: vitamin A, 8000 UI; vitamin D, 2500 UI; vitamin E, 8 UI; vitamin K, 2 mg; vitamin B12, 0.002 mg; riboflavin, 5.5 mg; pantothenate of calcium, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; thiamine, 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg. [1] *Leucana leucocephala* leaf meal [2] *Moringa oleifera* leaf meal.

Table 2. Chemical analysis of *Leucaena leucocephala* (LLM) and *Moringa oleifera* (MOLM) leaf meals.

Leaf meal	DM %	Nutrients content (on dry matter basis)						
		CP%	ADF%	NDF%	Ca %	P %	Ash %	GE(Kcal/kg)
LLM	91.0	23.61	25.69	40.38	1.62	0.18	8.27	4731
MOLM	91.22	19.76	27.11	44.42	2.13	0.24	9.61	4456

Dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), gross energy (GE), calcium (Ca), available phosphorus (P)

RESULTS

The results showed considerable amounts of CP (23.61 %) and GE energy (4731 Kcal/kg DM) in LLM. The corresponding values in MOLM amounted 19.76% CP and 4456 Kcal/kg DM, respectively. In addition, they contain good levels of calcium and phosphorus. The volumes of dietary treatments (Table 3) increased linearly with the increase in LLM and MOLM levels in the diets.

The results of feed and nutrients intake of RIR hens fed on diets containing different levels of LLM and MOLM are showed in tables 4 and 5, respectively. The dietary treatments had no significant effect on the ingested feed, dry matter (DM), organic matter (OM), gross energy (GE), crude protein (CP) or neutral detergent fiber (NDF) in both experiments, while the acid detergent fiber (ADF) consumption increased linearly with the increase of either LLM or MOLM levels.

Table 3. The volume averages (of 100 g) of the experimental diets contained *Leucaena leucocephala* (LLM) or *Moringa oleifera* (MOLM) leaf meal.

Experiments	Dietary treatments				SEM	*P*- value		
	0%	5%	10%	15%		Linear	quadratic	Cubic
Exp 1. LLM diets volumes (ml)	142.85	151.42	157.53	163.68	1.20	0.0070	0.7830	0.8673
Exp 2. MOLM diets volumes(ml)	141.91	157.01	168.82	179.47	1.66	0.0050	0.8366	0.6751

SEM: Standard error of mean, P: Probability

Table 4. Feed and nutrients intake of Rhode Island Red Hens fed diets containted *Leucaena leucocephala* leaf meal (LLM).

Nutrients	LLM treatments				SEM	*P* –value		
	0%	5%	10%	15%		Linear	quadratic	Cubic
FI (g/d)	106.81	105.50	101.25	98.56	4.18	0.1307	0.8698	0.8117
DM (g/d)	95.65	94.76	91.81	89.37	3.76	0.2054	0.8384	0.8801
OM (g/d)	82.74	82.91	80.71	78.37	4.57	0.4597	0.7862	0.914
GE (Kcal/d)	371.73	380.07	357.08	359.87	20.76	0.5307	0.8939	0.5408
CP (g/d)	17.27	17.28	17.05	16.89	0.95	0.754	0.9282	0.9441
ADF (g/d)	7.52	8.89	9.47	9.41	0.50	0.0096	0.165	0.9448
NDF (g/d)	51.98	55.14	54.30	59.90	3.16	0.1146	0.7007	0.4652

SEM: standard error of mean, P: probability**,** FI: feed intake, DM: dry matter, OM: organic matter, GE: gross energy, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber

Table 5. Feed and nutrients intake of Rhode Island Red Hens fed diets contained *Moringa oleifera* leaf meal (MOLM).

Nutrients	MOLM treatments				SEM	*P* –value		
	0%	5%	10%	15%		Linear	quadratic	Cubic
FI (g/d)	113.61	4.68	111.22	107.43	4.68	0.3411	0.7409	0.9629
DM (g/d)	102.20	4.21	99.99	96.95	4.21	0.3687	0.79	0.9612
OM (g/d)	88.87	3.68	87.59	84.56	3.68	0.3973	0.6928	0.9646
GE (Kcal/d)	408.75	17.45	412.31	379.52	17.45	0.9859	0.8181	0.923
CP (g/d)	19.81	0.75	17.87	16.91	0.75	0.0142	0.5353	0.4136
ADF (g/d)	8.04	0.41	10.15	9.85	0.41	0.0028	0.027	0.8954
NDF (g/d)	53.49	2.51	60.28	59.17	2.51	0.1455	0.1118	0.5591

SEM: standard error of mean, P: probability**,** FI: feed intake, DM: dry matter, OM: organic matter, GE: gross energy, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber

The results of apparent nutrients digestibility (tables 6 and 7) revealed a linear decrease for DM, CP, ADF, and OM with the increase of LLM and MOLM levels. MOLM treatments did not show any significant effect on GED, while it decreased linearly in LLM trial. The dietary treatments had no effect on NDF digestibility in both trials.

The GED coefficients were used to calculate the metabolizable energy (ME) of the experimental diets, which amounted to 2933, 2831, 2585, and 2600 Kcal/kg diet at 0, 5, 10 and 15% LLM, respectively. The corresponding ME values at the same levels of MOLM treatments were 3104, 3073, 2873 and 3025 Kcal/kg diet, respectively. The average ME intake amounted, 313, 300, 262 and 260 (Kcal/hen/d) at 0, 5, 10 and 15% LLM, respectively. Meanwhile, the ME intake was 353, 347, 330 and (Kcal/hen/d), at 0, 5, 10 and 15% MOLM, respectively.

Table 6. Apparent digestibility of Rhode Island Red Hen diets contained *Leucaena leucocephala* leaf meal (LLM).

Nutrients	Nutrients digestibility (%) of LLM treatments				SEM	*P* –value		
	0%	5%	10%	15%		Linear	quadratic	Cubic
DM	75.90	69.46	65.34	62.34	2.51	0.0004	0.498	0.9165
OM	80.89	74.36	70.88	66.76	2.22	0.0001	0.5913	0.7129
GED	84.28	78.59	73.31	71.49	1.90	0.0001	0.3197	0.7244
CP	60.95	47.54	45.21	39.35	5.56	0.0075	0.4776	0.5393
ADF	45.59	39.94	35.48	25.63	4.31	0.0021	0.6317	0.7399
NDF	83.01	81.73	80.53	79.44	1.42	0.0703	0.9478	0.9957

SEM: standard error of mean, P: probability, DM: dry matter, OM: organic matter, GED: Gross energy digestibility, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber

Table 7. Apparent digestibility of Rhode Island Red Hen diets contained *Moringa oleifera* leaf meal (MOLM).

Nutrients	Nutrients digestibility (%) of MOLM treatments				SEM	*P* –value		
	0%	5%	10%	15%		Linear	quadratic	Cubic
DM	83.97	75.34	72.90	72.94	2.20	0.0011	0.0587	0.7101
OM	84.04	80.50	77.86	78.03	2.63	0.0189	0.4872	0.8738
GED	86.29	83.77	80.15	81.22	2.19	0.0741	0.4195	0.5603
CP	70.30	63.96	62.57	56.70	4.96	0.0128	0.1411	0.6149
ADF	64.94	49.74	41.56	40.69	4.92	0.0009	0.1559	0.989
NDF	86.26	84.56	84.25	82.76	2.26	0.2937	0.9635	0.8016

SEM: standard error of mean, P: probability, DM: dry matter, OM: organic matter, GED: Gross energy digestibility, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber

DISCUSSION

The feed and nutrients intake of chickens is affected by the increased diet bulkiness (D'Mello, 1991). However high bulkiness results were observed in the diets of high levels of LLM and MOLM, no significant effects were detected on FI or nutrients intake (except for ADF) in both trials. The FI results were in agreement with FI results reported by Ayssiwede *et al*. (2010), who reported similar FI values of the indigenous Senegal hens fed diets with 0, 7, 14 or 21% LLM. When chickens receive diets with high bulkiness, which is commitment to high fiber content, they may increase their FI in order to meet their nutrients requirements as reported by Kakengi *et al*. (2007). Indeed, the hens are likely try to eat until they meet their energy demand (Smith, 1999). Low energy utilization in CF component of MOLM or LLM was reported (D'mello and Acamovic, 1989; Kakengi *et al*., 2007). As a solution, Kakengi *et al*. (2007) recommended the use of high energy materials when the MOLM is added in high levels in the laying hen's diets. In the current study, the oil was used to formulate Iso-caloric dietary treatments.

The obtained digestibility coefficients revealed a linear reduction in CP, DM, OM and ADF by the increase of MOLM and LLM levels. In addition, GED decreased linearly by the increase of LLM levels. In contrast of our results, Ayssiwede *et al*. (2010) reported feeding hens at 0, 7, 14 and 21% LLM and had no significant effect on utilization values of DM, OM, CP and energy. However, they concluded that the controversy of their results with others is due to the fact that they have treated the

LLM with additive ferric-sulphate to form a complex with mimosine, which led to eliminate its toxicity. Indeed, Mimosine is considered toxic for poultry and constitutes 7% of the total protein existed in *LLM* (Meulen *et al*., 1979; D'Mello and Acamovic, 1982). Detoxification of LLM could be possible by using ferric sulfate, but the low AME remains the major challenging problem with LLM (D'Mello and Acamovic, 1989), which could be associated with the decreased AME values obtained in LLM treatments in the current study. Bhatnagar *et al.* (1996) and Kakengi *et al.* (2007) showed that including high levels (10 and 15%) of LLM and MOLM in the diets of laying hens has contributed to low available energy and CP. Similar results with broilers are reported by Iheukwumere *et al*. (2008), where the utilization values of DM, CP and CF were lower in 10 and 15% Cassava leaf meal than its control. The obtained CP and DM digestibility values were comparable to those found by Ige *et al.* (2006), where the hens fed on 0, 5, 10, and 15% of gliricidia leaf meal, respectively.

The obtained ME (Kcal/kg diet) as well as the daily ME intake at all MOLM treatments were in the recommended ranges by NRC (1994), which reported to be amounted to 2900 Kcal/Kg diet; and 292 Kcal/hen/day. The corresponding values which were obtained at 10 and 15% LLM were lower than the recommended range, while the achieved values at 0 and 5% LLM levels were within the recommended range. That could indicate that the hens fed on 10% and 15% LLM of their diet could not meet their ME demands. The same conclusion was reported by D'Mello and Acamovic (1989).

The low digestibility of crude protein observed in high fiber diets is attributed to the indigestible cell wall which reduces the digestion and absorption of protein overall (Nyman *et al*., 1990). In addition, Bolton and Blair (1974) reported that up to 20 percent of the nitrogen in dried forages maybe present as non-protein compounds. Poor nutrient utilization observed could be in general due to the increased fiber content of the diets with higher LLM and MOLM levels, also attributed to the low capacity of the chicken digestive system in utilizing the high fibrous feeds. The caeca, which is the unique place for digesting plant fiber and where the major concentration of intestinal micro-organisms is found in mature birds, is very short. In addition, previous reports revealed no more than 25% of the total dry matter passing through the intestines enters the caeca (Son *et al.,* 2002; Odunsi *et al*., 2002 and Ige *et al*., 2006). Unlike ruminants, and even some species like pigs and rabbits, chickens lack the anatomic adaptations and the necessary digestive microorganisms to support processes of fermentation, which is necessary for efficient utilization of fibrous feeds in order to cover part of their nutrient needs (Esonu *et al*., 2006; Ige *et al*., 2006).

CONCLUSION

Including LLM or MOLM upto 15% of the diets of RIR hens had no effect on the ingested feed, DM, GE, CP, or NDF. According to the obtained digestibility coefficients of dietary nutrients, the incorporation of LLM or MOLM in the diets of Rhode Island Red hens could be recommended between 5 and 10% of the diet.

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