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Technical note

Effect of *Moringa oleifera* intake on productive and toxicological parameters in broiler chickens

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Abstract:

Poultry farming is common in rural Mexico in part because it provides extra household income. Alternative protein sources for poultry feed are needed for these systems. An evaluation was done of how 10% inclusion of *Moringa oleifera* leaf in diets for broilers affected productive and blood parameters. A proximal analysis, and Fe, Ca and tannins contents were quantified for moringa leaf flour. Four productive and six blood parameters (two proteins and four enzymes) were evaluated in Ross-308 broilers. Histopathological analyses were done of liver and kidney tissue. The moringa leaf flour contained 33.4% protein, and had high iron (19.7 mg/100 g) and calcium (2593.3 mg/100 g) contents, as well as low tannins content (24.4 mg CE/100 g). Compared to a control, daily weight gain decreased 12 % in the moringa leaf treatment while productivity index values decreased 20%. No differences were observed between the control and treatment in terms of protein content, and alanine aminotransferase and aspartate aminotransferase activities. However, differences in albumin, alkaline phosphatase and the glutamyl transpeptidase

levels suggest possible liver and renal damage in the moringa leaf treatment. These results were not confirmed in the histological analysis. Further research is needed using lower moringa leaf flour inclusion levels to compare to the present results and better define possible inclusion levels in poultry diets.

Key words: Broilers, Moringa, Productivity, Serology, Histopathology.

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Commercial poultry farming is a major industry in Mexico[1]. Poultry feed can include pastes from cottonseed, peanuts, safflower seeds, sesame seeds and coconut as sources of protein and other nutrients, although these are all lysine deficient. Soybean paste, in contrast, is a protein source with an optimal amino acids balance for animal feed[2]. Poultry farming, including growing of backyard chickens, is important in the country's rural communities since it provides families with meat and eggs as well as surplus birds for sale[3]. Greater research is needed on protein sources from locally available resources which can be added to the poultry feeds used in rural areas such as corn, food scraps and other materials.

The tree *Moringa oleifera* has been studied as a protein source for animals[4,5] and humans[6]. Native to India and Pakistan, it was introduced to Mexico[7]. The moringa leaf contains 20 to 30 % protein, 5.0 to 7.5 % fat and 25 to 31 % fiber[8,9]. It is also a good source of iron, calcium and vitamin C[9]. Due to the other phytochemicals it contains, moringa leaf has also been recommended for treating gastrointestinal ulcers[10], lowering cholesterol levels and as a source of antioxidants[11].

Moringa leaf has been used in feed for rabbits[12], goats[5], sheep[13] and cows[14], among other domestic species. Only minimal research has been done on the use of moringa leaf as an ingredient in feed for broilers. Studies have been done evaluating its effect on intestine morphology and the condition of other internal organs[15,16], breast fatty acids content[17], tibia strength and mineral content[18], animal weight, hematology and immune response[4,19]. Only two of these studies have evaluated moringa leaf's effect on weight gain[4,19], and one used *Moringa stenopetala*[4], a different species in the same genus.

The present study objective was to characterize nutritional content and vitamin C and condensed tannins levels in *Moringa oleifera* leaf flour, and evaluate its effect on productive and toxicological parameters when included in diets for broiler chickens.

Leaves were harvested from two-year-old *M. oleifera* trees in an orchard in the municipality of Gasca, in the state of Guanajuato, Mexico. Immediately after harvest the leaves were washed and disinfected with a 0.1% sodium hypochlorite solution followed

by sterile distilled water, and dried in the shade. The dried leaves were milled with a hammer mill (2.0-3.0 mm sieve) and stored at -20 °C until analysis and inclusion in broiler diets. Experimental animals were 180 ROSS-308 line broilers (39 ± 1.5 g initial weight), mixed, which were one-day old at experiment outset.

The animal trial was carried out at the Poultry Unit of the Livestock Area of the Zootechnical Station of the Autonomous University of Aguascalientes, in the municipality of Jesús María, in the state of Aguascalientes. The chickens were randomly selected to form two groups with three repetitions of thirty birds each. They were fed either a conventional feed (T1) or a conventional feed plus 10% moringa leaf flour (MF). Both diets were isoproteic and isoenergetic[20] (Table 1). Feed and water were freely available throughout the 42-day experimental period.

Table 1: Experimental diet ingredients and nutritional composition

	***Moringa oleifera* leaf flour (%)**			
	0	**10**	**0**	**10**
Ingredients (g/kg)	Broilers 1-21 days		Broilers 22-42 days	
Sorghum (8.5%)	600	560	685	640
Soy paste (46%)	335	280	250	200
Moringa leaf flour (33.4%)	0.0	100	0.0	100
Vegetable oil	25	20	25	20
Micro pollo eng 1[a]	40	40	0.0	0.0
Micro pollo eng 2[b]	0.0	0.0	40	40
Calculated[c]				
Metabolic energy/bird, kcal/kg	3150	3150	3200	3200
Crude protein, %	21.0	21.0	18.0	18.0
Total lysine, %	0.52	0.54	0.48	0.50
Digestible lysine, %	0.81	0.83	0.75	0.75
Digestible methionine, %	0.73	0.75	0.66	0.68
Digestible methionine+cysteine, %	0.23	0.24	0.20	0.21
Digestible threonine, %	0.13	0.14	0.11	0.12
Digestible tryptophan, %	4.0	4.4	3.5	4.0
Digestible arginine, %	0.884	0.902	0.824	0.411
Calcium, %	0.213	0.213	0.205	0.205
Available phosphorous, %	1.236	1.263	1.115	1.139
Na, %	1.137	1.163	1.029	1.050
Crudeber, %	0.350	0.451	0.311	0.824

[a] Initiation feed supplement (g/kg product): 12.6 L-lysine HCl; 56.6 methionine; 10.2 60% choline chloride (National);274 21% orthophosphate; 10 25% copper sulfate; 50 ground salt (Roche); 10 mineral oil 90 NF (Petroblanc) ; 10 Bacitracin B.M.D. 110; 169.4 soybean paste; 40 baking soda; 7.6 Ronozyme VP; 10 Sacox; 268 M-20 calcium; 60 V/init broiler plus NF/3*Ton; 7.5 Vip/Prem/A/Prem Lucantin yellow at 10; 4 Invivo/P/AYC/Prem Ronozyme Hiphos GT/1*Ton.

[b] Mineral, vitamin and amino acid supplement (kg product): 10,000,000 IU vit A; 3,000,000 IU vit D3; 7,000 IU vit E; 1.5 g vit K; 4.4 g riboflavin;15 mg vit B12; 5.5 g pantothenic acid; 25 g niacin; 25 mg biotin; 1 g thiamine; 0.5 g folic acid; 32.5 g iron; 50 g manganese; 50 g zinc; 4.5 g copper; 150 mg selenium; 450 mg iodine; 150 g choline; 50 g antioxidant. Vehicle: 3 Kg.

[a] Finishing feed supplement (g/kg product): 25 L-lysine HCl; 1.8 L-threonine; 64.8 methionine; 64.8 60% choline chloride (National); 186 21% orthophosphate; 10 25% copper sulfate; 50 ground salt (Roche); 10 90 NF mineral oil (Petroblanc); 10 Bacitracin B.M.D. 110; 235.6 soybean paste; 30 baking soda; 5 Ronozyme VP; 11 Sacox; 5 Progen 20; 244 M-20calcium. 64 V/init broiler plus NF/3* Ton; 37.5 Vip/Prem/A/Prem Lucantin yellow at 10.

[b] Mineral, vitamin and amino acid supplement (kg product): 10,000,000 IU vit A; 3,000,000 IU vit D3; 7,000 IU vit E; 1.5 g vit K; 4.4 g riboflavin;15 mg vit B12; 5.5 g pantothenic acid; 25 g niacin; 25 mg biotin; 1 g thiamine; 0.5 g folic acid; 32.5 g iron; 50 g manganese; 50 g zinc; 4.5 g copper; 150 mg selenium; 450 mg iodine; 150 g choline; 50 g antioxidant.

[c] Calculated according to NRC (1994).

Proximal analysis of the moringa flour was done with established methods for protein (960.52), fiber (991.43 G, H), fat (920.85) and ash (923.03)[21]. Carbohydrate content was calculated by the percentage difference compared to the previous analyses. Iron and calcium content were determined by atomic absorption spectrometry[22], and vitamin C by high performance liquid chromatography (HPLC)[23]. Total phenols[24] and condensed tannins[25] contents were also determined.

Daily records were kept of feed provided and consumed. At the end of the experiment calculations were done of average weight (AW), daily weight gain (DWG), feed conversion rate (FCR) and the productivity index (PI)[26].

After 42 d blood samples were taken to quantify total proteins (TP) and albumin (ALB); as well as blood levels of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT)[27].

Blood samples were taken from three chickens selected from each of the three replicates of the two groups originally formed. These were killed by cervical dislocation following the Ethical Management and Humanitarian Sacrifice Guide of the Autonomous University of Aguascalientes. Samples (1 cm^3) of liver and kidney were taken for histopathological analysis[28]. In the liver, evaluations were done of the condition of the histo-architecture of the hepatic parenchyma, presence or absence of inflammation cells, the Möll space, the Disse space, the hepatic sinusoids and the bile ducts. In the hepatocytes evaluations were done of the nucleus-cytoplasm relationship, cell nucleus characteristics (pyknosis, karyolysis and karyorrhexis) and presence or absence of lipid vacuoles in the cytoplasm.

The data were processed with an analysis of variance (ANOVA) and Student t test with repeated measurements and a $P<0.05$. All statistical analyses were run with the Statistical Analysis System statistical package (SAS, 2001)[29].

The moringa leaf flour in the present study had a higher protein content (33.4%) than reported elsewhere for this species (20.0 to 29.0 %)[8,9,30] (Table 2). The higher protein content in the present results was probably because the reported data were from other moringa species and/or local environmental conditions affected protein content[9]. The remaining compounds were within ranges reported in the literature[8,9,30]. Moringa is known to be a good source of iron and calcium (Ca= 2.6 g; Fe= 19 mg/100g)[9], which is consistent with the present results (Table 2)[31]. Moringa may therefore contribute substantial amounts of calcium when added to animal feed. Its high iron content may assist in synthesis when added to broiler diets, and its vitamin C content could promote metabolism[32]; indeed, the combination of these compounds could contribute to this phenomenon.

Table 2: Proximate analysis (%), and mineral and vitamin C (mg/100 g, DM) and condensed tannins (mg CE/100 g, DM) contents in *Moringa oleifera* leaf

Compound	Content
Proximate chemical analysis (%)	
Protein	33.4 ± 0.72
Fiber	8.8 ± 0.70
Fat	8.1 ± 0.41
Ash	2.3 ± 0.46
Carbohydrates	47.4 ± 0.52
Minerals (mg/100 g DM)	
Iron	19.7 ± 1.07
Calcium	2593.3 ± 121
Vitamin C	63.5 ± 1.63
Tannins (mg CE/100 g, DM)	24.4 ± 0.92

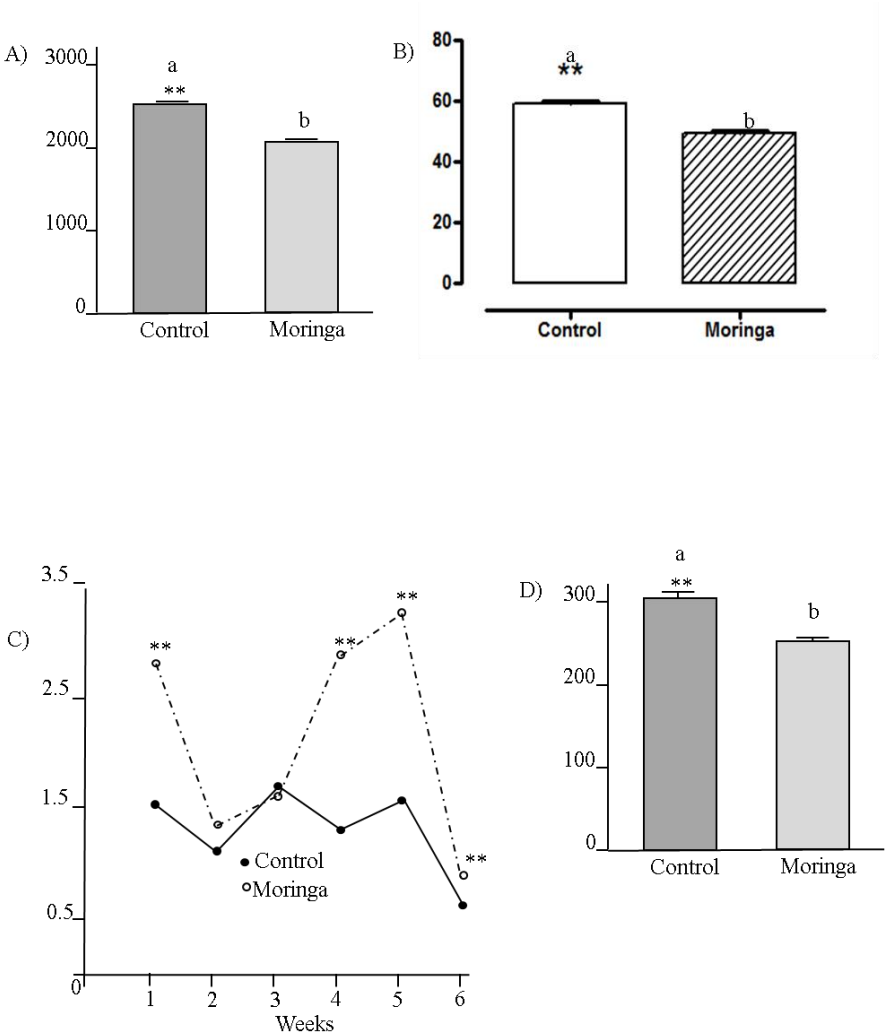
CE= catechin equivalents, DM= dry matter..

Use of sorghum with high tannin content (1,360 mg/100 g) in broiler diets is reported to reduce weight gain, feed intake and absorption of iron and calcium, among other minerals[33]. The moringa leaf flour used in the experiment had a very low tannin content, suggesting it did not have any antinutritional effect in the experimental animals.

Average final weight in the MF treatment was 414 g less than those in the control group (Figure 1A). This weight loss reflects the lower feed intake in the MF treatment (Table 3). Average total feed intake per replicate in each treatment was 123.35 kg in the control group and 107.19 kg in the MF group. This 16.16 kg difference ($P<0.05$) in the MF treatment can be attributed to higher dietary fiber intake. This contrasts with a study in which broilers were fed diets containing from 5 to 14 % flour from *M. stenopetala* in which the 5 to 11 % *M. stenopetala* diets exhibited no weight difference compared to the

control[4]. The difference between this study and the present results may be attributed to use of different moringa species in each. The difference in average weight in the present results was also reflected in the DWG results throughout the study (Figure 1B); at no time did DWG in the MF treatment exceed that in the control group.

Figure 1: Production parameters in the control and moringa flour treatment: A) Average weight at 42 d (g); B) Daily weight gain (g/d); C) feed conversion index (feed intake/weight); D) productivity index (daily weight gain) (viability)/(FCx10).



Asterisks indicate signficant difference (Student t, $P<0.05$, n=63).

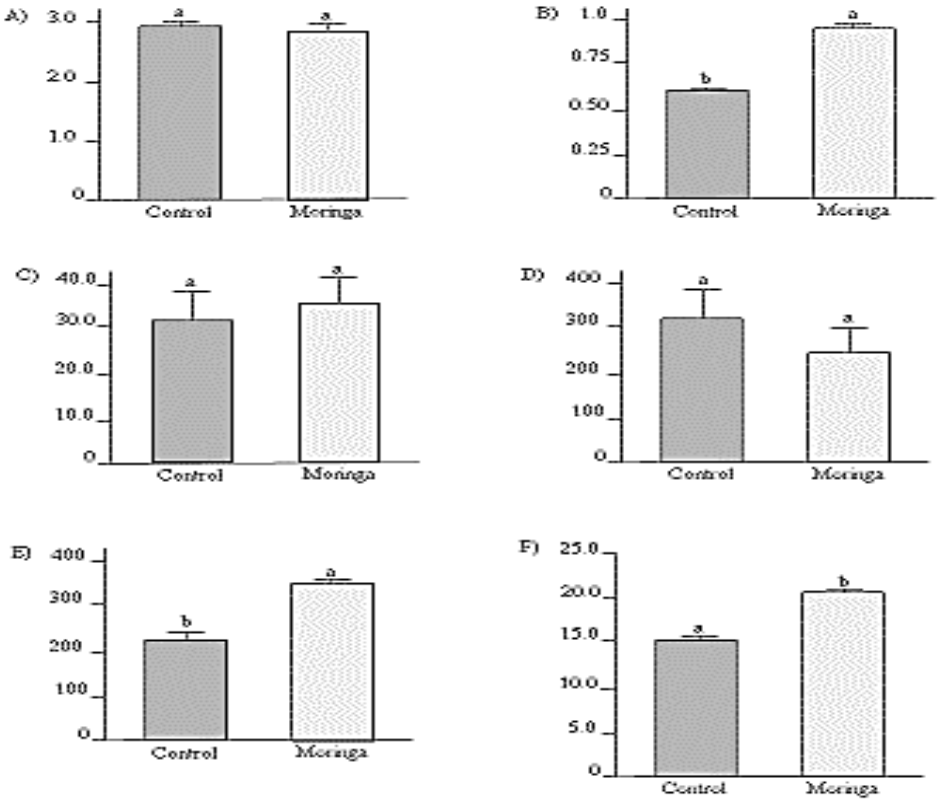
Table 3: Average feed intake (kg) in control and moringa flour treatment (n=30)

Age (days)	Control (kg)	Moringa (kg)
7	4.42 ± 0.425	4.04 ± 0.327
14	11.42 ± 0.915	9.13 ± 0.721
21	18.00 ± 0.740	12.49 ± 0.951
28	24.45 ± 1.198	16.77 ± 1.114
35	30.90 ± 1.255	27.65 ± 1.300
42	34.16 ±1.322	37.11 ± 1.625
	123.35 ± 7.255	107.19 ± 6.234

The lower the FCR the more efficiently a feed increases animal weight[26]. In the present results the FCR in the two treatments was similar only in the second and third weeks (Figure 1C). In a study conducted with *M. stenopetala*, no differences in FCR compared to the control were observed in broilers fed diets containing from 5 to 8 % moringa leaf[4]. However, at higher moringa inclusion levels the FCR increased, with the 14 % moringa leaf diet resulting in the lowest weight. Productivity index (PI) values were 21% higher in the control group than in the MF treatment, probably because average weight, DWG and FCR results for the latter treatment were lower (Figure 2D).

Total protein (TP) content did not differ between the treatments (Figure 2A), although albumin content was higher in the MF treatment (Figure 2B). However, in both cases TP levels were lower than the reported reference values for poultry (3.1 to 5.05 g/dl)[34]. The low globulin levels observed in both treatments in the present study may be associated with pathologies in the liver and/or kidney, or intrinsic factors typical of the studied animals.

Figure 2: Blood protein and enzyme activity levels in the control and moringa flour treatment: A) total proteins (g/dl); B) albumin (g/dl); C) alanine amino transferase (IU/L); D) aspartate amino transferase (IU/L); E) alkaline phosphatase (IU/L); and F) gamma glutamyl transferase (IU/L).



[ab] Different lowercase letters in the same parameter indicate significant difference (P<0.05 n=90).

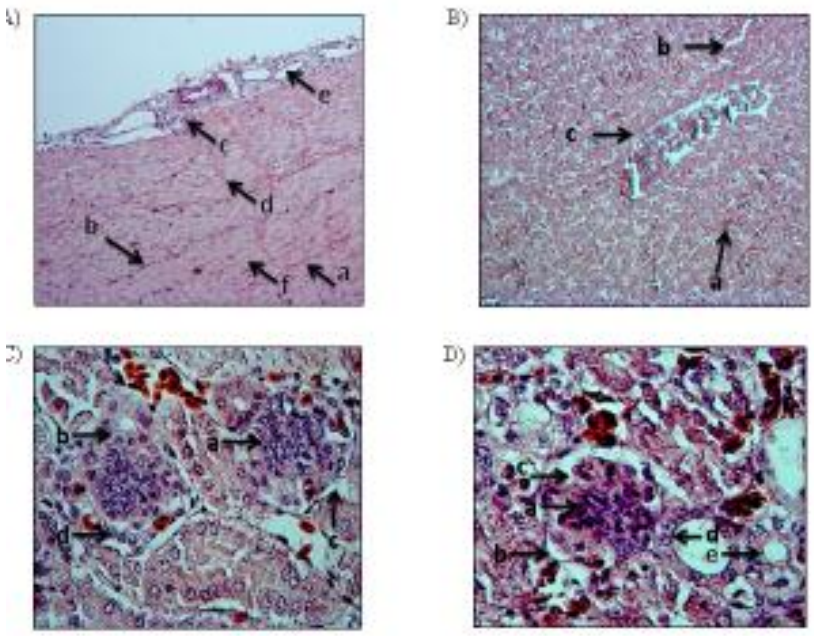
No differences (P>0.05) were observed in ALT and AST activities (Figure 2C and 2D). However, in the MF treatment the ALT level was 35.1 IU/L, near the reference maximum for this enzyme (9.5 to 37.2 IU/L)[35]. Values above this maximum for ALT suggest hepatic and renal damage[36]. The lack of a difference in AST is to be expected since chronic liver damage normally only causes subtle cell rupture, which manifests as normal or even decreasing AST levels[37], as was observed in the MF treatment (Figure 2D). Values for AST activity above 275 IU/L may be related to liver or muscle disturbances, while values above 800 IU/L strongly suggest severe liver damage[38]; this was not the case in the present study.

Activities for ALP and GGT differed between the treatments (Figure 2E and 2F). The reference ALP activity value is 600 IU/L[4], which is higher than occurred in both groups (Control and MF). Increases in ALP activity suggest bone growth, osteomyelitis and neoplasms[38], although it is not known if these problems arise at levels higher than the reference. The present results do not allow verification of whether this problem occurred in the MF treatment.

The membrane enzyme GGT is associated with a protein and linked to the level of amino acid metabolism. Increased GGT activity can be caused by inflammation, biliary cholestasis and bile duct hyperplasia[39]. A 25% increase in GGT activity was observed in the MF treatment (Figure 2F). Normal GGT values are between 0-10 IU/L[40]. Based on the reference values, both groups of birds apparently exhibited active liver lesion[41].

No micromorphological differences were apparent between the groups (Figure 3A and 3B) since the evaluated structures exhibited no congestion, inflammation, scar tissue, liquefaction, lipid vacuoles, pyknosis, karyolysis or karyorrhexis.

Figure 3: Histopathologies in the liver of the control (A) and the MF treatment (B), and in the kidneys of the control (C) and the MF treatment (B)



A) (a) histological architecture of parenchyma; (b) basophilic cells; (c) Möll space; (d) sinusoids, (e) bile ducts; and (f) hepatocytes.

B) (a) parenchyma; (b) sinusoids; and (c) centrilobular vein.

C) (a) renal glomeruli; (b) proximal tubule; (c) Bowman capsule; (d) glomerular visceral layer.

D) (a) renal glomeruli; (b) reduced urinary space; (c) filtration barrier; (d) apparently normal macula densa; (e) whole proximal tubule.

Histopathological analysis of the kidneys (Figure 3C and D) examined the glomeruli, Bowman's capsule, the urinary space, podocytes, the vascular and urinary poles, the filtration barrier, the proximal tubules, the Henle loop, the distal tubules, the juxtaglomerular apparatus, the collecting tubules and the renal interstitium. No micromorphological differences were apparent between the two groups since the evaluated structures showed no congestion or inflammation and their structures were complete.

Leaves from *Moringa oleifera* are a good source of protein and minerals. Addition of 10 % moringa flour to a broiler diet reduced production parameter values during the study period. Differences in protein and enzyme levels were detected although no visible toxic effects were apparent, which was confirmed in the histopathological studies. The higher GGT and ALP activity levels in the moringa flour treatment may be due to certain inflammatory processes in the bile ducts which occurred in both groups and were caused by factors other than consumption of moringa and feed. Establishing the proper inclusion levels of moringa leaf in poultry diets requires further research to compare with the present results.

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