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Inclusion of the *Moringa oleifera* leaf on immunological constants in broiler chickens

Inclusión de la hoja *Moringa oleifera* sobre constantes inmunológicas en pollos de engorda

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ABSTRACT

The national poultry production has acquired great importance for being an activity of high socioeconomic impact and generates high demand for ingredients. *Moringa oleifera* has acquired great importance as a nutritional supplement and medicinal plant; therefore, it could represent an alternative as an immune stimulant. The effects of the inclusion of the *M. oleifera* leaf on the immunological constants of broiler chickens were evaluated. 180 chickens (females) Cobb genetic line were used, distributed in 3 treatments: Control, M10, and M20. Intestinal morphometry and the content of IgY were determined at 21 and 42 days. A completely randomized statistical design was used: ANOVA and comparison of Tukey's means were performed. At 42 days, the height of villi of the duodenum of the Control group obtained the lowest values (1485 µm) with respect to M10 and M20 groups. The height of the jejunum villi was significantly higher ($P<0.05$) in the M10 group, with respect to the M20 and Control groups. In the depth of the jejunum crypts, there was a difference ($P<0.05$) between the Control and M20, the latter being the least. The IgY content at 21 days was significantly higher ($P<0.05$) in the M10 and M20 than in the Control. *M. oleifera* has a positive effect as an immunostimulant and can be used in animal feeding.

Keywords: Moringa, broiler chicken, intestinal morphometry, immunology.

RESUMEN

La producción avícola tiene gran relevancia por su alto impacto socioeconómico, genera alta demanda de ingredientes. *Moringa oleifera* ha adquirido gran importancia como suplemento nutricional y planta medicinal, por tanto, podría representar una alternativa como estimulante inmunológico. Se evaluaron los efectos de la inclusión de la hoja *M. oleifera* sobre constantes inmunológicas de pollos de engorda. Se utilizaron 180 pollos (hembras) línea genética Cobb distribuídas en 3 tratamientos: Control, M10 y M20. Se evaluó morfometría intestinal y contenido de IgY a los 21 y 42 días. Se utilizó un diseño estadístico completamente al azar, se realizó ANDEVA y comparación de medias de Tukey. A los 42 días, la altura de vellosidades del duodeno del Control obtuvo valores más bajos (1485 µm) con respecto a los grupos M10 y M20. La altura de vellosidades de yeyuno fue significativamente mayor ($P<0.05$) en M10, con respecto a M20 y Control. En la profundidad de criptas de yeyuno existió diferencia ($P<0.05$) entre Control y M20, siendo este último el menor. El contenido de IgY a los 21 días fue significativamente mayor ($P<0.05$) en M10 y M20 con respecto a Control. *M. oleifera* tiene efecto positivo como inmunoestimulante y puede utilizarse en alimentación animal.

Palabras clave: Moringa, pollos engorda, morfometría intestinal, inmunología.

INTRODUCTION

The poultry production sectors in developing countries face some problems, such as increased food costs; due to this, alternative sources have been sought in their diet that is available and not expensive. Poultry farming has a high impact in the economic and social spheres; since more than 60 % of the animal protein consumed in Mexico and the world comes from the poultry industry (SAGARPA, 2017).

Moringa oleifera is the genus of a tree belonging to the Moringaceae family (Kumar *et al*., 2013); it has been used mainly for human consumption, since it has acquired great importance as a nutritional supplement and medicinal plant; this brings as a consequence the increase in its cost; therefore, to be used in animal feed, it should be promoted that more trees are planted in the productions where it is required (Etalem *et al*., 2014). This plant is important as a forage crop due to its nutritional characteristics and its high yield in fresh biomass production (Padilla *et al*., 2014). The leaves of the genus *M. oleifera* are distinguished by their high content of macronutrients such as protein and energy; and micronutrients such as vitamins and minerals. However, it is worth mentioning that it also has phenols, anti-nutritional factors such as tannins, saponins, phytates and oxalates (Teteh *et al*., 2013).

One of the factors that it is important to take into account in animals is stress, which can affect the modulation of the immune response, among other things; which causes changes in the circulating immune cells in blood. The most common causes of stress in animals are due to feeding and environmental conditions. It has been proven that *M. oleifera* is a tree rich in vitamins, minerals and antioxidants, which promotes health and improves the immune response. A study was conducted to evaluate the effect of the *M. oleifera* leaf as an immunomodulator in mice, and it was shown to have significant potential as an immunomodulatory agent (Al-Majali *et al*., 2017).

It has been shown that the pro-inflammatory mediators produced during the inflammatory response aggravate the pathological development of some chronic diseases and showed that *M. oleifera* is a potent inhibitor of inflammation (Channarong *et al*., 2011)

Also, it is important to take into account the development of the intestine, which is reflected in the integrity of its morphology; it has a direct relationship with the growth and development of the animal. A study was conducted in which the effects of dust from the leaves of *M. oleifera* as a dietary supplement were evaluated to evaluate intestinal morphometry in rats without obtaining significant differences (Zvinorova *et al*., 2015). The intestinal mucosa development shows a relationship with the presence or absence of exogenous agents which is reflected in the change in height and density of the villi and in depth of the crypts, among others (Gomide, 2004).

Table 1. Inclusion levels of ingredients in initiation and completion diets.

Ingredient	Control		M10		M20	
	I	F	I	F	I	F
Oil	6.00	6.00	6.00	6.00	6.00	6.00
Sorghum	48.18	59.28	46.91	58.20	45.31	58.23
Soybean paste	35.92	26.34	32.80	24.50	30.50	22.00
Leaf of M. oleifera	-	-	4.50	3.00	8.50	5.50
Carbonate of Ca	1.21	1.10	1.00	0.97	0.79	0.88
Dicalcium phosphate	0.68	0.28	0.80	0.34	0.90	0.39
Premix	8.00	7.00	8.00	7.00	8.00	7.00

I = Initiation stage diet, F = Completion stage diet.

The importance of this work lies in looking for a viable alternative available from a food source, which has an effect on the immune system and can be incorporated into the diets of poultry production. It is expected that the inclusion of the *M. oleifera* leaf in broiler feed will have a positive effect on its immunological constants.

The objective of this work was to evaluate the effects of the inclusion of *M. oleifera* leaf on the immunological constants of broiler chickens.

MATERIAL AND METHODS

They were used 180 Cobb line chicks, one day old females were distributed in 3 treatments of 6 repetitions each and 10 replicates per repetition: 1. Control, 2. M10 (10 % inclusion of *M. oleifera*), 3. M20 (20 % inclusion of *M. oleifera*). They lived in pens of 1 m^2 per 10 birds (Cobb 500, 2013, Cobb 500, 2015). The diets were formulated at 95 % of that stipulated by the NRC (1994) (table 1).

For the microscopic morphometric evaluation, samples were taken from the duodenum and jejunum of the birds destined for humane euthanasia, at 21 and 42 days of age (SEMARNAT, 1995); the samples were preserved in 10 % formaldehyde. The paraffin inclusion technique was performed; subsequently, histological sections 4 microns thick and hematoxylin eosin staining were performed. The plates were read with micrometric objective microscopes, then the height of the villi and the depth of the intestinal crypts were measured; then the Motic Images Plus ® program was used (Rodríguez, 2012).

The experimental design was used completely at random; to then perform ANDEVA. When there was a statistical difference between the treatments, the Tukey test was performed to compare means with a confidence level of 95 %. The data was analyzed in the statistical package Minitab (2014) ®. 3 ml of blood was extracted from 3 birds per pen, at 21 and 42 days of age, and the samples were placed in collection tubes; these were allowed to stand until complete coagulation and then centrifuged at 3500 rpm for 10 min. Sera were separated and transferred to 1.5 ml microvials; then, two aliquots of each were made.

The quantification of IgY was carried out by means of a Sandwich ELISA method, the standards available in the kit were used; samples were added to the polystyrene microtiter wells (included in the kit) containing Anti-IgY antibodies adsorbed to the bottom, and allowed to bind for 30 min. Subsequently, four washes were made to remove proteins that did not bind; Anti-IgY antibody conjugated to horseradish peroxidase (HRP) was added at the dilution recommended by the kit, and incubated at 37 °C for the time indicated in the kit. Again, 4 washes were carried out and subsequently chromogenic substrate 3, 3', 5, 5' - tetramethylbenzidine (TMB) was added and incubated for 10 min in the dark. Finally, a solution was added to stop the reaction, and the absorbance was read; a filter for 450 nm was used in an ELISA reader. The amount of antibodies contained in the sample is directly proportional to the absorbance at 450 nm (MyBioSource®).

To perform the statistical analysis, a linear trend of coincidence with known data points was used; using the method of least squares to determine the values in ng/ml. Once these values were obtained, ANOVA was performed; when there was a statistical difference between the treatments, the Tukey test was used with a level of significance of 95 %.

RESULTS AND DISCUSSION

With respect to the height of villi in the duodenum at 21 days, the treatment that had the highest values was M20, with a value of 1620 µm, which was higher than the M10 group and Control at 156 and 366 µm, respectively. On the other hand, when comparing the Control group, it was lower by 210 µm with respect to the M10 group. The height of the villi of the jejunum showed a similar behavior, since the M20 group presented a value of 1537 µm, which was higher in 165 and 271 µm to the M10 and Control groups, respectively. The differences were statistically significant and are presented in Table 2. For the measurement of the crypt depth of the duodenum at 21 days, an opposite behavior was presented, since the M20 group obtained a value of 213 µm; which is smaller than Control and M10 by 38 and 44 µm, respectively; these values are statistically significant. In Jejunum there was no statistically significant difference between treatments; these results are shown in Table 2. At 42 days of age, in the height of villi of the duodenum, the Control group obtained a value of 1485 µm, which was higher by 170 and 160 µm to the groups M10 and M20, respectively; these differences are statistically significant. However, the height of the villi of the jejunum was significantly higher in the M10 treatment, with a value of 1579 µm compared to the M20 and Control treatments, which obtained 155 and 164 µm less, respectively; this difference is statistically significant (table 2).

However, for the depth measurement of duodenum crypts at 42 days of age, the same pattern was not present, since no statistically significant difference was found between the treatments; On the contrary, in the jejunum there was a statistically significant difference

Table 2. Height of villi and depth of intestinal crypts at 21 and 42 days

	Control	M10	M20	*P-Value-P*	EE
Height of villi at 21 days (µm)					
Duodenum	1263 [c]	1473 [b]	1629 [a]	*<0.001*	56.27
Jejunum	1266 [b]	1372 [b]	1537 [a]	*<0.001*	68.90
Depth of crypts at 21 days (µm)					
Duodenum	251 [a]	257 [a]	213 [b]	*<0.001*	15.30
Jejunum	262 [a]	227 [a]	258 [a]	*0.051*	18.33
Vellosity height at 42 days (µm)					
Duodenum	1485 [b]	1655 [a]	1645 [a]	*<0.001*	52.13
Jejunum	1415 [b]	1579 [a]	1424 [b]	*0.002*	61.98
Depth of crypts at 42 days (µm)					
Duodenum	193 [a]	177 [a]	181 [a]	*0.098*	9.52
Jejunum	190 [a]	176 [ab]	167 [b]	*0.015*	10.05

[a, b] Different literals per row indicate a statistically significant difference (P <0.05). EE = Standard error of the deviation

between the Control treatment and M20 with values of 190 and 167 µm, respectively (Table 2).

This tendency of behavior that increases the height of villi and decreases depth of crypts suggests a greater absorption of nutrients and leads to a better digestion (Lipismita *et al*., 2015). Zvinorova *et al*. (2015) measured intestinal villi height and depth of crypts in rats, diets with 14 and 20 % of *M. oleifera* were used and did not obtain significant differences between villus length, depth of crypts, nor in the proportion of both

When analyzing the results of the amount of IgY on day 21, the control treatment obtained a value of 92 ng/ml, lower by 85 and 121 ng/ml to groups M10 and M20; these values are statistically significant; however, between the M10 and M20 treatment there was no significant statistical difference. At 42 days, the Control group had a higher value of 52 ng/ml with respect to the M10 group this difference is statistically significant. However, there was no significant statistical difference between the Control and M20 treatments, as between the M10 and M20 treatments (Table 4). There are no works in which the amount of immunoglobulins in birds has been determined when using *M. oleifera*.

Table 3. Different inclusion levels of the *M. oleifera* leaf on the amount of IgY expressed in ng / mL at 21 and 42 days of age

Traetments	21 days	42 days
Control	92 [a]	160 [a]
M10	177 [b]	108 [b]
M20	213 [b]	140 [ab]
Valor P	*<0.001*	*0.048*
EE	29.13	30.10

[a, b] Different literals per row indicate a statistically significant difference (P <0.05). EE = Standard error of the deviation

CONCLUSION

Intestinal villi development was better in animals fed *M. oleifera*, since longer villi and shallower crypts were obtained. *M. oleifera* had a positive effect as an immunostimulant at 21 days, since it raises the levels of IgY in the blood.

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