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Antidiarrheal Effects of *Anacardium occidentale* L. Leaf Powder on Newborn Chicks

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ABSTRACT

To evaluate the antidiarrheal effects of *Anacardium occidentale* L. leaf powder, 45 two-day old White Leghorn hybrid chicks (L-33) were included in an experiment following a completely randomized design with three treatments, for 8 hours. The feed treatments consisted in basal diet (BD), BD + 1.0 % of *Anacardium occidentale* L. leaf powder, and BD + 1.5 % of *A. occidentale* leaf powder. The consumption of feeds accumulated and the initial and final live weights were determined. Besides, the incidence of diarrhea was calculated at 2, 4, 6 and 8 hours after inducing diarrhea with castor oil. Then, five chicks were sacrificed per treatment; the accessory organs of the immune system and the viscera were weighed. The intestinal pH was analyzed and the hematocrit levels were measured. The initial and final live weights, and feed consumption of the newborn diarrheal chickens remained stable ($P > 0.05$) among the treatments. Likewise, the liver, glandular stomach, muscular stomach, the relative weights of the small and large intestines, and the intestinal pH, did not have statistically significant variations ($P > 0.05$). Nevertheless, supplementation with the medicinal powder changed ($P < 0.05$) the relative weight of the pancreas, heart, thymus gland, and bursa of Fabricius. The T2 treatment (1.5 %) decreased the incidence of diarrhea significantly ($P < 0.05$) compared to the other treatments. However, the experimental treatments did not alter ($P > 0.05$) the concentration of hematocrits in the newborn chicks. Supplementation of 1.5 % of *Anacardium occidentale* L. leaf powder is recommended for use in newborn poultry with metabolic diarrhea.

Key words: *antidiarrheal, leaf, cashew nut, chicks, replacement*

INTRODUCTION

Diarrhea is characterized by the ejection of intestinal liquid contents that have remained in the digestive track for a short time. It is the most important symptom of the syndrome (Martínez, Martínez and Betancourt, 2015). Besides, it can be defined as poor absorption of water and electrolytes, mainly produced by intestinal infections (acute and chronic), food poisoning (internal or external), and by deficient nutritional management (Liu *et al.*, 2017).

The usual treatment to control diarrhea in poultry is based on sulfa drugs; however, this treatment is inefficient in birds with metabolic diarrhea (Más *et al*., 2017). Accordingly, functional and nutraceuticals (prebiotics, probiotics, organic acids, and medicinal plants) have been utilized alternatively for their safety and null residual (Liu *et al*., 2016).

In that sense, *Anacardium occidentale* L. can be used to cure and prevent human and animal diseases. The powder made from the leaves contains acceptable levels of electrolytes, sponnins, tannins, soluble protein, and phyto-sterols. It is also effective for the control of diarrheal syndrome in pigs, calves, rabbits, and humans. Besides, it has anti-inflammatory, analgesic, anti-tumoral, antimalarial, anticoagulant, hypoglucemic, antispasmodic, and astringent properties (Sousa de Brito, Pessanha de Arau, Liu and Harnly, 2007; Sokeng *et al*., 2007; Ayepola and Ishola, 2009; Martínez *et al*., 2013; Rosabal *et al*., 2017). Although many are the benefits of the plant, no reports have been published on the effects of its leaf powder on diarrhea, morphometry, and the intestinal pH of newborn poultry after the establishment of the diarrheal syndrome. The aim of this paper was to evaluate the antidiarrheal effect of the powder of *Anacardium occidentale* L. leaves on newborn poultry with metabolic diarrhea induced with castor oil.

MATERIALS AND METHODS

This research was made in the laboratory of the Center for Animal Production Studies (CEPA), Faculty of Veterinary Medicine, University of Granma, Cuba.

Leaves from three ± 12-year old trees of *Anacardium occidentale* L., collected in the Peralejo region, Bayamo, Granma province, Cu-

ba, with a flat topography and mostly red ferrallitic soil.

The leaves were identified in the Department of Botany, Faculty of Agricultural Sciences, University of Granma. The diversity, size and structure of leaves were considered during recollection (Martínez *et al.*, 2013).

The leaves were washed three times with distilled water to remove most impurities, then they were dried at room temperature in the shade, for three days. Later, the leaves were crushed, using a crushing mill with side blades, to 1 mm particle size.

A total of 45, two-day old hybrid White Leghorn (L-33) chicks took part in the experiment, following a completely randomized design with three treatments, for 8 h. Five chicks were placed per unit, on paper floor, with a density of nine chicks per square meter. The treatments did not include medication, or therapy of any kind throughout the experimental phase.

The treatments consisted in basal diet (BD, 1 % of *Anacardium occidentale* L. leaf powder, and DB, 1.5 % of *A. occidentale* leaf powder). To induce diarrhea, 1.0 ml of castor oil was administered per bird (Martínez, Martínez and Betancourt, 2015). The diets were formulated according to the Union of Companies of the National Poultry Corporation (UECAN, 20119). The ingredients and nutritional contribution of the diet are shown in Table 1. The results of Martínez *et al*. (2015) were considered for diet supplementation with *A. occidentale.*

Initially, the chicks fasted for 4 h, and water was supplied *ad libitum*. An electric heater was used, as well as lighting. After the induction of diarrhea, feed was administered *ad libitum* throughout the experimental phase.

The initial and final live weights were determined, using a Sartorius scale (± 0.1 g precision). Besides, accumulated feed consumption was calculated, following the supply-rejection method.

The incidence of diarrhea (ID) in the chicks was determined according to the formula suggested by Liu *et al*. (2016), ID (%)=number of diarrheal birds/number of chicks/×100, at 2, 4, 6, and 8 h following the induction of diarrhea.

Then, five chicks were sacrificed per treatment, by jugular vein exsanguination (Martínez *et al.*, 2013). The total viscera (liver, pancreas, and heart), the immune organs (Thymus gland, and bursa of Fabricius), and accessories (proventriculus and gizzard) were weighed, using a digital Sartorius scale (±0.1 g precision). After that, the relative weight of organs in relation to the live weight was calculated.

Several portions of the small and large intestines from the sacrificed birds were homogenized in a mortar. Then 2 g of the sample were mixed with 10 ml of distilled water, and vortexed for 2 min. The pH was determined using a digital potentiometer (Bantex 300A), calibrated with buffer solutions, pH 7 and 10. At the end of the experiment, 1 ml of blood was drawn from the jugular veins of five chicks/treatment, and the hematocrits were determined, according to Wintrobe.

The data were processed through simple variance analysis (Anova), using a completely randomized design. The Duncan´s test (1955) was performed whenever necessary to determine the differences among the means (SPSS, 22.1, for Windows).

RESULTS AND DISCUSSION

The initial and final live weights, and the feed consumption of newborn replacements laying hens (chicks) with diarrhea showed no statistical variability ($P > 0.05$) (Table 2). Presumably, the secondary metabolites in the powder caused no symptoms associated to anti-nutritional factors, whose excess might have reduced body weight and productive behavior.

However, the diarrheal syndrome caused a depression of final live weight in all the treatments (1.90 - 2.68 g, Table 2), possibly attributed to a decline in feed consumption in the experimental phase. In that sense, UECAN (2011) had reported that newborn chicks (1-2 days old) consume 12 g daily. However, the chickens used in this experiment consumed between 2.92 and 3.33 g in 8 hours, less than the recommended amount (4 g). Furthermore, unsaturation of the diarrheal syndrome caused excessive loss of water-soluble nutrients, like proteins, carbohydrates, and vitamins, which led to a reduction of live weight, especially in the newborn chicks, with low immunological and enzymatic activity.

Other authors, such as Martínez, Martínez and Betancourt (2012), and Martínez *et al*. (2013) in apparently normal newborn poultry increased live weight and feed consumption. It seems that the effectiveness of this natural product on diarrheal

poultry, first controlled the enteric disorder, and then had an effect on body and immune restoration, though further studies are necessary to confirm this hypothesis.

Table 3 shows that the relative weights of liver, glandular stomach, muscular stomach, small intestine, large intestine, and intestinal pH did not have significant differences ($P > 0.05$) among the treatments. Nevertheless, supplementation with this medicinal powder changed ($P < 0.05$) the relative weights of pancreas, heart, thymus gland, and the bursa of Fabricius.

The reduction in the relative weight of pancreas after supplementation of 1.5% of *A. occidentale*, demonstrated that high concentrations of the medicinal powder led to greater retention of intestinal contents. During the occurrence of diarrhea, a lot of pancreatic enzymes were lost, increasing the organ´s activity by stimulating the positive feedback mechanism. In that sense, Más *et al.* (2016) reported that optimum concentrations of *A. occidentale* leaf powder were required to achieve a preventive antidiarrheal response in pigs before weaning, thanks to the astringent and mucous protective effects that the leaves from this medicinal plant have (Rosabal *et al.*, 2017).

Heart weight increase of the control in comparison to the treatment with diet supplements (0.12-0.29 g) was possible thanks to greater activity in the circulatory system, caused by hemoconcentration and hypovolemia induced by the loss of liquid during diarrhea. Apparently, the experimental treatments with optimum doses showed decreased levels of liquid loss in the gastrointestinal tract, which proved that the prophylactic treatment with cashew nut leaves to diarrheal animals contributed to lower water and electrolyte loss.

Additionally, Table 3 shows an increase in the relative weight of immune organs (thymus gland and the bursa of Fabricius) in newborn chickens that consumed supplements based on *A. occidentale* leaf powder. Presumably, this medicinal plant stimulated the immune system of the chickens, as a response to a greater proliferation of pathogenic agents during the diarrheal syndrome, previously corroborated by Martínez *et al.* (2012). They claimed that the immunostimulating effect of this natural product in the diet of presumptively newborn poultry owed to the antioxidant capacity and stimulation of humoral immunity.

According to Martínez *et al.* (2015), *A. occidentale* decreased the intestinal pH in mammals, due to a decline in the losses of fixed bases in the feces, and increased proliferation of lactic acid bacteria and volatile fatty acids. However, these results (Table 3) in diarrheal chickens had no differences ($P > 0.05$) in intestinal pH. In another study, Martínez *et al.* (2017) achieved a similar response in apparently normal young chicks, after using 2.5% of *A. occidentale*. Birds are known to have a greater intestinal pH than mammals, due to the low proliferation of beneficial bacteria in the first days of life (Landoni and Albarellos, 2015), which might have influenced on the these results.

Fig. 1 shows the antidiarrheal effect of *A. occidentale* leaf powder on newborn chicks. In treatment T2 the values for that indicator significantly decreased 2 h after post-induction, in comparison to the control and supplementation of 1.0% leaf powder ($P < 0.05$). These results coincided with the reports made by Yusuf, Aliyu and Ndanusa (2009), and Martínez *et al.* (2015), who found a similar response in diarrheal rabbits and calves, after using liquid therapeutic solutions of *A. occidentale*, respectively.

The antidiarrheal capacity of some secondary metabolites of *A. occidentale* was possible thanks to the astringent action of the powder, especially tannins (Martínez *et al.*, 2013; Rosabal *et al.*, 2017). According to Gimeno (2004) the astringency of tannins is produced by hydrogen bridge bonding. However, Lichovnikova *et al.* (2015) and Barrios (2016) reported that the excess of this metabolite reduced absorption of sulphur aminoacids and iron, causing growth depression and iron deficiency anemia, respectively.

Moreover, Yusuf, Aliyu and Ndanusa (2009) recommended the use of *A. occidentale* in diarrheal rabbits because the activation of co-transporters of chlorine and sodium in the colon decreased the secretion of intestinal fluids. Likewise, Más *et al.* (2016) observed a decline in the diarrheal index of pre-weaning pigs, after using powder of *A.* occidentale leaves preventively. Other authors reported antibacterial, anti-inflammatory, astringent, and antidiarrheal effects of *A. occidentale* in pigs, calves, birds, and humans with infectious diarrhea (Martínez *et al.*,

2013; Rosabal *et al.;* 2017). It demonstrated that this medicinal plant could be used to fight the effects of diarrheal syndrome caused by different etiologies.

The concentration of hematocrits from newborn birds with diarrhea did not vary ($P > 0.05$) among the treatments (Fig. 2); however, the normal values were higher than the reports made by Causey (2000). This may be explained by the great loss of water and electrolytes during the diarrheal syndrome, bringing about hemoconcentration and increased HTC values. In that sense, Martínez *et al*. (2015) noted that the liquid administration of *A. occidentale* in diarrheal calves had a restoring effect on hematocrits and hemoglobin. Nevertheless, the experiment with diarrheal chickens did not have similar results. Ostensibly, the characteristic digestive physiology of the animal model studied, as well as greater hypotonic dehydration might have influenced on this result.

CONCLUSIONS

Supplementation of 1.5% of *A. occidentale* leaf powder had antidiarrheal effects on newborn chickens with metabolic diarrhea. Additionally, it stimulated the immune system without negative effects on production, intestinal pH, and hematocrits.

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Table 1. Composition and contribution to the diet of laying replacement chicks (Humid base)

Ingredients (%)	Inclusion levels
Corn meal	55.70
Soybean meal	37.60
Plant oil	0.90
Dicalcium phosphate	1.85
Calcium carbonate	2.50
BHT (Antioxidant)	0.01
DL-Methionine	0.15
L-Lysin	0.04
Salt	0.25
Pre-mix	1.00
Contribution (%)	
ME (MJ/Kg DM)	12.13
Gross protein	21.00
Lysin	1.20
Methionine+cystine	0.80
Calcium	1.50
Available phophorous	0.48
Ethereal extract	2.40
Gross fiber	3.90

[1]Each kg contains: vit. A, 10 x 10^6 U.I.; vit. D_3, 1.5x 10^6 U.I.; vit. K_3, 2 100 mg; vit. E, 10 000 mg; thiamine, 800 mg; riboflavine. 2 500 mg; ac. pantothenic, 10 000 mg, pyridoxine, 2 500 mg; folic acid, 250 mg; biotin, 100 mg; vit. B_{12}, 15 mg; magnesium, 60 000 mg; copper,8 000 mg; iron, 60 000 mg; zinc, 50 000mg; selenium, 200 mg; iodine, 800 mg; cobalt, 500 mg; Antioxidant, 125 000 mg.

Table 2. Effect of *A. occidentale* leaf powder on the productive behavior of replacement laying birds with diarrhea

Indicators	Diet supplementation of *A. occidentale*			SE±	P value
	TT	T1	T2		
Initial live weight (g)	36.20	34.18	35.35	0.679	0.612
Final live weight (g)	33.52	32.88	33.45	0.441	0.547
Feed consumption (g)	2.92	3.15	3.33	0.143	0.164

Table 3. Effect of A. occidentale leaf powder on the relative weight of digestive organs, viscera, immune organs, and accessories of newborn chickens with diarrhea

Relative weight (%)	Diet supplementation of *A. occidentale*				P value
	TT	T1	T2	SE±	
Liver	3.89	3.59	3.35	0.183	0.163
Pancreas	0.24[a]	0.24[a]	0.18[b]	0.011	0.002
Heart	1.02[a]	0.73[b]	0.90[ab]	0.085	0.009
Thymus gland	0.18[b]	0.24[a]	0.24[a]	0.011	0.003
Bursa of Fabricius	0.06[b]	0.12[a]	0.12[a]	0.010	0.001
Glandular stomach	1.01	0.85	0.90	0.069	0.199
Muscular stomach	8.55	6.83	7.20	0.672	0.344
Small intestine	5.26	4.62	4.78	0.295	0.318
Large intestine	1.13	0.98	1.08	0.161	0.789
pH SI	6.72	6.73	6.85	0.047	0.855
pH LI	6.84	6.90	6.89	0.030	0.080

[a,b] Means with different letters in the same row differ from P < 0.05. SI: Small intestine; LI: large intestine

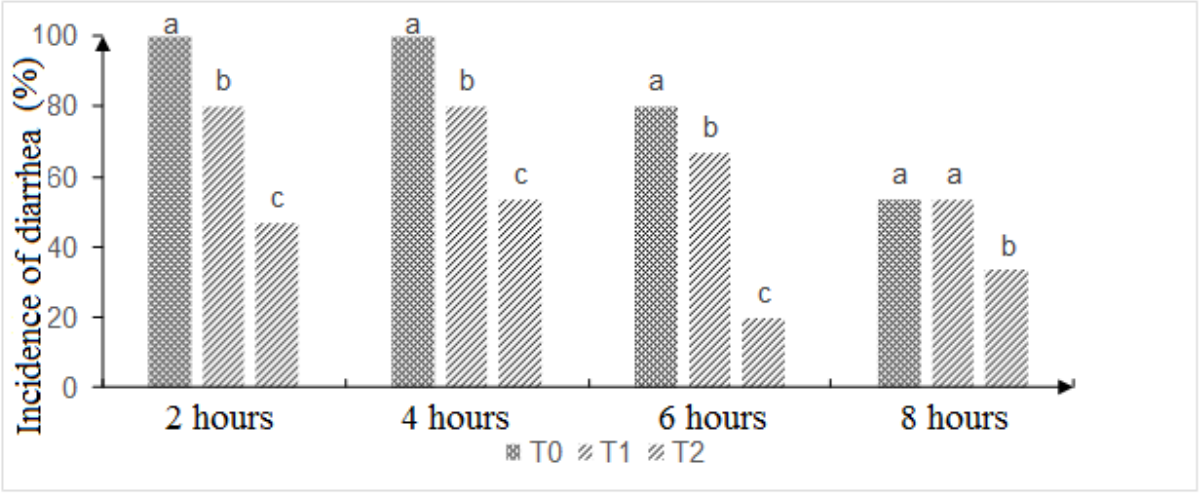

Fig. 1. Effect of *A. occidentale* leaf powder on the diarrheal index of newborn chickens
[a,b,c] Columns with different letters at the same time, differ statistically from P < 0.05

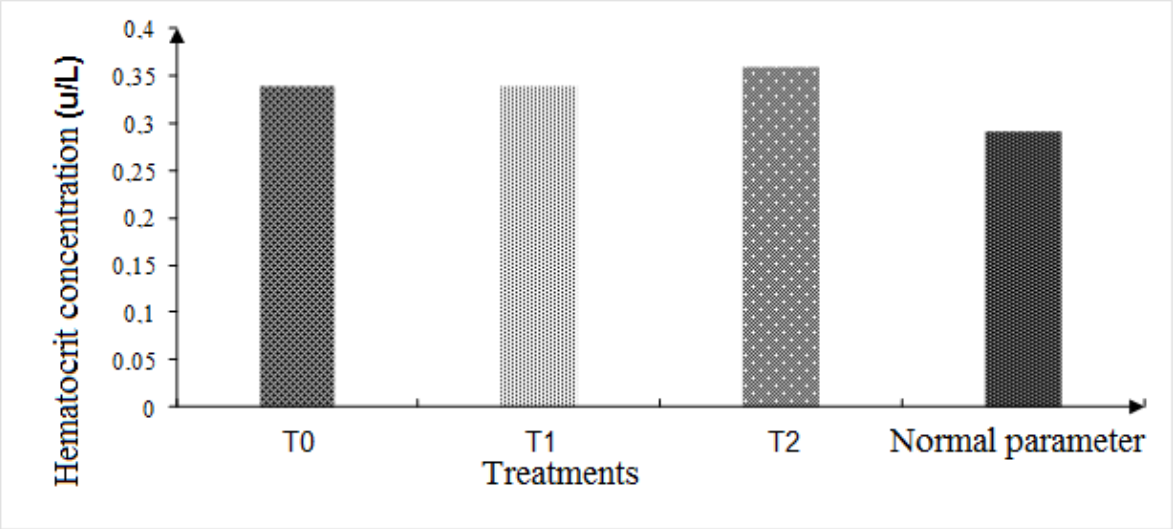

Fig. 2. Effect of A. occidentale leaf powder on hematocrit concentration of newborn birds