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Antidiarrheal Effect 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 chicks (L-33 hybrid) were studied for 8 hours. A completely randomized design with three treatments was applied. The treatments consisted in a basal diet (BD) + 1.0 % leaf powder of *Anacardium occidentale*, and BD + 1.5 % leaf powder of *A. occidentale.* The accumulated food consumption and initial and final live weights were determined. Additionally, the occurrence of diarrhea was calculated 2, 4, 6, and 8 hours after inducing diarrhea with castor oil. Then, five birds were sacrificed per treatment and the accessory and immune organs, and all the viscera were weighed. The intestinal pH and hematocrits were analyzed. The initial and final live weights, and food consumption of diarrheal newborn birds remained stable ($P > 0.05$) among the treatments. Likewise, the relative weights of liver, glandular stomach, muscular stomach, and small and large intestines did not have statistical differences ($P >0.05$). However, supplementation with the medicinal powder modified ($P <0.05$) the relative weight of pancreas, heart, thymus, 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 newborn birds. The supplementation of 1.5% leaf powder of *Anacardium occidentale* L. is recommended for newborn chicks with metabolic diarrhea.

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

INTRODUCTION

The most important symptom of diarrheal syndrome is the ejection of intestinal liquid contents that have remained in the digestive track for a short time (Martínez, and Martínez and Betancourt, 2015). Besides, it can be defined as poor absorption of water and electrolytes, produced by intestinal infections mainly (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 nutraceutical foods (prebiotics, probiotics, organic acids, and medicinal plants) have been utilized alternatively for their safety and null residuals (Liu *et al*., 2016).

Accordingly, *Anacardium occidentale* L. is a plant that can be used to cure and prevent human and animal diseases. The powder made from the leaves contains acceptable levels of electrolytes, saponins, tannins, soluble protein, and phytosterols. It is also effective to control the diarrheal syndrome in pigs, calves, rabbits, and humans. Additionally, it has anti-inflammatory, analgesic, anti-tumor, antimalarial, anticoagulant, hypoglycemic, 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 birds with the establishment of the diarrheal syndrome.

MATERIALS AND METHODS

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

The leaves of approximately 12 year-old trees of *Anacardium occidentale* L were collected in

the Peralejo region, Bayamo, Granma province, Cuba, in an area with flat topography and mostly red ferralitic soil.

The leaves were identified in the Department of Botany, at the Faculty of Agricultural Sciences, University of Granma. Leaf diversity, size, and structure were taken into account during collection (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 hammer mill with side blades (1 mm particle size).

Forty-five, two-day old White Leghorn chicks (L-33 hybrid) chicks were used 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 a basal diet (BD) + 1 % leaf powder of *Anacardium occidentale*, and BD + 1.5 % leaf powder of *A. occidentale.* To induce diarrhea, 1.0 ml of castor oil was administered per chick (Martínez, and Martínez and Betancourt, 2015). The diets were formulated according to the National Network of Poultry Farms (UECAN, 2011). The ingredients and nutritional contribution of the diet are shown in Table 1. The results of Martínez *et al.* (2015) were weighed for diet supplementation with *A. occidentale* L. acting as a nutraceutical.

Initially, the chicks fasted for 4 h, and water was supplied *ad libitum*. Electric heating and lighting were provided. After the induction of diarrhea, the food was administered *ad libitum* throughout the experimental phase.

The initial and final live weights were determined, using a Sartorius scale (± 0.1 g precision), and the accumulated food consumption was calculated, following the acceptance-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 chicks/number of chicks × 100 in 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). Later, the relative weight of organs was calculated in relation to the live weight.

Several portions of the small and large intestines from the sacrificed birds were homogenized in mortar. Then 2 g of the sample were mixed with 10 ml of distilled water and it was vortexed for 2 min. 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 for hematocrit determination, according to Wintrobe.

The data were processed through simple analysis of variance (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 food consumption of newborn replacements of layer 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 (Savón, Scull, Orta, and Martínez, 2007).

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 food consumption during the experimental phase. In that sense, UECAN (2011) reported that newborn chicks (1-7 days old) consumed 12 g daily. The experimental chickens consumed between 2.92 and 3.33 g in 8 hours, less than the recommended amount (4 g). Furthermore, insaturability 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) found that diet supplementation with 0.5% *A. occidentale* in seemingly normal newborn birds increased live weight and food consumption. It seemed that the effectiveness of this natural product in diarrheal birds controlled the enteric disorder at first, and then influenced the body and immune restoration of the animal model, 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. However, supplementation of the medicinal powder altered (P >0.05) the relative weight of pancreas, heart, thymus, and bursa of Fabricius.

The reduction in the relative weight of pancreas after supplementation with 1.5% of *A. occidentale* showed that high concentrations of the medicinal powder induced retention of the intestinal contents at a larger scale. A lot of pancreatic enzymes are lost during the occurrence of diarrhea; which increases the activity of pancreas through stimulation of the positive feedback mechanism. In that sense, Más *et al*. (2016) reported the need for optimum concentrations of leaf powder of *A. occidentale* to induce a preventive antidiarrheal effect on pigs before weaning, thanks to the astringent and mucous protective effects that the leaves from this medicinal plant have (Rosabal *et al*., 2017).

Increased heart weight of the control in comparison to the treatment with diet supplements (0.12-0.29 g) owed to a greater activity in the circulatory system, which was caused by hemoconcentration and hypovolemia induced by the loss of liquid during diarrhea. It seems that the experimental treatments with optimum doses cut down liquid loss in the gastrointestinal tract, which demonstrated that the prophylactic treatment with cashew nut leaf powder in animals with acute diarrhea contributed to a decrease in water and electrolyte losses.

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

According to Martínez *et al.* (2015), *A. occidentale* reduced the intestinal pH in mammals, due to a decline in the losses of fixed bases of feces, and increased proliferation of lactic acid bacteria, and volatile fatty acids. However, these results (Table 3) in diarrheal chickens showed no differences (P >0.05) in intestinal pH. In a similar study, Martínez *et al*, (2012) achieved a similar response in seemingly young normal chicks, after using 2.5% of *A. occidentale*. Birds are known to have a greater intestinal pH than mammals, due to low proliferation of beneficial bacteria on the first days of life (Landoni and Albarellos, 2015), which may have influenced these results.

Fig. 1 shows the antidiarrheal effects of *A. occidentale* leaf powder on newborn birds. 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 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*.

The antidiarrheal capacity of some secondary metabolites of *A. occidentale* owes to the astringent action of the powder, particularly tannins (Martínez *et al*., 2013; Rosabal *et al.*, 2017). According to Gimeno (2004), tannin astringency is produced by protein hydrogen bond network. However, Lichovnikova *et al.* (2015) and Barrios (2016) reported that the excess of this metabolite reduced absorption of sulfur amino acids 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 chlorine and sodium co-transporters in the colon decreased the secretion of intestinal fluids. Más *et al.* (2016) observed a decline in the diarrheal index of preweaned pigs, after using the powder of *A. occidentale* leaves preventively. Other authors

noted the 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 demonstrates that this medicinal plant could be used to counterattack the diarrheal syndrome caused by different etiologies.

The concentration of hematocrits from newborn birds with diarrhea did not vary (P >0.05) among the experimental treatments (Fig. 2). However, they were higher than the normal values reported 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, Más *et al*. (2015) pointed out that the administration of liquid *A. occidentale* to diarrheal calves had a restitutive effect on the normal hematocrit and hemoglobin values. 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 this result.

CONCLUSIONS

The supplementation with 1.5% of leaf powder of *A. occidentale* L. had antidiarrheal effects on newborn birds with metabolic diarrhea. Additionally, it stimulated the immune system without affecting the productive indicators, intestinal pH, and hematocrits.

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Table 1. Composition and diet contribution to replacement layer chicks (Humid base)

Ingredients (%)	Inclusion levels
Corn meal	55.70
Soybean meal	37.60
Vegetable oil	0.90
Dicalcium phosphate	1.85
Calcium carbonate	2.50
BHT (Antioxidant)	0.01
DL-methionine	0.15
L-lysine	0.04
Salt	0.25
Pre-mix[1]	1.00
Contribution (%)	
ME (MJ/Kg DM)	12.13
Crude protein	21.00
Lysine	1.20
Methionine+cystine	0.80
Calcium	1.50
Available phosphorous	0.48
Ethereal extract	2.40
Crude fiber	3.90

1. Each kilogram contains: vit. A, 10 x 10 6 I.U.; vit. D3, 1.5x 106 I.U.; vit. K3. 2 100 mg; vit. E, 10 000 mg; thiamine, 800 mg; riboflavin 2 500 mg; pantothenic acid, 10 000 mg, pyridoxine, 2 500 mg. folic acid, 250 mg; biotin, 100 mg; vit. B12, 15 mg; manganese, 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.

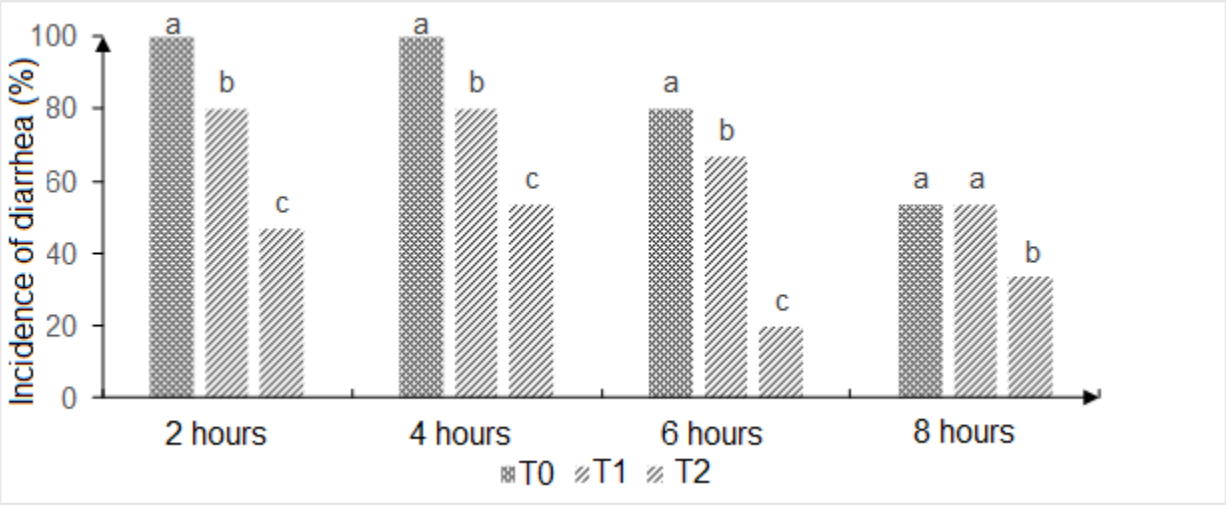
Table 2. Effect of leaf powder of *A. occidentale* on the productive behavior of replacement layer flocks with diarrhea

Indicators	Diet supplementation with *A. occidentale*			SE±	P value
	T0	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
Food consumption (g)	2.92	3.15	3.33	0.143	0.164

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

Relative weight (%)	Diet supplementation with *A. occidentale*				P value
	T0	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 unequal letters in the same row differ significantly ($P < 0.05$).
SI: Small intestine; LI: large intestine



a, b, c: columns with unequal letters at the same time differ statistically (P <0.05).

Fig. 1. Effect of leaf powder of *A. occidentale* on the diarrheal index on newborn birds

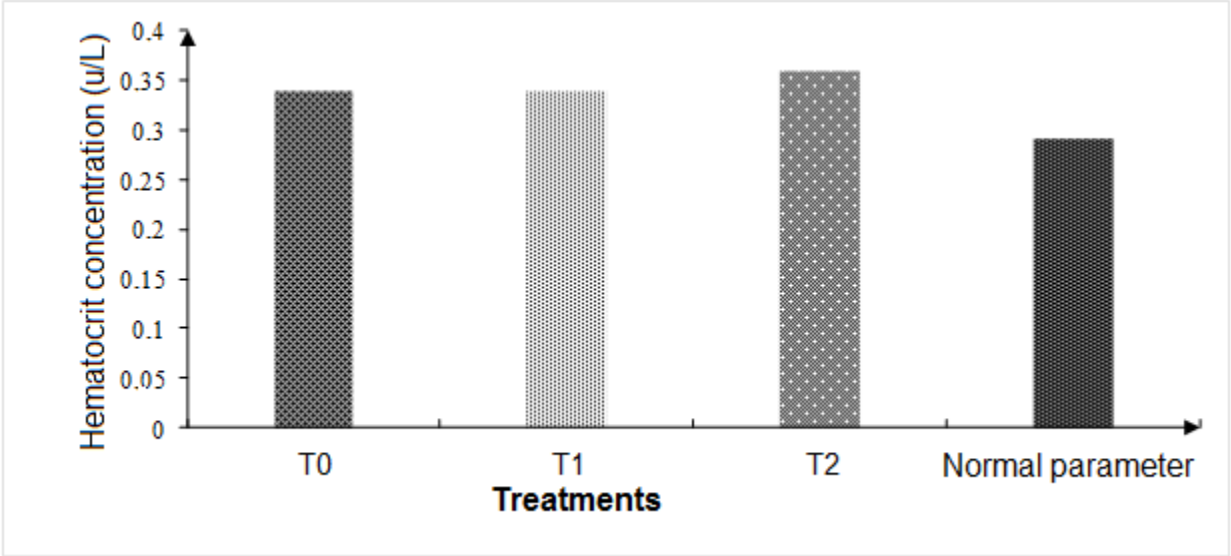


Fig. 2. Effect of leaf powder of *A. occidentale* on the hematocrit concentration of newborn birds