



## The effect of the ethanol extract from the *Dracontium spruceanum* rhizome on hematologic and biochemical profiles and performance parameters of broiler chickens

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### Abstract

The objective of the research was to evaluate the antioxidant capacity and effect of the ethanol extract from the *Dracontium spruceanum* rhizome (EERDs) on the blood, biochemical and productive parameters of chickens. To do so, ninety, male, Cobb 500 broiler chickens were used. Once the dehydrated extract was obtained, it was placed in the drinking water at concentrations of 0.0, 0.35 and 0.70 mg/mL of EERDs. The birds were distributed into three treatments, five repetitions and each repetition had six chickens. The variance analysis was done with the statistical program InfoStat and the averages of the treatments were analyzed with the 5% Tukey test. The results showed that chickens that consumed drinking water with EERDs presented ( $p < 0.05$ ) greater concentrations of hematocrit, hemoglobin and erythrocytes; meanwhile, the serum protein and the glucose did not change ( $p > 0.05$ ). The daily food consumption diminished ( $p < 0.05$ ) and the DWG and FRC were not influenced ( $p > 0.05$ ) by the consumption of EERDs in the drinking water. It is concluded that the consumption of EERDs by broiler chickens from 1 to 35 days of age produces greater concentrations of the levels of red blood cells and diminishes the feed intake.

**Keywords:** *Dracontium spruceanum*; hematocrit; productive performance; antioxidant capacity.

### 1. Introduction

The intensive production of domestic animals, conditions them to stressful oxidative processes, due to the handling conditions such as the confinement, the breeding density, productive stage, etc. (Huerta-Jimenez *et al.*, 2005; Koknaroglu and Akunal, 2013).

Oxidative stress occurs in all animal species, above all, in those with early growth, like the broiler chicken, which due to its physiological characteristics and intense metabolic activity, is permanently producing oxidative substances (DrÖge, 2002). To overcome this demand, the first line of action of the organism is to resort to its endogenous antioxidant reserves and the use of nutrients to alleviate the negative effects.

To improve the adverse effects of oxidative

stress and the productive indices, traditionally, we resort to the use of pharmaceuticals which, on one hand, develop microorganism's resistant to the drugs and on the other hand, generate residuals in the derived products that could have collateral effects for the consumer (Bistoletti *et al.*, 2011; Sakai *et al.*, 2016). Due to this, at present the industry is introducing natural antioxidants to eliminate the factors that negatively affect production (Jahanian and Mirfendereski, 2015; Boostani *et al.*, 2015; Gerasopoulos *et al.*, 2015; Soltani *et al.*, 2016).

*Dracontium spruceanum* is a plant in the Peruvian Amazon which is characterized by its antioxidant and immunomodulatory properties (Giovannini and Howes 2017; Benavides *et al.*, 2009; Napolitano *et al.*, 2011; Lovera *et al.*, 2006); due to this, its

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use in the feeding of broiler chickens could improve their well-being and health. The objective was to determine the effect of the ethanol extract from the *Dracontium spruceanum* rhizome in the well-being and health of broiler chickens under an intensive rearing system, through the evaluation of the *in vitro* antioxidant activity, blood profiles and productive parameters.

## 2. Materials and methods

### Obtaining the Ethanol Extract from the *Dracontium spruceanum* Rhizome

For this, a 200 g sample of the rhizome, dried and crushed, was placed in a jar, later ethanol was added at 75% to reach one liter and it was left macerating for forty-eight hours. Later, the suspension was stirred and filtered with cotton. The filtered substance, containing the extract, was submitted to evaporation of the ethanol, using a rota vapor and finally, finished drying in an oven at 65 °C. The ethanol extract obtained was packaged and stored.

### Determining the antioxidant activity *in vitro*

The antioxidant capacity of the ethanolic extract from *D. spruceanum*, was evaluated using the method of sequestering the free radical 1,1 diphenil-2-picrylhidrazyl (DPPH) (100  $\mu$ M) (Brand-Williams *et al.*, 1994; Sánchez-Moreno, 2002), causing a reaction with 25, 75, 125, 175 and 250  $\mu$ g/mL of EERDs; the readings were done in a UV light spectrophotometer and Electron Corporation visual, Genesys-6 model, with a 515 nm filter, every thirty seconds for a total time of ten minutes.

The percentage of inhibition of the DPPH obtained by each of the concentrations was used to determine the Inhibition Coefficient (IC<sub>50</sub>), which was expressed in  $\mu$ g/mL, indicating the necessary quantity of the aqueous atomized extract from *D. spruceanum* to inhibit, at 50%, the DPPH radical.

### Experimental Animals

The work was done with ninety baby male chickens from the Cobb Vantress 500 breed, with an average weight of 46.11  $\pm$  1.6 g; which were distributed into three treatments, with five repetitions and each repetition with six birds, which were bred under similar handling and feeding conditions. Three balanced diets were formulated, the composition of which was according to the requirements of the breed (Rostagno *et al.*, 2011).

### Design and Statistical Analysis

The hematological profiles were submitted to a completely randomized design CRD

with a factorial arrangement of 3 x 2 + 1 (three levels of EERDs x 2 ages + 1 control). The productive performance parameters were submitted to a completely randomized design (CRD) with three treatments, five repetitions and each repetition with six chickens. The variance analyses were done with the statistical program InfoStat (Universidad Nacional de Cordova, 2016) and the averages were compared using the Tukey test (5%).

## 3. Results and discussion

### Antioxidant Activity of the Ethanol Extract from the *Dracontium spruceanum* Rhizome

The inhibition capacity was related to the concentration of EERDs, with T1 (250  $\mu$ g/mL) showing the greatest free radical inhibition capacity ( $p < 0.05$ ) (Table 1).

**Table 1**

Effect of the Ethanol Extract from the *Dracontium spruceanum* Rhizome (EERDs) on the *in vitro* inhibition of DPPH

Concentration de EERDs	Absorbance 515nm	Inhibition (%)
T1: 250 $\mu$ g/mL	0.260 $\pm$ 0.01	71.92 <sup>e</sup>
T2: 175 $\mu$ g/mL	0.403 $\pm$ 0.01	56.56 <sup>d</sup>
T3: 125 $\mu$ g/mL	0.577 $\pm$ 0.01	37.79 <sup>c</sup>
T4: 75 $\mu$ g/mL	0.752 $\pm$ 0.01	18.88 <sup>b</sup>
T5: 25 $\mu$ g/mL	0.858 $\pm$ 0.01	7.41 <sup>a</sup>

Different letters in the same column indicate statistical differences (SNK 5%).

By the linear equation, it was determined that the concentration of EERDs required to inhibit the DPPH at 50% was 164.37  $\mu$ g/mL (Figure 1). The treatments with 25  $\mu$ g/mL and 250  $\mu$ g/mL generate an inhibition of 7.41 and 71.92%, respectively; showing that the greater the concentration of EERDs, the greater the inhibition of the DPPH radical. Velandia (2009), did a test on the antioxidant activities, with fraction and extracts from *Dracontium croatti*, at different concentrations (1-100  $\mu$ g/ml), with butanol, on the retention effect of the DPPH; obtaining an IC<sub>50</sub> with 25.12  $\mu$ g/ml of the extract. This difference could be associated to the specie of *Dracontium*.

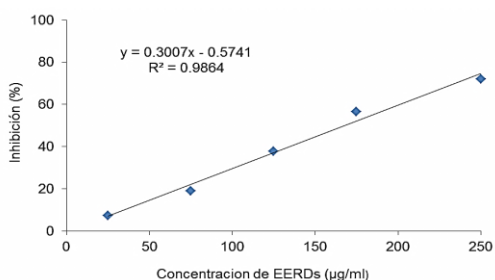
The antioxidant activity of *Dracontium spruceanum* could be associated to the presence of highly unsaturated nuclei such as polyphenols, flavonoids and quinones (Rivera, 2012), as well as to the presence of aromatic compounds like sterols, triterpenoids and alkaloids (Rengifo, 2007). In a similar study with an aqueous atomized extract from *Uncaria tomentosa*, it was found that the inhibition of the DPPH radical was 88.17% with 250  $\mu$ g/ml of the extract (Sandoval, 2012).

**Table 2**

Hematological and biochemical profiles of broiler chickens under the effect of EERDs in the drinking water at different age

Factors	Hematocrit (%)	Hemoglobin (g/dL)	Erythrocytes (x10 <sup>6</sup> /uL)	Serum Protein (g/dL)	Glucose (g/dL)
EERDs Age	0.027	0.008	0.012	0.978	0.111
E x A	0.011	0.001	0.001	0.007	0.012
	0.002	0.032	0.049	0.411	0.756
cv (%)	9.57	19.35	21.83	18.78	6.47
<b>Level of EERDs</b>					
0.00 mg/mL	24.84b	8.12b	1.28b	2.33	195.0
0.35 mg/mL	25.53a	8.98b	1.72a	2.29	205.0
0.70 mg/mL	26.54a	10.39a	1.89a	2.27	200.0
<b>Age in Days</b>					
1 day	25.73ab	6.87c	0.743c	2.49a	192.0b
14 days	26.37a	8.31b	1.36b	2.44a	196.0b
28 days	24.61b	10.44a	2.08a	2.10b	206.0a

abc: Different letters in the same column indicate statistical differences (SNK 5%). EERDs: Ethanol extract from the *Dracontium spruceanum* rhizome.



**Figure 1.** Inhibition coefficient (IC<sub>50</sub>) of DPPH due to the action of the ethanol extract from the *Dracontium spruceanum* rhizome (EERDs).

### Hematological and Biochemical Profiles

The hematocrit, hemoglobin and total erythrocyte profiles of the experimental chickens increased as the level of EERDs in the drinking water ( $p < 0.05$ ) increased. However, the serum protein and glucose profiles were similar between treatments ( $p > 0.05$ ) (Table 2).

The hematocrit increased at twenty-eight days old ( $p < 0.05$ ) as the level of the EERDs increased in the drinking water; these results resemble the study by Sandoval (2012), who reported a progressive increase in the percentage of hematocrit in broiler chickens every time the IC<sub>50</sub> from the aqueous atomized extract of cat's claw was increased in the drinking water.

The level of hemoglobin increased at fourteen days of age ( $p < 0.05$ ) as the levels of EERDs increased in the drinking water (Tables 2 and 4). This also increased with the increase in the birds age from the control group and in the group with 0.35 mg/mL of EERDs ( $p < 0.05$ ). These results are similar to those reported by Sandoval (2012), meanwhile, Reátegui-Inga et al., (2012) reported an increase in hemoglobin at forty-eight days old.

This profile also increased as an effect of the age of chickens up to fourteen days only in the control group, ( $p < 0.05$ ) (Tables 2 and 3). This could be associated to the fact that the antioxidant effect of the EERDs on the cells allows for a lengthening of the life of the erythrocytes, covering up the effect of age on erythrocytes life span in the treatment groups chickens; contrasting with the physiological patterns which are characterized by a progressive increase in the level of hematocrit each time that the chickens increase in age (Vásquez et al., 2012).

**Table 3**

Hematocrit profile (%) under the effect of EERDs level in the drinking water and age of Chickens

Level of EERDs	Age of Chickens		
	1 day	14 days	28 days
0.00 mg/m	25.73A	26.90A	21.90Bb
0.35 mg/m		25.61	25.44a
0.70 mg/mL		26.58	26.50a

AB, ab: Different capitalized letters in the same row and lowercase letters in each column indicate statistical differences (SNK 5%). EERDs: Ethanol extract from *Dracontium spruceanum* rhizome.

**Table 4**

Hemoglobin profile (g/dL) under the effect of EERDs level in the drinking water and age of Chickens

Level of EERDs	Age of Chickens		
	1 day	14 days	28 days
0.00 mg/m	6.87B	6.99 Bb	10.10A
0.35 mg/m		7.82 Bb	10.15A
0.70 mg/mL		10.12 a	10.67

AB, ab: Different capitalized letters in the same row and lowercase letters in each column indicate statistical differences (SNK 5%). EERDs: Ethanol extract from the *Dracontium spruceanum* rhizome.

The number of erythrocytes in broiler chickens increased ( $p < 0.05$ ) as the dose of the EERDs in the drinking water increased at 28 days (Tables 2 and 5). The level of erythrocytes also increased with the increase of the age, as much in the control group as in the groups with 0.35 and 0.70 mg/mL of EERDs ( $p < 0.05$ ).

**Table 5**

Total Erythrocytes (x10<sup>6</sup>/μL) under the effect of EERDs levels in the drinking water and age of Chickens

Level of EERDs	Age of Chickens		
	1 day	14 days	28 days
0.00 mg/m	0.743 C	1.29 B	1.82 Ab
0.35 mg/m	1.42 B	2.02 Ab	
0.70 mg/mL	1.38 B	2.41 Aa	

AB, ab: Different capital letters in the same row and lowercase letters in each column indicate statistical differences (SNK 5%). EERDs: Ethanol extract from the *Dracontium spruceanum* rhizome.

The level of hematocrit, hemoglobin and the number of erythrocytes of broiler chickens are within the normal values (Reece, 2015). The increase in hemoglobin, hematocrit and total erythrocytes as the EERDs increases may be related with those described for *Uncaria tomentosa*. The numerous groups of compounds present in this plant have protective effects on erythrocytes and diminish the levels of hemoglobin oxidation and the lipid peroxidation; as well as lowering the levels of ROS and hemolysis provoked by 2-4 dichlorophenol in human erythrocytes (Bors et al., 2011; Bucowska et al., 2012). The polyphenols, principal components of the *Uncaria tomentosa* extracts, could act, not just sequestering free radicals and inhibitors of lipid peroxidation, but also have the capacity to interact directly with biological membranes, causing them to be more resistant to oxidative alterations (Dreifus et al., 2010). These same mechanisms permit human erythrocytes to induce an increase in membrane thickness, followed by an increase in size and morphological variation (Bors et al., 2012). In the present study, it was found that an interaction exists, between the dose of EERDs in the drinking water and the age of chickens, on the levels of hematocrit, hemoglobin and erythrocytes ( $p < 0.05$ ) (Tables 2, 3, 4 and 5). The results show that the levels of hematocrit, hemoglobin and total erythrocytes, in general, increase with the age increase and the increase in the level of EERDs, which could be associated to physiological mechanisms of increase in oxygen demand by the muscular mass for metabolism (Skovgaard et al., 2010) and antioxidant and immunomodulatory activity of *Dracontium* (Giovannini and Howes, 2017; Benavides et al., 2009; Napolitano et al., 2011), similar to those produced by *U. tomentosa* (Vielma, et al., 2014; Wagner, et al., 1985). The age of chickens influenced ( $p < 0.05$ ) the levels of serum protein and glucose, denoting that chickens at one and fourteen-day old presented a greater concentration of serum protein ( $p < 0.05$ ) than the chic-

kens at twenty-eight days old (Table 2). These results contrast with serum protein levels produced by the physiological mechanisms (Eckersall, 2008); different results from those were reported by Sandoval (2012). On the contrary, the glucose resulted at a greater level in the chickens of twenty-eight days old than those at one and fourteen days old ( $p < 0.05$ ). This data contrasts the results of González et al. (2001), who showed a lowering of the serum glucose level in broiler chickens as age increased.

### Productive Parameters

The daily feed intake (DFI) diminished as a result of the EERDs increase in the drinking water ( $p < 0.05$ ) during the finishing phase (22-35 days) and during the total rearing phase (1-35 days) (Table 6). However, the daily weight gain (DWG) and the feed rate conversion (FRC) of the male broiler chickens during the initial, growth, and finishing phases and the total period, were not influenced by the level of EERDs in the different productive phases of the broiler chickens ( $p > 0.05$ ).

**Table 6**

Productive parameters of broiler chickens under the effect of different levels of EERDs

Initial Phase: 1 to 7 days old					
Trat.	IW	FW	DWG	DFI	FRC
0.00 mg/mL	46.00	212.00	28.00	28.00	1.04
0.35 mg/mL	45.00	207.00	27.00	27.00	1.03
0.70 mg/mL	47.00	213.00	28.00	29.00	1.04
p-value	0.15	0.52	0.52	0.84	0.99
cv (%)	3.10	3.57	4.57	12.67	3.19
Growth Phase: 8 to 21 days old					
0.00 mg/mL	212.00	1028.00	58.00	97.00	1.66
0.35 mg/mL	207.00	1029.00	59.00	96.00	1.64
0.70 mg/mL	213.00	994.00	56.00	94.00	1.69
p-value	0.52	0.26	0.26	0.38	0.78
cv (%)	3.57	3.33	4.20	3.19	6.63
Finishing Phase: 22 to 35 days old					
0.00 mg/mL	1028.00	2242.00	87.00	159.00a	1.83
0.35 mg/mL	1029.00	2193.00	84.00	156.00ab	1.87
0.70 mg/mL	994.00	2227.00	86.00	155.00 b	1.80
p-value	0.26	0.58	0.58	0.042	0.62
cv (%)	3.33	3.33	6.15	1.62	6.10
Total Period: 1 to 35 days old					
0.00 mg/mL	46.00	2247.00	63.00	108.00a	1.72
0.35 mg/mL	45.00	2170.00	61.00	107.00ab	1.76
0.70 mg/mL	47.00	2245.00	63.00	105.00 b	1.68
p-value	0.15	0.29	0.29	0.039	0.39
cv (%)	3.10	3.51	3.58	1.61	4.55

a,b: Different letters in the same column indicate statistical differences (SNK 5%). EERDs: Ethanol extract from the *Dracontium spruceanum* rhizome. IW: initial weight, FW: final weight, DWG: daily weight gain, DFI: daily feed intake, FRC: feed rate conversion.



These results could be correlated to the toxic effects of certain plants with similar nutraceutical characteristics to *Dracontium*, as is the case of *U. tomentosa*, for which toxic effects have been found in rats, mice and fish (Méndez et al., 2014; Ibrahim et al., 2009; Cala and Kochenborger, 2015), and associated with the initial phase of chickens (Sandoval, 2012).

Notwithstanding, the lack of effect of the DFI lowering on the weight gain of chickens could also be attributed to the fact that *Dracontium* possesses similar characteristics to those reported for *U. Tomentosa*, which improve the length of the intestinal villi and as a result the improvement in the absorption of nutrients (Yunis-Aguinaga et al., 2015); thus, countering the potential toxic effects attributed, in the same manner, to this plant.

#### 4. Conclusions

The ethanol extract of the *Dracontium spruceanum* rhizome at a concentration of 164.37 µg/mL inhibited 50% the DPPH; at the same time, the levels of hematocrit, hemoglobin and total erythrocytes increased, while the daily food consumption decreased and there was no effect on the daily weight gain and food rate conversion in the broilers. Other research to clarify potential toxic effects and promoters of intestinal health from *D. spruceanum* should be done.

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