



Influence of dietary artemisinin supplementation on productive performance and haematological parameters of broiler chickens

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ABSTRACT - In the present study, we aimed to assess the toxicity of artemisinin on the haematological system and its effect on the productive performance of broiler chickens. Eighteen-day-old chickens were randomly divided into four groups of 30 chickens (three replicates of 10 broilers/group): control group and three experimental groups: ART5 - diet with 5 ppm of artemisinin; ART50 - diet with 50 ppm of artemisinin; and ART500 - diet with 500 ppm of artemisinin. Artemisinin enhanced the productive performances of broiler chickens at the lowest concentration (5 ppm), but at the highest concentration (500 ppm), it negatively affected weight gain and the feed conversion ratio. The performance characteristics of the chickens whose diets were supplemented with 50 ppm artemisinin were similar to those of the control group. Additionally, 5 ppm artemisinin in feed did not significantly affect the haematological parameters of the chickens, but 50 and 500 ppm artemisinin induced a gradual decline of the total leukocytes, lymphopenia, monocytosis, and eosinopenia, and the highest concentration caused anaemia. Artemisinin at a low concentration could be used as a feed additive in the poultry industry to improve organic broiler production performance with no serious side effects.

Key Words: animal nutrition, growth promoter, toxicity

Introduction

Artemisinin is a sesquiterpene lactone isolated from the herb *Artemisia annua*, which has been used with success for many centuries in traditional medicine to treat fever and jaundice and to heal wounds, eye infections, and skin diseases. The effects of artemisinin have been studied in many diseases, such as cancer and infectious or parasitic diseases, and artemisinin is used as a combination therapy against drug-resistant malaria (Naeem et al., 2014; Sadiq et al., 2014).

Different researchers have studied the effects of artemisinin and *A. annua* on chicken coccidiosis

(Allen et al., 1997;1998; Youn, 2001; Arab et al., 2006; del Cacho, 2010; de Almeida, 2012; Drăgan et al., 2014). Coccidiosis is an economically devastating disease for the poultry industry. The development of drug resistance to all known anticoccidial drugs and consumer concerns regarding drug residues have increased the interest of the scientific community in searching for alternative means of coccidiosis prevention. Botanicals, natural feedstuffs, and artemisinin are intensively studied compounds (Chapman, 1997; Allen and Fetterer, 2002, Abbas, 2012a; Abbas, 2012b).

Despite the extensive research on using artemisinin in coccidiosis control, its effect on chickens has not been sufficiently investigated. Arab et al. (2009) showed that chickens treated with a single oral dose of artemisinin exhibited neurological signs, liver, kidney, and brain degeneration, especially at very high dosages, and presented a dose-dependent reduced feed intake. Shahbazfar et al. (2011) showed that after chronic intake of artemisinin, the chickens exhibited anaemia, dose-dependent decreases in haematocrit and red blood cells, and mild lesions in liver, kidney, and brain. The authors concluded that at therapeutic dosages, artemisinin causes no serious side effects.

Although artemisinin has been proven to be safe in chickens at therapeutic dosages, the negative effect on the

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haematological system and the effect on the productive performance of chickens needs further investigation.

Material and Methods

Ross 308 broiler chickens were purchased at one day of age and were housed in metal cages. Feed and water were provided *ad libitum* and the lighting programme was continuous. From 1-18 days of age, the chickens received standard broiler starter feed without the addition of anticoccidials (Table 1).

Veterinary conditions regarding the protection of the animals used in this research were complied with all national and EU standards and legislation. All experiments were approved by the Research Bioethics Commission of UASMV Cluj-Napoca (case no. 4/19.09.2013).

Artemisinin powder (min. 98% purity) was purchased from Intatrade Chemicals GmbH, Germany, and was introduced in the feed in concentrations of 5, 50, and 500 ppm

(5, 50, and 500 mg artemisinin/kg feed), according to the dosages used in a previously published manuscript of the anticoccidial effect of artemisinin (Pop et al., 2015).

At 18 days old, broilers were divided into four experimental groups of 30 chickens each, with three replicates of 10 individually marked chickens. On the same day (day 0 of the experiment), the experimental feed was introduced into their diet for 28 days (until the age of 46 days). The groups were as follows: a control group, which received standard broiler grower feed without the addition of anticoccidials (Table 1); and three experimental groups: ART5 - diet with 5 ppm of artemisinin; ART50 - diet with 50 ppm of artemisinin; and ART500 - diet with 500 ppm of artemisinin. On day 0 and after 14 and 28 days of continuous administration of experimental feed, blood samples were aseptically collected from the ulnar vein of each chicken from the first replicate into heparin-containing tubes (50 IU/mL); a 2.0 mL syringe and 23G needle were used for the collections.

Blood samples were tested for red blood cells (RBC) count, haemoglobin concentration, packed cell volume (PCV), the total and differential white blood cells (WBC) counts, and the values of derived erythrocyte constants: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC).

The count of the total number of RBC and WBC was carried out manually by the haemocytometer method using Natt-Herrick modified by Prochaska dilution liquid (Ognean and Cernea, 2011).

The concentration of haemoglobin was determined by the spectrophotometer method (ELISA Bio-Rad 1100 microplate reader); the absorbance at 540 nm was measured after adding 4% ammonia solution to the blood samples.

The PCV was established using the microhaematocrit method. Briefly, capillary tubes were filled 2/3 with anticoagulated blood, the unfilled ends were sealed with clay, and they were centrifuged at 12,000 rpm for 5 min. The PCV values were determined with the aid of a microhaematocrit reader.

The MCV, MCH, and MCHC were calculated using the following formulas:

$$\text{MCV } (\mu\text{m}^3) = [\text{PCV} \times 10] / \text{RBC}$$

$$\text{MCH } (\text{pg}) = [\text{Hb} \times 10] / \text{RBC}$$

$$\text{MCHC } (\text{g/dL}) = [\text{Hb} \times 100] / \text{PCV}$$

Differential WBC counts were performed by the microscopic examination of blood smears; 100 successive WBC/sample were counted and identified, and the results were reported as the percentages of different WBC (Samour, 2006).

Table 1 - Composition of basic diets of broiler chickens at 1-46 days of age

	Starter (1-17 days)	Grower (18-46 days)
Dry matter (g/kg)	879.0	885.7
Crude protein (g/kg)	199.6	175.0
Crude fat (g/kg)	28.4	45.7
Crude cellulose (g/kg)	49.9	33.3
Ash (g/kg)	-	8.8
Metabolisable energy (Mj/kg)	11.65	12.60
Methionine (g/kg)	3.8	4.4
Threonine (g/kg)	-	6.3
Methionine + cysteine (g/kg)	7.1	8.0
Tryptophan (g/kg)	2.3	1.9
Lysine (g/kg)	9.7	11.5
Ca (g/kg)	9.6	10.0
P (g/kg)	6.8	7.0
Na (g/kg)	1.2	1.5
Zn (mg/kg)	50.36	70.13
Cu (mg/kg)	8.63	12.02
Fe (mg/kg)	43.16	60.11
Mn (mg/kg)	71.94	100.19
I (mg/kg)	0.72	1.00
Se (mg/kg)	0.14	0.20
Betaine (mg/kg)	-	200.60
Vitamin A (IU/kg)	10152.00	12096.00
Vitamin D3 (IU/kg)	2538.00	3024.00
Vitamin E (mg/kg)	33.84	40.32
Vitamin K3 (mg/kg)	2.54	3.02
Vitamin B1 (mg/kg)	2.50	3.02
Vitamin B2 (mg/kg)	5.92	7.06
Ca d-pantothenate (mg/kg)	10.15	12.10
Vitamin B6 (mg/kg)	4.23	5.04
Vitamin B12 (mg/kg)	0.02	0.03
Biotin (mg/kg)	0.10	0.12
Niacin (mg/kg)	33.84	40.32
Folic acid (mg/kg)	0.85	1.01
Choline (mg/kg)	398.40	-
Antioxidant (mg/kg)	128.00	128.00

At the end of the experiment, the chickens were euthanized by cervical dislocation and chicken necropsies were performed.

Bone marrow samples were collected from the chickens in the first replicate for both histological and cytological evaluations (Elmore, 2006). For the histopathological examination, the proximal tibiotarsus (Campbell, 1994) was bilaterally harvested, cleaned of connective tissue and skin, and trimmed longitudinally into small pieces (approximately 1 × 1 cm with 0.2-cm thickness). After 72 h of fixation in 10% neutral-buffered formalin, the samples were decalcified for three weeks in a 1:1 mixture of 8% formic acid and 8% hydrochloric acid (Prophet, 1992). When decalcification was completed, the tissues were dehydrated using an isopropyl alcohol gradient (70%, 90%, 95%, and 100%), clarified in xylene and embedded in paraffin wax of high-melting temperature following a routine processing protocol (Prophet et al., 1992). After solidification, the paraffin blocks were trimmed and kept at room temperature until use. Tissue sections were cut from each paraffin block at 2-3- μ m thickness with a rotary microtome (Leica RM 2125) and were stored overnight at 37 °C on a thermostatically controlled slide warmer. Tissue sections were routinely stained with haematoxylin and eosin (H&E) and examined using an Olympus BX41 microscope.

During histopathological examination, the proportions of granulocytic to erythroid cells (or Myeloid to Erythroid ratio) were assessed (Wakenell, 2010; Reagan et al., 2011).

The bone marrow smears were produced using touch imprints and the push-slide technique with sectioned proximal tibiotarsus before it was trimmed for histopathology. After fixation, the smears were stained using the Wright-Giemsa technique.

Bright field microscopy was performed using a 3.2-megapixel resolution Olympus UC30 Digital Camera and processed with the Olympus Stream Basic image analysis software (Olympus Stream Basic Package).

The amount of consumed feed was monitored daily per cage and the chickens were weighed individually on days 0, 14, and 28 of the experiment to determine the weight gain, feed intake, and feed conversion ratio.

Statistical analysis was performed using Medcalc software, version 15.8. The normal distribution of the data was verified by Shapiro-Wilk test and in the case of normally distributed data, independent samples T-test was performed, which consisted of an F test for equal variances; depending on the variance, T-test (equal variances) or Welch-test (unequal variances) was used. Mann-Whitney tests were used for data that were not normally distributed. The statistical interpretation of the histopathological and cytological data was performed by ANOVA test using the Origin 8.5 software. Differences were considered significant at a P-value less than or equal to 0.05.

Results

At the first blood sample collection (day 0), the number of erythrocytes was higher in the groups treated with 5 and 500 ppm artemisinin compared with that of the control group ($P \leq 0.01$). At the second collection, the total number of erythrocytes increased significantly in all experimental groups, but there were no significant differences between groups. At the third blood sample collection, the RBC count had similar values between the control and ART5 groups; the ART50 group had fewer erythrocytes compared with the control group ($P = 0.03$), and the chickens from ART 500

Table 2 - Effect of different concentrations of artemisinin on erythrogram parameters of broiler chickens compared with control group

Blood sampling	Group	RBC ($\times 10^{12}/L$)	PCV (%)	Hb (g/dL)	MCV (μm^3)	MCH (pg)	MCHC (g/dL)
First	Control	1.88 \pm 0.70a	27.3 \pm 0.67b	5.12 \pm 0.15a	155.99 \pm 12.96a	28.86 \pm 2.28a	18.71 \pm 0.67a
	ART5	3.37 \pm 0.36b	29.7 \pm 0.58a	5.18 \pm 0.15a	91.54 \pm 9.14b	17.03 \pm 2.00b	18.67 \pm 0.84a
	ART50	2.06 \pm 0.36ac	27.5 \pm 0.70b	5.37 \pm 0.33a	102.32 \pm 25.87ab	20.32 \pm 5.65b	19.71 \pm 1.23ab
	ART500	3.04 \pm 0.44bc	27.9 \pm 0.64ab	6.28 \pm 0.23b	114.51 \pm 15.22b	24.23 \pm 3.49ab	21.08 \pm 0.68b
Second	Control	5.94 \pm 1.11a	28.6 \pm 0.55ab	5.14 \pm 0.26a	62.08 \pm 9.75a	11.40 \pm 2.02a	17.94 \pm 0.86a
	ART5	4.94 \pm 0.77a	29.0 \pm 0.50a	6.51 \pm 0.50b	72.43 \pm 9.91a	16.03 \pm 2.47a	22.49 \pm 1.68ab
	ART50	4.55 \pm 0.99a	26.9 \pm 0.85b	5.85 \pm 0.57ab	96.01 \pm 22.15a	19.80 \pm 4.16a	21.66 \pm 1.79ab
	ART500	5.04 \pm 1.05a	28.7 \pm 0.68ab	6.63 \pm 0.54b	81.58 \pm 14.24a	18.78 \pm 3.83a	23.20 \pm 1.89b
Third	Control	3.76 \pm 0.25a	26.0 \pm 1.12a	5.46 \pm 0.36a	72.48 \pm 5.11a	15.26 \pm 1.63a	20.87 \pm 1.46a
	ART5	3.35 \pm 0.32ab	25.8 \pm 0.70a	4.68 \pm 0.26a	84.13 \pm 9.11ab	15.86 \pm 2.53ab	18.22 \pm 1.08a
	ART50	3.13 \pm 0.23b	25.2 \pm 0.80a	4.51 \pm 0.39a	83.90 \pm 5.63a	14.91 \pm 1.55ab	18.03 \pm 1.62a
	ART500	2.23 \pm 0.14c	23.3 \pm 1.12a	4.28 \pm 0.71a	106.49 \pm 6.20b	20.61 \pm 3.49b	18.67 \pm 3.27a

RBC - red blood cells; PCV - packed cell volume; Hb - haemoglobin; MCV - mean corpuscular volume; MCH - mean corpuscular haemoglobin; MCHC - mean corpuscular haemoglobin concentration; ART5 - diet with 5 ppm artemisinin; ART50 - diet with 50 ppm artemisinin; ART500 - diet with 500 ppm artemisinin.

Values with no common letters in a column and periods of blood sampling were significantly different ($P < 0.05$).

Results are expressed as means \pm standard error of the mean.

group had a very low number of erythrocytes ($2.23 \times 10^{12}/L$) (Table 2).

Before the administration of artemisinin, the ART5 group had a higher value of PCV than that of the control group ($P = 0.01$). In day 14 of the experiment, all the groups had similar percentages of PCV. In correlation with the values recorded for the RBC counts, at 28 days after the administration of experimental feed, all the groups exhibited lower PCV values, and the chickens of the ART500 group had the lowest values compared with those of the control group ($P = 0.1$) (Table 2).

At the first blood collection, the concentration of haemoglobin values were similar in all groups except the ART500 group, which had a higher haemoglobin concentration ($P = 0.0005$). This trend persisted at the second blood sampling as well ($P = 0.02$), but in the third collection, the ART500 group had a lower haemoglobin concentration compared with that of the control group ($P = 0.09$) and also with those of the other two samplings ($P \leq 0.02$) (Table 2).

The value of MCV decreased significantly at the second sampling and slightly increased at the third blood collection in all experimental groups. In the first sampling, the chickens from all artemisinin-treated groups had a lower value of MCV compared with the control group and this difference was more significant in the ART5 and ART500 groups ($P \leq 0.05$). At the second sampling, the values recorded did not significantly differ among groups. At the final recording, the highest value was recorded for the ART500 group ($106.49 \mu m^3$; $P = 0.0008$). The same trend was observed for MCH. The MCHC values were similar in all groups, except for the ART500, which had a higher MCHC value at the first and second blood samplings ($P \leq 0.02$) (Table 2).

At the first two blood samplings, the total number of WBC was higher in the experimental groups than in the control group and the only WBC values from the ART5 group at the first collection were significantly different ($P = 0.006$). After 28 days of administration of artemisinin in the feed of chickens, the WBC values recorded for all experimental groups were lower than those for the control group ($P \leq 0.4$). In terms of dynamics, the control group had similar values at all three blood samplings, while in the groups treated with artemisinin, the total number of WBC was significantly decreased from the first sampling until the last ($P \leq 0.05$). The ART5 group had the most dramatic decrease of WBC ($P < 0.001$), although the lowest value recorded was in the ART500 group after 28 days of experimental feed administration (Table 3).

Regarding the different types of leukocytes, the groups treated with 5 or 50 ppm artemisinin had a progressive decrease in their heterophil percentage from the first to last blood sampling, with the lowest value recorded for the ART50 group (17.4%) at the third blood collection ($P = 0.02$). However, the heterophil percentage of the chickens that received 5 ppm artemisinin in their feed was similar to that of the control group at the second and third samplings. The ART500 group had similar values of heterophil percentages at all three collections, but these values were only significantly lower than those recorded for the control group at the third blood sampling ($P < 0.05$) (Table 3).

The percentage of lymphocytes was significantly decreased from the first blood sampling until the last sampling in all groups treated with artemisinin ($P \leq 0.05$). Although the control group had a lower percentage at the second collection, this reduction was recovered in the third blood sampling. The highest reduction in the lymphocyte

Table 3 - Effect of different concentrations of artemisinin on leukogram parameters of broiler chickens compared with control group

Blood sampling	Group	WBC ($\times 10^9/L$)	Differential WBC (%)				
			Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
First	Control	13.9 \pm 1.03a	24.6 \pm 4.20a	40.5 \pm 4.81a	20.8 \pm 3.93a	13.5 \pm 1.86a	0.6 \pm 0.40a
	ART5	22.3 \pm 2.32b	28 \pm 3.56a	36.6 \pm 3.66a	23.3 \pm 4.53a	11.3 \pm 1.85a	0.8 \pm 0.61a
	ART50	15.7 \pm 1.43ab	25.3 \pm 3.20a	45.7 \pm 2.64ab	14.7 \pm 1.77a	13 \pm 1.51a	1.3 \pm 0.65a
	ART500	17.1 \pm 1.06b	19.9 \pm 3.34a	52.3 \pm 2.89b	16.3 \pm 2.32a	10.4 \pm 1.54a	1.1 \pm 0.57a
Second	Control	13.8 \pm 2.39a	23.2 \pm 2.98a	27.8 \pm 4.77a	31.5 \pm 4.17a	17.5 \pm 4.37a	0a
	ART5	16.7 \pm 2.20a	22.6 \pm 3.49a	22.3 \pm 3.96a	33.4 \pm 3.96a	21.7 \pm 2.70a	0a
	ART50	18.3 \pm 2.78a	20.6 \pm 3.62a	31.3 \pm 4.44a	23.9 \pm 2.54a	24.1 \pm 4.27a	0.1 \pm 0.10a
	ART500	15.5 \pm 1.74a	21.5 \pm 3.06a	25.7 \pm 4.67a	30.1 \pm 2.76a	22.7 \pm 2.86a	0a
Third	Control	13.0 \pm 1.45a	22.0 \pm 1.18a	38.38 \pm 1.95a	12.5 \pm 2.07a	26 \pm 2.23a	1.13 \pm 0.40a
	ART5	11.2 \pm 0.39a	23.0 \pm 1.06a	29.8 \pm 1.00b	23.9 \pm 1.00b	23.3 \pm 2.54ab	0b
	ART50	11.3 \pm 0.56a	17.4 \pm 0.60b	30.8 \pm 1.44b	38.2 \pm 1.32c	13.6 \pm 1.35c	0b
	ART500	10.2 \pm 0.99a	19.11 \pm 0.70b	25.33 \pm 1.69c	38.89 \pm 1.26c	16.67 \pm 1.41bc	0b

WBC - white blood cells; ART5 - diet with 5 ppm artemisinin; ART50 - diet with 50 ppm artemisinin; ART500 - diet with 500 ppm artemisinin.

Values with no common letters in a column and periods of blood sampling were significantly different ($P < 0.05$).

Results are expressed as means \pm standard error of the mean.

percentage was recorded for the ART500 group, but this group had a higher percentage of lymphocytes at the first blood sampling compared with the control group ($P = 0.05$). After 28 days of artemisinin administration, the percentage of lymphocytes in the chickens from all experimental groups was significantly lower than that in the control group ($P \leq 0.005$) (Table 3).

The percentage of monocytes in the control group was increased at the second blood sampling and was dramatically decreased at the third sampling. This aspect was also recorded in the chickens treated with 5 ppm artemisinin, but in the third blood sampling, the percentage of monocytes in this group was significantly higher than that of the control group ($P = 0.0004$). In the two other artemisinin-treated groups, the percentage of monocytes increased gradually from the first to the third blood sampling and had similar values, but these values were much higher than those of the control group at the third collection ($P \leq 0.0005$) (Table 3).

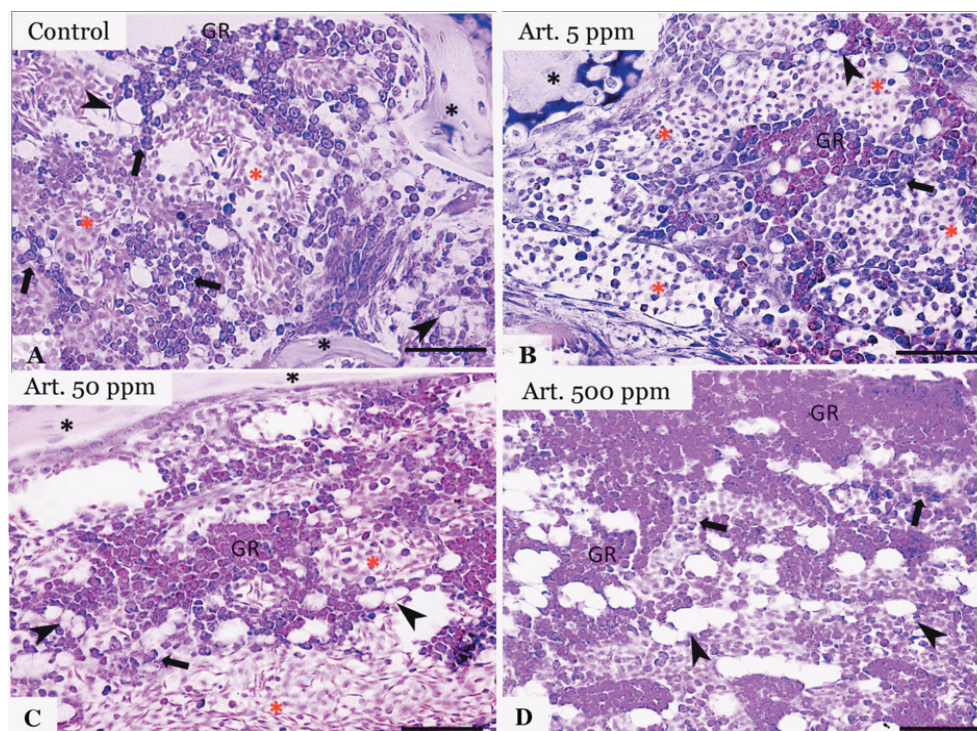
The eosinophil percentage in the chickens treated with 50 and 500 ppm artemisinin was significantly lower than that of the control group at the third blood sampling ($P \leq 0.002$) (Table 2).

Regarding the basophil percentage, there were only significant differences between the control group and the experimental groups at the third blood sampling ($P \leq 0.01$) and in this case, there were no basophils detected in the artemisinin-treated groups (Table 3).

The myeloid to erythroid ratio was higher for group ART5 than for ART50 (2:1 and 2.1:1, respectively, compared with 1.6:1 for the control group and 1.7:1 for the ART500 group), but the difference was not statistically significant ($P = 0.4$). Except for the moderately increased number of myeloid precursors, no other changes were apparent (Figures 1 and 2).

For all the periods recorded, the weight gain of the chickens treated with 5 ppm artemisinin was higher than that of the chickens from the control group, but the difference was significant only at the first and third samplings ($P = 0.01$). The group treated with 50 ppm artemisinin had similar values to the control group, while the chickens treated with 500 ppm artemisinin had significantly reduced weight gains compared with those of the control group ($P \leq 0.05$) (Table 4).

During the experiment, the chickens treated with 5 ppm artemisinin consumed a higher amount of feed than the



In all images, abundant venous sinuses (indicated by red asterisks) delineate islands and sheets of haematopoietic cells that are admixed in various proportions with mature adipocytes (indicated by arrow heads). A moderately increased number of myeloid precursors can be observed for the ART50 (image C) and ART500 (image D) groups compared with the control group (image A).

GR - myeloid cells (granulocytic/myeloid); arrows indicate cells from the erythroid cell line.

Black asterisks indicate the trabecular bone, lined by flattened or cuboidal osteoblasts and sparse osteoclasts.

H&E stain, objective x40. Scale bar = 75 μ m.

Figure 1 - Representative histological images of the bone marrow following chronic oral intake of 5, 50 or 500 ppm artemisinin compared with the control group.

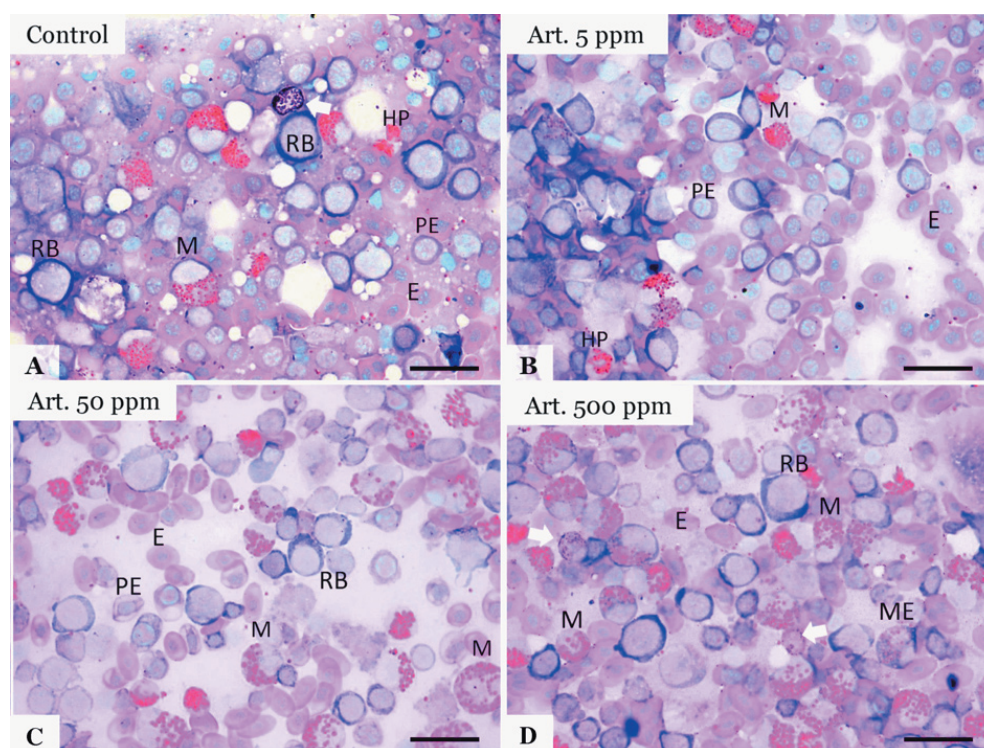
control group, but this difference was significant only at the second and third samplings ($P \leq 0.001$). The ART50 group had values that were similar to those of the control group during the experiment. The chickens treated with 500 ppm artemisinin had a lower feed intake compared with that of the control group at all the three samplings ($P \leq 0.03$) (Table 4).

In the first sampling recorded, the feed conversion ratio was superior for the group treated with 5 ppm artemisinin ($P = 0.0005$), while the group treated with 500 ppm artemisinin had an inefficient use of feed compared with that of the control group ($P = 0.02$). In the period from 14-28 days, all the groups had similar values of FCR. For

the total period of recording, the groups treated with 500 ppm artemisinin had the worst FCR ($P = 0.2$), while the chickens that received 5 ppm artemisinin had a better FCR compared with that of the control group ($P = 0.1$) (Table 4).

Discussion

The poultry industry has benefited from rapid development, mainly due to advances in genetics, nutrition, and health management. Diet formulations are especially important because they can increase broiler performances or can cause great economic losses in the meat industry (Alvarenga et al., 2015).



E - mature erythrocyte; PE - polychromatic rubricytes and erythrocytes; M - myelocyte; ME - eosinophilic myelocytes; RB - rubriblast; HP - heterophil. Wright-Giemsa stain, objective x100. Scale bar = 20 μ m.

Figure 2 - Cytological images of bone marrow following the chronic oral intake of 5, 50, and 500 ppm artemisinin compared with the control group.

Table 4 - The effect of different concentrations of artemisinin on production performances of broiler chickens compared with control group

Group	Weight gain			Feed intake			Feed conversion ratio		
	0-14 days	14-28 days	0-28 days	0-14 days	14-28 days	0-28 days	0-14 days	14-28 days	0-28 days
Control	43.44 \pm 1.30a	42.41 \pm 1.60ab	42.92 \pm 1.26a	114.52 \pm 0.04a	124.41 \pm 0.12a	119.47 \pm 0.07ab	2.71 \pm 3.10a	3.07 \pm 1.43a	2.89 \pm 1.85a
Art5	48.40 \pm 1.42b	46.71 \pm 1.59a	47.56 \pm 1.28a	117.35 \pm 0.03b	143.30 \pm 0.12a	130.33 \pm 0.06a	2.43 \pm 1.40a	3.09 \pm 2.85b	2.75 \pm 1.47b
Art50	42.76 \pm 1.70a	41.49 \pm 2.11ab	42.33 \pm 1.64a	113.34 \pm 0.05a	126.40 \pm 0.11a	119.87 \pm 0.06ab	2.66 \pm 1.88a	3.12 \pm 4.02a	2.88 \pm 2.59a
Art500	33.71 \pm 1.21c	38.07 \pm 1.47b	35.89 \pm 1.16a	98.13 \pm 0.11c	111.48 \pm 0.12a	104.81 \pm 0.11b	3.02 \pm 5.16b	3.07 \pm 4.52c	3.04 \pm 4.38c

ART5 - diet with 5 ppm artemisinin; ART50 - diet with 50 ppm artemisinin; ART500 - diet with 500 ppm artemisinin.

Values with no common letter in a column are significantly different ($P < 0.05$).

Results are expressed as means \pm standard error of the mean.

Despite advances in drug development, broilers are still exposed to many infectious or parasitic diseases that produce losses with morbidity or even mortality. Searching for alternative substances for disease control, particularly natural products, is highly encouraged not only for solving the problem of drug accumulation in the meat, but also for their beneficial effects on the gut microbiota or for their immunomodulatory properties, resulting in improved production performances (Sugiharto, 2014).

Artemisinin, the main bioactive compound of the herb *A. annua* and a powerful antimalarial drug, has been extensively studied for its effects against coccidiosis in broiler chickens. Although it has been proven that it can be used as an alternative in coccidiosis control, its effects on chicken health are not well known.

In the present study, at the lowest concentration, artemisinin improved weight gain and the feed conversion ratio and the chickens had a higher feed intake. The chickens treated with 50 ppm artemisinin had similar weight gains and feed conversion ratios as the chickens from the control group, which were fed with standard feed. However, artemisinin at the 500-ppm concentration caused reduced weight gain, inefficient feed conversion, and a lower feed intake. These negative effects are likely due to the higher quantity of the compound, which may give the feed a bitter taste and reduce its palatability, similar to what happens in children treated for malaria with artemisinin-based combination therapies (Yeka and Harris, 2010). In a single-oral dose study, Arab et al. (2009) administered 10, 50, 250, 1250, and 2500 mg artemisinin/kg feed to 30-day-old chickens and noticed the same dose-dependent reduction in food intake. In contrast, Shahbazfar et al. (2011) did not report any negative effects on the weight gain or food intake, but they used lower doses of 17, 34, 68, and 136 ppm for 36 days. In this study, the tendency for a decreased number of erythrocytes, haemoglobin concentration, and PCV in the chickens supplemented with 500 ppm artemisinin suggests the evolution of anaemia. Based on the increased values of MCV, we can conclude that it is a macrocytic anaemia (Aslinia et al., 2006). Shahbazfar et al. (2011) noticed the clinical signs of anaemia in chickens fed 68 and 136 ppm artemisinin, including a significant reduction of the haematocrit in blood tests and of the RBC count, especially at higher concentrations of artemisinin.

In the present study, the white blood cells followed a decreasing trend in all chickens that were fed the artemisinin diets, which corresponded with lymphopenia, monocytosis, and eosinopenia in the chickens supplemented with 50 ppm and 500 ppm artemisinin. This leukogram pattern is consistent with a stress leukogram due to

endogenous steroid release in stressful conditions (Maxwell, 1993, Schmidt, 2015). This result may imply that dietary supplementation with a higher concentration of artemisinin is a stressor to the chickens.

In animal experiments, artemisinin and its derivatives have frequently produced the inhibition of erythropoiesis and this seems to represent a sensitive target for artemisinins (Efferth and Kaina, 2010). In the present study, there were sufficient numbers of erythroid precursors detected during the bone marrow evaluation for all experimental groups. Contradictory studies show that artemisinins can enhance or inhibit leukocyte functions in human or animal trials (Efferth and Kaina, 2010).

Conclusions

Artemisinin improves the productive performance of broiler chickens and has no serious side effects; thus, at 5 ppm concentration, it is suitable for use as a feed additive in the poultry industry, in particular for organic broilers.

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References

- Abbas, R. Z.; Colwell, D. and Gilleard, J. 2012a. Botanicals: an alternative approach for the control of avian coccidiosis. *World's Poultry Science Journal* 68:203-215.
- Abbas, R. Z.; Iqbal, Z.; Khan, A.; Sindhu, Z. U. D.; Khan, J. A.; Khan, M. N. and Raza, A. 2012b. Options for integrated strategies for the control of avian coccidiosis. *International Journal of Agriculture and Biology* 14:1014-1020.
- Allen, P. C.; Danforth, H. D. and Augustine, P. C. 1998. Dietary modulation of avian coccidiosis. *International Journal of Parasitology* 28:1131-1140.
- Allen, P. C. and Fetterer, R. H. 2002. Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clinical Microbiology Reviews* 15:58-65.
- Allen, P. C.; Lydon, J. and Danforth, H. D. 1997. Effects of components of *Artemisia annua* on coccidia infections in chickens. *Poultry Science* 76:1156-1163
- Alvarenga, R. R.; Rodrigues, P. B.; Zangeronimo, M. G.; Oliveira, E. C.; Mariano, F. C. M. Q.; Lima, E. M. C.; Garcia Jr, A. A. P.; Naves, L. P. and Nardelli, N. B. S. 2015. Validation of prediction equations of energy values of a single ingredient or their combinations in male broilers. *Asian-Australasian Journal of Animal Sciences* 28:1335-1344.
- Arab, H. A.; Mardjanmehr, S. H.; Shahbazfar, A.; Rassouli, A.; Abdollahi, M. and Nekouie, O. 2009. Toxicopathologic effects of artemisinin in broiler chickens following a single oral dose: An LD50 study. *International Journal of Poultry Science* 8:808-812.

- Arab, H. A.; Rahbari, S.; Rassouli, A.; Moslemi, M. H. and Khosravirad, F. 2006. Determination of artemisinin in *Artemisia sieberi* and anticoccidial effects of the plant extract in broiler chickens. *Tropical Animal Health and Production* 38:497-503.
- Aslinia F.; Mazza J. J. and Yale S. H. 2006. Megaloblastic anemia and other causes of macrocytosis. *Clinical Medicine & Research* 4:236-241.
- Campbell, T. W. 1994. Hematology. p.176-197. In: *Avian medicine: principles and application*. Ritchie, B. W.; Harrison, G. J. and Harrison, L. R., eds. Wingers Publishing, Lake Worth, FL.
- Chapman, H. D. 1997. Biochemical, genetic and applied aspects of drug resistance in *Eimeria* parasites of the fowl. *Avian Pathology* 26:221-244.
- de Almeida, G. F.; Horsted, K.; Thamsborg, S. M.; Kyvsgaard, N. C.; Ferreira, J. F. and Hermansen, J. E. 2012. Use of *Artemisia annua* as a natural coccidiostat in free-range broilers and its effects on infection dynamics and performance. *Veterinary Parasitology* 186:178-187.
- del Cacho, E.; Gallego, M.; Francesch, M.; Quílez, J. and Sánchez-Acedo, C. 2010. Effect of artemisinin on oocyst wall formation and sporulation during *Eimeria tenella* infection. *Parasitology International* 59:506-511.
- Drăgan, L.; Györke, A.; Ferreira, J. F.; Pop, I. A.; Dunca, I.; Drăgan, M.; Mircean, V.; Dan, I. and Cozma, V. 2014. Effects of *Artemisia annua* and *Foeniculum vulgare* on chickens highly infected with *Eimeria tenella* (phylum Apicomplexa). *Acta Veterinaria Scandinavica* 56:22.
- Efferth, T. and Kaina, B. 2010. Toxicity of the antimalarial artemisinin and its derivatives. *Critical Reviews in Toxicology* 40:405-21.
- Elmore, S. A. 2006. Enhanced histopathology of the bone marrow. *Toxicologic Pathology* 3:666-686.
- Maxwell, M. H. 1993. Avian blood leucocyte responses to stress. *World Poultry Science Journal* 49:34-43.
- Naeem, M.; Idrees, M.; Singh, M.; Khan, M. M. A. and Moinuddin. 2014. *Artemisia annua*: A Miraculous Herb to Cure Malaria. p.27-49. In: *Artemisia annua - Pharmacology and Biotechnology*. Aftab, T.; Ferreira, J. F. S.; Khan, M. M. A. and Naeem, M., eds. Springer-Verlag Berlin Heidelberg.
- Ognean, L. and Cernea, L. C. 2011. Practical applications in animal physiology. Ed. AcademicPres, Cluj-Napoca.
- Pop, L.; Györke, A.; Tăbăran, A. F.; Dumitrache, M. O.; Kalmár, Z.; Magdaş, C.; Mircean, V.; Zagon, D.; Balea, A. and Cozma, V. 2015. Effects of artemisinin in broiler chickens challenged with *Eimeria acervulina*, *E. maxima* and *E. tenella* in battery trials. *Veterinary Parasitology* 214:264-271.
- Prophet, E. B.; Mills, B.; Arrington, J. B. and Sobin, L. H. 1992. Laboratory methods in histotechnology. Armed Forces Institute of Pathology-American Registry of Pathology, Washington, DC. p.53-58, 128-130, 134-136, 156-158, 170, 177-178.
- Reagan, W. J.; Irizarry-Rovira, A.; Poutout-Belissent, F.; Bolliger, A. P.; Ramaiah, S. K.; Travlos, G. and Walter, G. 2011. Best practices for evaluation of bone marrow in nonclinical toxicity studies. *Veterinary Clinical Pathology* 40:119-134.
- Sadiq, A.; Hayat, M. Q. and Ashraf, M. 2014. Ethnopharmacology of *Artemisia annua* L.: A review. p.9-25. In: *Artemisia annua - Pharmacology and biotechnology*. Aftab, T.; Ferreira, J. F. S.; Khan M. M. A.; Naeem, M., eds. Springer-Verlag Berlin Heidelberg.
- Samour, J. 2006. Diagnostic value of hematology. p.587-609. In: *Clinical Avian Medicine*. v.2. Harrison, G. and Lightfoot, T., eds. Spix Publishing, Palm Beach, FL, USA.
- Schmidt S. 2015. Top 5 Leukogram Patterns. Available at: <<http://www.cliniciansbrief.com>>. Accessed on: July 22, 2015.
- Shahbazfar, A. A.; Mardjanmehr, S. H.; Arab, H. A.; Rassouli, A. and Abdollahi, M. 2011. Effects of artemisinin in broiler chickens following chronic oral intake. *Tropical Animal Health and Production* 43:843-849.
- Sugiharto, S. 2014. Role of nutraceuticals in gut health and growth performance of poultry. *Journal of the Saudi Society of Agricultural Sciences*. doi: <http://dx.doi.org/10.1016/j.jssas.2014.06.001>.
- Wakenell, P. S. 2010. Hematology of chickens and turkeys. p.958-966. In: *Schalm's veterinary hematology*. Weiss, D. J. and Wardrop, K. J., eds. Blackwell Publishing Company, USA.
- Yeka, A. and Harris, J. C. 2010. Treating uncomplicated malaria in children: comparing artemisinin-based combination therapies. *Current Opinion in Pediatrics* 22:798-803.
- Youn, H. J. and Noh, J. W. 2001. Screening of the anticoccidial effects of herb extracts against *Eimeria tenella*. *Veterinary Parasitology* 96:257-263.