

Piperine as a phytogetic additive in broiler diets

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Abstract – The objective of this work was to determine the effect of piperine as a phytogetic additive in chicken broiler diet. Seven-day-old male chicks were randomly allocated in four experimental treatments (n = 24), with four replicates (n = 6). The piperine was added to diets at concentrations of 0, 60, 120, and 180 mg kg⁻¹ for 35 consecutive days. The following were evaluated: biochemical, hematological and histopathological parameters; performance and carcass yield. Histomorphometric analyses were also carried out. The addition of 120 and 180 mg kg⁻¹ of piperine did not alter broiler body weight and feed conversion, whereas 60 mg kg⁻¹ of piperine interfered positively in both parameters from 36 to 42 days of age and significantly increased the absorption surface of the duodenum and the ileum. No macroscopic alteration in organ size and color was observed in the broilers fed diets with the evaluated concentrations of piperine. The supplementation of 120 and 180 mg kg⁻¹ of piperine is toxic to liver tissue and reduces the absorption surface of the jejunum. The diet supplemented with 60 mg kg⁻¹ of piperine is safe.

Index terms: *Piper nigrum*, performance, phytogetic additive, sanitation.

Piperina como suplemento fitogênico na dieta de frangos de corte

Resumo – O objetivo deste trabalho foi determinar o efeito da piperina, como suplemento fitogênico, na dieta de frangos de corte. Pintos com sete dias de idade foram distribuídos aleatoriamente em quatro tratamentos (n = 24), com quatro repetições (n = 6). A piperina foi adicionada na ração nas concentrações de 0, 60, 120 e 180 mg kg⁻¹ por 35 dias consecutivos. Foram avaliados: parâmetros bioquímicos, hematológicos e histopatológicos; desempenho e rendimento de carcaça. Também foram realizadas análises histomorfométricas. A adição de 120 e 180 mg kg⁻¹ de piperina não alterou o peso corpóreo e a conversão alimentar dos frangos, enquanto 60 mg kg⁻¹ de piperina interferiram positivamente em ambos os parâmetros, de 36 a 42 dias de idade, e aumentaram significativamente a superfície de absorção do duodeno e do íleo. Não foi observada alteração macroscópica no tamanho e na coloração dos órgãos dos frangos alimentados com as rações com as concentrações de piperina avaliadas. A suplementação com 120 e 180 mg kg⁻¹ de piperina é tóxica ao tecido hepático e reduz a superfície de absorção do jejuno. A dieta suplementada com 60 mg kg⁻¹ de piperina é segura.

Termos para indexação: *Piper nigrum*, desempenho, aditivo fitogênico, sanidade.

Introduction

The practice of using antimicrobial feed additives, such as antibiotics and chemotherapy drugs, as growth promoters was banned in livestock and poultry diets in 2006 by the European Union (Castanon, 2007). Therefore, a worldwide search for equally effective alternatives, without negative impacts on animal welfare and consumer health, was initiated. Herbal extracts, spices, and some of their components (phytogetic additives) are possible natural alternatives to the use of antibiotics as growth promoters in broiler diets. Phytogetic feed additives are plant-derived products

used in animal feeding to improve the performance of agricultural livestock and may satisfy the increasing concerns of consumers, since they prove to be safe and effective. Phytoetics may also minimize the serious environmental problem of bacterial resistance caused by the use of antibiotics as growth promoter additives (Perić et al., 2009). Phytoetics can improve the consumption and conversion of food, and the digestibility and weight gain of broiler chickens. However, the action mechanisms of these additives is not yet clear (Perić et al., 2009; Scheuermann et al., 2009). Studies on the application of phytoetics in broiler nutrition show inconsistent – positive or

negative – results on broiler performance (Botsoglou et al., 2002; Ertas et al., 2005; Cross et al., 2007; Ocak et al., 2008).

Piperine (1-piperoyl-piperidine) is a major alkaloid component of black (*Piper nigrum* Linn.) and long (*Piper longum* Linn.) pepper, and is responsible for their pungent and biting taste (Dogra et al., 2004). These pepper species have been used as seasoning in cookery and as an ingredient in alternative medicine for the treatment of several illnesses, such as bronchitis and asthma (Singh, 1992). Among its chemical-biological activities, piperine exhibits antimicrobial (Reddy et al., 2004), anti-inflammatory (Pradeep & Kuttan, 2004) and antioxidant (Mittal & Gupta, 2000) properties. It also increases the bioavailability of certain drugs in the organism (Karan et al., 1999) and acts as a chemopreventive factor against pro-carcinogens activated by cytochrome P-450 (Reen et al., 1997).

According to Kohlert et al. (2000), the active principles of phytogetic additives are absorbed in the intestine by enterocytes and are quickly metabolized by the body. Piperine induces alterations in membrane dynamics and permeation characteristics, as well as the synthesis of proteins associated with cytoskeletal function, resulting in an increase in the small intestine absorptive surface (Khajuria et al., 2002). The administration of piperine by gavage (170 mg kg⁻¹ of body weight) or intraperitoneally (85 mg kg⁻¹ of body weight) to male albino rats was efficiently absorbed (about 97%), and its passage through the intestine indicated that the highest concentration in stomach and small intestine occurred at approximately 6 hours. Only traces (less than 0.15%) were detected in the serum, kidney, and spleen from 30 min to 24 hours (Bhat & Chandrasekhara, 1986). The rapid metabolism and the short-life of piperine indicate a low risk of accumulation in the tissue. Cardoso et al. (2009), while working with broiler chickens, found that orally administered piperine did not interfere on weight gain or liver relative weight. However, liver histopathological changes were observed in a dose-dependent manner, indicating that 1.12 mg kg⁻¹ of piperine, with oral route of administration, is nontoxic for broiler chickens, as previously reported for rats and mice (Dogra et al., 2004; Gagini et al., 2010).

Besides being a natural compound that does not produce detected residues in the animal or in their derived products, piperine is easily isolated in great

amounts and has shown interesting biological effects in studies developed in mammals.

The objective of this work was to determine the effect of piperine as a phytogetic additive in chicken broiler diet.

Materials and Methods

The experiment was carried out in 2010 at the Universidade Federal Rural do Rio de Janeiro (UFRRJ), RJ, Brazil. The piperine was obtained according to Ikan (1991), in 5–7% yield and 98% purity, determined by gas chromatography-mass spectrometry (GC-MS), with melting point of 128–129°C and spectrometric data (1:00 and 13C NMR for infrared spectroscopy and MS) similar to that reported in the literature (Araújo-Júnior et al., 1997; Siddiqui et al., 1997). The piperine was incorporated in the diets at concentrations of 0, 60, 120, and 180 mg kg⁻¹. A vertical mixer with a 500-kg capacity was used for the homogenization of the feed treatments, and mixing was done 15 min after the last ingredient was added. Micronutrients were premixed, and the different concentrations of piperine were incorporated to the basal diet instead of a fixed amount of the inert material, without altering the composition of the diet. The concentrations were determined in a previous study on the bioavailability of orally administered piperine (Cardoso et al., 2009).

The experiment was approved by the Research Ethics Committee of the UFRRJ and was carried out with 96 one-day-old male Cobb chicks housed in an experimental shed. At seven days of age, with the intestine able to metabolize fat-soluble substances, such as piperine, the chicks were randomly allocated to four experimental treatments, with four replicates of six chicks each, in a completely randomized design. The treatments used were: non-supplemented control diet, and diet supplemented with 60, 120, and 180 mg kg⁻¹ of piperine. The diets (Table 1) were made based on nutrient requirement recommendations (Rostagno et al., 2005), and food and water were provided ad libitum. The chicks were kept in a standard environmental condition with natural light. The corn used in the diets was sent to the Mycotoxicology Laboratory of the Veterinary Institute of the UFRRJ for mycotoxin analysis. The treatments were administrated for 35 consecutive days, and the broilers were weighed and slaughtered at end of the experiment according to

the rules established by the Ministério da Agricultura Pecuária e Abastecimento.

The following production indexes were determined at initial (8-21 days), growing (22-35 days), final (26-42 days) and total period (8-42 days of age): feed intake, weight gain and feed conversion ratio. Mortality, body weight and carcass yield at 42 days of age.

For hematological tests, the blood was collected with EDTA from the wing vein of 12 randomly selected broilers from each treatment. Red blood cells, white blood cells, and hematocrit were determined according to Jain (1993). The hemoglobin concentration was measured by the method of oxyhemoglobin (Campbell & Dein, 1984), and total plasmatic protein was determined by refractometry (Coles, 1984). The mean corpuscular volume and the mean corpuscular hemoglobin concentration were calculated according to Wintrobe (1934).

For biochemical tests, the blood was collected without EDTA from the wing vein of 12 randomly selected broilers from each treatment. Urea, creatinine,

aspartate transaminase (AST), alanine transaminase (ALT), and gamma glutamyl transpeptidase (GGT), from the serum, were analyzed using a commercial kit (Bioclin S.A., Belo Horizonte, MG, Brazil).

For histopathological analysis, the proventriculus, gizzard, liver, small intestine, and kidney from six broilers of each treatment were analyzed. The tissues were fixed in 10% neutral buffered formalin solution and embedded in paraffin wax. All histological analyses were performed on 5.0- μ m sections (four cross-sections for each sample), mounted on slides, and stained with haematoxylin and eosin.

The histomorphometric analysis of the small intestine was performed with four randomly selected broilers from each treatment. The whole small intestine and some samples from the duodenum, the jejunum, and the ileum were collected, and the slides were prepared as previously described. The histomorphometric examination (villi height and width, as well as crypt depth) was performed by light microscopy, and the measurement was done using a public domain image analysis software (ImageJ, National Institute of Mental Health, Bethesda, MD, USA). The results were obtained from the average of ten adjacent villi.

All values were expressed as mean \pm standard deviation. Means were compared by the analysis of variance, followed by the Kruskal Wallis or the Student-Newman-Keuls test, at 5% probability. The software package GraphPad Prism 5.0 was used for statistical analysis.

Results and Discussion

Piperine did not promote significant effects in the production indexes in the initial and growing periods. In the final period, from 36 to 42 days of age, the broilers receiving the diet supplemented with 60 mg kg⁻¹ of piperine showed better weight gain and feed conversion rate, at 5% probability. However, this was not reflected in the total period (Table 2). The supplementation of piperine in broiler diets does not cause any loss in production parameters, which can be improved in the final period.

The highest concentration of piperine (180 mg kg⁻¹) significantly reduced the total number of leukocytes (Table 3). In this treatment, there was a decrease of heterophils in comparison to the control. Broiler chickens fed diet supplemented with piperine showed significant decrease of monocytes with dose-dependent

Table 1. Composition and nutritional values of chicken broiler diets.

Ingredient (kg)	8-21 days	22-35 days	36-42 days
Corn	60.73	65.38	68.85
Soybean meal	33.92	29.92	25.925
Soybean oil	1.603	1.85	1.85
Dicalcium phosphate	1.763	1.884	1.457
Limestone	0.82	0.78	0.745
Salt	0.447	0.425	0.4
Choline chloride	0.04	0.04	0.04
DL-Methionine	0.223	0.227	0.213
L-Lysine	0.177	0.227	0.265
L-Threonine	0.037	0.052	0.063
Vitamin supplement ⁽¹⁾	0.1	0.052	0.05
Mineral supplement ⁽²⁾	0.05	0.1	0.1
Cocciostatic ⁽³⁾	0.05	0.05	-
Starch (inert material)	0.03	0.03	0.040
Piperine	-	-	-
Total	100	100	100
Calculated value (%)			
Crude protein	20.65	19.1	17.74
Metabolizable energy (kcal kg ⁻¹)	2,980	3,050	3,100
Methionine	0.5453	0.5257	0.4964
Methionine/Cystine	0.871	0.833	0.788
Calcium	0.878	0.81	0.751
Available phosphorus	0.439	0.405	0.374
Sodium	0.213	0.201	0.191

⁽¹⁾Composition per kg of product: 7,500 UI of vitamin A; 2,500 UI of vitamin D3; 18,000 mg of vitamin E; 1,200 mg of vitamin K3; 1,500 mg of vitamin B1; 5,500 mg of vitamin B2; 2,000 mg of vitamin B6; 12,500 mcg of vitamin B12; 67 mg of biotin; 10 g of pantothenic acid; 35 g of nicotinic acid. ⁽²⁾Composition per kg of product: 120 g of Mn; 13 g of Cu; 60 g of Fe; 100 g of Zn; 2,500 mg of I; 500 mg of Se. ⁽³⁾5.0 g of semduramicin.

characteristics. However, 60 and 120 mg kg⁻¹ of piperine did not decrease white blood cell count. When performing hematological analyses of mice, Dogra et al. (2004) found that concentrations of piperine (2.25 and 4.5 mg kg⁻¹) caused a significant reduction in total leukocyte counts; in the differential leukocyte count,

there was an increase in the percentage of neutrophils. Piperine at 120 mg kg⁻¹ significantly decreased the mean corpuscular hemoglobin concentration, when compared to the other treatments and the control. No significant differences were observed among treatments as to the other evaluated parameters.

Table 2. Production indexes of chicken broiler fed diets supplemented with different concentrations of piperine during each growth phase and the entire experimental period⁽¹⁾.

Parameter	Piperine (mg kg ⁻¹)				CV (%)
	0	60	120	180	
	8–21 days				
Feed intake (g)	1,020±30.92a	1,036±8.70a	976.00±40.44a	970.40±22.67a	1.84
Weight gain (g)	700.10±39.53a	668.50±35.30a	662.50±23.50a	655.20±14.41a	4.17
Feed conversion rate	1.50±0.10a	1.59±0.10a	1.49±0.05a	1.51±0.08a	3.54
	22–35 days				
Feed intake (g)	2,138±219.70a	2,029±139.10a	2,103±203.50a	2,027±1,690a	8.77
Weight gain (g)	1,213±39.46a	1,166±64.37a	1,174±73.55a	1,187±71.86a	5.27
Feed conversion rate	1.76±0.16a	1.74±0.10a	1.80±0.26a	1.71±0.08a	8.31
	36–42 days				
Feed intake (g)	1,543±99.82a	1,458±102.80a	1,456±113.50a	1,476±105.00a	7.11
Weight gain (g)	674.60±107.70a	829.30±73.32b	699.20±34.24a	688.30±59.04a	9.57
Feed conversion rate	2.32±0.25a	1.77±0.165b	2.09±0.204a	2.15±0.08a	8.40
	8–42 days				
Feed intake (g)	4,701±275.30a	4,525±196.00a	4,535±76.58a	4,473±239.10a	4.31
Weight gain (g)	2,587±136.30a	2,663±105.10a	2,536±93.44a	2,531±127.30a	4.48
Feed conversion rate	1.82±0.05a	1.70±0.08a	1.79±0.09a	1.77±0.06a	3.85
Mortality	0	0	0	0	
Body weight (kg)	2.83±0.20a	2.82±0.19a	2.80±0.19a	2.77±0.26a	7.50
Carcass yield (%)	71.88±3.96a	72.11±2.31a	73.42±3.17a	72.47±2.29a	4.05

⁽¹⁾Means±standard deviation followed by equal letters do not differ by the Student-Newman-Keuls test, at 5% probability.

Table 3. Hematological and biochemistry parameters of chicken broiler fed diets supplemented with different concentrations of piperine⁽¹⁾.

Parameter	Piperine (mg kg ⁻¹)				CV (%)
	0	60	120	180	
Red blood cell x 10 ⁶ µL ⁻¹	2.68±0.36a	2.81±0.31a	3.04±0.38a	2.83±0.38a	12.59
Hematocrit (%)	30.17±2.86a	30.17±3.01a	30.50±2.32a	30.33±2.64a	7.90
Hemoglobin (g dL ⁻¹)	9.10±0.65a	9.73±0.90ab	8.78±0.71ac	9.18±0.61abc	7.79
Mean corpuscular volume (fL)	114.40±17.80a	108.40±14.56a	101.50±11.09a	108.00±10.20a	12.36
Mean corpuscular hemoglobin (gm dL ⁻¹)	30.33±2.70a	32.47±3.96a	28.90±2.85b	30.41±2.34a	9.67
Total plasmatic protein (g dL ⁻¹)	3.42±0.25a	3.48±0.30a	3.53±0.27a	3.48±0.28a	7.90
White blood cell x 10 ³ µL ⁻¹	31.08±5.57a	29.75±4.77a	28.17±5.29a	25.92±3.37b	16.43
Lymphocytes x 10 ³ µL ⁻¹	16.77±2.20a	18.89±3.50a	17.33±3.50a	17.13±2.89a	17.17
Heterophils x 10 ³ µL ⁻¹	10.60±3.40a	9.12±1.57ab	9.02±2.59ab	7.27±1.81b	25.72
Monocytes x 10 ³ µL ⁻¹	2.53±1.13a	1.77±0.75b	1.69±0.60b	1.45±0.31b	30.69
Eosinophil x 10 ³ µL ⁻¹	0.42±0.13a	0.24±0.07a	0.40±0.10a	0.27±0.04a	26.05
Aspartate aminotransferase (U L ⁻¹)	200.40±91.07a	227.60±70.37a	286.80±81.85b	265.80±83.93b	34.11
Alanine aminotransferase (U L ⁻¹)	38.17±13.98a	49.42±14.54a	45.83±13.58a	40.33±11.52a	31.06
Gama glutamyltransferase (U L ⁻¹)	12.83±4.90a	15.00±3.08a	13.75±3.34a	13.58±3.63a	27.41
Urea (mg dL ⁻¹)	5.27±1.42a	5.00±1.54a	4.90±2.18a	4.27±1.68a	35.36
Creatinine (mg dL ⁻¹)	0.50±0.11a	0.45±0.09a	0.47±0.10a	0.49±0.11a	20.84

⁽¹⁾Means±standard deviation (n = 12) followed by equal letters do not differ by the Student-Newman-Keuls and the Kruskal-Wallis test (for monocytes and eosinophils), at 5% probability.

At the concentrations of 120 and 180 mg kg⁻¹, piperine increased AST, indicating hepatocyte lesions due to alterations in cellular membrane permeability (Kaneko, 1989). The activity of AST is considered a sensitive, but not specific, marker of hepatocyte disorder in most bird species (Schmidt et al., 2007). The other hepatic enzymes and renal function were in the normal range for broiler chickens and did not vary among the treatments (Table 3).

To investigate whether piperine is able to modify the ultrastructure of small intestinal epithelium, increasing its absorption surface, histomorphometric analyses of the duodenum, the jejunum, and the ileum were carried out (Table 4).

The effect of piperine was not homogeneous on the intestinal mucosa. Dietary supplementation of piperine for 35 days influenced the measurements of segments of the small intestinal villi. Piperine (60 mg kg⁻¹) increased the area (height x width) of the villi in the duodenum and the ileum, as well as the absorption surface in these segments of the small intestine (Table 4). The treatments 120 and 180 mg kg⁻¹ of piperine reduced the absorption surface of the jejunum. There was no modification in the duodenum and the ileum, except for the treatment with 120 mg kg⁻¹ of piperine in the ileum segment. There was a reduction in crypt depth in the duodenum and the jejunum in all concentrations of piperine, when compared to the control. However, the same was not observed in the ileum.

Khajuria et al. (2002) related piperine (10 mg kg⁻¹ of body weight) to alterations in membrane dynamics and permeation characteristics, and to the induction in the synthesis of proteins associated with cytoskeleton function, which increased the small intestine absorptive surface, in a time-dependent manner, up to 2 hours in rats.

The gastrointestinal system is affected by black pepper and piperine, which have a definite effect on intestinal motility, anti-diarrhea property, and on the ultrastructure of intestinal villi, improving the absorbability of nutrients (Takaki et al., 1990; Bajad et al., 2001). According to Samanya & Yamauchi (2002), increased villus height is paralleled by an increased digestive and absorptive function of the intestine due to the increased absorptive surface area, the expression of brush border enzymes, and the nutrient transport systems.

In fact, growth promoters are able to modify the morphology of the small intestine. This could be attributed to the fact that the promoters reduce the growth of many pathogenic or nonpathogenic intestinal organisms. The reduction of the inflammatory reactions at the intestinal mucosa leads to the increase of the villus area and of the functions of secretion, digestion, and absorption of nutrients by the mucosa (Miles et al., 2006).

Although there were a few controversial reports regarding the safety of black pepper or piperine as food additives (Srinivasan, 2007), the examination of the proventriculus, gizzard, liver, kidney, and small

Table 4. Surface absorption in small intestine of chicken broiler fed diets supplemented with different concentrations of piperine⁽¹⁾.

Fragment of small intestine	Piperine (mg kg ⁻¹)				CV (%)
	0	60	120	180	
Duodenum					
Villi height (µm)	1,344±151.70a	1,323±189.30a	1,350±288.20a	1,304±194.20a	15.36
Villi width (µm)	190.00±45.70a	229.20±52.56b	198.60±70.05a	203.70±62.71a	28.26
Crypt depth (µm)	416.90±86.89a	332.40±94.94b	317.10±64.52c	356.40±96.03bc	19.03
Surface absorption (mm ²)	0.25±0.06a	0.32±0.09b	0.29±0.10a	0.29±0.11a	24.46
Jejunum					
Villi height (µm)	1,738±230.80a	1,024±140.80b	1,092±138.40b	1,012±138.40b	15.68
Villi width (µm)	160.20±44.55a	179.10±39.68a	161.30±43.30a	145.70±41.04a	26.04
Crypt depth (µm)	234.00±47.54a	218.40±21.93ab	168.60±34.71b	196.20±29.09b	19.07
Surface absorption (mm ²)	0.24±0.04a	0.21±0.03a	0.17±0.04b	0.15±0.03b	20.02
Ileum					
Villi height (µm)	523.10±120.50a	752.90±114.0b	909.00±155.30c	755.10±73.84b	16.26
Villi width (µm)	129.50±37.13a	144.10±42.23b	168.30±49.97b	111.10±32.97a	28.92
Crypt depth (µm)	127.70±36.18a	181.30±40.04b	246.10±60.83c	175.50±55.80b	26.27
Surface absorption (mm ²)	0.06±0.02a	0.12±0.03b	0.16±0.06b	0.08±0.02a	29.03

⁽¹⁾Means±standard deviation followed by equal letters do not differ by the Student-Newman-Keuls test, at 5% probability. Data are expressed by the group for 15 adjacent villus of 4 broilers per group.

intestine did not indicate any alterations in the size and the color of the parenchyma and the mucosa of organs or the presence of lesions when different piperine

doses were added to the feed. However, mononuclear infiltration was observed in the hepatic tissue (Figure 1), randomly disposed forming nodules with

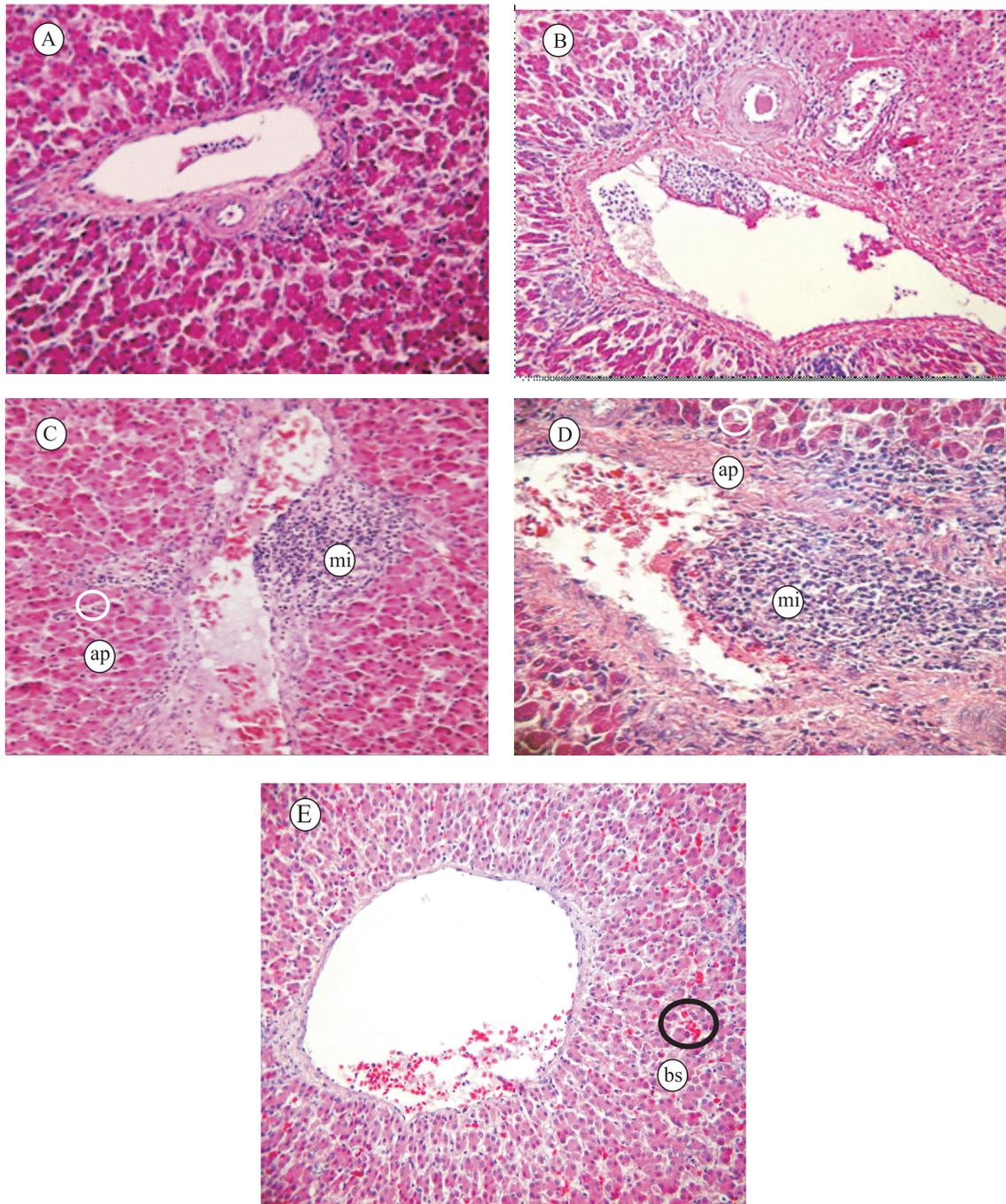


Figure 1. Sections of hepatic tissue of chicken broiler fed diets supplemented with different concentrations of piperine, stained with haematoxylin and eosin, and examined by an optical microscope (Olympus CH30), at 200 x, coupled with a Nikon digital camera (Coolpix 4300). A, control 0 mg kg⁻¹ of piperine; B, C, and D, concentrations of 60, 120, and 180 mg kg⁻¹ of piperine, respectively; E, 180 mg kg⁻¹ of piperine, showing dilatation of blood sinusoids (bs). Mononuclear infiltration (mi) randomly disposed forming nodules, and hepatocytes showing small dense nuclei – apoptosis process (ap) – near the portal space at 120 and 180 mg kg⁻¹ of piperine.

predominance of macrophages and rare heterophils near the portal space, with dose-dependent characteristics. The sinusoids in the control and in the treatments with 60 mg kg⁻¹ of piperine showed almost normal architecture, although there was a dilatation of blood sinusoids in the broiler fed diets with 180 mg kg⁻¹ of piperine. In addition, many hepatocytes showed small dense nuclei (apoptosis process) near the portal space, at 120 and 180 mg kg⁻¹.

Conclusions

1. The supplementation of 60 mg kg⁻¹ of piperine in broiler diet increases weight gain and feed conversion rate at 36 to 42 days of age, and significantly increases the absorption surface in the duodenum and the ileum.

2. The concentration of 180 mg kg⁻¹ of piperine is toxic to leukocytes, promoting leucopenia, whereas the decrease of monocytes is observed in all concentrations of piperine and is dose-dependent.

3. The supplementation of 120 and 180 mg kg⁻¹ of piperine is toxic to liver tissue and reduces the absorption surface of the jejunum.

4. The diet supplemented with 60 mg kg⁻¹ of piperine is secure.

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