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Original article

Effects of supplementing graded levels of methenamine in drinking water on growth performance, gut microbiota, organs histology and haemato-biochemical profile of broiler chickens

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ABSTRACT

The residual effects of antibiotics in meat products can induce resistance to pathogens that may lead to health problems to the consumers. In this regard, much attention has been paid towards growth activators order than antibiotics in livestock breeding. This study was designed to evaluate the effects of graded levels of methenamine in drinking water on growth performances of broiler chickens. A total of 72 three-weeks old sexed broiler chicks were assigned to six experimental treatments in a completely randomized design. Methenamine was incorporated at 0.5, 1, 1.5 and 2 g per liter of water and compared to an antibiotic medicated diet as positive control and to a ration without any supplement as negative control. Feed intake significantly ($p<0.05$) increased in birds fed on methenamine compared to the positive control diet, while live body weight and weight gain of broiler fed 1.5 g and 2 g methenamine/L were significantly ($p<0.05$) higher compared to the negative control diet. Feed conversion ratio significantly ($p<0.05$) decreased with 2 g methenamine/l compared to the negative control diet. Inclusion of 1 to 2 g methenamine/L significantly ($p<0.05$) increased coliform counts compared to the positive control diet, while *salmonella* count

significantly ($p<0.05$) decreased with 1 and 1.5 g methenamine/L. The serum content in ASAT, ALAT, Urea, total cholesterol and HDL-cholesterol were significantly ($p<0.05$) affected irrespective of the inclusion level of methenamine compared to the negative control diet. RBC, Hgb, MCH and PLT respectively increased significantly ($p<0.05$) with 1.5 g and 2 g methenamine /L while MCV, MCHC and PCV decreased ($p<0.05$) irrespective of the level of incorporation of methenamine in drinking water compared to the negative control treatment. In conclusion methenamine can be incorporated up to 2 g/L of drinking water to improve growth without any adverse effects on haemato-biochemical profile and organs histology of broilers chickens.

1. Introduction

Due to the resistance developed by microorganisms and the increasing public concern about drug residues in animal products, research has been focused on the natural alternatives to antibiotics feed additives (Ciza et al., 2019). The most investigated alternatives include probiotics and prebiotics (Tuohy et al*.,* 2005), organic acids and enzymes (Gunal et al., 2004; Yang et al., 2009), spices (Kana et al*.*, 2017a; Ebile et al. 2018a), essential oils (Ngouana et al., 2017), metals ions such as silver (Dongwei et al*.,* 2009; Yemdjie et al*.*, 2017), amino acid salt such as monosodium glutamate (Khadiga et al., 2009; Gbore et al., 2016; Ciza et al., 2019) and non-protein amino acid such as methenanine (Affset, 2009; EFSA, 2015).

Methenamine is an antimicrobial drug used in the treatment of urinary tract infections, prophylaxis and bacteriuria in human (Williams et al., 2012; Ahrens et al., 2013; Al-Badr and Al-Shaikh, 2013; Hickling and Nitti, 2013). It is relatively harmless and decomposes into formaldehyde and ammonia under acidic conditions or in the presence of proteins (EFSA, 2014). The bactericidal properties of formaldehyde fight against infection (Mohebbi-Fani et al., 2002; Papich, 2016). In avian medicine, methenamine is often dispense in the treatment of nephritis, pyelitis, pyelonephritis, ascitis, edema and hepatitis. It also has diuretic and anti-bleeding properties for the treatment of sulpha-drug intoxication, infectious bursal disease and coccidiosis (Hadi et al., 2013). In animal feed it is used as a preservative to improve the silage process of feed for pigs, cattle, sheep, goats, rabbits and horses at a maximum rate of 600 mg / kg of fresh material (Affset, 2009; EFSA, 2015) and as cheese preservative at a rate of 25 mg/kg (EFSA, 2014; 2015). According to AFSA (2004), the antimicrobial properties of formaldehyde have been widely exploited for the microbial decontamination of stored feeds, mainly fishmeal. At a dose of 660 mg per kilogram of feed, formaldehyde is able to eliminate or reduce the number of pathogenic bacteria such as *Escherichia coli* and *Salmonella typhimurium* (Afsset, 2009). Furthermore, the high reactivity of formaldehyde with amine groups makes it possible to protect soybean, rapeseed and/or sunflower seed meal proteins from microbial degradation (Afsset, 2009). Based on the above properties, we believed that methenamine could be used to balance intestinal microbiota with positive consequences on growth performance in poultry. The aim of this study was to assess the effects of graded levels of methenamine on growth performance, intestinal microbial load, liver and kidney histology and haemato-biochemical parameters of broiler chickens.

2. Materials and methods

2.1. Area of study

This study was carried out at the Teaching and Research Farm of the Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon. This farm is located at an altitude of 1420 m above sea level, between latitude 5°26'N and longitude 10°26'E with an equato-guinean climate, annual temperatures vary between 10°C and 25°C. The annual Rainfall ranges from 1500 to 2000 mm, the wet season lasts from mid-March to mid-November and the dry season from late November to mid-March.

2.2. Methenamine

Merck brand methenamine (Darmastadt, Germany) used in this study was bought (imported) from Germany. It is in the form of white crystals very soluble in water.

2.3. Experimental birds

A total of 72 three-weeks - old Cobb 500 strain broiler chicks of average weight 472 g were divided into 6 groups of 12 chicks each. Each group was subdivided into six replicates of 2 chicks (1 males and 1females in each replicate). The chicks were housed per couple in cages at a density of 2 chicks/0.12 m^2 up to 49 days of age. Antistress and vitamins were given once a week. Feed and water were offered *ad libitum.*

2.4. Dietary treatments

Dietary treatments consisted of supplementing control diet (T0) (Table 1) with 1 g of Doxcyclin/kg of feed as positive control (T0+), 0.5 g, 1 g, 1.5 g, and 2g of methenamine/L of drinking water. Each experimental diet including the control was fed to 12 chicks (6 males and 6 females) replicated 6 times (each couple representing an experimental unit) in a completely randomized design.

Table 1
Chemical composition of experimental diet.

Ingredients (%)	**Finisher phase**
Corn	60.00
Wheat bran	12.00
Cotton seed cake	1.00
Fishmeal	5.00
Soybean meal	16.00
Shell	1.00
Premix 5%*	5.00
Total	100.00
Calculated chemical composition	
Metabolic energy (kcal/kg)	3000.90
Crude protein (%)	20.20
Energy/protein	148.90
Calcium (%)	1.16
Phosphorus (%)	0.53
Calcium/Phosphorus	2.18
Lysine (%)	1.20
Methionine (%)	0.44
Price of kg (FCFA)	254.50

*Premix 5%: crude protein= 40%, Lysine= 3.3%, Methionine=2.40%, Calcium=8%, Phosphorus=2.05%, metabolizable energy= 2078 kcal/kg.

2.5. Data collection

2.5.1. Growth parameters

Data was collected after every seven day on feed intake (FI), live body weight (LBW), from which, weight gain (WG) and feed conversion ratio (FCR) were calculated. At 49 days old, 10 birds per treatment were randomly selected, fasted for 24 hours, weighed, slaughtered and eviscerated to evaluate carcass characteristics as preceded by Kana et al. (2017a). Dressing percentage was calculated by dividing the carcass weight by the live body weight of the bird and the result was expressed as a percentage. The length of the intestine was measured with the cut done from the start of the duodenal loop to the end of the cloacal. The density of the intestine was calculated by dividing the intestine weight by its length (Kana et al., 2017a).

2.5.2. Haematological-biochemical and histological parameters

Blood was collected in heparinised and non-heparinised test tubes for the analysis of haematological and biochemical parameters respectively. Haematological parameters including white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb), haematocrit (HCT) and platelets (PLT) were analysed using Genius electronic hematocymeter (Model KT6180S/N 701106101557).

For biochemical analysis, collected blood samples were stored at room temperature and after 24 hours, the serum was collected and preserved at -20°C for the evaluation of serum content in protein, albumin, globulin, triglyceride, total cholesterol, HDL and LDL-cholesterol, Aspartate aminotransferase (ASAT), Alamine-aminotransferase (ALAT), urea and creatinine using Chronolab® commercial kits.

Liver and kidney samples were randomly sliced from each treatment and fixed by immersion in formol solution for 2 weeks. Tissues were dehydrated in graded levels of ethanol, xylene and embedded in paraffin. Sections of 5 µm were stained with hematoxylin-eosine for histological observations (40X magnification).

2.5.3. Microbial count

At the end of the trial, faeces samples were collected from the cloaca using an antiseptic scovel from 4 birds per treatment. The number of colony of lactic acid bacteria, *Escherichia coli* and *Salmonella* were counted in an specific culture media (MRS Agar for lactic acid bacteria, Mac Conkey Agar for *E. coli* and SS Agar for *salmonella* respectively) as proceeded by Ciza et al. (2019).

2.6. Statistical analysis

Data collected were submitted to one-way analysis of variance using the Statistical Package for Social Science (SPSS 20.0). Significant differences between treatment means were separated using Duncan's Multiple Range test. Probability values less than 0.05 were consider as significant.

3. Results and discussion

Table 2 summirized the effects of graded levels of methenamine on feed intake, live body weight, weight gain and feed conversion ratio. The inclusion of graded levels of methenamine in drinking water significantly ($p<0.05$) increased feed intake compared to the positive control diet (T0+). The increased trend in feed intake observed in this study could be attributed to the odourless and sweet taste of methenamine which may have enhanced the palatability of feed. The present result contradicted the findings of Goorzardi and Nanekarani (2013) and Kana et al. (2017a) who reported that introducing 1%, 2% onion extracts in drinking water and 2%, 4% *D. glomerata* in feed respectively did not have any significant effect on feed intake of broiler chickens in the starter phase. These authors explained the results by the strong odour and bitter taste of their additives.

Live body weight (LBW) and weight gain (WG) of broiler birds fed on 1.5 g and 2 g of methenamine per liter of drinking water were significantly ($p<0.05$) higher compared to the negative control diet but similar to the other treatments. This improvement could be due to the fact that formaldehyde from methenamine was able to reduce intestinal microbial count, and to make a strong reactivity with proteins protecting it from microbial degradation (Hadi et al., 2013). These properties could lead to better health and greater availability of some nutrients for the host thus, improved weight gain. The present result corroborates the results of Ciza et al. (2018) who reported a significant increase in LBW and WG of broilers supplemented with 2 mg of monosodium glutamate /kg of feed. In addition, Kana et al. (2017b) reported a significant ($p<0.05$) increase on live body weight and weight gain when 0.2% and 0.4% of *Tetrapleura tetraptera* fruit powder was introduced in the feed of broiler chicks. The present result contradicts the results of Della et al. (1970) who reported a significant reduction of body weights with the inclusion of 1% of methenamine in rat's drinking water.

Feed conversion ratio (FCR) of broiler birds fed on the negative control diet was significantly ($p<0.05$) higher compared to broilers fed on the positive control diet, but did not differ with birds fed on 0.5 g and 1 g of methenamine per liter of drinking water. The FCR of Birds fed on the positive control diet and 2 g of methenamine per liter of drinking water were 11.85% lower ($p<0.05$) compared to the negative control diet. Feed conversion ratio (FCR) of broiler birds receiving methenamine in water was comparable to that of birds fed on the positive control diet but significantly ($p<0.05$) lower compared to the negative control diet. This result is in close agreement with the findings of Kana et al. (2017a) who recorded a decrease in FCR with the inclusion of 0.2% and 0.4% *D.*

glomerata in broilers feed and Ebile et al. (2018b) who recorded a decrease in FCR with the inclusion of 2, 4 and 6 g\kg of *D. glomerata* in japanese quail's feed. These results contradicted the findings of Abdulkarimi et al. (2011) and Noman et al. (2015) who recorded no marked effect of thymus and garlic extracts respectively when added to broiler's drinking water. This difference could be due to the anti-nutritional factor found in the seeds which are absent in methenamine.

Table 2
Effects of graded levels of methenamine on growth performance.

	Treatments						
Growth parameters	**T0**	**T0⁺**	**T0.5**	**T1**	**T1.5**	**T2**	**p**
Feed intake (g)	3787.17 ±166.50^{ab}	3607.00 ±122.30^{b}	3983.00 ±179.37^{a}	3999.58 ±143.34^{a}	3919.70 ±147.51^{a}	3920.40 ±205.85^{a}	0.002
Live body weight (g)	1977.33 ±130.19^{b}	2097.25 ±123.02^{ab}	2103.00 ±125.17^{ab}	2128.42 ±104.64^{ab}	2219.75 ±147.10^{a}	2240.00 ±133.48^{a}	0. 017
Weight gain (g)	1505.52 ±130.19^{b}	1625.44 ±123.02^{ab}	1631.19 ±125.17^{ab}	1656.61 ±104.64^{ab}	1747.94 ±147.10^{a}	1768.19 ±133.48^{a}	0.017
Feed conversion ratio	2.53 ±0.16^{a}	2.23 ±0,14^{b}	2.45 ±0.22^{ab}	2.43 ±0.22^{ab}	2.25 ±0.20^{b}	2.23 ±0.20^{b}	0.035

a, b: Means with the same superscript on the same row are not significantly different (p>0.05). T0= basal diet; T0+= T0+ 1g antibiotic; T0.5: T0+0.5g Methénamine in water; T1: T0+1g Methénamine in water; T1.5: T0+1.5g Methénamine in water; T2: T0+2g Methénamine in water, p= probability.

Table 3
Effects of methenamine on carcass characteristics and relative weight of broiler's organs.

Carcass characteristics	Treatments						
	T0	**T0⁺**	**T0.5**	**T1**	**T1.5**	**T2**	**p**
Carcass yield (%)	71.00±2.09	71.65±0.99	72.35±1.31	72.00±1.76	71.98±1.95	72.73±2.35	0.380
Relative weight of organs							
Head (% LW)	2.37±0.17	2.33±0.26	2.36±0.19	2.28±0.24	2.30±0.21	2.33±0.19	0.926
Leg (% LW)	4.02±0.60	3.80±0.26	3.78±0.41	3.77±0.48	4.01±0.40	3.74±0.31	0.497
Liver (% LW)	2.00±0.16^{b}	2.51±0.41^{a}	2.01±0.31^{b}	2.06±0.23^{b}	1.94±0.30^{b}	2.06±0.38^{b}	0.002
Heart (% LW)	0.50±0.09	0.54±0.06	0.57±0.06	0.51±0.05	0.47±0.06	0.52±0.09	0.078
Pancreas (% LW)	0.24±0.06^{a}	0.23±0.07^{a}	0.25±0.05^{a}	0.24±0.07^{a}	0.16±0.06^{b}	0.24±0.03^{a}	0.016
Abdominal fat (% LW)	2.16±0.85	1.97±0.44	2.07±0.55	2.05±0.62	1.95±0.50	2.06±0.55	0.977

a, b: Means with the same superscript on the same row are not significantly different (p>0.05). T0: basal diet; T0⁺: T0+ Antibiotic; T0.5: T0+0.5g Methenamine in drinking water; T1: T0+1g Methenamine in water; T1.5: T0+1,5g Methenamine in wate; T2: T0+2g Methenamine in drinking water; p= probability.

Carcass characteristics and relative weight of organs expressed in proportions of body weight are summarized in Table 3. There was no significant (p>0.05) effect among treatment groups for carcass yield compared to the negative and positive control diets. However, broiler birds fed 2 g of methenamine per liter of water recorded a non significant increased in carcass yield by 2.43% as compared to the negative control diet. This result agrees with the findings of Emadi and Kermanshi (2006) and Kana et al. (2011) who respectively showed that turmeric feed additive and Canarium charcoal had no effect on broiler's carcass yield and relative weight of organs. Della et al. (1970) recorded no difference in organs weight by incorporating 1% of methenamine in rat's drinking water.

Data on the effects of graded levels of methenamine on digestive organs of broilers are presented in table 4. The gizzard and intestinal density did not vary significantly (p>0.05) between treatments suggesting that the additive had no effect on the gizzard and intestinal density. This corroborates the findings of Kana et al. (2017a) who stipulated that the addition of *D. glomerata* in the feed of broilers at 0.2 % and 0.4% had no marked effect on the gizzard. In the same line, adding 2% powder of cumin seeds (Berrama et al., 2017) and 1 mg, 2 mg, 4mg of Monosodium Glutamate (Ciza et al., 2019) in broiler's diet had no significant (p>0.05) effect on the gizzard weight and intestinal density. These results are in contradiction with the findings of Mafouo et al. (2019) who showed that intestine density significantly (p< 0.05) increased with 20 g of neem oil/kg compared to antibiotic. Noman et

al. (2015) and Islam et al. (2017), stated that 1% garlic extract increased gizzard weight when administered in broiler's drinking water. Likewise, Kana et al. (2017b) reported an increased in gizzard weight of broiler chickens when 0.2% and 0.4% *Tetrapleura tetraptera* fruit powder were incorporated in feed compared to antibiotic administration.

The intestinal microbial count of broilers fed on graded levels of methenamine is presented in table 5. No significant (p>0.05) effect of methenamine was recorded on lactic acid bacteria count. Although not significantly different, lactic acid bacteria count tend to increase with increasing levels of methenamine in drinking water. This could be explained by the presence of formaldehyde produced by dissolution of methenamine which reduced the number of pathogenic bacteria in the profit of lactic acid bacteria. Formaldehyde is able to eliminate or reduce the number of pathogenic bacteria such as *Escherichia coli* and *Salmonella typhimurium* (Afsset, 2009). This result supports the findings of Hosseinzader et al. (2014) who reported that introducing 750, 1000, 1250 ppm of coriander extracts in water and 1.5%, 2%, 2.5 % in feed respectively increased lactic acid bacteria population in the gut of broiler chickens. Meanwhile, *Salmonella* population decreased significantly (p<0.05) compared to the positive and negative control treatments. This might be due to the antimicrobial property of methenamine which is considered to arise from formaldehyde. Formaldehyde attacks pathogenic bacteria on their amino protein groups thus altering their nature and activity of the groups (Affset, 2009). The present result obtained confirms the findings of Ebile et al. (2018a) who reporter that *E. coli* and *Salmonella* counts markedly decreased with antibiotic and *D. glomerata* administration in feed and water compared to the negative control. Hosseinzader et al. (2014) observed that introducing coriander extract and powder in water and feed respectively reduced significantly the population of *E. coli* in the microflora of broiler chicks.

Table 4
Effects of graded levels of methenamine on digestive organs of broilers.

	Treatment						
Digestive organs	**T0**	**T0$^+$**	**T0.5**	**T1**	**T1.5**	**T2**	**P**
Gizard (% LW)	1.58 ±0.18	1.56 ±0.16	1.73 ±0.34	1.65 ±0.17	1.55 ±0.18	1.62 ±0.24	0.465
Intestinal Weight (g)	65.20 ±10.04	65.10 ±11.17	67.90 ±9.62	68.60 ±11.82	71.30 ±6.29	65.50 ±10.94	0.705
Intestinal Length (cm)	205.20 ±20.53	205.10 ±21.30	205.00 ±22.19	204.20 ±17.55	210.30 ±18.19	201.30 ±20.13	0.957
Intestinal density (g/cm)	0.32 ±0.036	0.32 ±0.05	0.33 ±0.04	0.33 ±0.04	0.34 ±0.03	0.32 ±0.03	0.669

T0: basal diet; T0$^+$: T0+Antibiotic; T0.5: T0+0.5g Methenamine in water; T1: T0+1g Methenamine in water; T1.5: T0+1.5g Methenamine in water; T2: T0+2g Methenamine in water, p= probability.

Table 5
Effects of graded levels of methenamine on gut microbial load of broilers.

Bacteria load	**Treatments**						
log10 (cfu)	**T0**	**T0+**	**T0.5**	**T1**	**T1.5**	**T2**	**p**
Lactobacillus	7.94±0.90	8.31±0.66	7.85±0.11	8.00±1.21	8.58±0.49	9.01±0.31	0.219
Coliforms	8.64±0.95^{ab}	7.35±0.40^{c}	7.88±0.33^{bc}	9.09±0.47^{a}	9.47±0.12^{a}	9.07±0.58^{a}	0.000
Salmonella	7.20±0.14^{a}	7.35±0.29^{a}	7.16±0.22^{a}	1.90±0.00^{b}	1.80±0.00^{b}	7.20±0.14^{a}	0.000

a, b: Means on the same row with different superscripts are significantly different (p<0.05). T0: basal diet; T0$^+$: T0+ Antibiotic; T0.5: T0+0.5g Methenamine in drinking water; T1: T0+1g Methenamine in water; T1.5: T0+1.5g Methenamine in water; T2: T0+2g Methenamine in drinking water, p= probability.

Table 6 summirized the haematological parameters of broiler chickens fed on graded levels of methenamine via drinking water. Blood acts as a pathological reflector of health status of the animal (Etim et al., 2014) and animals with a good blood composition are susceptible to present good performances (Isaac et al., 2013). In the present study, WBC was not significantly (p>0.05) affected by the test substance. RBC and Hgb, MCH and PLT significantly (p<0.05) increased with 1.5 g and 2 g/l methenamine compared to the control treatments, while MCV,

MCHC and PCV respectively increased and decreased ($p<0.05$) irrespective of the level of incorporation of methenamine in water compared to the negative control treatment. Oleforuh et al. (2015) and Islam et al. (2017) reported that an increase in RBC and Hgb contents in blood is an indication of improved oxygen carrying capacity of the cells which translates a better availability of nutrients to the birds consequently affecting their well-being. This results show no significant ($p>0.05$) different in WBC compared to the control diets. This corroborates the findings of Onu (2010) who observed no significant effect on WBC when 0.25% garlic, ginger and a combination of garlic ginger were incorporated in finisher broiler's feed. Also, Toghyani et al. (2010) when supplementing broiler chickens with thyme powder did not have any marked effect on WBC counts. The present results are in agreement with the findings of Al-Kassie et al. (2011) who observed a significant decrease in PCV when 0.25%, 0.5%, 0.75% and 1% hot red pepper was introduced in broiler's diet compared to the negative control diet. Oleforuh et al. (2015) reported a significant increased in RBC and Hgb compared to the negative control treatment when 50 ml ginger, garlic and a combination of ginger-garlic were incorporated in broiler's drinking water. The present results are in contradiction with the findings of Noman et al. (2015) and Islam et al. (2017) who reported that incorporating 1% and 2% garlic extracts in broiler's drinking water had no marked effects on RBC and Hgb.

Table 6
Effects of graded levels of methenamine in drinking water on haematological parameters of broilers.

Haematological parameters	Treatments						
	T0	T0$^+$	T0,5	T1	T1,5	T2	p
WBC (10^3/µl)	90.16±7.75	86.08±7.98	84.48±5.50	86.30±4.73	85.06±4.9	84.03±4.35	0.652
RBC (10^6/µl)	2.12±0.33^{cd}	2.39±0.42^{bc}	2.70±0.27^{ab}	2.58±0.42^{abc}	2.88±0.22^{a}	1.87±0.33^{d}	0.001
Hgb (g/dL)	13.36±2.31	11.50±2.24	12.75±1.95	13.20±2.20	14.76±1.07	12.07±1.66	0.145
MCV (%)	20.60±5.43^{b}	34.64±7.68^{a}	31.60±7.47^{a}	35.35±8.01^{a}	36.75±4.28^{a}	29.84±5.83^{a}	0.008
MCH (pg)	63.73±6.85^{ab}	51.30±7.39^{c}	55.63±1.56^{bc}	53.98±6.62^{c}	50.58±4.69^{c}	66.28±10.54^{a}	0.004
MCHC (g/dL)	67.05±10.09^{a}	32.48±2.52^{b}	42.43±14.80^{b}	36.83±4.87^{b}	39.95±5.14^{b}	41.94±8.21^{b}	0.000
PLT (fL)	57.00±13.77^{bc}	62.00±17.20^{bc}	108.50±5.50^{a}	50.00±9.27^{c}	70.00±4.97^{b}	122.00±14.45^{a}	0.000
PCV (%)	0.08±0.02^{b}	0.42±0.22^{a}	0.16±0.01^{b}	0.07±0.02^{b}	0.07±0.02^{b}	0.20±0.06^{b}	0.000

a, b, c, d: Means with the same superscript on the same row are not significantly different ($p>0.05$). T0: basal diet; T0$^+$: T0+ Antibiotic; T0.5: T0+0.5g Methenamine in water; T1: T0+1g Methenamine in drinking water; T1.5: T0+1.5g Methenamine in water; T2: T0+2g Methenamine in drinking water; p= probability; WBC= White Blood Cell; RBC= Red blood cell; Hgb= Haemoglobin; MCV= Mean corpuscular volume; MCH= Mean corpuscular haemoglobin; MCHC= Mean corpuscular haemoglobin concentration; PCV= Packed cell volume.

As summarized in table 7, no significant ($p>0.05$) effects was observed among treatments for serum contents in total proteins, HDL-cholesterol, creatinin, albumin, globulin and albumin/globulin ratio compared to the control diets. Serum contents in ALAT (alanine amino-transferase) and ASAT (aspartate amino-transferase), are used to evaluate liver function, the increase in their activities are related degenerations of hepatocytes or liver damage irrespective of its origin (Fatemi et al., 2005; Vahdatpour and Babazadeh, 2016). The present study showed that the concentration of ASAT and ALAT was significantly ($p<0.05$) higher in birds fed 1 g and 2 g of methenamine per liter of water compared to the negative control diet but were within normal range for broiler birds. With respect to the negative control treatment, methnamine inclusion in water had an upward trend in the serum content in ALAT. These results are similar to those of Kana et al. (2017b) in which an increase in ASAT and ALAT was observed when 2 g/kg of fruit, bark and a combination of fruit-bark of *Afrostyrax lepidophyllus* were administered to broilers through feed. Moreover, Kana et al. (2017a) reported an increase in these parameters when 0.2% and 0.4% *D. glomerata* were given to broilers through feed. This observation contradicts the finding of Rehman et al. (2011) who reported that feeding broilers with a mixture of aqueous extracts of medicinal plants induced a reduction in ALAT and ASAT ratio.

In the same line, the findings of Al-Shuwaili et al. (2015) revealed that adding 5% spices of ginger, garlic and cinnamon in the ration of turkey lower the serum content in ASAT and ALAT. This contradiction can be explained by the multitude active compounds in the mixture of the extracts used by these authors which could have affected liver function. The examination of the histological section of the liver (figure 1) showed no damage suggesting that ALAT content increased without reaching the critical level indicating no cell destruction. This is in agreement with the study of Ciza et al. (2019) who recorded no damage of histological structure of the liver of broiler's supplemented with 2 mg of MSG/kg. In contradiction, the finding of Yemdjie et al. (2017) on chelating effect of

silver nitrate by chitosan in broiler chickens reported that, the histological structure of liver revealed the presence of macro necro hepatic steatosis.

Table 7
Effects of graded levels of methenamine in drinking water on biochemical parameters of broilers.

Biochemical paramètres	Treatments						
	T0	T0+	T0,5	T1	T1,5	T2	p
ASAT (IU/L)	195.13±34.11^{c}	237.30±14.42^{b}	116.96±3.38^{d}	284.67±57.62^{a}	121.80±13.08^{d}	269.21±17.45^{ab}	0.000
ALAT (IU/L)	14.00±2.23^{d}	29.31±2.59^{c}	29.58±2.56^{c}	29.53±2.18^{c}	35.44±3.94^{b}	45.79±4.86^{a}	0.000
Créatinine (mg/dL)	0.02±0.01	0.02±0.01	0.02±0.00	0.02±0.01	0.02±0.01	0.02±0.01	0.801
Urea (mg/dL)	8.31±0.86^{c}	11.08±1.33^{b}	9.32±1.26^{c}	8.18±1.53^{c}	16.64±1.83^{a}	11.46±1.33^{b}	0.000
Total protéines (g/dL)	2.76±0.26	2.72±0.39	3.34±1.00	2.77±0.65	2.96±0.66	2.86±0.48	0.383
Albumin (g/dL)	1.48±0.14	1.39±0.17	1.74±0.52	1.66±0.25	1.62±0.15	1.62±0.18	0.284
Globulin (g/dL)	1.24±0.24	1.40±0.31	1.34±0.63	1.55±0.32	1.27±0.20	1.25±0.2	0.663
Ratio A/G	1.25±0.35	1.02±0.21	1.41±0.45	1.11±0.25	1.30±0.23	1.34±0.36	0.369
Triglycérides (mg/dL)	50.08±3.30^{b}	55.23±7.52^{b}	70.91±17.91^{a}	53.72±9.03^{b}	43.06±9.46^{b}	48.93±7.14^{b}	0.004
Total cholestérol (mg/dL)	105.45±2.10^{b}	138.46±4.98^{a}	135.73 ±14.08^{a}	138.61±16.55^{a}	127.38±11.94^{a}	131.82±17.89^{a}	0.003
HDL-cholestérol (mg/dL)	22.18±4.61	24.40±1.43	25.00±1.97	21.28±2.56	27.03±4.04	25.50±5.36	0.077
LDL-cholestérol (mg/dL)	91.19±6.21^{d}	128.89±9.58^{abc}	111.21±2.70^{c}	132.00 ±15.09^{ab}	113.84 ±19.75^{bc}	139.24±14.20^{a}	0.000

a, b, c, d: Means with the same superscript on the same row are not significantly different (p>0.05). T0: basal diet; T0^{+}: T0+ Antibiotic; T0.5: T0+0.5g Methenamine in water; T1: T0+1g Methenamine in water; T1.5: T0+1.5g Methenamine in water; T2: T0+2g Methenamine in water; p: probability.

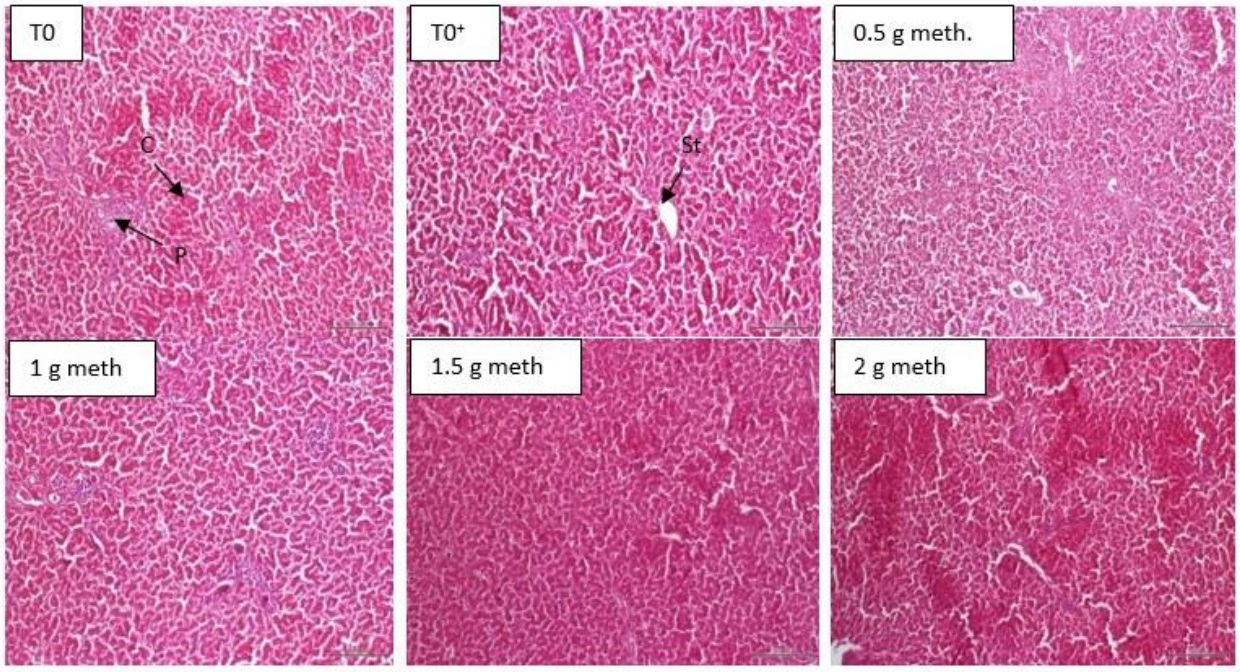

Fig. 1. Histological structure of the liver of broiler chickens as affected by graded levels of methenamine (C: conjonctive tissues; p: portal spaces; St: steatonecroses; magnification: 400X).

No marked (p>0.05) effects on total protein, globulin, A/G ratio and albumin levels was recorded in this study suggesting that methenamine did not cause any deficiency in protein. This corroborates the results of Al-Shuwaili

et al. (2015) which showed that incorporating ginger, garlic and cinnamon in turkey's ration had no marked effects on total protein and albumin levels. Moreover, Kana et al. (2017a) recorded a non-significant effect when *D. glomerata* at the level of 0.2% and 0.4% were introduced in broiler's feed. But the present results contradict the results obtain by Oleforuh-Okoleh et al. (2015) that showed a significant increase in the levels of total protein and albumin when 50 ml of aqueous extract of garlic, ginger and a combination of garlic-ginger were incorporated to broiler's drinking water.

Serum content in urea was significantly ($p<0.05$) higher with 1.5 g of methenamine per liter of drinking water. This result agrees with the finding of Ciza et al. (2019) who reported a significant increase in urea of broiler chickens supplemented with 1 mg of MSG. The increase in urea level could reveal kidney dysfunction. However, histological sections of the kidneys (figure 2) of chickens fed with graded levels of methenamine showed no mark of lesions. This suggests that, the increasing concentration of serum urea did not reach the level which could be harmful to the kidney. This study is in disagreement with the result of Yemdjie et al. (2017) who reported that, the chelation of $AgNO_3$ by chitosan has no significant effect on urea serum concentration of broilers. This same author also reported that, the histological section of kidney revealed a disorganization of the glomerular structure and atresies in chickens fed on unchelated $AgNO_3$.

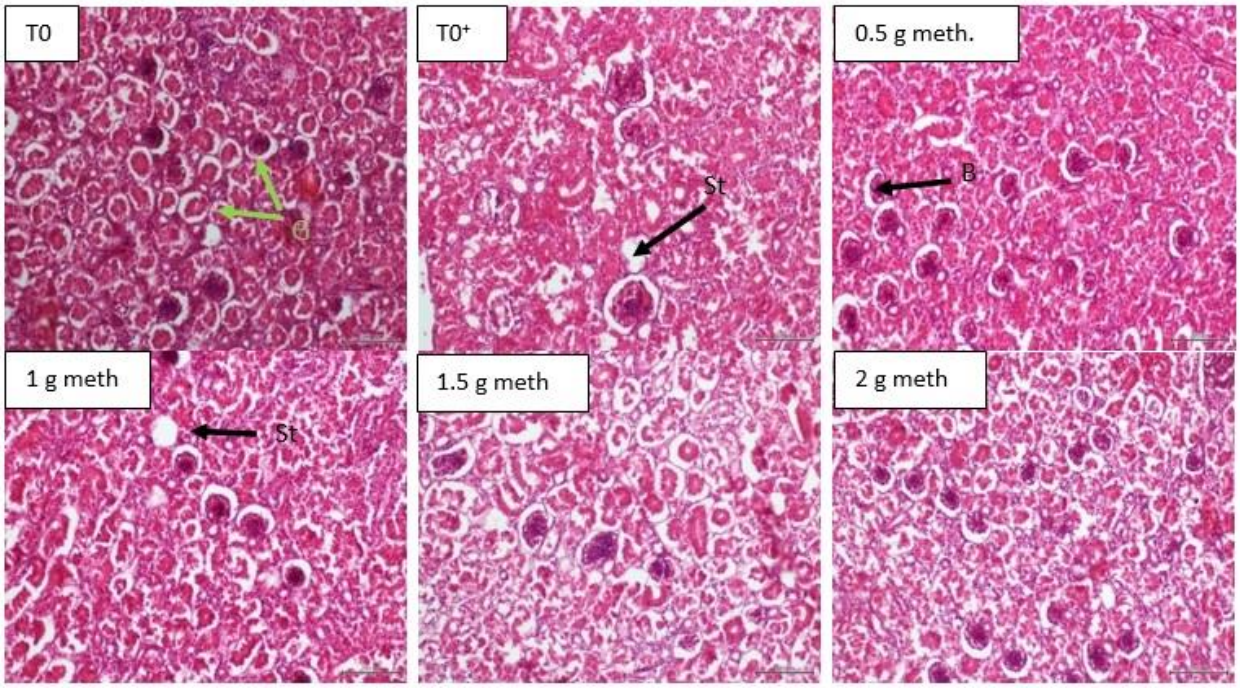

Fig. 2. Histological structure of the kidney of broiler chickens as affected by graded levels of methenamine (G: Glomerules; St: Steatoses; B: Bowman capsules; Magnification: 400X).

4. Conclusion

This study revealed that the inclusion of methenamine in drinking water improves growth performances without any adverse effects on the organs histology, haematological and biochemical paramaters of broilers. Methenamine can effectively be used in chickens up to 2 g/L of drinking water as alternative to antibiotic growth promoter.

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