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Adding hepatoprotective plants to beverage water: an alternative to conventional drugs for broiler production

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ABSTRACT

The present work was to study the effects of *Desmoduin adscendens, Khaya grandifoliola, Xylopia phloiodora* extracts on growth parameters and selected blood parameters of broilers chickens. A total of 252 broiler chicks were randomly distributed into 4 groups. Chickens of control batch received commercial hepatoprotective (Hepaturyl 1 g/l) and the experimental groups received a formulation based on 3 extracts hepatoprotective plants at a concentration of 200, 100 and 50 mg/kg body weight. The mortality rate of control and experimental groups was 6.3% and 4.7% respectively. Average weight of batches at day 48 was, 2.6 for the control, 2.7 for the batch 2, 2.6 batch 3 and 2.5 kg batch 4 with an average consumption index ranging from 1.6 for the control and 1.7 for the experimental groups. Liver function in broilers was not altered (The values of alanine aminotransferase and aspartate aminotransferase were 5-25 IU/l and 50-350 IU/l respectively). Cholesterolemia, proteinemia and the triglyceridemia increased with the age of the animals (0.6- 4.1 mmol / l; 21-83 g / l; 0.3- 3.8 g / l respectively). The kinetics of the humoral immune response against infectious bursal disease was not influenced. This work has shown that the use of the formulation as hepatoprotective in chick drinking water shows results similar to those of commercial hepatoprotectors.
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Keywords: Broilers, hepatoprotective plants, hepatic functioning, growth performances, immune response.

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INTRODUCTION

The poultry sector, especially modern poultry, has emerged in recent years as an attractive solution to meet the growing demand for animal protein within the African population (Ayssiwede et al., 2009). Drivers for this growth include: human population growth, greater purchasing power and urbanization. The poultry sector, especially modern poultry, has emerged in recent years as an attractive solution to meet the growing demand for animal protein. Thus, poultry farming, because of its strengths (species with a short cycle, easy to produce, relatively low price, the absence of religious prohibitions against it), occupies a place of choice in the livestock sector in Cameroon (Dupraz et al., 2009). The current scope of poultry farming in Cameroon depends on the particular dynamics of the various farming systems present in the field. Intensive rearing and increased density of poultry on farms are predisposing factors for the emergence of different diseases. Nevertheless, current control methods are essentially based on prophylactic methods. However, to improve the health status of poultry, food additives (antibiotics, probiotics, enzymes, hepatoprotectants and many others) are incorporated into their diet or drinking water (Tayeb et al., 2009; Seal et al., 2013; Tubéry et al., 2015; Roger & Ducrot, 2017)

The use of antibiotics as growth promoters has been banned in recent decade because of development of antibiotic resistant bacteria and their residue in animal products and the potential harmful effects on human health. Therefore, there is increasing interests to find alternatives for antibiotics in recent years. Pro and prebiotics, enzymes, acidifiers, phytogenic and herbal products have been investigated as alternatives to antibiotic in animal feed (Yang et al., 2009). Many beneficial effects for bioactive herbal derivatives and essential oils (EO's) has been reported including increase in digestive enzymes secretion and activities, improving gut health by selectively growth stimulating effects on useful bacterial species and inhibition of pathogens and improving immune system status (Jang et al., 2007; Bolukbasi et al., 2008; Rahimi et al., 2011; Kouam et al., 2017). Therefore, EO of herbs could be considered as a potential replacement for antibiotic growth promoters. Therefore, the traditional pharmacopoeia, advocates the use of several plant species with therapeutic properties, which may have effects on the digestion and immune system of broilers. These plant-derived substances have the advantage of good liver function, an immunomodulatory effect, the minimization of some of the side effects of modern drugs, and more importantly the reduction of the cost of therapy (Taheri et al., 2005; Namvari et al., 2011).

Thus, the aqueous extract of the bark of *Khaya grandifiolia* has shown a favorable effect on the reduction of blood cholesterol and glucose levels in the rat (Stephen et al., 2009). Its extract on the other hand, demonstrated significant activity ($p < 0.05$) in the activities of plasma AST, ALT and a decrease in plasma ALP, hepatic AST and 'ALT compared to control after 1 week of administration in rats (Bumah & Agbedahunsi, 2010). The methylene chloride / methanol mixture extract of the bark exhibited hepatoprotective and immunomodulatory potentialities, the methylene chloride / methanol fraction from the crude extract showed *in vitro* hepatoprotective activity (Njayou et al., 2004; Owona et al., 2013). The antioxidant activity and hepatoprotective properties of this plant have also been demonstrated (Njayou et al., 2013). Also, Xylopia p*hloiodora* leaves and stem bark extracts showed hepatoprotective activities at doses equivalent to 2.5 g plant / kg, since serum levels of ALAT and ASAT in the extract treated rats were,, according to the investigators, significantly weak compared to control CCl_4 -injured rats (Moundipa et al., 2007). Moreover, *Desmoduin adscendens* enjoys an excellent reputation and is used for various purposes by naturopaths and other traditional practitioners because of its hepatoprotective and immunomodulatory role in both human medicine and animal medicine (Magielse et al., 2013; Tubéry et al., 2015; Chuisseu et al., 2020).

These beneficial effects observed in previous studies can be an asset to boost

production in farms, hence the justification of this study. The objective of this study was to determine the effects of the inclusion of a hepatoprotective phyto-formulation in drinking water, on the production performance of broiler chickens.

MATERIALS AND METHODS

Materials

With regard to the animal material, the study was carried out on 252 one-day-old Cobb 500 broiler chicks from the AGROCAM hatchery in Bafoussam. The plant material was a basic formulation of 3 hepatoprotective plants (*Desmoduin adscendens, Khaya grandifoliola and Xylopia phloiodora*) that have been prepared at the Laboratory of Galenic Pharmacy of the Higher Institute of Health Sciences of *Université des Montagnes*.

The botanical identification of the plants was done at the Cameroon National Herbarium, where voucher specimens were kept under the reference numbers 6425-RF-CAM for *D. adscendens*, 23434 YA for *K. grandifoliola* and 10259/SRF CAM for *X. phloiodora*.

Methods

Sanitary prophylaxis

The building was emptied and cleaned. The disinfection was carried out with TH_4 a commercial product based on quaternary ammoniums. A crawl space of 15 days has been respected. Before the arrival of the chicks for the experiment, the litter was distributed on the ground and disinfected with the disinfectants mentioned above.

Chickens and experimental treatments

The experiment started with 252 chicks (Cobb 500), in a completely random test with 4 treatments and 63 chickens per treatment. All animals received a standard diet for broilers (Table 1).

However, batch 1 chickens received a commercial hepatoprotectant (Hepaturyl, the active ingredient of this product is sorbitol) administered at 1 g/l according to the manufacturer's dosage, while those in the experimental groups received the formulation based on 3 hepatoprotective plant extracts at 50, 100 and 200 mg/kg body weight respectively for group 2, 3 and 4, respectively, during days 2-5, 14-18 and 35-38 in drinking water.

Clinical and zootechnical follow-up

The vaccination program which included vaccination against infectious bronchitis virus, Newcastle disease and Gumboro disease, was implemented by the hatchery. The animals were observed daily. Clinical signs were noted and autopsies performed on dead animals. For weight monitoring, ten chickens were chosen by random draw and weighed each weekend until the 7th week. Data on zootechnical parameters were recorded and calculated. For each of the Batchs, the mortality rate (MR) and the consumption index (CI) were calculated and converted into a performance index (PI).

Biochemical and immunological parameters

During the experiment, 10 subjects from each batch were randomly selected for blood sampling. This operation was carried out on D7, D14, D21, D28, D35, D42 and D48. Three (3) to five (5) ml of blood were collected from the right wing vein of the subjects, in dry hemolysis tubes, allowed to stand for 30 min, then transported to the laboratory and centrifuged at 3000 rpm for 10 min. The fresh serum obtained was recovered using a micropipette and divided into aliquots that were stored in the freezer at -20 °C for biochemical and immunological assays. The levels of total cholesterol, triglycerides, total proteins and transaminase activity (ALAT and ASAT) were determined. The anti-Gumboro antibody assay was performed by indirect ELISA. The principles of the analytical methods and the references of the techniques used for biochemical and immunological analyzes are summarized in Table 2.

Statistical analysis

The statistical analysis was performed using R version 5.0 software to calculate the mean ± Standard Error of the Mean of the different variables of the study. The comparison of the means of the variables between the experimental groups and the control group was performed by the Student's t-test and $p < 0.05$ was considered statistically significant.

Table 1: Composition of the standard diet for broilers, distributed by the company *Société des Provenderies du Cameroun*.

Determined components (%)	Food		
	Start-up (1-21 days)	**Growth (21-35 days)**	**Finish (35-48 days)**
Raw protein	Min 22	Min 20	Min 19.5
Fat	Min 6	Min 6	Min 8
Humidity	Max 12	Max 12	Max12
Crude fiber	Max 3.5	Max 3.6	Max 4
Ashes	Max 5	Max 5	Max 5.4

Min= Minimum ; Max= Maximum

Table 2: Analytical methods.

Parameter		Analysis Method	
Organic elements	**Total protein**	Colorimetric, biuret	Inmesco Kit
	Total Cholesterol	Enzymatic, Colorimetric	Inmesco Kit
	Triglycerides	Enzymatic, Colorimetric	Inmesco Kit
Enzymes	**ALAT**	Kinetics.IFCC, UV-37 °C	Inmesco Kit
	ASAT	Kinetics.IFCC, UV-37 °C	Inmesco Kit
Immunology	**Antibody titration against Gumboro disease**	Indirect ELISA	BioChek″ - ref : CK113 IBD‴

UV= Ultraviolet, IFCC= International Federation of Clinical Chemestry and Laboratory Medecine, ALAT= Alanine Aminotransferase, ASAT= Aspartate Aminotransferase, IBD=Infectious bursal disease.

RESULTS

Zootechnical parameters

The MR in the four batches, for the total period of the breeding ranged from 1.6 to 9.5% with an average of 5.1%. It shows that the highest MR was recorded in lot 4, which received the highest dose of the formulation. The average weight of batches at day 48 varies from 2.5 to 2.7 kg with an average of 2.6 kg for the whole band. During the seven weeks we did not observe a significant difference between the weights of the subjects of the experimental batch and those of the control batch.

We note, however, a non-significant improvement in the weights of the chickens in weeks 2 and 3 in weeks 6 and 7. The overall CI was 1.71, with values ranging from 1.69 to 1.75. We generally notice that there is no significant difference in daily food consumption in different batches. The incorporation of hepatoprotective plant extracts into the drinking water improves the feed intake of birds from the third week to the end of the experiment. The PI has been calculated for all Batches and ranges from 281.70 to 321.22. No significant difference in the ponderal monitoring of the poultry of the

different lots. The results obtained in each batch are shown in Table 3.

Biochemical analyses

Serum alanine aminotransferase (ALAT) rate

ALAT is an enzyme whose determination of serum UI/l allows evaluating and monitoring the functioning of the liver (Samali et al., 2012). Normal values are around 5 to 25 IU/l in chicken. Figure 1 show the serum alanine aminotransferase rate obtained in the different batches at regular time intervals. The comparison of the means ± standard obtained between the four batches shows non-statistically significant differences (p=0.406). We noted that ALAT activity was high at W3, W4 and W6 which corresponds to periods of dietary change. This variation remains normal around 5-25 IU/l

Serum aspartate aminotransferase (ASAT) rate

ASAT is an enzyme whose serum rate is used to assess the state of the liver but also the functioning of the heart. Its usual normal values are between 50-350 IU/l in the chicken. Figure 2 show the serum aspartate aminotransferase rate obtained in the different batches at regular time intervals. The values obtained and compared with each other do not show statistically significant differences (p=0.6005). ASAT activity was high at W2, W4 and W6, which corresponds to periods of dietary change. This variation remains normal around 50-350 IU/l.

Total cholesterol

Figure 3 show the variation in cholesterol rate obtained in the different batches at regular time intervals. Normal values are around 0.6- 4.1 mmol / l. The cholesterolemia observed in our study increases with the age of the animals. However, from one group to another for the same period, no significant differences are noted (p=0.9324).

Triglyceride rate

Normal values are around 0.3- 3.8g / l. Figure 4 shows the variation in triglyceride rate of animals from different groups during the experiment. Triglyceridemia observed in our study increased with age of animals. However, we noted a decrease in W 5 triglyceride levels in the 4 lots followed by an increase to W 7 (Figure 4). However, from one group to another and for the same period, no significant differences are noted (p=0.4323) in Figure 4.

Rate proteins

Proteins are the main component of cells, accounting for more than 50% of their dry weight. Normal values are around 21-83 g/l. The proteinemia observed in our study increases with the age of the animals (Figure 5). However, from one group to another for the same period, no significant differences (p=0.8611) are noted in Figure 5.

Immunological result

Like natural infection, vaccination induces a humoral and cellular immune response. The protection provided by current vaccines relies mainly on the induction of neutralizing antibodies (antibodies capable of neutralizing pathogens or facilitating their phagocytosis and elimination). The nature and intensity of the response will vary depending on two parameters: the type of vaccine administered (live or inactivated) and host factors. Figure 6 shows the kinetics of the antibodies against infectious bursal virus of the different groups throughout the experiment. The statistical treatment did not show a significant difference between the batches at W1 until S7 (p=0.2258). On the other hand, at W4, we notice an improvement in the antibody titer of lot 2 compared with the control group. The first 3 weeks are marked by a decrease in the titer of the antibodies in the 4 batches, then an exponential growth of the antibody level up to the W4 for the lot 2 and the W5 for the batch 1, 3 and 4. A fall in antibody titer at week 6 was noted (Figure 6).

Table 3: Zootechnical parameters of batches.

Batches	Hepatoprotective treatment	Dosage	MR (%)	CI	ALW	PI
1	Hepaturyl	1g/l	6.3	1.69	2.65	305.80
2	Phyto-formulation	50 mg/kg	3.2	1.72	2.74	321.22
3	Phyto-formulation	100 mg/kg	1.6	1.75	2.68	314.04
4	Phyto-formulation	200 mg/kg	9.5	1.70	2.54	281.70

ALW = Average live weight; CI = Consumption Index; MR= Mortality Rate; PI = performance index.

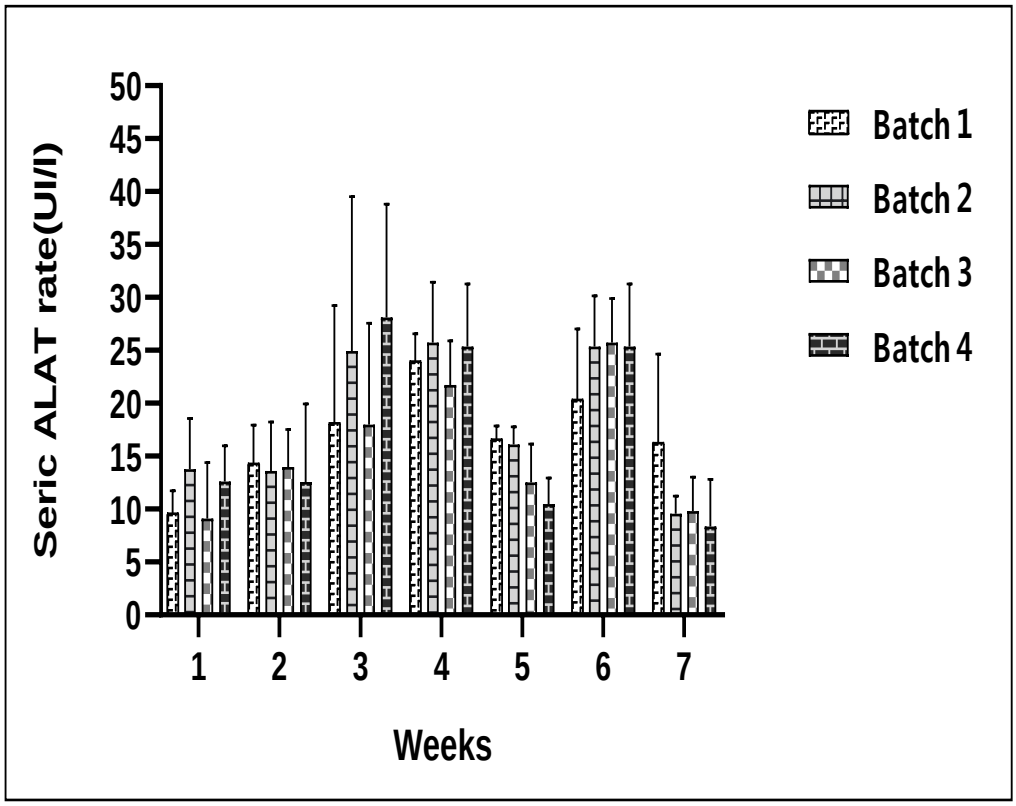

Figure 1: Variation in serum ALAT rate during chicks rearing. Results are represented as means ± standard deviation. No Significant difference (p=0.406) compared to control group. Batch 1 =control, Batch 2, 3, 4= experimental groups.

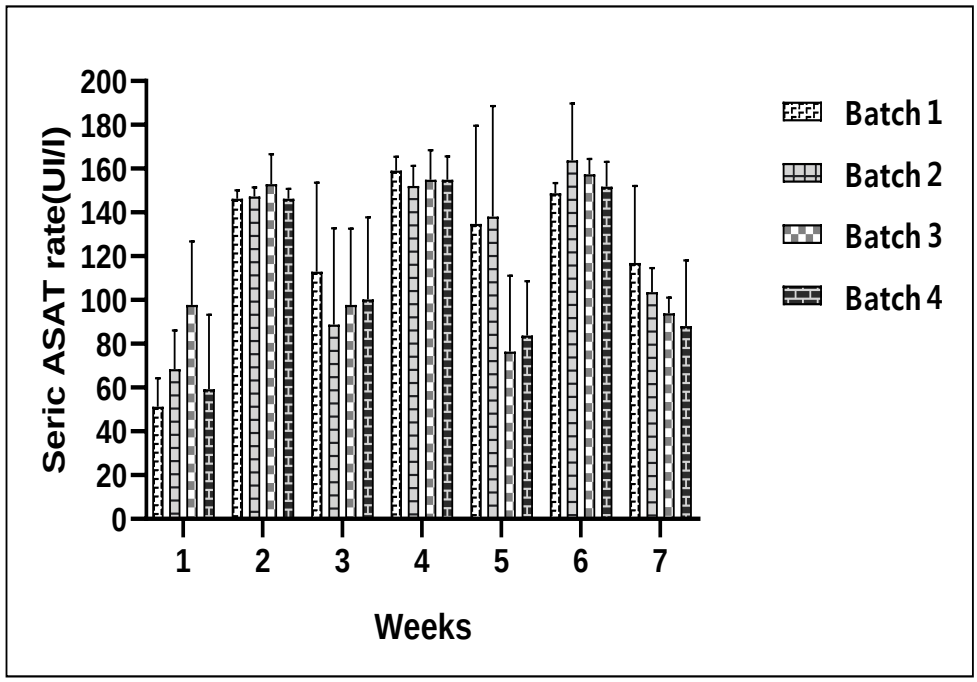

Figure 2: Variation in serum aspartate aminotransferase rate during chick rearing. Results are represented as means ± standard deviation. No Significant difference (p=0.6005) compared to control group. Batch 1 =control, Batch 2, 3, 4= experimental groups.

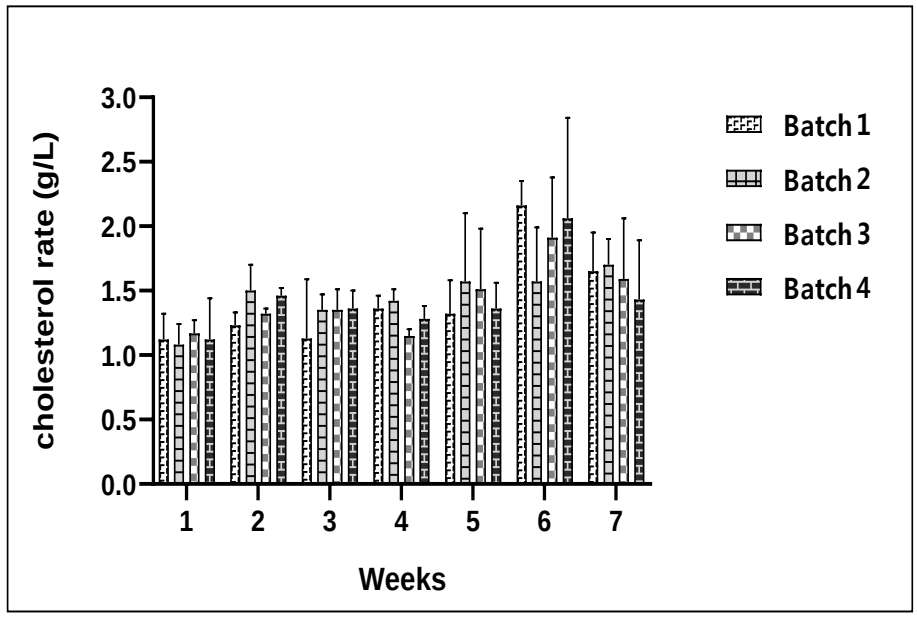

Figure 3: Variation in total cholesterol rate during chick rearing. Results are represented as means ± standard deviation. No Significant difference (p=0.9324) compared to control group. Batch 1 =control, Batch 2, 3, 4= experimental groups.

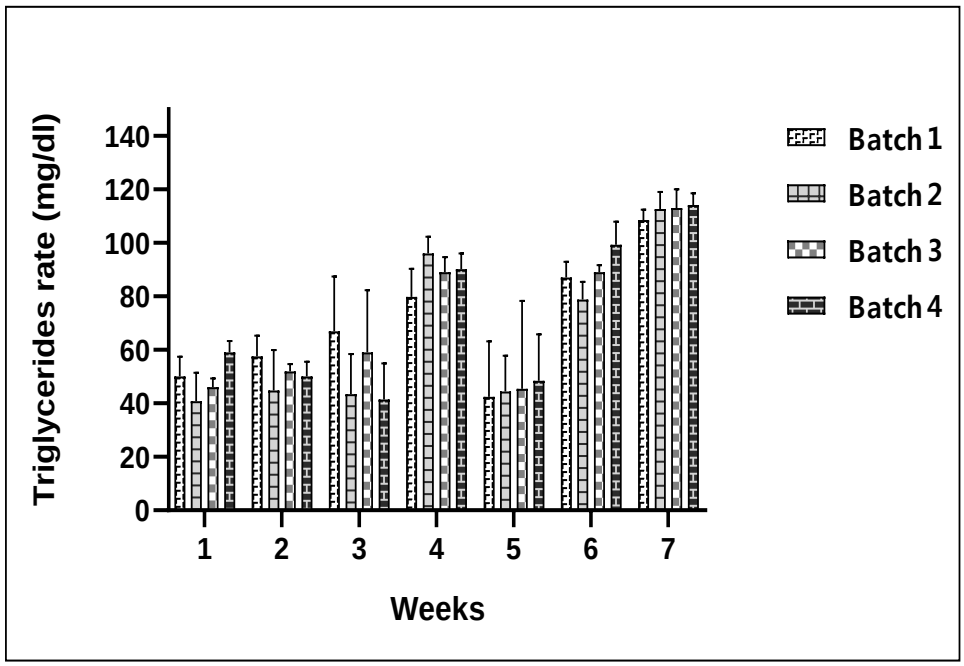

Figure 4: Variation in serum triglyceride levels during chick rearing. Results are represented as means ± standard deviation. No Significant difference (p=0.4323) compared to control group. Batch 1 =control, Batch 2, 3, 4= experimental groups.

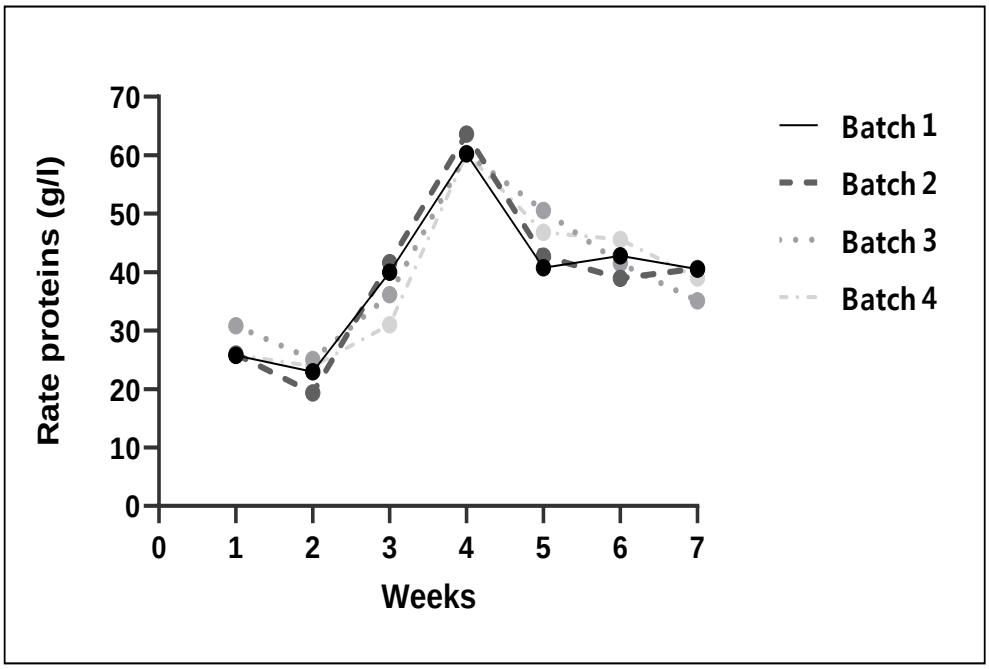

Figure 5: Variation in serum proteins levels rate during chick rearing. Results are represented as means ± standard deviation. No Significant difference (p=0.8611) compared to control group. Batch 1 =control, Batch 2, 3, 4= experimental groups.

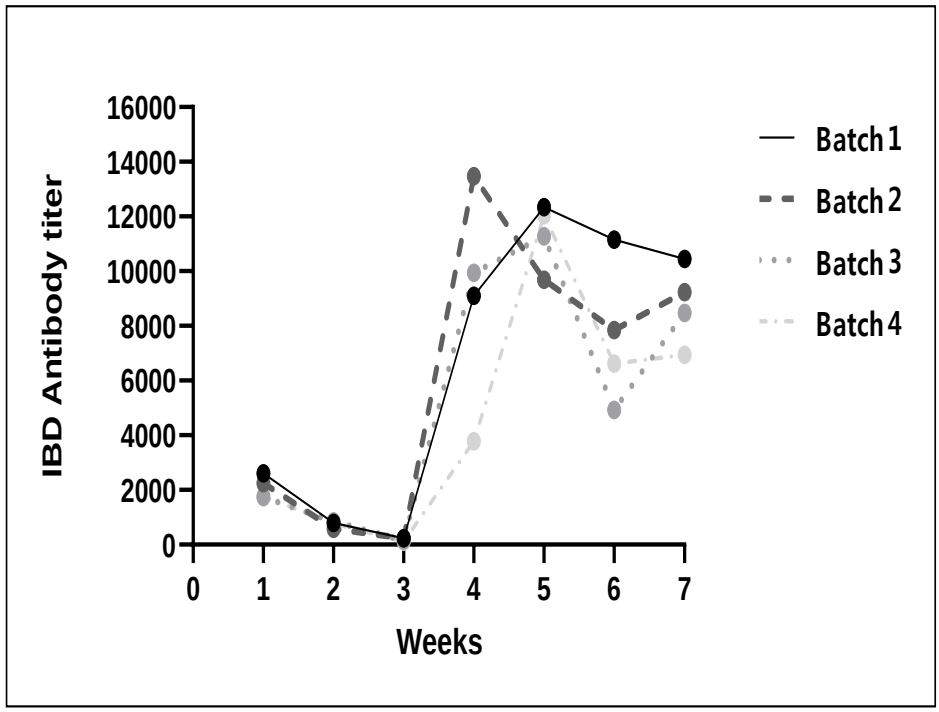

Figure 6: Variation of antibody titer against infectious bursal disease during chick rearing. Results are represented as means ± standard deviation. No Significant difference (p=0.2258) compared to control group. Batch 1 = control, Batch 2, 3, 4 = experimental groups.

DISCUSSION

The MR recorded during the experiment was 5.1%. We found that it was higher in batch 4 (9.5%). However, it was low in batch 2 (3.2%) and 3 (1.6%) as compared to control batch. The antioxidant and hepatoprotective properties of the formulation may have given some resistance to the chicks of these experimental batches (Feher & Lengyel, 2011). It's during the start-up phase that the MR was highest for the four lots. This can be explained by the stress during transportation and handling during the installation of the chicks (Simonin, 2018). The formulation has no adverse effect on the zootechnical performance of subjects in experimental batches compared to the control lot. In poultry, the use of hepatoprotective agents has beneficial effects on food consumption and feed efficiency. These results are similar to those obtained by Teguia et al. (2002) who showed that the dry leaves of *Desmodium sp* resulted in a drop in the consumption index of 6% in the starter food and 10% in the growth food (Teguia et al. 2007).

The results of the activity of ALAT and ASAT obtained indicated a normal activity in all the batches, which highlights the hepatoprotective activity of these plant extracts. ALAT is the most specific transaminase in the liver because of its hepatic and muscular localization, whereas ASAT is more specific in the kidneys, heart, skeletal muscles and liver. In contrast, ASAT activity increased with the age of the subjects in our study. This could have been due to an increase in metabolic activity of the liver and muscle development. The difference between individuals in each lot could be due to age-related physiological changes (Slominski, 2011; Kouam et al., 2017).

An increase in cholesterol levels in each of the experimental and control batches as a function of age was observed. According to the studies of Grizzle et al. (2009), charcoal

does not reduce cholesterol levels during all rearing phases (Grizzle et al., 2009). The results obtained show similar blood triglyceride values in the four batches ($p>0.05$). These show that hepatoprotective plants have no adverse effect on the blood triglyceride content. The lipid content of blood represented by triglycerides and cholesterol is identical in the experimental lot and control lot. This can be because the studied hepatoprotective plant extract and commercial hepatoprotective both have a cholesterol-lowering property that may be due to inhibition of hepatic cholesterol synthesis and their ability to deconjugate bile salts (Mamoudou et al., 2014). The protidemia observed during our study increases with the age of the animals. However, from one group to another and for the same period, no significant difference is noted. These show that hepatoprotective plants have no adverse effect on the protein content of the blood.

Like natural infections, vaccination induces a humoral and cellular immune response. The protection provided by current vaccines relies mainly on the induction of neutralizing antibodies (antibodies capable of neutralizing pathogens or facilitating their phagocytosis and elimination). The nature and intensity of the response will vary depending on two parameters: the type of vaccine administered (live or inactivated) and host factors (Dao et al., 2001). However, the statistical analysis did not show a significant difference between the antibody titers of the W1 to W7 batches ($p>0.05$). This can be justified by the fact that the injected vaccine strain, gaining the Fabricius stock exchange, multiplies and prevents the penetration of wild viruses by an interference phenomenon. This multiplication did not significantly increase the weight of the Fabricius bursa in our study. The primary vaccination was carried out on D1, by nebulization, at the same time as vaccination against Newcastle disease and infectious bronchitis. The recall took place on D7 and the beginning of the samples on D7. This is why the antibody titers are not very high at this time. In addition, the persistence of antibodies of maternal origin probably limits the production of vaccine antibodies (Dao et al., 2001). A W4; and W5, 14 days and 28 days after the booster, the titers of the vaccine antibodies are much higher. Thus, Taheri et al., (2005) and Aghdam et al. (2011) respectively demonstrate the effect of propolis oil extract and the effect of propolis alcohol extract on the immune system of the chicken (Namvari et al., 2011; Taheri et al., 2005). The same is true for Dao et al. (2001) when studying a vaccination program in Viet Nam against Newcastle disease, Gumboro disease, and avian infectious bronchitis (Dao et al., 2001).

However, the study conducted by Cường & Bích, (2018) confirms the independence and non-interaction of the vaccine against Gumboro disease from that directed against Newcastle disease and infectious bronchitis (Cường & Bích, 2018).

Conclusion

Medicinal plants are widely used in Africa and developing countries. They are nowadays natural substances used in various fields. It is therefore vital to look for high efficiency, low toxicity and low cost plants. In order to reduce the cost of production, we proposed to determine the effect of the inclusion of a hepatoprotective phyto-formulation in drinking water on broiler production performance. In general, the results have demonstrated that the use of the formulation as a hepatoprotectant in chicks' drinking water shows similar results to those of commercial hepatoprotectants. These hepatoprotective plants (*Desmodium adscendens, Xylopia phloiodora* and *Khaya grandifoliola*) in combination therefore do not present at this stage of experimentation significant differences in the parameters studied.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

PDDC, ANN, LT, FMK and JN designed the experiment; PDDC, RCN, ANN,

BFNS, AFK and ED carried out the experiments; PDDC, RCN, CD, BRTG wrote the manuscript; PDCD, RCN and CD analyzed data; PFM and JN supervised the work. All authors revised the manuscript critically for important intellectual content, and approved to be published.

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