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Effects of *Moringa Oleifera* leaves powder and *Aloe vera* gel on biochemical and haematological parameters of *Coturnix Japonica* quail

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Abstract

This study was conducted with the objective of evaluating the effects of *Moringa oleifera* leaves powder and *Aloe vera* gel on the biochemical and haematological parameters of quails. For this purpose, 147 quails were divided into 7 batches of 21 subjects (12 males and 9 females) receiving respectively 0% ; 2% ; 5% and 10% of *Moringa oleifera* leaves powder and 2% ; 5% and 10% of *Aloe vera* gel were used. After 8 weeks of treatment, the blood of a sample of 3 males and 3 female's quails was taken from each batch for biochemical and haematological analysis. The analyses were done using automatics Mindrays analysers according to different assays methods. Following the analyses, no significant differences were observed between the blood parameters of males and females quails. The haematological analyses showed that only the white blood cell count varied. Thus, this rate was meaningfully higher in the quails of the MO5% batch by 55.16% (P=0.040), the MO10%, batch by 51.77% (P=0.041) and the AV10% batch by 104.45% (P=0.009) compared to the control batch. As for the biochemical parameters, the analyses revealed no significant difference between those of the treated quails and the controls, except for the MO10% lot, which had an increased blood glucose level of 72.73% (P=0.032) compared to the controls. The addition of *Moringa oleifera* leaves powder or *Aloe vera* gel up 10% to the feed had no adverse effect on the health of the quails.

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Introduction

In breeding, antibiotic additives have been used for 50 years to improve growth performances and neutralize the decline in productivity related to poor house hygiene, stress, or poor feeding (Moser *et al.,* 2003). However, antibiotic use can lead to potential public health risks due to the development of antibiotic resistance mechanisms by the bacteria in animals and humans (Gay *et al.,* 2017). In addition, Madrid *et al.* (2003**)** showed some undesirable factors caused by the use of antibiotics as growth in poultry feed. As an alternative, researchers have turned their attention to aromatic and medicinal plants as a replacement for antibiotics (Deschepper *et al.,* 2003). *Moringa oleifera* and the *Aloe vera* are medicinal plants known to be rich in vitamins, minerals, amino acids and fatty acids (Razis *and al.,* 2014). They are also known for their therapeutic properties (Anwar *and al.,* 2007). These plants would be good food complements and immune system stimulants for farm animals such as quails. Quails are often fed chicken feed, which does not allow them to express their full potential because of their low protein content compared to the Quail's needs (Vali, 2009). It would still be wise to verify the toxicity of these plants in these animals. Thus, this study was conducted to evaluate the effects of *Moringa oleifera* leaves powder and *Aloe vera* gel on biochemical and haematological parameters of quails.

Materials and methods

Study area

This test took place in the animal house (*Vivarium*) of the Superior Normal School (S.N.S) located in Cocody-Abidjan. Abidjan is a city located into the South-East of Ivory Coast between 5°10 and 5°30 North latitude and 3°45 and 4°21 West longitude (Saley *and al.,* 2009). The climate in this area is equatorial with about 2000 to 3000 mm of rains spread over 2 seasons from May to July for the big rainy season and from October to November for the short rainy season (Tapsoba, 1995). The average temperature is between 24,2 and 27,4°C and the relative humidity is generally between 78 and 87% (Kablan, 2016).

Plant material

Fresh *Moringa oleifera* leaves were collected in Abidjan (Ivory Coast) in the township of Cocody at the Felix Houphouet-Boigny university. The leaves were transported, washed with water and then spread evenly on aluminum foil at the Biology and Health laboratory of the University Felix Houphouet-Boigny (UFHB). The leaves were dried at the laboratory temperature (17 to 21 °C), then ground in a blender to obtain a powder. For *Aloe vera*, the plants were purchased from a gardener on the outskirts of the UFHB. The leaves were taken, washed and peeled with a knife. The gel obtained was washed to remove the sticky sap, then mixed to obtain a liquid.

Animal material

The experiment started with 180 quails of 7 days of age and an average weight of 7.4 g. These quails, without distinction of sex, were divided into 3 groups of 30 control quails (control batch), 25 quails receiving 2% of *Moringa oleifera* leaves powder (MO2% batch), 25 quails receiving 5% of *Moringa oleifera* leaves powder (MO5% batch), 25 quails receiving 10% of *Moringa oleifera* leaves powder (MO10% batch), 25 quails receiving 2% of *Aloe vera* gel (AV2% batch), 25 quails receiving 5% of *Aloe vera* gel (AV5% batch) and 25 quails receiving 10% of *Aloe vera* gel (AV10% batch). At 21 days of age, the quails were sexed and the number was reduced to 21 quails (12 males and 9 females) per batch.

Progress of the experiment

The quails were fed with an industrial feed containing 16% crude protein and 2620 Kcal of energy. Three feed rations were formed by adding and mixing *Moringa oleifera* leaves powder to the basal feed at 2%, 5% and 10%, respectively.

These rations were randomly distributed to obtain 3 treatments (MO2%, MO5% and MO10%) repeated 3 times each. The mixed *Aloe vera* gel was added to the drinking water of the quails at respective percentages of 2%; 5% and 10% to obtain 3 treatments (AV2%, AV5% and AV10%) repeated 3 times each. In addition to these different treatments, a control group was

given neither *Moringa oleifera* leaves powder nor *Aloe vera* gel. The blood of 3 quails per batch and per sex was taken for analysis after the sacrifice of the quails to 8 weeks of treatment. The different groups of quails were kept in similar environments: the average temperature of the room was 26°C (23 to 28°c) with a humidity of 79% HR (74 to 94%).

Bood collecting

Blood was collected from the quails just after they were killed by decapitation. The collection was done in 2 types of tubes: the tubes containing the anticoagulant EDTA (ethylene diamine tetraacetic acid) for the blood count (CBC) and the dry tubes. The blood in the dry tubes was centrifuged using a Vidas Biomerieux/France centrifuge at 3000 rpm for 5 minutes, and then the serum was collected for the determination of biochemical parameters.

Haematological analysis

The levels of red blood cells, white blood cells, hematocrits, platelets and hemoglobin were determined on blood samples collected in a tube containing the anticoagulant EDTA.

The analyses were performed according to the standard methods of Baker *and al.* (1985) using a Mindray BC-5380 China automatic analyzer.

Biochemical analysis

The biochemical dosages in the sera were all performed by a Mindray BS-200E, China automatic analyzer. The dosages methods differed according to the biochemical parameter sought.

The protocol for each dosage was pre-established and was then incorporated into the instrument during the dosage. Dosages concerned glucose, triglycerides, total cholesterol, urea, uric acid, creatine, AST, ALT, total protein, total bilirubin and conjugated bilirubin. Thus, the blood glucose determination was done according to the test enzymo-colorimetric test of Trinder (1969) and improved by Dingeon. Triglycérides levels were determined by the enzymo-colorimetric test (Young *and al.,* 1975). The total cholesterol was determined by the principle of separation of cholesterol from its esters in lipoproteins by detergents. Urea and uric acid were determined as a kinetic dosage within a defined time interval (Kaplan *and al.,* 1988) and creatinine was determined measured by Henry's colorimetric method. Aspartate aminotransferase (AST) was determined by the colorimetric method of Karmen (1955). ALT was determined by the colorimetric method recommended by the International Federation of Clinical chemistry (IFCC) (Bergmeyer and Horder, 1980). Total protein was determined by the Biuret method (Doumas *and al.,* 1981). Total and conjugated bilirubin were determined on the basis that they react with sulfanilic acid to form azobilirubin.

Statistical analysis

Statistical analyses of the experimental results were performed using GraphPad Prism 5.01 software (Microsoft, USA). The values are presented as mean ± standard error. The data were evaluated by the one-way ANOVA analysis method followed by Tukey's multiple comparison test at the 5% level to assess the significance of the observed differences. If $P<0.05$ the difference between the values is considered significant and if $P>0.05$, this difference is not significant. The values of the treated Quail's batches were compared only to the values of the control quails.

Results and discussion

The analysis of blood parameters showed no significant difference between males and females quails of different batches ($P=0.999$).

Haematological parameters

White and red blood cells: The red blood cell levels of the different treated batches were not significantly different from the levels of the quails of the control batch (P=0.999). As for the white blood cell count, it was significantly higher in the quails of the MO5%, MO10% and AV10% batches, respectively, by 55.16% (P=0.040), 51.77% (P=0.041) and 104.45% (P=0.009) compared to that of the controls (Table 1).

Table 1. White and red blood cells levels of japanese quails receiving *Moringa oleifera* leaves powder and *Aloe vera* gel.

Lots	RBC (×10^6 µL-1)	*P-value*	WBC (×10^3 µL-1)	*P-value*
Witness	3.30±0.20		128.00±2.06	
MO2%	3.33±0.21	0.998	120.90±9.33	0.978
MO5%	3.27±0.90	0.998	198.60±1.55*	0.040
MO10%	3.53±0.55	0.999	194.26±5.77*	0.041
AV2%	3.46±0.16	0.999	141.50±0.88	0.967
AV5%	3.32±0.99	0.998	145.90±29.85	0.967
AV10%	3.34±0.37	0.998	261.70±8.45*	0.009

Values are means± MSE (n=3/batch). For the values without (*), *P* is not significant ; MO2% : batch of the quails receiving 2% of *Moringa oleifera* leaves powder MO5% : batch of the quails receiving 5% of *Moringa oleifera* leaves powder ; MO10% : batch of the quails receiving 10% of *Moringa oleifera* leaves ; AV2% : batch of the quails receiving 2% of *Aloe vera* gel *;* AV5% : batch of the quails receiving 5% of *Aloe vera* gel ; MO10% : batch of the quails receiving 10% of *Aloe vera* gel ; RBC : Red blood cell ; WBC : White blood cell.

Hemoglobin, platelets and hematocrits: Hemoglobin, platelet and hematocrit levels of treated quails were not significantly different from those of control quails (Table 2).

Table 2. Hematocrit, platelet and hemoglobin levels of japanese quails receiving *Moringa oleifera* leaves powder and *Aloe vera* gel.

Lots	HCT (%)	*P-value*	PLT (×10^3 µL-1)	*P-value*	HGB (g/dL)	*P-value*
Witness	57.1±5.77		5.67±0.58		10.74±0.65	
MO2%	54.57±3.18	0.890	5.33±0.58	0.999	10.2±0.80	0.999
MO5%	53.63±5.43	0.890	4.67±1.16	0.999	11.17±0.95	0.999
MO10%	51.7±2.1	0.900	5.33±0.58	0.999	10.73±0.83	0.999
AV2%	56.2±1.59	0.889	5.33±0.58	0.999	11.50±0.20	0.999
AV5%	56.5±2.1	0.889	5.33±1.16	0.999	11.20±0.70	0.999
AV10%	54.6±3.71	0.890	5.00±1.00	0.999	11.4±0.54	0.999

Values are means± MSE (n=3/batch). For the values without (*), *P* is not significant;: HCT : Hematocrit, PLT : Platelet ; HGB : Hemoglobin.

Biochemical parameters

Blood glucose: The difference in blood glucose levels was not significant between the treated and control quails except forth MO10% quail, which had a 72.73% increase in blood glucose levels compared to the control (Table 3).

Table 3. Serum glucose and lipid concentration of Japanese quails receiving *Moringa oleifera* leaves powder and *Aloe vera* gel.

Lots	Glucose (g/L)	*P-valuer*	Triglyceride (g/L)	*P-value*	Cholesterol (g/L)	*P-value*
Witness	1.76±0.05		1.38±0.25		2.92±0.12	
MO2%	2.17±0.13	0.988	1.40±0.40	0.999	2.81±0.27	0.999
MO5%	1.69±0.08	0.999	1.60±0.2	0.999	2.81±0.62	0.999
MO10%	3.04±0.68*	0.032	1.22±0.08	0.999	2.34±0.07	0.999
AV2%	1.93±0.17	0.999	1.20±0.10	0.999	2.75±0.21	0.999
AV5%	2.05±0.05	0.988	1.33±0.25	0.999	2.42±0.13	0.999
AV10%	1.85±0.25	0.999	1.67±0.06	0.999	2.87±0.74	0.999

Values are means± MSE (n=3/batch). For the values without (*), *P* is not significant.

Cholesterol and triglyceride levels: Cholesterol and triglyceride levels of quails fed Moringa oleifera leaves powder were not significantly different from those of the control lot. Similarly, all batches of AV quails had cholesterol and triglyceride levels that were not significantly different from those of the control batch (Table 3). Uremia, creatine and uric acid levels: There was no significant difference in blood uremia creatinemia and uric acid levels between the quails fed Moringa oleifera leaves powder or Aloe vera gel and the control quails (Table 4).

Table 4. Serum concentration of renal parameters of Japanese quails receiving *Moringa oleifera* leaves powder and *Aloe vera* gel.

Lots	Urea (g/L)	*P-value*	Uric acid (g/L)	*P-value*	Creatine (mg/L)	*P-value*
Witness	0.18±0.01		21.67±0.58	0.999	3.67±0.58	0.999
MO2%	0.15±0.02	0.999	20.33±1.53	0.999	2.67±0.58	0.999
MO5%	0.16±0.01	0.999	21.67±0.58	0.999	3.67±0.58	0.999
MO10%	0.17±0.03	0.999	22.00±1.00	0.999	3.00±1.00	0.999
AV2%	0.26±0.03	0.999	22.00±1.00	0.999	3.33±0.58	0.999
AV5%	0.19±0.02	0.999	20.67±0.58	0.999	3.68±1.16	0.999
AV10%	0.24±0.07	0.999	21.67±0.58	0.999	4.00±1.00	0.999

Values are means± MSE (n=3/batch). For the values without (*), *P* is not significant.

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT): The analyses revealed no significant difference between the AST and ALT of quails from all treated batches and those of control quails (P=0.999) (Table 5).

Table 5. Serum AST and ALT concentration of Japanese quails receiving *Moringa oleifera* leaves powder and *Aloe vera* gel.

Lots	AST (UI/L)	*P-value*	ALT (UI/L)	*P-value*
Witness	222.30±52.44		30.37±5.56	
MO2%	222.3±75.35	0.999	36.33±2.52	0.999
MO5%	201.70±67.88	0.999	33.63±3.56	0.999
MO10%	215.70±63.66	0.999	35.83±5.11	0.999
AV2%	205.00±79.70	0.999	36.33±4.04	0.999
AV5%	207.00±75.36	0.999	34.33±4.04	0.999
AV10%	215.00±61.65	0.999	36.33±5.03	0.999

Values are means± MSE (n=3/batch). For the values without (*), *P* is not significant.

Protein and total/conjugated bilirubin levels: Moringa oleifera leaves powder and Aloe vera gel did not cause significant changes in the protein and bilirubin levels of treated quails compared to control quails (P=0.999) (Table 6).

Discussion

The analysis of the haematological and biochemical parameters makes it possible to assess the health status of the animals. Indeed, the analysis of the haematological parameters measured in the quails did not reveal any significant change in the red blood cell, haematocrit, blood platelet and haemoglobin levels between the treated quails and the controls. In contrast, a significant increase in the white blood cell count of quails from the MO5%, MO10% and AV10% batches compared to the control batch was observed. This high rate of white blood cells could be explained by a stimulation of the non-specific defense of the immunity of the quails by the *Moringa oleifera* leaves

powder and the *Aloe vera* gel at a certain quantity (5 and 10%). Indeed, *Moringa oleifera* leaves powder contains tannins (Chikh and Idir, 2016) which possess immunostimulant activities (Feldman *et al.*, 1999). In addition, *Aloe vera* gel contains a polysaccharide called acemannan that strengthens the lymphatic system. Indeed, this sugar has immunostimulant properties recognized. It stimulates the action of the bone marrow and the multiplication of lymphocytes and the white globules cells that protect the body (Tizard and Ramamoorthy, 2004).

Table 6. Serum protein and bilirubin concentration of Japanese quails receiving *Moringa oleifera* leaves powder and *Aloe vera* gel.

Lots	Total protein (g/L)	*P-value*	Total bilirubin (mg/L)	*P-value*	Conjugated bilirubin (mg/L)	*P-value*
Witness	40.67±4.51		4.67±1.16		1.55±0.05	
MO2%	39.67±6.51	0.999	5.33±1.16	0.999	1.26±0.30	0.999
MO5%	33.67±2.52	0.999	4.67±1.16	0.999	1.11±0.13	0.999
MO10%	35.67±5.51	0.999	5.00±1.00	0.999	1.04±0.06	0.999
AV2%	48.00±3.00	0.999	5.33±1.16	0.999	1.23±0.50	0.999
AV5%	36.00±2.00	0.999	5.33±0.58	0.999	1.28±0.31	0.999
AV10%	41.00±10.00	0.999	5.01±1.00	0.999	1.45±0.42	0.999

Values are means± MSE (n=3/batch). For the values without (*), *P* is not significant.

The biochemical analysis concerned the analysis of glucose level, lipids, kidney and liver parameters. Indeed, the evaluation of carbohydrate metabolism involves the analysis of blood glucose levels (Lelevich and Popechits, 2010). Thus, in this study, blood glucose levels didn't vary between the different treated batches and the witnesses, except in the MO10% batch, where it showed an increase compared to the control.

Despite this increase, all blood glucose levels observed in Quail in this study were within the blood glucose standards for poultry developed by Sholtz *et al.* (2009). The blood glucose levels obtained in this study are also close to those of quails in the works of Khaksar *et al.* (2012**)** and Tufan *et al.* (2015).

As for cholesterol levels, the treatments applied to the quails didn't affect them. Moreover, the cholesterol levels in this study corroborate those observed by Tufan *et al.* (2015) in control female quails of 6 to 7 weeks of age. However, lower cholesterol levels (1.3 g/l) were reported by El Yamany *et al.* (2008). Like cholesterol levels, triglyceride levels were not affected by the treatments. Khaksar *et al.* (2012) and Bensalah (2016) obtained the same triglyceridemia from their studies on Japanese quails.

Regarding the concentration of urea, uric acid and creatinine, it is an important marker for the diagnosis of renal function in mammals (Mukinda *et al.,* 2010). These parameters are present in very small amounts in avian plasma, and determination of their levels is generally considered to be of little value in detecting kidney disease but can be used as a sensitive indicator of dehydration (Kaneko, 2008). However, studies by Lumeij (1987) showed that uremia and, to a lesser extent, creatinine with an unchanged plasma uric acid concentration were good indicators of pre-dysfunction of the kidneys in pigeons (poultry). The determination of these different renal parameters in this study didn't reveal any significant difference between treated quails and the witnesses. The values recorded are close to those obtained by Scholtz *et al.* (2009).

The decrease in plasma proteins could reflect chronic damages (Kaneko, 2008). Indeed, most plasma proteins can act as indicators of the capacity of liver synthesis. When there is cellular damage, the capacity to synthesize the proteins is reduced and when the extent of the damage increases, the levels of these

proteins in plasma tend to decrease. In this study, the blood protein levels of the treated Quail's batches were almost similar to those of the control batch. The values are in agreement with those of A-Ghally and Abd EL-Latifs (2007) and Krupakaran (2013), which are within the physiological norms of poultry. However, lower values than that of this study were obtained by Tufan *et al.* (2015). And this observation would be explained by the fact that the quails were in the early stages of reproduction, thus producing higher amounts of hormone, which would raise the level of some parameters in the blood.

This is confirmed by the observations of Kabir (2013) that the high level of protein in the blood in poultry is due to the secretion of testosterone, growth hormone and œstrogen at the beginning of egg production.

ALT and AST are part of a group of enzymes that catalyzes the interconversion of amino acids and oxyacids by an amino group transfer. They are of great clinical importance (Hochleithner, 1994). However, the addition of *Moringa oleifera* leaves powder and *Aloe vera* gel to Quail's diet didn't affect their levels in this study. ALT and AST levels in this study were similar to those observed in control quails in the studies of Babazadeh *et al.* (2011).

Conclusion

From the study the addition of *Moringa oleifera* leaves powder or *Aloe vera* gel up to 10% to the feed of the quails doesn't cause any adverse effect on the levels of biochemical and haematological parameters of the latter. These plants extracts can therefore be used as feed supplements for quails (*Coturnix japonica*). They don't compromise the health of the quails but would stimulate their immunity to a certain percentage.

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ABBREVIATIONS

ALT: Alanine aminotransferase
AST: Aspartate aminotransférase
CBC: Blood count
EDTA: Ethylene Diamine Tetraacetic Acid
MSE: Mean standard error
RBC: Red blood cell
SNS: Superior Normal School
UFHB: University Felix Houphouet-Boigny
WBC: White blood cell