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Research Paper

Molecular and Genetic Characterization of Infectious Bronchitis Viruses Isolated from Commercial Chicken Flocks in Egypt between 2014 and 2016.

Setta A, Salem HM, Elhady M, El-Hussieny A and Arafa AS.

J. World Poult. Res. 8(1): 01-07, 2018; pii: S2322455X1800001-8

ABSTRACT

Infectious Bronchitis is one of the major viral diseases affecting poultry causing severe economic losses. The prevalence of Infectious Bronchitis viruses was studied in commercial chicken farms in Egypt between 2014 and 2016. A total of 1722 organ samples (trachea, kidney, caecal tonsils and lungs) were collected from 246 problematic flocks, showing respiratory signs and considerable mortalities, from 13 governorates throughout the examination period and were then subjected to molecular analysis using real-time reverse transcription-polymerase chain reaction. Data from this study have shown a high prevalence (75.6%) of Infectious Bronchitis virus in Egyptian farms. Infections mixed with other respiratory viruses were frequently observed, including very virulent Newcastle disease, Low pathogenic avian influenza, H9N2 and High pathogenic avian influenza H5N1 with 27.9%, 25.7% and 17.1%, respectively with higher detection percentages observed in the winter season. Phylogenetic analysis of 19 selected positive Infectious Bronchitis virus has revealed Infectious Bronchitis virus genotypes closely related to variant II strains Eg/12120S/2012, IS/885, IS/1494, with 4 isolates was clustered in a new group. In conclusion, the present study provides further updates on the circulation and co-circulation of Infectious Bronchitis virus in commercial Egyptian flocks. The continuous existence of field variant Infectious Bronchitis virus in commercial chicken's farms in Egypt emphasizes the need for regular monitoring of Infectious Bronchitis with updating the control and vaccination strategies.

Keywords: Infectious bronchitis, Genetic characterization, Poultry, Prevalence, Epidemiology, Sequencing.

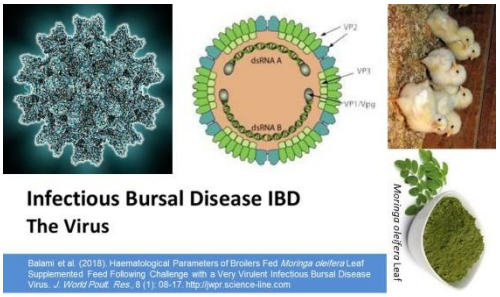
[Full text-PDF] [XML]

Research Paper

Haematological Parameters of Broilers Fed Moringa oleifera Leaf Supplemented Feed Following Challenge with a Very Virulent Infectious Bursal Disease Virus.

Balami AG, Ndahi JJ, Gadzama JJ, Enam SJ, Chiroma MA, Abdu PA, Wakawa MA, Aluwong T and Oladele SB.

J. World Poult. Res. 8(1): 08-17, 2018; pii: S2322455X1800002-8



ABSTRACT

A study was conducted to evaluate the haematological parameters of broilers fed *Moringa oleifera* leaf supplemented feed and had been challenged with a very virulent infectious bursal disease virus. Two hundred and forty day-old Ross 308 hybrid broiler chicks were assigned into four groups (A,B,C,D) of 60 chicks each in a deep litter type housing. While groups A and B were fed with formulated broiler starter and finisher containing 5% *Moringa oleifera* leaf, groups C and D were fed with formulated broiler starter and finisher without *Moringa oleifera* leaf. Groups A and C were vaccinated intramuscularly against infectious bursal disease (IBD) at 14 and 21 days of age, and Newcastle's disease at 18 days of age. Groups A, B and C were intraocularly challenged at 35 days of age with 0.05 ml of a live vv IBDV. Groups B and D served as positive and negative control respectively. Blood was collected from 10 broilers in each group via the wing vein at 35, 38 and 42 days of age for various haematological indices. A significant decrease in the values of lymphocytes counts in group B at 38 days of age was observed. Heterophil / lymphocyte ratio had significantly decreased ($P < 0.05$) in group D at 38 days of age. Packed cell volume significantly decreased ($P < 0.05$) at 38 days of age in groups A, B, C and D and subsequently increased ($P < 0.05$) by 42 days of age in groups B, C and D. Red blood cell count was significantly decreased ($P < 0.05$) in group B and C at 38 days of age, while haemoglobin concentration significantly increased ($P < 0.05$) at 42 days of age in group B and D. Feeding broilers with5% MOL supplemented diet without vaccination did not prevent vvIBDV from causing a decrease in lymphocyte count 3 dpi in broilers of group B.

Keywords: *Moringa oleifera* leaf, vvIBDV, PCV, Lymphocyte, Haemoglobin

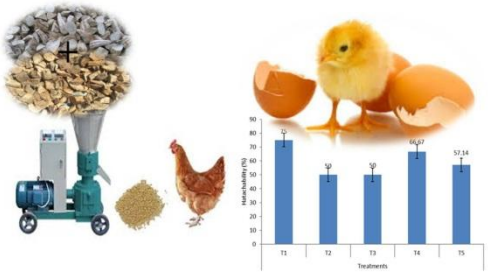
[Full text-PDF] [XML]

Research Paper

Reproductive Performances of a Cameroonian Dual-Purpose Local Chicken Strain Fed Pelleted Diets Containing Graded Levels of Cassava and Sweet Potato Meal as an Energy Substitute for Maize.

Keambou TC, Vukiesu TC, Toukala JP, Tedongmo AMY, Soares RJ, Lisita F, Hako TBA , Kana JR, Defang FH and Ndamunkong KN.

J. World Poult. Res. 8(1): 18-24, 2018; pii: S2322455X1800003-8



Keambou TC, Vukiesu TC, Toukala JP, Tedongmo AMY, Soares RJ, Lisita F, Hako TBA , Kana JR, Defang FH and Ndamunkong KN (2018). Reproductive Performances of a Cameroonian Dual-Purpose Local Chicken Strain Fed Pelleted Diets Containing Graded Levels of Cassava and Sweet Potato Meal as an Energy Substitute for Maize. *J. World Poult. Res.*, 8 (1): 18-24.

ABSTRACT

The continuous rising price of maize due to an increasing competition between humans and livestock requires palliative measures to sustain animal production. cassava-sweet potato meal combination can be used as a substitute for maize in feeding chicken. This study aimed at improving poultry productivity through the enhancement of the reproductive performances of Cameroon Kabir chickens fed pelleted diets of graded levels inclusion of cassava-sweet potato meal as an energy substitute for maize.315 Kabir chickens (270 hens and 45 rosters) of 23 weeks of age, were randomly allocated to five treatments T1, T2, T3, T4 and T5 with graded levels of cassava-sweet potato meal as energy substitute for maize, and eggs were collected for the evaluation of laying performances and characteristics. Fertility and hatchability were also evaluated across four successive batches of incubations. The eggs' weight was significantly ($P < 0.05$) different between treatments at weeks 2, 4, 5 and 12, highly significant ($P < 0.01$) at week 9, and very highly significant ($P < 0.001$) at week 6, 7, 8 and 10. The highest number of eggs laid, egg weight and mass were recorded in chicken receiving 25% (T2) replacement of maize with cassava and sweet potato meal, followed by T4 (75%), T5 (100%), T3 (50%) while T1, receiving control diet without cassava and sweet potato meal performed less for all the parameters. Generally, the trend of the feed conversion ratio was decreasing with increasing the inclusion level of cassava and sweet potato meal. The egg index showed significant differences in weeks 6 and 12, while week 2 showed high significant difference between the treatments. T2 (25%) recorded the highest fertility, while animals receiving control ration without maize substitution recorded the highest hatchability. In general, incorporation of 25% of fifty-fifty percent weight to weight of cassava and sweet potato meal can be recommended for reproduction in chicken without affecting neither the hatchability nor the physical characteristics of the eggs, though hatchability will require better attention.

Keywords: Reproduction, Local chicken, Cameroon, Cassava-sweet potato

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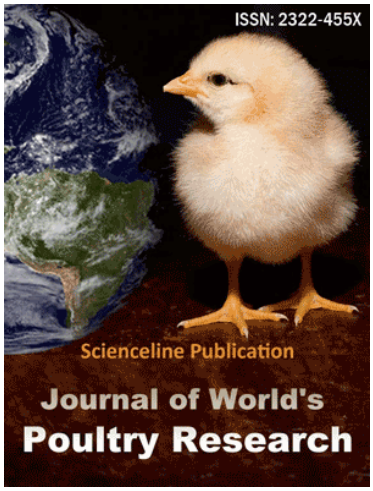

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Molecular and Genetic Characterization of Infectious Bronchitis Viruses Isolated from Commercial Chicken Flocks in Egypt between 2014 and 2016

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ABSTRACT

Infectious Bronchitis is one of the major viral diseases affecting poultry causing severe economic losses. The prevalence of Infectious Bronchitis viruses was studied in commercial chicken farms in Egypt between 2014 and 2016. A total of 1722 organ samples (trachea, kidney, caecal tonsils and lungs) were collected from 246 problematic flocks, showing respiratory signs and considerable mortalities, from 13 governorates throughout the examination period and were then subjected to molecular analysis using real-time reverse transcription-polymerase chain reaction. Data from this study have shown a high prevalence (75.6%) of Infectious Bronchitis virus in Egyptian farms. Infections mixed with other respiratory viruses were frequently observed, including very virulent Newcastle disease, Low pathogenic avian influenza, H9N2 and High pathogenic avian influenza H5N1 with 27.9%, 25.7% and 17.1%, respectively with higher detection percentages observed in the winter season. Phylogenetic analysis of 19 selected positive Infectious Bronchitis virus has revealed Infectious Bronchitis virus genotypes closely related to variant II strains Eg/12120S/2012, IS/885, IS/1494, with 4 isolates was clustered in a new group. In conclusion, the present study provides further updates on the circulation and co-circulation of Infectious Bronchitis virus in commercial Egyptian flocks. The continuous existence of field variant Infectious Bronchitis virus in commercial chicken's farms in Egypt emphasizes the need for regular monitoring of Infectious Bronchitis with updating the control and vaccination strategies.

Keywords: Infectious bronchitis, Genetic characterization, Poultry, Prevalence, Epidemiology, Sequencing.

INTRODUCTION

Infectious bronchitis (IB) is an acute, highly contagious disease of chickens caused by coronavirus infection with a major impact to the poultry industry worldwide (Al-Shekaili et al., 2015). While IB virus (IBV) infections in chicks are often manifested with respiratory signs and decrease in feed conversion, infections in mature and layer chickens commonly resulting in urogenital tract affection and sharp decrease in egg production (Cavanagh,

2007; Chen et al., 2010 and Butcher et al., 2011). Chickens and recently pheasants are well-known as main natural hosts for IBV (Ignjatovic and Sapats, 2000).

The morbidity rate in IBV-infected flocks, may reach as high as 100%, while the mortality rate often depends on the presence of secondary infections, flock age, immune status, management and other environmental factors. In young chickens, the mortality rate is typically 25-30% but it can reach 80% relying on the virulence of the IBV infecting strain and the presence of other complicating viral and bacterial agents. Even though all age groups of chicks are susceptible to IBV, young chicks are more susceptible and vulnerable to infections than older ones (Cavanagh and Gelb, 2008).

The coronavirus of domestic chickens is worldwide distributed (Cavanagh, 2007) while in Egypt, IB variants have been firstly reported since 1950s with an identification of variant IBV strain revealed to be closely related to the Dutch strain D3128 (Sheble et al., 1986 and Eid, 1998). The presence of multiple genotypes of IBV has been also reported in Egypt and strains related to the Israeli variants (IS/885 and IS/1494/06) as well as those related to the 793-B genotypes (4/91 and CR 88) have been identified in commercial poultry farms (Abdel-Moneim et al., 2012; El-Mahdy et al., 2010 and Selim et al., 2013). In Egypt, IB and its co-infections with endemic virulent viruses of Newcastle Disease (ND) and Avian Influenza (AI) continue to present a constant threat to the profitability of the poultry industry (ELbayoumi et al., 2013; Selim et al., 2013, Osman et al., 2015 and Kiss et al., 2016). Hence, the present investigation was carried out to provide an update on the epidemiological situation of IB infection in commercial chicken farms in Egypt through molecular analysis of the S1 gene.

MATERIALS AND METHODS

Sampling

In this study, a total of 1722 organ samples were collected from diseased commercial chicken flocks exhibiting respiratory manifestations with variable mortalities during March 2014 to February 2016. Tissue samples from trachea (1107 samples), kidney (178), caecal tonsils (369) and lungs (68) were collected from 246 chicken farms from 13 governorates in Egypt. Samples were transferred on ice to the laboratory and were kept frozen at -80°C until use. From each farm, up to 5 organ samples from each organ type were pooled and subjected to RNA extraction, cDNA synthesis and Real Time Polymerase Chain Reaction (RT-PCR).

RNA extraction

Total RNA for RT-PCR was extracted from 50-100 mg of liquid nitrogen-homogenized samples. The extraction of viral RNA was performed using a QiaAmp viral RNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. RNA was then kept frozen at -80°C until use.

Viral detection by RT-PCR

Real-time RT-PCR for IBV as well as Very Virulent ND (vvND), Highly Pathogenic Avian Influenza Virus (HPAIV) H5N1 and Low Pathogenic AIV (LPAIV) H9N2 was performed using Qiagen one step RT-PCR Kit (Qiagen, GmbH, Hilden, Germany) using strata gene thermal cycler according to the manufacturer's instructions. Target gene amplification, RT-PCR cycling conditions, probe and primer sequences were previously described (Callison et al., 2001; Wise et al., 2004; Slomoka et al., 2007; Ben Shabat et al., 2010).

Genetic characterization of IBV

The genetic analyses of the S1 gene of Egyptian IBV sequences used for comparison in this study were obtained from GenBank and were available for the national center for biotechnology Information Infectious bronchitis viruses resource (https://www.ncbi.nlm.nih.gov/). Sequence identities were calculated using DNA star software (Thompson et al.,1994) and the phylogenetic tree of the nucleotides sequence were constructed using Mega5 (Tamura et al., 2011) and as previously described by Selim et al. (2013).

Ethical approval

This research work did not involve the introduction of any intervention in/on birds, but direct collection of tissues and organs from freshly dead birds was carried out in strict accordance with the recommendations of institutional guidelines for the care and use of laboratory animals. Also, dead chickens were humanly handled.

RESULTS AND DISCUSSION

The present study provided further updates on the molecular characterization of avian infectious bronchitis viruses circulating during 2014-2016 with highlighting the other respiratory viruses which severely complicate infections in poultry farms in Egypt.

Clinicopathological picture of sampled chicken flocks

In this study and throughout the two years survey time, samples were collected from problematic flocks

exhibiting respiratory signs and considerable mortalities reaching in some infected flocks to more than 60%. Clinical manifestations include gasping, rales, respiratory sounds and peeping, ruffled feather, depression and whitish droppings. Post-mortem picture involved tracheal congestion and exudation, tracheal and bronchial casts, rhinitis, serositis (pericarditis, perihepatitis and air sacculitis), splenic and hepatic congestion, salpingitis (in laying chickens) and nephritis with deposition of the ureates in renal tissue and ureters. This is in line with previous literature which reported significant clinical outcomes and gross lesions following respiratory viral infections in chickens (El-Mahdy et al., 2010 and Hassan et al., 2017)

Prevalence of IBVs in commercial poultry farms

Here we report the prevalence of IBVs in commercial poultry farms in Egypt over two years of sampling ranging between 2014 and 2016. Data presented in this study has shown a very high detection rate of IBVs (75.6%) in chicken flocks in Egypt. As shown in Diagram 1, the detection rate of IBVs was highly influenced by sampling season. While a very low detection rate was observed during summer months, the high incidence rate of IBVs was observed during winter (reaching 100%) followed by autumn and spring. Increased detection of IBVs during cold weather could be impacted by many factors, including improper farm ventilation and litter management as well as inadequate biosecurity measures and natural air movements between farms (Abdel-Moneim et al., 2012).

Incidence of IBVs with other avian respiratory viruses

Prevalence of IBVs either alone or in combination with other respiratory viruses commonly complicating poultry farms was also determined (Diagram 2). Whilst the high prevalence of IBVs were seen in problematic Egyptian chicken's flocks, sole IBV infection represented 19.4% and, interestingly, the majority of cases was complicated with one of other avian viruses, including very virulent ND (27.9%), LPAI H9N2 (25.7%) and HPAI H5N1 (17.1%). Involvement of more than two viruses was also detected in a low rate compared double infection cases, although devastating losses were often reported. Previous literature has demonstrated that the prevalence of avian respiratory viruses in Egyptian broiler chicken flocks during 2012-2014, with increased incidence of IBV and H9N2 mixed infections, representing 41.7% (Hassan et al., 2016). In the present study, the incidence of IBV-H9N2 mixed infections was 25.7% during 2014-2016. This reduction in IBV-H9N2 mixed infections could be influenced by the increased coverage of inactivated H9N2 vaccines in different sectors of poultry production. However, clinical observations and mortality rates have significantly increased during the last few years with IBV mixed infections. Indeed, a recent study has shown that experimental co-infection of IBV and LPAI H9N2 resulted in sever clinical outcome and mortality with an increase in the H9N2 shedding from infected chickens (Hassan et al., 2017).

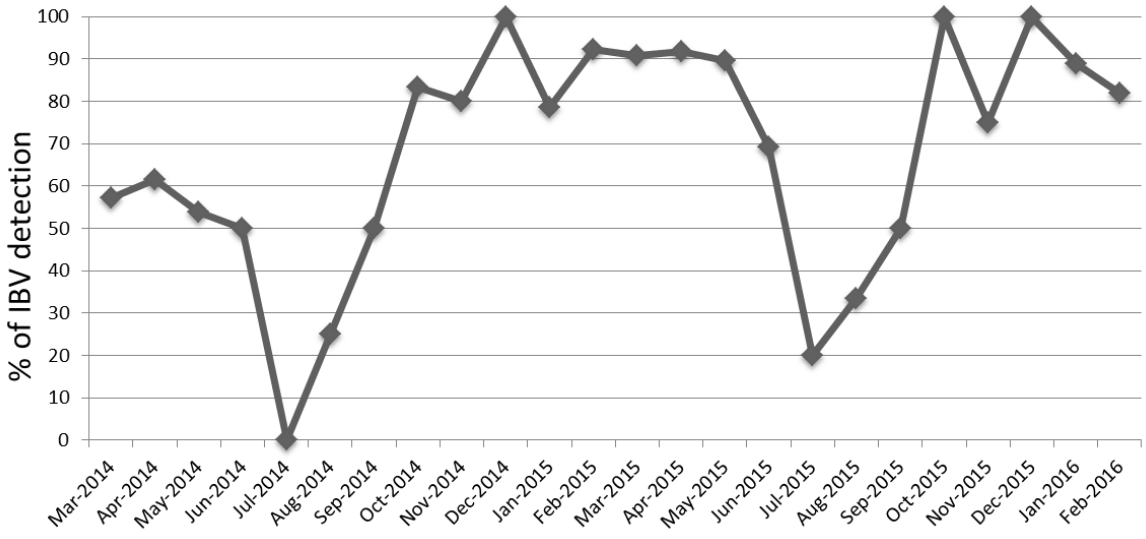

Diagram 1. Prevalence of infectious bronchitis in commercial chicken farms in Egypt during 2014 -2016

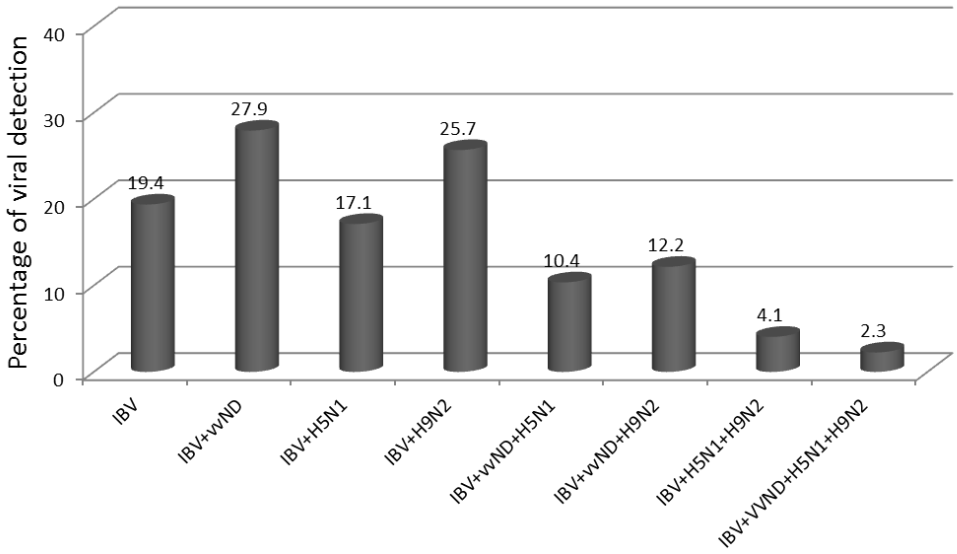

Diagram 2. Incidence of IBV detection with other avian respiratory viruses in commercial chicken flocks during 2014-2016**.** Where IBV is Infectious Bronchitis virus, vvND is very virulent Newcastle Disease, H5N1 is highly pathogenic Avian Influenza H5N1 and H9N2 is low pathogenic Avian Influenza H9N2

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                                10        20        30        40        50
                    ....|....|....|....|....|....|....|....|....|....|...
variant-2           FVYKQSDFMYGSYHPKCNFRPETINNGLWFNSLSVSLAYGPLQGGCKQSVFNNRATCX
6/82                .I..A..Y.......S.K..L.................G...I.........A......
UK/123/82           .I..E..Y.......S.K..L.......X..P.....G...I.........E......
strain D207         .I..A..Y.......S.K.KL.................G...I.........A......
isolate J2          .R.VE.Y...........S..L.................G.................M....
CK/CH/LDL/98I       .R.VE.............S..L.................G.................M....
Q1                  .R.VE.............S..L.................G.................M....
JF24                ....E.N.................N.............I.....H........KG.....
H120                ....E.N........S....L.................I..............SG.....
IBV-QXIBV           ....A..........S.S.......S...........T..............SGK....
IBV-M41             ....E.N........S....L.................I..............SG.....
IBV-CR88121         ....P..........H.K....N.............T...I.........S......
IB-VAR2-06          ...................D.....................................S......
IS/1494/06          ...................D.....................................S......
Eg/CLEVB-2/IBV/012  ...................D.....................................S......
Israel/720/99       ....A..............D.......................P..............
IS/885              ....A..............D.....................................
Eg/12120s/2012      ....P..........Q.D.....................................
Eg/12197B/2012      ....P..........Q.D.....................................
Eg/1265B/2012       ....P..........Q.D.....................................
CV-28-23            ....P..........Q.D..Q.............F.....................
CV-28-2T            ---------------XSD.S..........KNI....E......DG..CM.SHS....
CV-28-13K           ---------------XRD...........CIKT....HQ.K....SEEHCMNS.S....
CV-28-14            ---------------XSD.S..PL........I.......R.....G.......K....
CV-28-22            ---------------XRD.S..SL......QN...IHQ.R.....GE..MYKHS....
CV-28-35            --------X......Q.D.....................................S......
CV-28-31            ....P..............S.....................T..............S.K....
CV28-28             ....P..........Q.D.....................................S......
CV-28-38            ....P..........Q.D.....................................S......
CV-28-41            ....P..........Q.D.....................................S......
CV-28-26            ....P..........Q.D.....................................S......
CV-28-48            ------------H..QSD.....................................
CV-28-4             ....P..............S.....................T..............S.K....
CV-28-7             ....P..........Q.D..Q..................................S......
CV-28-10            ....P..........Q.D..Q..................................
CV-28-2K            ....P..............S..T.....................T.................K....
CV-28-4K            ....P..............S.....................T..............S.K....
CV-28-17            ....P..........Q.D.....................................
CV-28-18            ....P..........Q.D.....................................
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Diagram 3. Amino acid alignment of partial S1 gene from 19 selected IBVs with other previously reported and reference Infectious Bronchitis Virus

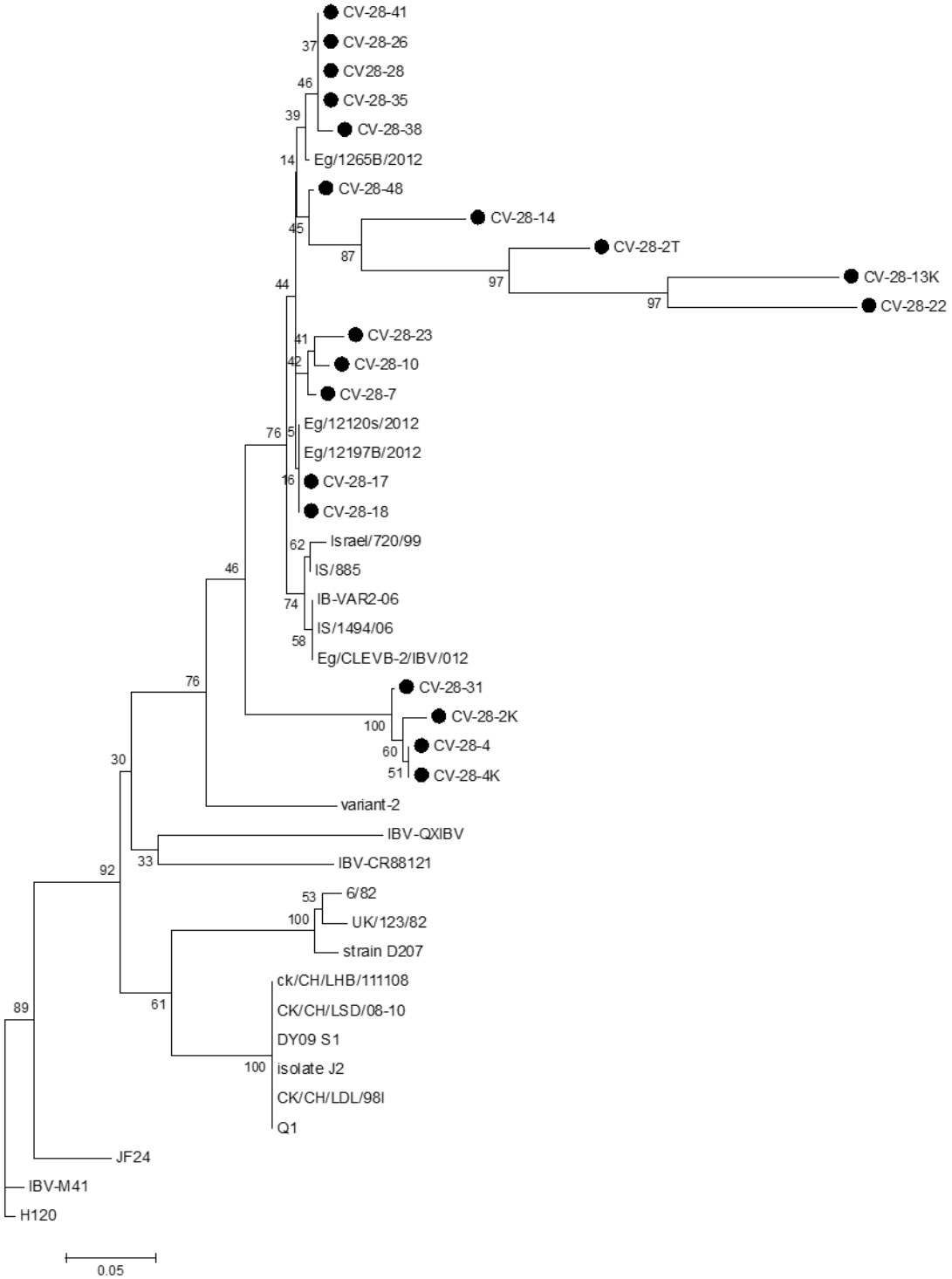

Diagram 4. Phylogenetic tree of 19 selected Infectious Bronchitis Virus generated from S1 genes of Egyptian IBVs detected during 2014-2016, the previously reported IBVs and other selected reference IBVs

S1 sequencing and phylogenetic analysis

Selected IBV positive samples were further examined for genetic identification. Amino acid alignment of partial S1 gene from 19 samples with other previously detected and reference IBV viruses For IBV is seen in Diagram 3. The obtained results have shown that all the 19

IBVs were genotyped as a variant type closely related to variant II-like strain. These viruses were then subdivided into two main groups; 15 viruses closely related to Eg/12120S/2012, IS/885 and IS/1494 like strains, while 4 viruses (CV-2K, CV-4, CV-4K and CV-31) were clustered separately in another group as seen in Diagram 4. Previous investigations have also shown the prevalence of variant IBVs closely related to Israeli variants (IS/1494/06 and IS/885/00) and original Egyptian variant strain (Egypt/Beni-Suif/01) (Abdel-Moneim et al., 2012; Selim et al., 2013; Zanaty et al., 2016). The uncontrolled field circulation of various IBV variants in chickens is highly relevant from the epidemiological point of view since possibility of emergence of new variants is highly expected. Indeed, recent study of genome sequencing of IBV has shown the emergence of a new QX-like strain in Sudan from ancestor ITA/90254/2005 genotype (Naguib et al., 2016). In this study, no evidence of detection of other IBV variants in the tested positive samples although previous investigators have recorded the presence of Q1-like IBV strain in Egyptian poultry during 2012-2013 (Abdel-Sabour et al., 2017).

CONCLUSION

The present paper provides an update about the molecular epidemiology of IBV circulating, and challenging, commercial poultry flocks in Egypt. The presented data have shown genetic makeup of field IBV is closely related to variant II strains. These variants have been detected from problematic flocks with severely impacted performance parameters, pointing out the significant role of complicating viral pathogens, mainly vvND and LPAIV H9N2. With no doubt, enhancing biosecurity measures, regular IBV monitoring and updating the vaccination schemes are crucial components to control IBV infection in commercial chickens in Egypt, and perhaps elsewhere in the region.

DECLARATIONS

Acknowledgments

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Competing interests

The authors declare that there is no conflict of interest.

Consent to publish

All persons gave their informed consent prior to their inclusion in the study.

Author`s contributions

All authors participated in making the design, support with sampling and surveillance work, interpretation of results and writing the paper.

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Haematological Parameters of Broilers Fed *Moringa oleifera* Leaf Supplemented Feed Following Challenge with a Very Virulent Infectious Bursal Disease Virus

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ABSTRACT

A study was conducted to evaluate the haematological parameters of broilers fed *Moringa oleifera* leaf supplemented feed and had been challenged with a very virulent infectious bursal disease virus. Two hundred and forty day-old Ross 308 hybrid broiler chicks were assigned into four groups (A,B,C,D) of 60 chicks each in a deep litter type housing. While groups A and B were fed with formulated broiler starter and finisher containing 5% *Moringa oleifera* leaf, groups C and D were fed with formulated broiler starter and finisher without *Moringa oleifera* leaf. Groups A and C were vaccinated intramuscularly against infectious bursal disease (IBD) at 14 and 21 days of age, and Newcastle's disease at 18 days of age. Groups A, B and C were intraocularly challenged at 35 days of age with 0.05 ml of a live vv IBDV. Groups B and D served as positive and negative control respectively. Blood was collected from 10 broilers in each group via the wing vein at 35, 38 and 42 days of age for various haematological indices. A significant decrease in the values of lymphocytes counts in group B at 38 days of age was observed. Heterophil/lymphocyte ratio had significantly decreased ($P<0.05$) in group D at 38 days of age. Packed cell volume significantly decreased ($P<0.05$) at 38 days of age in groups A, B, C and D and subsequently increased ($P<0.05$) by 42 days of age in groups B, C and D. Red blood cell count was significantly decreased ($P<0.05$) in group B and C at 38 days of age, while haemoglobin concentration significantly increased ($P<0.05$) at 42 days of age in group B and D. Feeding broilers with5% MOL supplemented diet without vaccination did not prevent vvIBDV from causing a decrease in lymphocyte count 3 dpi in broilers of group B.

Keywords: *Moringa oleifera* leaf, vvIBDV, PCV, Lymphocyte, Haemoglobin

INTRODUCTION

Haematology has been defined as the study of blood. It is an important part of clinical pathology as well as diagnostic process. Haematological analysis has been widely used as indices in assessing the health status of an animal (Graczyk et al., 2003; Uchegbu et al., 2010) and the resultant changes are important in assessing the response of animal to various pathological, nutritional and physiological situations (Akpodiete and Ologhobo 1998; Khan and Zafar 2005; Owen et al., 2009).

Poultry diseases such as infectious bursal diseases (Panigraphyet al., 1986; Juranova et al., 2001), avian

coccidiosis (Koinarski et al., 2001), Newcastle disease (ND) (Galindo-Muniz et al., 2001; Oladele, et al., 2012), fowl typhoid (Kokosharov and Todorova 1987; Barde et al., 2015) and Mycoplasmosis (Branton et al., 1997; Burnham et al., 2003; Ahmed et al., 2015) greatly influence haematological parameters of avian species.

Differential leucocytes are used as indicators of stress response and are sensitive biomarkers that are vital for immune functions. Bacterial and viral diseases affect the total number of white corpuscles thus; the proportion among the various types of white corpuscles and the percentages of the different types of white corpuscles in healthy birds, have therefore been modified in sick birds (Wendy and Jean-Marc 1992; Post et al., 2007; Muhammad and Oloyede, 2009; Barde, et al., 2015). Infectious bursal disease virus has been reported to cause alterations in different haematological parameters of poultry (Zeryehun, et al., 2012). In birds, clinical signs of illness are often delicate; therefore, clinical chemistry is essential to assess cellular changes (Ritchie, et al., 1994).

Moringa oleifera leaf (MOL) in the diets of broilers has been shown to have a significant effect on the blood parameters of broilers (Oghenebrorhie and Oghenesuvwe, 2016), and Makanjuala et al. (2014) had earlier observed a numerical increase in the values of Parked cell volume (PCV), Red blood cell (RBC) and haemoglobin (HB) of broilers fed with MOL supplemented diets. The absences of hematological values of broilers fed *Moringa oleifera* leaf (MOL) supplemented diet and then challenged them with vvIBDV demand this study. Therefore, this study was aimed at assessing the effect of dietary MOL feed supplement on haematological parameters of broilers challenged with a very virulent infectious bursal disease virus (vvIBDV).

MATERIALS AND METHODS

Study location

The study was conducted at the Poultry Research Unit of the Faculty of Veterinary Medicine, Ahmadu Bello UniversitySamaru, Zaria, Kaduna state, Nigeria.

Ethical approval

Approval this research was sorted from the ethics committee of the Ahmadu Bello University, Zaria and guidelines for the care and humane handling of animals were strictly followed throughout the study (FASS, 2010).

Collection and processing of *Moringa oleifera* leaf

Moringaoleifera leaf was harvested during the early flowering stage of the plant from orchards in Potiskum, Yobe State. The stem together with the branches of the Moringa trees were cut down and spread out under shade for it to dry at room temperature for 5 days. The MOL were then manually separated from the stem and branches by hand and grounded into powder using a milling machine.

Mineral analysis

The analysis of the mineral content of MOL was carried out based on the procedure of AOAC (1990) to determine minerals such as calcium, phosphorus, magnesium, iron, sodium, zinc, copper, selenium, potassium, and manganese components of the leaf.

Phytochemical analysis

Qualitative and quantitative analysis of MOL was done, based on the method described by Sofowora (1993), to ascertain the presence of tannins, phytates, saponins, oxalates, cyanides, alkaloids, carbohydrates, flavonoids, steroids, terpenoids, phenols and phylobatanins.

Proximate analysis

The method described by the AOAC (1990) for the proximate analysis of the MOL was used to verify the proportion of carbohydrates, crude protein, fats, fibre, ash, moisture and metabolizable energy.

Feed formulation and analysis

Following shade drying of MOL, it was milled with a hammer mill and sieved with 3 mm mesh to obtain *Moringaoleifera* leaf meal. A 22% and 20% crude protein broiler starter and broiler finisher mash respectively were formulated [with 5% MOL (Olugbemi, et al., 2010) forming part of the feeds ingredients for group A and B] using Pearson square and milled in a toll mill in Zaria. The feed was subjected to analysis based on the method described by the AOAC (1990) in the Feed analysis laboratory of the Department of Animal Science, Ahmadu Bello University Zaria, to determine the level of metabolizable energy, crude protein, crude fibre, moisture, ash content, and dry matter.

Experimental chicks and housing

A total of 240 day-old Ross 308 hybrid broiler chicks were obtained from a reputable commercial hatchery located in Yola, Nigeria. The chicks were brooded in a conventional open-sided house which was properly disinfected before the arrival of the chicks (deep litter system of management with wood shavings as litter material, feeders and drinkers were provided) with cyclic temperatures. The chicks were individually weighed with

a weighing balance and assigned in a completely randomised design into four different groups A, B, C and D of 60 chicks each (each pen has a floor space of 3×4 m). A 100-watt bulb was provided in each of the compartment to supply light and heat during brooding.

Experimental design

Groups A and B were fed with broiler starter and finisher diets each containing 5% MOL, while groups C and D were fed with broiler starter and finisher feed without MOL. Groups A, B and C were challenged at 35 days of age with a vvIBDV. While group B served as the positive control, group D served as the negative control. All the groups were fed for 49 days (7 weeks) with feed and water provided *ad libitum*.

Vaccines and vaccination

Inactivated vaccines against IBD (Virsin 122, oil emulsion, Biovac limited, Isreal, Batch 1- 382222) and inactivated vaccines against ND (oil emulsion Komorov strain, Biovac limited, Isreal, Batch 1- 422222) were obtained from a reputable Veterinary Pharmaceutical supplier in Jos, Nigeria. Broilers in groups A and C were vaccinated on the thigh muscles intramuscularly with 0.5 ml of killed IBD vaccine on 14 and 21 days of age respectively, while vaccination against ND was done with a killed ND vaccine (0.5 ml) on the thigh muscles intramuscularly on 18 days of age.

Challenge infectious bursal disease virus

At 35 days of age, all the broilers in groups A, B and C were challenged intraocularly with 0.05 ml of a live vvIBD virus. The IBD virus used for the challenge was a field strain of vvIBDV obtained from previously vaccinated layers that died of natural outbreak of IBD. Sixty five per cent of commercial cockerels were inoculated at 30 days of age with 50 µl of bursal suspension (v/w) in phosphate buffer saline (PBS) (pH 7.4) died. One millilitre of bursal suspension (v/w) in PBS (pH 7.4) contained 10^{-976} CID_{50} of IBDV.

Collection and processing of blood

Blood samples of the broilers were collected when the chicks were 35, 38 and 42 days of age from all the groups for haematological assay. On each blood collection day, 10 broilers from each group that had been previously randomly selected and marked were bled via the brachial vein using a 25-gauge sterile needle on a plastic disposable 5 ml syringe. Two ml of blood were collected after the birds were properly restrained by an assistant and then emptied into a commercially available sample bottle containing ethylene diamine tetra acetic acid for haematology. Prior to this, the area around the brachial vein was swabbed with 70% methanol to obtain optimal disinfection and to allow for easy access to the vein and for the collection of blood. Each of the sample bottles were properly labelled using a permanent marker. Direct contact with blood was avoided by the use of hand gloves and laboratory coat.

Determination of packed cell volume

The Packed Cell Volume (PCV) was determined using a standard technique as described by Rehmanet al. (2003). Non-heparinized capillary tubes were filled up to about ¾ of its length from one end and the second end was heat-sealed using Bunsen burner. The blood in the sealed capillary tubes was then centrifuged for 5 minutes at 4,383 x *g* using the Saitexiangyi TG12MX® Microhaematocrit centrifuge machine. Then the proportion of cells in the total volume of blood was measured and recorded as a percentage using the Hawksley® Micro-haematocrit reader.

Estimation of haemoglobin concentration

Haemoglobin concentration was assayed colorimetrically as cyanmethemoglobin (Drabkin, 1945). Five ml of hemiglobincyanide (HICN) (Drabkin) solution were measured using a 5 ml syringe into plastic test tubes. Twenty µl of blood was measured using a micropipette and was added to the Drabkin solution in the test tube and properly mixed by gently shaking the test tube. It was centrifuged at 1,509 × *g* for 15 minutes to separate the empty Red Blood Cell (RBC) from interfering with the reading. The supernatant was separated into a sample bottle. The supernatant was absorbed into the haemoglobin meter (XF–C, China). After the wiggling pump stops working, the value displayed on the screen was recorded in g/dl as the haemoglobin concentration.

Determination of red blood cell and total white blood cell count

RBC and Total White Blood Cell (TWBC) counts were determined with the Natt-Herrick solution (1:200 dilution) and the improved Neubauer haemocytometer (Campbell and Ellis, 2007) as both counts can be prepared directly from the same sample placed in the haemocytometer.

The heparinised blood samples were slightly agitated and the RBC diluting pipette was used to pipette the blood to the 0.5 marking. The tip of the pipette was cleaned properly using a tissue paper without touching the distal opening of the pipette tip with tissue, as this will causea

capillary shift of blood into the tissue. The diluting solution (Natt-Herrick) was also pipette to the 101 marking (1:200) without entirely immersing the pipette tip into the diluting fluid. The mixture was well shaken for 1 minute to obtain equal distribution then emptied into a clean sample bottle. The Neubauer haemocytometer and cover slip were cleaned using a dry, lint free cloth. The cover slip was properly placed on the haemocytometer.

The mixture of Natt-Herrick solution and the blood sample was then agitated a little and a capillary tube was used to withdraw a small aliquot. Both sides of the haemocytometer were filled up (charged) by gently touching the intersection between the cover slip and haemocytometer with the loaded capillary tube avoiding air bubbles and under-filling or over-filling, and then left for 5 minutes for the cells to settle down. The light microscope (Olympus-XSZ-107BN), at low power magnification (×40) was used to view the cells and counting was done using the tally counter.

For TWBC count, the WBC in the four outer large squares of the haemocytometer were counted and calculated using the formula:

$N/20 = WBC \times 10^9$ /l

Where N = Number of WBC counted in the four outer large squares (or in 64 small squares).

For RBC count, the cells contained in the four corners and central squares in the mid-section of the haemocytometer were counted. Following the "L" rule: cells that touch the centre triple lines of the ruling on the left and the bottom sides were counted but the cells that touch the centre triple lines of the ruling on the right and the top sides were not counted. The RBC count was calculated using the formula:

$N/100 = RBC \times 10^{12}$ /l

Where N = Number of RBC was counted in the five squares in the mid-section of the haemocytometer (or in 160 squares). Note that both charged sides of the haemocytometer were counted for both the RBC and TWBC and the average calculated.

Preparation of smears for differential leucocyte count and thrombocytes estimation

From the blood sample collected in all the birds, a pair of smears for each blood sample was made. A small drop of blood was immediately used for the preparation of blood smears each using the standard slide-to-slide technique. The air-dried smears were properly labelled using a pencil on the frosted end of the slide and then fixed in a fixing jar containing 70% methanol for 3 minutes and air-dried.

Staining was done by flooding the smears with Wright-Giemsa stain for 3 minutes. An equal amount of Sorensen's buffer (pH of 6.8) was added then mixed gently by blowing using a pipette until green metallic sheen forms on the surface. The smear was allowed to stand for further 6 minutes. The smears were rinsed with the Sorensen's buffer and allowed to stand for a minute for differentiation. The stained slides were then washed copiously with the Sorensen's buffer and the back of the smears were wiped with tissue paper to remove the excess stain and allowed to air dry. The slides were then neatly packed into a slide box until viewing.

Examination of the stained blood smears for differential leucocytes count was done using a light microscope (Olympus-XSZ-107BN) under high-power magnification with oil immersion (×1, 000). One hundred WBC were counted and classified based on their morphologic features (Campbell 1988; Hawkey and Dennet 1989; Campbell and Ellis, 2007). The counting was done using the Marble® Blood Cell Calculator. The differential WBC count was then expressed as a percentage of the individual cell group. The percentage of each cell was then converted into absolute numbers by reference to the total WBC using the formula:

$$\frac{\text{Percentage of WBC counted} \times \text{TWBC}}{100} = \text{Absolute Number} \times 10^9 \text{ /l}$$

An estimated thrombocyte count was obtained from the stained blood film using the same formula for the indirect estimation of total WBC (Campbell, and Ellis, 2007). Valid and reliable results were not obtained where there was evidence of thrombocytes clumping. The absolute number of thrombocytes was estimated by using the formula:

$$\frac{\text{Number of thrombocytes counted}}{100} \times \text{TWBC} = \text{Absolute Thrombocytes} \times 10^9 \text{ /l}$$

Data analyses

The results of the haematological values were expressed as means ± standard deviation. They were further subjected to repeated measure one way analysis of variance (ANOVA) followed by post-hocDunnett's control test for multiple comparison. Analyses are considered significant at $p < 0.05$ using Statistical Package for Social Science (IBM SPSS version 20) for windows.

RESULTS

Haematology results had shown significant decrease in the values of PCV in group A at 38 ($P<0.05$) and 42 ($P<0.05$) days of age. The values of PCV were also

significantly decreased in group B (P<0.05), C (P<0.05) and D (P<0.05) at 38 days of age, but had significantly increased (P<0.05) by 42 days of age in group B (Table 1). Haemoglobin concentration significantly increased in group B (P<0.05) and group C (P<0.05) at 38 days of age and decreases significantly (P<0.05) at 38 days of age in group D (Table 2). The value of RBC significantly (P<0.05) decreased at 38 days of age and increased significantly (P<0.05) at 42 days of age in group B (Table 3), while TWBC count was observed to significantly increase (P<0.05) between groups A and C, and B and C at 42 days of age (Figure 1).

The result of this study also showed a significant increase (P<0.05) in the values of eosinophils count among broilers in group B at 42 days of age, but no significant increase (P>0.05) was however observed among broilers in groups A, C and D at 35, 38 and 42 days of age (Table 4). A significant decrease (P<0.05) was observed in lymphocyte count at 38 days of age in group B (Table 5). The values of heterophil/lymphocyte (H/L) ratio had significantly (P<0.05) decrease at 38 days of age and a significant (P<0.05) increase was observed between 35 and 42 days of age in group D (Table 6).

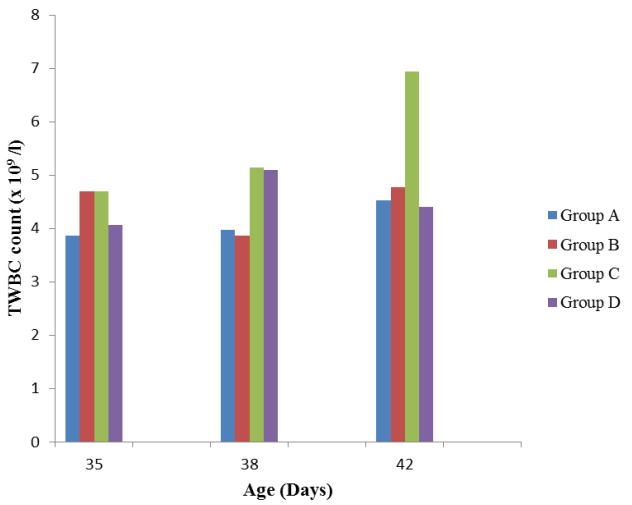

Figure 1. Mean (± SD) total white blood cell count of broilers fed 5% *Moringa oleifera* leaf feed supplement (A and B), vaccinated (A and C) with killed infectious bursal disease vaccine at 14 and 21 days old and challenged (A, B and C) at 35 days old with very virulent infectious bursal disease virus

Table 1. Packed cell volume of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups / Age in days	A n = 10	B n = 10	C n = 10	D n = 10
	Mean (± SD) packed cell volume (%)			
35	28.60 ± 1.77^{a}	26.30 ± 1.94^{a}	25.22 ± 2.28^{a}	25.50 ± 3.54^{a}
38	21.80 ± 3.46^{b}	21.10 ± 1.91^{b}	21.44 ± 1.33^{b}	23.30 ± 3.06^{a}
42	19.90 ± 2.73^{b}	23.80 ± 3.01^{c}	22.67 ± 1.87^{b}	26.80 ± 2.04^{c}
F statistics	25.528	12.585	8.952	3.028
P value	0.0000	0.001	0.003	0.079

n = total number of birds sampled, Mean (± SD) = standard deviation of the mean; Means having different superscripts alphabet on the same column differ significantly P<0.05

Table 2. Haemoglobin concentration of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups / Age in days	A n = 10	B n = 10	C n = 10	D n = 10
	Mean (± SD) haemoglobin concentration (g/dl)			
35	10.55 ± 2.21	10.01 ± 1.77^{ac}	9.81 ± 0.97^{a}	13.38 ± 1.47^{a}
38	10.78 ± 1.95	10.32 ± 0.93^{b}	11.39 ± 1.17^{b}	11.14 ± 1.47^{a}
42	10.77 ± 1.50	12.06 ± 2.04^{ac}	11.77 ± 2.00^{b}	13.38 ± 1.47^{c}
F statistics	0.053	5.499	4.280	8.924
P value	0.940	0.015	0.040	0.015

n = total number of birds sampled, Mean (± SD) = standard deviation of the mean; Means having different superscripts alphabets on the same column differ significantly P<0.05

Table 3. Red blood cell count of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups / Age in days	A n = 10	B n = 10	C n = 10	D n = 10
	Mean (± SD) red blood cell count (× 10^{12}/L)			
35	2.44 ± 0.44	2.49 ± 0.34[ac]	2.25 ± 0.26	2.44 ± 0.63
38	2.01 ± 0.42	1.78 ± 0.35[b]	1.79 ± 0.52	2.19 ± 0.82
42	2.04 ± 0.49	2.19 ± 0.51[ac]	2.09 ± 0.29	2.10 ± 0.51
F statistics	2.210	8.011	3.399	0.542
P value	0.140	0.006	0.077	0.572

n = total number of birds sampled, Mean (± SD) = standard deviation of the mean; Means having different superscripts alphabets on the same column differ significantly P<0.05

Table 4. Eosinophil count of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups / Age in days	A n = 10	B n = 10	C n = 10	D n = 10
	Mean (± SD) eosinophil count ×10^9 /L			
35	0.05±0.06	0.01 ±0.02[a]	0.15± 0.17	0.16 ±0.21
38	0.10±0.12	0.03±0.03[a]	0.19± 0.24	0.12± 0.12
42	0.12± 0.12	0.05 ±0.05[b]	0.13±0.11	0.09 ±0.12
F statistic	4.205	7.25	0.389	0.570
P value	6.07	0.025	0.618	0.556

n = total number of birds sampled, Mean (± SD) = standard deviation of the mean; Means having different superscripts alphabets on the same column differ significantly P<0.05

Table 5. Lymphocyte count of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups / Age in days	A n = 10	B n = 10	C n = 10	D n = 10
	Mean (± SD) lymphocyte count (x 10^9 /L)			
35	3.2 ± 0.26	3.87 ± 1.52[a]	3.5 ± 0.34	2.2 ± 0.44
38	3.1 ± 0.32	2.67 ± 1.26[b]	4.1 ± 0.40	4.0 ± 0.60
42	3.6 ± 0.62	3.77 ± 0.82[a]	4.7 ± 0.70	3.3 ± 0.51
F statistic	0.335	1.338	1.165	2.160
P value	0.653	0.023	0.328	0.153

n = total number of birds sampled, Mean (± SD) = standard deviation of the mean; Means having different superscripts alphabets on the same column differ significantly P<0.05

Table 6. Heterophil/lymphocyte ratio of broilers fed 5% *Moringaoleifera* leaf supplemented feed

Groups / Age in days	A n = 10	B n = 10	C n = 10	D n = 10
	Mean (± SD) heterophil/lymphocyte ratio			
35	0.15±0.05	0.13±0.06	0.19±0.07	0.68± 0.42[a]
38	0.22±0.20	0.18±0.10	0.14±0.06	0.17±0.07[b]
42	0.22±0.10	0.19±0.09	0.20±0.12	0.22±0.11[b]
F statistic	0.877	1.511	2.442	11.568
P value	0.387	0.251	0.118	0.007

n = total number of birds sampled, Mean (± SD) = standard deviation of the mean; Means having different superscripts alphabets on the same column differ significantly P<0.05

DISCUSSION

The significant decrease in PCV and RBC observed in groups A, B and C 3 dpi in the current study could be due to anaemia resulting from haemorrhage that is usually associated with IBD infection. The findings in the present study are in accord with those of Skeeles et al. (1980) and Moss (1999). Additionally, this finding also agrees with the works of Panigraphyet al. (1986) and Kassim, (2014) who in their separate studies reported a significant reduction in the values of PCV and RBC 5 dpi with vvIBDV, when a 4 weeks old broilers and cockerels were challenged respectively. But it is however, contrary to the findings of Oladeleet al. (2005) who reported an increase in PCV of broilers challenged with vvIBDV at 32 days of age. The significant increase in PCV at 7 dpiob served in group B could suggest that minerals such as iron that were found in high quantity in the MOL used for supplementing the feed fed to broilers in group B may have possibly helped in increasing their PCV. Significant increase in the values of PCV was also reported in broilers fed MOL supplemented diet (though not challenged with vvIBDV) (Tijjani et al., 2015; Emmanuel, 2016; Oghenebrorhie and Oghenesuvwe, 2016).

The increase in the concentration of haemoglobin observed in groups B and C could indicate polycythaemia that could be due to dehydration, which is a characteristic finding in IBD (Gross, 1989). It could also imply that the 5% MOL supplemented diet and the feeds without MOL were rich in iron which is responsible for the synthesis of haemoglobin. Hassan et al. (2016) also reported an increase in the values of Hb concentration when MOL was supplemented in the diet of broiler. The increase in TWBC count observed in group C could be a response to subclinical bacterial infections such as *E. coli.* Infectious bursal disease has been reported to increase the susceptibility to various bacterial infections (Niki, 1996; Shane, 1997). The significant increase observed in the values of eosinophils in group B was probably as a result of an increase from an initial absence of eosinophils at 35 days of age (pre infection with vvIBDV).

The significant decrease observed in the values of lymphocyte in group B, showed a marked lymphopenia, 3 dpi with vvIBDV. It is well known that viral infections in birds are associated with lymphopenia (Jain, 1986). This is because IBDV causes the destruction of B-lymphocytes within the bursa of Fabricius before their migration into the blood stream, thus causing the reduction in the number of lymphocytes in the blood (Weiss and Kaufer-Weiss, 1994). After infection with IBDV, an increase in the percentage of lymphocyte without lysosomes and a decrease in lymphocytes with large single and large multiple lysosomes have been reported 3 dpi (Klucinski, et al., 1984). The finding of this work agrees with that of Oladele et al. (2005) and Kassim, (2014) who separately reported lymphopenia 6, 12, and 48 hours, and a subsequent, increase in lymphocyte counts between 120 and 144 hours post infection with vvIBDV in 4 weeks old broilers and cockerels respectively. The result of this study implies that despite feeding broilers with 5% MOL supplemented feed; the vvIBDV was able to cause destruction of B-lymphocytes.

Increase H/L ratio has been used as an important indicator of stress in birds (Gross and Siegel, 1983). Stress in birds which may vary from food or water deprivation, temperature extremes, constant light or diseases usually elevates the number of heterophils and depresses the number of lymphocytes (Gross, 1989; Mcfarlanje, and Curtis, 1989). The highly significant increase in H/L ratio observed in broilers of group D may not necessarily be due to the above mentioned stressors, because broilers used for this study were not deliberately disturbed in anyway. However, it was observed that broilers in group D had more number of males than females, though not deliberately apportioned. This high number of males could probably be the reason for the higher H/L ratio observed. This finding is in agreement with the findings of Al-Murraniet al. (1997) where he reported that male (cock) broilers had a higher H/L ratio when compared to female (Hen) broilers and suggested that, the additional stress of higher body weight in males must have attributed to the increase in the H/L ratio.

CONCLUSION

It was concluded that, 5% MOL supplemented diet without vaccination did not prevent vvIBDV from causing a decrease in lymphocyte count 3 dpi in broilers of group B. Supplementation of broiler feed with 5% MOL and vaccination with a killed IBD vaccine did not prevent a decrease in PCV and RBC, but cause an increase in Hb concentration3dpi with vvIBDV in groups A and B, respectively.5% MOL supplemented diet with or without vaccination did not cause an increase in the heterophil to lymphocyte ratio in groups A and B.

DECLARATIONS

Author's contribution

All authors participated in making the design, performing the experiment, analyses of the data, and writing of the manuscript.

Consent to publish

All the authors have consented to the publication of this paper.

Competing interest

The authors declare that they have no competing interests.

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Reproductive Performances of a Cameroonian Dual-Purpose Local Chicken Strain Fed Pelleted Diets Containing Graded Levels of Cassava and Sweet Potato Meal as an Energy Substitute for Maize

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ABSTRACT

The continuous rising price of maize due to an increasing competition between humans and livestock requires palliative measures to sustain animal production. cassava-sweet potato meal combination can be used as a substitute for maize in feeding chicken. This study aimed at improving poultry productivity through the enhancement of the reproductive performances of Cameroon Kabir chickens fed pelleted diets of graded levels inclusion of cassava-sweet potato meal as an energy substitute for maize.315 Kabir chickens (270 hens and 45 rosters) of 23 weeks of age, were randomly allocated to five treatments T1, T2, T3, T4 and T5 with graded levels of cassava-sweet potato meal as energy substitute for maize, and eggs were collected for the evaluation of laying performances and characteristics. Fertility and hatchability were also evaluated across four successive batches of incubations. The eggs' weight was significantly ($P<0.05$) different between treatments at weeks 2, 4, 5 and 12, highly significant ($P<0.01$) at week 9, and very highly significant ($P<0.001$) at week 6, 7, 8 and 10. The highest number of eggs laid, egg weight and mass were recorded in chicken receiving 25% (T2) replacement of maize with cassava and sweet potato meal, followed by T4 (75%), T5 (100%), T3 (50%) while T1, receiving control diet without cassava and sweet potato meal performed less for all the parameters. Generally, the trend of the feed conversion ratio was decreasing with increasing the inclusion level of cassava and sweet potato meal. The egg index showed significant differences in weeks 6 and 12, while week 2 showed high significant difference between the treatments. T2 (25%) recorded the highest fertility, while animals receiving control ration without maize substitution recorded the highest hatchability. In general, incorporation of 25% of fifty-fifty percent weight to weight of cassava and sweet potato meal can be recommended for reproduction in chicken without affecting neither the hatchability nor the physical characteristics of the eggs, though hatchability will require better attention.

Key words: Reproduction, Local chicken, Cameroon, Cassava-sweet potato

INTRODUCTION

According to FAO (2014), WFP (2016) and FAO (2018), cereal prices have risen more than their five-year average in Africa. There is an increasing competition for maize between humans and livestock populations, requiring palliative measures to be taken. With the present trend of rising prices of animal feedstuff, greater attention is being paid to the search for safe and cheap alternative and locally available feedstuff, by-products from agriculture and industry, especially in the rural areas of the developing countries where farmers cannot afford the

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expensive commercial feed for livestock (Okereke, 2012; World Economic Forum, 2018).

Cassava and sweet potato can be used as a substitute for maize at high level in diets for all species of livestock, provided that they are supplemented with a nitrogen source (Heuze et al., 2015) and sulphur amino acids such as methionine and cysteine. Their fibre contents are also low, which makes cassava roots highly digestible for livestock.

Cassava and sweet potato products have been used in feeding chicken (Ladokun et al., 2007; Adewolu, 2008; Nguyen et al., 2010; Afolayan et al., 2012; Etchu et al., 2013; Khalid et al., 2013; Oyewumi,2013; Beckford and Bartlett, 2015) with limited levels of inclusion. However, no work has been done yet, on using a combination of cassava and sweet potato as energy substitute for maize to produce pellets for chicken production in Cameroon.

Apart from the hydrocyanic acid content, one of the greatest limitation in the use of cassava or sweet potato root meal is their dustiness causing crop impaction and irritation of the respiratory tract of animals, but feed pelleting could be a solution (Chhay et al., 2003; Ukachukwu, 2005). Furthermore, transformation of mash feed into pellets reduces feed wastage with an added advantage of improving digestibility and ease of incorporation of additives and drugs when necessary.

Cassava and sweet potato are potential substitutes for energy source which are not fully explored in animal feed in Cameroon. On the other hand, though the banning of imported frozen chicken has boosted poultry production in Cameroon, indigenous species are underutilized, probably due to their low productivity. The Cameroon Kabir chicken is a dual purpose locally adapted strain, phenotypically comparable to it common indigenous relatives but with superior performances in term of meat and egg production. It also displays good adaptability to low input production systems. Its meat and eggs have better organoleptic characteristics and most appreciated by consumers. It can be used to boost the productivity of the family poultry farming in rural Africa.

This study is aimed at contributing to family poultry productivity by evaluating the reproductive performances of Cameroon Kabir chickens fed on pelleted diets containing graded levels of cassava and sweet potato meal as an energy substitute for maize.

MATERIALS AND METHODS

The study was carried out at the Green Gold Agro-Venture experimental farm, located in Buea-Cameroon (4° 10’ 57” N and 9° 18’ 40.55” E). A total of 315 Kabir chickens (270 hens and 45 rosters) of 23 weeks of age, were randomly allocated to five treatmentsT1, T2, T3, T4 and T5 are defined as follows:

T1:100% maize, no substitution of Cassava and Sweet Potato Meal (CASPM), control diet.

T2: 25% replacement of maize with CASPM (50% cassava and 50% sweet potato meal by weight)

T3: 50% replacement of maize with CASPM (50% cassava and 50% sweet potato meal by weight)

T4: 75% replacement of maize with CASPM (50% cassava and 50% sweet potato meal by weight)

T5: 100% replacement of maize with CASPM (50% cassava and 50% sweet potato meal by weight)

Animals within the treatments were balanced for weight within each sex, each treatment having 63 Kabir chickens in three replications of 18 hens and 3 roosters each. The composition and bromatological values of the diets are summarized in the table 1. The various diets were then pelletized to 6 mm in diameter at 80°C for five minutes, allowed to cool down, sun dry, then packaged into pre-labelled bags and stored in a dry environment.

Table 1. Formulation of various diets using the least costful ingredients

Treatments / Ingredient	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
Maize	54	40.5	27	13.5	00
Cassava	00	6.75	13.5	20.25	27
Sweet potato	00	6.75	13.5	20.25	27
Wheat bran	16	15	14	10	08
Soya bean cake	07	07	08	08	10
Fish meal	03	04	05	08	08
Palm kernel	06	05	05	05	06
Oyster shell	07	07	07	05	07
Bone meal	02	03	02	05	02
Layer concentrate	05	05	05	05	05
Total	100	100	100	100	100
Calculated bromatological composition					
Energy (Kcal/kg)	2796	2769	2779	2731	2719
Protein (%)	16.68	16.04	16.07	16.42	16.32
Fat content (%)	3.89	3.59	3.33	3.14	2.77
E/P ratio	167.63	172.63	172.93	166.32	166.61
Lysine (%)	0.75	0.75	0.79	0.87	0.88
Methionine (%)	0.29	0.29	0.29	0.31	0.30
Calcium (%)	4.16	4.60	4.38	4.95	4.70
Phosphorus (%)	0.89	1.07	0.96	1.56	1.09

E/P ratio = Energy/Protein Ratio

The chicken houses were disinfected using the conventional protocol in force in poultry farms in Cameroon, and the floor was covered with a deep litter of wood shaving. Water and feed were offered ad libitum, and each chicken house was provided with laying nests. The prophylaxis plan was that applied to layers. The adaptation period lasted for 3 weeks, during which chickens had received the control pelleted diet.

During the laying period, eggs were collected early in the morning and at 3:00 pm. They were cleaned, the internal and external characteristics immediately evaluated using a sample of them, and the remaining stored in labelled trays for a maximum of seven days for incubation. The parameters included concerned laying performances, characteristics of eggs, fertility and hatchability. Fertility and hatchability were evaluated across four successive batches of incubations.

Statistical analysis

All the data collected were analysed using Microsoft excel and Graph PadInStat version 3.10. The data was analysed using the following systematic approach. The data were submitted to one-way analysis of variance for the comparison of means at 95% confidence interval, and Duncan multiple range test was used for separation of these means in case of significant difference.

RESULTS

The reproductive performances of Kabir chickens had been significantly (P<0.05) affected by the treatments.

Evolution of eggs weight

The evolution of eggs weight laid of Kabir chickens fed the diets containing graded levels of CASPM as an energy substitute for maize is summarized in table 2.

No significant (P<0.05) difference in egg weight was observed in week one and three across the treatments. However, at weeks 2, 4, 5 and 12 and 6, 7, 8 and 10 egg weight was significantly higher (P<0.01) and (P<0.001) respectively. The highest number of eggs laid, egg weight and mass (table 3) were recorded with chicken fed diet T2 containing 25% replacement of maize with CASPM, followed by T4 (75%), T5 (100%), T3 (50%) while T1, receiving no substitution performed less for all the parameters. Generally, the feed conversion ratio was found to decrease with increasing inclusion level of CASPM, but not in a regular trend, with T1 having the highest one, followed by T3, T2, T5 and T4 respectively. Table 3 presents the number of eggs laid, mean egg weight, egg mass and Feed Conversion Ratio (FCR) during the experimental period.

The weekly evolution of eggs' diameter and length was significantly (P<0.05) affected by the dietary treatments (table 4 and 5). No significant difference (P<0.05) was observed on egg length for week 1, 3, 4, 5, 8, 9 and 12 among treatments, and for the diameter only for weeks 3 and 4. However, egg length was significantly (P<0.05) higher at the 2nd, 6th, 7th and 9thweek for chickens fed diets T1, T2, and T3compared to the rest of the treatments. At week 11, egg length was significantly (P<0.05) higher for chickens fed diets T2, T3 and T4 compared to treatments T5 and the control (T1). Table 6 displays values of egg index of Kabir chicken's fed pelleted rations containing graded levels of cassava and sweet potation inclusions as energy substitute for maize. No significant (P<0.05) difference was observed among treatments for egg shape index for the 1st, 3rd, 4th, 5th, 7th, 8th, 9th, 10th and 11th weeks. However, egg shape index was significantly (P<0.05) higher in the2^{nd} week with T4 and T5, in the 6^{th} week with T2 and T3 and 12^{th}week with T3, T4 and T5.

The egg fertility of the Kabir chicken fed pelleted diets containing graded levels of CASPM meal is presented in table 7. All the treatments had shown a mean fertility level of greater than 80%. Treatment T2 recorded the highest mean % fertility, followed by T4, T3, T5 and T1. It was noted that there were no marked differences in fertility between the treatments. What about CV?

Table 2. Evolution of eggs weight by Kabir chickens fed pelleted diets containing graded levels of cassava and sweet potato meal as an energy substitute of maize in Cameroon

Egg Weight (g) / Weeks	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)	*P* Value
Week 1	45.8±0.89	49.2±1.59	50.4±3.12	62.31	*No record*	0.23
Week 2	48.0±2.20^{b}	52.2±1.20^{c}	47.8±0.69^{b}	47.6±0.30^{b}	44.8±1.01^{a}	0.01*
Week 3	51.9±0.51	55.2±3.58	50.4±0.45	48.6±1.20	48.4±1.35	0.15
Week 4	52.1±2.00^{b}	52.3±1.62^{b}	48.8±0.78^{a}	49.2±1.08^{a}	47.5±0.83^{a}	0.043*
Week 5	52.4±1.92^{b}	52.0±1.26^{b}	51.7±1.00^{ab}	50.5±2.18^{ab}	48.3±0.81^{a}	0.024*
Week 6	55.6±0.12^{b}	53.3±1.07^{b}	51.5±1.39^{ab}	49.8±0.44^{a}	48.5±0.93^{a}	0.0003***
Week 7	55.9±1.48b	56.9±1.88^{b}	56.3±1.16^{b}	51.5±0.67^{ab}	49.1±0.70^{a}	<0.0001***
Week 8	54.9±1.41^{b}	55.3±0.39^{b}	55.4±1.78^{b}	51.1±0.88^{ab}	49.4±0.60^{a}	<0.0001***
Week 9	52.3±1.42^{ab}	55.6±0.52^{b}	54.6±1.16^{b}	50.7±0.79^{a}	50.0±1.34^{a}	0.0016**
Week 10	53.8±1.08^{ab}	56.2±0.43^{b}	55.4±1.84^{b}	50.7±1.05^{a}	50.4±0.86^{a}	<0.0001***
Week 11	45.4±1.46^{a}	57.1±1.89^{c}	52.4±3.25^{b}	51.5±1.11^{b}	46.4±1.01^{a}	0.0016**
Week 12	50.4±0.81^{a}	55.4±0.66^{b}	52.5±1.17^{ab}	53.4±0.88^{ab}	No record	0.021*
Mean egg weight	51.6 ± 1.15	54.2±1.34	52.3±1.48	51.4±0.88	48.3±0.94	--
CV	2.23	2.47	2.83	1.71	1.94	--

*P< 0.05, **P< 0.01, ***P< 0.001, ^{abcd} Treatments within a row (week) having the same letter are not significantly different, no record; indicates periods where no eggs were collected before onset of laying and during the resting period (confirm by observation of some hens molting and others brooding), CV= coefficient of variation

Table 3. Total eggs laid, mean egg weight and egg mass of Kabir chickens fed on pelleted diets containing graded levels of cassava and sweet potato meal as an energy substitute of maize in Cameroon

Parameter \ Treatments	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
Total eggs laid	204	441	294	432	414
Mean egg weight (g)	51.6 ± 1.15	54.2±1.34	52.3±1.48	51.4±0.88	48.3±0.94
Egg mass (kg)	10.5±0.23	23.9±0.59	15.4±0.43	22.2±0.38	20.0±0.39
FCR	5.10	2.82	3.39	2.46	2.51

FCR = food conversion ratio

Table 4. Diameter of Kabir eggs fed pelleted diet containing graded levels of cassava and sweet potato meal as an energy substitute of maize in Cameroon

Weeks \ Egg Diameter (cm)	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)	*P* Value
1	3.9±0.02	3.98±0.01	4.10±0.07	4.38	*No record*	0.014*
2	4.08±0.03^{b}	4.10±0.03^{b}	3.90±0.69^{a}	4.05±0.02^{ab}	4.00±0.03^{a}	0.028*
3	4.11±0.009	4.17±0.07	4.10±0.08	4.06±0.03	4.02±0.06	0.76
4	4.12±0.05	4.15±0.05	4.02±0.03	4.11±0.02	4.01±0.05	0.24
5	4.15±0.04^{ab}	4.17±0.03^{b}	4.12±0.03^{ab}	4.09±0.06^{a}	4.02±0.03^{a}	0.042*
6	4.21±0.02^{b}	4.22±0.03^{b}	4.19±0.02^{ab}	4.06±0.02^{a}	4.03±0.03^{a}	<0.0001***
7	4.19±0.04^{b}	4.27±1.88^{b}	4.23±1.16^{b}	4.15±0.04^{ab}	4.05±0.03^{a}	<0.0001***
8	4.19±0.04^{b}	4.24±0.01b	4.23±0.05^{b}	4.11±0.02^{ab}	4.07±0.03^{a}	0.0003***
9	4.15±0.03^{b}	4.35±0.10^{b}	4.19±0.04^{b}	4.09±0.02^{a}	4.17±0.09^{b}	0.0006***
10	4.14±0.02^{b}	4.24±0.01^{b}	4.23±0.06^{b}	4.11±0.03^{b}	4.09±0.04^{a}	0.0002***
11	3.93±0.03^{a}	4.36±0.08^{c}	4.17±0.85^{b}	4.11±0.03^{b}	3.81±0.20^{a}	0.0005***
12	3.96±0.02^{a}	4.22±0.01^{b}	4.12±0.06^{ab}	4.14±0.02^{b}	No record	0.0029**
Mean egg diameter	4.09±0.03	4.21±0.2	4.13±0.26	4.12±0.02	4.03±0.06	--
CV	0.73	4.75	6.29	0.48	1.48	--

*P< 0.05, **P< 0.01, ***P< 0.001, ^{abcd} Treatments within a row (week) having the same letter are not significantly different**,** no record; indicates periods where no eggs were collected before onset of laying and during the resting period (confirm by observation of some hens molting and others brooding, CV = coefficient of variation

Table 5. Length of Kabir eggs fed pelleted diet containing graded levels of cassava and sweet potato meal as an energy substitute of maize in Cameroon

Weeks \ Egg Length (cm)	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)	*P* Value
1	5.32±0.08	5.56±0.14	5.44±0.21	5.90	*No record*	0.42
2	5.52±0.02^{b}	5.50±0.09^{b}	5.68±0.12^{b}	5.17±0.04^{ab}	5.04±0.15^{a}	0.02*
3	5.49±0.04	5.57±0.16	5.54±0.05	5.25±0.08	5.35±0.07	0.08
4	5.54±0.09	5.43±0.05	5.43±0.04	5.36±0.05	5.40±0.05	0.31
5	5.45±0.11	5.39±0.05	5.50±0.04	5.27±0.12	5.36±0.03	0.06
6	5.69±0.02^{b}	5.42±0.03^{b}	5.43±0.05^{b}	5.40±0.03^{a}	5.36±0.03^{a}	0.009**
7	5.64±0.06^{b}	5.58±0.07^{b}	5.57±0.05^{b}	5.38±0.05^{a}	5.34±0.02^{a}	<0.0001***
8	5.61±0.08	5.43±0.10	5.54±0.06	5.44±0.04	5.38±0.04	0.056
9	5.49±0.11	5.48±0.03	5.56±0.04	5.41±0.05	5.40±0.04	0.18
10	5.59±0.06^{b}	5.56±0.03^{b}	5.58±0.05^{b}	5.38±0.05^{a}	5.41±0.05^{a}	0.0072**
11	5.22±0.01^{a}	5.58±0.02^{b}	5.41±0.12^{ab}	5.52±0.05^{b}	5.32±0.04^{a}	0.0012**
12	5.59±0.09	5.58±0.06	5.45±0.033	5.54±0.04	No record	0.64
Mean egg length	5.51±0.06	5.51±0.07	5.51±0.07	5.42±0.05	5.34±0.05	--
CV	1.09	1.27	1.27	0.92	0.94	--

*P< 0.05, **P<0.01, ***P< 0.001,^{abcd} Treatments within a row(week) having the same letter are not significantly different, no record; indicates periods where no eggs were collected before onset of laying and during the resting period (confirm by observation of some hens molting and others brooding, CV = coefficient of variation

Table 6. Egg shape index of Kabir chicken fed pelleted diet containing graded levels of cassava-sweet potato meal as an energy substitute of maize in Cameroon

Egg shape index / Weeks	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)	*P* Value
1	73.3±0.00	71.7±2.82	75.3±2.43	74.2±0.00	*No record*	0.72
2	73.9±0.95^{ab}	74.7±3.29^{ab}	68.8±4.74^{a}	76.3±5.22^{b}	79.6±6.25^{c}	0.037*
3	74.8±1.25	75.2±3.49	74.2±6.03	77.4±2.81	75.9±5.85	0.51
4	74.4±1.31	76.4±2.70	74.1±3.07	76.8±2.50	74.5±5.86	0.25
5	76.2±2.41	77.4±2.47	75.1±3.01	77.9±5.19	74.9±4.18	0.21
6	74.1±0.60^{a}	77.9±2.04^{b}	77.2±1.41^{b}	75.1±3.11^{ab}	75.2±3.34^{ab}	0.01*
7	74.4±2.23	76.6±1.75	75.8±1.61	77.2±4.59	75.7±0.00	0.11
8	74.7±2.80	78.5±4.71	76.3±1.53	75.7±2.83	75.8±4.05	0.19
9	76.8±3.43	79.5±7.29	75.4±1.68	76.2±2.40	77.2±6.33	0.17
10	74.1±1.24	76.3±2.50	75.9±1.99	76.5±1.90	75.6±4.00	0.13
11	75.3±0.60	77.9±6.32	77.0±1.88	74.4±1.15	71.7±8.90	0.07
12	70.9±2.78^{a}	75.7±3.19^{b}	75.7±2.25^{b}	74.7±2.32^{b}	No record	0.02*
Mean egg length	74.4±1.63	76.5±3.54	75.1±2.63	76.0±2.83	75.6±4.88	--
CV	2.19	4.63	3.50	3.72	6.45	--

*P<0.05, **P< 0.01, ***P< 0.001, ^{abcd} Treatments within a row(week) having the same letter are not significantly different, No record; indicates periods where no eggs were collected before onset of laying and during the resting period (confirm by observation of some hens molting and others brooding), CV = coefficient of variation

Table 7. Percentage fertility of Kabir eggs fed pelleted diet containing graded levels of cassava and sweet potato meal as energy substitute of maize in Cameroon

% Fertility / Batches	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
1	74.0	84.6	90.0	88.9	94.4
2	100.0	100.0	75.0	84.6	90.5
3	100.0	92.3	88.9	88.2	58.3
4	50.0	87.5	100.0	93.3	100.0
Mean	81.0±24.0	91.1±6.7	88.5±10.3	88.8±3.57	85.8±18.7
CV	29.7	7.37	11.6	4.02	21.8

CV = coefficient of variation

Table 8. Yolk characteristics of Kabir chicken fed pelleted diet containing graded levels of cassava and sweet potato meal as an energy substitute of maize in Cameroon

Treatments / Parameters	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)	*P* Value
Weight (g)	15.39±0.34	16.29±0.35	16.56±0.50	14.94±0.64	15.90±0.48	0.13
Length (mm)	43.43±1.09	41.80±2.21	43.40±0.94	37.21±2.55	37.29±3.45	0.16
Width (mm)	10.09±0.90	10.48±0.53	10.30±0.58	10.49±0.92	12.07±0.35	0.20
Yolk index	23.39±9.20	27.03±10.47	24.12±6.52	29.22±14.57	29.26±12.6	0.47

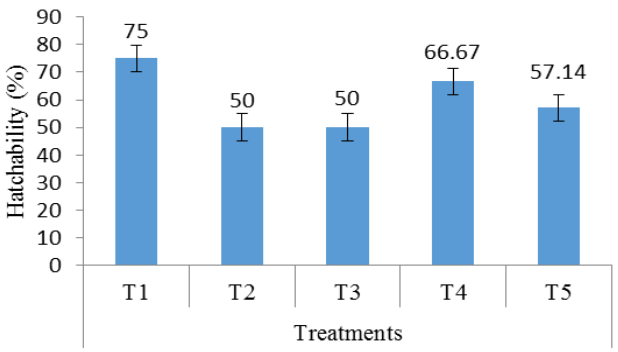

Figure 1. Hatchability of eggs laid by Kabir chickens fed on pelleted rations containing graded levels of Cassava and sweet potato meal as an energy substitute of maize in Cameroon.

Hatchability

The hatchability of eggs from Kabir chicken fed pelleted rations with graded levels of CASPM meal as an energy substitute of maize is presented in figure 1. The control diet had the highest hatchability, followed by T4, T5, T2 and T3. All the groups receiving CASPM meal had fertilities lower than 75%. The yolk characteristics of eggs laid by Kabir chicken fed on pelleted diet containing graded levels of cassava and sweet potato meal as an energy substitute of maize is presented in table 8. Statistical analysis carried on the Kabir egg yolk parameters showed no significant ($P<0.05$) difference among treatments.

DISCUSSION

The significant ($P<0.05$) influence on the number of egg laid, egg weight and egg mass as compared to the control diet after substitution of maize with pelleted diets is in agreement with Kana et al. (2015) and May Galon et al. (2017), however it disagreed with the results of Aina and Fanimo (1997) and Salami, Odunsi (2003) and Aderemi et al. (2012) who observed that laying performances of layers decline with increasing levels of cassava root meal in the diet. The differences observed could be attributed to possible differences of the texture of the experimental diets. In fact, it is known that the texture or and the form of presentation of feed significantly affects the digestibility of the feed and in so doing, affecting the production performances. The breed used can also be a determining factor of variation. Of cause, local chicken adapts easier to various feed texture as it is the case in backyard production systems as compared to intensively selected chickens. Further, Kana et al. (2015) in his findings revealed that local chicken could tolerate up to 100% replacement of maize by cassava root meal without any adverse effect on laying performances.

The feed conversion ratio was higher in birds fed control diets. This is in conformity with Becford and Barlett (2015). This feed conversion ration difference ranging from 33.5 to 51.7% according to the level of CASPM could be of significant importance to producers as it could potentially be translated into reducing feed cost.

The mean fertility levels of the incubated eggs for all experimental treatments were all above 80%, but the chicken fed control diet still displayed the least performance, though having the best hatchability confirming that CASPM improves laying performances of chickens without a negative effect on its hatchability. A high fertility is an asset in animal breeding as rapid genetic progress can be achieved through artificial insemination using a roster with a high breeding value.

The hatchability levels of the incubated fertile eggs for all experimental treatments varied from 50% to 75%. T1 had the highest hatchability followed by T4, T5, T3 and T2. This is in conformity to what was reported by King'ori et al., (2010) for Kenyan local chicken eggs hatchability ranging from 66-73%. But also lower than the 80-90% reported for exotic hybrid egg and meat strains according to Moald (2013). Differences in hatchability between different research results can be explained by common environmental effect like the diet but, also the type of incubator used, altitude, temperature or additive genetic effect. Kabir egg yolk analysis was found to be insignificantly different between treatments. This agreed with Aina and Fanimo (1997).

CONCLUSION

The study has shown that the incorporation rate of 25% of fifty-fifty percent weight to weight of Cassava and sweet potato meal can be recommended as a good substitute of maize in the chicken diet raised for reproduction. Irrespective of the incorporation rate, CASPM improves the reproduction performances without affecting the hatchability as well as the physical characteristics of the eggs. Therefore, these diets can be highly recommended pending complementary studies on the cost effectiveness of CASPM-based diets.

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Author's contributions

Christian Keambou, Raquel soares, Frederico and Ndamukong designed, monitored and supervised the study, Vukiesu, Toukala, Tedongmo implemented the farm work, collected organised, analysed the data and drafted the first manuscript. Defang, Hako and Kana did the drafting and review of the manuscript. All the authors edited and approved the manuscript.

Consent to publish

All the authors gave their informed consent prior to their inclusion in the study.

Competing interests

The authors declare that they have no competing interests.

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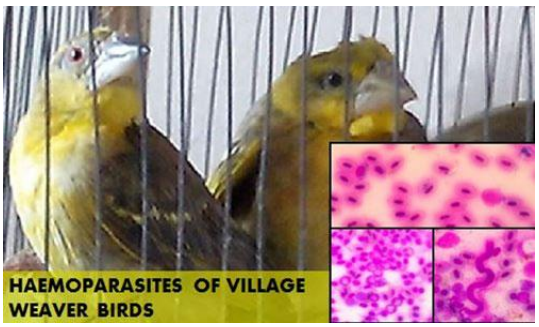

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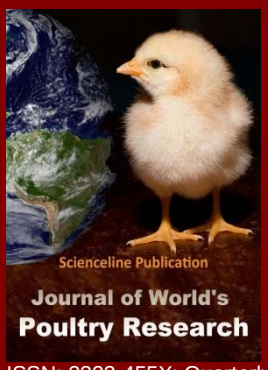

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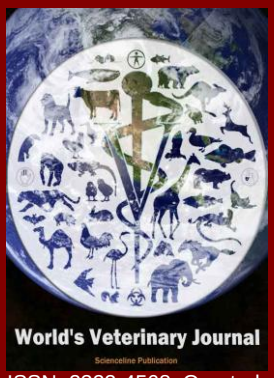

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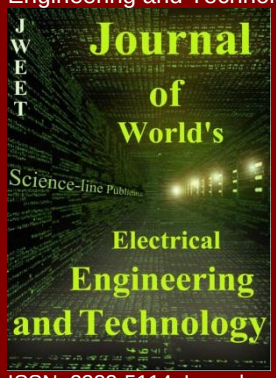

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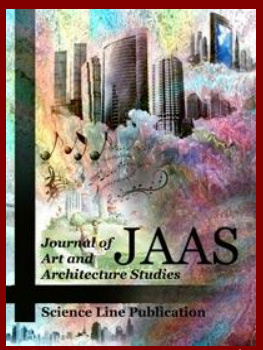

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