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EFFECT OF AQUEOS EXTRACT OF *Momordica balsamina* AND *Cucumis metuliferus* FRUITS ON NEWCASTLE DISEASE IN PULLETS

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ABSTRACT
The study was conducted to determine the effect of aqueous extract of Mormordica balsamina and Cucumis metuliferus fruits on Newcastle disease (NCD) in pullets. Ninety (90) pullets were used for the study. The pullets were divided into prophylactic and curative groups comprising 10 birds per group. For prophylactic groups aqueous extract of the fruits at concentration of 2.5g and 5.0g per 100litres of water were given to the pullets ad-libitum for two weeks and then challenged with Newcastle disease virus kudu 113 strain via intra ocular route while for curative groups the pullets were challenged with the virus and then treated with the aqueous extracts of the fruits at 2.5g and 5.0g per 100litres of water ad-libitum. Data collected were analyzed using one way analysis of variance (ANOVA) using Statistical Analysis Software version 9.1. The results showed that M. balsamina at 2.5g/100litres of water had very good curative abilities which recorded lower clinical signs, mortality, postmortem lesions, higher antibody titer and higher white blood cell activity with a statistically significant ($p<0.05$) association while the same fruit at 5.0g/100litres of water had the best results for prophylactic treatments recording least clinical signs and mortality rates and highest antibody titers, highest white blood cell count which was statistically significant ($P<0.05$). It is therefore recommended that Mormodica balsamina can be recommended at 2.5g/100litres and at 5g/100liters of water for the control and prevention of Newcastle disease respectively to farmers and that further studies should be carried out to establish optimum concentration levels for the aqueous and other (methanolic and ethanolic) extracts of the fruit for achievement of best prophylactic and curative effect.

Keywords: Newcastle disease, *Mormordica balsamina, Cucumis metuliferus* and pullets.

INTRODUCTION

Poultry production is the fastest growing segments of the agricultural subsector in Nigeria due to the increased demand of animal protein with the ever growing population (Salami *et al*., 1989). However, one of the constraints to the development of the poultry industry is outbreak of diseases (Salami *et al*., 1989) one of which is Newcastle disease (ND). Newcastle Disease Virus (NDV) causes a highly infectious neurological, respiratory or enteric disease of poultry.

Plants have been used for various purposes since prehistoric times (Lawrence and Bennett, 1995; Evans, 2009) and medicinal herbs are being increasingly studied by pharmacological researchers (Sinclair, 1998). Reports of various plants used in the treatment of diseases have been documented in Nigeria (Alawa *et al*., 2008; Sofowora, 2008), Togo (Beloin *et al*., 2005), South Africa (Rabe and van Staden, 1997; Lin *et al*., 1999), Uganda (Hamill *et al*., 2003), Kenya (Fabry *et al*., 1998; Matu and van Staden, 2003), Ethiopia (Gedif and Hahn, 2003), India (Harsha *et al.,* 2003; Nandagopalan *et al*., 2011), Belize (Camporese *et al*., 2003), Turkey (Yeşilada *et al*., 1995), Columbia (Ritch-Krc *et al*., 1996), Panama (Gupta *et al*., 2005), Italy (Guarrera, *et al*., 2005), Mexico (Hernández *et al*., 2003), Australia (Semple *et al*., 1998). According to the World Health Organization (WHO) more than 80% of the world's populations rely on traditional medicine for their primary healthcare, majority of which use plants or their active principles (Gupta *et al.,* 2005).

*Cucumis metuliferus***,** locally called 'gautar kaji' in hausa language, belongs to the family *Cucurbitaceae* and is a monoecious, climbing, annual herb that can be grown practically anywhere, provided the season is warm (Benzioni *et al*., 1993). It is commonly known as African horned cucumber, melano, Jelly melon, and kiwano. The fruits occur in two forms: the bitter and non-bitter forms, which occur mostly in the wild state. The non-bitter form has been found to be less toxic and has also been widely cultivated (Enslin *et al.*, 1954).

Momordica balsamina Linn commonly known as African pumpkin (or African cucumber), Balsam apple (or Balsam pear) and locally called "*Garahuni*" (Hausa Language), also belongs to the family *Cucurbitaceae*. A macerate of the whole plant to which salt has been added is used in Senegal as a galactogogue and to increase milk yield of cows (Burkill, 1985). The fruit mixed with olive or almond oil is used for the treatment of pile; for festers, inflammations, swellings, yaws, burns, intermittent fever, burning sensation of sole, night blindness, diabetes, asthma and cough (Burkill, 1985). The fruit has emetic and cathartics effects. Roodt (1998) reported various medicinal uses of *Momordica balsamina*. The fruit extract contains alkaloidal components which have antiviral properties reverted the negative effects of Newcastle Disease virus in chicks when injected at 600 mg/kg (Raza *et al.*, 2015)

Newcastle disease still remains a serious economic challenge to all segments of the poultry industry because of its high morbidity and mortality rates (Alexander, 2003). The only control method so far still remains vaccination, which does not confer 100% immunity in all vaccinated birds. Vaccine breaks and failures are also a major problem. It has also been reported that vaccination itself may cause disease and reduced growth in vaccinated birds (Alexander, 2003). Moreover

outbreaks have been reported in vaccinated populations (Alexander, 2003).

Investigations show that local farmers in the study area claim to use *Cucumis metuliferus* and *Momordica balsamina* fruits to treat their birds for various diseases including Newcastle disease by immersing the whole fruits in drinking water of birds (Alhaji, 2017). The need to test the above theory and the need to find a cheaper and more accessible means to the local farmer for the treatment and prevention of Newcastle disease thus preventing huge economic loses informed this research.

MATERIALS AND METHODS

Study area

The study was conducted at the Poultry Unit of the Department of Animal Science Teaching and Research farm, Federal University Dutsinma, Katsina State, Nigeria. Dutsinma lies within the coordinates of latitude 12.45N and longitude 7.49E and also on the altitude of 605m above the sea level (Isah, 2009). Generally, the climate varies considerably according to months and seasons of the year namely a cool dry (harmattan) season from December to February, a hot dry season from March to May, a warm wet season from June to September; and a less marked season after rains characterized by decreasing rainfall and gradual lowering of temperature during the months of October to November, (Ati *et al*., 2010).

Experimental design

The research adopted a Completely Randomized Design with 9 treatments, each treatment/group were made up of 10 birds. Day old pullets were sourced from reputable hatchery (Olam® Hatcheries). They were brooded under strict biosecurity measures to prevent entry of pathogens. The birds were vaccinated against Infectious bursal disease at day seven of the arrival but not against Newcastle disease and they were fed with chick-mash till 8weeks when experiment started. Experimental groups consisted of Curative, Prophylactic and Control groups. Aqueous solutions of both *M balsamina* and *C metuliferus* were made at 2.5grams per liter of water and 5grams per liter of water. The curative group was made up of groups- CM2.5g/l, CM5.0g/l, CC2.5g/l and CC5.0g/l where CM indicates curative mormodica and CC curative cucumis. They were initially challenged with the Newcastle disease virus Kudu 113 strain at 8weeks and treated with aqueous extracts of *Cucumis metuliferus* and *Momordica balsamina* ad libitum in drinking water at above concentrations as soon as clinical signs were observed. The prophylactic groups, PM2.5g, PM5.0g, PC2.5g and PC5.0g, were treated with aqueous extracts of *Cucumis metuliferus* and *Momordica balsamina* ad libitum in water for 2weeks and then subsequently challenged with Newcastle disease virus Kudu 113 strain to observe if the treatments would prevent or reduce the intensity of infection. The control group was challenged with the Newcastle disease virus Kudu 113 strain but was not treated with any extracts at 8 weeks of age.

Study parameters

The clinical signs, mortality and post mortem lesions were observed and recorded daily according to the method of Agang, 2014.

Laboratory analysis

Blood was collected via wing vein of birds at week two of the experiment and at week four of the experiment. Whole blood was collected for Heamogram/blood picture and the serum for Heamagglutination inhibition test.

Data analysis

Data were subjected to oneway ANOVA (analysis of variance) using Statistical Analysis Software version 9.1. P values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Clinical signs

The clinical signs in pullets challenged with Newcastle disease for curative groups are presented in Tables 1. Signs observed included: Gasping, sneezing, watery greenish diarrhea, anorexia, torticollis, gasping, depression, swelling of the head, paralysis and emaciation.

Table 1: Clinical Signs in pullets challenged with Newcastle Disease Virus (Curative groups)

Treatments	COG	WGD	TTC	ANX	GSP	DPR	SOH	PLY	EMC
CM 2.5g	-	+	-	+	-	+	-	-	-
CM 5.0g	-	+	-	+	+	+	-	+	+
CC 2.5g	+	+	+	+	+	-	-	+	+
CC 5.0g	+	+	+	+	+	-	-	-	+
Control	+	+	+	+	+	+	+	+	+

COG – Coughing, WGD – Watery greenish diarrhoea, TTC- Torticollis, ANX – Anorexia, GSP – Gasping, DPR – Depression, SOH – Swelling of the head, PLY – Paralysis, EMC – Emaciation

For curative groups CM2.5g showed the least clinical signs of anorexia, watery green diarrhea and depression while the control group were most severely affected recording all clinical signs (Table 1).

Table 2 shows the clinical signs for the prophylactic groups. For the prophylactic groups PM5g recorded the least clinical signs while PC5g showed most clinical signs (Table 2). Ibrahim and Tanya (2001) and Bukar-Kolo*et al*., (2006) both reported Newcastle disease to be the major poultry diseases affecting pullets and its management and high cost of orthodox drugs lower pullet's production too.

Groups CM2.5g and PM5g both treated with aqueous extract of *Mormodica balsamina* at 2.5g/liter and 5g/liter recorded the least clinical signs. This is not unexpected as Hassan and Umar (2006) reported that M balsamina has high calcium, phosphorus and vitamins. These contents would naturally be expected to translate to healthy birds. Agang (2014) reported increase in weights of birds treated with *M. balsamina* when compared to untreated groups.

Table 2: Clinical Signs in pullets challenged with Newcastle Disease Virus (Prophylactic groups)

Treatments	COG	WGD	TTC	ANX	GSP	DPR	SOH	PLY	EMC
PM 2.5g	+	+	-	+	+	+	-	+	+
PM 5.0g	-	+	-	+	+	+	-	-	+
PC 2.5g	+	+	+	+	+	+	+	+	+
PC 5.0g	-	+	+	+	+	+	-	-	+
Control	+	+	+	+	+	+	+	+	+

COG – Coughing, WGD – Watery green diarrhea, TTC- Torticollis, ANX – Anorexia, GSP – Gasping, DPR – Depression, SOH – Swelling of the head, PLY – Paralysis, EMC –Emanciation

Mortality Rates

The mortality rates of pullets challenged with Newcastle disease virus is recorded in Table 3. Group M2.5g that was treated with *Mormodica balsamina* recorded the least mortality of 2 birds followed by M5g with 3 birds while the *Cucumis* groups had the highest mortality rates of 4birds for curative groups. For prophylactic groups C5g and M5g had the least mortality rates of 5 birds each while M2.5g showed the highest mortality rate of 7 birds.

The mortality rates observed were not surprising since the clinical signs reflected similar findings and it is anticipated that higher mortality would occur in groups with more serious clinical signs and vice versa. Wakamatsu (2006) worked on a similar isolate of NDV as Kudu 113 strain used in this study and reported 100% mortality post infection. Agang (2014) also reported similar results of lower mortality in *M. balsamina* treated groups.

Table 3: Mortality rates of pullets challenged with Newcastle Disease Virus

Treatments	Curative groups	Prophylactic groups
M 2.5g	2	7
M 5.0g	3	5
C 2.5g	4	7
C 5.0g	4	5
Control	10	9

Post Mortem Lesions (Curative Group)

The post mortem lesions of pullets challenged with Newcastle disease virus are indicated in Tables 4 and 5. Post mortem lesions observed included haemorrhage in the intestine, haemorrhage in trachea, haemorrhage on proventriculus, bile enlargement, cloudy air sack, haemorrhage on glandular surface of proventriculus and necrotic areas in caecal tonsils. Findings for post mortem lesions followed a similar pattern with group CM2.5g showing the least post mortem lesions (Heamorrhage on proventiculus, cloudy air sacs and necrotic areas on cecal tonsils) while CC5.0g showed most serious post mortem lesions for curative groups with all signs recorded observed in this group (Table 4). For prophylactic groups PC2.5g showed most serious signs when compared with other groups recording all signs except bile duct enlargement (Table 5).

The post mortem lesions seen may be attributed to the route of challenge (Ocular) which may enhance the clinical signs and gross lesions observed in most cases as the Newcastle disease virus replicates in the Haderian gland before spreading to other sites. This is in agreement with the work of Agang (2014) with *M. balsamina* on pullets, who reported that severe clinical signs, mortalities and lesions observed showed low level of protection in untreated groups.

Table 4: Post Mortem Lesions of pullets challenged with Newcastle Disease Virus (Curative groups)

Treatments	HII	HIT	HOP	BE	ASC	HGSP	NCT
CM 2.5g	-	-	+	-	+	+	+
CM 5.0g	-	+	+	+	+	+	+
CC 2.5g	+	+	+	+	+	+	-
CC 5.0g	+	+	+	+	+	+	+
Neg. control	+	+	+	+	+	+	+

HII – Heamorrhage in intestine, HIT – Heamorrhage in trachea, HOP – Heamorrhage on Proventriculus, BE – Bile duct enlargement, ASC – Cloudy air sac, HGSP – Heamorrhage on glandular surface of proventriculus, NCT – Necrotic areas on cecal tonsils

The phytochemical analysis of the fruit pulp extract of Momordica balsamina shows the presence of alkaloids, tannins, saponins and flavonoids (Bakari *et al.*, 2012). Tannins may be employed medicinally in antidiarrheal, haemostatic, and antihemorrhoidal compounds. The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders (Bakari *et al.*, 2012). Alkaloid produces analgesic, anti-inflammatory and adaptogenic effects which help to develop resistance against disease and endurance against stress (Mgbojikwe *et al*., 2019). A number of flavonoids have been shown to have anti-inflammatory effects and to strengthen blood vessels. Flavonoids have been investigated for possible anti-inflammatory effects and anti-viral property (Mgbojikwe *et al*., 2019).These attributes of *Mormodica balsamina* may explain the less severe post mortem lesions observed in *Mormodica* treated groups.

Table 5: Post mortem lesions of pullets challenged with Newcastle Disease Virus (Prophylactic groups)

Treatments	HII	HIT	HOP	BE	ASC	HGSP	NCT
PM 2.5g	+	+	+	-	+	+	-
PM 5.0g	-	+	+	-	+	+	+
PC 2.5g	+	+	+	-	+	+	+
PC 5.0g	-	+	+	-	+	+	+
Neg. Control	+	+	+	+	+	+	+

HII – Heamorrhage in intestine, HIT – Heamorrhage in trachea, HOP – Heamorrhage on Proventriculus, BE – Bile duct enlargement, ASC – Cloudy air sac, HGSP – Heamorrhage on glandular surface of proventriculus, NCT – Necrotic areas on cecal tonsils

Heamatology

The blood pictures of pullets challenged with Newcastle Disease Virus is presented in Table 6. Blood pictures showed highest total white blood cell counts and packed cell volumes for group CM2.5g in curative groups while CM5g showed the least TWBC.

The higher white blood cell count seen from blood pictures for group CM2.5g showed higher white blood cell activity ($17.33*10^9$/l) and thus higher chances of the immune system fighting off the infection than other groups (CC2.5g, CC5g, CM2.5g and the control group). This can be seen to be translated in the lower clinical signs and mortality rates of the groups with higher TWBCs.

Table 6: Blood Pictures of pullets challenged with Newcastle Disease Virus (Curative groups)

Parameters	CC2.5g	CC5g	CM2.5g	CM5g	SEM
PCV	31.00[a]	24.33 [b]	43.67 [c]	34.33 [a]	2.62926
HGB (g/dl)	10.20 [a]	8.07 [a]	13.80 [b]	11.67 [a]	0.81666
TP	4.73 [a]	3.53 [a]	4.10 [a]	4.33 [a]	0.15333
TWBC x 10^9/l	14.27 [a]	14.33 [a]	17.33 [b]	13.20 [a]	0.85076
TRBC x 10^{12}/l	5.23 [a]	4.23 [a]	7.03 [b]	5.93 [a]	0.37389
HETERO (%)	13 [a]	9 [b]	13 [a]	11 [a]	1.03494
LYMPHO (%)	82 [a]	89 [a]	85 [a]	87 [a]	1.47745
MONO (%)	0	2	1	0	0.39841
EOSINO (%)	0	0	0	2	0.23231
BASO (%)	0	0	0	0	0.00000
BAND (%)	3	0	1	0	0.40237

Means with the same letter are not significantly different (P>0.05).

The blood pictures of pullets challenged with Newcastle Disease Virus is presented in Table 7. For prophylactic groups PM5g had the highest TWBC while group PC5g showed the least TWBC count.

Agang *et al.* (2019) reported increased in feed consumption and activity in pullets treated with *Mormodica balsamina.* They attributed this to high amount of calcium, phosphorus and vitamin in ash (11.10%) of *Mormodica balsamina*. Hassan and Umar (2006) also mentioned that *Mormodica balsamina* has high calcium, phosphorus and vitamins. These minerals and vitamins could be the reason for the increased total white blood cell count, PCV, Heamoglobin and Total protein seen in CM2.5g group and PM5g groups for curative and prophylactic groups respectively.

Table 7: Blood Pictures of pullets challenged with Newcastle Disease Virus (Curative groups)

Parameters	PC2.5g	PC5g	PM2.5g	PM5g	SEM
PCV	24.33 [a]	25.33 [a]	16.67 [b]	24.00 [a]	1.11469

HGB (g/dl)	7.97 [a]	8.40 [a]	5.60 [b]	8.03 [a]	0.35017
TP	6.53 [a]	4.80 [b]	4.60 [b]	3.83[b]	0.32403
TWBC x 10^9/l	14.40 [a]	9.87 [b]	12.80 [a]	20.03 [c]	1.02003
TRBC x 10^{12}/l	3.97 [a]	4.07 [a]	3.27 [a]	4.00 [a]	0.12794
HETERO (%)	14 [a]	11 [b]	11 [b]	11 [b]	1.02291
LYMPHO (%)	84 [a]	85 [a]	84 [a]	85 [a]	1.37737
MONO (%)	2	3	2	3	0.47676
EOSINO (%)	0	0	2	0	0.23503
BASO (%)	0	0	0	0	0.00000
BAND (%)	0	1	0	1	0.16330

Means with the same letter are not significantly different (P>0.05).

Heamagglutination Inhibition

Heamagglutination inhibition titres of pullets challenged with Newcastle disease virus (Kudu 113) are presented in Tables 8 and 9. For curative groups CM2.5 showed highest antibody titre up to dilution rate of 1 in 128 (Table 8) while CC5g recorded lowest titre of 1 in 8. For prophylactic group PM5 recorded the highest antibody titer of I in 64 while PC2.5g had the lowest tire of 1 in 8 (Table 9).

Antibody titers for heamagglutination inhibition tests were indicated by highest titre levels at which agglutination became absent thus indicating no antigen antibody reaction at that titre. All groups showed an initial positive response. This could be attributed to maternal derived antibodies. Similar results of maternal antibodies conferring protection in all groups have been reported by Agang *et al., 2019;* Allan *et al*., 1978 and Verma *et al*., 1985. The results for HI were not surprising as the groups with highest antibody titres had lowest recorded the lowest clinical signs in curative and prophylactic groups respectively as well as mortality and postmortem lesions. Agang (2014) reported that mean antibody titres showed significant increased from day-old up to the 7th week in groups treated with *M.balsamina*. The initial rise in antibody titer is attributed to maternal derived antibodies and this is obvious as even pullets in the negative control group showed initial agglutinations.

Table 8: Heamagglutination Inhibition titres of pullets challenged with Newcastle Disease Virus (Curative groups)

Treatment	1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048
CC5g	+	+	+	+	-	-	-	-	-	-	-	-
CC 2.5g	+	+	+	+	+	-	-	-	-	-	-	-
CM5g	+	+	+	+	+	+	-	-	-	-	-	-
CM 2.5g	+	+	+	+	+	+	+	+	-	-	-	-
Control	+	+	+	-	-	-	-	-	-	-	-	-

Table 9: Heamagglutination Inhibition titres of pullets challenged with Newcastle Disease Virus (Prophylactic groups)

Treatment	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048
PC5g	+	+	+	+	+	+	-	-	-	-	-	-
PC2.5g	+	+	+	+	-	-	-	-	-	-	-	-
PM5g	+	+	+	+	+	+	+	-	-	-	-	-
PM2.5g	+	+	+	+	+	-	-	-	-	-	-	-
Control	+	+	-	-	-	-	-	-	-	-	-	-

CONCLUSION AND RECOMMENDATION

The study showed better curative and prophylactic effects at concentration of M2.5g while the same plant at concentration of M5g showed best prophylactic ability.

It is thus recommended that aqueous extract of *Mormodica balsamina* at 2.5g/100litres and 5g/100liters of water should be used for the control and prevention of Newcastle disease respectively by local poultry farmers. Further studies should be carried out to establish optimum concentration levels for the aqueous and other (methanolic and ethanolic) extracts of the fruit for achievement of best prophylactic and curative effect.

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PLATE I: *Cucumis metuliferus* Fruit

PLATE II: *Momordica balsamina* Linn Fruit