EFFECT OF *XYLOPIA AETHIOPICA* FRUIT POWDER ON HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF FINISHER BROILER CHICKENS

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ABSTRACT

This study was carried out to determine the effect of Xylopia aethiopica fruit powder on broiler chickens' haematological and serum biochemical indices. A total of two hundred broiler chickens (Abor acre) at twenty- eight days of age was used for the experiment. Five experimental diets were formulated and labelled T_1, T_2, T_3, T_4 and T_5. Treatment one contained no X. aethiopica fruit powder while T_2, T_3, T_4 and T_5 contained X. aethiopica fruit powder at 100, 200, 300 and 400 g per 100 kg feed respectively. The broiler chickens were divided into five groups of forty birds and each group was assigned one of the treatment diets in a completely randomised design (CRD). Each group was further divided into four replicates of ten birds per replicate. Feed and water were supplied ad libitum. Blood was collected for haematological and Serum biochemistry analysis on the (28) twenty-eight days of the experiment. The blood samples collected were analysed for haematological parameters and Serum biochemical parameters. The results showed that the diets did not significantly affect all the haematological parameters ($p>0.05$). There are significant reductions ($p<0.05$) in the values of cholesterol, triglyceride, low-density lipoprotein, and very low-density lipoprotein, as the level of X. aethiopica fruit powder increased in the diet. In contrast, a significant increase ($p<0.05$) was observed in high-density lipoprotein. Therefore, it may be concluded that including X. aethiopica fruit powder at 400 g/100 kg, feed enhanced Serum biochemical parameters in broiler chickens.

Keywords: Broiler chicken, *Xylopia aethiopica*, Haematology, Serum biochemical indices

INTRODUCTION

The quest to meet the outrageous demand for animal protein by the teeming populace has called for the development and expansion of the poultry industry. Broilers are good sources of quality protein and there is a burning desire to increase its production in terms of quality and quantity of meat (Isikwenu *et al*., 2014). This can be achieved by raising the nutritional status and safeguarding the health and welfare of broiler species.

Antibiotic growth promoters have been used to facilitate the fast growth of broilers and reduce the incidence of diseases (Onunkwo *et al*., 2019). Despite these advantages concerns were raised and a series of critics arose on the effect of indiscriminate use of antibiotics which resulted in cross-drug resistance and accumulation of its residues in tissues of poultry. The consumption of these products posed a serious risk to human health and led to it being banned by the European Union in 2006 and the United States Department of Agriculture (Belal *et al*., 2017).

Furthermore, the high cost of antibiotics growth promoters, the technicalities involved in their usage, and scarcity especially to farmers in rural areas later gave rise to the massive use of natural grow enhancers such as plants and their extract (herbs and spices) (Manyi-Loh *et al*., 2018). Herbs and spices are useful to man because of their nutritional and medicinal properties (Czarra, 2009). Some of these useful herbs and spices are indigenous to the environment, and available at a lower cost, with limited side effects or no residues in their products. These spices and herbs are known to improve food palatability through their aroma (Dougkas *et al*., 2019), improve digestion, and immune system response and possess antibacterial, antiviral, anti-inflammatory and antioxidant properties (Greathead, 2003; Jamroz *et al*., 2003). *Xylopia aethiopica* (Dunal) A. Rich (Magnoliales: Annonaceae) is commonly known as Selim pepper, Negro Pepper, or African pepper. It is locally referred to as *Ata* (Ibibio), *Uda* (Igbo), and *Eeru* (Yoruba), and may be used as a natural feed additive in poultry diets. *X. aethiopica* is an aromatic tree that grows up to 15 – 30 m in height and has a diameter of about 60 – 70 cm. It is native to the lowland rainforest and moist fringe (Orwa *et al*., 2009; Erhirhie and Moke, 2014). The fruit is dark brown with several pods and each pod contains 5 – 8 kidney-shaped fruit grains of approximately 5 mm in length (Ngumbau *et al*., 2020). *X. aethiopica* has been reported to possess anti-microbial, anti-inflammatory, anti-oxidant and anti-bacterial characteristics. It contains phytochemical compounds such as tannin (0.79%), saponins (1.23%), phytate (0.40%), oxalate (0.21%), and flavonoid (0.5%) (Solomon *et al.,* 2022). The proximate composition of *X. aethiopica* indicated that it contains: crude protein (6.21%), either extract (7.62%), crude fibre (15.31), ash (5.24%), and nitrogen-free extract (67.61%) (Okon *et al*., 2022).

Blood is a specialized body fluid in animals that delivers essential substances such as nutrients and oxygen to the cells and transports metabolic waste products away from these same cells (IQWiG, 2006). Blood assessment gives an in-depth knowledge of the physiological, nutritional, and pathological state of any organism. Blood evaluation offers important information for the diagnosis and treatment of diseases. Thus, therefore raises the need to formulate diets containing *X. aethiopica* to assess its impact on different blood parameters. Thus, the objective of this study was to determine the effect of varying levels of *X. aethiopica* on the blood profiles of broiler chickens.

MATERIALS AND METHODS

Experimental Site: The study was conducted at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Science, Akwa Ibom State University, Obio-Akpa Campus. Obio-Akpa area lies between latitude 5^0 17 and 5^027 North of the Equator and longitude 7^021 and 5058 East with temperatures ranging between 24 – 25^0C, an average annual rainfall ranging from 3500 – 5000 mm and a relative humidity of between 60 – 90% (Essien, 2021).

Procurement and Processing of Xylopia aethiopica: The *X. aethiopica* fruit was purchased from the local market in Abak, Akwa Ibom State. The fruits were washed and sundried for seven days. The dried samples were ground into powder using a manual blender (Corona 1016, Landersy Y CIA, South Africa) to produce *X. aethiopica* fruit powder which was later packaged and stored in cellophane bags under room temperature till usage.

Experimental Diet: Five experimental finisher broiler diets were formulated and labelled T_1, T_2, T_3, T_4 and T_5. T_1 had no *X. aethiopica* fruit powder while T_2, T_3, T_4 and T_5 contained *X. aethiopica* at 100, 200, 300 and 400 g/100 kg feed respectively. The ingredients and nutrient composition of the experimental broiler diet are presented in Table 1.

Management of Experimental Birds: A total of two-hundred-day-old (Abor acre) strains of broiler chickens were purchased from a reputable poultry distributor in Uyo, Akwa Ibom State. The house was washed, disinfected and left to air dry for one week before the arrival of day-old chicks. The birds were raised in a brooder for two weeks and fed an *X. aethiopica*-free diet for four weeks.

At 28 days of age, the birds were divided into five groups of forty birds. Each group was assigned to one of the experimental diets in a completely randomised design (CRD). Each group was subdivided into four replicates of ten birds each and housed in pens measuring 2 x 2 m^2.

Table 1: Ingredient and nutrient composition of experimental broiler finisher diets incorporated with varied levels of *Xylopia aethiopica* fruit powder

Ingredients	T_1 (0 g XFP)	T_2 (100 g XFP)	T_3 (200 g XFP)	T_4 (300 g XFP)	T_5 (400 g XFP)
Maize	55.00	55.00	55.00	55.00	55.00
Soyabean meal	20.00	20.00	20.00	20.00	20.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Wheat offal	9.00	9.00	9.00	9.00	9.00
Palm kernel cake	8.00	8.00	8.00	8.00	8.00
Bone meal	4.00	4.00	4.00	4.00	4.00
Common salt	0.25	0.25	0.25	0.25	0.25
Min/vit premix*	0.25	0.25	0.25	0.25	0.25
l-lysine	0.25	0.25	0.25	0.25	0.25
l-methionine	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated chemical composition (%DM)					
Crude protein	21.37	21.37	21.37	21.37	21.37
Ether extract	4.32	4.32	4.32	4.32	4.32
Crude fibre	3.96	3.96	3.96	3.96	3.96
Ash	5.25	5.25	5.25	5.25	5.25
NFE	65.10	65.10	65.10	65.10	65.10
ME (Kcal/kg)	2911.02	2911.02	2911.02	2911.02	2911.02

*XFP = Xylopia aethiopica fruit powder, DM = Dry matter, NFE = Nitrogen free extract, *To provide the following per kg of feed, Vitamin A 10,000 IU, Vitamin D3 2000 IU, Vitamin E 12 mg, Vitamin K 2 mg, Vitamin B1 1.5 mg, Vitamin B2 4 mg, Vitamin B6 1.5 mg, Vitamin B12 12 mg, Niacin 15 mg, Pantothenic acid 5 mg, Folic acid 5 mg, Biotin 2 mg, Choline chloride 100 mg, Manganese 75 mg, Zinc 5 mg, Iron 2 mg, Copper 5 mg, Iodine 10 mg, Selenium 2.0 mg, Cobalt 5 mg, Anti-oxidant 125 mg*

The system of management was a deep lifter system and wood shavings were used as litter material. Feed and water were provided *ad libitum*. All routine medications and vaccines were administered as when due. Strict sanitation was adopted and litters were changed at appropriate times. The trial lasted for 28 days.

Haematological and Serum Biochemical Studies: On the 56th day of the experiment four birds were randomly selected from each treatment and used for haematological and blood chemistry analysis. Five (5 ml) of blood samples were collected from each of the birds from their wing vein using a sterilised syringe and transferred into bottles treated with Ethylene diamine tetra acetic acid (EDTA) for the determination of haematological parameters. packed cell volume (PCV) was determined using the capillary haematocrit centrifuge and haematocrit reader as described by Coles (1986). Haemoglobin (Hb) concentration, red blood cells (RBC), and white blood cells (WBC) were determined by cyanomethaemoglobin methods as described by Coles (1986). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated thus: MCV (%) = PCV x 10/RBC, MCH (%) = Hb x 10/RBC, and MCHC (%) = Hb x 100/PCV.

Another 5 ml of the blood sample was transferred into anticoagulant-free bottles and was used to determine blood biochemical components such as total protein was determined by the biuret method (Reinhold, 1953), albumin was determined by bromocresol green method (Doumas *et al.*, 1971). Serum globulin was estimated based on the difference between the concentrations of total protein and albumin. Cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein were determined based on the methods described by Baker *et al.* (1998). Alanine aminotransferase (AST), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) were determined using Cobas Mira Automatic Analyzer (Roche Diagnostic System, Basel, Switzerland) at 37^0 with the aid of commercial kits. Sample readings were performed using a spectrophotometer with a light wavelength adequate for each test.

Statistical Analysis: The data from the various parameters measured were subjected to analysis of variance (ANOVA) using SAS (1999) software and significant means were separated using the Duncan Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The result of haematological indices of broiler chickens fed diets containing varying levels of *X. aethiopica* fruit power indicated that there were no significant differences (p>0.05) in the values of all the haematological parameters determined (Table 2). A progressive numerical increase was observed in the values of RBC as the level of *X. aethiopica* fruit powder increased in the diet. T_5 had the highest RBC value followed by T_4, T_3, T_2 and T_1. Similar findings have been reported by Elagib *et al.* (2013), and Uzochukwu *et al.* (2019) for broilers and cockerels fed diets containing garlic and *X. aethiopica* at 5 and 3% levels respectively. The slight numerical increase observed in the value of RBC counts may be attributed to the high iron content in *X. aethiopica* fruit which must have stimulated blood formation. However, the value fell within the reference range (2.88 – 4.12 x 10^6 mm) reported by Mitruka and Rawnsley (1977). The PVC values obtained in this study were higher than the value (28.00%) reported by Akinola and Eqwuanumku (2017) for laying hens fed a diet containing *X. aethiopica* fruit power. Haemoglobin (HB) values were within the normal range (7.40 – 13.10 g/dL) reported by Mitruka and Rawnsley (1977). The increase in RBC, PCV and HB values obtained in this research suggests a beneficial synergistic effect of the phenolic compound on erythropoiesis.

The MCV, MCH, and MCHC were not significantly affected (p>0.05) by the diets. MCV, MCH, and MCHC are important blood parameters whose values are used to assess the presence and magnitude of anaemia (Tvedten, 2010). The values for the three parameters were within the reference range of 90 – 14 fl and 26 – 40 fl reported by Bounous *et al.* (2000) and Talebi *et al.* (2005) respectively.

White blood cell (WBC) count values were not negatively influenced by the diets. WBCs are immune cells that protect the blood against disease and pathogen invasion. A decrease in WBC indicates suppression of leukocytes and their production from bone marrow which may be a result of infection or regeneration of anaemia (Adefisan *et al.*, 2020). Elevated leucocytes are considered a risk factor for cardiovascular and ischaemic disease. Since anaemia is associated with hypoxia and ischaemia, it may be possible that it may affect white blood cells (Singh, 2010). *X. aethiopica* has been shown to possess immune stimulating potentials through its content of flavonoid, one of the bioactive components of *X. aethiopica*. This result may be an indication that *X. aethiopica* powder has the potential to boost the phagocytic actions of white blood cells. The values recorded for WBC in this research were within 10.30 x 10^3 mm^3 reported by Uzochukwu *et al.* (2019) for *X. aethiopica* fruit powder. Lymphocytes; heterophils monocytes and eosinophils were not significantly influenced (p>0.05) by the diets. The values for lymphocytes fell within the reference range of 45 – 70% and 45 – 75% reported by Tvedten (2010) and Oleforuh-Okoleh *et al.* (2015) respectively. There was a trace of basophil in all the treatments. Basophils are small cells that raise alarm when infectious agents invade the body. They secrete chemicals such as histamine, a marker of allergic diseases that helps to control the body's immune system (Chirumbolo *et al.*, 2018).

The results of the serum biochemical indices of broiler chickens fed *X. aethiopica* fruit powder indicated that there were no significant differences (p>0.05) in the values for total protein across treatments (Table 3). Serum protein is associated with protein quality and quantity in the diets. Serum protein serves as a source of replacement for tissue protein, it acts as a buffer in acid-base balance and aids in the transportation of blood constituents such as vitamins, iron copper, hormones, lipids, and enzymes (Ayodele and Funmilayo, 2013). The result for serum protein is in line with the report of Ayodele *et al.* (2021) who recorded non-significant serum protein values in broiler chickens fed turmeric powder.

Table 2: Haematological indices of broiler chicken fed dietary inclusion of varied levels of *Xylopia aethiopica* fruit powder

Parameter	T_1 (0 g XFP)	T_2 (100 g XFP)	T_3 (200 g XFP)	T_4 (300 g XFP)	T_5 (400 g XFP)
Red blood cell ($x10^6$/HL)	2.55 ± 0.04	2.81 ± 0.08	2.92 ± 0.09	3.01± 0.10	3.34± 0.12
Haemoglobin (g/dL)	12.04 ± 0.03	12.13 ± 1.22	12.21 ± 0.00	13.01± 1.25	3.04± 0.10
PCV %	35.75 ± 0.13	35.81 ± 4.08	36.10± 3.04	36.05± 3.03	36.26± 4.63
MCV (fl)	91.34 ± 0.09	90.23 ± 8.18	90.55± 13.18	91.10± 13.24	91.51± 13.28
MCH (pg)	37.28 ± 0.31	36.54 ± 4.19	36.78± 3.09	37.35± 3.11	37.42± 3.11
MCHC (g/dL)	33.51 ± 0.30	34.00 ± 3.29	33.63 ± 3.02	34.12 ± 3.04	33.51 ± 3.06
WBCL x $10^3/\mu$	8.75 ± 0.21	9.23 ± 0.79	9.59 ± 0.74	9.71± 0.75	9.21± 0.70
Lymphocyte (%)	61.25 ± 0.06	61.51 ± 6.14	61.65 ± 5.15	62.45 ± 5.15	61.51 ± 5.14
Heterophils (%)	30.15 ± 0.03	30.35 ± 3.60	30.43 ± 3.60	31.02 ± 3.98	30.51 ± 3.96
Monocytes (%)	4.05 ± 0.04	4.31 ± 0.16	4.25 ± 0.13	4.41 ± 0.13	4.81 ± 0.15
Basophils (%)	0.00	0.00	0.00	0.00	0.00
Eosinophils (%)	2.35 ± 0.02	2.21 ± 0.06	2.50 ± 0.04	2.23 ± 0.04	2.75 ± 0.07

XFP = Xylopia aethiopica fruit powder, PCV = Packed Cell Volume, MCV = Mean Corpuscular volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration

Table 3: Serum biochemical indices of broiler chicken fed dietary inclusion of varied levels of *Xylopia aethiopica* fruit powder

Parameter	T_1 (0 g XFP)	T_2 (100 g XFP)	T_3 (200 g XFP)	T_4 (300 g XFP)	T_5 (400 g XFP)
Total protein (g/dL)	5.15 ± 0.03	5.07 ± 0.02	5.13 ± 0.03	5.13 ± 0.03	5.03 ± 0.02
Albumin (g/dL)	2.84 ± 0.01	2.67 ± 0.01	2.41 ± 0.01	2.55 ± 0.01	2.52 ± 0.01
Globulin (g/dL)	2.31 ± 0.01	2.40 ± 0.01	2.82 ± 0.02	2.59 ± 0.02	2.51 ± 0.01
AST (μ/L)	80.12 ± 0.21	79.56 ± 0.19	80.31 ± 0.20	80.01 ± 0.20	79.81 ± 0.19
ALT (μ/L)	25.51 ± 0.13	25.02 ± 0.12	24.51 ± 0.13	25.11 ± 0.13	24.67 ± 0.12
ALP (μ/L)	34.56 ± 0.14	34.11 ± 0.13	35.00 ± 0.15	34.75 ± 0.14	35.34 ± 0.14
Cholesterol (mg/dL)	140.31 ± 2.04^e	121.44 ± 1.10^d	117.03 ± 1.09^c	102.41 ± 1.06^b	91.02 ± 1.07^a
Triglyceride (mg/dL)	150.51 ± 2.00^e	138.56 ± 2.00^d	124.01 ± 1.18^c	111.53 ± 1.16^b	90.10 ± 1.06^a
HDL (mg/dL)	60.02 ± 0.20^a	70.25 ± 0.22^b	74.04 ± 0.23^c	76.68 ± 0.25^d	84.21 ± 0.29^e
LDL (mg/dL)	55.95 ± 0.16^d	54.06 ± 0.5^c	28.58 ± 0.03^b	16.86 ± 0.04^a	16.08 ± 0.04^a
VLDL (mg/dL)	32.05 ± 0.12^e	27.51 ± 0.12^d	24.31 ± 0.11^c	22.01 ± 0.09^b	18.61 ± 0.05^a

XFP = Xylopia aethiopica fruit powder, ALT = Alanine Aminotransferase, ALP = Alkaline Phosphatase, AST = Aspartate Aminotransferase, HDL = High-Density Lipoprotein, LDL = Low-Density Lipoprotein, VLDL = Very Low-Density Lipoprotein, $^{a-e}$ = means along a row with different letter superscripts are significantly different ($p<0.05$)

The similarity in value suggests adequate protein intake by birds and non-inhibition of protein metabolism. However, the values for serum protein obtained in this research fell within the reference range of 2.5 – 5.5 g/dL reported by Knottnerus *et al.* (2002), Thrall (2007) and Bogusławska-Tryk *et al.* (2012) for Broiler chickens.

Albumin and globulin were not negatively influenced ($p>0.05$) by the diets. The values recorded for albumin fell within the reference range of 2.10 – 3.45 g/dL reported by Mituka and Rawnsley (1977) and 1.54 – 3.2 g/dL reported by Nworgu *et al.* (2007).

Albumin's function is the transporting and storage of a wide variety of ligands e.g. fatty acids, calcium, bilirubin and hormones such as thyroxin. High concentrations of albumin in the blood usually indicate dehydration while low concentrations may be a result of inadequate functioning of the liver due to malnutrition and infection (Esubonteng, 2011).

Globulins are a group of proteins in the blood serum or stream that help to regulate the function of the circulating system. A reduction in globulin level prevents blood clotting and transportation of nutrients to the muscles. The values for globulin fell within the reference range (2.13 – 3.02 g/dL) reported by Adeyomo (2008).

There were no significant differences ($p>0.05$) in the values of the liver enzymes; AST, ALT, and ALP across the treatments. AST, ALT, and ALP values suggest the presence of non-toxic substances in the diets and that the diets were devoid of anti-nutritional substances. The result corroborates with the findings of Emadi and Kermanshahi (2007) where birds fed turmeric powder recorded non-significant values for AST, ALT, and ALP. These results disagreed with the report of Ekine *et al.* (2020) who recorded a significant increase in AST, and ALT values of broiler chicken-fed turmeric powder. Also, Ayodele *et al.* (2021) reported a significant increase in ALP values but a decrease in AST value in broiler chickens fed 2% turmeric and clove powder. AST and ALT are important in the determination of the proper functioning of the liver. ALP is produced by the intestinal mucosa, liver, bone and kidney (Aikpitanyi and Eqweh, 2020). Higher levels of ALP were observed when there is increased osteoblastic activity involving the formation and mineralization of bone (Lumeji, 2008). The values obtained for ALP in this research were within the normal range recommended for poultry (24.50 – 44. 40 NW) (Mitruka and Rawnsley, 1977).

Significant differences ($p<0.05$) were observed in cholesterol and triglyceride values. Cholesterol and triglyceride values decreased as the level of *X. aethiopica* fruit powder increased in the diets. The highest significant values ($p<0.05$) were recorded in T_1 (control). The reductions in cholesterol and triglyceride levels may be attributed to the antioxidant properties of *X. aethiopica* which inhibit lipid peroxidation. *X. aethiopica* fruit has been reported to contain several powerful anti-oxidants such as glutathione superoxide dismutase, vitamin C and Vitamin E (Solomon *et al*; 2022).

The results for cholesterol agreed with the reports of Aikpitanyi and Eqweh (2020) who reported a significant decrease in values for cholesterol in broiler chickens fed diets containing ginger and black pepper. Also, the decrease in triglyceride values agreed with the findings of several authors (Ekine *et al*, 2020; Ayodele *et al.,* 2021; Essien and Udoh, 2021) who recorded significant reductions in triglyceride levels when broiler chickens were fed diets containing *Tetrapleura tetraptera,* ginger and black pepper, turmeric and clove respectively. High-density lipoprotein values increased significantly across treatment with an increase in *X. aethiopica* fruit powder. High-density lipoprotein also known as 'good' cholesterol helps to get rid of excess cholesterol in the body. HDL picks up cholesterol from other lipoproteins and body cells and returns them to the liver to be reused or eliminated. The increase in HDL value in this study may be attributed to the enhanced hypocholesterolemic mechanism and hypolipidemic action of *X. aethiopica* as a phytogenic feed additive (Tosheska Trajkovska and Topuzovska, 2017).

Significant reductions ($p<0.05$) were observed in the values of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) with chicken-fed *X. aethiopica* fruit powder. The T_1 (control) group recorded the highest significant values ($p<0.05$) for LDL and VLDL. LDL has been reported to be the major transporter of cholesterol in the bloodstream and is considered bad cholesterol. LDL carries fats out of the liver into the blood vessels and this action seems to enhance arterial cholesterol disposition (Prasad *et al*., 2009). The significant decrease ($p<0.05$) recorded in LDL and VLDL in this study suggests *X. aethiopica* fruit powder is a potential hypolipidemic agent. *X. aethiopica* fruit is rich in antioxidants such as flavonoids and vitamins (Lee and Choe, 2018). This seems to be the contributing factor to the decrease in LDL and VLDL in broiler chickens. However, the average LDL levels in broiler chickens in this study fell within the normal range (16.08 mg/dL) reported by Basmacioğlu and Ergül (2005). The average VLDL levels fell within the normal range of 18.61 mg/dL which is between 3 – 44 mg/dL reported by Stevens (1996).

Conclusion: The use of *X. aethiopica* fruit powder as a feed additive in a broiler diet at 400 g/100 kg feed had a beneficial effect on some serum biochemical parameters such as cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein and very low-density lipoprotein.

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