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THE RESPONSE OF BROILER CHICKENS TO TWO DIFFERENT PHYTOGENIC FEED ADDITIVES AS POTENTIAL NATURAL GROWTH PROMOTERS

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ABSTRACT
Globally, the poultry industry has been facing increasing pressure to produce improved quality, healthy and safe meat and egg. This has kindled research interest in using natural feed additives. This experiment was conducted to evaluate the response of broiler chickens to two different phytogenic feed additives, namely, African black pepper (Piper guineense) and Negro pepper (Xylopia aethiopica) as potential natural growth promoters. Two hundred and ten Cobb 500 broiler chicks were randomly allotted to seven treatments with three replicates of ten birds each. Treatment 1 was the control diet without Xylopia aethiopica and Piper guineense. Treatments 2, 3 and 4 were diets supplemented with 4g, 8g and 12g of Piper guineense each per 1kg of feed, respectively, while treatments 5, 6 and 7 were diets formulated with 4g, 8g and 12g of Xylopia aethiopica per 1kg of feed. The study lasted for seven weeks. The phytochemical screening of Piper guineense showed moderately present (++) phenols and trace amounts (+) of flavonoids. Xylopia aethiopica, had flavonoids in high concentration (+++), whereas phenols, alkaloids, tannins and oxalate were moderately present (++). Notably, both spices had no cyanide. The dietary supplementation with phytogenic feed additives significantly improved weight gain, final live weight and feed conversion ratio. Treatment 7 produced the best result, closely followed by Treatment 5. Remarkably, birds fed diets containing the phytogenic additives responded positively with a significant ($p<0.05$) enhancement in growth performance. These spices can be utilized as potent phytogenic growth promoters in broiler production.

Keywords: *phytogenic additives; growth promoter; spices; performance status; broilers*

INTRODUCTION

There is a growing public health concern about the quality and safety of poultry products such as meat and eggs because of the residual effects of synthetic antibiotics in these products and the antimicrobial resistance to antibiotics. The expansion of antibiotic-resistant pathogenic microbes has always been the negative effect of using sub-therapeutic antibiotic concentrations in poultry feed (Hussein *et al*., 2020; Ricke *et al*., 2020). There has been increasing pressure on the poultry industry to produce healthy and safe chicken meat and egg, which has ignited research interest in using natural feed additives, like phytogenic feed additives.

Globally, different types of spices have been used as food additives for several years (Akbarian *et al*., 2012). Spices are mainly used for seasoning, flavouring and colouring in food preparation and cooking. Plant-derived additives such as spices, herbs and other plant materials and extracts incorporated in animal feed to im-

prove performance are called phytogenic feed additives (PFA) (Windisch *et al*., 2008; Karaskova *et al*., 2015). Phytogenics are also referred to as phytobiotics, and these have been used since time immemorial but with little knowledge of their mode of action in humans and animals. Notwithstanding, recent advances have been made to elucidate their roles and effects on living organisms (Alloui *et al*., 2014). According to Karaskova *et al*. (2015), phytogenic additives are a potential alternative to antibiotics as they enhance many vital processes in the animal body. The gradual withdrawal from synthetic antibiotics supplementation in feeds as growth stimulants have increased research interest in discovering and developing alternative supplements, management measures and dietary modifications targeting improvement in animal productivity, health and welfare (Abdelli *et al*., 2021).

The use of PFA as an antioxidant is not only limited to improvement in poultry health but as well as for the oxidative stability of their products, particularly meat. It is worth noting that the antioxidant activities of plant-based feed additives can immensely contribute to dietary lipids protection from oxidation (Alloui *et al*., 2014). Additionally, these phytogenics exhibits hypolipidemic property associated with improving the shelf-life of various animal products. Notably, this hypolipidemic property is used to produce lean meat (Prabakar *et al*., 2016).

Phytogenic products like herbs and spices have been utilized for many years as therapeutics for humans and in ethno-veterinary interventions. However, in recent times their usage in the feed industry is gaining ground (Azodo *et al*., 2021). Several researchers have reported the beneficial properties of Mediterranean spices for use as phytobiotics as a possible alternative to antibiotics in poultry production. Their use has enhanced productive performance and the general well-being of poultry birds (Sahib *et al*., 2022).

Negro pepper is a spice and a rich source of phytochemicals and possesses free radical quenching properties. Thus, it could be used as a potential natural antioxidant in food systems (Okechukwu-Ezike and Oly-Alawuba, 2020; Aliyu *et al*., 2022). Phytochemical constituents of Negro pepper are phenols, alkaloids, tannins, oxalates, flavonoids and saponins, which are linked with various biological and pharmacological properties. It is also a good source of zinc, magnesium, calcium, potassium and iron (Solomon *et al*., 2022).

Broiler chickens that had diets supplemented with Negro pepper recorded better growth performance in terms of body weight gain and feed conversion ratio when compared to those on synthetic antibiotics. Negro pepper has growth-promoting potential and can be used as a natural replacement for antibiotics in broiler production (Isikwenu and Udomah 2015a; Isikwenu and Udomah 2015b).

Black pepper is one of the most extensively used spices (McCormick Science Institute, 2022). It is valued for its distinctive biting effect, linked to piperine, an active compound in black pepper. Piperine is an alkaloid which exerts a stimulatory effect on the digestive enzymes of the pancreas, boosting digestive capacity and markedly reducing the gastrointestinal food passage time. It also can enhance the bioavailability and absorption of some herbal and conventional drugs (Srinivasan, 2007; Meghwal and Goswami 2013).

Generally, most of the previous studies carried out by researchers on these phytogenics were not based on livestock nutrition and performance but were majorly focused on their chemical composition, medicinal and pharmacological properties which mainly involved *in vitro* and not *in vivo* experimentations. Therefore, this research was conducted to assess broiler chickens' response to two different phytogenic feed additives as potential growth promoters.

MATERIALS AND METHODS

Location of the study

This research study was carried out at the Poultry Unit of the Research Farm of the Department of Animal Production, School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State, Nigeria.

Source of the test ingredients (phytogenic additives)

Xylopia aethiopica and *Piper guineense* were procured from Kure ultra-modern market, Minna, Niger State, Nigeria. The test ingredients were sorted to remove debris that might be present and were later separately grounded to pow-

der using a hammer mill.

The proximate analyses of the test ingredients
The proximate analyses of *Piper guineense* and *Xylopia aethiopica* were carried out as described by AOAC (2000). The compositions that were evaluated are moisture content, crude protein, ether extract, crude fibre and ash, while nitrogen free extract was estimated by difference that is; by subtracting the moisture, crude protein, crude fibre, ether extract and ash contents from 100%. The dry matter was obtained by subtracting the moisture content value from 100%.

The procedure for proximate analysis is as follows:
Moisture: This was determined by drying a sample in an oven at 105°C until a constant weight is reached. The dry matter was obtained by subtracting the moisture content value from 100%.

Crude protein: The Kjeldahl method was used in the determination of the crude protein content of the samples. In this method, the samples were digested with sulfuric acid, and the ammonia that is released was then distilled and titrated with a standard acid solution.

Ether extract: The ether extract or the crude fat content was determined by extracting the fat from the sample with a solvent, such as petroleum ether. The crude fat was then weighed and expressed as a percentage of the total sample weight.

Crude fibre: This was determined by weighing 2-3 g of the sample into a crucible and 25 ml of 1.25% sulfuric acid was added to the crucible. The crucible was heated in a water bath at 80°C for 30 minutes, after cooling the crucible, 25 ml of 1.25% sodium hydroxide solution was added and heated in a water bath at 80°C for another 30 minutes. The crucible was allowed to cool down and the weighed. The difference in weight between the initial weight and the final weight is the crude fibre content.

Ash: The ash content was determined by burning of samples in a muffle furnace at 550°C. The ash was then weighed and expressed as a percentage of the total sample weight.

Phytochemical screening of the spices
The phytochemical screening analysis of *Piper guineense* and *Xylopia aethiopica* was conducted and the procedure for the tests is outlined as follows;

Phytate
The method is a modified indirect colorimetric method that depends on an iron-phosphorus ratio of 4:6. The method is based on the ability of standard ferric chloride to precipitate phytate in dilute HCl extract of the sample. The phytate is precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding 5 ml of 1M NaOH. The precipitate is dissolved with hot 3.2M HNO_3 and the absorbance is measured immediately at 480 nm. A standard curve of different $Fe(NO_3)_3$ concentrations is plotted against the corresponding absorbance of the spectrophotometer to calculate the ferric iron concentration. The phytate phosphorus is calculated from the concentration of ferric iron assuming a 4:6 iron: phosphorus molar ratio (Wheeler and Ferrel, 1971).

Oxalate
The method is a qualitative test for the presence of oxalate ions. The test is based on the reaction of oxalate ions with potassium permanganate ($KMnO_4$) in dilute sulfuric acid (H_2SO_4). The reaction produces a pink colour, which indicates the presence of oxalate ions (Brown, 2009).

Anthocyanins
The presence of anthocyanins was determined by adding 2 ml of the sample extract with 2 ml of HCl. The test is based on the reaction of anthocyanins with acids to form pink-red colour. The addition of ammonia to the pink-red solution causes the colour to change to purplish-blue which indicates the presence of anthocyanins (Harborne, 1998).

Cyanide
The qualitative test for the presence of cyanide ions was conducted by preparing a blank solution by adding 5 ml of distilled water to a 10 ml volumetric flask. 1 ml of the sample solution was pipetted into a 10 ml volumetric flask. Afterwards, 4 ml of distilled water was added to the sample solution. 1 ml of ferric chloride solution

and 1 ml of hydrochloric acid solution were also added to the sample solution. The solution was well mixed and allowed to stand for 5 minutes. The absorbance of the solution was measured at 410 nm using a UV-V is spectrophotometer. A positive test is indicated by the formation of a blue precipitate or greenish-blue colour. The intensity of the colour is proportional to the concentration of cyanide ions in the sample (Vogel, 1996).

Tannins
To determine the presence of tannins, 0.5g of the dried powdered sample was boiled in 20 ml of distilled water in a test tube and filtered. Afterwards 0.1% ferric chloride ($FeCl_3$) solution was added to the filter. The appearance of brownish green or a blue-black colouration indicates the presence of tannins in the test sample. The brownish-green or blue-black colour is due to the formation of a complex between the tannin molecule and the ferric ion (Harborne, 1998).

Terpenoids
One millilitre of the sample extract was added to 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, the formation of a reddish-brown precipitate indicates the presence of terpenoids in the plant. The reddish-brown precipitate is due to the formation of a complex between the terpene molecule and sulfuric acid (Harborne, 1998).

Flavonoids
The Alkali Reagent Test as outlined by Harborne (1998) was used in the determination of the presence of flavonoids in the sample, 0.5g of the sample was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Yellow colouration was observed thus indicating the presence of flavonoids. The yellow colour is due to the deprotonation of the flavonoid molecule, which exposes the conjugated double bonds in the molecule.

Steroids
The qualitative test for detecting the presence of steroids in the sample is known as the Liebermann-Burchard Reaction as described by Harborne (1998). This procedure involved the measurement of 0.5 ml of the sample extract, which was then mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Saponins
The test used for the determination of the presence of saponins is known as the Foam Test or the Emulsion Test, the involves the measurement of 2.0 g of the powdered sample boiled in 20 ml of distilled water in a test tube in a boiling water bath and filtered. Measurement of 10 ml of the filtrate was done and then mixed with 5 ml of distilled water and shaken vigorously. The formation of stable foam indicated the presence of saponins. A drop of olive oil was added to the foam and shaken again, the formation of emulsion confirms the presence of saponins (Harborne, 1998).

Alkaloids
The method employed for the determination of alkaloids is known as the Picric acid test or Mayer's test. The sample extract of 0.5ml was measured and stirred with $5cm^3$ of 1% aqueous HCI on a steam bath, few drops of the picric acid solution were added to $2cm^3$ of the extract. The formation of a reddish-brown precipitate confirmed the presence of alkaloids (Harborne, 1998).

Cardiac glycoside
For the determination of cardiac glycosides, a 10% extract of the sample was mixed with 10 ml of freshly prepared Baljet's reagent (95 ml of 1% picric acid + 5 ml of 10% NaOH). After an hour, the mixture was diluted with 20 ml of distilled water and the absorbance was measured at 495 nm. For the preparation of the standard curve, 10 ml of different concentrations (12.5-100 mg/l) were prepared. The production of a yellow-orange complex with Baljet's reagent confirms the presence of cardiac glycosides or its lack of production confirms its absence in the sample (Solich *et al*., 1987).

Phenols
The qualitative test for the presence of phenols in a sample is called the Liebermann-Burchard

Test. The sample solution of 1 ml was measured into a test tube and 1 ml of concentrated sulfuric acid was then added to the sample solution in the test tube. This was then followed by the addition of 1 ml of a solution of amyl alcohol and sodium nitrite to the test tube. A positive test was indicated by the formation of a blue or green colour. The intensity of the colour is proportional to the concentration of the phenol in the sample (Singleton and Rossi, 1965; Liebermann and Burckhardt, 1882).

Experimental design

A total of two hundred and ten (210) one-day-old Cobb 500 broiler chicks were randomly allotted to seven experimental treatment groups, and each dietary treatment had three replicates of ten birds. A completely randomized design was used in the experiment. Treatment 1 was the control diet without the test ingredients (phytogenic additives). Treatments 2, 3 and 4 had diets supplemented with 4g, 8g and 12g of *Piper guineense* per 1kg of feed, respectively. While treatments 5, 6 and 7 had diets formulated with 4g, 8g and 12g of *Xylopia aethiopica* per 1kg of feed. The experimental feeding trial was conducted for seven weeks. The single-phase feeding method was used in this experiment (Table 1).

Table 1: Composition of experimental diet in a single-phase feeding

Ingredient	Percentage (%)
Maize	44.10
Maize offal	12.00
Full-fat soybeans	36.65
Fish meal	4.00
Limestone	0.58
Bone meal	1.82
Vitamin premix	0.25
Salt	0.25
Methionine	0.25
Lysine	0.10
Total	100
Calculated	
Crude protein (%)	22.15
Energy (kcal/kg ME)	3048.74

***ME** = Metabolizable energy*

Nutritional profile of the experimental diet

The nutritional profile of the diet used was determined by proximate analysis, the average nutrient content of the diet was recorded as follows; dry matter 93.40%, crude 23.10%, crude fibre 5.25%, ether extract 5.00%, and ash 5.75%. The nitrogen free extract content of 54.30% was obtained by subtracting the moisture content, crude protein, crude fibre, ether extract and ash contents from 100%.

Management of experimental birds

At the onset of the experiment, the chicks were weighed to obtain their initial weights, and afterwards, they were randomly distributed to the seven treatment groups. The broilers were reared on deep litter. Wood shavings were used as litter material. All the experimental birds were allowed free access to feed and water on an *ad libitum* basis. The diets were formulated to meet National Research Council (NRC) recommendations (NRC, 1994). The birds were raised under standard management conditions (Khan, 2020). Birds were vaccinated against the prevalent diseases following the recommended vaccination schedule. The first dose of Gumboro vaccine was administered at week one against Gumboro disease, and at week two the first dose of Lasota vaccine was administered to the birds against Newcastle disease. When the birds were three weeks old the second dose of Gumboro vaccine was given. The last dose of Lasota vaccine was administered in the fourth week. The feeding trial was carried out for seven weeks. The measurement of feed intake was conducted daily, while the weighing of birds was done weekly.

Data analysis

All data collected during the experiment from the measured parameters were subjected to one-way analysis of variance (ANOVA) using IBM SPSS version 23.0. The significant means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

Proximate composition of *Piper guineense* and *Xylopia aethiopica*

The proximate composition of *Piper guineense* and *Xylopia aethiopica* is presented in Table 2. In this experiment, *Piper guineense* had a crude protein of 11.98% and a value of 5.02% for crude fibre, which is close to the crude protein

value of 12.20% and the value of 5.32% crude fibre as reported by Udofia and Alozie (2015). These results imply that *Piper guineense* could be a potential feed supplement. The results of this experiment also suggest that *Piper guineense* could be a valuable additional source of nutrients in the diet as confirmed by the findings of Udofia and Alozie (2015). *Xylopia aethiopica* had 10.20% moisture, 8.40 % crude protein, 5.25% ether extract, 6.50% crude fibre, 5.50% ash and 64.15% nitrogen free extract. Okon *et al*. (2022) reported an ash content of 5.24%, which is close to the value of 5.50% obtained for *Xylopia aethiopica* in this study. The moisture content of 10.20% for this spice was slightly lower than the value of 10.74% obtained by Udofia and Alozie (2015). These results imply that *Xylopia aethiopica* could be a potential alternative to antibiotics in that it provides nutrients alongside other benefits, contrary to the use of antibiotics which do not provide nutrients.

Table 2: Proximate composition of *Piper guineense* and *Xylopia aethiopica*

Nutrients	*Piper guineense* (%)	*Xylopia aethiopica* (%)
Moisture content	7.60	10.20
Crude protein	11.98	8.40
Crude fibre	5.02	6.50
Ash	4.00	5.50
Ether extract	4.42	5.25
Nitrogen free extract	66.98	64.15

Phytochemical analysis of *Piper guineense* and *Xylopia aethiopica*

The phytochemical composition of *Piper guineense* and *Xylopia aethiopica* powder is depicted in Table 3. *Piper guineense* had moderately present phenols (++), while saponins, alkaloids, tannins, cardiac glycosides, terpenoids and flavonoids, oxalate and phytate were present in trace amounts (+). In *Xylopia aethiopica*, flavonoids were present in high concentration (+++), whereas phenols, alkaloids, tannins and oxalate were moderately present (++). *Xylopia aethiopica* had trace amounts (+) of saponins, glycosides and phytate. However, results showed the absence of cyanide in both spices.

In this study, the presence of saponins and alkaloids in *Piper guineense* was observed to be in trace amounts (+), which is in line with the findings of Udofia and Alozie (2015). While in *Xylopia aethiopica* moderate concentration (++) of phenol was observed, while oxalate and glycoside were found to be in trace amount (+), which was in line with the reports of Okon *et al*. (2022). However, for flavonoids, these authors recorded moderate concentration (++), whereas a high concentration (+++) was observed in this study.

Piper guineense has trace amounts of saponins and alkaloids, which are both compounds with antimicrobial properties. The implication is that *Piper guineense* could help to prevent bacterial infections in broiler chickens. The presence of moderate amounts of phenols suggests substantial free radical scavenging activity of *Piper guineense.*

Xylopia aethiopica has a moderate concentration of phenol, which is also a compound with antioxidant and antimicrobial properties. In addition, *Xylopia aethiopica* has high levels of flavonoids, which are also antioxidants that can help to protect broiler chickens from oxidative stress and disease.

Table 3: Phytochemical analysis of *Piper guineense* and *Xylopia aethiopica*

Phytochemicals	*Piper guineense*	*Xylopia aethiopica*
Saponins	+	+
Alkaloids	+	+ +
Phenols	+ +	+ +
Tannins	+	+ +
Steroids	+	+
Cardiac glycosides	+	+
Terpenoids	+	-
Flavonoids	+	+ + +
Cyanide	-	-
Anthocyanins	-	+
Oxalate	+	+ +
Phytate	+	+

absent (-), trace presence (+), moderately present (++) and highly present (+++)

The overall implication of the results of this experiment suggests that both *Piper guineense* and *Xylopia aethiopica* could be potential feed additives for broiler chickens because of their valuable phytochemical composition.

Growth performance of broilers fed diets supplemented with phytogenic additives (*Piper guineense and Xylopia aethiopica*)

The growth performance of broilers fed diets supplemented with phytogenic additives (*Piper guineense and Xylopia aethiopica*) is shown in Table 4. Broiler chickens fed diets containing phytogenic feed additives showed positive responses. As observed from the results obtained, the birds recorded significant improvement in weight gain, final live weight and feed conversion ratio than birds in the control treatment group.

Hernandez *et al*. (2004) reported that using aromatic plants in poultry diets has a stimulatory influence on digestion by increasing the production of digestive enzymes and the utilization of digestive products through enhanced liver function. According to Effiong and Ochagu (2019), broiler diets can be supplemented with African black pepper seed meal at 4g/1kg of feed (0.40%) during the brooding phase for optimum performance, however from this present research, African black pepper was successfully included up to the level of 12g per 1kg of feed that is 1.20% throughout the feeding trial of seven weeks with no adverse effect on the performance of birds. Piperine, an active component of black pepper, has been linked with the ability to enhance digestive enzyme activities. The improved performance of birds fed diets supplemented with black pepper may be associated with its stimulatory effect on digestive enzymes for a better digestion process (Abou-Elkhair *et al*., 2014).

Treatment 7 (G7) produced the best result, closely followed by Treatment 5 (G5). Probably, the high concentration (+++) of flavonoids in *Xylopia aethiopica* may be responsible for this improved performance. Remarkably, the dietary effect of these spices generally led to a significant ($p<0.05$) enhancement in the performance status of broiler chickens. Solomon *et al.* (2022) concluded from their experiment that including

Table 4: Growth performance of broilers fed diets supplemented with phytogenic additives (*Piper guineense and Xylopia aethiopica*)

Parameters	G1	G2	G3	G4	G5	G6	G7	SEM	P-Value	Sig
Initial weight (g)	86.00	86.00	86.67	86.33	86.00	86.00	86.33	0.225	0.987	NS
Final weight (g)	1868.67^{b}	1942.67^{ab}	2025.17^{ab}	1918.33^{ab}	2117.83^{a}	1964.00^{ab}	2151.50^{a}	31.821	0.024	*
Average weight gain (g)	1782.67^{b}	1856.67^{ab}	1938.50^{ab}	1832.00^{ab}	2032.00^{ab}	1869.66^{ab}	2065.00^{a}	32.293	0.030	*
Average feed intake (g)	3221.20	3093.97	2859.03	2929.63	2985.43	2902.03	3031.27	57.557	0.735	NS
Feed conversion ratio	1.81^{b}	1.66^{ab}	1.47^{a}	1.59^{a}	1.47^{a}	1.56^{a}	1.47^{a}	1.276	0.012	*

Note: *Means bearing different superscripts within the same row differ significantly*
NS*=Not significant*
Sig *= Significance*
** = P < 0.05*
SEM *= Standard error of mean*
The treatment groups were designated as follows:
G1 *= diet supplemented with 0% (without phytogenic additives (control)*
G2 *= diet supplemented with 4g of Piper guineense per 1kg of feed*
G3 *= diet supplemented with 8g of Piper guineense per 1kg of feed*
G4 *= diet supplemented with 12g Piper guineense per 1kg of feed*
G5 *= diet supplemented with 4g Xylopia aethiopica per 1kg of feed*
G6 *= diet supplemented with 8g Xylopia aethiopica per 1kg of feed*
G7 *= diet supplemented with 12g Xylopia aethiopica per 1kg of feed*

0.40% of Negro pepper powder in the diet of broiler chickens improved growth performance and did not adversely affect the carcass characteristics and internal organs of the broiler chickens. It is worth mentioning that these authors recommended a 4g inclusion level of Negro pepper per 1kg of feed; because that was the highest inclusion level they used in their experiment. But in this study, 4g/1kg of feed was the starting inclusion level. It was, however, discovered from this experiment that Negro pepper can be included up to 12g/1kg of feed for optimum growth performance of broiler chickens

CONCLUSION

The spices used in this research as potential phytogenic feed additives in broiler diets produced a notable positive effect on their growth performance as reflected in their body weight gain, the conversion ratio, and the final live weight when compared with the control diet without the spices. Based on the experimental findings from this study, it was concluded that Negro pepper could be used as a natural growth promoter and for enhancing the performance of broilers. Thus, the addition of 12g Negro pepper per 1kg of feed (Treatment 7) is therefore recommended in the diet of broiler chickens since it recorded the best performance. A further research study may be required to ascertain the active component responsible for the improved performance and their specific mode or mechanism of action. In addition, future research may consider examining the effect of using higher inclusion levels of these spices than that used in this present study on the performance of birds, separately or in combination.

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