

*Original Research Article*

## **Influence of dietary manipulation on coping with stress in pig production**

Olufemi Adebukola **Adebisi**<sup>1</sup>, Isaac Oluseun **Adejumo**<sup>1,2</sup>, Elizabeth Abiola **Ajayi**<sup>1</sup>, Emmanuel Ikemefuna **Okiwelu**<sup>1</sup>

<sup>1</sup>*Department of Animal Science, University of Ibadan, Nigeria*

<sup>2</sup>*Helix Biogen Institute, Nigeria*

**Correspondence to:**

**I.O. Adejumo**, Department of Animal Science, University of Ibadan, Nigeria;

e-mail: isaac.ade@helixbiogeninstitute.org; oa.adebisi@ui.edu.ng; +2348066446246; +2348108149676

### **Abstract**

Pigs for commercial production are often housed intensively because of its benefits, such as proper management of the animals, record-keeping, feed and water provision and management. However, intensive systems of production may also produce stress in animals, arising from operations such as weaning, feed reduction or manipulation, and vaccination. In Africa, pig farmers feed cassava peels to pigs to reduce the cost of production despite its low protein content and anti-nutritional factor (hydrocyanide). However, little is known about the effect of this feed change in terms of stress on weaners. In this study, we replaced maize with cassava peels and supplemented the diets with a fixed amount of tryptophan (Trp) to investigate its effect on performance, haematology, behavioural response of pigs to weaning, and their response to metabolic stress. Crossbred weaner pigs were used and randomly assigned to three treatments in a completely randomised design. Each treatment had three replicates and each replicate had three animals. The feeding trial lasted for four weeks. Dietary manipulations did not influence the growth performance characteristics and behavioural response of the animals. Packed cell volume, haemoglobin, red blood cell counts, and neutrophils were significantly ( $p < 0.05$ ) higher for 20% cassava peel as a replacement for maize but lower for a 40% cassava peel-based diet when compared with the control group. Feeding cassava peels as a replacement for maize in weaner pigs may be carefully considered, although further studies in this direction are recommended.

**Keywords:** cassava; corticosterone; cost; diets; haematology; peels; performance; pigs; stress; tryptophan

### **INTRODUCTION**

In commercial pig production, intensive system of production is mostly adopted because of some of its benefits, such as proper management of the animals, record-keeping, feed and water provision and management. However, intensive system of production may also produce stress in animals (Barnett et al., 2001). Stress is a term that could mean different things in different fields of human endeavors. Simply put, stress could mean any social imbalance or disequilibrium producing discomforts in the social structure within

a society or population (Fink, 2009). Stress could be described as “the non-specific response of the body to any demand for change” (Selye, 1936). It may also mean the perception of threat with accompanying discomfort, anxiety, difficulty in adjusting to it and emotional tension or trauma (Fink, 2009). Stress may further be described as any stimulus or response provoking the release of adrenal secretions (adrenal glucocorticoids and adrenocorticotrophic hormone) (Martínez-Miró et al., 2016). However, a supposedly universal definition may be any biological response or stimulus brought about when an individual (humans

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or animals) perceives a threat to its homeostasis (Moberg, 2000).

The impact of dietary supplementation of neuromediators on reducing the negative impacts of the stress response has been documented. Inclusion of neuromediators in pig diets might reduce the adverse effect of the stress response and reduce production losses associated with weaning (Sterndale et al., 2020). Having a molecular weight of 204.22 g/mol and a molecular formula of  $C_{11}H_{12}N_2O_2$  tryptophan (Trp) is the largest proteinogenic amino acid in the cell. Being an essential amino acid, it is not synthesised in animals and must thus be provided in the diets. Trp is hydrophobic and aromatic. Phenylalanine, tyrosine and Trp are the three aromatic amino acids that minimally contain a six-membered benzene ring in their side chains. The side chain of Trp is indole, which is complex. It is a six-membered benzene ring fused to a five-membered pyrrole ring with an integrated NH group. Trp can also be viewed as a derivative of alanine, having an indole substituent on the  $\beta$  carbon (Barik et al., 2020). Trp is required for normal growth and it serves as an *in vivo* precursor for bioactive compounds such as serotonin, nicotinamide (vitamin B6), melatonin, tryptamine, kynurenine, 3-hydroxykynurenine, and quinolinic and xanthurenic acids (Li et al., 2006; Le Floc'h and Seve, 2007; Poletto et al., 2010; Valros et al., 2015; Friedman, 2018).

During their lifetime, pigs encounter many stressors, including separation from the dam at weaning and changes in feed/feeding systems (Puppe et al., 1997; Martínez-Miró et al., 2016). Stress has been observed as “a process with multifactorial causes, producing an organic response that generates negative effects in the health and production of the animals affected” (Martínez-Miró et al., 2016). Pigs are highly productive. They are omnivores and compete with humans for food. They are fed varieties of food/feed but their performance depends to a large extent on the quality of their feed. Protein and energy sources are the most expensive feed ingredients for monogastric animals, considering the quantities required. Feed cost is increasingly rising, caused by several factors, which has made sourcing for alternative feed ingredients (less competed for by human beings) necessary, especially in areas where food security is currently a challenge.

In Africa, pig farmers feed cassava peels to pigs to reduce the cost of production despite its low protein content and anti-nutritional factor (hydrocyanide). It has recently been reported that the production of cassava in Nigeria stands at over 59 million tons per year (Garba et al., 2020). It has been documented

that agro-waste could serve as a potential source of secondary raw material for energy and material recovery (Santos et al., 2022). Their suitability for energy and material recovery could be traced to their lignocellulosic content (Santos et al., 2022; Adeniyi et al., 2022). Cassava has been reported to contain anti-nutritional factors which may include cyanide, phytate, tannin and oxalate. The anti-nutritional factors in cassava are harmful to human health. Cyanogenic glycosides are the most important of them, which break down to release toxic hydrogen cyanide gas in a process known as cyanogenesis, which may lead to chronic health problems unless appropriately processed (Montagnac et al., 2009; Zekarias et al., 2019; Mariam and Woldeyes, 2020). However, little is known about the effect of this feed change in terms of stress to weaners. In this study, we replaced maize with cassava peels and supplemented the diets with Trp to investigate its effect on performance, haematology, behavioural response and corticosterone levels of pigs to weaning and metabolic stress.

## MATERIALS AND METHODS

### Management of experimental animals

The feeding trial was carried out at the Swine Unit of the University of Ibadan Teaching and Research Farm, Nigeria. The experiment was approved by the Animal Care and Ethics Committee of the Department of Animal Science, University of Ibadan, Nigeria (ANSUI/PS/09/21). Crossbred (Large White  $\times$  Landrace) weaner pigs (4.5–4.7 kg) were used and randomly assigned into three treatments in a completely randomised design (CRD). The experimental animals were individually numbered, weighed and allotted to treatments in CRD in such a way that the difference in the average initial body weight within each treatment and across treatments was not significantly different (Adejumo et al., 2021). Each treatment had three replicates and each replicate had three animals. The feeding trial lasted for four weeks. The cassava peels used for the study were obtained from a reputable farm in Ogbomoso, Nigeria. The standard feed ingredients were procured from a reputable feed miller in Ibadan, Nigeria. The animals were injected with Ivomec® (Ivermectin) subcutaneously against endo- and ectoparasites and were properly housed and fed with a standard maize-based diet ( $T_1$ ), 20% cassava peel inclusion (replacement for maize) ( $T_2$ ) and 40% cassava peel-based diets ( $T_3$ ). The animals had unrestricted access to feed and water throughout the experiment.

**Chemical determination and collection of data**

The proximate analysis of the diet was determined using AOAC (1990). The composition of the diet is shown in Table 1. Weekly feed intake (FI) and body weight gain (BWG) were measured and feed efficiency (g/kg) was calculated from the values of FI and BWG. At the end of the feeding trial, blood samples were collected according to treatments early in the morning to minimise stress on the animals. The blood was collected into a serum tube through the jugular vein puncture method into two sample bottles using a sterilised needle and syringe. The blood samples (n = 27) for haematology were analysed for packed cell volume (PCV), haemoglobin (Hb), red blood cell counts (RBC), white blood cell counts (WBC), etc. The blood samples for serum analysis (corticosterone) were allowed to clot before centrifuging to obtain the serum. The blood was centrifuged at 1008 × g for 10 min to separate the serum. The serum samples were collected into micro tubes and stored at -20 °C until

the determination of corticosterone concentration by chemiluminescent microparticle immunoassay using IMMULITE 1000 automated immunoassay system (Cinquanta et al., 2017).

The behaviours of the weaned pigs in the groups were video-recorded continuously for one hour per day for two consecutive days a week and it was done for four weeks. All behavioural data were obtained from video images that were digitally recorded. The behavioural time values presented are the means and standard errors of the relative frequencies of each behavior, calculated from the results obtained from each observation of each group (Rhim, 2012; Rhim et al., 2015). The feeding, manipulative, sleeping and lying, agonistic, and other behaviours were observed. The frequency and duration of these pig behaviours were recorded by video. Behavioural ethnology is presented in Table 2. Agonistic behaviour was defined as the encounter between two pigs involved in physical contact, and started with the first physical contact and ended with submissive behaviour (escape) being shown by one

**Table 1.** Gross composition of experimental weaned pig diet (g/kg DM)

Feed ingredients	Treatments		
	Treatment 1 (T <sub>1</sub> )	Treatment 2 (T <sub>2</sub> )	Treatment 3 (T <sub>3</sub> )
Maize	40.00	20.00	0.00
Soybean meal	10.00	10.00	10.00
Cassava peels	0.00	20.00	40.00
Wheat	15.00	15.00	15.00
Groundnut cake	15.00	15.00	15.00
Bone	01.00	1.00	1.00
Palm kernel cake	13.00	13.00	13.00
Lysine	0.25	0.25	0.25
Methionine	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Palm oil	5.00	5.00	5.00
Tryptophan	0.25	0.25	0.25
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>

Treatment 1 = 40% maize + 0% cassava peels + 60% fixed ingredients  
 Treatment 2 = 20% maize + 20% cassava peels + 60% fixed ingredients  
 Treatment 3 = 0% maize + 40% cassava peels + 60% fixed ingredients  
 Source: Self (obtained in this study)

**Table 2.** Behavioural ethnology

Behavior	Description
<b>Ingestive behaviour</b>	Feeding and drinking
<b>Aggressive behaviour</b>	Head thrusting, biting, chasing or fighting with another pen or littermate
<b>Manipulative behaviour</b>	Belly nosing, nosing and mounting other pen or littermates
<b>Exploratory behaviour</b>	Investigating the surroundings such as nosing the floor, scrapping the floor with one of the forelegs, nosing or nibbling of pen fixtures
<b>Lying behaviour</b>	Lying on the side or belly with eyes open, not performing any other described behaviour
<b>Sleeping behaviour</b>	Lying on the side or belly with eyes closed, not performing any other described behaviour

Source: Adapted from Bolhuis et al. (2005); Middelkoop et al. (2019)

of the opponents or when they both move away or separate from each other (Borberg and Hoy, 2009; Samarakone and Gonyou, 2009; Krauss and Hoy, 2011; Rhim, 2012).

**Experimental design and statistical analysis**

The experimental design of the study was completely randomised. Data obtained were analysed using the one-way analysis of variance and significant means were separated using Duncan's Multiple Range test, and the general linear model of the SAS package.

**RESULTS**

The proximate composition of the feed samples is shown in Table 3. The diets were isonitrogenous and isocaloric. The dry matter was similar with T<sub>1</sub> obtaining the highest numerical value while T<sub>3</sub> obtained the least value. The ash content ranged between 5.45 and 6.65 mg/g. T<sub>2</sub> obtained the highest numerical crude protein content while T<sub>3</sub> had the least value. Values for crude fat were also similar. Table 4 shows the effect of the varying levels of dried cassava peels on the growth performance of weaned pigs. There were no significant (*p* > 0.05) differences among the treatments for growth

**Table 3.** Proximate analysis of the feed %

	Treatment 1	Treatment 2	Treatment 3	SEM
Moisture content	7.55	7.75	8.30	0.057
Dry matter	92.45	92.25	91.70	0.057
Ash content	6.65	5.45	6.20	1.517
Crude fat	13.85	14.15	13.55	0.258
Crude protein	18.46	19.25	17.14	0.390
Crude fibre	5.85	6.45	5.65	0.298

Treatment 1 = 40% maize + 0% cassava peels + 60% fixed ingredients  
 Treatment 2 = 20% maize + 20% cassava peels + 60% fixed ingredients  
 Treatment 3 = 0% maize + 40% cassava peels + 60% fixed ingredients  
 Source: Self (obtained in this study)

**Table 4.** Growth performance contents of weaner pigs fed cassava peels as a replacement for maize

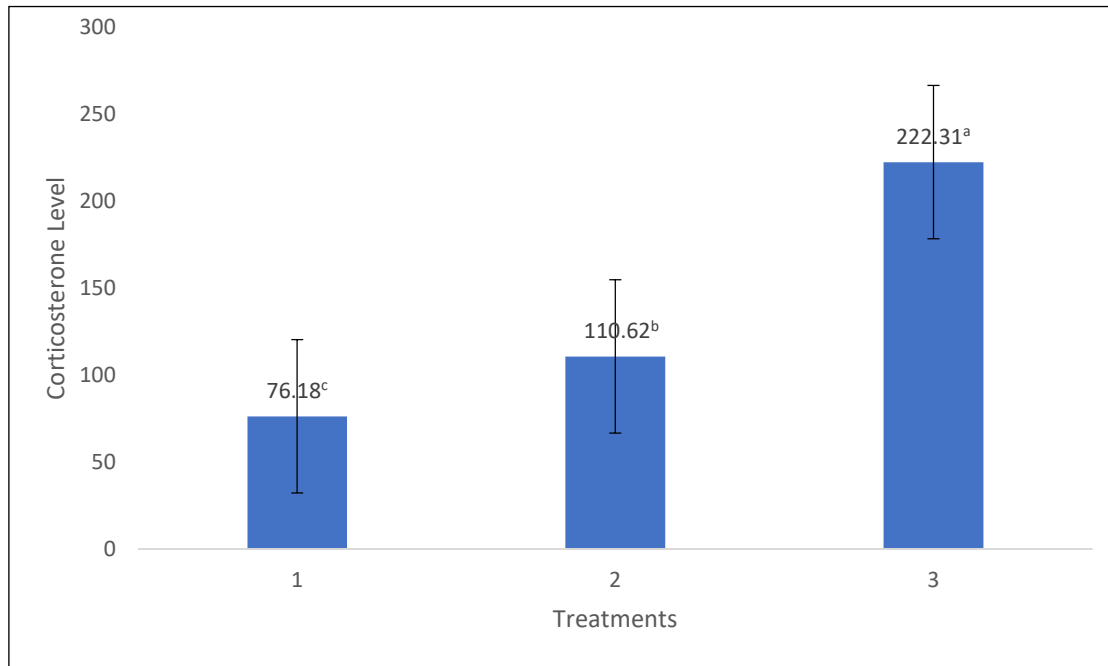
Parameters	Treatment 1(T <sub>1</sub> )	Treatment 2(T <sub>2</sub> )	Treatment 3(T <sub>3</sub> )	SEM
Initial weight (kg)	4.69	4.65	4.54	0.05
Final weight (kg)	8.27	8.12	7.65	0.06
Weight gain (kg)	3.58	3.47	3.10	0.04
Feed intake (kg)	10.78	10.88	10.14	0.04
Feed efficiency (g/kg)	332.10	318.93	305.72	10.77

Treatment 1 = 40% maize + 0% cassava peels + 60% fixed ingredients  
 Treatment 2 = 20% maize + 20% cassava peels + 60% fixed ingredients  
 Treatment 3 = 0% maize + 40% cassava peels + 60% fixed ingredients  
 Source: Self (obtained in this study)

**Table 5.** Haematology contents of weaner pigs fed cassava peels as a replacement for maize

	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	SEM
PCV%	35.67 <sup>b</sup>	38.67 <sup>a</sup>	30.33 <sup>c</sup>	0.11
Hb (g/dL)	11.43 <sup>b</sup>	12.47 <sup>a</sup>	9.90 <sup>c</sup>	0.05
RBC (×10 <sup>6</sup> /mm <sup>3</sup> )	5.59 <sup>b</sup>	6.13 <sup>a</sup>	4.75 <sup>c</sup>	0.01
WBC (×10 <sup>6</sup> /mm <sup>3</sup> )	3.48 <sup>c</sup>	4.82 <sup>a</sup>	3.78 <sup>b</sup>	0.32
Platelets (×10 <sup>6</sup> /mm <sup>3</sup> )	79.33	79.67	79.33	11.03
Lymphocytes (%)	51.67 <sup>b</sup>	48.33 <sup>b</sup>	58.00 <sup>a</sup>	0.20
Monocytes (%)	3.67 <sup>a</sup>	2.67 <sup>b</sup>	4.00 <sup>a</sup>	0.05
Eosinophils (%)	4.00	4.00	3.33	0.07
Neutrophils (%)	40.67 <sup>b</sup>	44.00 <sup>a</sup>	34.67 <sup>c</sup>	0.14

T<sub>1</sub> = 40% maize + 0% cassava peels + 60% fixed ingredients  
 T<sub>2</sub> = 20% maize + 20% cassava peels + 60% fixed ingredients  
 T<sub>3</sub> = 0% maize + 40% cassava peels + 60% fixed ingredients  
 Different superscripts within the same row indicate significant (*p* < 0.05) difference  
 PCV = packed cell volume; Hb = haemoglobin; RBC = red blood cell counts; WBC = white blood cell counts.  
 Source: Self (obtained in this study)



**Figure 1.** Corticosterone levels of weaner pigs fed cassava peels in replacement for maize  
 Treatment 1 = 40% maize + 0% cassava peels + 60% fixed ingredients  
 Treatment 2 = 20% maize + 20% cassava peels + 60% fixed ingredients  
 Treatment 3 = 0% maize + 40% cassava peels + 60% fixed ingredients  
 Source: Self (obtained in this study)

**Table 6.** Behavioural measurements (in minutes) of weaner pigs fed cassava peels as a replacement for maize (morning)

	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	SEM
<b>Feeding</b>	8.17	9.00	7.94	0.11
<b>Sleeping/Lying</b>	0.00	0.00	0.00	0.00
<b>Manipulation</b>	0.39	0.56	0.39	0.06
<b>Aggression</b>	0.11 <sup>b</sup>	0.61 <sup>a</sup>	0.55 <sup>ab</sup>	0.03
<b>Explorative</b>	2.89	3.11	2.00	0.25

Source: Self (obtained in this study)

**Table 7.** Behavioural measurements (in minutes) of weaner pigs fed cassava peels as a replacement for maize (afternoon)

	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	SEM
<b>Feeding</b>	5.72	6.00	5.83	0.11
<b>Sleeping/Lying</b>	3.72	3.44	3.56	0.12
<b>Manipulation</b>	2.00	1.39	1.11	0.07
<b>Aggression</b>	0.44	0.50	0.28	0.04
<b>Explorative</b>	1.39	1.00	1.67	0.16

T<sub>1</sub> = 40% maize + 0% cassava peels + 60% fixed ingredients  
 T<sub>2</sub> = 20% maize + 20% cassava peels + 60% fixed ingredients  
 T<sub>3</sub> = 0% maize + 40% cassava peels + 60% fixed ingredients  
 Source: Self (obtained in this study)

performance characteristics of the experimental animals. No mortality was recorded across the treatments for the experimental animals.

The haematological variables of weaner pigs fed cassava peels as a replacement for maize are shown in Table 5. Platelets and eosinophils were not significantly ( $p > 0.05$ ) different across the treatments. Packed cell volume (PCV), haemoglobin (Hb), red blood cell counts

(RBC), white blood cells counts (WBC) and neutrophils were significantly ( $p < 0.05$ ) higher for T<sub>2</sub>, but lower for T<sub>3</sub> when compared with the control group (T<sub>1</sub>), except for WBC which was higher for T<sub>3</sub> than the control group. Monocytes were significantly ( $p < 0.05$ ) lower for T<sub>2</sub> when compared with the control (T<sub>1</sub>) group. However, the values for both T<sub>1</sub> and T<sub>3</sub> were similar. Lymphocytes were significantly ( $p < 0.05$ ) higher for T<sub>3</sub>

(58.00%) when compared with  $T_1$  and  $T_2$  groups, which were similar. Pigs fed on  $T_3$  obtained the highest value of corticosterone followed by  $T_2$ , while  $T_1$  obtained the least value (Figure 1). Behavioural measurements of weaner pigs fed cassava peels as a replacement for maize for morning and afternoon are presented in Tables 6 and 7, respectively. There were no significant ( $p > 0.05$ ) differences observed across the treatments for both morning and afternoon measurements.

## DISCUSSION

The present study shows that despite the variations in the cassava content of the various diets, there was relative adequacy of protein in all the diets. This is notwithstanding the very poor protein content of cassava meal (Gomez et al., 2018). The result of the present study for growth performance characteristics agrees with the findings of Adebisi et al. (2017) who recorded no significant differences in the weight gain and average feed intake if weaner pigs were fed with cassava peels and plantain peels at levels 0, 60% cassava peels and 60% plantain peels.

In the present study values obtained for white blood corpuscles were lower than the values reported in the previous study by Etim et al. (2013), which might be a result of the breed, season, age, and fasting (Afolabi, 2010). Eheba et al. (2008) posited that a decrease in WBC count, however, reflected a fall in the production of defensive mechanisms to combat infection. The result showed that there were significant reductions in PCV, RBC counts, Hb concentration and neutrophils in  $T_3$ . Based on the findings in the present study, pigs in  $T_1$  and  $T_2$  could be said to have better haematological values than those in  $T_3$ . The values obtained were similar to earlier values reported by previous authors (Radostits et al., 2000). WBC values were higher for cassava peel-based diets, which may be an indication of stress. It is known that any form of stress can increase WBC. Etim et al. (2013) previously noted stated that low values in pig haematological parameters might be due to malnutrition. In a previous study by Adeschinwa et al. (2011) Hb and RBC of pigs were increased by the inclusion of enzyme, and replacement of the maize content of the basal diet with cassava peels significantly reduced both parameters. The authors, however, recommend further studies in this direction to unravel the subject matter presently being discussed, that is, the adverse effect of cassava peels on haematological and physiological parameters of pigs.

Pigs are very adaptable to their environment, but nutritional status may affect their response to environmental stimulus. Even though a fixed level

of Trp was included in all diets, maize has a higher Trp content than cassava. However, in the present study pigs' behavioural displays were not significantly different between treatments. Previous studies reported a decreased aggressive behaviour among mice when their water was supplemented with Trp (Li et al., 2006; Janczak et al., 2001). Also, Martínez-Trejo et al. (2009) reported that Trp supplementation in the diets of piglets positively affected some of their behaviour, however, it did not affect their productive performance, which is similar to the growth performance of pigs in the present study.

## CONCLUSION

Pigs are known to easily adapt to their environment but it is becoming clearer that their nutritional quality or status might influence their response to stimulus including stress. Cassava peels are fed to pigs mainly to reduce the cost of production. However, supplementing cassava peel-based diets with Trp might help reduce the effect of stress on the animals as a result of feed amendment. Therefore, it can be concluded from this study that:

1. Cassava peel-based diets supplemented with a fixed amount of Trp did not influence the growth performance of weaner pigs.
2. Haematological variables seem compromised for pigs fed a 40% cassava peel-based diet as a total replacement for maize, supplemented with Trp.
3. Cassava peel-based diets supplemented with a fixed amount of Trp did not affect pigs' behaviour when compared with a maize-based diet.
4. As a coping strategy for metabolic stress that diet manipulation might have brought on the pigs, a cassava peel-based diet as a total replacement for maize raised the corticosterone level of the experimental animals.
5. Based on the findings on corticosterone, the complete replacement of maize with cassava peel in the diets of piglets may be carefully considered, although this may be subjected to further studies.

## CONFLICT OF INTEREST

The authors declared no conflicts of interest concerning the research, authorship, and publication of this article.

## ETHICAL COMPLIANCE

This experiment was conducted in line with the approval of protocol by the Animal Ethics and Care Committee of the Department of Animal Science, University of Ibadan, Nigeria by the ethical standards of the Institutional and/or National Research Committee

and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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