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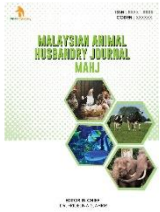

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RESEARCH ARTICLE

IMPACT OF DIETARY SUPPLEMENTAL ASCORBIC ACID ON ORGAN WEIGHT, VILLI MORPHOMETRY, ORGANOLEPTIC ATTRIBUTES AND ORGAN HISTOLOGY OF WEANED PIGS

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ABSTRACT

This study aimed at evaluating the impact of dietary supplemental ascorbic acid on organ weight, villi morphometry, organoleptic attributes, and organ histology of weaned pigs using thirty-two Large white-Landrace crossbred with an average weight of 12.32±0.59 kg. The pigs were grouped into four treatments with eight replicates each. Diet C_0 was the basal diet (control). Diets C1, C2, and C3 contained the basal diet with 1g/kg, 2g/kg and 3g/kg of ascorbic acid respectively for seven weeks. The weight of liver, kidney, spleen, heart, and lungs were significantly different (p<0.05) with pigs offered ascorbic acid-supplemented diet having higher values. Also, the villus height, villus width and muscle thickness were significantly influenced (p<0.05) with pigs fed diet C3 having the highest value for villus height and width. The organoleptic properties did not differ significantly (p>0.05). The jejunum villi histology showed that pigs fed diets C_0 and C1 were essentially normal while those fed diets C2 and C3 had moderate enteritis. No observable lesions were recorded in the structure of the nephron. Moreover, the hepatic histology of pigs fed diet C1 was essentially normal while those fed diets C2 and C3 showed normal hepatocytes but with moderate sinusoidal congestion. It can be concluded that ascorbic acid supplementation caused increased organ weight, aided villus height and width, and reduced muscle thickness with moderate sinusoidal congestion at up to 3g/kg with no deleterious effects on organoleptic attributes.

KEYWORDS

Ascorbic Acid, Blood, Villus, Sensory Properties, Liver.

1. INTRODUCTION

Different types of feed additives have been commercialized for use in swine nutrition. These additives influence the well-being, metabolism, and performance of swine (Ferronato and Prandini, 2020). Common additives are amino acids, vitamin/mineral premix, antibiotics, parasiticides or antihelminthics, pro and prebiotics, acidifiers (organic and inorganic acids), botanicals or phytogenics, enzymes, phytase, and flavours (Feed additive compendium, 2004; Ojediran et al., 2021). Amino acids such as methionine, lysine, and threonine were added to the feed to make up for their inadequate supply in feed because they are the first three limiting amino acids in pig diets. Vitamin/mineral premix is added to feed to provide essential vitamins and minerals, which are insufficient in feedstuffs. Antibiotics are added to feed to combat or prevent diseases and to improve health status. Parasiticides are used to deworm pigs against internal worms and feed-grade ectoparasite formulation are available against lice and mange. Probiotics consist of living bacteria that modulate the gut microflora while prebiotics selectively stimulate the activities of favorable bacteria in the gut. Acidifiers reduce the stomach pH against gram-negative bacteria to increase protein digestion and absorption. Botanicals such as plant part derivatives stimulate feed consumption and have antimicrobial, antiparasitic, or antioxidative properties (Radzikowski and Milczarek, 2022). Enzymes help in the digestion process. Phytase increases the bioavailability of phosphorus. Flavours mask off-odours and enhance the smell or taste of the feed. Antibiotics had been banned in some countries.

Ascorbic acid is a natural phytochemical in fruits and vegetables and is chemically identical to the synthetic form (Carr and Vissers, 2013). The former is not easily quantifiable when offered to livestock. Mammals are known to be able to synthesize some portion of ascorbic acid in the liver but reports of improved performance had been recorded with supplementation (Capo et al., 2021; Hieu et al., 2022). It had been observed to enhance immune response, act as anti-stress, aid oxidation processes, improve productivity, and modulate physiological processes and crucial biological processes in electron transfer, enzyme co-factor, and activities. It also plays a role in collagen production.

Digestive organs respond to the feed ingested or non-feed materials in various ways (Ojediran et al., 2021). A group researchers reported pigs had enlarged liver pancreas, and spleen when enzymes were not added to fibrous feeds (Agyekum et al., 2012). There may be hyper or hypo-secretions of digestive fluids owing to the feed taken by pigs in the digestion processes (Wenk, 2001). Some researchers proved that ascorbic acid a had protective role on the liver when perfluorooctanoic acid was used to induce hepatic steatosis (Li et al., 2022). Also, some researchers showed the ameliorative effect of ascorbic acid on the hepatotoxic effect of two analgesics (Al-Hayder et al., 2022). Liver and kidney weights are indicators of toxicity (Shittu et al., 2016). Increased weight of the liver in response to the detoxification process with increased metabolic rates since the liver is the first organ than receives metabolized macromolecules through the hepatic portal vein from the small intestine (Ojediran et al., 2020).

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A group researchers reported that ascorbic acid lowers the gastro-intestinal pH, promoting proteolytic enzymes like acidifiers (Kim et al., 2005). Mroz, believed that acidifiers substrates epithelial mucosa synthesis thus affecting villi morphology (Mroz, 2005). Some researchers also proved that ascorbic acid positively influence the production of red blood cell which may affect the level of myoglobin in the pork (Ojediran et al., 2022). Increased myoglobin content would influence the pork colour, which thus dictates consumer perception (Karthika et al., 2016). Moreover, also established that increased ascorbic acid influences fat deposition in pigs (Ojediran et al., 2022). This also would affect consumers' judgment of the pork.

There is dearth of information on the effects imposed by the use of ascorbic acid on weaned pigs. This study purposed to appraise the impact of dietary supplemental ascorbic acid on organ weight, villi morphometry, organ histology and organoleptic attributes of weaned pigs.

2. Materials and Methods

2.1 Experimental Site, Test Ingredient, and Experimental Design

The trial was performed between July and August, 2021 at the Piggery Unit of the University's Research Farm, Ogbomoso. Ogbomoso lies on longitude and latitude 4°16' E and 8°10' N respectively at about 300 meters altitude, with a mean temperature of about 26.8 ^{0}C and 1247 mm precipitation in the derived savannah zone. The experiment conforms to the University's Animal Research Ethos. The ascorbic acid was obtained from Animal Health, Member Bv, 17A, ZA Venter-Holland (colour – white, physical consistency – coated powder). Large white-Landrace crossbred weaners, (n=32, age=7 weeks, average weight=12.32±0.59 kg) were used for the dietary trial. They were grouped into 4 treatments with 8 replicates each, housed in open-sided pens with concrete floors. Feed and water were given without restriction. They were acclimatized for seven days before the commencement of the feeding trial, which lasted for 49 days.

The basal diet: (i) was isocaloric and isonitrogenous and was referred to as the control diet (C_0) with no ascorbic acid added. It contained 15% cashew kernel meal, 57% palm kernel meal, 18.30% corn bran, 8.00% soybean meal, 1.00% limestone, 0.20% lysine, 0.25% premix, and 0.25% salt. The energy of the feed had 11.36MJ, 19.93% crude protein, 6.35% ether extract, 8.70% crude fibre, 0.97% calcium, 0.38% available phosphorus, 0.87% lysine, and 0.40% methionine.

(ii). Diet C1= Diet C_0 + ascorbic acid at 1g/kg diet;

(iii). Diet C2= Diet C_0 + ascorbic acid at 2g/kg diet; and

(iv). Diet C3: basal diet + ascorbic acid at 3g/kg diet.

The pigs were allotted to the dietary groups in a completely randomized design.

2.2 Data Collection

2.2.1 Organ Weight of Weaned Pigs fed Diet Supplemented with Ascorbic Acid

At the end of the experiment, four pigs per replicate were selected randomly for organ evaluation. The pigs were weighed before slaughtering and properly bled. They were eviscerated and the following internal organs were removed and weighed separately on an electronic sensitive scale. The spleen, kidney, liver, lungs, and heart were expressed in relation to the live weight.

2.2.2 Villi Morphology of Weaned Pigs fed Diet Supplemented with Ascorbic Acid

Histo-morphometric characteristics of the jejunum including the villus length, and width, crypt depth, and width were determined. The samples were first dissected and labeled appropriately and fixed in 10% neutral buffered formalin as described by Yaghobfar and Kalantar, (2017) before being further processed in an automatic tissue processor.

2.2.3 Organoleptic Assessment of Weaned Pigs fed Diet Supplemented with Ascorbic Acid

A portion of the ham of slaughtered pigs were excised and tagged. They were cooked for 15 minutes in nylon containers, allowed to cool, and served as coded samples. The organoleptic evaluation was done as described by using 10 regular meat consumers as panelists. A 9-point hedonic scale served as the descriptor (Ojediran et al., 2019).

2.2.4 Histological Study of Weaned Pigs fed Diet Supplemented with Ascorbic Acid

Samples of the liver and jejunum were appropriately fixed in 10% neutral buffered formalin (in properly labeled histological bottles) and were processed in an automatic tissue processor, embedded in paraffin wax, and sectioned at 5 microns on a rotary microtome mounted on glass slides stained with eosin-haematoxylin dye as described (Carson and Christa, 2009). The stepwise protocol for the automatic tissue processor for histological examination slide was as described (Akpokodje et al., 2005).

2.3 Statistical Analysis

The various data were subjected to analysis using 1-way ANOVA with SPSS 16v. A significant level was at 5%. The significant mean values were separated by DMRT (Duncan, 1955).

3. Results

3.1 Organ Weight of Weaned Pigs fed Diet Supplemented with Ascorbic Acid

Organ weight of weaner pig fed diet supplemented with ascorbic acid is presented on Table 1. Significant differences ($p < 0.05$) were observed in liver, kidney, spleen, heart and lungs. Pigs fed diet C1-C3 had higher liver, kidney, spleen, heart and lungs weight than those fed the control diet C_0. The values were not significantly different ($p>0.05$) among pigs offered diets C1-C3 but were significantly different ($p<0.05$) from those fed diet C_0. The highest values were recorded in Pigs fed diet C3.

Table 1: Organ Weight of Weaned Pigs fed Diet Supplemented with Ascorbic Acid (% of Live Weight)

Parameter	C_0	C1	C2	C3	SEM	P-VALVE
Liver	2.08^b	2.60^a	3.17^a	3.12^a	0.14	0.01
Kidney	0.29^b	0.40^a	0.47^a	0.45^a	0.27	0.05
Spleen	0.10^b	0.10^a	0.16^a	0.14^a	0.01	0.03
Heart	0.26^c	0.30^b	0.38^{ab}	0.43^a	0.02	0.00
Lungs	0.65^b	0.80^a	0.97^a	0.94^a	0.04	0.01

^{abc} Mean in same row with different superscript are significantly different ($P < 0.05$)

3.2 Villi Morphometrics of Weaned Pig fed Diet Supplemented with Ascorbic Acid

Table 2 shows the villi morphometrics of weaned pigs fed diet supplemented with ascorbic acid. The result below shows that the villus height, villus width, and muscle thickness were significantly influenced ($p<0.05$) while crypt depth and crypt width were not significantly different ($p>0.05$). The villi height and width were highest in pigs fed diet C3 and lowest in diet C_0 ($p<0.05$). However, the muscle thickness was highest in pigs fed diet C_0 while those fed ascorbic acid supplemented diets were reduced.

Table 2: Villi Morphometrics of Weaned Pigs fed Diet Supplemented with Ascorbic Acid

Parameters (µm)	C_0	C1	C2	C3	SEM	P-VALVE
Villus height	574.00^b	762.00^b	618.00^b	894.00^a	64.80	0.00
Villus widtht	133.56^b	141.27^{ab}	134.83^{ab}	150.26^a	6.27	0.02
Crypt depth	895.35	839.66	889.60	862.43	14.04	0.51
Crypt width	115.68	121.45	134.34	128.92	3.24	0.19
Muscle thickness	442.44^a	384.65^b	379.47^b	399.65^b	10.19	0.01

^{abc} Mean in same row with different superscript are significantly different ($P < 0.05$)

3.3 Organoleptic Properties of Weaner Pigs fed Diet Supplemented With Ascorbic Acid

Organoleptic properties of weaner pigs fed diet supplement with ascorbic acid is shown on *Table 3*. All the parameters including color, flavor, tenderness, juiciness, texture, and overall acceptability were not significantly influenced (p>0.05) by the dietary treatments.

Table 3: Organoleptic Properties of Weaned Pigs fed Diet Supplemented with Ascorbic Acid

Parameters	C_0	C1	C2	C3	SEM	P-VALVE
Colour	6.50	6.90	5.90	6.30	0.20	0.39
Flavour	4.30	5.10	5.30	5.10	0.29	0.62
Tenderness	5.50	5.30	6.00	5.70	0.30	0.69
Juiciness	5.30	5.00	4.40	5.80	0.29	0.39
Texture	5.30	4.80	5.50	6.10	0.31	0.53
Overall acceptability	7.00	6.60	6.70	6.20	0.26	0.75

^{abc}Mean in same row with different superscript are significant different (P < 0.05)

3.4 Jejunum Villi Histology of Pigs fed Diet Supplemented with Ascorbic Acid

Plates 1-4 showed the jejunum villi histology of pigs fed diet supplemented with ascorbic acid respectively for pigs fed diets C_0-C3. Micrograph (x100) of pigs fed diets C_0 and C1 were essentially normal while those fed diets C2 and C3 had moderate enteritis. No observable lesions were recorded in the micrographs showing nephrons structure of pigs fed ascorbic acid supplemented diets.

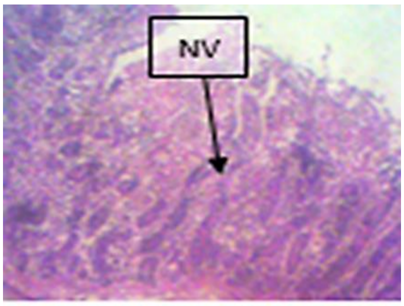

Plate 1: Micrograph showing the villi structure of weaned pig fed diet C_0.

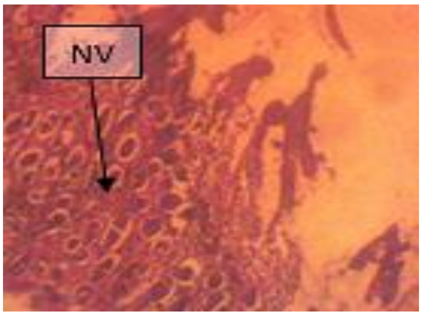

Plate 2: Micrograph showing the villi structure of pigs fed diet C1.

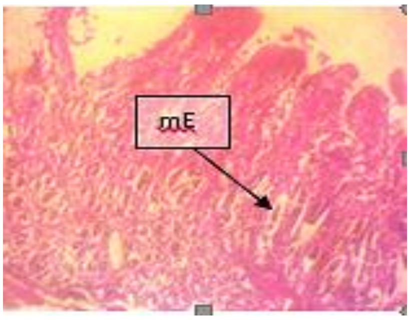

Plate 3: Micrograph showing the villi structure of weaned pig fed diet C3.

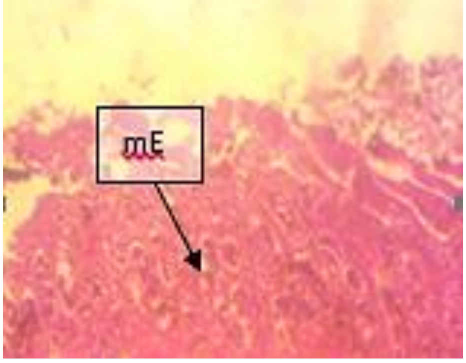

Plate 4: Micrograph showing the villi structure of pig fed diet C4.

3.5 Hepatic Histology of Pigs fed Diet Supplemented with Ascorbic Acid

Plates 5-8 shows the hepatic histology of pigs fed diet supplemented with ascorbic acid respectively for pigs fed diets C_0-C3. Micrograph (x100) of pigs fed diet C_0 showed normal hepatic portal vein and hepatocytes while those fed diet C1 was essentially normal hepatocytes. Pigs fed diet C2 and C3 showed normal hepatocytes but with moderate sinusoidal congestion.

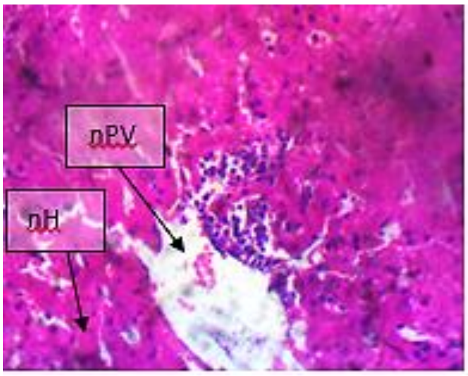

Plate 5: Micrograph showing the hepatic structure of weaned pig fed diet C_0.

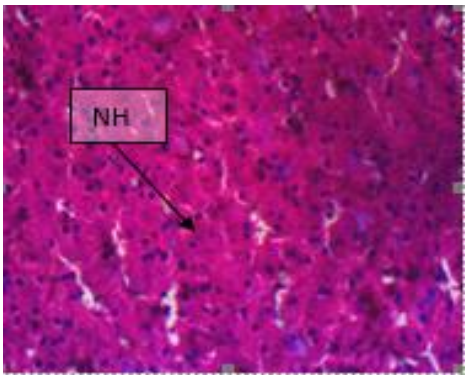

Plate 6: Micrograph showing the hepatic structure of weaned pig fed diet C1.

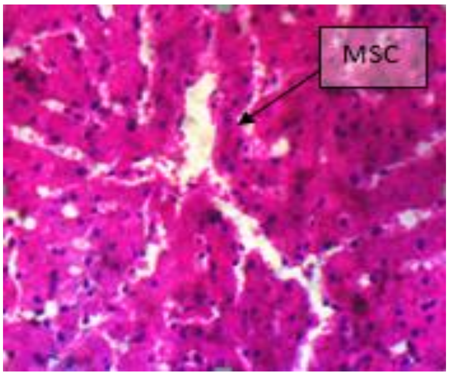

Plate 7: Micrograph showing the hepatic structure of weaned pig fed diet C2

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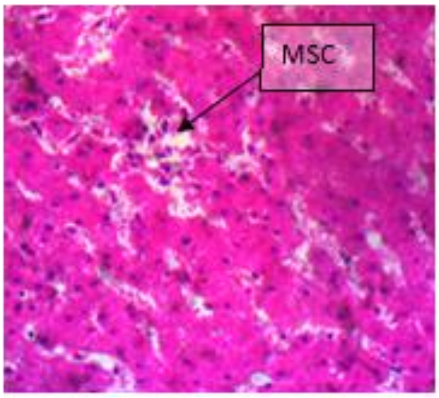

Plate 8: Micrograph showing the hepatic structure of weaned pig fed diet

4. Discussion

4.1 Organ Weight of Weaned Pigs fed Diet Supplemented with Ascorbic Acid

It is observable that the weight of liver, kidney, spleen, heart and lungs increased in diets supplemented with ascorbic acid. A group researchers attributed the increase in weight of liver and kidney to toxicity or anti-nutritional factors in feed (Shittu et al., 2016). However, this might not be the case in this study. Some researchers also reported pigs fed fibrous feed had enlarged liver, pancreas, and spleen (Agyekum et al., 2012). That is connected to hyper or hypo-secretions of digestive fluids owing to the feed taken by pigs for digestion processes (Wenk, 2001). Ascorbic acid had been reported to increase metabolic activities, thus may have influenced the weight of internal organs like liver, kidney, heart, lungs and spleen. In addition, explained that ascorbic acid supplementation supports the synthesis of fat, cholesterol, and triglyceride in weaned pigs (Partanen and Mróz, 1999; Ojediran et al., 2022). The cholesteric effect of ascorbic acid may have played out in the weight of these organs. A group researcher found a relationship between heart disease and elevated cholesterol and triglyceride levels (He et al., 2004). The result of this study differs from the observation of when enzymes were added to the diet of weaned pigs (Ojediran et al., 2021). Spleen is associated with haematopoietic elements, immune function and lymphatic cell. The increased spleen weight further established the immune enhancement capacity of ascorbic acid.

4.2 Villi Morphometrics of Weaned Pig fed Diet Supplemented with Ascorbic Acid.

The villus height and width improved with ascorbic acid supplementation. This proved that ascorbic acid improved absorption of nutrients by increasing the surface area needed. This is similar to the observation (Ojediran et al., 2017). Nkukwana confirmed that villi respond to feed form (Nkukwana, 2014). A group researcher postulated that reduction in transit time effects an increase in villi height through microbial activities (Hedemann et al., 2006). This can be linked to the acidifier effect of ascorbic acid against gram negative bacteria. Acidifiers acts as a substrate for epithelial mucosa influencing villi morphology (Mroz, 2005). Nevertheless, the muscle thickness reduced in pigs fed ascorbic acid supplemented diet. Ascorbic acid helps in electron transfer and also the enzyme cofactor ability helps increase enzyme activity needed for digestion and absorption of nutrient at enzyme active site (Timberlake, 2015). Ascorbic acid does not influence the organoleptic properties of pigs as observed in this study. This showed that consumer preference was not influenced by the meat colour, flavour, tenderness, juiciness, texture and acceptability (Hanczakowska et al., 2015). Some researchers indicate that supplementation with oregano essential oil improved the sensory quality of meat (Cheng et al., 2017).

4.3 Villi and Hepatic Histology of Weaned Pig fed Diet Supplemented with Ascorbic Acid.

The observed villi and hepatic histology in this study revealed that the level of ascorbic acid supplementation influenced these organs moderately at 2g/kg diet and 3g/kg diet. The increase in the villus height and width in this study corroborated the findings on histology. May and Harrison reported that ascorbic acid causes proliferation of endothelial cells (May and Harrison, 2013). The absence of sweet gland in pig makes them prone to stress. However, Ascorbic acid is reported to contribute to energy supply by corticosterone biosynthesis (Ahmadu et al., 2016). This may have resulted in sinusoidal congestion since liver stores energy in the form of glycogen established that ascorbic acid at high levels resulted in fat and cholesterol deposition in weaned pig (Hieu et al., 2022; Ojediran et al., 2022).

Stress generates reactive oxygen species (ROS) but ascorbic acid antioxidant capacity alleviates the ROS effects by protecting cells against free radicals by increasing antioxidant markers such as superoxide dismutase, total antioxidant capacity, and glutathione peroxidase activities which are enhanced in the liver (Yang et al., 2017; Zhang et al., 2019). However, noted that a high dose of ascorbic acid supplementation above 1g/kg had shown to have adverse effects on the production of the enzymes (Hieu et al., 2022). This is capable of influencing the hepatocytes. In situations of toxicity from chemicals or drugs, the protective role of ascorbic acid had been reported in rat and guinea pig (Singh and Rana, 2010; Ghosh et al., 1999; Al-Hayder et al., 2022).

Moreover, they attributed it to the antioxidant properties of ascorbic acid as it protects the DNA from ROS generated from the stress. Nevertheless, there is dearth of information on the effects imposed by the use of ascorbic acid in the absence of the use of harmful chemicals or drugs. In addition, there are no information on the effect of ascorbic acid supplementation in weaned pigs on organs.

4.4 Organoleptic Properties of Weaner Pigs fed Diet Supplemented with Ascorbic Acid

The observed result showed that ascorbic acid does not adversely influence colour, flavor, tenderness, juiciness, texture and overall acceptability. Observations on colour and flavor was unlike the report of (Ojediran et al., 2022). The ascorbic acid does not influence consumer judgment on colour, thus the myoglobin content was not influenced unlike when fed pigs with biscuit dough (Ojediran et al., 2019). Also, this result showed that taste and other parameters which cumulate to overall acceptability was not influenced. This suggests that the varying inclusion level of ascorbic acid had no deleterious effect on the sensory evaluation of the pork.

5. Conclusion

It can be concluded that ascorbic acid supplementation increased organ weight like liver, kidney, spleen, heart, and lungs. It also aided jejunum villus height and width but reduced the muscle thickness. Ascorbic acid influenced the liver with moderate hepatic sinusoidal congestion at up to 3g/kg with no deleterious effects on organoleptic properties such colour, flavor, tenderness, juiciness, texture and overall acceptability). Thus, ascorbic acid supplementation at up to 3g/kg increases organ metabolic rate, villus absorption potential but with moderate hepatic sinusoidal congestion.

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