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*Full Length Research*

# Comparative study on the prebiotic effects of Inulin and aqueous extract of *Vernonia amygdalina* on growth and intestinal morphometry of broiler chicken (Cobb strain)

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**ABSTRACT:** Antibiotic misuses aimed at improving growth performance in poultry production have resulted to widespread of antimicrobial resistance (AMR) in poultry. Recently, prebiotics are accepted as preferred alternatives. The aim of this study was to compare the prebiotic activities of a known and commercially available substance, inulin and a locally available plant, *Vernonia amygdalina* (bitter leaf) on growth performance and intestinal morphometry of broiler chicken. Sixty day-old broiler chicken (Cobb strain) employed in this study were acclimatized for three weeks and randomly divided into six treatment groups and a control. The treatment groups were further divided into the Inulin group treated with 5, 10 and 20 mg/kg of Inulin and the Vernonia group treated with 50, 100 and 200 mg/kg of *Vernonia amygdalina* (VA) aqueous extract. After three weeks of prebiotic administration, samples were collected to ascertain effects on body weight, intestinal length and histomorphological measurements of the intestinal villi and crypts were determined. Results showed that body weight did not differ significantly ($p>0.05$) with both prebiotic administration but a higher weight gain of 1.216 kg and 1.334kg were observed with 20 mg/kg inulin and 100 mg/kg VA administration respectively. Administrations of both prebiotics were not found to significantly affect intestinal length measurement ($p>0.05$). However, they were found to significantly increase ($p<0.05$) the intestinal villus height and crypt depth in the treatment groups when compared to the control group. Increased villus height, which may suggest enhanced absorptive function, were improved at dosing broilers with 20 mg/kg inulin and 100 mg/kg VA. Concluding, the effects of both prebiotics; inulin and *Vernonia amygdalina aqueous* extract on enhancement of growth performance and intestinal function are comparable. Where the effect of inulin was observed to be dose dependent, the effects of *Vernonia amygdalina* aqueous extract was observed to decline above 100 mg/kg.

**Keywords:** Prebiotics, bitter leaf, histomorphometry, poultry production, intestinal villi and crypt.

## INTRODUCTION

Profitable poultry production depends on efficient conversion of natural resources into edible products like meat and egg (Mottet and Tempio, 2017). Over the years, this has been achieved by the addition of antibiotics in sub-therapeutic dose which improves conversion of feed to meat, intestinal health, and inhibition of pathogenic organisms (Gaskins et al., 2002). Addition of antibiotics in poultry feeds has resulted in widespread of antimicrobial resistant strains – AMR (Nhung et al., 2017), drug residue in poultry products affecting human health (Mund et al., 2016) and distortion of the normal micro flora, thereby affecting growth (Awad et al., 2009). As a result of these constraints associated with antibiotic misuse in birds, there is a growing research on antibiotic alternatives by employing bio-substances that will balance the gut bioflora to achieve intestinal health (Andremont, 2000); increase

the villi height of the small intestine to achieve increased absorptive surface area (Heak et al., 2017).

The alternatives to antibiotics widely researched upon are probiotics and prebiotics. These are biological substances speculated to increase disease resistance and enhance growth in animals and their combination as symbiotics have been found to effectively improve performance of broilers (Pelicano et al., 2015). Unlike probiotics, which are direct fed microbial (DFM), utilized to improve animal performance by maintaining the normal microflora of host animals (Adriani et al., 2019), prebiotics are indigestible food that act in the gut by selective stimulation and growth of beneficial microflora (Chen et al., 2016).

Prebiotics are non-digestible oligosaccharides which exert its beneficial effects by enhancing the growth of beneficial microflora in the gastrointestinal tract (Baurhoo et al., 2007). For a compound to be classified as prebiotic, it must selectively increase the growth of bifidobacteria and other lactic acid producing bacteria (Wiseman, 2012); and must reach the colon undigested. Additionally, a prebiotic should not add any organism to an existing colony of bacteria, instead they provide nourishment for existing flora, allowing the colony to grow naturally and flourish (Sekhon and Jairath, 2010). Commercially available prebiotics include Insulin-type fructans, lactulose, fructooligo-saccharides, isomaltooligo-saccharides, and galactooligosaccharides (Chen et al., 2015). Manitol, xylitol, Sorbitol and Lactulose are other prebiotic candidate (Ezeonu et al., 2016).

The prebiotic fiber, inulin is a naturally occurring fructan found in many plants such as onion, garlic, barely, wheat etc. Chicory root and Jerusalem antichoke are particularly rich in inulin. It is one of the most studied compounds for its prebiotic activity and this is employed in the fortification of foods such as cereals, yoghurts because of its various health benefits (Kleessen et al., 2007). Inulin supplementation in poultry feed has been reported to positively affect the intestinal histomorphology, enhancing absorptive capacity in birds (Bucław, 2016). Enhancement of lipid metabolism and hormonal regulation are other beneficial effects associated with inulin supplementation in poultry diet (Nabizadeh, 2012).

*Vernonia amygdalina* (commonly known as bitter leaf) is shrub found in most African countries used for different medicinal purposes against common tropical diseases such as helminthosis, bacterial infections, malaria and diabetes (Oyeyemi et al., 2017). Its supplementation as Bitter Leaf Meal (BLM) in poultry diet have been reported to possess anticoccidia activity (Banjoko et al., 2018), improve haematological parameters (Oleforuh-Okoleh et al., 2015) and increase feed conversion ratio (Durunna et al., 2011).

However, there is dearth of information on the prebiotic potential of *Vernonia amygdalina* as regards to its effect on digestive anatomy of broiler chicken. This study therefore, is aimed to evaluate and compare the prebiotic potentials of purified inulin and locally available plant, *Vernonia amygdalina* as growth promoter in poultry production, and also, to ascertain their effects on intestinal morphometry of broiler chicken. The results from this study will reveal if this locally available plant has a comparable prebiotic effect to inulin which could promote its use for profitable poultry production.

## MATERIALS AND METHOD

### Study location

This research was conducted at the experimental animal house of the Department of Veterinary Anatomy, University of Nigeria, Nsukka, Nigeria. This is located between latitude 06.86°N and longitude 07.40°E of the tropical savanna with a mean annual rainfall range of 1500 to 2250 mm. It has its mean temperature and relative humidity set at 28°C and 63.6%, respectively.

### Source of materials

Inulin was purchased from FoodInc with a CAS no 900-80-5. Leaves of *Vernonia amygdalina* was collected from a private farm in Nsukka town, Enugu State, Nigeria. A taxonomist from the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka authenticated the plant, and assigned a herbarium Voucher number: UNH/001.

### Preparation of aqueous plant- leaf extracts

The aqueous extracts of *Vernonia amygdalina* was prepared as described by Arhoghoro et al. (2009), with slight modifications. The washed and air- dried brittle *V. amygdalina* leaves were pulverized using the traditional pounding method. The pulverized leaves were then soaked in one litre of distilled water and left to stand for 24 hours. This infusion was filtered through Whatman No. 1 filter paper. The resulting filtrate was evaporated to a sticky paste under a constant stream of cool air. Working solutions of the concentrated extracts were obtained by dissolving a calculated gram of the extract with 10 mls of sterile water in order to achieve a particular dosage for each of the experimental animal group.

### Phytochemical analysis of crude extract

Phytochemical analysis of the obtained *V. amygdalina* extract was carried out at the Department of Pharmacognosy, University of Nigeria, Nsukka. Alkaloids, glycosides, flavanoids, reducing sugars, steroids and terpanoids, phenols, tannins and carbohydrates in the

extract were determined using standard procedures as described by Trease and Evans (1996).

### Experimental animals

Forty nine (49) day-old broiler chicks (Cobb strain) purchased from Agrited hatchery, Ibadan, were used for this study. Management protocols in raising day old chicks were employed by brooding at a temperature range of 33 to 35°C with the provision of adequate feed and water for seven (7) days. The experimental animals were fed commercial broiler starter from day 0 to week 4, then broiler finisher from week 5 to Week 7 (Supreme Feeds, Nigeria Limited®). Clean drinking water was provided *ad libitum* throughout the experimental period and vaccination schedule as presented in Table 1 was strictly adhered to. The use of these birds was in accordance with the guidelines on the use of animals for research as set by the Institutional Animal Care Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

### Experimental design

After the birds were brooded for twenty-one (21) days, they were randomly divided into six (6) treatment groups and a control group with seven (n=7) birds in each group. The treatment groups were designated INL (5 mg/kg inulin), INM (10 mg/kg inulin), INH (20 mg/kg inulin), VAL (50 mg/kg *Vernonia amygdalina*), VAM (100 mg/kg *Vernonia amygdalina*) and VAH (200 mg/kg *Vernonia amygdalina*), corresponding to a graded concentration of Inulin and *Vernonia amygdalina* aqueous extract respectively. The animals in the control group were drenched with distilled water at the rate of 0.5 ml/100g of body weight. Dosing was done every morning (7:00-9:00 AM) for 28 days using a syringe. Weight of the birds in each group were taken weekly using a kitchen scale and the determined mean weight was used to calculate the concentration (mg/ml) of the inulin and the *Vernonia amygdalina* extract that was given to each group according to their dosage within a particular week.

### Weight gain determination

After brooding for twenty-one (21) days and random assignment in groups, the body weight of the broilers in each group were taken using a kitchen scale and their mean weight recorded as the weight for week 0. Following administration of the prebiotic substances (inulin and *Vernonia amygdalina* aqueous leaf extract), the mean body weight was determined and recorded for week 1, week 2, week 3 and week 4 at the end of the experiment The body weight gain for each group was then determined as thus:

**Table 1.** Vaccination schedule for the experimental animals.

| Day | Vaccine administered | Disease prevented |
|---|---|---|
| 0 | Hitchner B1 (i/o) | Newcastle disease |
| 8 | Gumboro | Infectious Bursal disease |
| 13 | Lasota | Newcastle disease |
| 21 | 2nd Gumboro | Infectious Bursal disease |
| 27 | 2nd Lasota | Newcastle disease |

Body weight gain = Week 4 mean body weight – Week 0 mean body weight

### Sample collection and intestinal measurements

After the treatment period, five birds were selected randomly from each group, euthanized by $CO_2$ asphyxiation, and dissected exposing the different segments of the digestive system starting from the tongue to the colorectum. The length of the different segments of the intestines (small intestine, caecum and colorectum) were measured after dissecting them out as described by Wang et al. (2016).

### Histomorphological samples, light microscopy and measurements

Five gram (5 g) of tissue samples from the duodenum, jejunum, ileum, caecum, and colorectum were taken for histomorphological studies. These tissue samples were fixed by immersion in Bouins fluid for 16 hours. The processing involves dehydration (70 to 100% absolute alcohol), clearing using xylene, paraffin embedding, sectioning (5 to 6 µm using microtome), and staining with hematoxylin and eosin stain. The tissue sections were then examined using Leica light compound microscope (Leica DM750).

The villus height, crypt depths and thickness of the tunica muscularis were measured with an eyepiece graticle, which was calibrated using a stage micrometer under x 40 magnification. Photomicrographs were captured using Moticam Image plus 2.0 digital camera (Motic China Limited) attached to a computer.

### Statistical analysis

Obtained data were analyzed with a One-way Analysis of Variance (ANOVA) and post hoc comparison with the Duncan test using SPSS version 21. Results were presented as Mean ± Standard deviation (SD). A probability level of less than 5% ($p<0.05$) was considered significant.

**Table 2.** Phytochemical constituents of *Vernonia amygdalina* aqueous extract.

| Parameter determined | Cold water extract | Remark |
|---|---|---|
| Alkaloid | - | Absent |
| Tannin | ++ | Moderately present |
| Saponin | +++ | Strongly present |
| Glycosides | ++ | Moderately present |
| Flavanoids | - | Absent |
| Steroids | ++ | Moderately present |
| Phenol | - | Absent |
| Carbohydrates | ++ | Moderately present |
| Reducing Sugars | ++ | Moderately present |
| Oil | ++ | Moderately present |

**Table 3.** Effects of different doses of the prebiotics, inulin and aqueous extract of *Vernonia amygdalina* on body weight.

| Week | Body weights (KG) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Inulin group | | | *Vernonia amydaline* group | | | Control (Distil water) | p-values |
| | INL (5mg/kg) | INM (10mg/kg) | INH (20mg/kg) | VAL (50mg/kg) | VAM (100mg/kg) | VAH (200mg/kg) | | |
| Week 0 | 0.624±0.14 | 0.631±0.18 | 0.626±0.17 | 0.653±0.49 | 0.639±0.22 | 0.632±0.25 | 0.646±0.15 | 0.842 |
| Week 1 | 0.820±0.18 | 0.849±0.24 | 0.882±0.18 | 0.893±0.66 | 0.859±0.19 | 0.750±0.30 | 0.758±0.21 | 0.573 |
| Week2 | 1.272±0.30 | 1.244±0.26 | 1.402±0.31 | 1.370±0.82 | 1.483±0.50 | 1.279±0.43 | 1.213±0.47 | 0.743 |
| Week 3 | 1.524±0.52 | 1.553±0.75 | 1.604±0.06 | 1.581±0.12 | 1.757±0.12 | 1.538±0.08 | 1.509±0.49 | 0.595 |
| Week 4 | 1.742±0.80 | 1.769±0.14 | 1.842±0.104 | 1.703±0.17 | 1.973±0.16 | 1.649±0.14 | 1.678±0.07 | 0.287 |
| Weight gain | 1.118 | 1.138 | 1.216 | 1.050 | 1.334 | 1.017 | 1.032 | |

## RESULTS AND DISCUSSION

Phytochemical analysis of aqueous extract of *Vernonia amydaline* revealed its constituents; tannins, saponins, glycoside, steroids, carbohydrates, reducing sugar and oil. Qualitatively, saponin was found to be the phytochemical constituent strongly present while others are present in a moderate amount as seen in Table 2. This result is in accordance with the work of Imaga and Bamigbetan (2013) on the biochemical assesment of aqeous extract of *Vernonia amydaline.* However in their work, flavanoids and alkaloid phytochemicals were present unlike in this study where it was seen to be absent. This could be explained to be due to the extraction method employed. Cold extraction method was used in the preparation of the extract in this study while hot infusion method was used in their work. In support of this, Ramirez-Rodrigues et al. (2011) have reported that phenolic constituents of phytochemicals are better extracted with hot water which results to their higher yield. The phytochemicals, saponin and carbohydrates as reported by Ezeonu et al. (2016) escapes digestion in the upper gastrointestinal tract and are only fermentable at the colon, which is an important property of prebiotics.

Although the mean body weight of the experimental animals across the study groups did not significantly differ throughout the experimental period (Table 3), it was observed that the groups treated with the graded concentration of inulin and *Vernonia amygdalina* aqueous extract posess a slightly higher weight gain compared to that of the control group that received distilled water. The broilers treated with inulin, however appear to have a slight dose dependent increase in weight gain where the group dosed with 20 mg/kg have a higher weight gain of 1.216 kg. Oritiz et al. (2009) in their work, reported that supplementation of inulin to broilers has not rewarded significant response to weight gain which supports this current study. On the contrary, the mean weight gain changes of broilers that received the aqueous extract of *Vernonia amygdalina* did not occur in a concentration dependent fashion where a higher weight gain of 1.334 kg was observed with the study group dosed with 100 mg/kg of the aqueous extract. This current study is in aggrement with the findings reported by Nwogwugwu et al. (2016) where in feed supplementation of broilers with *Vernonia amygdalina* caused a slight increase in their body weight. In the reports of Adaramoye et al. (2008), the slight increase in weight gain associated with *Vernonia amygdalina* supplementation is suggested to be as a result of its effect in enhancing nutrient digestion and assimulation through its action on digestive enzymes. However, in this study, it was observed that the study

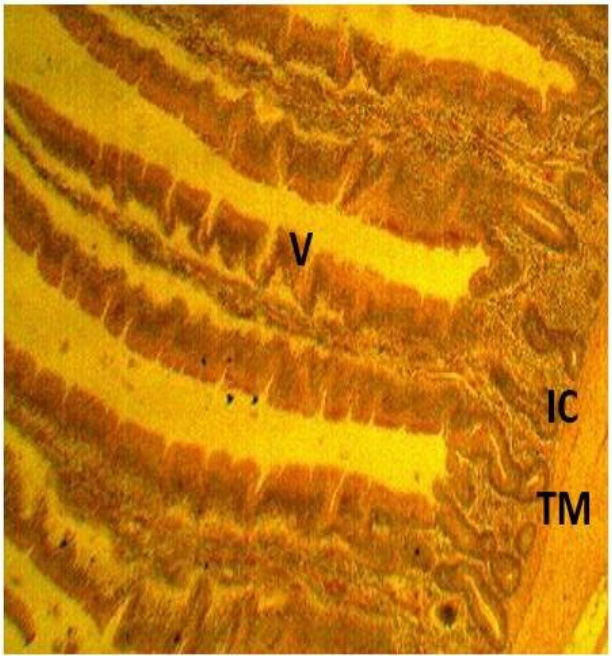


**Figure 1.** Photomicrograph of duodenum of a broiler chicken dosed with 20 mg/kg of inulin showing the; Villi (V), Intestinal crypts (IC) and the Tunica Muscularis (TM). H & E x4. The intestinal villi appears healthy and long with an undulating surface.

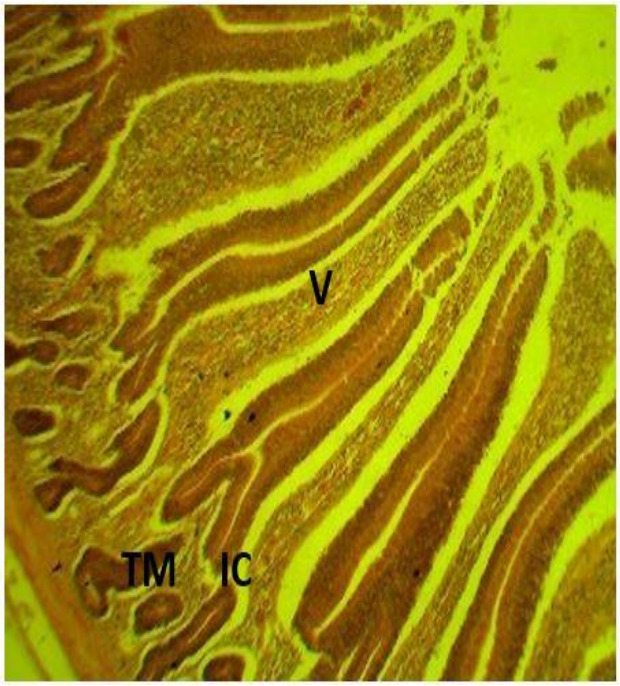


**Figure 2.** Photomicrograph of jejunum of a broiler chicken dosed with 20 mg/kg of inulin showing the; Villi (V), Intestinal crypts (IC) and the Tunica Muscularis (TM). H & E x4. The Jejunum have a normal appearing villi with deep intestinal crypts.

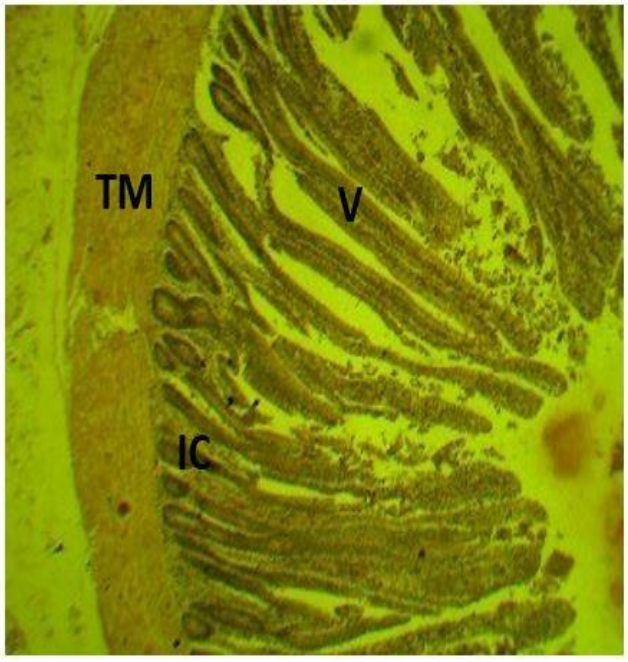


**Figure 3.** Photomicrograph of ileum of broiler chicken dosed with 20 mg/kg of inulin showing the; Villi (V), Intestinal crypts (IC), Tunica Muscularis (TM). H & E x4. The ileal villi are long and closely packed together with deep intestinal crypts.

group administered 200 mg/kg of the aqueous extract of *Vernonia amygdalina* have a slightly lower mean weight gain of 1.017 kg. Arhoghoro et al. (2009) in their work reported a decrease in weight gain in bird treated with high doses of *Vernonia. amygdalina* which is in agreement with the findings in this study. This might be as a result of the presence of anti-nutritive factors such as alkaloids, saponines, tannins and glycosides as reported by Yacout (2016).

The histological appearance of the small intestine (duodenum, jejunum and Ileum) of broilers in the various treatment groups and the control are shown in Figures 1 to 9. Generally, broilers treated with inulin showed a normal and healthy appearance of the duodenal, jejunal and ileal villi and crypt depth (Figures 1 to 3). The duodenal villi of the broilers that received inulin treatment appear to have healthy long appearing villi with an undulating surface (Figure 1). This arrangement of the villi is speculated to be more effective in nutrient absorption according to the reports of Yamauchi et al. (2010). The jejunal and illeal villi of the inulin administered broilers appear long with its normal appearing intestinal crypts just like the duodenal villi (Figures 2 and 3). This long appearing intestinal villi as observed with inulin dosed broilers is positively correlated with absorptive function because of a large surface area for nutrient absorption as opined by Rehman et al. (2008). Similarly, the duodenal villi of broilers administered with *Vernonia amygdalina*

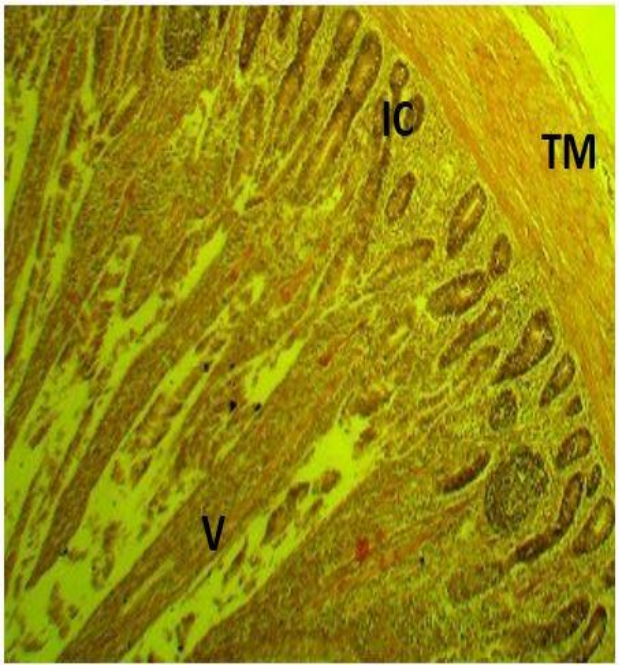


**Figure 4.** Photomicrograph of duodenum of a broiler chicken dosed with 200 mg/kg of *Vernonia amygdalina* aqueous leaf extract showing the; Villi (V), Intestinal crypts (IC), Tunica Muscularis (TM). H & E x4. There are areas of desquamation of the epithelial sheet of the villi.

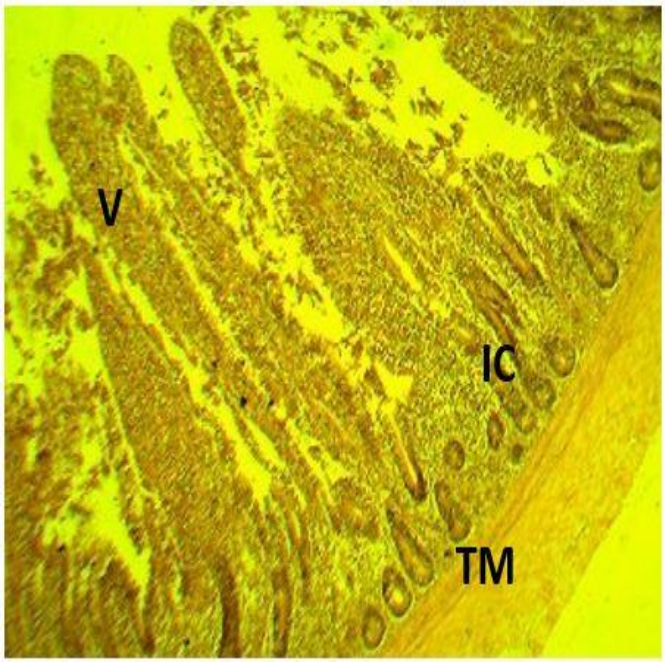


**Figure 6.** Photomicrograph of the ileum of broiler chicken dosed with 200 mg/kg *Vernonia amygdalina* aqueous leaf extract showing the; Villi (V), Intestinal crypts (IC), Tunica Muscularis (TM). H & E x4. Areas of desquamated villi epithelial sheet can be observed.

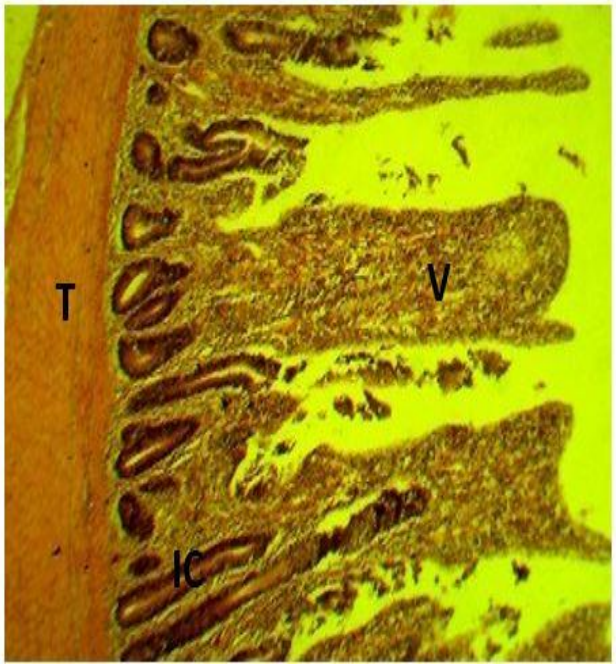


**Figure 5.** Histology of the jejunum of a broiler chicken dosed with 200 mg/kg *Vernonia amygdalina* aqueous leaf extract showing the; Villi (V), Intestinal crypts (IC), Tunica Muscularis (TM). H & E x4. There is an increased depthness of the intestinal crypts and shortened villi.

aqueous extract as shown in Figure 4 is observed to have a relatively long normal appearing villi with a deep intestinal crypts. However, there is somewhat appearance of desquamation of the epithelial sheet of the villi and also their associated deep crypt which is suggestive of increased epithelial cell turn over and enhanced mitosis as reported by Bucław (2016). However, the Jejunal villi of the broilers administered with 200 mg/kg of *Vernonia amygdalina* aqueous extract as seen in Figure 5 was observed to have a shortened intestinal villi which looks desquamated. This could be as a result of the anti-nutritive factors in this plant extract which at this high concentration will cause a decrease in enzymatic activity and bioavailability of nutrients required for the growth of intestinal villi as suggested by Yacout (2016). For the control group, the duodenal, jejunal and illeal villi are relatively shorter in appearance when compared with those of the broilers dosed with inulin and *Vernonia amygdalina* aqueous leaf extract (Figures 7 to 9). The duodenal histology of the broilers in the control group appeared to have disorganized villi (Figure 7). As suggested by Xu et al. (2003), the disorganization of the villi could be linked to increased inflammation of the mucosa caused by intestinal pathogen due to poor immune defenses, resulting in shortening of villi, which could lead to poor nutrient absorption.

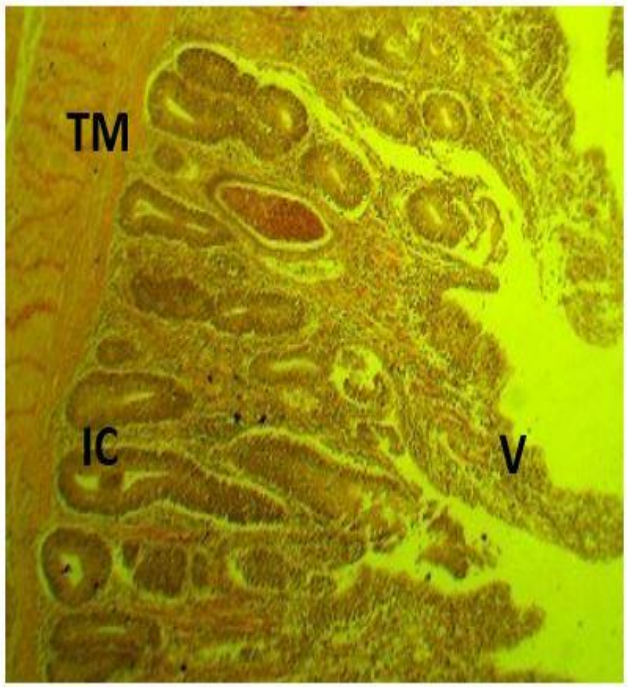


**Figure 7.** Photomicrograph of the duodenum of a chicken (control group) showing the; Villi (V), Intestinal crypts (IC), Tunica Muscularis (TM). H & E x4. The villi are disorganized with large intestinal crypts.

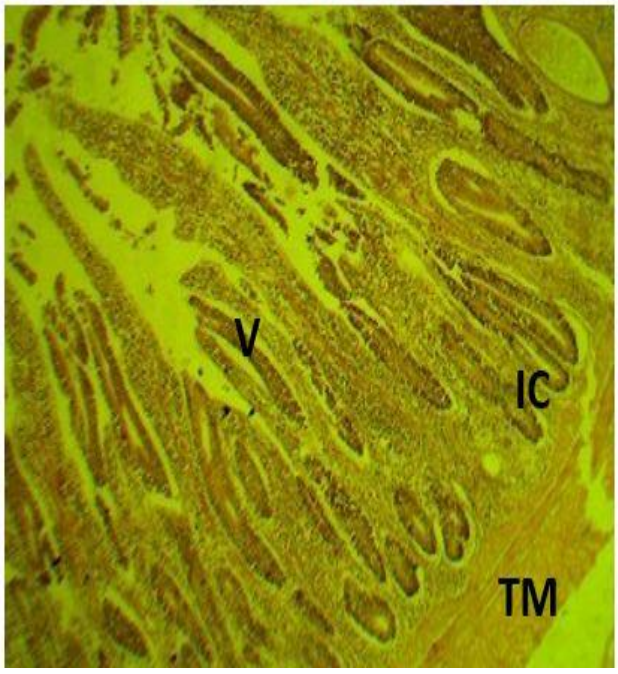


**Figure 8.** Photomicrograph of the jejunum of a chicken (control group) showing the; Villi (V), Intestinal crypts (IC), Tunica Muscularis (TM). H & E x4. Presence of abundant intestinal crypts with areas of desquamated intestinal villi epithelial sheet can be observed.

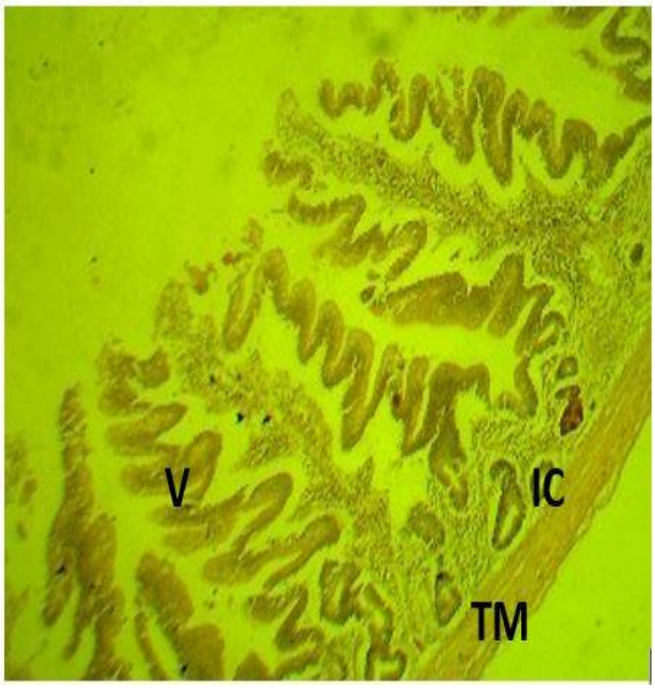


**Figure 9.** Photomicrograph of the ileum of a chicken (control group) showing the; Villi (V), Intestinal crypts (IC), Tunica Muscularis (TM). H & E x4.

The result presented in Table 4 shows that administration of inulin and *Vernonia amygdalina* aqueous leaf extract significantly increased ($p<0.05$) the duodenal, jejunal and ileal villus height. The duodenal villus height of birds treated with graded concentration of inulin appears to increase in a dose dependent fashion where the broilers that received the highest dosage of inulin of 20 mg/kg (INH group) have a higher mean duodenal villus height of 1142.500 ±41.50 µm. On the contrary, the mean duodenal villus height of broilers treated with aqueous extract of *Vernonia amygdalina* did not occur in dose concentration fashion where the group dosed with 100 mg/kg (VAM group) have a higher villi height of 1245.000±18.10 µm while increasing the dose up to 200 mg/kg resulted in a lower villus height of 876.000±39.99 µm. Similar to the results observed with duodenal villus height, a higher jejunal villus height of 418.500±8.59 was observed with the group dosed with 20 mg/kg of inulin while the VAM group dosed with 100 mg/kg of *Vernonia amygdalina* aqueous leaf extract have a higher mean jejunal vili height of 1083.000±65.88 µm. For ileal villus height, the groups treated with 20 mg/kg of inulin, 100 mg/kg of *Vernonia amygdalina* and 200 mg/kg of *Vernonia amygdalina* appeared to have a significantly higher mean illeal villi height of 862.500±16.89, 788.000±22.19 and 855.500±15.02 µm respectively when compared with that of the control with mean illeal villus height of 683.500±17.49 µm. This finding supports the work of

**Table 4.** Effects of different doses of the prebiotics, inulin and aqueous extract of *Vernonia amygdalina* on villus height.

| Groups | Villus height measurements (µm) | | |
|---|---|---|---|
| | Duodenum | Jejunum | Ileum |
| INL (5mg/kg of Inulin) | 744.500±22.03$^{a}$ | 756.000±38.79$^{ab}$ | 666.500±13.66$^{a}$ |
| INM (10mg/kg of Inulin) | 990.000±50.73$^{b}$ | 900.000±27.31$^{bc}$ | 674.500±15.16$^{a}$ |
| INH (20mg/kg of Inulin) | 1142.500±41.56$^{c}$ | 1128.500±36.62$^{d}$ | 862.500±16.89$^{c}$ |
| VAL(50mg/kg of VA) | 835.000±26.83$^{a}$ | 825.500±26.66$^{ab}$ | 683.500±10.14$^{a}$ |
| VAM (100mg/kg of VA) | 1245.000±18.10$^{c}$ | 1083.000±65.88$^{d}$ | 788.000±22.19$^{b}$ |
| VAH (200mg/kg of VA) | 876.000±39.99$^{ab}$ | 1039.500±21.08$^{cd}$ | 855.500±15.02$^{bc}$ |
| Control (Distilled water) | 740.000±16.43$^{a}$ | 712.500±18.193$^{a}$ | 683.500±17.49$^{a}$ |

Mean within a column with different superscript (a, b, c) are significantly different ($p<0.05$).

**Table 5.** Effects of different doses of the prebiotics, inulin and aqueous extract of *Vernonia amygdalina*on crypt depth.

| Groups | Crypt depth measurements (µm) | | |
|---|---|---|---|
| | Duodenum | Jejunum | Ileum |
| INL (5mg/kg of Inulin) | 366.500±16.86$^{a}$ | 305.500±13.95$^{ab}$ | 173.500±15.63$^{ab}$ |
| INM (10mg/kg of Inulin) | 410.500±15.38$^{ab}$ | 335.000±7.66$^{bc}$ | 202.000±12.70$^{bc}$ |
| INH (20mg/kg of Inulin) | 486.500±15.46$^{bc}$ | 418.500±8.59$^{d}$ | 141.000±6.84$^{a}$ |
| VAL (50mg/kg of VA) | 486.000±14.68$^{bc}$ | 379.000±8.76$^{cd}$ | 195.500±6.75$^{bc}$ |
| VAM (100mg/kg of VA) | 584.000±29.70$^{d}$ | 407.500±18.61$^{d}$ | 256.000±11.15$^{d}$ |
| VAH (200mg/kg of VA) | 495.500±14.07$^{c}$ | 394.500±13.69$^{d}$ | 222.500±14.33$^{cd}$ |
| Control (Distilled water) | 434.00±19.92$^{abc}$ | 255.500±10.60$^{a}$ | 143.500±8.28$^{a}$ |

Mean within a column with different superscript (a, b, c) are significantly different ($p<0.05$).

Rehman et al. (2008), where in-feed inulin supplementation in birds was found to significantly increase the height of different segments of intestinal villi. However, in the reports of Awad et al. (2011), it was shown that in feed supplementation of inulin in broilers only significantly increased the villus height of the duodenum and not other segments of the small intestine. Similarly, Ofongo–Abule and Ohimain (2019) in their works reported that *Vernonia amygdalina* extract supplementation to broiler chicken significantly increased the ileal villus height which supports the findings in this current study. As suggested by de los Santos et al. (2005), the mechanism of increase in intestinal villi upon supplementation with these prebiotic substances could be as a result of gut fermentation of these indigestible substances which led to the production of short chain fatty acids that can stimulate cell mitosis and also aid in the modulation of enteric microflora which will subsequently lead to a decrease in microbial load and a reduction in villi desquamation, thereby increasing the villus height. Rehman et al. (2008) opined that intestinal structure strongly affects its function and as such, any alteration in villi structure will have an effect in nutrient absorption since villi growth is positively correlated with absorptive function of the intestine.

In this study, the significant increase in intestinal villi as a result of inulin and *Vernonia amygdalina* aqueous leaf extract supplementation correlated with the findings on mean body weight where they appear to be slight higher in the treatment group, which is suggestive of a better absorptive function.

On crypt depth measurements **(**µm**)**, significant changes ($p<0.05$) were observed within the treatment groups and when the treatment groups were compared with the control group (Table 5). The birds treated with 10 and 20 mg/kg of inulin have a mean duodenal crypt depth of 410.500±15.38 and 486.500±15.46 µm respectively which is significantly higher when compared with the control group with the mean duodenal crypt depth of 434.00±19.92 µm. Comparatively, the birds dosed with 50, 100 and 200 mg/kg of *Vernonia amygdalina* aqueous extract have a significantly higher mean duodenal crypt depth of 486.000±14.68, 584.000±29.70 and 495.500±14.07 µm respectively when compared with that of the control group. The mean jejunal crypt depth of the control group (255.500±10.60 µm) was found to be significantly lower when compared with the mean crypt depth of the groups treated with inulin and *Vernonia amygdalina* aqueous extract. Unlike the other treatment group which was observed to have a significantly higher mean illeal crypt depth, the group treated with 20 mg/kg of inulin was found to have a mean ileal crypt depth of 141.000±6.84 µm which did not differ significantly with that of the control group with

**Table 6.** Effects of different doses of the prebiotics, inulin and aqueous extract of *Vernonia amygdalina (VA)* on intestinal length.

| Groups | Intestinal length (CM) | | |
|---|---|---|---|
| | **Small intestine** | **Caecum** | **Colorectum** |
| INL (5mg/kg of Inulin) | 182.500±7.50 | 20.400±1.50 | 10.550±0.45 |
| INM (10mg/kg of Inulin) | 172.300±6.70 | 19.050±0.45 | 10.700±1.30 |
| INH (20mg/kg of Inulin) | 195.500±4.90 | 21.150±0.45 | 8.400±0.20 |
| VAL (50mg/kg of VA) | 167.850±31.35 | 20.000±3.50 | 9.750±1.75 |
| VAM (100mg/kg of VA) | 162.400±4.80 | 20.250±0.15 | 9.650±2.35 |
| VAH (200mg/kg of VA) | 184.600±1.60 | 20.150±0.85 | 9.350±0.85 |
| Control (Distilled water) | 191.950±25.55 | 22.550±1.55 | 9.500±1.50 |
| P-value | 0.722 | 0.818 | 0.913 |

the mean ileal crypth depth of 143.500±8.28 µm. This finding affirms the work of Rehman et al. (2008) where it was reported that inulin supplementation in broilers significantly increased thier crypt depth. Awad et al. (2006) suggested that an increase in crypt depth is suggestive of a faster cell turn over which enhances villi growth. Therefore, the increase in intestinal crypt depth as seen in this study could be as a result of the prebiotic substances administered, which directly or indirectly aids in accelerating intestinal cell division as opined by Setiawan et al. (2018). The observed increase in mean crypt depth of the treatment group directly correlates with the significant increase in villus height of the groups administered with the prebiotics as observed in this study. On the contrary, this study disagrees with the report of Awad et al. (2011) where it was observed that inulin supplementation in birds significantly reduced their jejunal and ileal crypt depth.

No significant difference ($p>0.05$) was observed in associating intestinal length changes with the administration of inulin and Vernonia *amygdalina* aqueous leaf extract in broilers (Table 6). This is in agreement with the report of Ortiz et al. (2009) where inulin supplementation did not significantly affect intestinal morphological measurements in birds. However, the work of Chen et al. (2005) where in-feed supplementation of inulin at the rate of 10 g/kg increased the length of small intestine and colon is in contrary with the results in this study. This variable effects associated with inulin supplementation could be due to its plant source, inclusion level, environmental condition and animal characteristics as suggested by Verdonk and Shim (2005). In a similar context, it was also observed in this study that *Vernonia amygdalina* aqueous extract administration to broiler chicken did not cause any significant change ($p>0.05$) in the length of the small intestine, caecum and colorectum. This is supported by the work of Nwogwugwu et al. (2016) where in their study reported that administration of *Vernonia amygdalina* plant extract did not produce any significant changes both in intestinal length and weight in broiler chickens.

## Conclusion

This study conducted concludes that both prebiotics (Inulin and *Vernonia amygdalina* aqueous leaf extract) have the potential to increase the weight of birds through stimulation of villus growth and increase in crypt depth for better absorption of nutrients. The locally available *Vernonia amygdalina* leaf extract is comparable to inulin as regards to its effects in the intestinal anatomy of broiler chicken where both act in a similar pattern. However, unlike in inulin administration to broiler chicken where concentration dependent effects was observed (ie. the higher the dose, the corresponding increase in effect), the effects associated with the administration of *Vernonia amygdalina* aqueous leaf extract was observed to decline above 100 mg/kg. Therefore, administration of *Vernonia amygdalina* aqueous leaf extract to about 200 mg/kg will cause a reduction in the desirable effects of this prebiotics on digestive anatomy of broiler chicken.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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