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# EFFECT OF *Vernonia Amygdalina* LEAF MEAL EXTRACT IN DRINKING WATER ON POST-WEANING DIARRHEA OCCURRENCE IN PIGLETS

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## ABSTRACT

This study was aimed at determining the effect of *Vernonia amygdalina* leaf meal (VALM) extract in drinking water on the faecal microbial load on post-weaning diarrhea occurrence in piglets using different agar. Forty-five piglets of Large white X Duroc breed were given *Vernonia amygdalina* (VA) through water infusion. Three levels of 0.0g, 1.2g, and 2.4g of VA per 1000ml of clean drinking water designated as $T_1$, $T_2$ and $T_3$ were used. Nine piglets weaned at different ages of $2^{nd}$, $3^{rd}$, $4^{th}$, $5^{th}$, and $6^{th}$ week of age were randomly allotted to three treatments with each treatment replicated three times with a piglet per replicate in a completely randomized design. The different agar used are Macconkey Agar, Eosin Methylene Blue (EMB) Agar, and Nutrient Agar. These agars were used for 4days each to determine the faecal microbial load. The experiment lasted 28 days. Results showed that the bitter leaf contain high Alkaloids, medium Tannins, Saponins Flavonoids, and low amounts of Terpenoids and Phenols while Cardiac Glycosides were not determined. Bitter leaf showed the power of antibacterial activity for the reduction of faecal microbial load. Bitter leaf as a feed additive in the drinking water of piglets caused a significant ($P<0.05$) reduction in the faecal microbial load for piglets given 1.2g and 2.4g of VA per 1000ml of drinking water. It can be concluded that bitter leaf extract in drinking water can be used as phyto-additive for piglets to reduce faecal microbial load.

**Keywords**: *Vernonia amygdalina*; piglets; post-weaning diarrhea; antimicrobial; phyto-additive

## INTRODUCTION

The anti-microbial, anti-inflammatory, and anti-oxidative potential of herbal plants can affect pig performance by improving digestive tract function. Herbs can also provide many functions in the pig's body system (Hernández *et al.*, 2004). Antibiotic and antibacterial medications are still used in the pig industry for several purposes as therapeutic, prevention, or as traditional growth promoters (Diarra, 2014). Alternative phytogenic additives improve several principal processes in the livestock's body. They are also applicable in the food industry due to their antibacterial properties (Karásková, 2015).

Antimicrobial peptide (AMP) a component of bitter leaf (*Vernonia amygdalina*) defense system can be isolated from roots, seeds, flowers, stems, and leaves, and has activity against phytopathogens, as well as against pathogenic bacteria in humans (Nawrot, 2014). Wang and Wang (2004) and Hammami *et al.* (2009) reported a study comparing the primary and tertiary structures of plant antimicrobial peptides showed that 33% of plant peptides had activity against bacteria, and these antibacterial peptides were composed of cysteine and/or glycine residues. Plant AMP is considered a good drug because of its chemical properties combined with biological specificity such as antibodies (Craik, 2018). Plant AMP exhibits broad-spectrum antibiotic activity against pathogenic bacteria, fungi, enveloped viruses, and parasites (Havenga, 2019; Marcocci, 2018; Li *et al.*, 2021).

Bitter leaves (*Vernonia amygdalina*) are used in tropical Africa for multiple purposes, especially in culinary and traditional medicine for malaria, hepatitis, diarrhea, venereal disease, diabetes, digestive problems, skin disorders, coughs, constipation, and in wounds treatments (Ajebesone and Aina, 2004). The administration of 50 mL/L of bitter leaf water extract in drinking water did not cause any adverse effect on performance, reducing total cholesterol, LDL, and glucose in broiler blood plasma (Oleforuh-Okoleh, 2015).

Traditionally, much attention has been directed justifiably to the role of phytochemicals in animal nutrition. Phytochemicals such as alkaloids, tannins, phenol, saponin, cardiac glycosides, terpenoids, and flavonoids are known to have anti-inflammatory, anti-cancer, antibacterial, and antifungal activities. However, there is no information about the antimicrobial activity of bitter leaves. This study was to determine the effect of *Vernonia amygdalina* (VA) leaf extract in water on the faecal microbial load on post-weaning diarrhea occurrence in piglets using different agar.

## MATERIALS AND METHODS

### Experimental Site

This study was conducted in the Piggery Unit of the Faculty of Agriculture and Agricultural Technology Teaching and Research Farms, Legacy Campus, Benson Idahosa University, Benin City, Edo State. It is located between Latitude $6^0$ and 11'48"N of the Equator and Longitude $5^0$39'21" E of the Greenwich Meridian, in the rainforest zone, with a temperature of

$27.6^0C$. Benin City has an average annual rainfall and relative humidity of 2162mm and 72.5% respectively.

**Source and preparation of *Vernonia amygdalina* (VA)**

Fresh *Vernonia amygdalina* (bitter leaf) was harvested from the Delta Steel Company Housing Complex, Warri, Delta State. The leaves were dried at room temperature while retaining the greenish colouration, and then ground into *Vernonia amygdalina* leaf meal (VALM). The bitter leaf extract was prepared by soaking 1.2 and 2.4g of the ground bitter leaf meal in 1 litre of boiled water overnight (12 hours) respectively. This was filtered in the morning and the measured quantity of filtrate according to the experimental treatment was added to 1000ml of pure drinking water and served to the piglets. The treatment was made available on a daily basis.

**Management of Experimental Piglets**

A total of forty-five piglets were used for the study. The piglets used were the offspring of Large white X Duroc breed. The piglets were randomly picked from synchronized farrowed sows and their piglets were weaned at different ages of $2^{nd}$, $3^{rd}$, $4^{th}$, $5^{th}$, and $6^{th}$ week of age. Nine piglets per week were randomly allotted to three treatments with each treatment replicated three times with one piglet per replicate in a completely randomized design (CRD). The experimental treatment consisted of nine piglets maintained on 0.0g VALM/1000ml of water ($T_1$), 1.2g VALM/1000ml of water ($T_2$), and 2.4g VALM/1000ml of water ($T_3$) for seven consecutive days. The experiment lasted seven days each week. Faecal samples were collected from each treatment for 4 days and 3 agar (Macconkey Agar, Eosin Methylene Blue (EMB) Agar, and Nutrient Agar) were used to determine the microbial load on each piglet given the different levels of VA. There was no mortality recorded throughout the experimental period.

The experimental piglets were fed *ad libitum* on a commercially (Top feed) formulated starter diet and Palm Kernel Cake (PKC) combined in equal proportion.

**Phytochemical Screening of *Vernonia amygdalina* and Data Collection**

Phase 1 was the analysis of *Vernonia amygdalina* leaf meal which was subjected to a standard chemical test for the detection of alkaloids, tannins, phenol, saponin, cardiac glycosides, terpenoids, and flavonoids.

The powdered sample of *Vernonia amygdalina* leaf was carefully screened for alkaloids, tannin, saponin, phenol, and flavonoid by following the standard procedure described by Nagaraju *et al.* (2019), Hidayathula *et al.* (2011), Igbinosa *et al.* (2009) and Kaur *et al.* (2015).

**Faecal Matter Analysis**

Phase 2 involves the collection of faecal samples from each piglet in the treatment. The samples were taken to the laboratory for analysis. The faecal matter from the piglets were collected and analyzed for microbial load on the last four consecutive days after weaning for each week during the experimental period.

**Statistics**

The data collected on the different microbial loads using the agars were subjected to a one-way analysis of variance, and then the differences between the treatment means were compared using the Least Significant Difference Test. All statistical analysis was performed with IBM SPSS Statistics 24 software.

## RESULTS

Table 1. Quantitative Phytochemical Analysis of Bitter Leaf (*Vernonia amygdalina*) Meal.

| Parameters | (%) |
|---|---|
| ALK | 4.31 |
| TAN | 6.34 |
| SAP | 3.26 |
| FLAV | 0.68 |
| GLY | ND |
| TERP | 1.40 |
| PHEN | 0.48 |

ALK = Alkaloids; TAN = Tannins; SAP = Saponins; FLAV = Flavonoids; GLY = Cardiac Glycosides; TERP = Terpenoids; PHEN = Phenols; ND = Not Determined

Table 2: Qualitative Phytochemical Screening of Bitter Leaf (*Vernonia amygdalina*) Meal.

| Parameters | (%) |
|---|---|
| ALK | +++ |
| TAN | ++ |
| SAP | ++ |
| FLAV | ++ |
| GLY | ++ |
| TERP | - |
| PHEN | + |

**NOTE:** + = Trace; ++ = Medium; +++ = High.

**Table 3.** Feacal Microbial Load of piglets weaned at 2 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Nutrient Agar

| Parameters | Dietary Groups | | | S.E.M |
|---|---|---|---|---|
| | TRT 1 0g VALM | TRT 2 1.2g VALM | TRT 3 2.4g VALM | |
| Day 1 | 525.000[a] | 347.000[b] | 405.000[c] | 0.346 |
| Day 2 | 542.000[a] | 289.000[b] | 385.000[c] | 0.345 |
| Day 3 | 551.000[a] | 230.000[b] | 329.000[c] | 0.339 |
| Day 4 | 593.000[a] | 205.000[b] | 268.000[c] | 0.316 |

[abc]Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT-Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VALM/1000ml of water, SEM.– Standard Error of Mean.

Table 3 shows the faecal microbial load of piglets weaned at 2 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Nutrient agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments the highest (593.000) microbial load on Day 4 and TRT 2 has the lowest (205.000) microbial load also on Day 4.

Table 4. Feacal Microbial Load of piglets weaned at 2 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Macconkey Agar.

| Parameters | Dietary Groups | | | S.E.M |
|---|---|---|---|---|
| | TRT 1 0g VALM | TRT 2 1.2g VALM | TRT 3 2.4g VALM | |
| Day 1 | 528.000[a] | 356.000[b] | 415.000[c] | 0.376 |
| Day 2 | 549.000[a] | 289.000[b] | 305.000[c] | 0.346 |
| Day 3 | 582.000[a] | 210.000[b] | 249.000[c] | 0.344 |
| Day 4 | 595.000[a] | 195.000[b] | 228.000[c] | 0.422 |

Abc Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT-Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VAlM/1000ml of water, SEM.– Standard Error of Mean.

Table 4 shows the faecal microbial load of piglets weaned at 2 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Macconkey agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (528.000, 549.000, 582.000, and 595.000) microbial load and TRT 2 has the lowest (356.000, 289.000, 210.000 and 195.000) microbial load from day 1 to day 4.

**Table 5.** Feacal Microbial Load of piglets weaned at 2 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Eosin Methylene Blue (EMB) Agar.

| Parameters | Dietary Groups | | | S.E.M |
|---|---|---|---|---|
| | TRT 1<br>0g VALM | TRT 2<br>1.2g VALM | TRT 3<br>2.4g VALM | |
| Day 1 | 551.000[a] | 349.000[b] | 432.000[c] | 0.345 |
| Day 2 | 563.000[a] | 328.000[b] | 402.000[c] | 0.147 |
| Day 3 | 574.000[a] | 281.000[b] | 387.000[c] | 0.241 |
| Day 4 | 603.000[a] | 256.000[b] | 331.000[c] | 0.322 |

[abc]Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT-Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VAlM/1000ml of water, SEM.– Standard Error of Mean.

Table 5 shows the faecal microbial load of piglets weaned at 2 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Eosin Methylene Blue agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (551.000, 563.000, 574.000, and 603.000) microbial load and TRT 2 has the lowest (349.000, 328.000, 281.000 and 256.000) microbial load.

**Table 6.** Feacal Microbial Load of piglets weaned at 3 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Nutrient Agar.

| Parameter | Dietary Groups | | | S.E.M |
|---|---|---|---|---|
| | TRT 1<br>0g VALM | TRT 2<br>1.2g VALM | TRT 3<br>2.4g VALM | |
| Day 1 | 521.000[a] | 302.000[b] | 395.000[c] | 0.241 |
| Day 2 | 545.000[a] | 259.000[b] | 346.000[c] | 0.344 |
| Day 3 | 568.000[a] | 232.000[b] | 301.000[c] | 0.238 |
| Day 4 | 583.000[a] | 165.000[b] | 254.000[c] | 0.416 |

[abc] Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT-Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VAlM/1000ml of water, SEM.– Standard Error of Mean.

Table 6 shows the faecal microbial load of piglets weaned at 3 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Nutrient agar. There was a significant difference ($p>0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (521.000, 545.000, 568.000, and 583.000) microbial load and TRT 2 has the lowest (302.000, 259.000, 232.000 and 165.000) microbial load from day 1 to day 4.

**Table 7.** Feacal Microbial Load of piglets weaned at 3 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Macconkey Agar.

| Parameters | Dietary Groups | | | S.E.M |
|---|---|---|---|---|
| | TRT 1<br>0g VALM | TRT 2<br>1.2g VALM | TRT 3<br>2.4g VALM | |
| Day 1 | 518.000[a] | 315.000[b] | 409.000[c] | 0.341 |
| Day 2 | 529.000[a] | 246.000[b] | 354.000[c] | 0.146 |
| Day 3 | 536.000[a] | 218.000[b] | 301.000[c] | 0.244 |
| Day 4 | 541.000[a] | 171.000[b] | 232.000[c] | 0.142 |

[abc] Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT-Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VAlM/1000ml of water, SEM.– Standard Error of Mean.

Table 7 shows the faecal microbial load of piglets weaned at 3 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Macconkey agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest

(518.000, 529. 000, 536.000, and 541.000) microbial load and TRT 2 has the lowest (315.000, 246.000, 218.000 and 171.000) microbial load.

**Table 8.** Feacal Microbial Load of piglets weaned at 3 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Eosin Methylene Blue (EMB) Agar.

| Parameters | Dietary Groups | | | S.E.M |
|---|---|---|---|---|
| | TRT 1<br>0g VALM | TRT 2<br>1.2g VALM | TRT 3<br>2.4g VALM | |
| Day 1 | 546.000[a] | 327.000[b] | 412.000[c] | 0.145 |
| Day 2 | 552.000[a] | 297.000[b] | 372.000[c] | 0.342 |
| Day 3 | 569.000[a] | 265.000[b] | 302.000[c] | 0.421 |
| Day 4 | 578.000[a] | 184.000[b] | 251.000[c] | 0.242 |

[abc]Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT-Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VAlM/1000ml of water, SEM.– Standard Error of Mean.

Table 8 shows the faecal microbial load of piglets weaned at 3 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Eosin Methylene Blue agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (546.000, 552.000, 569.000, and 578.000) microbial load and TRT 2 has the lowest (327.000, 297.000,265.000 and 184.000) microbial load from day 1 to day 4.

**Table 9.** Feacal Microbial Load of piglets weaned at 4 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Nutrient Agar.

| Parameters | Dietary Groups | | | S.E.M |
|---|---|---|---|---|
| | TRT 1<br>0g VALM | TRT 2<br>1.2g VALM | TRT 3<br>2.4g VALM | |
| Day 1 | 455.000[a] | 195.000[b] | 310.000[c] | 0.426 |
| Day 2 | 479.000[a] | 137.000[b] | 295.000[c] | 0.374 |
| Day 3 | 487.000[a] | 118.000[b] | 232.000[c] | 0.438 |
| Day 4 | 492.000[a] | 94.000[b] | 197.000[c] | 0.216 |

[abc]Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT-Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VAlM/1000ml of water, SEM.– Standard Error of Mean.

Table 9 shows the faecal microbial load of piglets weaned at 4 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Nutrient agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (455.000, 479.000, 487.000, and 492.000) microbial load and TRT 2 has the lowest (195.000, 137.000, 118.000 and 94.000) microbial load.

**Table 10.** Feacal Microbial Load of piglets weaned at 4 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Macconkey Agar.

| Parameters | Dietary Groups | | | S.E.M |
|---|---|---|---|---|
| | TRT 1<br>0g VALM | TRT 2<br>1.2g VALM | TRT 3<br>2.4g VALM | |
| Day 1 | 485.000[a] | 296.000[b] | 394.000[c] | 0.145 |
| Day 2 | 492.000[a] | 245.000[b] | 352.000[c] | 0.231 |
| Day 3 | 498.000[a] | 208.000[b] | 281.000[c] | 0.362 |
| Day 4 | 503.000[a] | 173.000[b] | 215.000[c] | 0.426 |

[abc]Means along the same row with different superscripst are significantly different.VA- *Vernonia amygdalina* TRT-Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VAlM/1000ml of water, SEM.– Standard Error of Mean.

Table 10 shows the faecal microbial load of piglets weaned at 4 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Macconkey agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (485.000, 492.000, 498.000, and 503.000) microbial load and TRT 2 has the lowest (296.000, 245.000, 208.000 and173.000) microbial load from day 1 to day 4.

**Table 11.** Feacal Microbial Load of piglets weaned at 4 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Eosin Methylene Blue (EMB) Agar.

| Parameters | Dietary Groups | | | S.E.M |
|---|---|---|---|---|
| | TRT 1 0g VALM | TRT 2 1.2g VALM | TRT 3 2.4g VALM | |
| Day 1 | 520.767$^{a}$ | 318.667$^{b}$ | 398.433$^{c}$ | 0.178 |
| Day 2 | 532.500$^{a}$ | 298.900$^{b}$ | 362.900$^{c}$ | 0.465 |
| Day 3 | 546.100$^{a}$ | 235.900$^{b}$ | 322.033$^{c}$ | 0.342 |
| Day 4 | 566.600$^{a}$ | 189.800$^{b}$ | 281.000$^{c}$ | 0.341 |

$^{abc}$Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT-Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VALM/1000ml of water, SEM.– Standard Error of Mean.

Table 11 shows the faecal microbial load of piglets weaned at 4 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Eosin Methylene Blue agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (520.767, 532.500, 546.100, and 566.600) microbial load and TRT 2 has the lowest (318.667, 298.900, 235.900 and 189.800) microbial load compared to piglets on TRT 3.

**Table 12.** Feacal Microbial Load of piglets weaned at 5 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Nutrient Agar.

| Parameters | Dietary Groups | | | S.E.M |
|---|---|---|---|---|
| | TRT 1 0g VALM | TRT 2 1.2g VALM | TRT 3 2.4g VALM | |
| Day 1 | 435.167$^{a}$ | 192.900$^{b}$ | 284.600$^{c}$ | 0.412 |
| Day 2 | 448.667$^{a}$ | 185.900$^{b}$ | 253.200$^{c}$ | 0.323 |
| Day 3 | 457.233$^{a}$ | 162.000$^{b}$ | 222.933$^{c}$ | 0.331 |
| Day 4 | 486.233$^{a}$ | 101.933$^{b}$ | 205.333$^{c}$ | 0.265 |

$^{abc}$Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT-Treatment, Trt 1–0g VALM/1000ml of water, Trt 2–1.2g VALM/1000ml of water, Trt 3 – 2.4g VAlM/1000ml of water, SEM.– Standard Error of Mean.

Table 12 shows the faecal microbial load of piglets weaned at 5 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Nutrient agar. There was a significant difference ($p>0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (435.167, 448.667, 457.233, and 486.233) microbial load and TRT 2 has the lowest (192.900, 185.900, 162.000 and 101.933) microbial load when compared to piglets on TRT 3.

**Table 13.** Feacal Microbial Load of piglets weaned at 5 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Macconkey Agar.

| Parameters | Dietary Groups<br>TRT 1<br>0g VALM | <br>TRT 2<br>1.2g VALM | <br>TRT 3<br>2.4g VALM | S.E.M |
|---|---|---|---|---|
| Day 1 | 450.833[a] | 240.922[b] | 358.900[c] | 0.423 |
| Day 1 | 462.066[a] | 218.900[b] | 308.967[c] | 0.341 |
| Day 1 | 479.000[a] | 182.000[b] | 276.324[c] | 0.286 |
| Day 1 | 488.000[a] | 98.933[b] | 203.600[c] | 0.372 |

[abc]Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT- Treatment, Trt 1–0g VALM/1000ml of water, Trt 2–1.2g VALM/1000ml of water, Trt 3–2.4g VALM/1000ml of water, SEM.– Standard Error of Mean.

Table 13 shows the faecal microbial load of piglets weaned at 5 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Macconkey agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (450.833, 462.066, 479.000, and 488.000) microbial load and TRT 2 has the lowest (240.922, 218.900, 182.000 and 98.933) microbial load when compared with TRT 3 from day 1 to 4.

**Table 14.** Feacal Microbial Load of piglets weaned at 5 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Eosin Methylene Blue (EMB) Agar.

| Parameters | Dietary Groups<br>TRT 1<br>0g VALM | <br>TRT 2<br>1.2g VALM | <br>TRT 3<br>2.4g VALM | S.E.M |
|---|---|---|---|---|
| Day 1 | 492.767[a] | 289.933[b] | 371.433[c] | 0.167 |
| Day 2 | 506.500[a] | 271.000[b] | 342.900[c] | 0.433 |
| Day 3 | 518.100[a] | 215.500[b] | 289.033[c] | 0.285 |
| Day 4 | 538.600[a] | 160.800[b] | 231.000[c] | 0.336 |

[abc]Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT- Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VALM/1000ml of water, SEM.– Standard Error of Mean.

Table 14 shows the faecal microbial load of piglets weaned at 5 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Eosin Methylene Blue agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (492.767, 506.500, 518.100, and 538.600) microbial load and TRT 2 (289.933, 271.000, 215.500 and 160.800) has the lowest microbial load.

**Table** 15**.** Feacal Microbial Load of piglets weaned at 6 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Nutrient Agar.

| Parameters | **Dietary Groups**<br>TRT 1<br>0g VALM | <br>TRT 2<br>1.2g VALM | <br>TRT 3<br>2.4g VALM | S.E.M |
|---|---|---|---|---|
| Day 1 | 415.800[a] | 180.867[b] | 262.233[c] | 0.368 |
| Day 2 | 426.600[a] | 169.067[b] | 221.700[c] | 0.458 |
| Day 3 | 431.233[a] | 143.533[b] | 206.133[c] | 0.233 |
| Day 4 | 451.000[a] | 129.300[b] | 198.533[c] | 0.178 |

[abc]Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT- Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VALM/1000ml of water, SEM.– Standard Error of Mean.

Table 15 shows the faecal microbial load of piglets weaned at 6 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Nutrient agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (415.800, 426.600, 431.233, and 451.000) microbial load and TRT 2 has the lowest (180.867.867, 169.067, 143.533 and 129.300) microbial load.

**Table** 16**.** Feacal Microbial Load of piglets weaned at 6 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal using Macconkey Agar.

| Parameters | Dietary Groups | | | S.E.M |
|---|---|---|---|---|
| | TRT 1 0g VALM | TRT 2 1.2g VALM | TRT 3 2.4g VALM | |
| Day 1 | 421.500$^{a}$ | 210.933$^{b}$ | 302.200$^{c}$ | 0.233 |
| Day 2 | 434.200$^{a}$ | 194.567$^{b}$ | 256.333$^{c}$ | 0.246 |
| Day 3 | 446.467$^{a}$ | 166.533$^{b}$ | 219.733$^{c}$ | 0.362 |
| Day 4 | 464.000$^{a}$ | 106.333$^{b}$ | 201.433$^{c}$ | 0.216 |

$^{abc}$Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT-Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VAlM/1000ml of water, SEM.– Standard Error of Mean.

Table 16 shows the faecal microbial load of piglets weaned at 6 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Macconkey agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (421.500, 434.200, 446.467, and 464.000) microbial load and TRT 2 has the lowest (210.933, 194.567, 166.533 and 106.333) microbial load when compared to TRT 3 from day 1 to 4.

**Table 17.** Feacal Microbial Load of piglets weaned at 6 weeks of age given bitter leaf (*Vernonia amygdalina*) meal using Eosin Methylene Blue (EMB) Agar.

| Parameters | **Dietary Groups** | | | S.E.M |
|---|---|---|---|---|
| | TRT 1 0g VALM | TRT 2 1.2g VALM | TRT 3 2.4g VALM | |
| Day 1 | 451.762$^{a}$ | 258.133$^{b}$ | 362.100$^{c}$ | 0.392 |
| Day 2 | 463.900$^{a}$ | 230.500$^{b}$ | 340.766$^{c}$ | 0.248 |
| Day 3 | 471.533$^{a}$ | 148.700$^{b}$ | 213.633$^{c}$ | 0.321 |
| Day 4 | 483.167$^{a}$ | 92.533$^{b}$ | 196.600$^{c}$ | 0.246 |

$^{abc}$Means along the same row with different superscripts are significantly different.VA- *Vernonia amygdalina*, TRT-Treatment, Trt 1 – 0g VALM/1000ml of water, Trt 2– 1.2g VALM/1000ml of water, Trt 3 – 2.4g VALM/1000ml of water, SEM.– Standard Error of Mean.

Table 17 shows the faecal microbial load of piglets weaned at 6 weeks of age given varying levels of bitter leaf (*Vernonia amygdalina*) meal for seven consecutive days using Eosin Methylene Blue agar. There was a significant difference ($p<0.05$) in the microbial load of the piglets across the treatments for Days 1, 2, 3, and 4 with TRT 1 having the highest (451.762, 463.900, 471.533, and 483.167) microbial load and TRT 2 has the lowest (258.133, 230.500, 148.700 and 92.533) microbial load.

**DISCUSSION**

The data of Alkaloids, Tannins, Saponins, Flavonoids, Cardiac Glycosides, Terpenoids, and Phenols were presented in Table 1. It shows that these phytochemicals in *Vernonia amygdalina* leaf meal (VALM) are present in amounts that were able to confer medicinal value. The phytochemical analysis carried out indicated that alkaloids were highly present, tannins saponins, and flavonoids were moderately present while phenols and terpenoids were slightly present, and cardiac glycosides were not determined in the VALM. The presence of these phytochemicals in VALM confirms the report by Egharevba *et al.* (2014) that VALM possesses flavonoids, alkaloids, tannins, phenols, and terpenoids which can be extracted from leaves. The presence of alkaloids in plants is characterized by its bitter taste. *Vernonia amygdalina* leaves are characteristically bitter, hence, the name bitter leaf. This further affirms what Chiemela *et al.* (2015) reported that alkaloids are one of the most significantly bitter substances known.

The effect of *Vernonia amygdalina* leaf meal on faecal microbial load of piglets weaned at two weeks through six weeks using different agars for a period of four days followed a definite trend with *Vernonia amygdalina* inclusion. It was observed that TRT 2 had the lowest microbial load followed by TRT 3 but TRT 1 had the highest microbial load. This showed that the piglets conveniently drank more from TRT 2 and it could be attributed to the *Vernonia amygdalina* inclusion level (1.2%) which did not make the water too bitter and it enhanced the gastro-intestinal enzymes thus increasing the water intake as reported by Chiemela *et al.* (2015). This high volume of water intake gave the lowest faecal microbial load of the piglets on this treatment. This revealed that *Vernonia amygdalina* at this inclusion level has some beneficial effects on the disease management of piglets. This finding is in agreement with recent findings on plant–based vaccines for the protection of piglets against Enterotoxigenic *Escherichia coli* (ETEC) (Havenga *et al.,* 2019). A rice-based cholera vaccine expressing the choleratoxin (CT) subunit B (CTB) (Muco Rice-CTB) was tested in pigs for protection against LT-ETEC infection (Takeyama *et al.,* 2015). CTB-based vaccines can target not only F4-type but also F18-type ETECs, and this vaccine also induced maternal CTB-specific IgG and IgA in the colostrum and milk of sows after farrowing. CTB-specific antibodies were also secreted into the gut lumen of weaned pigs and reduced intestinal loop fluid accumulation upon ETEC challenge, indicating a protective effect of this vaccine against ETEC diarrhea (Luo *et al.*, 2016). Also, fibers improve intestinal health by promoting the establishment of a healthy bacterial community in the hindgut (Postma *et al.,* 2015). In addition to that, fibers improve intestinal morphology (Ferarra *et al.*, 2017) and prevent diarrhea formation by decreasing *Escherichia coli* counts in faeces (Uddin *et al.*, 2017). Furthermore, a study by Luo and colleagues compared the effect of hydrolyzable tannins (HT) to pharmacological doses of ZnO in post-weaning piglets. The utilization of HT could reduce diarrhea incidences at the same extent of 2000ppm of ZnO, and showed a synergistic tendency in the group supplement with both HT and ZnO.

A careful look at the microbial load of the faecal matter using Eosin – Methylene Blue (EMB) Agar, MacConkey Agar, and Nutrient Agar showed that EMB agar revealed the highest faecal microbial load followed by MacConkey agar irrespective of the weaning age. This means that EMB agar and MacConkey agar were able to isolate and revealed most of the Gram-negative bacteria that cause diseases such as E. coli, Salmonella, etc. This finding is supported by Tambaduo *et al.* (2016) who reported that Eosin-Methylene Blue agar is selective for gram-negative bacteria against gram-positive bacteria; also, Eosin – Methylene Blue (EMB) Agar is useful in the isolation and differentiation of the various gram-negative bacilli and enteric bacilli, generally known as coliforms and faecal coliforms respectively. Also, Lan *et al.* (2016) reported that MacConkey agar, like EMB agar, inhibits the growth of most gram-positive pathogens and it is commonly used to differentiate *E. coli* from other gram-negative pathogens. This equally explains the diarrhea experienced in piglets weaned at week 2 (TRT 1) and piglets weaned at week 4 (TRT 1). Piglet weaned at week 2 (TRT1) experienced diarrhea on the second day of the experimental period; also, piglet weaned at week 4 (TRT 1) experienced diarrhea on the fifth day of the experimental period. In both cases, following veterinary doctor's prescription, one capsule of Tetracycline and half tablet of Metronidazole per Os were administered to each of them orally without water but they were allowed to drink water immediately, three times daily for five days. There was improvement on the viscosity of the faecal matter on the third day, however, the administration of the antibiotic continued until the fifth day, and there was no sign of diarrhea at this point. Furthermore, irrespective of the agar used, as the weaning age was increasing, the microbial load in the faecal matter was reduced. This revealed that increased weaning age or late weaning of piglets reduces the faecal microbial load and in turn reduces the chances of piglets experiencing diarrhea and its consequences. This result is in consonance with the findings of Rhouma *et al.* (2017) who stated that many stress factors are associated with the weaning period. Moreover, studies investigating the profitability of weaning piglets based on their age had encouraged weaning piglets not earlier than 28 days of age to reduce the occurrence of post-weaning diarrhea (Luo *et al.*, 2017; Main *et al.*, 2014).

However, in all the weaning weeks, *Vernonia amygdalina* inclusion at 1.2% (TRT 2) always reduced the microbial load meaningfully, irrespective of the agar used. This showed that 1.2% *Vernonia amygdalina* inclusion level in piglets' drinking water is a practical and achievable approach to solving the problem of weaned piglet diarrhea, improvement in animal gut health, and reducing economic losses in piglets without promoting bacteria resistance.

This study is in agreement with the findings of Visschers *et al.* (2015) that in several countries, implementation of financial penalties for high anti-microbial users is proposed as a method to reduce antimicrobial usage and pig farmers would receive financial bonus when they use alternative methods or when they greatly reduce antimicrobial usage on their farms.

## CONCLUSION

Results showed that bitter leaf contain alkaloids, tannins, phenol, saponin, terpenoids and flavonoids in high and medium percentages. It showed the power of antimicrobial activity. Bitter leaf extract as additive in the drinking water of piglets caused a reduction in faecal microbial activities within 2 weeks through to 6 weeks. Therefore, bitter leaf extract in water is recommended for piglets.

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