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Amla extract's impact on pre-weaning piglet diarrhea: An investigation into the therapeutic potential of Indian Gooseberry (*Phyllanthus emblica* L.)

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Abstract The crude extract of Amla was found to be effective in treating pre-weaning piglet diarrhea. In this study, the group given a 25% extract concentration showed the most rapid recovery (4.80±0.57 days) and highest body weight gain (3240.00±45.83 g/piglet) over 15 days, outperforming the control group. Additionally, treatment costs were significantly lower in the 25% Amla crude extract group (0.039 USD per piglet) compared to the control group (0.052 USD per piglet). These findings suggested that Amla crude extract could be a cost-efficient alternative for managing pre-weaning piglet diarrhea, especially at a 25% concentration. Moreover, it addressed the issues of treatment expenses and antimicrobial resistance.

Keywords: *Emblica officinalis*, Diarrhea treatment, Antimicrobial resistance, Antibiotic, Antimicrobial resistance risk reduction

Introduction

Diarrhea is a common and significant health concern in pre-weaning piglets, causing substantial economic losses to the swine industry worldwide. The vulnerability of piglets to gastrointestinal disorders is particularly pronounced during the early stages of life, as their immune and digestive systems are still developing. The impact of diarrhea goes beyond its direct effects on piglet health; it also contributes to decreased growth rates, increased mortality rates, and the need for heightened veterinary interventions, ultimately affecting the overall profitability of pig farming operations (Jayaraman and Nyachoti, 2017).

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There has been growing interest in exploring natural alternatives to traditional pharmaceutical interventions to manage piglet diarrhea in recent years. One natural remedy that has gained attention for its potential therapeutic properties is the crude extract of Indian Gooseberry, scientifically known as Amla (*Phyllanthus emblica* L.). Amla or emblic myrobalan has been used in traditional medicine systems for centuries due to its wealthy bioactive constituents, including polyphenols, flavonoids, and vitamins (Variya *et al*., 2016).

Although Amla fruit has been used for human health for a long time (Treadway, 1994), there is limited understanding of its potential benefits for pre-weaning piglet diarrhea. However, studies have shown that dietary supplementation of Amla fruit can improve the growth performance of ruminants such as crossbred Holstein calves (Bostami *et al*., 2021), dairy claves (Nguse *et al*., 2023), buffalo calves (Patel *et al*., 2016), and lambs (Bostami *et al*., 2015). To bridge this knowledge gap, this study aims to investigate the effect of Amla crude extract on the occurrence and severity of diarrhea in pre-weaning piglets.

By exploring the potential therapeutic properties of Amla, we seek to contribute to the development of effective and sustainable management strategies for diarrhea in pig farming. Through rigorous experimentation and data analysis, we aspire to offer insights into the viability of Amla extract as a natural supplement for mitigating pre-weaning piglet diarrhea. Such knowledge could pave the way for innovative, eco-friendly, cost-effective solutions that enhance piglets' well-being and bolster the swine industry's sustainability. This study aimed to evaluate the impact of Amla extract on critical parameters, including the recovery period, cost, and body weight gain.

Materials and methods

Ethical approval

This study followed the guidelines in "The Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes", edited by the National Research Council of Thailand. The study was approved by the Animal Care and Use Committee, King Mongkut's Institute of Technology Ladkrabang (Approval number: ACUC-KMITL- RES/2023/010).

Animals, treatment, and experimental design

A total of 150 female piglets, a crossbreed of Landrace x Large White x Duroc, aged seven days and weighing between 2.10 and 2.20 kg, from the fourth

parity sow were obtained from a commercial farm in Kanchanaburi province, Thailand. The piglets were randomly assigned to five groups in a completely randomized design. The experiment consisted of three replicates, with each replicate comprising ten piglets. The groups included T1, an antibiotic control group receiving a daily dose of 1.0 ml colistin; T2, a group receiving Amla crude extract at a concentration of 67 % in normal saline; T3, a group receiving the extract at a concentration of 50 % in normal saline; T4, a group receiving the extract at a concentration of 33% in normal saline and T5, a group receiving the extract at a concentration of 25% in normal saline.

Formulating Amla crude extract

To create Amla extract, it used a blender to mix freshly cut Amla pieces with chilled, boiled water. For every 500 grams of fresh Amla, add 500 ml of cooled, boiled water. Then, filter was mixed through a white cloth. The extract was made using various concentrations of a standard saline solution that contained 5% dextrose and 0.45% sodium chloride for injection.

Criteria for identifying traits of piglets with diarrhea

The health of piglets was assessed and encompassed the feces quality, behaviour and overall appearance including the head, hair, and anus. The same individual was carried out a consistent visual evaluation for piglets exhibiting scours or diarrhea. The consistency of piglet feces was evaluated through visual inspection, employing specific criteria denoted by the following scoring categories (Figure 1) as Score 1: Feces are firm and well-formed, score 2: Feces are soft, retaining some shape but lacking firmness, score 3: Feces are semi-liquid, lacking shape but not entirely watery and Score 4: Feces are watery and fully liquid.

The evaluation of piglet health goes beyond feces scoring. General appearances are included the head, hair and anus were evaluated. Hair quality was assessed which based on three levels (Figure 2). Level 1 indicated smooth and lustrous hair, reflective of good health. Hair at this level appears well-groomed and shiny. Level 2 signified uneven and tangled hair, suggesting a moderate departure from ideal health. Hair might appear less sleek or tangled. Lastly, Level 3 signified erect and fluffy hair, which could indicate poor health.

Additionally, the color of the anus was offered insights into piglet health. Employing a similar scale is seen in Figure 3, Level 1 indicated a pale or faintly pink anus without signs of fecal soiling or diarrhea. The anus appeared to clean and unaffected. Level 2 suggested a slightly deeper shade of pink, indicating a

richer color. Although still within the normal range, it was accompanied by minor soiling of the hair around the tail or hindquarters. Level 3 denoted a distinctly deep red hue of the anus, potentially pointing to more significant issues.

Figure 1. Illustration of the fecal consistency score system for evaluating stool characteristics

Figure 2. Comparing health and hair traits scoring between diarrheic and healthy suckling pigs

At this level, there was the signs of soiling or crusting of the hair around the hindquarters and tail, along with damp, matted, and heavily coated feces.

Each animal's well-being was monitored twice daily, with body weight assessed every three days. This regular monitoring enabled the continuous evaluation of their overall health and growth progress throughout the experiment.

Figure 3. Assessment of anus color scores in diarrheic and healthy suckling pigs

Providing Amla crude extract to piglets exhibiting diarrhea symptoms

The study was conducted over 15 days, with the treatment commencing upon the onset of diarrhea symptoms in the piglets, identified by a feces score of 3. The crude extract of Amla was given twice daily in a dose of 3 ml, using a syringe. The extract's administration persisted until the faeces' consistency transitioned to a solid and adequately formed state, aligning with a score of 1.

Statistical analysis

The body weight gain data for 15 days and the recovery day were analysed using SAS statistics (SAS Institute, Cary, NC). Distinctions in outcomes between the control group (antibiotic treatment) and Amla extracts were evaluated utilizing one-way ANOVA. Determining noteworthy disparities among means was executed through Duncan's New Multiple Range Test (DMRT), employing a p-value of 0.05 as the threshold for statistical significance.

Results

The investigation unveiled crucial attributes of ill piglets before initiating treatment. These piglets exhibited a sunken abdomen and displayed an unsteady gait, contributing to health scores falling within the range of 2.0 to 2.2. Furthermore, their hair displayed irregularity, garnering scores ranging from 2.0 to 2.2. The anal region appeared as a dark pink hue, with scores spanning from 2.0 to 2.3. Additionally, the piglets frequently showed signs of diarrhea in their fecal output, characterized by creamy-white liquid droppings and reduced litter presence. The diarrhea severity score ranged between 3.3 and 3.6. These

discerned attributes serve as crucial benchmarks for evaluating the well-being of piglets prior to commencing treatment.

The findings found that nursing piglets afflicted with diarrhea exhibited diverse recovery durations, ranging from 4.80 to 7.51 days (Table 1) when subjected to two daily doses of Amla extract (T2-T5). In contrast, the colistin treatment (T1) necessitated 8.74 days for recuperation. Administering 67% Amla crude extract (T2) also led to a recovery time of 7.51 days, displaying no noteworthy disparity ($p>0.05$) compared to the colistin intervention. Notably, administering 25% to 50% of Amla crude extract emerged as the more efficacious approach for managing diarrheic nursing pigs, significantly curtailing the recovery period to 4.80 to 5.74 days compared to antibiotic treatment ($p<0.05$).

Table 1. Impact of Amla crude extract on diarrheic piglets

Treatments	Health score	Hair score	Anus score	Feces score	Recovery time (d)
T1	2.2	2.2	2.3	3.6	8.74 ± 2.50^{b}
T2	2.1	2.0	2.2	3.6	7.51 ± 1.18^{ab}
T3	2.0	2.0	2.0	3.4	5.74 ± 0.50^{a}
T4	2.0	2.0	2.0	3.3	5.69 ± 1.52^{a}
T5	2.0	2.0	2.0	3.4	4.80 ± 0.57^{a}
P value	-	-	-	-	0.045

* ^{a,b} Means within a column with different superscripts are significantly different ($P < 0.05$)

The investigation shifted to analyzing the weight gain patterns for 15 days, coinciding with the onset of diarrhea symptoms in piglets as identified by a feces score of 3. The investigation revealed that the body weight gain resulting from Amla extract treatment at 50% or lower was notably higher compared to the antibiotic treatment ($p<0.05$) (Table 2 and Figure 4). Among the Amla extract treatments, the highest body weight gain was observed in the group treated with 25% Amla extract.

The body weight was significantly ($P<0.05$) greater recorded in piglets treated with 25% Amla extract (3417.33 g) when compared to those treated with the antibiotic (1963.33 g). Furthermore, there were no statistically significant differences ($p>0.05$) in body weight among piglets treated with 50%, 33%, and 25% Amla extract.

Table 2. Effect of Amla crude extract on body weight gain during a 15-day period

Period (day)	Body weight gain (g)					P value
	T1	T2	T3	T4	T5	
1-3	263.33±63.51	346.66±101.16	363.33±226.79	403.33±116.76	360.00±65.57	0.077
4-6	220.00±192.87^{b}	390.00±200.75^{b}	483.33±145.03^{ab}	430.00±51.96^{ab}	550.00±265.14^{a}	0.044
7-9	410.00±101.49^{ab}	386.33±202.57^{b}	533.33±72.34^{a}	573.33±32.15^{a}	653.33±15.28^{a}	0.036
10-12	510.00±373.23^{b}	530.33±277.91^{b}	606.33±64.29^{b}	653.33±130.13^{b}	883.33±68.07^{a}	0.047
13-15	560.00±187.35^{b}	613.00±176.92^{b}	753.67±122.20^{b}	786.33±75.72^{b}	970.67±36.06^{a}	0.035
1-15	1963.33±775.91^{c}	2166.32±185.02^{bc}	2772.66±101.16^{ab}	2846.32±719.75^{ab}	3417.33±45.83^{a}	0.039

$^{*\,a,b,c}$ Means within a row with different superscripts are significantly different ($P < 0.05$)

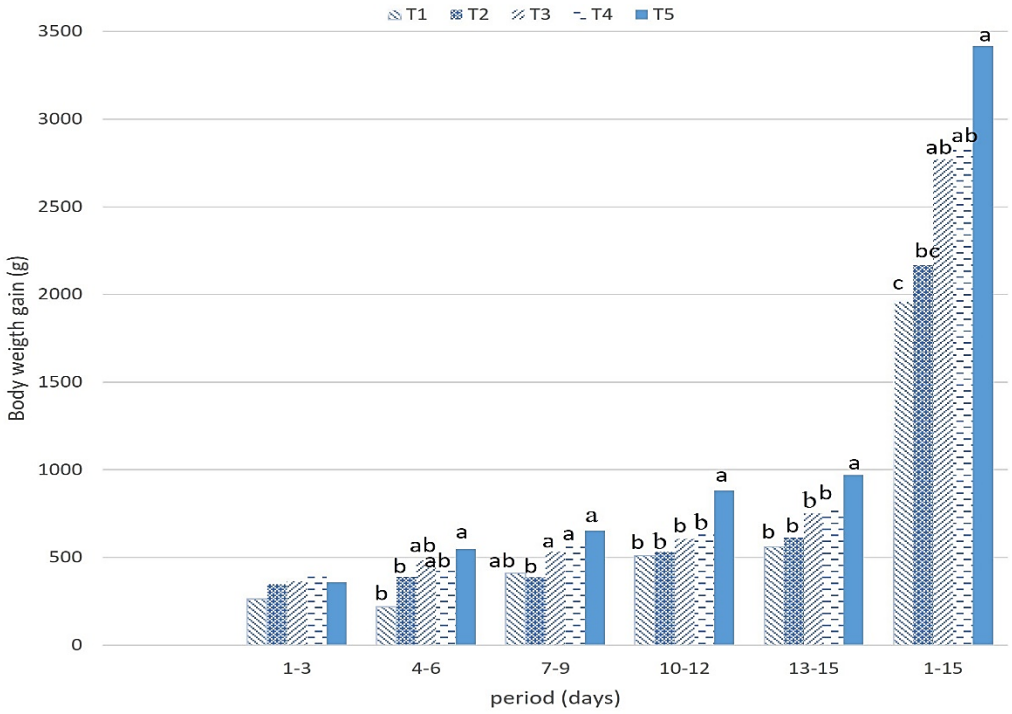

Figure 4. Impact of Amla on average body weight gain over 15 days. Groups labelled as T1 (antibiotic control with 1.0 ml colistin daily), T2 (67% Amla crude extract), T3 (50% Amla crude extract), T4 (33% Amla crude extract), and T5 (25% Amla crude extract). Different letters within the same period indicate statistically significant differences ($P < 0.05$)

Discussion

Post-weaning diarrhea is a common issue in pig farming that has traditionally been treated with feed antibiotics and zinc oxide (Lekagul, 2009). However, this practice contributes to antibiotic resistance in animals, which ultimately affects consumers. To address this issue, natural remedies can be used to treat diarrhea safely and sustainably. One good option is to use Amla, a fruit with a long history of being used to treat various diseases (Dasaroju and Gottumukkala, 2014). Amla fruit is cost-effective, easy to prepare, and safe for human consumption, making it an ideal remedy to incorporate into pig farming practices.

The use of Amla extract for managing pre-weaning piglet diarrhea has not been fully explored yet. While multiple studies have shown that supplementing Amla fruit can improve growth performance in ruminants, this is the first study conducted on pigs. The results further highlight the crucial role Amla fruit can play in reducing the occurrence and severity of diarrhea in pre-weaning piglets, filling the knowledge gap in this area. Our findings indicate that Amla crude extract is an effective and cost-efficient alternative for managing piglet diarrhea, especially when used at a 25% concentration. The group given the 25% concentration of Amla extract showed the most rapid recovery and highest body weight gain over 15 days, outperforming the antibiotic group. Additionally, treatment costs were significantly lower in the 25% Amla crude extract group compared to the antibiotic group.

The Amla extract is effective in managing piglet diarrhea due to its rich bioactive constituents, which include polyphenols, flavonoids, tannin, and vitamins (Vimala *et al*., 2011). These compounds possess antimicrobial and anti-inflammatory properties (Jain and Khurdiya, 2004; Yokozawa *et al*., 2007; Perianayagam *et al*., 2005; Dang *et al*., 2011; Muthuraman *et al*., 2011), which may help alleviate the symptoms of diarrhea and promote faster recovery. Tannins, which are present in Amla, have excellent antibacterial properties and can disrupt the structure of bacterial cell membranes, leading to the loss of cell membrane integrity and affecting the osmotic pressure changes at the bacterial cell membrane. This causes an imbalance in the nutrient exchange process, disrupting the normal processes of water absorption, ion transport, and other exchanges between cell membranes, leading to cell shrinkage and eventual collapse (Yokozawa *et al*., 2007; Perianayagam *et al*., 2005). Studies have shown that Amla is more effective against Gram-positive bacteria than fungi. Extracts from Amla have shown high inhibition zones when tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *S. cereus*, *Vibrio cholerae*, and *Candida albicans* (Saeed and Tariq, 2007; Khurana *et al*., 2019).

It is important to use Amla with caution when applying it to piglets, as it contains tannin. Tannin can help inhibit the growth of disease-causing microorganisms, but an excessive amount of it may have adverse effects on animal health. Previous reports have suggested that high levels of tannin can reduce the growth rate of animals or require increased food intake to maintain their body weight per unit of weight. This effect has been observed in various animals such as insects, pigs, chickens, and ducks (Yu *et al*., 2020). According to a recent study, piglets that were treated with a lower concentration of Amla extract (25%) had a greater body weight gain compared to those treated with a higher concentration. Additionally, it has been found that dietary tannin supplementation at levels of 125, 250, 500 and 1000 mg/kg can lead to a linear reduction in average daily gain and feed efficiency in weanling pigs (Smith *et al*., 2009). In addition, Nguse *et al*. (2023) conducted a study to evaluate the effect of amla fruit powder supplementation on the frequency of diarrhea and growth performance of dairy calves. Their research indicated that amla fruit powder supplementation had a quadratic effect on various parameters, including body weight, average daily gain, feed efficiency, body length, heart girth, and withers height. Notably, the highest values were observed in the group receiving 5 g/day of amla fruit powder, followed by the control group. In contrast, the lowest values were found in the group receiving 40 g/day of amla fruit powder supplementation. However, amla fruit powder supplementation did not improve body weight and average daily gain in calves compared to the control group. Amla fruit powder supplementation of 5 g/day ($P < 0.01$) and 10 g/day ($P = 0.03$) increased the frequency of fecal score "1" compared to the control group. Frequency of diarrhea in the Amla fruit powder supplementation 5 g/day (7.9%) was lower ($P < 0.05$) than all groups (11.1%, 14.3%, 22.6%, and 24.2% for 10, 20, and 40 g/day, respectively). According to a study conducted by Bostami *et al.* (2015) involving sheep, it was found that including Amla in their diet at concentrations between 0.4% to 0.8% resulted in significant improvements in both average daily gain and feed efficiency when compared to a basal diet ($p<0.05$). Additionally, supplementing the sheep's diet with Amla decreased the prevalence of diarrhea among them.

Furthermore, tannins help to reduce acidity in the stomach, which has implications for enzyme functionality. With a reduced acid-base balance, enzymes from bacteria are unable to function at their optimum capacity. Studies have reported that tannins efficiently interact and bind with enzymes when the acid-base balance is reduced. Therefore, bacterial enzymes are unable to function properly, leading to the eventual death of the bacteria. Diarrhea occurs when the tissues lining the large intestine become irritated, leading to excessive contractions and frequent bowel movements. Tannins can help alleviate this

irritation by binding with proteins in the superficial tissue and creating a coating that reduces bowel movements, ultimately stopping the diarrhea (Bhandari and Kamdod, 2012). In addition, tannins also break down proteins of disease-causing agents, contributing to their destruction and helping to eliminate the pathogens causing the illness.

Dehydration is a common issue that may occur due to diarrhea, as it leads to the loss of fluids and electrolytes. Amla, a fruit rich in vitamin C, gallic and ellagic acids, can help rehydrate the body by promoting water absorption and electrolytes in the intestines. It also helps in inhibiting the degradation of vitamin C. Moreover, Amla can stimulate the production of digestive enzymes, which can improve digestion and reduce the chances of diarrhea caused by indigestion or malabsorption.

The increased weight in piglets is mainly due to their fast recovery from illness, which is facilitated by the absorption of ample vitamin C in an easily assimilable form. This vitamin converts to dehydroascorbic acid, which further converts into diketogulonic acid which plays a vital role in growth, disease resistance, stress tolerance, inhibition of oxidation, and the metabolism and absorption of fats. Moreover, vitamin C helps in the synthesis of hormones in the adrenal glands, promotes the production of red blood cells, and aids in detoxifying foreign substances entering the body. It also enhances the antioxidant function within cells, which serves as a defense against internal oxidative stress (Fernandez-Duenas *et al*., 2008).

The cost of treatment was a significant factor to consider in the study. The control group required a considerably higher expenditure of 0.60 USD/100 doses until they recovered. In contrast, using Amla crude extract at concentrations ranging from 0.38 to 0.41 USD/100 doses showed potential cost benefits. This highlights the economic viability of Amla crude extract and its potential to provide significant economic advantages over traditional antibiotic treatments.

Excessively, using Amla extract as an alternative to antibiotics could help reduce the risk of antimicrobial resistance and address the growing concern of antibiotic overuse in animal agriculture. This is a cost-effective, eco-friendly, and straightforward way for farmers to treat pigs. However, it is important to note that our study had some limitations. For instance, we only evaluated the effectiveness of Amla extract in managing diarrhea in pre-weaning piglets. Therefore, our findings may not apply to other animal species or developmental stages. Additionally, it is worth noting that the active ingredients present in Amla may vary depending on several factors such as the season, amount of water and location. As a result, different factors may cause Amla to contain varying amounts of active ingredients which can impact its effectiveness in treating diarrhea in pigs, such as the location of being planted, and the amount of water

it receives. Furthermore, there could be a need for further studies to determine the ideal dosage and frequency of Amla extract supplementation for pre-weaning piglets. Moreover, further research could explore the long-term effects of Amla extract supplementation on piglet health and growth, as well as the potential impact on pork quality.

In conclusion, our study provided evidence supporting the potential of Amla crude extract as a natural and cost-efficient alternative for managing pre-weaning piglet diarrhea. Given the significant economic and health impacts of piglet diarrhea, using Amla extract could offer a promising solution that enhanced animal welfare, improved profitability, and reduced the risk of antimicrobial resistance in animal production.

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