

Efficacy of Green Papaya (*Carica papaya*) Fruit Juice against Intestinal Parasites of Newly Weaned Male Calves

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Abstract

Around 14.5% of the greenhouse gases causing climate change in the environment are released by livestock excrement. Calf deworming is one technique to prevent manure dispersion in the herd as it prevents diarrhea outbreaks. To evaluate the efficacy of *Carica papaya* in controlling coccidiosis and strongylids a total of 18 newly weaned calves (5 - 7 months old) were randomly distributed into three treatments in the Completely Randomized Design. Treatments were: T1 – Control (Amprolium); T2 – pure green unripe papaya fruit juice; T3 – green unripe papaya fruit juice with papaya powdered seeds. Fresh green unripe papaya fruits and ripe papaya were obtained from the local farmers of Balo-i, Lanao del Norte. After thoroughly washing the fruits, the seeds from the ripe papaya were collected, sundried for three days, and manually pulverized. The unripe green papayas on the other hand were chopped, blended, and filtered using a clean cloth. The extracted juice was then added to distilled water at a 1:1 ratio. After which, 100 grams of powdered seeds were added to the juice and blended again until the desired texture was achieved. The green papaya fruit juices were given once daily for 5 consecutive days at a rate of 10 mg/kg of the animal's body weight the same dosage as the control (amprolium). The fecal examination for quantitative and qualitative identification of internal parasite present was done during the days 0, 5, 7, and 21 post-incubation treatments. All the data gathered were analyzed using One-Way Anova run-in SPSS version 20. Results revealed that green unripe papaya fruit juice with or without added papaya powdered seeds significantly ($P \leq 0.05$) expelled higher counts of coccidia compared to the control. Efficiency analysis revealed that pure unripe papaya juices with or without added powdered papaya seeds were 78% effective at preventing coccidia and 61% effective at getting rid of strongylids.

Keywords: Anthelmintic; *Carica papaya*; Coccidia; In vivo; Strongylids

1. Introduction

Gastrointestinal (GI) parasitism is a disease caused by different genera of parasites that inhabit the digestive tract of cattle and sheep, causing inappetence, anemia, diarrhea, poor growth, and economic losses in the herds (Pinilla Leon *et al.*, 2019). Moreover, gastrointestinal parasites may often result in scouring, which increases the flow of manure to the environment. This manure contains phosphate, nitrogen, and methane, making its distribution troublesome. When it rains, high phosphorus levels in the soil can wash away

into nearby waterways, causing algal blooms that tint the water green and can release toxins that are dangerous to drinking water.

Among the nematodes, the most important and prevalent genera worldwide are those belonging to the *Strongylids* order, especially in tropical zones. According to Duval, (1996) among young calves' coccidiosis is the most common parasite. Infected calves may appear healthy or unthrifty accompanied by reduced feed efficiency (Constable *et al.*, 2018). Infestation can be easily detected with the

presence of oocysts in feces. It is usually sporadic during the rainy seasons constituting a major problem that returns periodically in almost all livestock herds (Stromberg & Gasbarre, 2006).

An excellent tool for parasite control is good pasture management and timely application of anthelmintic. For several years, patronage and the repeated use of conventional anthelmintic drugs such as albendazole, mebendazole, ivermectin, piperazine, and niclosamide have been linked to resistance development making deworming more difficult (Albonico *et al.*, 2004). Consequently, natural plant extracts have been investigated that can be used as anthelmintics to combat infestation in animals. As cited by Romero *et al.* (2018) these include *Lespedeza cuneta* (tannin-rich) reduced parasites in burdens in goats, *Mucuna pruriens L.*, paralyzes intestinal worms in lambs, and *Carica papaya L.* that inhibits the growth of *H. contortus* in ewes because of the benzyl isothiocyanate it contains.

Carica papaya Linn. (*Caricaceae*) is well known for its nutritional and medicinal values. The various plant parts are used in different health conditions (Priyadarshi & Ram, 2018). As cited by Guko *et al.* (2020) 'the fruits are used to treat indigestion (Krishna *et al.*, 2014), chronic diarrhea, ringworm infections, bleeding piles, and amoebic dysentery (Vij & Prashar, 2015). The stem bark, flowers, roots, and seeds have all been well documented for their use in the treatment of a variety of conditions, most notably helminth infections (Agarwal *et al.*, 2011; Ameen *et al.*, 2018; Vij & Prashar, 2015). According to Agyare *et al.* (2014), around 74% of traditional healers in Ghana used the plant for helminth infections. Several authors have well-documented the positive effects of the seeds, fruits, and leaves of papaya against internal parasite in vivo (Ameen *et al.*, 2018; Guko *et al.*, 2020; Roy, 2020). Recently, Roy (2012) reported that using different concentrations of *Carica papaya* against Indian adult earthworms found that a 5% concentration of papaya leaf extract provides the shortest time for paralysis compared to 2.5% and 1%. He also reported that the use of 1% and 2.5% leaf and steam extract increases death compared to the

standard drug albendazole. In addition, Guko *et al.* (2020) reported that *Carica papaya* leaf extracts contain tannin, while fixed oils were only present in the seeds. He also revealed that extracts from seeds were more effective than the extracts from the stem bark and leaves both in reducing paralysis and death times and that all extracts prepared were more effective than albendazole. Previously, papaya seeds have been reported to have effectivity against intestinal worms, *Haemonchus contortus*, *Oesophagostomum sp.*, *Trichostrongylus sp.*, and *Cooperia sp.* (Ameen *et al.*, 2018). The varied effects or anthelmintic effectivity of *Carica papaya* against internal parasites, in vivo, is affected by the differences in the chemical composition of the plant's various parts as well as the location where it is planted (Guko *et al.*, 2020). Furthermore, there have been very few in vivo research on the efficiency of *Carica papaya* in expelling internal parasites.

Thus, the current study attempted to assess the anthelmintic effectivity of *Carica papaya* fresh green unripe fruit juice with or without added powdered *Carica papaya* seeds in expelling coccidian and strongylids from newly weaned male calves in vivo.

2. Materials and methods

Prior to the conduct of the study, all the procedures were checked and approved by the University Veterinarian of Mindanao State University under Certification Number 012, 2008.

2.1 Experimental Animals and Treatments

Eighteen (18) newly weaned male calves, approximately 5-7 months old were randomly distributed into three (3) treatments following the completely randomized Design (CRD). These calves were found positive for coccidia and strongylids, they were placed in individual chutes and were acclimatized for 7 days prior to the administration of green papaya juices. Treatments were: T1 – control (amprolium), T2 – green unripe papaya fruit juice; T3- green papaya unripe fruit juice with powdered papaya seeds.

2.2 Preparation of Papaya juices

Local farmers in Balo-i, Lanao del Norte, provided fresh unripe, and ripe papayas. After carefully washing the samples, the seeds from ripened papaya were removed, sundried for at least three (3) days, and pulverized with a mill and pestle method described by Subandi & Anis Nurowidah (2019) with slight modification. Unripe green papaya, on the other hand, was sliced into tiny cubes and pureed using an electric blender. 100 grams of dried powdered papaya seeds were added to the papaya fruit juice for the groups that received it with seeds. Finally, 1:1 distilled water was added to the green papaya fruit juice.

2.3 Dosing and Administration

The papaya fruit juice (with or without powdered papaya seeds) were given to the male calves at a rate of 10 mg/kg of body weight. The concentration was maintained in accordance with the specified dosage for amprolium, which served as the control. The papaya fruit juices (with or without powdered papaya seeds) was administered orally using a syringe once daily for five (5) consecutive days. Oral treatments were given every 7:00 a.m., prior to morning feeding time.

2.4 Collection of Fecal Samples

Fecal samples were collected three times per day during days 0, 5, 7, and 21 of post administration trial. Fecal samples collected were immediately placed in a labeled container and transported to the Animal Disease Laboratory (ADDL), Central Mindanao State University College

of Veterinary Medicine, Musuan, Bukidnon for quantitative and qualitative fecalysis. The counts of eggs were reported as the number of worms per grams (EPG) of feces using McMaster technique and its modifications (Gordon and Whitlock, 1939; Whitlock, 1948; Ministry of Agriculture, 1986)

2.5 Data Collected

Data such as the qualitative and the quantitative identification of the fecal samples were collected during the day 0, 5th day, 7th day, and 21st day after five (5) consecutive days of papaya fruit juice administration. The efficacy of the papaya fruit juice (with or without papaya powdered seeds) was calculated using the formula below.

$$\text{Percent Efficiency} = \frac{\text{Egg output (1st trial)}}{\text{Initial egg output (before trial)}} \times 100$$

2.6 Statistical Analysis

All the data gathered were analyzed in ONE-WAY ANOVA run in SPSS version 20.0 with homogeneity of variance tested. Significant differences among treatments were analyzed using the Least Significant Difference (LSD) at $P \leq 0.05$.

3. Results and discussion

Table 1 shows the total number of coccidia discharged from male calves treated with fresh unripe papaya fruit juices. Based on the results, there was a significant difference ($P \leq 0.01$) in the number of coccidia eggs counted in the feces from the different treatments after several fecalysis in post-treatment periods.

Table 1. Number of expelled coccidia eggs from male calves (EPG)

Treatments	0 days	5 th day	7 th day	21 st day
Amprolium	5657 ^b	2,750 ^b	417 ^c	733
Papaya juice	9,576 ^a	7,000 ^a	2,167 ^a	333
Papaya juice + seeds	6,367 ^b	3,450 ^b	1,061 ^b	494
SD	123.1	211.0	118.3	225.2
SEM	2.6	3.3	1.4	1.0

^{a,b,c} Means significantly different at $P \leq 0.05$.

In the current study, the number of expelled coccidia from the different treatments showed that pure green papaya fruit juice and papaya fruit juice added with papaya powdered seeds were found to expel a higher ($P \leq 0.05$) number of coccidia compared to the control. In the first fecalysis (5th post-treatment), the highest number of coccidia expelled was observed in pure papaya fruit juice with 7,000 EPG followed by papaya fruit juice added with papaya powdered seeds of 3,450 EPG, while the lowest expelled coccidia was observed in the control with 2,750 EPG. In the second trial, similar observations were also noted. The highest expelled coccidia was still observed from the pure papaya fruit juice with 2,167 EPG, followed by papaya fruit juice with powdered seeds at 1,061 EPG and the control at 417 EPG. During the 3rd trial, papaya fruit juice with seeds had the highest number of expelled coccidia compared to papaya fruit juice and the control but this difference was non-significant ($P \geq 0.05$) based on ANOVA. From the three consecutive trials conducted, it was revealed that papaya fruit juice and papaya fruit juice added with powdered papaya seeds expelled a higher number of coccidia compared to the control.

Furthermore, when the percentage efficacy was compared between the control groups and the groups treated with papaya fruit juices, it was discovered that there was a slight difference, but it was non-significant ($P \geq 0.05$) based on ANOVA (shown in Table 2). The total efficiency of amprolium as the control was 68.93% while the two kinds of papaya fruit juices had 78% efficiency.

This observed efficiency of papaya fruit juices in expelling internal parasites was found to be consistent with the previously reported anthelmintic effects of papaya seeds, stem, and leaves (Ameen *et al.*, 2018; Guko *et al.*, 2020; Krishaet *et al.*, 2014; Waterman *et al.*,

2009). The higher number of expelled coccidia from the papaya fruit juice from this study can be attributed to the higher contents of benzyl isothiocyanate and papain found in unripe papaya (Kishore *et al.*, 2021). Unripe papaya, on the other hand, contains more latex, which aids in the expulsion of coccidia from calves' intestines (Devi Rajeswari, 2014). Some studies have also concluded that using papaya seeds is safe and effective in eliminating intestinal helminths (Okeniyi *et al.*, 2007). This could be due to their unusual chemical composition, which includes saponins, glycosides, and fixed oils, all of which have been reported to have anthelmintic properties (Garcia-Bustos *et al.*, 2019).

Aside from coccidia, the treatment of papaya fruit juices to the male calves also expelled several strongylids (shown in Table 3). Based on observations, a comparable ($P \geq 0.05$) number of expelled strongylids were found in male calves treated with papaya fruit juices over the control. In the first trial, calves treated with papaya fruit juice added with powdered seeds expelled the least number of strongylids with only 433 EPG, while pure papaya fruit juice has 1,400 EPG. The highest expelled strongylids were observed from the control groups with 1,500 EPG.

Moreover, on the second trial calves treated with papaya fruit juices obtained higher expelled strongylids compared to the control and on the last day of fecalysis, a higher ($P \leq 0.05$) number of strongylids were noted from the control compared to the papaya juice treated groups. Looking at the overall efficiency of the amprolium in expelling strongylids in the current study it was found to have 73.23% efficacy while papaya fruit juice has only 61.73%, and papaya fruit juice added with papaya powdered seeds has 61.12%. This finding suggests that papaya

Table 2. Percentage efficiency of green papaya fruit juice

Treatments	5 th day	7 th day	21 st day	Total efficacy
Amprolium	48.60	14.34	29.43	68.93
Papaya juice	57.53	17.82	2.74	78.11
Papaya juice + seeds	54.18	16.66	7.76	78.61

Table 3. Expelled strongylids from male calves treated with papaya fruit juices (EPG)

Trials	0 days	5 th day	7 th day	21 st day
Amprolium	3,960 ^b	1,500 ^a	667 ^c	733 ^a
Papaya juice	6,345 ^a	1,400 ^a	2,167 ^a	350 ^b
Papaya juice + seeds	2,580 ^c	433 ^b	1,061 ^b	83 ^c
SD	213.1	102.6	145.7	134.5
SEM	1.7	23.1	12.6	3.4

^{a,b,c} Means significantly different at $P \leq 0.05$.

fruit juice and papaya fruit juice mixed with papaya powdered seeds can still expel strongylids, though not as many as calves treated with amprolium. It was also noted that the addition of papaya seeds to the papaya fruit juice did not enhance the elimination of both strongylids and coccidia throughout the experimental trial. Guko *et al.* (2020) discovered that the paralysis and death of worms in vitro treated with *Carica papaya* were dose-dependent. They observed that increasing the concentration of papaya extract from 1 mg/mL to 5 mg/mL causes a significant decline in both the paralysis time. Several authors similarly reported that dosing affects worm paralysis and time of death using *Carica papaya* extracts (Ameen *et al.*, 2018; Mintah, 2017; Rupa & Jayanta, 2013). Chaudhary *et al.* (2012) claim that some phytochemicals such as tannins, glycosides, and alkaloids have exhibited activity against helminths. It has been reported that tannins produce helminthic activity by binding to free proteins in the gastrointestinal tract of the helminths or glycoprotein on the cuticle of the parasite leading to death (Chaudhary *et al.*, 2012). Another research reported that parasitized ruminants that grazed on forage with high tannin content had very low fecal egg count as compared to those who feed on low tannin content forage (Hoskim *et al.*, 1999; Simon *et al.*, 2012). Some phenolic compounds have also shown anthelmintic activity by uncoupling oxidative phosphorylation. This hinders energy production in the helminths causing death (Chaudhary *et al.*, 2012). Some alkaloids have also been reported to possess anthelmintic activity by acting on the central nervous system.

4. Conclusion

It is concluded that pure green unripe papaya fruit juice and green papaya fruit juice added with papaya powdered seeds at a dosage of 10 mg/kg body weight given daily for five consecutive days can effectively control coccidia and strongylids until 21 days with 78% and 61% efficiency, respectively. Hence, determination of the phytochemical analysis of green unripe papaya is recommended to further understand its effectiveness of papaya juice in controlling coccidia and other internal parasites from cattle.

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