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EFFECT OF *CARICA PAPAYA* SEEDS ON GASTRO-INTESTINAL PARASITES OF PULLET AND PRODUCTION PARAMETERS

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ABSTRACT: ***Parasitic diseases are known to impair poultry production. So, mean measure to cope with them is the use of veterinary pharmaceutical products those high cost and residues formation in eggs and meat lead to the use of others strategies like plant and plant product. Plants like Azadirachta indica, Combretum sp have been used by several authors to reduce helminthes load of pullets. The present study was carried out in order to evaluate anthelminthic effect of papaw seeds collected from fruits sellers, dried under ambient temperature and incorporated into feed. Two hundred (200) day-old chicks male Isa-brown reared up to 30 days were divided randomly into five groups (L0, L0.5, L1, L2 and LCP). L0, L0.5, L1 and L2 were respectively fed with diet containing 0%, 0.5%; 1 and 2% of papaw seed while pullets of Lcp group received Citrate of piperazine mixed to water. Results show that groups treated with Carica seed has obtained 100% of reduction rate while Lcp and L0 obtained respectively 69.23% and -623.07%. The chicks of L0.5 L1 and L2 were heavier than those of L0 and Lcp. It can be concluded that papaw seed reduces significantly parasitic population in pullets' gut.***

KEY WORDS: *Carica Papaya*, Egg per gram, Helminths, Pullet, Seed

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INTRODUCTION

Chicken gut can be contaminated by bacteria, viruses, protozoa and parasites such us worms (*Trichostrongylus sp, Heterakis sp, Ascaridia sp*, *Syngamus sp* etc.) which may negatively affect its health status and consequently production performance. Worm contamination is very frequent in tropical countries whether in modern or local poultry flocks. Among helminths, nematodes are the most important parasite group of poultry both in terms of number of species and extent of damage they cause; the main genera include *Capillaria*, *Heterakis* and *Ascaridia* (Jordan and Pattison, 1996; Ogbaje *et al.,* 2012). Their biological cycle starts with the consumption of eggs that grow in the gut to become mature parasites about four weeks later. Inside digestive tract, mature worms develop antiperistaltic movements, consume nutrients generated by digestion and produce species specific eggs allowing early qualitative and quantitative detection as well as being a key indicator of the parasitic status. Nutrient consumption by worms result in significant losses (Rabbi *et al.,* 2006) due to morbidity and mortality in chicken flocks (Ali *et al.*, 2006). Prior to mortality, growing chicks show low growth rate (Sven *et al.*, 2009) while hens have low egg production up to 25% lower than usual (Salifou *et al.*, 2009). Controlling worm population by regular anthelmintic treatment may avoid these detrimental effects. However, Multiple studies show that administration of veterinary pharmaceutical products may result in residues formation in egg and poultry meat and induces anthelmintic resistant strains of helminthes (Walter and Prichard, 1985; Dononghue, 2001; Youn and Noh, 2001; Hoque *et al.*, 2003; Kaplan, 2004; Borgsteede *et al.*, 2007; Beech *et al.,* 2011). This resistance development constitutes a real public health problem and together with the high cost of conventional anthelminthic, becomes a serious concern for researchers who therefore focus their investigations more on plants and plant products as an alternative for conventional anthelmintic. Indeed, Agbede *et al.* (1995) and Mpoamé *et al.*

(2008) have respectively used *Kalanchoe creneta* powder and ethanolic extract of *Carica papaya* seed and reported significant effects on coccidian population in local chicken digestive tracts. Soltner *et al.* (1996) have evaluated *Combretum sp* bark powder in layer mash and pointed out a significant drop of helminths eggs, mature *Capillaria sp* and *Heterakis sp* populations. Also, the use of *Azadirachta indica* seed cake by Ousmane (2012) and the latex of *Carica papaya* by Satyanarayanana *et al.* (1982), Satrija *et al.* (1995) and Adu *et al.* (2009) revealed significant reduction of parasitic load in chicken. In addition, Shaziya *et al.* (2012) reported effective activity of *Carica papaya* seed extract against larvae of *Ancylostoma canimum* in mice digestive tracts. These effects were also demonstrated by Satrija *et al.* (1994) in pigs and by Hounzangbe-Adote *et al.* (2005) in vitro on live-cycle of *Haemonchus contortus*. Mixing aqueous decoction of *Carica papaya* seeds to water, Mpaome *et al.* (2000), showed improved health in broiler chickens affected by *Ascaridia galli* infestation. Anthelmintic effects of *Carica papaya* reported by previous authors are attributed to benzyl isothiocyanate as its main active component (Kermanshai *et al.,* 2001). From previous reports, it is suggested that *Carica papaya* might help to control parasitic population in farm animals' digestive tracts. But, the use of latex and cake or aqueous and ethanolic extract of *Carica papaya* seeds seems rather complicated as a management tool for famers. The *Carica papaya* dried seed powder incorporation into feed may be a good alternative. Therefore, the objective of this study is to evaluate anthelmintic effect of *Carica papaya* seed powder and consequently, its effects on feed intake and growth rate of layer pullet.

MATERIALS AND METHODS

Experimental design

A total of 200 ISA Brown male (layer-type) chicks were reared during 10 weeks of age. During the first 4 weeks of age, there was no anthelmintic medication. Between 5 and

TABLE 1. Starter mash feed composition and treatment. L0, Standard diet group (no papaya seed); L0.5, Feed supplemented with 0.5% papaya seed; L1.0, Feed supplemented with 1.0% papaya seed; L2.0, Feed supplemented with 2.0% papaya seed; LCP, Positive control group receiving 5g of piperazine citrate per liter of drinking water once each month. PB is a poultry feed additive containing more than 20 enzymes combined with beneficial organic plant extracts of natural origin in a concentrated, easy to use and mix liquid form. It is used as a poultry drinking water additive for poultry broilers, layers and turkey grower applications. EMA refers to apparent metabolizable energy.

Feed stuffs	L_0	L_{CP}	$L_{0.5}$		$L_{1.0}$		$L_{2.0}$	
	0-8weeks	0-8 weeks	0-4 weeks	4-8 weeks	0-4 weeks	4-8 week	0-4 weeks	4-8 weeks
Maize	56%	56%	56%	55,72%	56%	55.44%	56%	54.88%
Wheat bran	11%	11%	11%	11%	11%	10.89%	11%	10.78%
Fish meal 40%	9%	9%	9%	8.95%	9%	8.91%	9%	8.82%
Soya seed	20%	20%	20%	19.9%	20%	19.8%	20%	19.57%
Oyster shell	1%	1%	1%	1%	1%	1%	1%	1%
Concentré5%	3%	3%	3%	2.98%	3%	2.96%	3%	2.95%
pawpaw seed	0%	0%	0%	0.5%	0%	1%	0%	2%
EMA (kcal)	2970	2970	2970	2021	2970	2025	2970	2032
PB (%)	20.18	20.18	20.18	29.72	20.18	29.75	20.18	29.81

TABLE 2. Grower mash feed composition and treatment. L0, Standard diet group (no papaya seed); L0.5, Feed supplemented with 0.5% papaya seed; L1.0, Feed supplemented with 1.0% papaya seed; L2.0, Feed supplemented with 2.0% papaya seed; LCP, Positive control group receiving 5g of piperazine citrate per liter of drinking water once each month.

Feed stuffs	L_0	L_{CP}	$L_{0.5}$	$L_{1.0}$	$L_{2.0}$
Maize	54%	54%	53,73%	53,46%	52,92%
Wheat bran	21%	21%	20,89 %	20,79%	20,58%
Fish meal 40%	9%	9%	8,95%	8,91%	8,82%
Soya seed	12%	12%	11,95%	11,88%	11,76%
Oyster shell	2%	2%	1,99%	1.98%	1,96%
Concentré 5%	2%	2%	1,99%	1.98%	1,96%
Pawpaw seed	0%	0%	0.5%	1%	2%
EMA (kcal)	**2779**	**2779**	**2783**	**2787**	**2795**
PB(%)	**17,63**	**17,63**	**17.68**	**17.72**	**17.82**

10 weeks of age, the birds were divided into 5 groups with 2 replications of 20 birds each. The replicates were randomly distributed over the poultry house. Negative control group was fed with standard diet (L_0), the positive control group received, for one day 5g of piperazine citrate per liter of drinking water each month or positive control group (Lcp) and other groups received papaya seed incorporation in the feed at 0.5% ($L_{0.5}$), 1% (L_1) and 2% (L_2). Every group had feed and water *ad libitum* and each diet was formulated to fit crude protein (CP) and metabolisable energy (ME) of birds during starter (Table 1) and grower (Table 2) stages.

Every two weeks, samples of chicken droppings from each group were collected and used to determine the number of worm's eggs per gram by the McMaster Technique. During experimental period, amount of feed consumption, body weight and feed conversion ratio were recorded weekly.

Incorporation of papaya seed powder in the feed

The seeds were collected freshly from ripe *Carica papaya* fruits and washed with clean water to remove dirt. They were sundried and later grinded into powdery with a moulinex. The feed was mixed with dry *Carica papaya* seed powder at 0.5%, 1 % and 2% incorporation level.

Helminthic presence and parasitic load evaluation

At 5, 6, 8 and 10 weeks of chicken age, bird's wet feces were collected to determine the presence of parasitic egg with method of floating enrichment and the parasitic load with Mac Master Method. The flotation method, which involved the use of saline solution (40%), was used to determine the helminth eggs present in fecal samples, while modified McMaster egg-counting technique was used for nematode eggs counts.

Five grams of feces was soaked and mixed in 100 mL of saturated solution of sodium chloride (specific gravity = 1, 18) and the mixing obtained was filtered. Then 50 mL of that solution were used to fill tube up to obtain of saturated solution of sodium chloride layer on the top. During 30 min. the tube was covered with blade against which eggs were attached. Blade was then took off and observed under stereo-microscope to determine the species of parasites represented by those eggs were observed.

Five grams of feces were soaked in 100 mL of saturated solution of sodium chloride and the mixing obtained was filtered to fill the two cells of Mac Master Blade. Five min. later, the blade was removed and observed at optical microscope to determine the Egg per Gram (EPG) of feces (Sum of amounts of eggs numbered in the two cells x 100) and the reduction rate of parasitic load at 5, 6, 8 and 10 weeks of chicken age. Parasite eggs were identified as described elsewhere (Soulsby, 1982; Zajac and Conboy, 2006; Foreyt, 2011).

Feed intake, body weight and feed conversion ratio determination

Feed intake and body weight were recorded weekly and body weight gain calculated. Feed intake was determined as the difference between the amount of feed given and remaining feed. The body weight gain was calculated as the difference between initial and final body weight. These data were used to determine feed conversion ratio by dividing feed intake by body weight gain.

Statistical analysis

The data obtained were processed with the statistical software Graph Pad PRISM 5. ANOVA model was used to analyze the effect of *Carica papaya* on parasitic load, parasitic reduction rate, feed intake, body weight and feed conversion rate. If the overall *F*-value was statistically significant ($p < 0.05$), further comparisons among groups were made according to Tuckey's test.

RESULTS

Effects of *Carica papaya* seed on parasitic load (EPG)

Table 3 shows the incidence of parasitic according to the treatments and age of the birds. Overall all the groups were naturally infected with worm at the beginning (egg per gram feces of initial fecal sample before *Carica papaya* seed medication was 65). Egg per gram feces (EPG) of negative control group L_0 increased from 4 to 10 weeks (65 at 4 to 470 at 10 weeks) irrespective of worm species. In opposite, in the groups treated with *Carica papaya* seed ($L_{0.5}$, L_1 and L_2) and Citrate of Piperazine (Lcp) significant reduction was observed ($p<0.05$). One week after incorporation of *Carica papaya* seed in chicken feed, important reduction were obtained in groups L_1 and L_2 with 0 EPG until 10 weeks while in L_{cp}, although

TABLE 3. Evolution of parasitic load (EPG) in droppings according to treatments and experimental stage. L0, Standard diet group (no papaya seed); L0.5, Feed supplemented with 0.5% papaya seed; L1.0, Feed supplemented with 1.0% papaya seed; L2.0, Feed supplemented with 2.0% papaya seed; LCP, Positive control group receiving 5g of piperazine citrate per liter of drinking water once each month. [a, b] Data sharing no common letter are different ($p<0.05$)

Age (week)	L_0	$L_{0.5}$	L_1	L_2	L_{CP}
5	1758,75±[c]	5,001,25±[b]	0,000,00±[a]	0,000,00±[a]	102,50±[b]
6	27511,43±[c]	2,000,50±[a]	0,000,00±[a]	0,000,00±[a]	122,50±[b]
8	38515,59±[d]	3,001,25±[b]	0,000,00±[a]	0,000,00±[a]	203,22±[c]
10	47017,38±[c]	0,000,00±[a]	0,000,00±[a]	0,000,00±[a]	302,8±[b]

TABLE 4. Ratio (%) of parasitic load reduction according to treatments and experimental stage.

Age (week)	L_0	L_{CP}	$L_{0.5}$	$L_{1.0}$	$L_{2.0}$
5	- 169,2	84,61	92,30	100	100
6	- 323,07	81,53	96,92	100	100
8	- 492,30	69,23	95,38	100	100
10	- 623,07	53,84	100	100	100

reduced, EPG was 10, 12, 20, and 30 respectively at 5, 6, 8 and 10 weeks. It appears in table 4 that groups treated with *Carica papaya* seed has obtained 100% of reduction rate while L_{cp} and L_0 obtained respectively 53.84% and -623.07% at 10 weeks of age.

Feed intake

Average daily feed consumption according to the treatment during the trial period is shown in table 5. In general, according to treatment, feed intake increased with age of chicken. Daily feed consumptions of chicken of groups $L_{0.5}$, L_1, L_2 and L_{cp} were comparable. Feed intake was in the following order: $L_0 > L_{0.5} = L_1 = L_2 = L_{cp}$ ($p < 0.05$)

Body weight, daily weight gains and feed conversion ratio

Figure 1 shows body weight up to 10 weeks of age according to treatment. Overall, weekly body weight increased from one to 10 weeks of age. With regard to treatments, body weights were similar for all groups during the first week of experiment. At week two of treatment, chickens that received 0.5% and 1 % ($L_{0.5}$, L_1) *Carica papaya* seed treatment and piperazine citrate group (Lcp) were heavier than those of negative control group L_0 and group L_2 ($p<0.05$). But, from week four onward chicks of $L_{0.5}$, L_1, and L_2 groups were heavier than those of L_0, and Lcp while at 10 weeks chickens of negative control group L_0 become lighter than the others ($p<0.05$). The same trend is observed about daily weight gain (figure 2) with the heaviest chicks in $L_{0.5}$ ($p < 0.01$) and the lightest chicks in L_{cp} and L_0 ($p < 0.05$) while those of L_1 and L_2 groups were similar but significantly heavier than L_0 and Lcp ($p < 0.05$). In opposite, the lowest feed conversion ratio (figure 3) was obtained in the groups of $L_{0.5}$, L_1, and L_2 and the highest in the control groups L_0 and Lcp ($p<0.05$).

TABLE 5. Average individual daily feed consumption according to treatments (g). [a,b] Data sharing no common letter are different ($p<0.05$)

Age (week)	L_0	L_{CP}	$L_{0.5}$	$L_{1.0}$	$L_{2.0}$
5	97,5±1,23^{a}	84,6±0,75^{a}	89,0±0,73^{a}	83,5±0,97^{a}	84,7±0,75^{a}
6	116,4±0,18^{b}	98,8±0,25^{a}	92,5±0,16^{a}	96,0±0,37^{a}	93,1±0,12^{a}
7	124,5±0,30^{b}	112,4±0,71^{a}	108,6±0,04^{a}	101,7±0,31^{a}	98,6±0,11^{a}
8	135.4±0,32^{b}	121.6±1,21^{a}	119.8±0,63^{a}	116.40±0,23^{a}	113.8±0,57^{a}
9	143.2±0,27^{b}	126.2±0,20^{a}	121.5±1,03^{a}	123.0±0,44^{a}	118.7±1,32^{a}
10	148,6±0,21^{b}	118,5±0,18^{a}	124,7±0,03^{a}	120,30±0,22^{a}	122,1±0,10^{a}

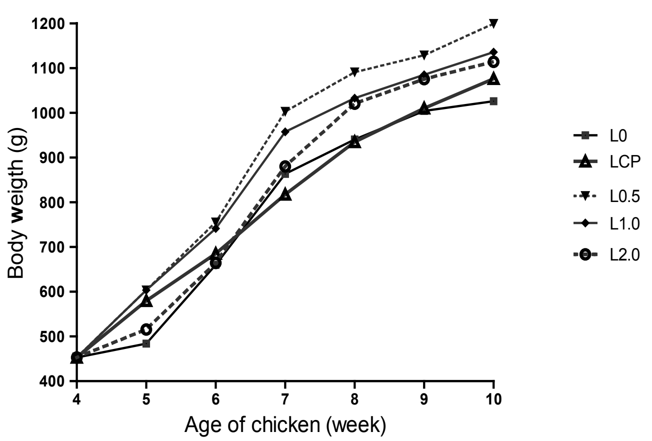


FIGURE 1. Effect of supplementation of feed with Papaya seed on weekly body weight gain. L0, Standard diet group (no papaya seed); L0.5, Feed supplemented with 0.5% papaya seed; L1.0, Feed supplemented with 1.0% papaya seed; L2.0, Feed supplemented with 2.0% papaya seed; LCP, Positive control group receiving 5g of piperazine citrate per liter of drinking water once each month.

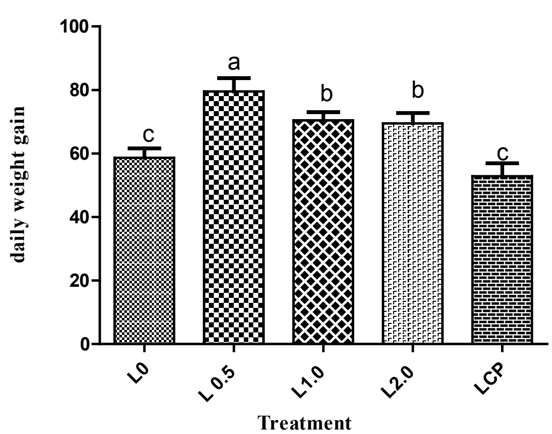
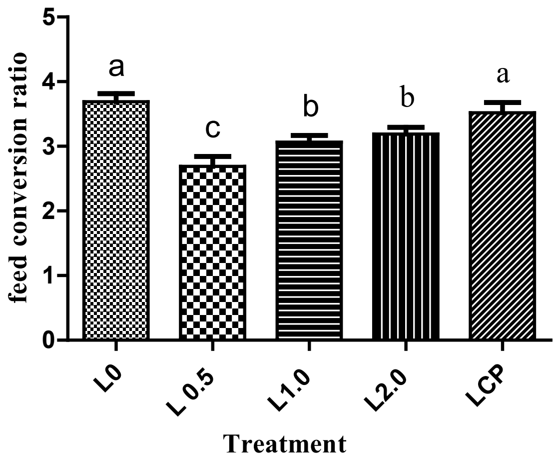


FIGURE 2. Effect of supplementation of feed with Papaya seed on daily body weight gain. L0, Standard diet group (no papaya seed); L0.5, Feed supplemented with 0.5% papaya seed; L1.0, Feed supplemented with 1.0% papaya seed; L2.0, Feed supplemented with 2.0% papaya seed; LCP, Positive control group receiving 5g of piperazine citrate per liter of drinking water once each month; a: $p < 0.01$ (L0.5 when compared with other groups); b: $p < 0.05$ (L1.0 or L2.0 when compared with L0 and LCP); c: $p < 0.05$ (L0 and LCP when compared with other groups).

FIGURE 3. Effect of supplementation of feed with Papaya seed on feed conversion ratios. L0, Standard diet group (no papaya seed); L0.5, Feed supplemented with 0.5% papaya seed; L1.0, Feed supplemented with 1.0% papaya seed; L2.0, Feed supplemented with 2.0% papaya seed; LCP, Positive control group receiving 5g of piperazine citrate per liter of drinking water once each month; a: $p < 0.05$ (L0 and LCP when compared with L0.5, L1.0 or L2.0); b,c: $p < 0.05$ (L0.5, L1.0 or L2.0 when compared with L0 and LCP).



DISCUSSION

Carica papaya seed incorporated in poultry feed and Piperazine citrate mixed with water reduce parasitic load of *Trichostrongylus sp*, *Heterakis sp*, *Ascaridia sp* and *Syngamus sp* and improve chicken performances. Every incorporation level of *Carica papaya* seed was more effective than Piperazine Citrate. The dose of Piperazine Citrate recommended by producer and medical prophylactic program, revealed lesser efficacy if compared with *Carica papaya* seed. During the rearing period, *Carica papaya* seed showed a significant and a dose dependent effect on worm eggs with 100% reduction from one week to 6 weeks after treatment at 1 and 2% of incorporation as claimed by Bauri *et al.* (2015). This EPG reduction can be linked to the destruction of mature worms in poultry gut. According to Kumar *et al*. (1991) and Kermanshar *et al.* (2001), worm destruction property of *Carica papaya* seed is due to benzyl isothiocyanate and papain on different parts including seeds of the plant. Explaining the mode of action of these active components, authors pointed out that energy metabolism and motility of the parasites was inhibited by benzyl isothiocyanate and their cuticle destruction by papain. The combination of paralyzing effect and worm cuticle destruction effect results in fast eggs evacuation leading to 100% of egg reduction rate at 1 and 2% incorporation. Moreover, daily use of *Carica papaya* seed can prevent other eggs ingested by bird to grow out and reach mature stage in the gut. In opposite, Piperazine Citrate acts only, according to Del Castillo *et al.* (1963), by motility inhibition. So eggs enclosed in the body of paralyzed worm should be evacuated out slowly of the gut resulting in a lower egg reduction rate as observed. Moreover, its monthly administration can result in fast reinfestation of bird. However, studies of Ketzis *et al.* (2006) and Hoque *et al.* (2006) have pointed out this motility inhibition is a crucial process for inducting worm mortality hence conferring anthelmintic property to medicinal plants as well as anthelmintic drug. The low weight gain and the higher feed intake shown by the negative control group can be attributed to nutriment competition and peristaltism perturbation from the high number of parasites in the gut. Their destruction by *Carica papaya* seed in groups $L_{0.5}$, L_1 and L_2 has resulted in significantly higher chick weight gain and better feed efficiency. Our results confirm those reported by Yvore (1978) who showed detrimental effects of *Eimeria adenoides* on turkey's growth performance. The similarity between weight gain and feed efficiency of groups L_0 and L_{CP} shows that the Piperazine Citrate producer recommendation (5g/l of water during a day) is not sufficient to cure bird of worm infestation. So, the posology can be readapted to our farming conditions for about three days consecutive administration. In conclusion, daily administration of *Carica papaya* seed improved performance parameters through gastrointestinal helminthes elimination. *Carica papaya* seed powder can be used in poultry farms as alternative to pharmaceutical deworming products.

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