TechHub Journal ISSN 2810-2800

TechHub Journal Vol. 8, pp.1-12 (2026)
ISSN: 2810-2800
www.techhubresearch.com

Comparative Assessment of Chili Pepper and Amprolium on Apparent Digestibility and Prevention of Coccidiosis in Broilers

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ABSTRACT

A trial was conducted for 56 days to assess the efficacy of Chili Pepper (CP) as an alternative additive to synthetic coccidiostat on performance, apparent digestibility and prevention of coccidiosis in broilers. Completely Randomized Design (CRD) was used to allot 210 a-day old Hybro chicks to three dietary treatments with seven replicates of ten birds each. Diet 1 which is a Positive Control (PC) supplemented with amprolium. Diet 2 was a Negative Control (NC) without amprolium and chili pepper, while Diet 3 was supplemented with 1.25% chili pepper.

Results showed that at starter phase an Average Feed Intake (AFI) and Average Body Weight Gain (ABWG) were similar in birds fed with diet 1 (PC) amprolium supplemented type, and diet 3 (CP) chili pepper supplemented type compared with diet 2 (NC) without amprolium and chili pepper supplementation. At finisher phase, feed : gain ratios observed in birds fed diet 1 (1.96), and birds fed diet 3 (1.98) were better when compared with those birds fed diet 2 (2.12). Percentage mortality (5.71%) was higher ($p<0.05$) in diet 2 as compared with diets 1 and 3 (2.85%) (1.42%), respectively. At the starter phase, a similar trend in apparent digestibility was observed throughout all treatment groups while at the finisher phase, higher ($p<0.05$) values of 88.31%, 78.11%, 76.91%, 78.88% and 64.44% were recorded for dry matter, crude protein, crude fibre, ether extract and ash, respectively, for diet 3, compared to diets 1 and 2.At both starter and finisher phases, oocytes shedding per gram of faeces (OPG) in diets 1 (61.8and 40.6) and diet 3 (70.1 and49.3) were lowered ($p<0.05$) compared to diet 2 (83.6 and98.9), respectively. These findings indicate that supplementation of chili pepper at 1.25% in feed improved production performance, characterized by faster growth rate, higher nutrient digestibility, reduced mortality and coccidia load, in broilers chickens.

Key words: *Amprolium, oocysts shedding, nutrient digestibility, mortality, broilers*

INTRODUCTION

One of the major threats to poultry industry in the developing countries is chicken Eimeria (coccidiosis diseases) (Abd El-Hack *et al*., 2021). The disease was considered to be an infectious protozoan disease, caused by the genius Eimera, under which common species such as (*E.acervulina, E. tenella, E. maxima, E. necatrix, E. brunetti, E. mitis, E. praecox)* affects an alimentary tract of chickens, to produce diarrhea, poor feed intake, poor feed to gain ratio, reduced weight gain and in severe cases, mortality (Pop, L., *et al.,* 2015, and Abebe and Gugsa, 2023*).* In poultry the most prevalent species are *E. acervulina, E.tenella and E. maxima* (Windhorst, 2016), of which *E. tenella* and *E. necatrix* are highly pathogenic. They cause hemorrhagic, diarrhea and being responsible for

TechHub Journal Vol. 8, pp.1-12 (2026)
ISSN: 2810-2800
www.techhubresearch.com

greatly lower weight gain and considerable mortality risk. Sick birds tend to cluster and isolate themselves in small groups, their feathers are rough and dirty, they eat less or even no longer eat, and they drink very little water (Gazoni*et al.,* 2020; Maric *et al*., 2021). Moreover, Poultry feed might be the source of human illness, resulting from the consumption of poultry products, because a wide range of chemicals can enter the feed production system, intentionally or unintentionally by Gururajam *et al*. (2021). Some veterinary drugs, such as antibiotics and coccidiostats are routinely included in poultry feeds as additives (Dembitsky, 2022). In meat-producing birds, the problem of drug residues in meat are overcome by providing a withdrawal diet containing no drugs for seven to ten days prior to slaughter. However, the possible development of microbial resistance due to the use of antimicrobials in animal diets and vaccine efficacy which is variable has become a major public concern in recent years. As a result, the use of in-feed antibiotics is either banned or restricted in the poultry industries of developed countries (Akinduro *et al.,*2020).With the restricted use or ban of dietary antimicrobial agents we must explore new ways to improve and protect the health status of farm animals, to guarantee animal performance and to increase nutrient availability. This precondition demands the efficient use of all available resources of traditional and modern technologies, also of feed additives in a responsible way (Mosobolaje *et al.,* 2019; Adedoyin *et al.,* 2023). There is no doubt that today's world food production must grow without increasing the environmental waste load (Adedoyi*n et al*., 2016).

Meanwhile, various alternative strategies have proven their effectiveness in coccidiosis control with potential stimulatory effects on immunity and performances. Such as usage of natural feed additives (Adedoyin *et al.,* 2023),and the derived essential oils and plant extracts (Abd El-Ghany *et al*., 2021, Naqvi, et al. 2023). In particular, artemisinin made by aerial parts of *Artemisia annua* and piperine and capsaicin in chili pepper (CP) had been proven to be effective against intestinal dysfunction in poultry. The way of acting of artemisinin most likely implies the manufacturing of free radicals (esters) due to cleavage of its endo-peroxide bridge resulting in the inhibition of "reactive oxygen species" (ROS) coccidian sarco (Tadewos, *et al.,* 2023). Similarly, Abd El-Ghany *et al*. (2021) reported cases of better feed to gain ratio and lower mortality in broilers fed HRP supplemented diets. Although, the control of this disease is based mainly on chemoprevention using coccidiostat additives and more recently on immunization (Lillehoj *et al.,* 2018). Furthermore, the exploration of groundbreaking (innovative) effective anti-coccidia is needed in order to keep in control this destructive disease (Pop, *et al*., 2015, and Abdelheg, *et al*., 2015). The present study therefore aimed to assess the comparative efficiency of amprolium and chili pepper (CP) on performance, apparent digestibility, and reduction on coccidia load in broiler chickens.

MATERIALS AND METHODS

Experimental Diets

The sun-dried chili pepper (CP) used in this experiment was obtained in large quantity from Maya market in Ibarapa Area and was then ground into powder. Diet 1 served as a positive control (with amprolium 0.6g/kg diet) diet 2 served as negative control (without amprolium and (CP) supplementation) and diet 3 was supplemented with 1.25% chili pepper.

Table 1: Nutrients composition of commercial broiler hybrid diets (g/100g)

Nutrients	Starter	Finisher
Dry matter	89.4	89.3
Moisture	10.8	10.5
Crude protein	22.5	20.01
Ether extract	5.1	3.8
Crude fibre	4.3	3.6
Ash	5.0	6.0
ME Kcal/kg	3000.8	3100.1
Phosphorus	0.45	0.44
Calcium	1.2	1.2
Methionine	0.56	0.52
Lysine	1.2	1.2

ME: Metabolisable Energy

Experimental Birds and Management

A total of 210-day old Hybro broiler chicks were used in the present study. Birds were allocated into 3 treatments, each with seven replicates using a Completely Randomized Designed (CRD). Birds were generally vaccinated against Newcastle disease and infection bursal disease in the 1st, 10th and 21st day. Also, birds fed with positive control (PC) diet 1 were medicated with antibiotics, Amprole-200 and vitalyte as outlined by Olomu (2003). In contrast birds, fed with negative-control diet 2 were provided only with vitalyte, while diet 3 was supplemented with 1.25% chili pepper (CP). Broilers were raised on the floor litter. Fresh feed and water were provided ad libitum. Feed intake, feed : gain, weight gain, and mortality were weekly recorded, and were used as an indicators of birds' performance. Duration for the experiment was 8 weeks. Feed : gain (FGR) was calculated as follows:

$$FGR = \frac{Feedintake}{Bodyweightgain}$$

Apparent Nutrient Digestibility (AND)

A digestibility trial was conducted on days 28 and 56 of the study to determine the apparent nutrient digestibility (AND). Four birds per replicate (28 birds/treatment) was randomly selected and housed separately in appropriate metabolism cages fitted with individual feed troughs, water troughs and facilities for separate excrete collection. Birds were acclimatized for 2days prior to the

TechHub Journal Vol. 8, pp.1-12 (2026)
ISSN: 2810-2800
www.techhubresearch.com

commencement of 5 days collection period. A known weight of feed (slightly above the respective daily requirements) was fed to the birds housed in individual metabolic cages. Excreta collected per bird per day (collected daily twice) were stored in air-tight bags and stored at – 180^c till analyzed. The frozen excreta per bird were thawed, pooled for each treatment, oven dried (60^{0}C) for 48h, and then ground to 0.5mm size. The proximate concentration of feed and dried excreta samples was determined using the standard method of AOAC (2020). The AND was calculated according to the equation below: Digestibility (%) = 100 - $\left[\frac{Nutrient\ in\ feed\ (\%)}{Nutrient\ in\ faeces\ (\%)} \times 100\right]$

Parasitological and Immunological Index

On the 28th and 56th days, oocytes counts were determined in 10gram of excreta collected along the day. Samples were placed in separate airtight storage bags, mixed thoroughly and kept refrigerated. Then, they were firstly ten-fold diluted in a tap water and the resulted solutions were further diluted in saturated NaCl solution at a ratio of 1/10 (floating technique) and oocytes per gram of faeces was determined by duplicate counts of duplicate faecal slurry from each specimen using McMaster chamber method; results presented as the number of oocytes per gram of excreta (Abdelheg *et al.,* 2015).

Statistical Analysis

Data obtained was subjected to analysis of variance (ANOVA) with SAS software SAS/STAT, 2020). Duncan Multiple range tests (1965) was also carried out to separate subclass means.

TechHub Journal ISSN 2810-2800

TechHub Journal Vol. 8, pp.1-12 (2026)
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Table 2: Production of broiler starter and finisher fed diets containing different Additives

	Starter Phase				Finisher Phase			
Diets + Additive	**Av. feed intake g/b/d**	**Av. body weight gain g/b/d**	**Feed: Gain**	**Mortality (%)**	**Av. Feed intake g/b/d**	**Av. Body weight gain g/b/d**	**Feed: Gain**	**Mortality (%)**
Diet 1- PC – with Amprolium)	43.08	32.80	1.31^{ab}	1.42^{b}	84.6^{ab}	43.14^{a}	1.96^{b}	2.85^{b}
Diet 2-NC- without Amprolium and CP	42.64	30.01	1.43^{a}	2.85^{a}	73.81^{c}	35.03^{ab}	2.12^{a}	5.71^{a}
Diet 3- (CP) – supplemented type	45.1	33.03	1.36^{ab}	1.42^{b}	88.46^{a}	44.61^{a}	1.98^{b}	1.42^{bc}
SEM±	2.08	3.39	0.08	0.19	9.91	1.39	0.20	0.87

Adapted from Adedoyin et al. (2019)
abc… means within a column with different superscript are differ, SEM±: standard error of the means, g/b/d: grams/bird/day; CP: chili pepper, PC: positive control, NC: negative control

Table 3: Apparent Nutrient Digestibility of broiler starter and finisher fed diets containing different additives

Treatment Phases	**Diet 1 PC-with amprolium**	**Diet 2 NC-without amprolium and (CP)**	**Diet 3 Chili-pepper supplemented type**	**SEM±**
Days 1 - 28 Starter Phase				
Dry Matter	78.37^{ab}	70.50^{c}	85.11^{a}	3.71
Crude Protein	64.86^{ab}	60.01^{c}	70.07^{a}	2.91
Crude Fibre	65.11	60.11	67.18	2.08
Ether Extract	63.88^{b}	60.01^{c}	76.72^{a}	4.77
Ash	58.99	50.91	62.83	3.10
Days 29 - 56 Finisher Phase				
Dry Matter	82.01^{ab}	80.11^{c}	88.31^{a}	5.01
Crude Protein	69.11^{b}	62.31^{c}	78.11^{a}	5.81
Crude Fibre	68.22^{b}	63.14^{b}	76.91^{a}	4.1
Ether Extract	60.44^{b}	61.56^{b}	78.88^{a}	3.01
Ash	60.07	60.11	64.44	3.01

abc… means within a row showing same superscript do not differ significantly (P>0.05).
SEM±: standard error of means, CP: chili pepper, PC: positive control, NC: negative control

Table 4: Oocytes excretion (x10^3) per g of faeces in broiler starter and finisher fed diets containing different Additives

Treatment Phases	Diet 1 PC-with Amprolium	Diet 2 NC-without amprolium and CP	Diet 3 Chili-pepper supplemented type	SEM±
Starter	61.7^c	83.6^a	70.18^b	9.1
Finisher	40.6^{bc}	91.1^a	43.10^b	3.3

abc… means within a row showing the same superscript do not differ, SEM±: standard error of the means, PC: Positive Control, NC: Negative Control, CP: chili pepper

RESULTS AND DISCUSSION

Data obtained for the both phases on Average Feed Intake (AFI), Average Body Weight (ABW), Feed: Gain ratio (FGR) and mortality are in (Table 2). At starter phase, birds that were fed with the diet 1 (Positive Control (PC)- with Amprolium) and diet 3 (chili pepper (CP)-supplemented type) had a numerical increase in AFI and ABWG compared to diet 2 (negative control (NC) – without amprolium and chili pepper). It is noteworthy that feed-intake at both starter and finisher phases in broilers fed with chili pepper (CP) were increased despite that studies have established that CP is the only plant that produces the alkaloid-capsaicin.

About 50% of its active elements are capsaicin (8-methyl-N-vanillyl-6-nonemide), the main active compound responsible for the intense effects of CP varieties and the main component prompting the hot flavor (Abd El-Hack *et al.,* 2021). Highly pungent tastes may be experienced as unpleasant in other animal such as rabbit. Meanwhile, broiler cannot perceive the biting taste of capsicum due to the lack of receptors specific for capsaicin binding, thereby enabling a high concentration of capsicum in broiler diets used for this study (Puvaca *et al*., 2015). Therefore, the addition of small ratios of chili pepper into experimental diets in previous studies (Zheng *et al.,* 2017, Akinduro*, et al*, 2020) may not be adequate to represent the mechanisms of action clearly. However, capsaicin influences taste and can also protect the gastrointestinal mucosa layer against injuries due to drugs or irritants, so the addition of chili pepper to the diet influences broilers feed consumption.

At finisher phase however, additives added, significantly ($P<0.05$) influenced better feed: gain ratio of (1.96) in birds fed diet 1 (PC –with Amprolium) and (1.98) in birds fed diet 3 (with chili pepper (CP) -supplemented) compared with (2.12) in birds fed diet 2 (NC – without amprolium and chili pepper respectively. It might be posited that the alkaloid capsaicin can enhance the digestive enzyme activity, bile acid secretion, improve feed intake, and feed digestibility and finally leading to an improved feed: gain ratio in CP based diets. The mortality rate (5.71%) was the highest in diet 2 (NC) – without amprolium and chili pepper. This could be attested to the effects of dietary and nutrition availability. Pop, *et al.* (2015) reported that the pathogenic microbial floral in the small intestine compete with host for nutrients while at the same time inhibiting the binding of the bile acids to the pertinent substances, which resulted to decrease in the digestion of fats and fat-soluble vitamins. This led to decrease in an essential nutrients absorption and increase in disease rate. Similarly, the nutrients digestibility of broilers fed diets containing different additives were shown in Table 3. At the starter phase, nutrient digestibility's were similar generally in diets 1 (PC – with amprolium) and 3 (with chili pepper), whereas at (finisher phase) a significantly ($P<0.05$) improved nutrients digestibility were recorded in birds fed diet 3 compared to those birds fed diet 1 (PC – with amprolium) and diet 2 (without amprolium and chili pepper). This result suggests that the supplementation of amprolium or chili pepper (CP) in isolation cannot be able to influence a significant digestibility of nutrients at the starter phase. The bioactive components of amprolium or CP, feed intake/feed composite, age/specie of animal and as well as synergistic effect between those factors play a major role.

At the finisher phase on the other hand, nutrients digestibility's obtained in birds fed diet 3 (with hot red pepper) 88.31, 78.11, 76.91, 78.88 and 64.44% were for dry matter, crude protein, crude fibre, ether extract, and ash respectively. The observed results showed that chili pepper was capable of promoting a substantial flow of bile acid, and boosting an activity of feed-degrading enzymes stemming from the pancreas and intestinal mucosa (Attia *et al.,* 2017). Similarly, the exhibition of an increased crude protein, crude fibre and ash digestibility for this group suggests that enhancement was made due to the composite mixture of pepper and feed components in broilers fed diet 3 (Lukas *et al.,* 2018; Oso *et al.,* 2019) compared to those birds fed diets 1 and 2. In chili pepper, capsaicin alkaloid ate was reported to be an effective digester. It was characterized by neurotronic and antimicrobial activities. It can decrease lipid peroxidation, with an enhanced bio-pharmacological properties of which included antioxidant, anti-inflammatory, antiallergenic, and anti-carcinogenic activities. In another mode of action, capsaicin protects the gastric mucosa through the afferent stimulation of nerve endings. More so, close to 85% of capsaicin is absorbed by passive diffusion, mainly in the jejunum, which improves the absorption nutrients in feeds by broilers (Munglang and Vidyarthi, 2019) and (Reda *et al.,* 2020). Such an advancement may be partly related to a reduction in heat stress emanating from a high concentration of vitamin C as reported (Attial, *et al.,* 2017). This might be that chili pepper is a good natural feed additive for improving digestibility in broiler chickens. Data from (Table 4) that showed the oocytes excretion per gram of faeces were significantly higher in birds fed diet 2 (NC) compared with others throughout the experimental trial. This might confirm the anti-coccidia activity of the used products, which means that the use of a natural coccidiostat based (chili pepper) in the diet 3 improves immunity and reduces the values of oocytes per gram of faeces. Various bioactive compounds have been used as antiviral, and antimicrobial medium in human health (Lillehoj *et al*., 2018). The phenolic compounds like piperine and capsaicin in pepper is claimed to be their ability to disintegrate bacteria cell membranes and penetrate the bacteria cells. This process is associated with killing of pathogenic bacteria directly and partitioning the bacteria into their lipid contents and mitochondria (Dembitsky, 2022). The lipid interaction interferes with bacterial metabolism, by breaking the cell wall, and membrane permeability, leading to extensive leakage of critical molecules and ions from the bacterial cells of such harmful bacteria. Again, the phenolic and coumarin component of medicinal plant has be seen to allow the passage of molecules through cytoplasmic membrane to both hydrogen and potassium ion, and this mechanism has been claimed to have an effect on the metabolic process (biological reactions) such as decreasing the intracellular pH and ATP concentration thus resulting in the bacterial cell wall lysis (Adhikari *et al*., 2020, and Dembitsky, 2022). Moreover, the deformation of cellular membrane, inhibition of ATPase activity, and release of intracellular ATP is an occurrence eventually lead to the death of the coccoid cells such as merozoites preventing secondary bacterial proliferation (Mnisi, Mlambo and Gila *et al.,* 2023). Aristide *et al*., (2017) established that generally a high number of more than 100,000 oocytes/g has a clear diagnostic value, whereas a very low numbers of less than 10,000 oocytes/g do not require treatment. Tadewos et al., (2023) reported the good sources of bioactive phytochemicals in hot red pepper like phenolic compounds such as capsaicin, luteolin, and quercetin, provitamin A; Vitamins E and C; carotenoids, and β–carotene with their radicals scavenging ability in the diets, along with the stimulated digestive enzymes seem to have stabilized intestinal microflora, thus inactivating the colonization and proliferation of coccidia cells. In another cell-based assays, to explore the chemo-preventive potential of capsaicin have been reported that the capsicum pepper has a wide-ranging effect on isolated bacterial strains due to the bacteriostatic and bactericidal activity of the capsaicin derivatives t-cinnamic and caffeic acids respectively (Srinivasan, 2015). In addition, methyl cinnamate has been used as a additive in an in-vitro and in-vivo disease challenge trials against coccidiosis. An in vitro followed by an in- vivo study has shown a higher stimulation of (purified recombinant protein) antibody response in E. tenella and cinnamate group compared with only E. tenella group (Moore, 2016, Lee *et al.,* 2019). Follow-up experiment, a morphological modification of intestinal mucus cells and altered expression of metabolism-related intestinal genes such as IL ~ 1β, IL – 6, IL ~ 15 and IFN-y mRNA were found to be elevated therefore reducing E. acervulina-induced and E. maxima-induced body weight loss as well as E. acervulina oocyst shedding. Such optimization in above cytokines, after supplementation of diets with capsaicin-methyl-cinnamate, might prove as a fresh opportunity to help increase in coccidiocides (Adhikari *et al.,* 2020).Again, this might be a result of an effective biosurfactant mixture in the gastro-intestinal motor, that transit disturbances associated with Eimeria life cycle as well as sporogony in the gastro intestinal tract of broiler. Such compounds are supportive to revitalize cell-mediated immunity resulting in an increase in natural killer cells, macrophages, CD4, CD8 T lymphocytes cells, and cytokines such as IFN-y and IL-6 that increased the host immunity against coccidiosis by stimulating the innate as well as the adaptive (humoral) resistant response. However, they do not completely eliminate the parasites from the intestine of those birds fed diets 1 and 3.

Various researchers Adhikari, *et al* (2020) and Abd El-Hack *et al.,* (2021) also attested that coccus stays in GIT for almost 6-7 ½ months to a year. Microscopic examination will reveal large numbers of schizonts. Moreso, white spots visible through the outer (mucosal) surface of the intestine indicate colonies of schizonts, and the thickening of an affected section of the small intestine. Sometimes, however, the control is aimed at preventing the build-up of coccidia to the stage when disease occurs, but retaining the level of infection which allows partial continuation of the life cycle so that a degree of antigenicity is maintained. Therefore, as given above, preventive measures aimed at complete elimination of the protozoan are no longer recommended, but efforts should be redirected at the reduction of protozoan numbers by the use of coccidiostat as alluded by (Gazoni *et al*., 2020).This study is therefore necessary for effective prevention or decision-making in a healthy livestock production as suggested by FAO, (2019), and Ahmad, Yu YH, and Hsiao *et al*., (2022).

CONCLUSION AND RECOMMENDATION

In this study, the use of chili pepper at 1.25% inclusion level in broiler diets reduces coccidiosis infections. Chili pepper as a natural (organic) feed additive led to significantly improved weight gain, percentage nutrient digestibility, lowered feed : gain as well as percentage mortality compared to synthetic coccidiostat.

Owing to the safety of chili pepper (CP), it is thus recommended for further studies and adoption at commercial levels

DECLARATIONS

CONFLICTS OF INTEREST: The authors affirm that they have no conflict of interest.

COMPLIANCE WITH ETHICAL STANDARDS: All experimental methodology were carried out in conformity with the approved guidelines for Animal Research by Nigeria Institute of Animal Science (NIAS)

ACKNOWLEDGEMENT

The authors are grateful to the Veterinary Clinic Staff of the University of Ibadan, Ibadan, Nigeria for their technical assistance.

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www.techhubresearch.com

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