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RESEARCH ARTICLE

BIOSYNTHESIS AND ANTICOCCIDIAL EFFICACY STUDIES ON SILVER NANOPATICLES COATED WITH FRUIT EXTRACT OF *MORINDA CITRIFOLIA* IN BROILER CHICKENS (VEN COBB 400)

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ABSTRACT

Coccidiosis, inflicting a huge production and economic losses to poultry industries is great concern due to its prevalence by shifting virulence from less to more pathogenic strains or species of coccidia. Control of coccidiosis is difficult due to emergence of drug resistance and several imitations of available anticoccidial vaccine leads to frequent disease outbreaks, strategic control and alternative approaches are being used and practiced in control of coccidiosis. Present study carried out to assess anticoccidial efficacy of SNPsCMcFE (Silver Nanoparticles coated with *Morinda citrifolia* Extract) against *E. tenealla* induced coccidiosis in broiler chicks (Ven cob 400). Total 120, one-day-old broiler chicks assigned and divided into six groups, each group comprised of 20 chicks employed in this study. Infection was given orally in groups T2 to T6 by single low dose sporulated oocysts of *E. tenella* (@ $20x10^3$ per chick) and subsequently given treatments through water for 28 days were served as infected and treated groups. Group T1 was healthy control. Group T2 was infection control. Group T3 standard drug control given amprolium sulfate @ 125 ppm for eight days through water. Group T4 treated with SNPs @ 15 ppm. Group T5 was administered SNPsMcFE @ 15 ppm and Group T6 birds treated with fruit extract of *Morinda citrifolia* (McFE) @ 300 ppm. General performance, mortality, oocytes counts and lesion scores were perform for all groups. Results showed 100% morbidity and no mortality among birds of infection and treatment groups. Group T2 birds were inactive, depressed reduced body weight, weight gain, feed, water intake, anemic and revealed signs of ruffled feather, semisolid feaces, bloody and blood mixed mucoid diarrhea. Severity of signs and symptoms were greater on day 4th day PI followed by 6th and 7th, there were significant improvement in clinical signs and symptoms, performance, OPG count and lesions score in all infected treated birds compared to control on 9th day PI onwards. In groups treated with SNPsMcFE and others lesions score was graded as +1 against +2 of infected untreated birds in control group T2 suggested mild infection. Fruit extract *M. citrdiloia* treatment showed 100% efficacy in terms of OPG counts and improved condition after 7th day PI (28 day of age) onwards, SNPsMcFE treatment was more effective than SNPs and less than McFE, where anticoccidial activity found highest with the fruit extract. Efficacy of SNPsMcFE was comparable to amprolium. No caecal lesions or fecal oocytes were found in birds treated with *M. citrdiloia* extract and healthy uninfected untreated control birds (Group T1). Among all OPG count were in order of treatment SNPs, > SNPsMcFE > amprolium, whereas lesion score were SNPsMcFE > amprolium > SNPs, McFEs as compared to infected untreated control (T2). In conclusion, the anticoccidial activity of SNPsMcFE and SNPs were moderate and for McFE found at par, which could be possible alternative coccidiostat, obviously subtle infection at low doses of *E. tenella* are needs further rectification with moderate and higher doses on large scale assessment for guaranteed efficacy against coccidiosis in broiler chickens (ven cobb 400).

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INTRODUCTION

In poultry coccidiosisis is caused by seven major pathogenic strains or species of Eimeria coccidian (Allen and Fetterer, 2002), is a diseases of economical importance due to huge production and economical losses to the poultry industries and small stakeholders. The chickens, turkeys, geese and ducks is specifically affected by pathogenic strains (McDougald and Fitz-Coy, 2008).

Clinical signs of coccidiosis in broiler chickens are characterize by poor performance, diarrhea and gross intestinal lesions, the hemorrhagic diarrhea also observed in chickens when infected with *E. tenella,* a highly pathogenic strain of genus *Eimeria*. The severity of disease varied from mild to moderate and moderate to severe (Chapman, 2009) involving multiple factors (Ruff, 1999), hence disease results in enormous production and economic losses due to morbidity and mortality by highly pathogenic strains to the poultry. The annual production and medication cost for controlling

coccidiosis reported to be higher in the United States (Chapman, 2009), worldwide it was in excess of US$ 3.2 billion (Dalloul and Lillehoj (2006). In India, total annual losses due to coccidiosis were around 1.14 billion rupees for the year 2003-04 (Beraa *et al.,* 2010). Incomplete protection, overwhelming resistance to available anticoccidials frequently encountered disease outbreaks, provokes new sustainable and viable alternatives for coccidiosis control and prevention (Innes and Vermeulen, 2006). Development of resistance in coccidians are very similar to that resistance develop in bacteria to available antibiotics, this leads a strong incentives in developing a new strategy in control of coccidials (Kyriacou *et al.,* 2004). The ionophores inclusions in diets of poultry as feed supplement is traditionally practiced (Chapman *et al.,* 2010), silver salts also used as feed additive in poultry since 1950s, however cost of silver is not affordable and competitive with antibiotics (Jeong *et al.,* 2005) forfeited use of silver. Currently available anticoccidial drugs are found less effective due to shifting the virulence also several limitations of live coccidial vaccines is problem in control of coccidiosis. Alternatively phytonutrients are evolved to fight coccidiosis in poultry, with the advent of nanotechnology could possible to target sites of action for most beneficial effects of conventional therapies. In this view, it was thought that using matters (nanomaterials) of size 1 and 100 nm particles may have beneficial effects in control of coccidiosis, as such small structures are having equivalent size to small biomolecules were significantly influence economy and affects every spare, including the agriculture, medicine and environment (Discher, 2006). Colloidal silver nanoparticles of size below 200 nm were proven their higher antimicrobial effects (Atiyeh *et al.,* 2007) are being used as antimicrobial agents against illness of various microorganisms (Navarro *et al.,* 2008; Spruill, 2006).

Silver nanoparticles, currently a promising and emerging alternative to feed supplement owes to their characteristic properties, very limited studies demonstrate biological effects of nano-silver in poultry. Although traditionally use herbal medicines have beneficial effects are unable to reach to target sites at appropriate concentration desired benefits and requires longer time to achieve desired concentration at sites of action. With advent of nanotechnology it could possible to produce sizable nanoparticles for delivery device (such as silver nanoparticles) at target site of action. The present study intended to evaluate the antioccidial efficiency of silver nanoparticles (alone and coated with fruit extract *M. citridolia*) with other treatments against *E. tenella* induced coccidiosis, being a predominant organism to develop pathogenesis in broiler chickens.

MATERIALS AND METHODS

Location of Experiment and Climate

The work was carried out at instructional poultry farm belongs to the Department of Poultry Science, College of Veterinary and Animal Sciences, Parbhani, located 457 meters above sea level on Deccan plateau at 18.45^0 and 20.01^0 of North latitude 76.13^0 to 77.29^0 have assured rainfall is average 774.59 mm, where environment temperatures normally range 22°C to 42°C. The month of May is the hottest month of the year.

Plant Materials: Collection, Authentication and Processing

Morinda citrifolia fruits collected directly from plants maintained at Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra (India) and the fresh *Azadirachta indica* leaf were collected in months of May-June. They authenticate from the expert Botanist, the voucher specimen of Noni fruit preserved for future reference purpose in the department. These plant materials clean in tap water, fruits were chopped into thin slices (2-5 mm diameter) was dried at room temperature in the laboratory and crushed to obtain powder materials mechanically using electric grinder and commercial flower mill. Ethanolic extract of Noni fruit was obtained by conventional maceration techniques, the aqueous extract of *A. indica* leaf by the simple hot extraction techniques (Tiwari *et al.,* 2011). Briefly, 200 gm fruit powder of Noni was dissolved in 2.0 L hydroethanolic solvent (50% v/v) was left for 48 hrs for maceration at room temperature, while maceration it was intermittently shaken manually and mechanically with help of mechanical shaker. Solvent extractives (macerates) strain through muslin cloth, then filtered through Whatman No. 42 filter paper, the filtrates collected and transfer to Petri plates was air-dried, thus the residue obtained was again dried in hot air oven (43 ^{0}C) to remove completes moistness. Extract (dried residues) was store airtight in desiccators and used as and when required in this study. Similarly, aqueous extract of *A. indica* was obtained, 5 g leaf powder was dissolved in 100 ml double distilled water (5% w/v), boiled for 10 minutes on water bath, then cool. It was diluted fivefold with distilled water was used in ratio 1:9 ratio with AgNO3 aqueous solution for synthesis of SNPs.

Synthesis of Silver Nanoparticles (SNPs)

0.17 g silver nitrate granules (Emplura®, Mercks Specifications Pvt. Ltd) dissolved in 1 L double distilled water (1 mM solution) in a conical flask. Previously prepared aqueous solution of *A. indica* leaf extract (5% w/v) was use in ratio 1:9 by leaf broth (5 ml) and silver nitrate (AgNO3) (45 ml) were mixed, warmed for 10 minutes on water bath (45 ^{0}C), then cool at room temperature. The color changes observed in aqueous solution matrix faint yellow color to brown and later gray color qualitatively confirmed synthesis of SNPs (Panigrahi, 2012).

Coating SNPs with *Morinda citrifolia* Fruit Extract

For synthesis of SNPs, previously prepared colloidal aqueous SNPs solution was used as starting materials in coating procedure, the SNPs coated with Noni fruit extract by pursuing similar procedure was used for synthesis of SNPs, where instead using aqueous silver nitrate salts solution was used hydrocolloidal silver nanoparticles, which was already prepared using A. indica leaf extract. Colloidal SNPs (9 ml) aqueous solution was mixed 9:1 ratio using 1 ml fruit extract aqueous solution of *M. citrifolia* (hydroethanolic extract (6 ml of 5 % w/v). It was mixed while continuously stir solution with magnetic stirrer and shaken intermittently with the mechanical shaker, then, warm at 45^0C on water bath for 15 minutes, cool at room temperature. Apparent change in color brown to clear brown color of coated solution matrix confirms surface coating SNPs with the fruit extract of *M. citrifolia*.

Characterization of SNPs and SNPsMcFE

Further characterization SNPs done by UV/Vis spectrophotometer (Systronics- 118) recording the absorbance (OD values) against water as blank at wavelengths350 to 600 nm, The maximal absorbance peak (SPR) for each samples

obtained by plotting the absorbance (OD) against light path (wavelengths). These test samples were morphometrically characterized by Scanning Electron Microscope (Model: JOEL-JSM 5600) and TEM images (Hitachi H-7500) at specified magnifications according to procedures described by Bozzola and Russell (1998) The shape and size of nanoparticles were analyzed, at Ruska Labs, Rajendranagar, Hyderabad (AP), India and obtained SEM and TEM images.

Phytochemical Analysis of Noni fruit Extract:

Qualitative pnytochemical analysis of test hydroethanolic extract of *Morinda citrifolia* fruit was done by employing standard phytochemical tests protocols as suggested by Harbourn (1976 and1998) Trease and Evans (2001). Results shown in (Table 9) and interpreted based on color development and intensity of color or ppts or ring formation was graded as (-) no change or negative, (+) poor, (++) good, (+++) moderate and (++++) intense.

Experimental Animals:

One-day-old, 120 broiler chicks, ven cobb 400 strain were procured from M/s, Kulswamini Poultry Farm Pvt. Ltd, Parbhani (Maharashtra). They were maintained under standard management conditions on deep litter system were provided brooding for first seven days of age, thereafter divided into 6 different groups of 20 chicks in each group.

Experimental Grouping and Treatments

Total 120, day old broiler chicks were assigned into six different groups, of 20 chicks in each group. All infected groups were received treatments through drinking water for 28 days, except in group T3 was treated with amprolium, a reference standard drug for initial 8 days. Coccidiosis experimentally induced in Group T2 to T6 by oral gavages *E. tenella* oocysts @ $20x10^3$ on 21st day of age (day of start experiment). Group T1 serves as healthy control. Group T2 was infection control. Group T3 was postive control treated with amprolium sulfate @ 125 mg/L through water for eight post infection days. Group T4 was given SNPs treatment @ 15 mg/L. Group T5 treated with SNPsMcFE @ 15 mg/L. Group T6, treated with fruit extract *M. citrifolia* at 300 ppm. All chicks were maintained on uniform managemental conditions on deep litter system were provided rice husk as bedding materials, and offered mesh type *ad-lib* diet, specially prepared free from coccidiostat drugs, but is was procured from commercial source. Diets provided daily at once at morning hours according to stages of growth viz. pre starter (first 2 weeks of age), starter (2-4 weeks of age) and finisher (4 weeks onwards). All birds in all groups allowed free access to drinking water. Other routine vaccination and managemental was provided, except the first vaccination against Marek's disease, only schedule treatments given and no any other medications were provided. For individual identifications and differentiations of birds among replicates aluminum leg bands (National Band & Tag Co.) encoded with different serial numbers used.

Procurement and Propagation of *E. tenella* Oocysts

Pure clone culture of *Eimeria tenella* oocytes procured from Research Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, U.P. (India). In pilot experiment virulence of test culture was ensured for infectivity (virulence) conducted on five, 14-day-old broiler chicks, gavages at 20 x 10^3 per chick at 0.5 ml saline solution directly into the crop. The infective dose was prepared and titrated before administration using Mc Master counting method. The required doses after confirmation of infection were prepared. Apparent signs of illness observed were dullness, depression, hemorrhagic diarrhea with mucus shreds in challenged chicks. Semisolid drops from infected chicks were collected in 2.5% potassium dichromate, incubate for 48 hrs later examined under low (10x) and high (40x) power object were typically showed the presence of oocytes and schizonts.

Dose Titration for Oocytes:

The infective dose was calculated using following formula to induce *E. tenella* infection as per procedure of Mc Master counting method was described by Conway and McKenzie (2007).

$$\text{OPG count} = \frac{N}{0.15} \times \text{volume} \times 0.1$$

Where, N = number of oocysts counted, 0.15 = volume of the McMaster counting chamber, Volume = 100 ml of water in which fecal materials (litter) was soaked, and 0.1 = correction factor for 10 g of litter material originally taken.

$$\text{Oocytes dose per bird} = \frac{\text{Number of oocytes per gram}}{\text{No of birds in pen}}$$

Experimental induction of infection

Experimental coccidiosis was induced by oral garages of sporulated oocysts of *E. tenella* on 21st day of age, all birds in group T2 to T6 were depressed up to 24 hrs following oral infection of oocysts triturated in volume 1 ml in normal saline, equivalent dose of saline water also offered to uninfected untreated control group. The fecal samples were retrieved On after 5th and 6th day PI were sporulated in 2.5 % potassium dichromate and harvested the oocytes, the infective dose (@ 20 x 10^3 oocysts per chick) was prepared in volume 1.0 ml per to all chick in normal saline used to induce further infection to all birds of experimental groups (Group II to VI). Feed was restricted to all birds for overnight before challenge and after 30 minutes after challenge in each group. Infection dose was given at morning hours before allowing feeds or water.

Sample collection and intervals

Fecal samples were obtained from individual chick were collected a fresh in plastic container on 6th, 7th and 8th day of post infection (i.e. 27th 28th and 29th of age), total 144 samples were from eight randomly selected birds of each group were preserved in 2.5% potassium dichromate solution and kept under refrigeration (4-5 ^{0}C) for 7 days. Likewise, the fecal oocytes shedding and OPG counts were determined.

Performance

Performance parameters such as body weight, weight gain and FCR were determined were recorded daily within each group, feed conversion ratio was calculated taking ratio of amount of

feed consumed (intake) to the actual weight gain in same birds. All birds were observed for clinical signs and symptoms of coccidiosis, on 4th, 6th, 7th, and 8th days of post infection, also noted behavioral changes if any in all groups, The mortality among all experimental groups recorded daily throughout experiment period, if any.

OPG Count

Fecal oocytes from fecal samples were recovered by flotation technique (Pellerdy (1974), wet slides microscopically examined and characterized the oocysts on morphology, basis was identified and characterized, hence OPG count determined by Mc Master Chamber method as described by Conway and McKenzie (2007), and expressed in hundred numbers. Total 144, faecal samples over a period, each of 24 h in groups directly collected from 8 birds of each group on 6th, 7th and 8th day of post infection. It was placed in separate airtight plastic container and thoroughly homogenised with a domestic mixer, then kept refrigerated until assess oocyst counts. The homogenized samples ten-fold diluted with tap water, further diluted with saturated NaCl solution at 1:10 ratio was used for oocyst count by McMaster chamber method, and presented the oocyst counts as the numbers of oocysts per bird.

Lesion Score

To assess severity of coccidial infection, total eight birds from each group were randomly selected and sacrificed on 9th day post infection (Singh, 2009), gross abnormalities were examined and recorded along with caecal lesion score for eight chicks in each group. Total 48 chickens were sampled from six different groups were bleed for lesions score on 9th days of post infection according to technique by Johnson and Reid (1990). Lesions score assigned 0 to 4, where 0 score was corresponding to normal status of bird with no gross lesions, Score 1, showed small scattered petechiae, score 2 was designated for numerous petechiae, score 3 is indicative the extensive hemorrhage and the score 4 was assign for extensive hemorrhage, dark colour to caecal intestine and to the dead birds.

Statistical analysis

Data on various parameters was subjected to analysis of variance (ANOVA) as per the method suggested by Panse and Sukhatme (1967) using FRBD or CRD and interpreted for statistical significance of treatment mean, effects were disclosed at a probability level of $P < 0.05$ in order to determine the statistical differences between means.

RESULTS AND DISCUSSION

Plant Extraction

Noni fruit powder (moderate size) extractability in hydroethanolic solvent (50% v/v) was 27.93 %. Which was higher than earlier reported extractability of noni fruit powder in organic solvents (Nayak and Mengi, 2010), in similar experiment Mandukhail *et al.,* (2010) was reported 20 % extractability of noni fruit powder in 70 % ethanolic solvent and similar extractability of noni fruit powder in 95% ethanol solvent showed in other study by Haque and Rao (2013). These reports are in close approximation with our results, however differences in extractability as reported in our study might be due differences in extraction methods, type of solvents and materials were used in extraction process.

Phytochemical Analysis

The phytochemical tests (qualitative) revealed presence of alkaloids, carbohydrates, flavonoids, glycosides, antraquinone, phenols, proteins, amino acids, saponnins, steroids, tannins, terpenoids (diterpenoid and triterpenoid), emodin, coumarine and quinone compounds in the hydroethanolic fruit extract of *M. citrifolia* (Table 9). The presence of these constituents in the Noni fruit extract found our study is clearly indicated quantum of phytochemicals in Noni fruit extract positively, where presence of several constituents in fruit extract was reported in several earlier studies. The presence of saponins, triterpens, steroids, cardiac glycosides (Sharma and Arya (2011), flavonoids, glycosides, proteins, saponnins, (Rao *et al.,* 2014) coumarine (Jain *et al.,* 2012) streroids (Assi at al. (2015) phenols, tannins, alkaloids, terpenoids, reducing sugar carbohydrates (Assi at al. (2015) in hydroethanolic extract of Noni fruit powder reported. Nayak and Mengi (2010) showed the presence of several phytochemical compounds in hydroalcoholic fruit extract of Noni. The medicinal properties and therapeutic uses of different consents of Noni have been reported in other studies; Chung *et al.,* (1998) showed tannins as antimicrobial compounds and also shown antioxidant activity (Rievere *et al.,* 2009). Terpenoids and tannins as anticancer agent (Auginaldo *et al.,* 2005) and tannins as astringent Okwu and Josiah (2006) reduce inflammation of mucous membrane and useful in faster wound healing. Glycosides as blood pressure lowering agent and the cardiac glycosides as in congestive heart failure and cardiac arrhythmiasacts. The phenolic compounds released from glycosides are toxic to microbes reported to possess the antiapoptic, antiaging, anticarcinogen, anti-inflammatory, antiatherosclerosis properties were useful in cardiac protection and improving endothelial functions. Tannins as antibacterial explain its activity by binding to prolin rich proteins in pathogens interfering protein synthesis also act as potent inhibitors of hydrolytic enzymes. Flavoniods glycosides and tannins found in seeds with variable concentrations revealed antioxidant, antimicrobial, anti-inflammatory, antiallergic, anticancer, antineoplastic activities is valuable in intestinal disorders. Sum up different properties Noni fruit extract observed in our study with potent anticoccidal activity might be due these constituents in positive therapeutic outcome against *E. tenella* induced coccidiosis in broiler chickens.

Silver Nanoparticle:

Preliminary synthesis of SNPs using the leaf extract of *A. indica* and silver nitrate solution (1mM) revealed change in color from faint yellow to brown and later gray color (Fig 3 and 4). Similar observations are reported in earlier studies (Banerjee *et al.,* (2014), however development of gray color subsequently after 2 hours of reaction time is not reported so far, hence it could be the first report observed in present study. It could possibly due greater sizes of SNPs (83 nm to 200 nm) is evident by EM and SEM (Fig 5a, 5b and 6a, 6b) analysis. The changes in color of aqueous solution were qualitatively confirmed synthesis of SNPs. Absorbance spectra (SPR) maximal at 430, 435 nm (light path) for SNPs and SNPsCMcFE against 3.012 and 3.683 OD values respectively, this was further confirmation for synthesis of SNPs (alone and coating SNPs with fruit extract of *M. citrifolia*).

Table1. Signs and symptoms of *E. tenella* infection in five different infected groups of broiler chickens (21-day age) treated with SNPs and associated treatments

Post Infection Days	Group	Signs and symptoms	Severity
4th	T2	Reduced feed and water intake, lack of activity. ruffled feathers, blood mixed semisolid feaces, mucus in blood	++++
	T3	Reduced feed and water intake, lack of activity. ruffled feathers, semisolid feaces mixed with blood and mucus	++++
	T4	Reduced feed and water intake, lack of activity. ruffled feathers, semisolid feaces mixed with blood and mucus	++++
	T5	Reduced feed and water intake, lack of activity. ruffled feathers, semisolid feaces mixed with blood and mucus in blood	++++
	T6	Reduced feed and water intake, lack of activity. ruffled feathers, semisolid feaces with blood mixed and mucus	++++
5th and 6th	T2	Reduced feed and water intake, lack of activity. ruffled feathers, semisolid feaces mixed with blood, birds were anemic.	+++++
	T3	Reduced feed and water intake, lack of activity. ruffled feathers, blood mixed semisolid feaces and mucus in blood	+++
	T4	Reduced feed and water intake, lack of activity. ruffled feathers, blood mixed mixed semisolid feaces	+++
	T5	Reduced feed and water intake, lack of activity. ruffled feathers, blood mixed semisolid feaces	+++
	T6	Reduced feed and water intake, lack of activity. ruffled feathers, blood mixed semisolid feaces	+++
7th	T2	Reduced feed and water intake, inactive, ruffled feathers, blood mixed semisolid feaces, birds were anemic	++++
	T3	Reduced feed and water intake, lack of activity. ruffled feathers, blood mixed semisolid feaces	+++
	T4	Reduce feed and water intake, activity, ruffled feathers, semisolid feaces, birds anemic	++
	T5	Reduce feed and water intake, birds inactive, ruffled feathers, semi-solid feaces, anemic birds	++
	T6	Reduced feed and water intake, bird inactivity. ruffled feathers, semisolid feaces	+

Note: Less sever (+), Sever (++), Moderate (+++) and Intense (++++) are denotes severity of signs and symptoms of caecal coccidiosis:

Table2. Effect of Silver nanoparticles coated with *Morinda citrifolia* fruit extract on performance following low dose infection of *E. tenella* in broiler chicks (Ven cob 400) (21-day age).

Group	Weekly Body weight (g) (Mean ± SE)				
	Initial (14st age)	21st Day	28th Day	35st Day	42th Day
T1	304.4b	570.8c	883.25	1220.85a	1566.4a
	±28.84	±35.5	±28.525	±32.965	±32.4
T2	318.1ab	594.6bc	895.4	1076.55d	1367.7c
	±13.19	±19.32	±15.66	±31.995	±49.66
T3	317.8ab	598.5ab	898.7	1190.6b	1540.75a
	±12.22	±23	±28.15	±37.7	±43.15
T4	321.2a	604.3a	906.25	1125.35c	1476.15b
	±15.44	±21.7	±36.8	±35.62	±71.57
T5	330.65a	586.6abc	891.85	1181.5b	1489.95b
	±9.155	±40.18	±34.58	±36.25	±61.05
T6	330.95a	576.4bc	897.2	1233.05a	1579.35a
	±12.27	±42.42	±43.54	±43.26	±42.255
Stat	S	S	NS	S	S
CD±SE	14.764	14.102	0.00	29.196	40.202±
	±5.263	±8.592	±9.464	±10.408	14.331
CV	7.343	6.529	4.727	3.974	4.263

(n=20)Superscripts with similar denominators are not significantly different at 5% levels.

Table 3. Mean Values ± SE of weekly weight gain in different treatment groups of broiler chickens infected with E. *tenella* oocytes (20x10^3 per bird)

Group	Weekly weight Gain (g) (Mean ± SE)			
	21st Day	28th Day	35st Day	42th Day
T1	266.40	312.45	337.60	345.55
	±40.04	±19.54	±13.66	±10.275
T2	276.50	300.80	181.15	291.15
	±20.7	±13.5	±35.05	±35.85
T3	280.70	300.20	291.90	350.15
	±27.36	±10.88	±46.19	±41.05
T4	283.10	301.95	219.10	350.80
	±25.18	±26.065	±54.09	±67.86
T5	255.95	305.25	289.65	308.45
	±41.06	±20.075	±60.82	±68.25
T6	245.45	320.80	335.85	346.30
	±43.16	±27.68	±2.68	±50.74
Stat	NS	NS	S	S
CD±SE	NS ± 6.879	NS ± 6.087	33.342 ± 11.885	42.143 ± 15.023
CV	16.684	8.870	19.267	20.232

(n=20

Ultramorphometric characterization of SNPs (alone and coated with fruit extract) by TEM and SEM analysis were showed mostly the spherical and triangular, a hexahydral shapes of nanoparticles varied 80 nm to 200 nm sizes. The color changes in aqueous colloidal solution during synthesis of SNPs with *A. indica* leaf extract as observed in our study is reported by Nivedhitha *et al.,* (2015) and several other researchers in their studies (). The particles size of SNPs in our study are corroborates with report of Firdhouse and Lalitha (2015) showed spherical shape and size 50-200 nm had SPR maxima at 427 nm, similarly other study shown 43 nm size SNPs were prepared using *A. indica* leaf. There are reports showed synthesis of SNPs using leaf extract of *A. inica* produced 200 nm size particles with varying shapes likes triangular, pentagons, hexagons, predominantly the spherical shapes, while color changes of aqueous colloidal solution matrix from faint light yellow to yellowish brown, later reddish-brown and finally brown. According to this study maximal absorption (SPR) of SNPs was between 425 to 475 nm (Banerjee *et al.,* (2014) and showed synthesis of SNPs was due to reduction of silver ions by *A. indica* leaf extract within first 15 minutes and thereafter particles were stable. In similar study Rudra *et al.,* (2014) demonstrated synthesis of SNPs by ethanolic fruit extract of *M. citrifolia* , where various functional groups of compounds of Noni fruit extract involved in reduction of silver ions to silver nanoparticles were uniform spherical in shapes. Reidy *et al.,* (2013) review different shapes of coated SNPs with citrate, polymer, peptides, sugar and mentioned during synthesis and coating the plant extracts with SNPs showed 100 nm size particles that influenced SPR depending on size of particles. Siromani and Daniel (2011) reported silver ions coated with hydrophilic polymers or biocompatible molecules were interacted with aldehyde group of substrates in aqueous solution and produced stable dispersion of SNP, and when interact with hydroxyl group were formed molecular colloidal matrix. These reports clearly showed plant extracts are useful in synthesis and coating SNPs, where silver ions produce stable dispersion and colloidal solution of SNPs in aqueous medium.

General Parameters

Table 1. depicts signs and symptoms of coccidisosis in broiler chickens were infected with *E. tenella*. There was significant improvement in clinical signs and symptoms on 7th PI days by treatment of fruit extract of *Morinda citrifolia* (group T6), followed by SNPsMcFE, and SNPs (group T5 and T4) than amrpolium sulfate (T3) as compared to uninfected untreated control (T2). On the 5th and 6th PI days moderate improvement was noted and on day 4th there was no improvement in all treated groups, significantly declined signs and symptoms and improved condition observed in group T6, followed by

SNPsMcFE and SNPs on 7th day PI. Similar clinical signs and symptoms shown in Mediterranean poultry infected with *Eimeria tenella* in earlier studies (Mallia, 1999; Pankiewicz and Leska, 2011), In other study shown significant recovery and improvement in coccidian infected birds when treated with amprolium (Maurice Pitesky, 2012). Thangarasu Muthamil selvan, *et al.,*(2016) in his review mentioned that the saponin compounds presents in several plants have shown anticoccidal activity by binding to sterol molecules causing disruption of cell membrane integrity leading parasitic death. Presence of saponins was also found in fruit extract of Noni in our study might be responsible in clinical recovery of coccidiosis in treated birds, Chauke and Siebrits (2012) showed non-significant effect of SNPs in chickens infected with *Eimeria tenella*. These reports are clear indications and are in consonance with earlier studies for moderate efficacy of SNPs and SNPsMcFE, however potent activity of McFE in improving clinical signs and symptoms of coccidiosis was noted in this study is additional findings not reported so far in previous studies. However, very few studies are reported the antcoccidial activity of Noni fruit in poultry and no detail reports available so far on this plant.

Body weight

Average weekly body weights of six different groups of broiler birds are presents in Table 2. The body weight in group T1, T2, T3, T4 T5, T6 on day 0, 21st, 28th, 35th, 42nd age raged between 304.4 to 1566.4, 570.8 to 604.3, 883.25 to 906.25, 1076.55 to 1233.05, 1367.7 to 1579.35 g respectively. The body weight was significant different before and after infection on respective interval days and found to be significant within the groups and between the treatments intervals. Progressive increase in body weight during pre- infection period in all groups was indicates normal physiological growth and reduced body weight after post infection days was highly significant in birds of group T2 (infected untreated group). Body weight among all groups in order of treatments were T6>T3>T1>T5>T4>T2, suggesting the highest body weight in *M. citrifolia* (group T6), the SNPs (alone and coated) in group T4 and T5 significantly ($P<0.05$) reduced body weight was found lower than amprolium. The lowest body weight observed in-group T2, was the infected and untreated control. In our study SNPs found to reduced body weight these findings are contradicted with the report of Fondevila, *et al.*(2009) who showed polydispersed mixture of SNPs supplemented of size 60 to 100 nm up to 40 ppm level through diet for 14 days in pigs were decrease intestinal coliforms and responsible to increase weight gain in pig. Other studies have showed no effect SNPs on growth of chickens when administered in feed (Ahmadi, 2009) and or either no effects or negative effect on performance (decreased body weight, feed intake) when give through water (Ahmadi and Kurdestany, 2010; Ahmadi and Rahimi, 2011). Bhol and Schechter (2007) reported SNPs treatment in rats was decreased inflammation and gut inflammatory cytokines thus improved gut condition, which is contrasting to toxicity studies in rodents, that showed elevated serum levels of procytokines and anti-inflammatory cytokines (Park *et al.,* 2011), since the serological cytokine levels is quite variable and may not reflect local activity in gut. There was also controversy reports showed species difference and several other factors. In current study, reduced body weight due to SNPs could be possible because it affected the intestinal microbiota and suppressed feed intake in exposed birds, however fruit extract of *M. citrifolia* showed positive impact on body weight and also favors to increase body weight in SNPs and SNPsMcFE treated birds compared to infected untreated control (group T2).

Weight Gain

Average weekly body weight gains of six different groups of birds are presents in Table 3. The weight gains on day 21st, 28th, 35th and 42nd of age in the group T1, T2, T3, T4 T5 and T6 raged between 255 to 280.70, 300.80 to 320.80, 181.15 to 337.60 and 291.15 to 350.80 g respectively. In order of treatments T1>T6>T3>T5>T4>T2 and interval days (age) on 28th >42nd >21st >35th indicates the highest weight gain in-group T6 and lowest in T2 was on 28th day of age and lowest on 35th of age. The highest gain in T6 treated with M. citrifolia fruit extract, and lowest in group T4 treated with SNPs, moderate gains was observed in SNPsMcFE which was low than amprolium. Among all treatments, fruit extract of *M. citrifolia* was found the best in improving weigh gain in these birds. Decreased weight gain in group T2 (infected and untreated birds) due to *E. tenell* infection can be linked to increased caecal lesion scores (Conway *et al.,* 1990) is in agreement with previous study (Gergiev, *et al.,* 2006). The improved condition of birds by decreasing gut inflammatory cytokines (Bhol and Schechter, 2007) consequently decrease inflammation and improved the feed consumption and weight gain. The increased weight gain by SNPs also reported in pig due to decrease intestinal coliforms (Fondevila, *et al.*2009) corroborating moderate gain as observed in our study. Vadalasetty (2012) showed no significant difference in weight gain in broiler birds infected with *C. jejunii* was treated with 50 ppm SNPs. The lowest gain noted in group T5 (SNPsMcFE) in current study accordance to the reports of Chauke and Siebrits (2012) showed 15-ppm SNPs given during 13-27 days through water not affected weight gain in broiler chicks were infected with *E tenealla*. Dose dependent anticoccidial effects of many polyherbal formulations have been reported to improve the performance against *E.tenella* induced coccidiosis in broiler chicks and showed comparable anticoccidial efficacy of amproliumin (Zaman, *et al.,* 2012; Muhamad *et al.,* 2012, ShafiqUllah, 2013) in other study. The increased body weight gain observed in present context by *Morinda citrifolia* fruit extract consonance to the report of (Ola-Fadunsin and Ademola, 2014), who reported acetone leaf extract of *Morinda lucida given* in broiler chickens infected with Eimeria species was increased weight gain. Similarly reported the absence of caecal lesions in birds when treated *Morinda citrifolia* extract in other study, these earlier reports could be correlates with our observations, possibly the enhanced immune status and improved condition of treated birds may increased the weight gain.

Feed Intake

The feed intake in the group T1, T2, T3, T4, T5 and T6 during first two weeks (pre infection period) were range between 32.19 to 37.69 and 60.51 to 66.63 and post infection 73.32 to 102.68 and 76.96 to 125.35 g respectively (Table 4). Significant difference in the feed intake observed before and after infection was statistically significant when compared to control group. This indicates normal physiological growth progressively increased feed requirement according to stage of growth of birds, there was increased feed intake on second week and reduced during third week (35th day of age) in infected untreated control group T2 (73.22 g) compared to uninfected untreated control group T1 (102.68 g).

Table 4. Mean values ± SE of feed Intake (g/bird/day) in different treatment groups of broiler chickens (21-day age) after infection of *E. tenella* oocytes (20x10^3 per chicken) (n=20)

Group	Feed Intake (g/bird/day) (Mean ± SE)				Average	Stat
	21st Day	28th Day	35st Day	42th Day		
T1	32.19	60.51	102.68	124.68	80.01	Significant at 5 % levels (CD = 6.21, CV=15.872)
	±6.25	±9.87	±14.18	±11.84		
T2	33.06	66.63	73.22	76.96	62.47	
	±3.81	±10.32	±4.01	±5.13		
T3	33.46	65.33	82.05	125.35	76.55	
	±7.39	±10.42	±19.36	±18.14		
T4	34.75	62.15	93.64	120.08	77.66	
	±5.64	±11.70	±8.72	±14.15		
T5	34.06	60.96	86.69	122.06	75.94	
	±4.38	±11.32	±9.51	±16.07		
T6	37.69	61.34	98.81	115.18	78.26	
	±4.64	±10.93	±8.97	±24.37		

Table 5. Mean ± SE values of FCR in different treatment groups of broiler chickens with E. *tenella* oocytes (20x103 per bird) (n=20)

Group	FCR				Average	Stat
	21st Day	28th Day	35st Day	42th Day		
T1	0.85	1.36	2.13	2.53	0.893^{a}	Significant at 5 % levels (CD = 0.128), CV=15.872)
T2	0.84	1.55	2.83	1.85	0.863^{a}	
T3	0.83	1.52	1.97	2.51	0.789^{ab}	
T4	0.86	1.44	2.99	2.40	0.786^{ab}	
T5	0.93	1.40	2.10	2.77	0.730^{b}	
T6	1.07	1.34	2.06	2.33	0.695^{b}	

Note: Similar Superscripts denominators are not significantly different at 5 % levels of significance

Table 6. Mean values ± SE of water Intake (ml/bird/day) in different treatment groups of broiler chickens (49 day old) infected with E. *tenella* (n=20)

Groups	Water intake (ml/chick/day) (Mean ± SE)				Average	Stat
	21st Day	28th Day	35st Day	42th Day	171.311	Not Significant at 5 % levels (CD = 14.697)
T1	77.77	148.03	205.87	253.57	165.621	
	±3.21	±5.24	±5.91	±11.14		
T2	76.93	137.43	195.76	252.37	174.504	
	±3.07	±3.80	±7.27	±4.81		
T3	88.20	149.40	200.49	259.93	170.504	
	±4.06	±3.51	±6.46	±2.89		
T4	92.30	144.63	186.40	258.69	164.796	
	±3.15	±4.31	±8.15	±5.92		
T5	92.00	135.80	182.49	248.90	173.868	
	±3.00	±4.14	±8.70	±2.93		
T6	89.40	141.07	181.43	283.57	171.311	
	±2.95	±3.44	±8.45	±12.58		

Table 7. Fecal OPG count in different treatment groups of broiler chickens (vencobb 400) infected with *E. tenella* (n=8)

Group	OPG Count (Numbers)			Total Fecal Score (Numbers)	Average OPG
	Post infection days				
	6th	7th	8th		
T1	0.0^{b}	0.0^{b}	0.0^{b}	0.0	0.0
T2	62.5^{a}	175^{a}	37.5^{ab}	275 (100%)	13.75 (100%)
T3	37.5^{ab}	62.5^{b}	12.5^{a}	113 (41.09%)	5.65 (41.09%)
T4	62.5^{a}	25^{b}	0^{b}	87.5 (31.42%)	4.38 (31.85%)
T5	37.5^{ab}	37.5^{b}	25^{ab}	100 (36.36%)	5 (36.36%)
T6	0.0^{b}	0.0^{b}	0.0^{b}	0.0	0.0

Different superscripts denominations are significantly differs at P< 0.05 levels of significance.

Table 8. Effect of SNPs (alone and coated with McFE) on average caecal lesions score at against *E. tenella* infection in WLH broiler chickens at 32-day age

Group	Average Caecal Lesions (Mean ± SE) on 9th day PI			Lesion Score at 0 to 4 scale
	Treatments	Dose (mg/L)	Lesions	
T1	Healthy control	---	0^{c}±0.0	0
T2	Infection control	---	2.375^{a}±0.6	+2
T3	Infection + Amprolium	125	0.5^{c}±0.6	+1
T4	Infection + SNPs	15	1.5^{b}±0.7	+1
T5	Infection + SNPsMcFE	15	0.375^{c}±0.4	+1
T6	Infection + McFE	300	0^{c}±0.0	0
Stat	---	---	S	---
CV= 77.352	Significant at 5% (CD=0.618),			

Note:S=Significant, SNPs = Silver Nanoparticles, SNPsMcFE= SNPs coated with *Morinda citrifolia* fruit extract, McFE= *Morinda citrifolia* Fruit Extract

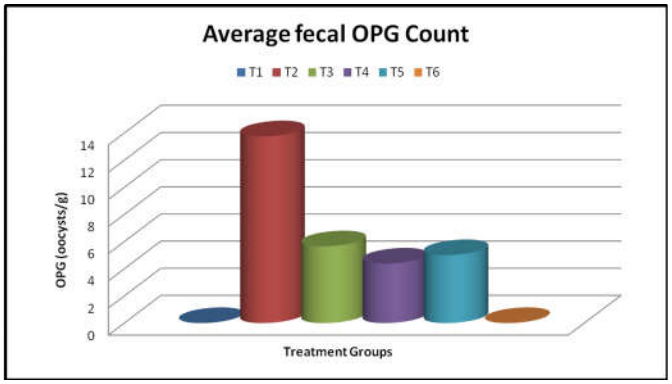


Figure 1. Average OPG count following treatment of SNPsMcFE compared to different treatments against *E. tenella* infection (20x103 oocysts per chick) in 21 day of broiler chickens (6th , 7th and 8th PI days)

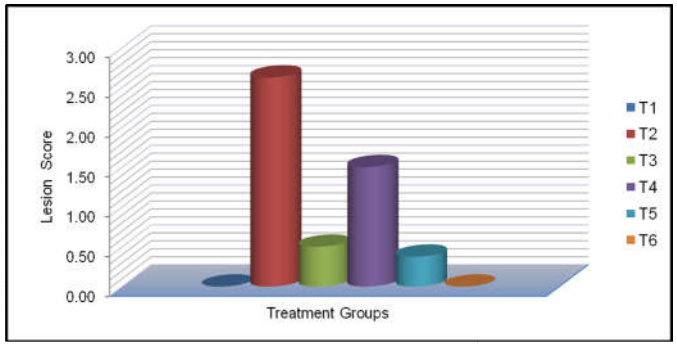
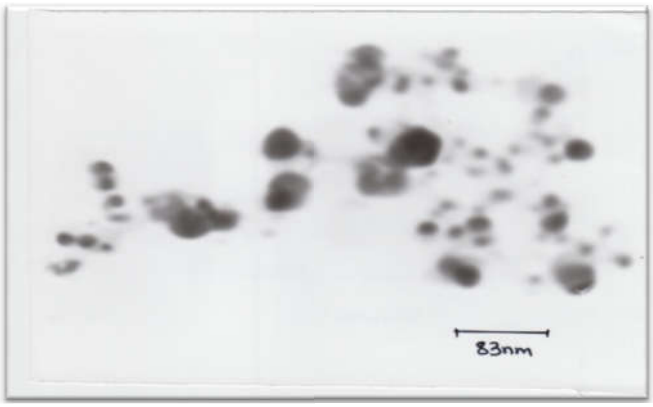


Fig. 2. SNPsMcFE on caecal lesion score against *E. tenella* infection in broiler chickens (vencobb 400) on 9th day PI at age 30 day compare with control

Fig.3. Shows change in color patterns in aqueous collodidal solution matrix during synthesis of SNPs and SNPsMcFE at different reaction times:
A: Aqueous solution of AgNo3 shows no color; B: Leaf extract *A. indica* indicates faint Yellow color; C: Colloidal aqueous solution of SNPs shows Brown color, indicating synthesis of SNPs; D: Aqueous solution of hydroethanolic fruit extract of M. citrifolia shows dark brown color and E: Aqueous colloidal solution of SNPs coated with fruit extract (SNPs McFE) shows brown color

During fourth week on 42nd day age, the feed intake in infected and untreated group T2 was significantly reduced (76.96 g) compared to uninfected and untreated birds in group T1 (124.68 g). Overall, in all infected and treated group the feed intake improved significantly and found higher in birds were treated with fruit extract of *Morinda citrifolia* (group T6). Lowest gain found in T5 group of birds were treated with SNPsMcFE. In all infected and treated groups, the feed intake were not restored as to that healthy control during post infection two weeks period and improved in groups treated

Fig. 4a. Aqueous colloidal solution of SNPs shows slight faint brownish yellow color after 30 minutes of reaction time 4 b. After 2 hours of reaction time while magnetic stirring, color of colloidal solution changed to gray



Fig, 5a. Shows Spherical SNPs of size 100 nM, (TEM at magnification 19300X, 19bar)

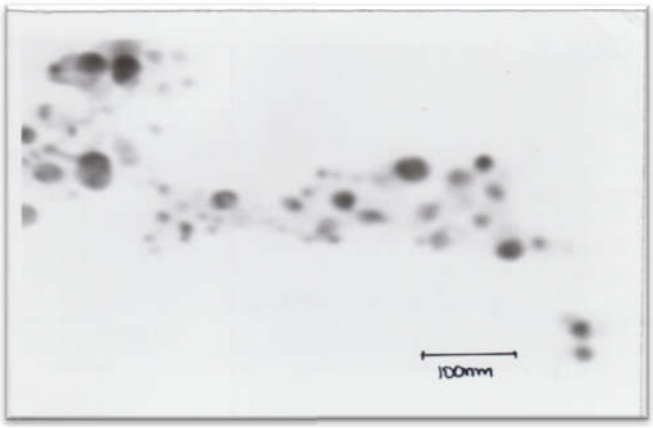


Fig. 5b. Shows mostly spherical particles of SNPsCMcFE, size 100 nM, (TEM, 19300X, 19 bar)

with SNPs and SNPsMcFE were significantly higher than fruit extract treated bird and found to be lower with the amprolium. Significantly increased feed intake observed in our study in group T6 might be due improved caecal lesion scores and enhanced immune status in treated birds, since, caecal region is reported as prime sites for immune response in birds. FAO (2008) mentioned growth-promoting effects of several medicinal plants including Noni improve performance and increased disease resistance in local chickens. Amongst all, Noni fruit extract was to be superior than other in improving feed intake observed in chickens infected with *E. tenella* were concurrently treated fruit extract of M. citrifolia. The wound healing effect and poor digestion following treatment of

Table 9. Phytochemical Analysis of hydroethanolic fruit extract of M. citrifolia

Active Principles	Test Applied	Results
Alkaloids	Dragendroffs	++++
	Hager	++++
	Iodine	-
	Mayer	++++
	Wagner	++++
Amino acids	Ninhydrine	++++
	Xanthoprotein	++
Carbohydrates	Benedict	++++
	Fehling	++++
	Molisch	++++
Flavonoids	NaOH test	+++
	Lead acetate	+++
	NH4 test	++++
Glycosides	Borntragers	++++
	Con H2SO4	++++
	Glycoside	++++
Glycosides	Keller-Killani	++++
(Cardiac)	Mollish	++++
Antraquinones	Antraquinone	+++
	Anthocyanins	+++
	Coumarine	++++
	Emodine	++++
	Quinone	++++
Phenols	Ellagic acid	++++
	Ferric chloride	++++
	Phenol	++++
Protein	Biuret	++++
Saponins	Foam	++++
	Froth	++++
Steroids	Liberman-Burchardss	++++
	Salkowski's	++++
Tannins	Salkowski	++++
	Ferric Chloride	+++
	Lead acetate	++++
Terpenoids	Acetic Anhydride	++++
Diterpenoids	Cu-acetate	++++
Tritepenoids	Liberman-Burchardss	++++

Noni fruit extract is reported by Rudra, *et al.,* (2014), antimicrobial effect of saponins constituent of the Noni fruit extract shown to binds with sterol molecules disrupts cell membrane and lysed oocysts of coccdial parasites, thus suppress the coccidial infection in chickens (Thangarasu Muthamilselvan, *et al.,* 2016). Pineda (2011). In other study showed the provision of nano silver in broilers was not affected feed intake. The reduced feed intake observed in our study with SNPs (alone and coated with McFE) could possibly attributed to larger size particles size and the oxidative damages by SNPs to the gastro intestinal mucosa leading to decreased absorption and utilization of feed, thus reduced the feed consumption.

Feed Conversion Ratio (FCR)

Average weekly FCR of birds in six different groups illustrates in Table 5. FCR of broiler birds in group T1, T2, T3, T4, T5, T6 on 21st, 28th, 35th, 42nd day age varies between 0.70 to 0.88, 0.87 to 2.47, 1.26 to 1.66, 1.98 to 2.96, 1.92 to 3.27, 2.43 to 3.74 respectively. In order of treatments FCR were T6>T5>T4>T3>T2>T1 and in order of interval days 35th >21st >42nd >28th, indicates the highest FCR in group T6 treated with *M. citrifolia* fruit extract and the lowest in amprolium treated group as compared to infected untreated control. However, in our study treatment of SNPs and SNPsMcFE revealed moderate FCR in treated birds compared to infected and untreated control (group T2). This finding was similar to earlier study that showed during starter phase reduced FCR

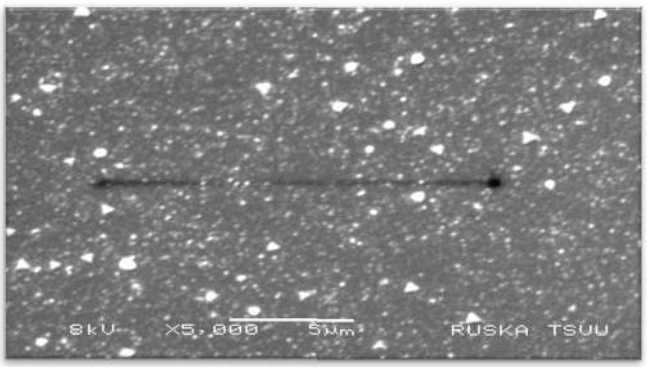


Fig. 6a. Shows different shape of SNPs mostly the spherical and triangular, hexahedral, cuboidal , size scale 5 µm (SEM, 5,000X, 8KV, scale)

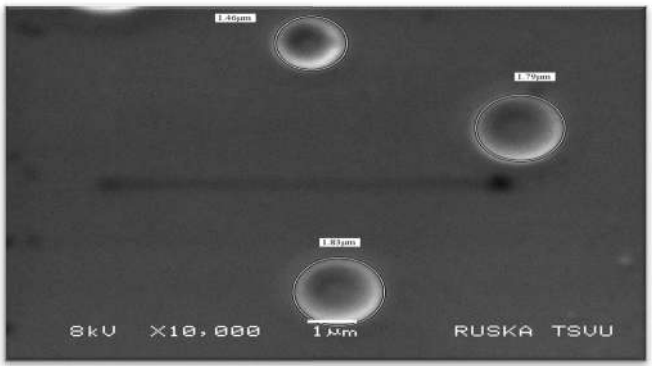


Fig. 6b. Shows spherical particles of SNPs coated with McFE, average size 1.93 µm, SEM, 5000 X, 8KV)

(better) and increased at the finisher phase in broiler chicks (Odunsi, *et al.,* (1999), Broiler chicks infected with *E. tenella* treated with SNPs reported to increase FCR (Jatau, *et al.,* (2014). The increased FCR observed in our study by SNPs (alone and coated with McFE) compared to infected untreated control is contradicting to the report of Ahmadi and Rahimi (2010) who did not observed significant effect of SNPs on FCR in broiler chicks were supplemented through diet. Similarly, reported the non-significant effect of nanosilver on growth of chicken embryos (Sawosa *et al.,* 2007). Differences in FCR observed in our study might be due to mode and level of SNPs of supplementation and the level of infection in treated birds where FCR was increased (reduce feed efficacy), the increased in FCR on second week PI in all groups of infected and untreated birds could be due to improved condition and recovery.

Water Intake

Table 6 shows average daily water intake of six different groups of broiler birds. Average water intake in group T1, T2, T3, T4, T5 and T6 on day 21st, 28th, 35th and 42nd of age were range between 77.77 to 253.57, 76.93 to 252.37, 88.20 to 259.93, 92.30 to 258.69, 92.0 to 2.48.90 and 89.40 to 283.57 ml per bird per day respectively. In order of treatments it was found to be T3>T6>T4>T1>T2>T5, and between interval days were 42nd >35th >28th >21st, indicating the highest water intake in group T3 in birds treated with amprolium sulfate. Lowest water intake with SNPsMcFE in group T5, which was significantly lower than healthy control (T1) and infected untreated control (T2). However, in group T6 and T4 treated with McFE and SNPs revealed moderate increase in water intake. The reduced water intake in group T2 observed in our study in accordance with earlier report of Williams (1996)

who showed reduced water intake after 4 to 6 days post infection of E. tenella *in chickens*. The reduced body weight phenomenon might be associated with reduced weight gains and poor food conversion ratio, irrespective to whether the birds were healthy or sick, and regardless to the degree of reduction of dietary intake, there was a more or less constant relationship between food and water consumption. Sun (2004) reported no change in water intake in coccidian vaccinated broiler chicks during first 15 to 28 days, birds were maintained on drug free diets. According to study of Reid and Pitois (1965), showed infected birds with caecal coccidiosis was decreased water intake. In similar study, Vadalashetty (2012) showed no significant effect of SNPs on water intake, also by supplemention with 50-ppm levels SNPs in diet of broilers infected *Campylobacter jejunii*. The provision of extract of noni at 7.5% is reported to improve the feed intake, weight gain, feed conversion and alo increased consumption of drinking water in treated broiler chickens (Ahmad and Elfawati, 2007) all these reports are in consonance with our findings.

Oocytes output (OPG count)

Table 7. Shows mean OPG counts of five different groups of ven cobb broiler birds on 6^{th}, 7^{th} and 8^{th} PI days. The oocytes counts (total scores) in group T2 (untreated control), T3 (reference drug amprolium treated), T4 (SNPs treated), T5 (SNPsMcFE treated) and T6 (McFE treated) birds on 6^{th}, 7^{th} and 8^{th} PI days were range between 62.5 to 175 (275), 0 to 62.5 (113), 25 to 37.5(87.5), 100 (36.36) and 0.00 (absent) respectively. The average OPG counts in these group were 13.75, (100%), 5.65 (41.39%), 4.38 (31.85%), 5.00 (36.63%) and 0.0 (No counts) respectively. On 6^{th} PI day, in the group T3 and T5 OPG count reduced than increased counts in groups T2 and T4, however in group T1 and T6 there was no oocytes were observed, which indicates that on 6^{th} day PI, SNPs treatments did not influenced OPG counts. OPG on this day significantly reduced by amprolium and SNPsMcFE when compared to infected untreated control (T2), and the fruit extract of *M. citrifolia* showed maximal activity with no oocysts counts on 6^{th} day PI day onwards. On 7^{th} day, OPG counts in group T3, T4 and T5 were significantly lowered compared to infected untreated control (T2) and there was absence of oocysts in group T6. On 8^{th} day PI no difference observed OPG count , in group T2 and T5 which was higher than T3 compared to uninfected untreated control (T1), however as usual no oocystes was observed in the group T6, and 9^{th} day onwards all birds in group (T2 to T6) were completely recovered, found healthy throughout experiment period. OPG score average in the group T1, T2, T3, T4, T5 and T6 were 0.0, 13.75. 5.65, 4.38, 5.00. 0.00 expressed in percent OPG counts as 0.00, 100, 41.09, 31.85, 36.36, 0.00 % respectively, suggesting the effectiveness of treatments against infected untreated birds (group T2), where oocysts count was 100%. Based on % OPG score effeteness of different treatments were T6>T4>T5>T3 against T2, that showed highest efficacy of *M. citrifolia* fruit extract and lowest for amprolium, a standard reference drug, whereas it was moderate for both the SNPs (alone and SNPs coated with extract).

Mortality

No mortality was observed in any of experimental group during course of 28 days experiment against low dose infection of *E. tenells* oocystes ($20x10^3$ oocysts) in Ven Cobb 400 broiler birds. After 9^{th} day PI onwards all birds were recovered, alert and active. Chauke and Siebrits (2012) also observed no morality among boiler chicks were challenged at 3.3 x 10^5oocysts dose of *E. tenella* on 21^{st} day of age were treated with SNPs up to 34 days of age. In similar study shown peak infection, clinical signs, caecal lesions and no mortality in broiler birds against coccidian oocysts infection at 20 to 25 x 10^3 per bird (Mondal *et al.,* 2011) these birds were completely recovered after 9^{th} day post infection. Absence of morality among low dose infection observed in our study is affirmative with these reports and clear indication of subtle coccidian infection can induce coccidiosis without mortality (subclinical infection) among exposed birds.

Lesion Score

For lesions scores six birds from each group were randomly sacrificed on 9^{th} day of post infection. Since, the *E. tenella* infection is specifically affects "caeca" is produced lesions in poultry, hence the "caeca" thoroughly examined interiorly the lumen and exteriorly wall of intestine for lesion score. The caeca opened by horizontal incision were examined caecal contents, mucosa revealed mild lesions. No lesions in other areas of gut except the caecal lesions were found were counted for average score and graded (numerical and graded lesion score) were determined (Johnson and Reid, 1990) in all infected and treated birds and compared with uninfected and untreated birds (group T1) is depicted in Table 8. Fig. 2. Average score for caecal lesions in group T2, T3, T4, T5 and T6 were 2.375, 0.5, 1.5, 0.375 and 0.0 respectively. Based on lesion score techniques graded as +2 for infected untreated birds (group T2) and +1 for all infected and treated birds of group T3 to T5. There was no lesions observed in group T6, on the contrarily significant higher lesion score was observed in group T2 when compared with SNPs (group T4), followed by SNPsMcFE (group T5). Significant reduced lesions score noted in amprolium treated birds (group T3) than SNPs, SNPsMcFE was similar to SNPsMcFE (group T5) and McFE (group T6) compares to group T2, where the lesion score was significantly reduced by these treatments, indicating the highest anticoccidial activity of McFE followed by SNPsMcFE, whereas the SNPs treatment was least effective did not improved lesions score. The effectiveness of SNPsMcFE was higher than amprolium, SNPs and lower than McFE, the McFE exhibited highest efficacy (100%) than all other treatments against *E. tenella,* All three treatments, except SNPs were satisfactory but not significantly different. Previous study showed combined treatment of amprolium and vitamin A and K in poultry shown significant recovery against coccidiosis in poultry (Maurice Pitesky, 2012). Comparable anticoccidial efficacy of amprolium with the acetic acid against coccidiosis in improving negative performance against coccidiosis in broilers is reported by Abbas *et al.,* (2011). The reduced lesion score in broiler chicks infected with *E. tenella* after SNPs treatment is reported by Chauke and Siebrits (2012), other studies showed polyherbal formulation and plant treatments in broiler chicks significantly decreased lesion score (Muhammad, *et al.,* 2012; Zaman, *et al.,* 2012; ShafiqUllah, 2013. Oyagbemi and Adejinmi, 2012). According to Karimy, *et al.,* (2013) noni leaf extract water-soluble granules treatment against *E. tenella* infection in broiler chicks was significantly decreased lesion score, where improvement in coccidial lesion score was dose dependent and significant at 300 mg/kg dose of extract, which is corresponding to the dose of McFE used in or study. The effectiveness of *M. citrifolia* fruit extract observed

in our study may be attributable to immunomodulatory and antioxidant properties. Several antioxidants, immunomodulatory compounds like iridoide, polysaccharides fractions (Hirazumi and Furusawa, 1999; Deng *et al.,* 2010) and other nutrients like amino acid, vitamins, minerals, co-enzymes, carbohydrates and alkaloids (Chan Blanco,*et al.,* 2006; Assi,*et al.,* 2015) were identified in the Morinda fruit extract that may directly or indirectly affect overall cell and tissues growths. The active chemical constituents such as flavonoids, saponins fatty acids, polysaccharides tannins reported to act as anticoccidial principles showed a strong anticoccidial activity (Abbas, *et al.,* 2012; Dhama, et al, 2015). These reports concurrence with our observation showed moderate anticoccidial activity of SNPsMcFE and low efficacy of SNPs was due to increased cellular entry and bioaccumulation of particles in tissues (Smith, *et al.,* 2012; Ahmdi and Kordestany, 2011). The enhanced free radical formation is accelerates the damages, whereas internalized particles and the extract coated SNPs produced less ROS and proinflmmatory cytokines, thus reduced inflammation or decreased cell viability was reported (Banerjee, 2010). In our experiment, colloidal SNPs might have reduced reactivity of silver ions, diminished undesirable effects, improves the performance, no mortality, thus affected administration of SNPs affects overall growth and development in treated birds.

Conclusion

Plant-mediated synthesis of SNPs is simple and economical means of nanoparticles production. Both, the SNPs (alone and coated with fruit extract of Noni) were showed moderate anticoccidial efficacy in terms of performance, fecal OPG counts and lesion score against *E. tenella* induced infection in broiler chickens, hence it could be promising anticoccidal agent in poultry. However, their anticoccidial evaluation at moderate and higher level of infection (single or mixed) is warrant with its safety concerns. In addition, the fruit extract of *M. citrifolia* showed potent anticoccidial efficacy could escort in control of caecal coccidiosis with better efficacy in chicken at 300 mg/L (300 ppm) if given in water for 28 days. In conclusion, the SNPs (alone and coate with McFE) can attenuate caecal coccidiosis in broiler chickens, moreover, treatment of McFE (300 ppm) through water found more effective than SNPs was given at 15 ppm levels (size 83 to 100 nm for SNPs and 100 to 200 nm for SNPsMcFE) against "caecal coccidiosis" in the broiler chickens.

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