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Effect of nanosilver as feed additive on performance, blood biochemistry, caecal microbiology and intestinal histomorphology of Vanaraja chicken

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ABSTRACT

A study was conducted to evaluate the effect of nanosilver (NS) as feed additive on performance, blood biochemistry and intestinal histomorphology of Vanaraja chicken. Chicks (180 day old) after one week of age were distributed randomly into five treatment groups of four replicates, each having 9 chicks. Treatment groups consisted of T1 (Control) fed basal diet without any additive and in T2, T3, T4 and T5, NS was added to the diet @ 20, 40, 60 and 80 mg/kg diet, respectively. Cumulative body weight gain also improved significantly with the inclusion of NS with highest gain in T5. There was no difference in the feed consumption among various groups including control. Feed conversion ratio was better in T5 compared to other groups. There was no difference in blood biochemical parameters among groups except for the protein levels which were highest in NS supplemented groups than control. The total viable and coliform counts decreased significantly in the group wherein NS was fed in the diet compared to the control. Villus height in different segments of small intestine improved significantly with the inclusion of NS in the diet with better values in T5 followed by T4. From the present study, it could be concluded that nanosilver up to 80 mg/kg level was effective in improving the growth performance, caecal microbiology and intestinal histomorphology of vanaraja birds.

Keywords: Blood biochemistry, Histomorphology, Nanosilver, Performance, Vanaraja

In Kashmir valley, the demand for poultry meat and eggs increases day by day due to increase in population and influx of immigrants, tourists, etc. To compensate this, researchers are exploring the new trends in poultry rearing techniques and innovations for increasing chicken meat and egg production without compromising the health and welfare of birds. In order to get maximum profitability in a shorter period, various feed additives are used in poultry ration such as antibiotics, phytogenics, etc.

Administration of subtherapeutic antibiotics in poultry has been used widely to increase weight gain, improve feed efficiency and reduce mortality and morbidity in birds (Zeng *et al.* 2015). Although they have been used in poultry for over 50 years, the use of antibiotic growth promoters has now declined due to consumer preferences and regulations resulting from concerns over the development of antibiotic resistance in bacteria (Forgetta *et al.* 2012). As such, the researchers are looking for other safe alternatives.

The potential for nano-materials to be used as supplements, medicines, and probiotics in animal feeding is being studied extensively. The nanomaterials have gained importance in the fields of animal nutrition, health and production because of the fact that due to their higher surface area-to-volume ratio, these particles act better than their bulky counterparts (Abdel-Moneim A-ME *et al.* 2021, 2022). NPs have been shown to increase production performance in animals, improves nutritional quality of poultry meat and egg (Li and Cao 2013). Different kinds of NPs are used in poultry feed like nano-silver, nano-chromium, nano-selenium, nano-gold, nano-germanium and nano-zinc. The use of nanosilver (NS) in chicken feed, water purification systems, and the cosmetic sector is common. It is very safe as feed additive in animal and poultry feeding due to its low absorption rate along the intestinal tract so less chances of toxicity (Hong *et al.* 2014). In view of the aforementioned facts, a research study was conducted to evaluate the effect of NS as feed additives on performance, blood biochemistry and intestinal histomorphology of Vanaraja chicken.

MATERIALS AND METHODS

Experiment site: The experiment was carried out in the Instructional Poultry farm of Division of LPM, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST-Kashmir. The birds were procured from the

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Experiment design and birds: The study was conducted in cages for a period of 8 weeks. A starter diet was supplied until the birds were 7 days old, and a finisher diet was fed thereafter. The birds in the control group were provided diet without NS. The diets were iso-nitrogenous and iso-caloric. The ingredient and chemical composition of control diets is shown in Table 1. All of the diets were made using the same batch of components and had the same composition. For 1 week, the chicks were brooded together and on 8th day, distributed into 5 treatments of 4 replicates, each having 9 chicks. Treatments consisted of T1 (Control) fed basal diet without any additive and in T2 T3 T4 and T5, NS was added to the diet @ 20, 40, 60 and 80 mg/kg diet respectively. The birds had unrestricted access to food and water and were vaccinated at prescribed time against prevalent diseases.

Table 1. Per-cent ingredient and proximate composition of experimental diets

Ingredient (kg)	Starter	Finisher
Maize	58	52
Soybean meal	38.1	37.1
Fish meal	0	5
Vegetable oil	0	2
Limestone powder	1.2	1.2
Di calcium phosphate	1.6	1.6
Common salt	0.35	0.35
D L Methionine	0.35	0.35
Lysine	0.1	0.1
Vitamin and mineral mixture	0.3	0.3
Total (kg)	100	100
Proximate composition		
Moisture (%)	9.76	9.32
Crude protein (%)	21.61	21.01
Crude fibre (%)	5.14	5.42
Ether extract (%)	4.13	4.28
Total ash (%)	7.35	5.76
Nitrogen Free Extract (%)	51.14	54.33
Metabolizable energy (kcal/kg)*	2974	3010.4

*Pauzenga's formula was used to calculate this (1985) ME = 37.5% NFE + 37.5% CP + 81% EE.

Performance study: Performance parameters, viz. weekly body weight gain (on individual basis), weekly feed consumption (on group basis) and weekly feed conversion ratio was calculated.

Blood biochemistry: At 8 weeks of age, blood biochemical studies were performed. The blood was obtained and stored in a slanting posture in sterile test tubes with no anticoagulant added. The tubes containing blood were incubated at 37°C for 1 h. Blood clots were shattered, and tubes were centrifuged at 3000 rpm for 30 min. The serum was pipette out in small tubes which were stored under deep freeze (at - 20 °C) until analysis. The parameters studied were glucose, total protein, cholesterol, SGPT and SGOT with the aid of auto analyser equipment by using respective biochemical kits.

Caecal microbiology: At the end of the trial, 2 birds per replicate were slaughtered and caecal contents were collected aseptically from and subjected to microbial analysis. 0.5 ml of intestinal contents were weighed and transferred to eppendorf tubes in an aseptic manner. 0.5 ml volume of 0.9% sterile normal saline was added to the tubes and mixed properly by vortex for obtaining 10^{-1} dilution. 0.5 ml of this diluted solution was transferred to another tube containing 4.5 ml of sterile 0.9% normal saline for obtaining 10^{-2} dilution. The procedure was repeated by taking 0.5 ml from 10^{-2} dilution to another tube with 4.5 ml sterilized 0.9% normal saline for obtaining 10^{-3} dilution. It was continued until required dilution was obtained. All the procedure was performed under Laminar Air Flow near flame creating practically efficient sterilized conditions. Bacterial colonies were counted by the pour plate method (Quinn *et al.* 1992). The average number of colonies was multiplied by reciprocal of the dilution factor and expressed as cfu/gram of contents.

Histomorphological study: For histomorphological changes, the representative samples of liver, small intestine and kidneys were collected from slaughtered birds into sterile containers containing 10% buffered formalin for fixation in order to prevent autolytic changes. The tissues were kept in capsules with proper labelling and then washing was done to drain out formalin. Dehydration was achieved by treating tissues with different grades of alcohol. Afterwards, the tissue samples were kept in benzene for the firmness in order to facilitate section cutting. The Casting of blocks was done with the help of L-molds and then section cutting was achieved by rotatory microtome. Afterwards, deparaffinization was done with the help of xylene and then the tissue was stained with haematoxylin and eosin to observe pathomorphological changes in the affected tissues (Luna 1968).

Statistical analysis: The data obtained were analysed by the Analysis of Variance (ANOVA) through SPSS (21.0) software considering replicates as experimental units. Duncan's Multiple Range Test (Duncan 1955) was used to test the significance of difference between means at $p<0.05$.

RESULTS AND DISCUSSION

There were no statistical ($p<0.05$) difference in the body weight gain of birds across various groups until 5th week. However, from 6th week onwards, a significant ($p<0.05$) improvement in the body weight gain was observed in groups fed NS in the diet (Table 2). Highest improvement was seen in T5 wherein 80 mg/ kg diet NS was used. These results are in agreement with Andi *et al.* (2011) who reported that utilising NS in broiler chicken boosted weight gain considerably. Hassan (2018) discovered that broilers fed NS gained more weight than control birds. Sawosz *et al.* (2007) found that Japanese quail provided with NS in drinking water had highest body weight than the control. Hang and Tra (2013) observed similar results in rabbits,

Table 2. Performance parameters of Vanaraja chicken fed diets supplemented with different levels of nanosilver

Parameter	T_1 (Control)	T_2 (20 mg/kg)	T_3 (40 mg/kg)	T_4 (60 mg/kg)	T_5 (80 mg/kg)
Body weight gain (g)					
1-3 weeks	227±7.09	225±9.88	232±4.38	229±6.78	239±5.05
1-5 weeks	591±11.98	599±6.38	607±5.44	616±16.99	626±12.28
1-8 weeks	1170^{c}±23.82	1192^{c}±18.53	1223^{bc}±29.86	1255^{ab}±21.73	1294^{a}±20.98
Feed intake (g)					
1-3 weeks	614±10.86	612±9.20	614±12.43	607±8.07	612±14.38
1-5 weeks	1378±21.65	1380±12.82	1382±13.31	1375±10.50	1383±15.21
1-8 weeks	3124±24.89	3125±21.45	3102±18.19	3110±27.14	3088±25.64
Feed conversion ratio					
1-3 weeks	2.70±0.05	2.74±0.11	2.64±0.09	2.65±0.08	2.57±0.09
1-5 weeks	2.33±0.08	2.31±0.02	2.28±0.03	2.26±0.14	2.22±0.08
1-8 weeks	2.68^{a}±0.08	2.62^{ab}±0.03	2.54^{ab}±0.11	2.48^{ab}±0.09	2.39^{b}±0.05

Means across rows bearing different superscripts differ significantly ($p<0.05$).

reporting that the body weight of rabbits offered NS in drinking water was higher than the control. The growth promoting effect has been attributed to the improvement in gut health by NS, leading to enhanced nutrient absorption and subsequent increase in weight gain of birds (Andi *et al.* 2011). However the results of the present study are in contrary to the findings of Ahmadi and Rahimi (2011) who studied the effect of NS on broiler performance and reported that Nanosilver supplementation had no significant effect on body weight gain of birds. Felehgari *et al.* (2013) also reported no significant difference in live body weight between birds fed NS based diets and control chicks. Variations in the size, dose, exposure time, and production process of NS could explain the inconsistent results found in different studies.

There was no statistical difference ($p<0.05$) in the feed consumption across groups (Table 2). Hassanabadi *et al.* (2012) reported similar results in their study on the effect of NS supplementation wherein they found that feed intake was same in all groups including control. Ahmadi (2012) also reported no change in the feed intake among NS groups and control in broiler chicken. However, Andi *et al.* (2011) reported that NS inclusion in the diet increased feed intake among birds than control. The cumulative feed conversion ratio (FCR) improved significantly ($p<0.05$) among various treatments with better FCR in T5 followed by T4 (Table 2). Andi *et al.* (2011) also reported better FCR in broiler chicken in NS supplemented groups than control. The improvement in the FCR could be attributed to the improved body weight achieved in the present study as a result of feeding NS based diets. In contrast, Hassanabadi *et al.* (2012) reported similar FCR among birds fed diets either supplanted with or without NS. Improvement in the FCR as observed in the present study could be related to the better weight gain observed by the birds as a result of NS supplementation.

The blood biochemical parameters like glucose, cholesterol, Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) did not differ statistically ($p>0.05$) among various treatments and control; however, a significant ($p<0.05$) change in the protein levels was seen across groups (Table 3). It has been reported that in-ovo injection of broiler eggs with NS had no effect on blood biochemical parameters of birds (Sikorska *et al.* 2010). Ahmadi (2009) observed that NS supplementation had no significant ($p<0.05$) effect on blood enzymes in broiler chicken. According to Andi *et al.* (2011), NS supplementation had no effect on ALT or AST levels in broiler birds. Furthermore, Sawosz *et al.* (2009) discovered that dietary NS supplementation in quail birds had no effect on the activity of ALT and AST or cholesterol. It can thus be perceived from the results obtained regarding ALT and AST that NS could be used up to 80 mg/kg level in Vanaraja birds without any harmful effect in poultry birds.

Birds fed NS supplemented diets had a significant ($p<0.05$) decline in total viable count and coliform count

Table 3. Blood biochemical parameters and caecal microbiology of Vanaraja chicken fed diets supplemented with different levels of nanosilver

Parameter	T_1 (Control)	T_2 (20 mg/kg)	T_3 (40 mg/kg)	T_4 (60 mg/kg)	T_5 (80 mg/kg)
Glucose (mg/dl)	167.34±9.59	173.11±5.40	165.76±10.39	168.67±11.79	172.20±9.90
Total protein (g/dl)	4.53±0.15	4.52±0.06	4.66^{ab}±0.02	4.71^{ab}±0.06	4.83^{b}±0.06
Cholesterol (mg/dl)	122.90±2.46	123.52±2.57	122.42±4.26	124.72±2.41	121.92±4.37
ALT(U/L)	14.92±0.12	14.92±0.25	15.10±0.10	15.25±0.06	14.85±0.34
AST(U/L)	95.82±1.33	97.67±1.87	96.72±1.74	98.15±2.07	97.10±0.89
Total viable count (cfu/g)	8.67^{c}±0.04	8.55^{c}±0.06	8.32^{b}±0.06	8.07^{a}±0.06	7.90^{a}±0.09
Total coliform count (cfu/g)	5.72^{c}±0.04	5.67^{c}±0.04	5.35^{b}±0.06	5.22^{b}±0.08	5.02^{a}±0.07

Means across rows bearing different superscripts differ significantly ($p<0.05$).

Table 4. Histomorphology of small intestine in Vanaraja chickens fed diets supplemented with different levels of nanosilver

Organ	Parameter(µm)	T1(Control)	T2(20 mg/kg)	T3(40 mg/kg)	T4(60 mg/kg)	T5(80 mg/kg)
Duodenum	Villus Height	1561.30^{a}±24.74	1576.90^{a}±19.39	1581.15^{a}±19.01	1661.52^{b}±28.13	1772.22^{c}±13.34
	Villus width	123.40±4.43	118.40±3.62	111.85±9.54	121.72±4.24	118.85±2.58
	Crypt Depth	210.62±10.70	203.07±16.49	181.50±29.39	206.50±5.84	234.17±17.38
Jejunum	Villus Height	1367.57^{a}±22.14	1374.85^{a}±27.38	1399.30±29.94	1450.60^{ab}±44.50	1505.92^{b}±15.83
	Villus width	105.82±3.24	128.47±14.68	130.32±15.89	106.62±10.09	109.12±8.08
	Crypt Depth	135.42±20.27	129.00±23.36	147.87±16.37	132.92±14.43	137.77±12.35
Ileum	Villus Height	1036.07^{a}±32.55	1043.62^{a}±18.51	1108.87^{ab}±15.47	1138.82^{b}±30.78	1228.57^{c}±34.21
	Villus Width	103.40±9.53	110.90±5.85	112.90±4.95	107.90±1.23	117.90±4.78
	Crypt Depth	125.42±11.93	132.92±8.66	137.92±9.62	127.92±10.46	142.92±12.45

Means across rows bearing different superscripts differ significantly ($p<0.05$).

than control (Table 3). As per Fondevila (2010), *in vitro* NS supplementation @ 25, 50, and 100 ppm reduced the amount of coliforms in ileal content, but had no effect on the proportion of *Lactobacillus* in birds. However, Sawosz *et al*. (2007) found that 25 mg/kg of NS supplied in quail drinking water increased the quantity of gram positive bacteria considerably when compared to the control group. According to several studies, the criteria involved in the antibacterial activity of NS particles includes a combination of both physical and chemical aspects, such as the size, shape and surface volume ratio of nanoparticles, as well as their production process (Badawy *et al.* 2011, Allaker 2012).

NS supplementation increased villus height significantly ($p<0.05$) in duodenum, jejunum, and ileum of birds (Table 4). Highly significant values were achieved in T5 group in all the 3 segments of small intestine where in 80 mg NS per kg diet was supplemented, followed by T4 group. The width of villus and crypt depth did not differ significantly ($p>0.05$) among treatments including control. According to Ahmadi *et al.* (2009), the height of intestinal brush borders increased when different levels of silver Nanoparticles ranging from 300, 600, and 900 ppm were used in the diet of broiler chicken. However, Ognik *et al*. (2015) reported that silver nanoparticles had no impact on jejunum histology in the chickens fed hydrocolloid NS (5 mg/kg/day diet) and lipid coated NS hydrocolloid (5 mg/kg/day diet). Mahmoud (2012) also reported that histological sections of duodenum wall showed no significant ($p>0.05$) changes in quails fed diets supplemented with NS. Increased villus height helps to enhance the absorptive surface area for better utilization of nutrients while as short or damaged villi impair the absorption of the intestine, which might lead to poor performance of birds (Samanya and Yamauchi 2002, Adil *et al.* 2010). Thus, improved villus height as observed in the present study would have resulted in better absorption of nutrients, thereby justifying the results of improved performance of birds fed NS in the diet.

From the present study, it is concluded that supplementation of Nanosilver at 80 mg/kg in the diet was effective in improving the growth performance, gut microbiology and histomorphology of intestines in Vanaraja birds.

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