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ORIGINAL ARTICLE

Nano-Selenium affects on duodenum, jejunum, ileum and colon characteristics in chicks: An animal model

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Abstract
Selenium has many roles on the functioning of humans and animals. Chicks as animal model can play an important role to distinguish the role/function of selenium. The objective of the present study was to test the effect of variable doses of nano-selenium supplement on the chicken gastrointestinal tract. A total of 180 male Ross chicks were divided into six groups, with three replicates for each group and ten birds *per* replicate. Group 1 was the control group and was fed standard feeding for full experiment. Groups 2-6 were given variable doses of nano-selenium supplement from 1^{st}-42^{nd} days of age; Group 2 (0.1 mg/kg), Group 3 (0.2 mg/kg), Group 4 (0.3 mg/kg), Group 5 (0.4 mg/kg) and Group 6 (0.5 mg/kg). The results showed a decrease in the weights of small and large bowels with simultaneous increase in their lengths. Optimum doses of supplementary nano-selenium for bowel development are suggested.

Keywords: *Function; Humans; Intestine; Nano Selenium; Nutrition.*

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INTRODUCTION

Selenium as a trace mineral is essential for animals, plants and humans [1, 2]. Schwarz and Foltz first described the preventive effects of selenium in liver necrosis and liver degeneration in a rat model [3]. This historical work caused great interest in the broader scientific community about selenium in animal and human physiological fields. Today it is generally known that selenium may modulate a broad spectrum of key biological processes andplay an important role in imunne system activity, and protect from oxidative damage, including carcinogenesis inhibition. On the other hand, it is also well-known that high selenium doses are dangerous causing several serious health problems.

In chicken breeding, the most important indicators are meat quality and egg quality. Chicken producers worldwide are trying to increase production performance together with reduction in breeding costs [4].

Koyuturk et al. demonstrated a selenium, vitamin E and vitamin C combination have therapy effect on ethanol-induced duodenal mucosal damages [5]. Meanwhile, some authors, for example Ojuawoand Keith studied the role of serum concentrations of zinc, copper and selenium in children with inflammatory bowel disease [6].

Littlethought is given to the possibility of influencing the organ system development, which are primarily responsible for chick growing. Small and large bowels are important part of the gastrointestinal system of human/animals with primary involvement in their development. Bowel development with correct villous enzyme activities is in connection with nutrition resorbtion and chicken health status. Some research works have documented the impact of seleniumon enterocytes

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[7, 8]. Davis et al. demonstrated se-enriched broccoli could decline the intestinal tumorigenesis for multiple intestinal neoplasia mouce [9]. So, we can hypothesise that selenium supplements will have some effects on the growth of bowels, including bowels mucosal epithelial cellular differentiation. Besides, several research studies document positive effects of selenium on chicken health [10, 11], including chicken breeding [12].

The aim of the current experiment is to test the effects of variable doses fornano-selenium (NS) supplement on the chicken gastrointestinal tract as an animal model.

MATERIALS AND METHODS

Ethics

This study was conducted in 2016 in a commercial poultry farm at Rasht, Iran. The experiment was approved at Ethics Committee of the Ilam Branch, Islamic Azad University. The study followed International Guidelines for Research (Directive 2010/63/EU).

Animals and housing

In total, 180 male Ross chickens were randomly divided into 6 treatments, with 3 replicates per treatment, with 10 birds for each replicate. One-day-old broilers were purchased and randomly divided into treatments with same body weights. Chicks were reared till 42 day of age based on the starter (1-21 days of age), and the grower (22-42 days of age) periods. Formula and chemical analysis of used diets is shown in Table 1. Broiler chickens received feeds and water *ad libitum* throughout the trials. Broilers were unable to feed from adjoining cages. The animals were housed in land cages with dimensions of 1.0 x 1.0 m. All broilers had a common environment. Thermo-neutral ambient temperature was maintained in accordance with standard brooding practices. Lighting program was conducted for 23h on 1-7 days of age and 40-42 days of age, and for 20 h/day, with four hours of darkness on 8 -39 days of age. Routine vaccination and de-worming was designed and administered by the farm veterinarian in co-operation with the regional veterinary authority. Vaccination consisted of infectious bronchitis (Infectious Bronchitis Virus (IBV) (H120); Razi Co, Iran) at day 1, Gamboro vaccination (Gamboro (IBD071IR); Razi Co, Iran) at days 14 and 23, Newcastle at days 8 and 21, and influenza at day 1.

Experimental design

A completely randomized design was used, with

Table 1. Feed ingredients and nutrient analysis of the basal diets.

Ingredients (%)	Starter (1st -21st day of age)	Grower (22nd-42nd day of age)
Corn	58.6	61.6
Soybean meal (44% CP)	36.2	33.5
Soybean oil	1.4	1.5
Calcium Carbonate	0.8	0.9
Gluten meal	0.65	0.16
Dicalcium Phosphate	1.30	1.15
NaCl	0.25	0.32
Mineral premix*	0.25	0.25
Vitamin premix**	0.25	0.25
DL-Methionine	0.25	0.30
L-Lysine hydrochloride	0.05	0.07
Total	100	100
Nutrient analysis		
Metabolisable Energy (kcal/kg)	2950	3000
Crude Protein (%)	21.0	20.0
Calcium (%)	0.95	0.90
Available Phosphorus (%)	0.47	0.45
Sodium (%)	0.17	0.15
Chloride (%)	0.18	0.17
Lysine (%)	1.12	1.05
Methionine (%)	0.48	0.45
Methionine + Cysteine (%)	0.80	0.75
Threonine (%)	0.74	0.70

*Supplied per Kg of mixture: 1,081 mg *trans*-retinol; 20 mg cholecalciferol; 4 mg α-tocopherol acetate; 800 mg menadione; 720 mg thiamine; 2,640 mg riboflavin; 4,000 mg niacin; 12,000 mg calcium pantothenate acid; 1,200 mg pyridoxine; 400 mg folic acid; 6 mg cyanocobalamin; 40 mg biotin; 100,000 mg choline; 40,000 mg antioxidant.
** Supplied per Kg of mixture: 39,680 mg manganese; 20,000 mg iron; 33,880 mg zinc; 4,000 mg copper; 400 mg iodine; 80 mg selenium; 1 mg excipient.

treatments containing different selenium levels, in three replicate pens of 10 chicks each, in a total of 18 experimental units. Commencing from day one, groups were created as follows. Group 1 (CON) : control birds, Group 2 (NS1) : 0.1, Group 3 (NS2) : 0.2, Group 4 (NS3) : 0.3, Group 5 (NS4) : 0.4 and Group 6 (NS5) : 0.5; (0.1, 0.2, 0.3, 0.4 and 0.5 are mg/kg of feed dietary nano-selenium from 1st-42nd days of age in Groups 2 to 6, respectively).

Gastrointestinal morphometry

At the 42nd day of age, 1 bird for each replicate was euthanized. These birds were used for measuring of carcass characteristics. Birds were fully pecked. Feet were separated from the carcass in the tibio-tarsal joint and intestinal segment dimensions were weighted and recorded. The length, width and diameter of duodenum, ileum, jejunum, and colon were measured. All dimensions were rounded to integers. Total weight of each segment was related to the totally eviscerated carcass. Ratios were calculated according to the following formula: [(weight of segment/eviscerated carcass weight) × 100].

Statistical analysis

Data were analyzed using GLM. The significance of the differences between group means was analysed using the ANOVA procedure, followed by a Tukey's post hoc test, using IBM SPSS Statistics 21 software [13]. *P* values ≤ 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

All the achieved results are summarized in Tables 2-5. Usually bowels dynamically react to nutrition. The most dramatic changes usually occur in the duodenum [14]. Our experiment also showed that selenium has the biggest impact on duodenum weight, duodenum length and duodenum width, as compared to the control group.

The results document the positive effect of selenium supplement on duodenum with weight decreasing and simultaneously length increasing. The results of jejunum and ileum show the same trends, but the measured levels are not so clear, comparing results of duodenum.

Also nano-selenium improved the function of the large bowel (colon). The increase in length, width, diameter, and weight of colon in nano-selenium chicken consumer groups was seen in the results.

Small and large bowel length increase is in

Table 2. Mean (±SEM) of duodenum characteristics at 42nd days of age in Ross 308 male broilers fed the different levels of dietary nano selenium from 1st-6th weeks of age*.

Dietary nano selenium (mg/kg)	Duodenum weight (gr)	Relative weight of duodenum (%)	Duodenum length (mm)	Duodenum width (mm)	Duodenum diameter (mm)
CON	20.627	0.876^{ab}	37.130	6.043^{b}	0.493
NS1	16.517	0.704^{ab}	37.313	6.723^{b}	0.460
NS2	16.670	0.730^{ab}	42.450	7.753^{ab}	0.493
NS3	21.827	0.936^{a}	44.370	7.557^{b}	0.527
NS4	21.133	0.921^{a}	41.877	9.463^{a}	0.463
NS5	15.600	0.653^{b}	46.280	6.663^{b}	0.527
P-value	0.088	.058	0.436	0.017	0.459
SEM	1.733	0.071	3.627	0.575	0.029

* CON: control, without supplementation, NS1: supplemented with nano-selenium at 0.1 mg/kg DM of feed, or at 0.2 mg/kg of feed (NS2), or at 0.3 mg/kg of feed (NS3), or at 0.4 mg/kg of feed (NS4), or at 0.5 mg/kg of feed (NS5).
Means within each column and week of age with no common superscript differ significantly at P<0.05.

Table 3. Mean (±SEM) of jejunum characteristics at 42nd days of age in Ross 308 male broilers fed the different levels of dietary nano selenium from 1st-6th weeks of age*.

Dietary nano selenium (mg/kg)	Jejunum weight (gr)	Relative weight of jejunum (%)	Jejunum length (mm)	Jejunum width (mm)	Jejunum diameter (mm)
CON	39.100	1.661	53.000	7.203^{c}	0.380
NS1	26.800	1.147	59.667	8.540^{ab}	0.430
NS2	35.053	1.549	62.000	8.827^{a}	0.420
NS3	32.573	1.403	66.000	8.553^{ab}	0.447
NS4	35.200	1.530	59.000	7.577^{bc}	0.460
NS5	38.020	1.605	58.000	8.213^{abc}	0.410
P-value	0.367	0.486	0.270	0.044	0.588
SEM	4.033	0.191	3.562	0.351	0.032

* CON: control, without supplementation, NS1: supplemented with nano-selenium at 0.1 mg/kg DM of feed, or at 0.2 mg/kg of feed (NS2), or at 0.3 mg/kg of feed (NS3), or at 0.4 mg/kg of feed (NS4), or at 0.5 mg/kg of feed (NS5).
Means within each column and week of age with no common superscript differ significantly at P<0.05.

Table 4. Mean (±SEM) of ileum characteristics at 42nd days of age in Ross 308 male broilers fed the different levels of dietary nano selenium from 1st-6th weeks of age*.

Dietary nano selenium (mg/kg)	Ileum weight (gr)	Relative weight of ileum (%)	Ileum length (mm)	Ileum width (mm)	Ileum diameter (mm)
CON	58.033	2.458	72.667	6.980^{b}	0.473
NS1	51.670	2.212	73.333	7.607^{b}	0.513
NS2	58.070	2.601	75.667	7.870^{ab}	0.530
NS3	53.137	2.282	68.967	7.883^{ab}	0.457
NS4	60.517	2.645	74.667	7.863^{ab}	0.557
NS5	66.097	2.796	73.000	8.947^{a}	0.590
P-value	0.962	0.971	0.930	0.035	0.596
SEM	12.032	0.552	5.848	0.340	0.058

* CON: control, without supplementation, NS1: supplemented with nano-selenium at 0.1 mg/kg DM of feed, or at 0.2 mg/kg of feed (NS2), or at 0.3 mg/kg of feed (NS3), or at 0.4 mg/kg of feed (NS4), or at 0.5 mg/kg of feed (NS5).
Means within each column and week of age with no common superscript differ significantly at P<0.05.

Table 5. Mean (±SEM) of colon characteristics at 42nd days of age in Ross 308 male broilers fed the different levels of dietary nano selenium from 1st-6th weeks of age*.

Dietary nano selenium (mg/kg)	Colon weight (gr)	Relative weight of colon (%)	Colon length (mm)	Colon width (mm)	Colon diameter (mm)
CON	11.433	0.482	10.203	7.430	0.617
NS1	10.440	0.448	12.670	8.153	0.697
NS2	9.777	0.432	11.500	9.133	0.717
NS3	9.103	0.396	8.233	6.900	0.650
NS4	12.693	0.552	12.167	10.267	0.710
NS5	13.703	0.581	12.967	10.133	0.730
P-value	0.592	0.684	0.417	0.205	0.393
SEM	2.015	0.091	1.720	1.071	0.041

* CON: control, without supplementation, NS1: supplemented with nano-selenium at 0.1 mg/kg DM of feed, or at 0.2 mg/kg of feed (NS2), or at 0.3 mg/kg of feed (NS3), or at 0.4 mg/kg of feed (NS4), or at 0.5 mg/kg of feed (NS5).
Means within each column and week of age with no common superscript differ significantly at P<0.05.

direct relationship with better resorbtion. These results indicate that this parameter is important for chicken breeding industry.

Gastrointestinal homeostasis is one of the conditions for normal chicken production. Several references prove that selenium deficiency can cause intestinal mucosal inflammation [15]. The works of Wang et al. show that selenium deficiency induces degranulation of mast cells in the jejunum of chickens with jejunal mucosal changes with vacuolisation and granulation of the epithelial cells [16].

This findings also documents the impact of seleniumon bowel mucosa development. Another study showed that dietary supplementation with optimum levels of selenium reduced negative consequences of necroticenteritis induced dimmunopathology [17]. One of the most important problems of poultry breeding is still in higher mortality during initial stages of the breeding. The main cause is heart failure [18, 19].

Another important gastrointestinal problem is increased sensitivity to enteral small bowel, malabsorption syndrome and large bowel infections [20]. These factors significantly affect the productivity and economy of breeding. Necrotic enteritis is one of the main problems of chicken breeders with significant mortality and economical impact on chicken breeding. Nutrition has a big impact to chicken health, including impact on mortality elimination.

Ascites syndrome is one of the most serious health problems of chicken rearing. It is the result of fat, muscle and connective tissue amount increasing in which the heart cannot push sufficient blood through the lungs, leading to ventricular hypertrophy. In our previous work, we have shown that feeding regime has significant effect on the ascites incidence syndrome in chicks [21]. In this sense, there is intensive effort to limit the intense chicken breeding and replace it with natural chicken breeding. This is in relationship with animal welfare [22]. Previous research would indicatedthat it is also supported by several European and non-European countries with financial support for animal breeding.

Selenium plays an important role in chick growing and here we have proved that selenium nano-doses influence small and large bowel growing. Some previously published articles document important impact of selenium for chicken breeding with imparted resistance to oxidative stress [23, 24] and protective effect of selenium to immunologic injury [25].

Some researchers have interpreted the positive results of intestinal indicesin the selenium receptor groups that selenium in addition to improving the composition and rate of intestinal microflora, can have a positive effect on the morphology of the intestine (duodenum, jejunum, ileum and colon) as an antioxidant [26].

CONCLUSION

This research documents the positive effects to several bowel parameters. The results suggest thatadding nano-selenium doses to chick nutrition would result in improved health and effecient adult chicken production.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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