Influence of Bacterial Organic Selenium on Blood Parameters, Immune Response, Selenium Retention and Intestinal Morphology of Broiler Chickens

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Abstract

Background Several studies indicated that dietary organic Se usually absorbed better than inorganic Se with high retention and bioavailability. Dietary Se as an antioxidant element affects the immune system and hematological status in animals. Therefore, the aim of this study was to evaluate the effect of dietary supplementation of bacterial selenium as organic source on hematology, immunity response, selenium retention and gut morphology in broiler chickens.Results The present results revealed that supplementation of inorganic Se was associated with the lowest level of RBC, HB, and PCV with significant difference than ADS18-Se. In the starter stage, both T2 and T5 were associated with the significantly highest IgG level compared to the basal diet, while all supplemented groups showed higher IgM level compared to control group. In the finisher phase, all Se supplemented groups showed significant (P 0.05) increases in IgG, IgA and IgM levels compared to T1. Birds fed bacterial-Se showed high intestinal villus height and better Se retention more than sodium selenite. Selenium of ADS18 had a superior action in improving Se retention compared to ADS1 and ADS2 bacterial Se.Conclusion Bacterial organic Se had a beneficial effect on villus height of small intestine led to high Se absorption and retention. Thus, caused better effect of Se on hematological parameters and immunity response.

Background

Immunity response and hematological parameters indicates the physiological status, general health condition of the animals and diagnoses many types of disease [1]. Dietary Se as an antioxidant element affects the immune system and hematological status in animals. Sodium selenite is more toxic than organic Se and causes PCV reduction, which is a signal of the presence of toxic elements that adversely affect the blood formation [2]. Moreover, Selenium is a vital element for stimulation of the immune system in chickens, it has been reported that Se deficient diets lead to cellular and humoral immunity damages [3]. Nutritional supplementation of selenium plays a vital role in the activity of multiple components of the animal immune system [4].

Selenium (Se) is base component of at least 25 selenoproteins that contribute in a regulation of various biological functions in the body. Recent studies are converted from focusing on Se toxicity to its essential nutritional effects. A huge number of researches were carried-out to evaluate the effects of Se on poultry nutrition and biological functions, and rapid discoveries were made to reveal the proper source of Se and the optimal level that must be added to the poultry diet. All recent studies demonstrated that the organic Se is more bioavailable than inorganic forms in poultry nutrition [5; 6], which may be due to the differences in the absorption and metabolism of organic and inorganic Se. Selenium absorption and retention in the body mainly depend on the quantity of ingested Se and its chemical form [7]. Organic Se is usually associated with higher absorption and retention compared to the inorganic form [8]. Due to their different absorption ways, organic Se is retained in the muscles tissues more than inorganic Se, and excreted less than it.

Previous studies showed that, dietary bacterial Se as an organic source improved tissue Se deposition, antioxidant status, and selenoproteins gene expression, and can be considered as an effective alternative source of Se in broiler chickens [9]. As such, the current study sought to examine the impact of various

bacterial sources of Se compared to the inorganic form on hematology, immunity response, selenium retention and gut morphology in broiler chickens.

Methods

Extraction of bacterial selenium content

In the current study, Se enriched bacterial strains identified as *Enterobacter cloacae* (ADS1), *Klebsiella pneumoniae* (ADS2), and *Stenotrophomonas maltophilia* (ADS18) were used as a source of bacterial organic Se. The stock culture of ADS1, ADS2, and ADS18 strains prepared at the Laboratory of Microbiology, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM) and the sonicated Se-enriched bacterial cells were produced according to procedure described by Dalia et al. [9]. The extraction of seleno protein from Se-enriched bacterial cells was carried out using dialysis technique The dialysis process was performed using dialysis sacks of flat width 25 mm, 12,000 Da, (Sigma-Aldrich) against deionised water, which was changed every 12h for a total of 96 hours to separate inorganic Se from organic form [10]. The content in the dialysis tube was lyophilised and then used as source of bacterial Se.

Birds and experimental procedure

The birds handling and use in this study was carried out in compliance with the research policy guidelines of UPM on Animal Welfare and Ethics. A total of 180 one-day-old (Cobb 500, Arkansas, USA) female broiler chicks averaging 40 ± 0.13 g were obtained from a commercial hatchery. The chicks were individually wing-banded on arrival at the farm. The birds were randomly divided into five treatments fed the basal diet (Table 1), each with six replicates of 6 birds per replicate. The dietary treatments consisted of the basal diet supplemented with (0.3 mg/Kg feed sodium selenite), (0.3 mg /kg feed ADS1 Se), (0.3 mg /kg feed ADS2 Se), and (0.3 mg /kg feed ADS18 Se) in addition to the basal diet treatment served as control group. Starter diet was offered from 0 to 3 weeks old and finisher from 4 to 6 weeks old. Water and feed were given *ad libitum* to all the chickens. Experimental birds were housed in UPM- farm (Ladang-2) using semi-closed system. Lightening was 12h per day. All the birds subjected to vaccination against bronchitis (IB) and Newcastle disease (ND) on day 7, and against infectious bursal disease on day 14 through the intraocular route.

Hematological parameters

On completion of the experiment, 12 birds from each dietary treatment (2 birds from each replicate) were selected at random, then weighted and sacrificed. The slaughter procedure was conducted at the Department of Animal Science slaughter house, Faculty of Agriculture, Universiti Putra Malaysia. The animals were humanely slaughtered by a licensed slaughter man. The procedure involved severing the carotid artery, jugular vein, trachea and esophagus. Blood samples were taken directly from the neck vein into a vacuum tube (BD Vacutainer®, NJ. USA) containing anticoagulant (EDTA) and directed to hematological analysis using hematology analyzer (CELL DYN 3700, Abbott USA).

Plasma immunoglobulin concentration

At days 21 and 42, 6 birds per treatment were randomly selected and blood samples were collected into vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA). Blood samples were mixed gently before storage in ice, followed by centrifuging at 3000 rpm for 15 min at 4 °C, and the plasma was stored at −80 °C until antibody analysis.

Plasma IgA and IgM were determined using (Chicken IgA ELISA, Immunology Consultants Laboratory, Inc. USA) and (Chicken IgM ELISA, Immunology Consultants Laboratory, Inc., USA), while Chicken IgG determined using (CEA544Ga, Enzyme-linked Immunosorbent Assay Kit, Cloud-Clone Corp., USA). All the analysis performed according to the procedure recommended by the manufacturer. The absorbance was measured at 450 nm wavelength using micro-plate reader (Infinite® 200 PRO, TECAN).

Selenium retention

Twelve representative samples from each batch of feed (starter and finisher diets) were collected randomly and kept at −20°C until further analysis. Total excreta collection was performed on days 17, 18, 19 and 20 for starter diet and on days 38, 39, 40, 41 for finisher diet. Feed and fecal samples were analyzed for Se concentration using ICP.MS. Determination of Se retention was calculated using mass balance method [11] as follows:

$$\text{Se retention (\%)} = \frac{(\text{Ingested Se} - \text{Excreted Se}) \times 100}{\text{Ingested Se}}$$

Ingested Se = daily feed intake × analytical feed Se concentration

Excreted Se = daily feces weight × feces Se concentration.

Histomorphology of small intestine

Intestinal morphology was done employing the method stated by Choe *et al.* [12]. The intestinal samples of 6 birds/ treatment were collected at days 21 and day 42. Approximately 5 cm segments of the ileum (midway between the Meckel's diverticulum and ileo-ceacal junction), middle portion of the duodenum (apex section), and jejunum (midway between the end point of duodenal loop and Meckel's diverticulum) were cut gently and washed with phosphate buffer saline (PBS) and fixed in 10% neutral buffered formalin. Then, the intestinal samples were dried for 16 h in an automatic tissue processor (Leica ASP 3000, Tokyo, Japan) and embedded in paraffin wax following a paraffin embedding system (Leica EG 1160, Japan). Each sample was cut at 4 µm with a rotary microtome machine (Leica RM 2155, Japan). Sections of size 4 mm were fixed on glass slides, heated at 57^{o}C until dried, and stained with hematoxylin and eosin (Appendix F). The distance from the tip of the villi to the villus crypt junction represented the villus height, while, crypt depth was described as the depth of the invagination between 2 villi and was determined employing Image-Pro Plus software as described by Touchette *et al.* [13]. A total of 5 villi sections per slide were evaluated in each of 6 replicate slides per intestinal sample (30 measurements for each sample) and studied with light microscope (Dialux, LeitzWetzlar, Germany) fitted with a digital camera (Laice, Germany).

Statistical analysis

An ANOVA was carried using six replicates per means. Differences between treatments were studied using one-way ANOVA [14]. Duncan test was used to establish the significant differences among the treatment groups at a significant level ($P < 0.05$). The data of Se retention was analysed using GLM procedure suitable for Completely Randomized Design [14]. Treatment differences were established by orthogonal contrasts

(1) Basal diet vs. Se supplemented diets,

(2) Sodium selenite vs. bacterial organic Se,

Values of $P < 0.05$ were accepted as significant.

Results

Hematological parameters

The effects of inorganic and bacterial organic Se on the whole blood parameters of 42-day-old broilers are shown in Table 2. The present results showed that supplementation of inorganic Se was associated with the lowest level of RBC, HB, and PCV, while T5 of bacterial organic Se resulted in the highest levels, the difference between T2 and T5 was significant. Moreover, T2, T3, and T5 showed significant ($P > 0.05$) reduction in WBC compared to the basal diet, while, no significant difference was observed between T1 and T4. Other investigated hematological parameters had no significant effect among different treatments ($P > 0.05$).

Plasma immunoglobulins

The effects of inorganic and bacterial organic Se on plasma IgG, IgA, and IgM concentration are shown in Table 3. In the starter stage, significant ($P \leq 0.05$) effect of dietary Se supplementation was observed, both T2 and T5 were associated with the highest IgG level with significant difference compared to the basal diet, while T3 showed higher IgA level compared to control group. Both Se sources associated with significantly higher IgM than the basal diet. In the finisher phase, all Se supplemented groups showed significant ($P \leq 0.05$) increases in IgG, IgA and IgM level compared to T1; however, no significant effect among the Se supplemented treatments were observed.

Selenium retention

Table 4 shows the Se retention in broiler chickens supplemented with inorganic Se and different sources of bacterial organic selenoprotein for 42 days. Selenium supplemented diets versus basal diet showed significant difference in ingested and excreted Se compared to negative control (T1). However, the percentage of Se retention showed significant difference in the finisher stage with insignificant effect in the starter stage when the basal diet was contrast to Se-supplemented diets. Moreover, bacterial organic Se in broiler feed resulted in a significant ($P < 0.05$) increase of finisher ingested Se in contrast to inorganic Se (T2).

In the starter stage, T1 was associated with the lowest ingested and excreted Se. All Se supplemented diets showed insignificant differences in the ingested Se, while the excreted part was the lowest significantly in T5 compared to other Se dietary groups. Besides that, retention percentage in this stage was significantly ($P < 0.05$) highest in T5 compared to other treatments. In the finisher diet, ingested Se was significantly different

among treatments, which were, 236.3, 705.6, 792.2, 819.9, and 920.4 µg/g in T1, T2, T3, T4, and T5, respectively. The excreted part showed the highest level in T4 and the lowest level in T1. All Se supplemented diets showed the same retention percentage, which was significantly higher than T1 that associated with high body weight in compare to control group (Fig 1).

Villus height and crypt depth of the duodenum, jejunum, and ileum

The villi height and crypt depth of the duodenum, ileum, and jejunum of birds fed inorganic and bacterial organic Se after 21 and 42 days of age are shown in Table 5. At starter stage, broilers that were fed a bacterial organic Se had significantly ($P<0.05$) higher duodenum, ileum, and jejunum villi height compared to those fed the basal diet. Supplementation of bacterial organic Se showed higher jejunum villi height compared to inorganic Se (T2), also in the duodenum villi height, T4 and T5 of bacterial organic Se were significantly ($P < 0.05$) higher than T2. On the other hand, no significant difference was observed in the ileum villi height between the birds fed inorganic and bacterial organic Se except in T3 of bacterial organic Se, which indicated lower villi height compared to T2. Furthermore, no significant differences were observed for crypt depth in duodenum, jejunum, and ileum among the treatments.

At finisher phase, birds fed diet T4 and T5 had significantly ($P < 0.05$) higher villus height in the duodenum than the inorganic Se and the basal diet. There were no significant differences ($P > 0.05$) among T1, T2, and T3 treatments for the duodenal villus height. Moreover, there were no significant differences for villi height in the ileum and jejunum among the treatments. Experimental diets had no effect on the duodenum, jejunum, and ileum crypt depth.

Discussion

Hematological indices are good indicators of the animals' physiological status and have positive correlation with the animals' nutritional status [15]. Results obtained by Okunlola *et al.* [16], demonstrated that Se levels had no effect on PCV, HB, RBC and WBC, while there were significant differences ($p<0.05$) in heterophyl and lymphocytes. According to Chen *et al.* [6] and Boostani *et al.* [17], different selenium sources had no effect on blood WBC, RBC, HB and PLT of broiler chickens. In contrast, Biswas *et al.* [18] and Fawzy *et al.* [19], reported that Se supplementation increased the erythrocytes counts in poultry and changed PCV and HB significantly, and this supports the finding of this study that, supplementation of bacterial organic Se of (T5), showed significantly highest level of RBC, HB, and PCV compared to sodium selenite. Also, the finding of the current study indicates a significant ($P > 0.05$) reduction in WBC in T2, T3, and T5 compared to the basal diet, with no significant differences in monocyte, eosinophil and basophil percentages, thus partially agreeing with Fawzy *et al.* [19] and Singh *et al.* [20], but contrast their finding that the Se supplementation enhances cell mediated immunity, and significantly increases WBC. In the present study, the WBC count of Se supplemented treatments was lower than that of the basal diet but still within the reference range according to Mitruka and Rawsley, [2]. In the present study, sodium selenite reduced RBC, PCV, and HB values compared to bacterial organic Se, although all levels are still in the normal range according to Schalm *et al.* [21] and Mitruka and Rawsley, [2]. Sodium selenite is more toxic than organic Se and the reduction of PCV level signals the presence of toxic element which adversely affects the blood formation [2]. On the other hand, in rats, dietary sodium selenite for more than one month was associated with decreases in RBC, PCV, WBC, and HB levels [22]. Also,

repeated selenite treatment may reduce hemoglobin synthesis and induce a condition of hypochromic anemia [23], this may be attributed to the potential production of reactive oxygen species associated with chronic selenite supplementation.

In the current study, both inorganic Se and bacterial organic Se of (ADS18) showed significant increase in IgG concentration in the starter phase compared to the basal diet, but bacterial organic Se of (ADS1) and of (ADS2) showed the highest IgA and IgM levels respectively, compared to other treatments. In the finisher stage dietary Se raised both IgG, IgA and IgM concentrations with no significant difference between inorganic and bacterial organic Se. These data are in line with Lu *et al.* [24], who reported that Se-enriched exo-polysaccharides (Se-ECZ-EPS) produced by *Enterobacter cloacae* Z0206 showed significant increase in serum antibody titers against Newcastle disease virus in birds treated with 840 mg/kg Se-ECZ-EPS. They also partially agree with the finding that supplementation of different nano- Se levels in broiler chickens had no effect on the serum IgG, IgM, and IgA of the starter phase, while the birds supplemented 0.3 mg/kg of nano-Se showed the highest IgG and IgM levels on day 42 [25]. Also, supplementation with organic and nano-Se resulted in an increasing IgM and IgG concentration compared to the other groups in oxidative and non-oxidative condition [17]. However, some studies revealed that supplementation of different Se sources (organic and inorganic) did not affect immunoglobulins IgG, IgA, and IgM concentrations in the gilt or piglet [26]. Furthermore, Chen *et al.* [6], stated that both organic and inorganic Se had no effect on serum immunoglobulins at days 21 and 42. Therefore, dietary Se supplementation played a higher role in promoting humoral immune status in the starter and finisher stages in broiler chickens, but the effect of Se source is no longer observed. In the present study, all birds were vaccinated, which contributed to the dietary component in promoting a greater antigenic stimulation and production of higher concentration of immunoglobulins. The fluctuation of immunoglobulin concentration observed between the two dietary stages may be attributed to the fact that the antigen that activated the immune response will decline and then most of the T cells will die, which indicates the feedback mechanism of immune response.

Generally, organic Se may have better bioavailability and more efficient retention in the body than sodium selenite [27]. Sodium selenite is absorbed less efficiently and excreted at a higher rate compared to organic Se [28]. A study conducted by Yoon *et al.* [29] to examine the effect of two sources of Se-yeast as an organic Se and sodium selenite in broiler chickens, revealed that organic Se sources were more bioavailable and retained more efficiently than sodium selenite. Also, Hu *et al.* [30], indicated that Se retention in the whole body was higher in the group fed nano-Se compared to the group fed sodium selenite. In the current study, broilers fed dietary organic Se of T5, which originated from (ADS18) bacterial strain, retained more ($P < 0.05$) Se in the body associated with less Se excretion than sodium selenite and other bacterial Se sources at week 3, although, at week 6, they also retained the highest Se level compared to other treatments but the difference was insignificant. The observed difference compared to sodium selenite may be due to selenite having an ability to be bound by mucosal tissues to become unavailable for transfer to the other tissues [31]. Also, because the efficiency of Se from organic source is related to SeMet content, it could be that organic Se of (ADS18) contains a high amount of SeMet. Therefore, it would be more interesting to investigate the type of Se in each bacterial strain, which could explain why other bacterial strain (ADS1 and ADS2) were not different from sodium selenite.

Measurement of intestinal villus height and crypt depth as morphometric characteristics are important to maintain normal small intestine for proper absorption of nutrients and preventing translocation of bacteria from the gut [32]. The present results showed that the supplementation of bacterial organic Se had beneficial effect on villus height in all parts of the small intestine (ileum, jejunum, and duodenum) of starter phase and especially in the duodenum part of the finisher phase. However, inorganic Se had no effect on the villus height compared to basal diet except in the starter ileum part. Moreover, both inorganic and bacterial organic Se showed no effect on the crypt depth. This result is partially in line with Zamani-Moghaddam *et al.* [33], who indicated that supplementation of nano-Se to broiler chickens had positive influence on villus height in all intestinal parts except the ileum, while the organic Se increased all morphometric parameters in the jejunum part. Ahmed *et al.* [34], reported that dietary organic Se of Se-yeast, had a significant effect on duodenum and jejunum villi height in goat, but did not affect the villus height of ileum. Moreover, a study of Read-Snyder *et al.* [35], showed that organic Se supplementation in the form of (Sel-Plex) was associated with the greater intestinal villus height compared with the control and sodium selenite-fed birds in both normal and virus-infected groups of broiler. The main function of the small intestine is the digestion and absorption of nutrients. It is well recognised that a shortening of the villi will minimise the surface area for nutrient absorption, and a deeper crypt indicates fast tissue turnover [36]. A shortened villus height and a greater crypt depth are directly correlated with rising enterocyte turnover [37]. On the other hand, dietary antioxidant played a very important role in the enterocytes protection from apoptotic oxidative stress and could improve their development [38]. Therefore, the improvement in the villus height in the current study may be due to the role of organic Se as an exogenous antioxidant factor, which may positively affect enterocytes viability via the active contribution of Se in intestinal glutathione peroxidase (GSH-Px2).

These findings suggest that bacterial organic Se has the potential role to improve the small intestine villus heights especially in the duodenum segment, which is the main part for Se absorption [39]. The observed difference from sodium selenite could be due to the selenite having the ability to be bound by mucosal tissues and thus become unavailable for transfer to the other tissues [31]. These observations suggest that the improved Se retention and assimilation efficiency observed in the birds fed bacterial organic Se particularly (ADS18) can be explained by improved integrity of the intestinal tract and possibly by the improved gut antioxidant status.

Conclusion

The findings of the current study indicate that basal diets supplemented with 0.3mg/kg of different sources of bacterial organic Se showed better effect on intestinal villus height as well as better Se retention than sodium selenite (inorganic source). Selenium extracted from ADS18 bacterial strain had a superior action in improving Se retention compared to ADS1 and ADS2 bacterial Se. Resulted improvement in Se retention caused significant enhancement in blood formation and serum antibodies response.

Abbreviations

Se, Selenium; ADS1*, Enterobacter cloacae*; ADS2, *Klebsiella pneumonia*; ADS18, *Stenotrophomonas maltophilia*; RBCs, Red blood cells; HB, Hemoglobin; PCV, Packed cell volume; IgA, Immunoglobulin A; IgG, Immunoglobulin G; IgM, Immunoglobulin M.

Declarations

Ethics approval and consent to participate

It is confirmed that Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia approved this study.

Consent for publication

"Not applicable"

Availability of data and material

All relevant data and materials are available in the main manuscript.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions:

A.M., A.A.S., L.T.C. and S.A.Q contributed to the original idea and design of the study. D.A.M. conducted the experiments and collected the data. All authors were involved in the manuscript preparation and approved the final manuscript.

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Tables

Table 1. Ingredients and nutrient content of the basal diet.

Ingredients	Starter %	Finisher %
Corn	52.5	56.250
Palm oil	5.00	6.00
Soybean meal (44% cp)	32.50	30.00
Fish meal (58% cp)	5.15	3.25
L-Lysine	0.25	0.25
DL-Methionine	0.25	0.25
Dicalcium phosphate 18% [a]	1.60	1.85
Calcium carbonate	0.60	0.35
Salt	0.30	0.30
Mineral Premix[b]	0.15	0.15
Vitamin Premix[c]	0.10	0.10
Toxin Binder[d]	0.15	0.15
Choline Chloride	0.10	0.10
Wheat pollard (QL)	0.135	1.00
Calculated nutrient content (g/kg DM) [e]		
ME (MJ/Kg)	12.9	13.20
Crude protein	22.04	20.09
Crude fat	7.57	8.004
Calcium	1.189	1.0440
Phosphorus	0.786	0.768
Avail. P for Poultry	0.472	0.450
Analyzed Se (mg/kg)[f]	<0.09	<0.09

[a] di calcium phosphate provides phosphorus and calcium in a ratio of 1:1.

[b]Mineral premix provided the following per kg diet: iron 120 mg, manganese 150 mg, copper 15 mg, zinc 120 mg, iodine 1.5 mg, and cobalt 0.4 mg.

[c]Vitamin premix provided the following per kg diet: Vitamin A (retinyl acetate) 10.32 mg, cholecalciferol 0.250 mg, vitamin E (DL-tocopheryl acetate) 90 mg, vitamin K 6 mg, cobalamin 0.07 mg, thiamine 7 mg, riboflavin 22 mg, folic

acid 3 mg, biotin 0.04 mg, pantothenic acid 35 mg, niacin 120 mg and pyridoxine 12 mg.

[d]Toxin binder contains natural hydrated sodium calcium aluminium silicates.

[f]The Se content measured using ICP.MS.

[e]The diets were formulated using feedlive International software (Thailand).

Table 2. Effect of inorganic and bacterial organic selenium on serum hematological parameters in broiler chickens.

Parameters	Dietary Treatments[1]					SEM	*P*	Ref
	T1	T2	T3	T4	T5			
RBC $\times 10^{12}$/L	2.47 ^{ab}	2.25 ^{b}	2.47 ^{ab}	2.47 ^{ab}	2.64 ^{a}	0.05	0.037	1.82-3.46
HB g/L	114.3 ^{ab}	106.6 ^{b}	117.5 ^{ab}	118.0 ^{ab}	125.5 ^{a}	2.1	0.011	79-159
PCV L/L	0.277 ^{ab}	0.258 ^{b}	0.282 ^{ab}	0.280 ^{ab}	0.300 ^{a}	0.01	0.025	0.25-048
MCV Fl	112.8	115.0	114.0	113.3	113.5	0.75	0.912	100-200
MCHC g/L	411.5	413.5	416.3	421.7	419.0	2.68	0.820	376-456
WBC $\times 10^{9}$/L	39.5 ^{a}	28.2 ^{bc}	21.4 ^{c}	37.3 ^{ab}	18.7 ^{c}	2.73	0.006	13.84-37.82
Hetro $\times 10^{9}$/L	23.8	18.8	12.4	21.6	12.2	1.78	0.128	1.68-25.42
Lymp %	18.8	21.5	27.0	25.3	18.5	1.36	0.170	12.34-32.78
Mono %	8.3	6.0	7.5	7.0	5.5	0.66	0.768	2.52-12.3
Eosin %	2.8	2.5	3.0	3.7	3.0	0.28	0.797	2.06-3.89
Baso%	8.5	3.8	5.0	6.7	7.5	0.93	0.489	4.67-9.86
Thrombo$\times 10^{9}$/L	9.1	2.4	2.6	1.1	1.8	0.3	0.277	0.95-11.82

[1]T1; basal diet, T2; basal diet + 0.3mg/ kg feed sodium selenite, T3; basal diet + 0.3mg/ kg feed ADS1 Se, T4; basal diet + 0.3mg/ kg feed ADS2 Se, T5; basal diet + 0.3mg/ kg feed ADS18 Se

[a,b,c] Means in the same row with different superscripts are significantly different. Ref; refrence values according to Haematology & Clinical Biochemistry Laboratory, Faculty of Vetrinary Medicine. UPM.

Table 3. Effects of inorganic selenium and different sources of bacterial organic selenium on plasma immunoglobulin levels in broiler chickens.

Parameters	Dietary Treatments[1]					SEM	P
	T1	T2	T3	T4	T5		
				DAY 21			
IgG (mg/mL)	133.1^{b}	364.6^{a}	298.5^{ab}	298.7^{ab}	372.0^{a}	33.38	0.032
IgA (ug/mL)	763.8^{b}	743.0^{b}	1335.7^{a}	754.8^{b}	1085.2^{ab}	70.78	0.022
IgM (ug/mL)	481.6^{c}	552.2^{ab}	502.7^{b}	608.4^{a}	508.5^{b}	37.13	0.041
				DAY 42			
IgG (mg/mL)	258.4^{b}	469.0^{a}	454.4^{a}	450.1^{a}	476.4^{a}	25.66	0.045
IgA (ug/mL)	1156.9^{b}	1294.5^{a}	1202.4^{a}	1193.4^{a}	1117.6^{a}	75.01	0.014
IgM (ug/mL)	690.5^{b}	840.9^{a}	760.1^{a}	719.3^{a}	709.0^{a}	27.63	0.007

[1]T1; basal diet, T2; basal diet + 0.3mg/ kg feed sodium selenite, T3; basal diet + 0.3mg/ kg feed ADS1 Se, T4; basal diet + 0.3mg/ kg feed ADS2 Se, T5; basal diet + 0.3mg/ kg feed ADS 18 Se

^{a,b,c} Means in the same column with different superscripts are significantly different.

Table 4. Effects of inorganic and bacterial organic Se sources on serum and tissues Se concentration, and selenium retention in broiler chickens.

Parameters	Dietary treatments [a]					SEM	*P* value		
	T1	T2	T3	T4	T5		Anova	B	O
0-21 days									
Ingested Se µg/g	101.05^{b}	403.91^{a}	413.81^{a}	406.80^{a}	407.65^{a}	32.82	<.0001	<.0001	0.0632
Excreted Se µg/g	45.71^{d}	179.95^{ab}	233.38^{a}	170.57^{b}	109.08^{c}	17.41	<.0001	<.0001	0.4766
Retention %	54.76^{b}	55.45^{b}	46.08^{b}	58.07^{b}	73.24^{a}	2.82	0.012	0.4694	0.4550
22-42 days									
Ingested Se µg/g	236.3^{e}	705.6^{d}	792.2^{c}	819.9^{b}	920.4^{a}	63.95	<.0001	<.0001	<.0001
Excreted Se µg/g	118.33^{c}	233.37^{b}	232.75^{b}	302.16^{a}	256.00^{ab}	17.62	0.001	<.0001	0.1841
Retention %	49.93^{b}	66.93^{a}	70.62^{a}	63.15^{a}	72.19^{a}	2.39	0.002	0.0002	0.6183

B = basal diet VS Se supplemented diets, O = organic Se VS inorganic Se, P < 0.05 = significant differences

$^{a-c}$ Means with different letter within a row differed significantly.

[a]T1; basal diet, T2; basal diet + 0.3mg/ kg feed sodium selenite, T3; basal diet + 0.3mg/ kg feed ADS1 Se, T4; basal diet + 0.3mg/ kg feed ADS2 Se, T5; basal diet + 0.3mg/ kg feed ADS18 Se.

Table 5. Effects of inorganic and bacterial organic selenium sources on villus height and crypt depth of the duodenum, jejunum, and ileum in broiler chickens.

Parameters	Dietary treatments [1]					SEM	*p*
	T1	T2	T3	T4	T5		
21 days							
Villi height µm							
Duodenum	914.0^{c}	930.9^{cb}	1020.5^{ab}	1109.6^{a}	1141.9^{a}	20.72	<.0001
Jejunum	458.23^{b}	514.12^{b}	599.33^{a}	622.76^{a}	593.07^{a}	14.63	0.0002
Ileum	326.53^{c}	484.97^{a}	402.35^{b}	430.14^{ab}	475.16^{a}	13.18	<.0001
Crypt depth, µm							
Duodenum	80.87	75.15	78.35	74.17	73.88	1.82	0.7199
Jejunum	72.07	71.63	70.75	71.83	73.21	1.51	0.9925
Ileum	75.36	75.59	70.80	72.69	71.82	2.21	0.9508
42 days							
Villi height µm							
Duodenum	1159.9^{c}	1163.9^{c}	1155.1^{c}	1200.1^{b}	1265.2^{a}	16.71	0.0360
Jejunum	619.74	625.57	685.19	731.08	696.85	16.53	0.1474
Ileum	550.62	575.92	576.10	588.88	599.52	13.92	0.8618
Crypt depth, µm							
Duodenum	97.78	99.68	94.99	93.10	92.91	2.36	0.8809
Jejunum	89.68	87.14	85.33	83.20	75.99	1.51	0.0610
Ileum	91.61	87.75	82.81	80.99	78.81	2.38	0.4477

[1]T1; basal diet, T2; basal diet + 0.3mg/ kg feed sodium selenite, T3; basal diet + 0.3mg/ kg feed ADS1 Se, T4; basal diet + 0.3mg/ kg feed ADS2 Se, T5; basal diet + 0.3mg/ kg feed ADS18 Se

[abc] Means with different letter within a row differed significantly.

Figures

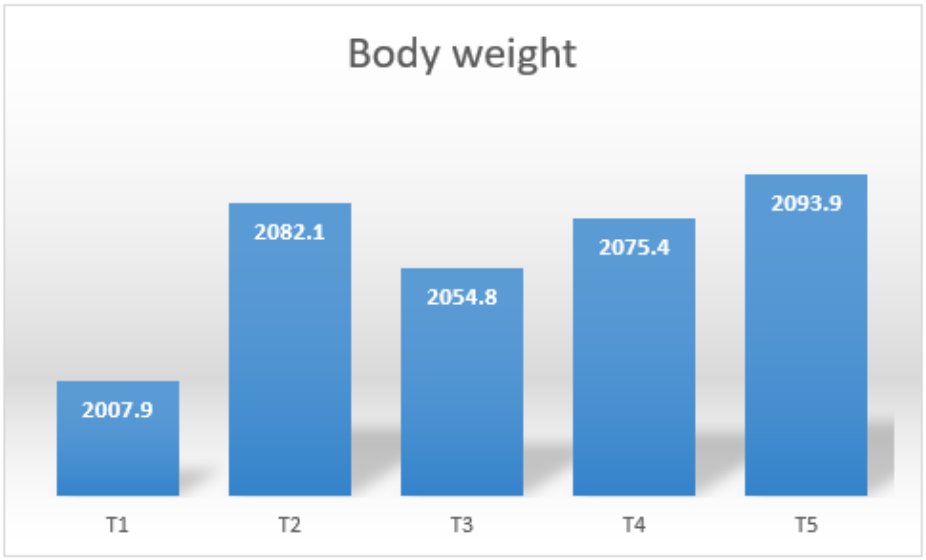

Figure 1

42-days body weight of broiler chicken. Treatments: T1; basal diet, T2 basal diet + 0.3 mg/kg sodium selenite, T3: basal diet + 0.3 mg/kg Se of ADS1, T4; basal diet + 0.3 mg/kg Se of ADS2, T5: basal diet + 0.3 mg/kg Se of ADS18. Bars with no common letter differ significantly ($P < 0.05$).

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