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Effect of organic trace mineral on gene expression of antioxidant enzyme and meat quality responsible enzyme in young chick

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ABSTRACT: Trace minerals play every role that associated with metabolism process and necessary for the growth of animals. In general, mineral premixes are produced in salts form of inorganic minerals to full fill mineral requirement of animal. However, inorganic minerals are relatively low availability in broilers. The organic mineral form has higher availability than the inorganic minerals. It can be used in lower doses without affecting production performance of broilers. This research is aimed to investigate the effects of organic mineral as a total replace to inorganic mineral on growth performance, antioxidant function and meat quality responsible enzyme on gene expression by using real-time PCR. Eight hundred, one day old, male broiler chicks of Ross 308 were divided into 4 dietary treatments including: T1) Positive control (PC) Diet with inorganic premix, T2) PC + organic minerals 500 g/tonne, T3) diet without inorganic Zn, Cu, Mn, Se, I and Fe in premix + organic minerals 750 g/tonne and T4) T3 + selenium yeast 200 g/ton. The trail ran between 1 and 21 days of age. Growth performance was not significantly different between treatments in terms of feed intake and mortality. For body weight gain and ADG, T2 and T1 showed no significant difference. However, these treatments produced higher body weight gain and ADG than T3 and T4 ($P<0.05$). The treatments showed a similar result on feed conversion ratio. The experiments on gene expression, muscle and liver samples immediately collected and perform to quantify the mRNA expression level of gene involved in meat quality and antioxidant function by real-time PCR. Supplementation of broiler diets with organic form of mineral was increased mRNA expression of a gene involved the glutathione redox cycle including glutathione peroxidase and glutathione S-transferase-α ($P<0.05$). Furthermore, organic trace mineral was decreased mRNA expression of a gene involved in meat quality to show that there is an increase in muscle development ($P<0.05$). Blood sample for Antioxidant enzyme measurement was selected form RNA expression measurement. GPx enzyme was measured by enzyme-linked immune sorbent assay (ELISA) kit. The concentration of glutathione peroxidase in antioxidant enzyme measurement was increased same with the result of mRNA expression ($P<0.05$). Thus, replacement of organic trace mineral supplements in broiler diets may be improve growth rate and meat quality for trace mineral supplementation of feed mixtures for poultry.

Keywords: organic trace mineral, broiler, quantitative PCR, antioxidant enzyme

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Introduction

Poultry are susceptible to stress induced as a response to common management practices as severe stressors such as high environmental temperature. These stressors can result in economic costs to producers that are associated with poor growth and disease (Jill et al., 2018). Modern commercial broiler produces more body heat due to their fast metabolism and growth rate, thus they have poor ability to adjust the environment fluctuations makes birds more sensitive to environmental temperature. Modern rapid-growing broiler strains are more susceptible to heat stress and oxidative damages than their ancestors.

Trace minerals are cofactors for cellular enzymes, transcription factors and thus participate in a wide variety of biochemical processes. Trace minerals are involved in the process of immune development and response, tissue and bone development and integrity, protection against oxidative stress, and cellular growth and division. Deficiencies in trace minerals can lead to deficits in any of these processes, as well as reductions in growth performance (James et al., 2010). These are required in the diet in only very small amounts. Trace minerals are the important association with the metabolism process and necessary for the growth of animals. In general, mineral supplements fed in the salt form of inorganic minerals to balance the levels of minerals in the diet (Bao et al., 2007) but inorganic minerals are relatively low utilization in broilers resulting in high doses of minerals in the excretion. The organic minerals are utilization higher than the inorganic minerals and can be used in lower doses than the inorganic minerals without affecting production performance of broilers. However, it can reduce the level of minerals in the excretion and result in mortality rates declined significantly (Nuzback et al., n.d.). In addition, an organic mineral also improves nutrients absorption in the gut improved and reduces the structure of the complex between minerals and other compounds.

Organic zinc led to an improvement in the activity of Cu/Zn superoxide dismutase and glutathione peroxidase (Ma et al., 2011) and reduced oxidative stress and improved some immune responses (Bun et al., 2011). The effect of organic and inorganic selenium on growth, meat quality, and antioxidant properties of broiler meat was studied by Yang et al. (2012). Serum GSH-Px activity in the experimental group was by 155.83% higher than that in the control. This is why it brings the research to study the effect of organic trace mineral on growth response and meat quality of broiler.

This Research is aimed to investigate the effects of the organic mineral total in dietary and inorganic mineral on growth performance, meat quality and gene expression of broilers. In the experiment will use six kinds of trace minerals Including zinc, manganese, copper, iron, selenium, and iodine. The experiments will divide into 2 parts; part1, the live performance during 1-21 DOA will be housed in the evaporative cooling system house. All diet will be corn-soybean meal diet. Part 2, the experiments on gene expression by using real time PCR will perform to quantify the expression level of the gene involved in meat quality and antioxidant function

Materials and Methods

Growth performance during 1-21 DOA

This animal use protocol has been submitted and reviewed by the KASETSART UNIVERSITY Institutional Animal Care and Use Committee (code ID# ACKU60-AGK-009) and found to be in accordance to the guidelines of animal care and use under the Ethical Review Board of the Office of National Research Council of Thailand (NRCT) for the conduction of the scientific research. All chicks were housed in a 12 m × 30 m curtain-sided, evaporative cooling system house with rice husk as litter material. The building house 40 identical pens separated by wire. Eight hundred, one day old, male broiler chicks of Ross 308 were divided into 4 dietary treatments show as **Table 1**. Each treatment consisted of 10 replications with 20 birds per replication. All diets were formulated to be isocaloric and isonitrogenous. Diets were formulated according to Thai industrial standards. All diet was corn-soybean meal diet. The trail was run between 1 and 21 days of age. Experimental diets were formulated and manufactured on the Kasetsart university Kamphaengsean campus. Feed in mash form and water were provided

Ad-libitum throughout the experiment. Twenty-three hours of light and one hour of darkness were provided to the birds throughout the experiment period 21 days. Body weights of all birds were recorded on 1 and 17 day (starter period). From these data, periodically gain in weights of the birds were calculated. The feed offered and the feed refused were recorded periodically for each replicate. On the basic of these data, periodically feed consumption per replicate and per bird were calculated. Feed conversion ratio were calculated. Number of death birds were recorded for mortality calculation.

Table 1 Ingredient corn-soybean meal diet and description of treatment

Ingredient name	**Treatment**			
	T1[1]	**T2[1]**	**T3[1]**	**T4[1]**
corn (%)	51.750	51.750	51.750	51.750
rice-bran oil-crude (%)	1.540	1.540	1.540	1.540
soybean meal 46% (%)	27.350	27.350	27.350	27.350
full fat soybean (%)	15.000	15.000	15.000	15.000
l- lysine (%)	0.190	0.190	0.190	0.190
dl-methionine (%)	0.280	0.280	0.280	0.280
choline chloride 60% (%)	0.003	0.003	0.003	0.003
monodicalciumphosphate21 (%)	1.722	1.722	1.722	1.722
calcium carbonate (%)	1.481	1.481	1.481	1.481
salt (%)	0.409	0.409	0.409	0.409
premix (inorganic minerals) (%)[2]	0.250	0.250	-	-
premix (without inorganic minerals) (%)[3]	-	-	0.250	0.250
organic minerals (Bioplex) (%)[4]	-	0.050	0.075	0.075
selenium yeast (Economase) (%)[5]	-	-	-	0.020
Amount of trace minerals				
iron (ppm)	40.000	47.500	11.250	11.250
copper (ppm)	8.000	10.500	3.750	3.750
zinc (ppm)	60.000	80.000	30.000	30.000
manganese (ppm)	80.000	100.000	30.000	30.000
iodine (ppm)	0.500	2.500	3.000	3.000
selenium (ppm)	0.100	0.250	0.225	0.525

[1]T1 - Positive control (PC) diet with inorganic premix
T2 - PC+ Organic minerals 500 g/tonne
T3 - Diet without inorganic Zn Cu Mn Se I and Fe in premix + Organic minerals 750g/tonne
T4 - T3 + selenium yeast 200 g/ton

[2]premix (inorganic minerals), 1 kg including vitamin A 4,800,000 IU, vitamin D 1,200,000 IU, vitamin E 6,000 IU, vitamin K3 0.6 g, vitamin B1 0.6 g, vitamin B2 2.2 g, vitamin B6 0.8 g, vitamin B12 0.004 g, nicotinic acid 10 g, pantothenic acid 4.8 g, Folic Acid 0.2 g, biotin 0.048 g, iron 16 g, zinc 24 g, copper 3.2 g, manganese 32 g, iodine 0.2 g, selenium 0.04 mg, cobalt 0.04 g

[3]premix (without inorganic minerals), 1 kg including vitamin A 4,800,000 IU, vitamin D 1,200,000 IU, vitamin E 6,000 IU, vitamin K3 0.6 g, vitamin B1 0.6 g, vitamin B2 2.2 g, vitamin B6 0.8 g, vitamin B12 0.004 g, nicotinic acid 10 g, pantothenic acid 4.8 g, Folic Acid 0.2 g, biotin 0.048 g, cobalt 0.04 g

[4]organic minerals (Bioplex), 1 kg including iron 1.5 g, zinc 40 g, copper 5 g, manganese 40 g, iodine 4 g, selenium 300 mg

[5]selenium yeast (Economase), 1 kg including selenium 1.5 g

Gene expression

Bird's sampling collection

The end of the experiment, 2 birds from each replication was euthanized with CO_2 and dissect by a trained team. Muscle and liver sample was immediately collect and divide parts was placed into cryopreservation tubes and then quickly placed into liquid nitrogen (-196°C) for preservation. The divided parts were placed to store at -80 °C prior to analysis.

Total RNA isolation

The total RNA isolated from liver and muscle tissue of five bird form each treatment use the Trizol reagent according to the manufacturer's instruction. The RNA concentration determined by measure ultraviolet light absorbance at 260 nm. Reverse transcription was carried out according to the kit first strand synthesis system for real-time quantitative PCR.

Real-time PCR

Real-time PCR were perform to quantify the mRNA expression level of myogenin (MyoG), myostatin (MSTN), rapamycin (mTOR), ribosomal S6 kinases 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4EBP1) from muscle tissue that involved in meat quality and the expression profiles of genes involved in antioxidant function (Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase-α (GSTα)) from liver tissue. The real-time quantitative PCR reaction was using iTaq™ Universal SYBR® Green Supermix. The real-time quantitative PCR data were analyzed by using the $2^{-\Delta\Delta CP}$ method and β-actin in muscle tissue and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in liver tissue were chosen as a reference to normalize the relative level of mRNA, the run was performing in triplicate. All primer show as **Table 2**.

Table 2 Primer of the gene involved in antioxidant function, meat quality, and housekeeping gene

Gene name	Sequence primer	Product size (bp)	Reference
Catalase	F : GGGGAGCTGTTTACTGCAAG	139	Yarru et al. (2009)
	R : TTTCCATTGGCTATGGCATT		
Superoxide Dismutase	F : AGGGGGTCATCCACTTCC	122	Yarru et al. (2009)
	R : CCCATTTGTGTTGTCTCCAA		
Glutathione peroxidase	F : TTGTAAACATCAGGGGCAAA	140	Yarru et al. (2009)
	R : TGGGCCAAGATCTTTCTGTAA		
Glutathione S-transferase-α	F : GCCTGACTTCAGTCCTTGGT	131	Yarru et al. (2009)
	F : CCACCGAATTGACTCCATCT		
Myogenin	F: GTGGGATGGTGATGCTGGAA	109	Qian et al. (2013)
	R: TTGGAGAGGAGTGGGAAAGGA		
Myostatin	F: GCTTTTGATGAGACTGGACGAG	173	Guernec et al. (2003)
	R: AGCGGGTAGCGACAACATC		
Rapamycin	F :GGTGATGACCTTGCCAAACT	220	Yuan et al. (2015)
	R :CTCTTGTCATCGCAACCTC		
Eukaryotic initiation factor 4E-binding protein 1	F : GCGAATGTAGGTGAAGAAGAG	176	Yuan et al. (2015)
	R : AACAGGAAGGCACTCAAGG		
Ribosomal protein S6 kinase 1	F : CAATTTGCCTCCCTACCTCA	146	Yuan et al. (2015)
	R : AAGGAGGTTCCACCTTTCGT		

Morphometry of villus

At the end of the experiment, 2 birds from each replication was euthanize with CO_2 and dissected by well trained staffs. Jajunum intestine sample was immediately collected and soused in formalin. After that, these tissue samples were sent to a laboratory for preparing permanent slides. The slides were viewed on an Olympus BH-2 microscope and digitised using video image soft- ware. Images were viewed (optical lens No. 4) to measure the crypt depth, villus width at the crypt–villus junction, villus height and villus apical width. Apparent villus surface area was estimated by trigonometry. Ten villi were assessed per sample. Villus height, crypt depth, villus basal width and villus apical width were measured using the ImageJ program.

Statistical Analysis

Analysis of variance all data will be conducted using the ANOVA of R project. Treatment effect will be considered to be significantly different at P<0.05. Variable having a significant F-test will be compared using Duncan's new multiple range test functions of R project.

Results and Discussion

Growth performance was shown in Table 3. Feed intake and average feed intake did not result in significant differences among the treatments including increasing the organic minerals in the normal feed (T2). For body weight gain and average daily gain, T2 and T1 did not differ but both were higher than T3 and T4 (P<0.05). A diet with total replacement of organic mineral (T3) resulted in a worse feed conversion ratio compared to all other treatments (P<0.05) and a decreased average body weight gain and average daily gain. The addition of selenium to the organic diet (T4) brought the growth performance approximately back to the control levels. Previous studies have reported that the use of organic trace minerals as a replacement for inorganic minerals in broiler diets did not affect performance with a possible advantage of reducing mineral (Bao et, al ,2007; Echeverry et al., 2016; Rattanavut and Yamuchi, 2010). These different results are likely caused by the different feeding period considered; Bao et, al (2009) reported that broiler chickens need more minerals in the 14-35 day period than they do in the 1-14 day period.

Table 3 Growth performance of broiler during 1-17 days of age

Parameters	Treatment				SEM	P-value
	T1[1]	T2[1]	T3[1]	T4[1]		
Initial weight (g)	45.58	46.07	45.53	45.60	0.079	0.062
Average body weight gain (g)	714.55^{ab}	722.62^{a}	690.05^{c}	705.66^{b}	3.126	<0.001
Average daily gain, ADG (g/b/d)	42.03^{ab}	42.51^{a}	40.59^{c}	41.51^{b}	0.184	<0.001
Feed intake (g)	858.35	864.10	851.99	850.18	3.298	0.265
Average feed intake (g/b/d)	50.49	50.83	50.12	50.01	0.194	0.266
Feed conversion ratio, FCR	1.20^{b}	1.20^{b}	1.24^{a}	1.21^{b}	0.004	0.001
Mortality (%)	0.00	0.00	0.00	0.00	-	-

a, b, c Mean values in a same row with no common superscripts are significantly different (P<0.05).

[1] T1 - Positive control (PC) diet with inorganic premix

T2 - PC+ organic minerals 500 g/tonne

T3 - Diet without inorganic Zn Cu Mn Se I and Fe in premix + organic minerals 750g/tonne

T4 - T3 + selenium yeast 200 g/ton

As shown in **Table 4**, glutathione S-transferase-α and glutathione peroxidase mRNA levels were enhanced significantly (P<0.05) in the liver tissue of broilers by organic trace mineral supplementation. However, organic trace mineral in the diet did not significantly (P>0.05) increase catalase and superoxide dismutase gene mRNA levels in broilers**.** The superoxide dismutase (SOD) catalyzes the conversion of superoxide anions to hydrogen peroxide is one of the primary enzymatic defenses against ROS. This downregulation in SOD gene expression could result in accumulation of superoxide anions within mitochondria, leading to oxidative stress. Catalase (CAT) is antioxidant enzymes within the peroxisomes and cytosolic, a powerful and potentially harmful oxidizing agent in the water and molecular oxygen. This down-regulation could lead to oxidative stress (Yarru et al., 2009). Gene expression among the 4 treatment groups for SOD and CAT was no statistical difference (P>0.05) because organic trace mineral not affect in the regulatory process of this gene.

As shown in **Table 4**, glutathione S-transferase-α (GSTα) conjugation of reactive xenobiotic metabolites with glutathione is an important step in detoxification and was mediated by GSTα. glutathione peroxidase (GPx) be involved in the conversion of hydrogen peroxide in the water and molecular oxygen. This down-regulation could lead to oxidative stress and selenium is a cofactor for the glutathione peroxidase (Rotruck et al., 1973). The gene expression of broilers using organic trace mineral for GSTα and GPx have increased the expression (P<0.05) because the expression of gene involved the glutathione redox cycle (**Table 4**) depends on the level of selenium. Se regulation of GSTα and GPx depending on which selenoprotein provided the selenocysteine insertion sequence, It has been proposed that a complex, composed of a Se-specific elongation factor, a selenocysteinyl-tRNA and selenocysteine insertion sequence mRNA stemloop structure.The selenoprotein complex is formed to mediate selenocysteine incorporation during translation. When Se is adequate, the formation of this Se-insertion complex could mask a unique endonucleolytic cleavage site resulting in increased mRNA level. (Sherri and Roger, 1997) The result the groups of using organic mineral for GSTα and GPx were increased the expression more than the groups of using inorganic mineral. Suggest that the chicken using organic mineral can be put selenium to use better than the chicken using inorganic mineral.

Table 4 The effect of organic minerals on the gene involved in antioxidant function

Gene name	T1[1]	T2[1]	T3[1]	T4[1]	SEM	P-value
Catalase	1.321±0.869	1.410±0.738	1.370±0.547	1.491±0.453	0.439	0.626
Superoxide Dismutase	1.072±0.318	1.160±0.290	1.111±0.186	1.303±0.219	0.066	0.085
Glutathione S-transferase-α	0.656±0.214^{a}	0.663± 0.136^{a}	1.489±0.831^{b}	1.808±1.133^{b}	0.594	<0.01 **
Glutathione peroxidase	0.532±0.068^{a}	0.578±0.153 ^{a}	1.045±0.264^{ab}	1.542±0.919^{b}	0.233	<0.01 **

a, b, c Mean values in a same row with no common superscripts are significantly different (P<0.05).

[1] T1 - Positive control (PC) diet with inorganic premix

T2 - PC+ organic minerals 500 g/tonne

T3 - Diet without inorganic Zn Cu Mn Se I and Fe in premix + organic minerals 750g/tonne

T4 - T3 + selenium yeast 200 g/ton

As shown in **Table 5**, the T3 and T4 treatments decreased myogenin and myostatin gene significantly (P<0.05). Myogenin is required for the fusion of myogenic progenitor cells to either new or previously existing fibers during the process of differentiation in myogenesis the myogenic progenitor cells firstly proliferate and differentiate extensively, and then decrease due to the number of myoblasts fused into multinucleated myotubes and myofibers, finally the muscle maintained its growth and maturation (Qian et al., 2013). The gene expression of broilers using organic trace mineral for myogenin was decreased expression (P<0.05), suggest that the broiler using organic trace mineral have myogenesis decrease it shows the muscle maintained growth and maturation better than the broiler using inorganic trace mineral.

Myostatin (MSTN), a member of the transforming growth factor-β superfamily, is expressed specifically in the muscle tissue and acts as a negative regulator of muscle development. The targeted invalidation of the myostatin (MSTN) gene has been shown to increase muscle development (Se-Jin and Alexandra, 2001). The gene expression of broilers using organic trace mineral for myostatin was down-regulation expression (P<0.05). Suggest that broiler using organic trace mineral have myostatin decrease it shows the muscle increase development better than the chicken using inorganic trace mineral.

Table 5 The effect of organic minerals on gene involved in meat quality

Gene name	T1[1]	T2[1]	T3[1]	T4[1]	SEM	P-value
Myogenin	1.332±0.931 [a]	0.948±0.537 [ab]	0.718±0.355 [b]	0.478±0.191 [b]	0.314	<0.01 **
Myostatin	1.032±0.457 [a]	0.928±0.399[ab]	0.844±0.441[ab]	0.600±0.229 [b]	0.147	0.0161 *
Rapamycin	0.854±0.371	0.754±0.425	0.742±0.302	0.633±0.323	0.121	0.188
Eukaryotic initiation factor 4E-binding protein 1	1.327±1.446	1.283±1.157	0.841±0.629	0.663±0.680	1.010	0.128
Ribosomal protein S6 kinase 1	0.713±0.298	0.825±0.197	0.637±0.339	0.594±0.238	0.185	0.0725

a, b, c Mean values in a same row with no common superscripts are significantly different (P<0.05).

[1] T1 - Positive control (PC) diet with inorganic premix

T2 - PC+ organic minerals 500 g/tonne

T3 - Diet without inorganic Zn Cu Mn Se I and Fe in premix + organic minerals 750g/tonne

T4 - T3 + selenium yeast 200 g/ton

As shown in **Table 5**, the T3 and T4 treatments did not significantly (P>0.05) decrease rapamycin (mTOR), ribosomal protein S6 kinase 1 gene (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) gene. mTOR or mechanistic target of rapamycin is a member of the phosphatidylinositol 3-kinase-related kinase protein family; it is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, autophagy, transcription. Rapamycin is a highly specific inhibitor of mTOR function; when complexes with its cellular receptor, FKBP12, the 12-kDa FK506-binding protein, is a ubiquitous abundant protein that acts as a receptor for the immunosuppressant drug FK506, binds tightly to intracellular calcium release channels and to the transforming growth factor β (TGF- β) type I receptor. Rapamycin binds directly to TOR to inhibit downstream signaling. mTOR also likely functions in a nutritional checkpoint, as its best characterized downstream targets, ribosomal protein S6 kinase 1 gene (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), are sensitive to amino acid levels and energy status (Yuan et al., 2015). mTOR exists in 2 protein complexes including the mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The protein mTORC1 is rapamycin-sensitive, stimulated by insulin, growth factors, energy signals (ATP level) and amino acids. Rapamycin signaling pathway is attenuated with aging (Huiling et al., 2014). Gene expression among the 4 treatment groups for mTOR, 4E-BP1 and S6K1 were not significantly different (P>0.05) because the 4 treatment groups were not different in the

level of insulin, growth factors, energy signals and amino acids of the feed

Rapamycin is a highly specific inhibitor of mTOR function; when complexes with its cellular receptor, FKBP12, the 12-kDa FK506-binding protein, is a ubiquitous abundant protein that acts as a receptor for the immunosuppressant drug FK506, binds tightly to intracellular calcium release channels and to the transforming growth factor β (TGF- β) type I receptor. Rapamycin binds directly to TOR to inhibit downstream signaling. mTOR also likely functions in a nutritional checkpoint, as its best characterized downstream targets, Ribosomal protein S6 kinase 1 gene (S6K1) and Eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), are sensitive to amino acid levels and energy status (Yuan et al., 2015). mTOR exists in 2 protein complexes including the mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The protein mTORC1 is rapamycin-sensitive, stimulated by insulin, growth factors, energy signals (ATP level) and amino acids. A rapamycin signaling pathway is attenuated with aging (Huiling et al., 2014). Gene expression among the 4 treatment groups for mTOR, 4E-BP1 and S6K1 were not significantly different (P>0.05) shows that, different source of minerals in the diet do not affect to rapamycin, eukaryotic initiation factor 4E-binding protein 1 and ribosomal protein S6 kinase 1 gene which corresponds to the report of Huiling et al. (2014); Yuan et al. (2015); report that rapamycin, eukaryotic initiation factor 4E-binding protein 1 and ribosomal protein S6 kinase 1 stimulated by amino acid levels, energy status, insulin and growth factors.

As shown in **Table 6**, the level of glutathione peroxidase measured by an enzyme-linked immunosorbent assay (ELISA). Level of glutathione was increased (P<0.05) in broiler fed the organic trace mineral compared with broiler fed the inorganic trace mineral because selenium is a cofactor for the antioxidant enzyme glutathione peroxidase (Rotruck et al., 1973). The results are consistent with the result of mRNA expression to showing increased mRNA in the liver tissue of broilers by organic trace mineral supplementation, suggest that the broiler using organic trace mineral have glutathione increase in the level of mRNA and can be translated into the protein, it shows the chicken using organic trace mineral increase the level of glutathione peroxidase enzyme to reduce free hydrogen peroxide resistance against oxidant stress in the broiler.

Table 6 Concentration of glutathione peroxidase

	T1	T2	T3	T4	SEM	P-value
Concentration of glutathion peroxidase (pg/ml)	124.43 ± 74.78 [c]	126.77 ± 84.15 [c]	364.58 ± 261.30 [b]	761.57 ± 473.02 [a]	85195.98	<0.001***

a, b, c Mean values in a same row with no common superscripts are significantly different (P<0.05).

[1] T1 - Positive control (PC) diet with inorganic premix
T2 - PC+ organic minerals 500 g/tonne
T3 - Diet without inorganic Zn Cu Mn Se I and Fe in premix + organic minerals 750g/tonne
T4 - T3 + selenium yeast 200 g/ton

The study of morphometry of villus showed that the increasing the organic mineral in the normal feeds increased (P < 0.005, **Table 7**) the villus height, villus width, villus surface area and villus height/crypt depth ratio compared with controls. However, the crypt depth was decreased with organic mineral supplementation when compared with that of the control. As shown in **Table 7**, the T3 and T4 treatments increased the villus height, villus width, villus surface area and villus height/crypt depth ratio compared with controls, it has been suggested that long villi result in an increased surface area that is capable of greater absorption of available nutrients (Rattanavut and Yamuchi, 2010). Higher villus height /crypt depth ratio has normally been associated with better harmony between cellular turnovers with respect to the height of the villi. However, the crypt depth was decreased by the T3 and T4 treatments compared with control. Lower crypt depth is indicative of a lower cellular turnover of the villi, with decreased demand of nutrients for production and maintenance of intestinal tissues that could lead to improved performance of the chicken (Echeverry et al., 2016).

Table 7 Morphometry of villus

Parameter	Treatment				SEM	P-value
	T1[1]	T2[1]	T3[1]	T4[1]		
Villus height (µm)	1004.66± 192.92[b]	1016.88± 218.04[b]	1084.51± 201.32[a]	1123.03± 112.30[a]	36862.69	<0.001**
Villus width (µm)	87.82± 18.354[bc]	85.57± 21.75[c]	93.94± 19.18[a]	92.340± 12.84[ab]	358.7892	0.0048**
Crypt depth (µm)	201.05± 48.27[b]	213.87± 48.92[a]	161.15± 33.52[c]	150.39± 32.25[c]	1998.945	<0.001**
Villus surface area (mm)	88.84± 28.46[b]	88.42± 33.07[b]	102.25± 29.64[a]	103.50± 21.25[a]	852.4399	<0.001**
Villus height/crypt depth ratio (µm)	5.26± 1.22[c]	4.73± 1.10[d]	6.99± 1.87[b]	7.73± 1.45[a]	2.382764	<0.001**

a, b, c Mean values in a same row with no common superscripts are significantly different (P<0.05).
[1]T1 - Positive control (PC) Diet with inorganic premix
T2 - PC+ Organic minerals 500 g/tonne
T3 - Diet without inorganic Zn Cu Mn Se I and Fe in premix + Organic minerals 750g/tonne
T4 - T3 + Selenium yeast 200 g/ton

Conclusion

In conclusion of mRNA expression, the addition of organic minerals improved the antioxidant status in broiler chickens. The supplement of different amounts of organic minerals in feed did not have negative effects on broiler meat production. The treatments fed by organic mineral increased GSTα and GPx significantly, but not significantly increase SOD and CAT, suggest that the chicken using organic mineral can be increase regulation process of oxidative stress in the broiler. T3 and T4 treatment substantially decreased level of Myogenin and myostatin gen, suggest that the chicken using organic mineral can be increase regulation process of cell growth, proliferation, maturation and protein synthesis in the broiler. For rapamycin (mTOR), ribosomal protein S6 kinase 1 gene (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), all treatments did not significantly decrease. Supplementation of broiler diets with different trace mineral sources did not appreciably affect live performance or carcass and meat quality. However, organic trace mineral seems to increase mRNA expression of gene involved the glutathione redox cycle and Concentration of glutathione peroxidase in Antioxidant enzyme measurement. Furthermore, organic trace mineral seems to decrease mRNA expression of Gene involved in meat quality to show muscle increase development and level of mineral in excreta. Thus, replacement of organic trace mineral supplements in broiler diets may be improve growth rate and meat quality for trace mineral supplementation of feed mixtures for poultry.

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