Effects of Dietary Manganese Methionine Supplementation on Production Performance, Egg Quality, and Serum Biochemical Indices in Laying Hens: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary manganese methionine supplementation on production performance, egg quality, and serum biochemical parameters in laying hens. A total of 480 commercial "Jinghong No. 1" laying hens at 53 weeks of age with similar production performance were randomly allocated into 5 groups, with 6 replicates per group and 16 hens per replicate. The hens were fed a corn-soybean meal basal diet (containing no manganese in the 5% premix). The control group received the basal diet supplemented with 60 mg/kg manganese sulfate (as manganese), while the experimental groups received the basal diet supplemented with 20, 40, 60, or 80 mg/kg manganese methionine (as manganese), respectively. The pre-trial period lasted 1 week, and the formal trial period lasted 9 weeks. The results showed: 1) The average daily feed intake of hens in the 40 mg/kg manganese methionine group was significantly higher than that of the control group ($P<0.05$), increasing by 3.23% compared to the control group; dietary manganese methionine supplementation had no significant effect on laying rate, average egg weight, or feed-to-egg ratio ($P>0.05$). 2) The Haugh unit, eggshell strength, and eggshell thickness in the 60 and 80 mg/kg manganese methionine groups were significantly higher than those in the control group ($P<0.05$), with Haugh unit increasing by 8.42% and 10.33% compared to the control group ($P<0.05$), eggshell strength increasing by 12.13% and 17.05% compared to the control group ($P<0.05$), and eggshell thickness increasing by 8.57% compared to the control group ($P<0.05$); dietary manganese methionine supplementation had no significant effect on albumen height or yolk color ($P>0.05$). 3) The serum uric acid content in the 80 mg/kg manganese methionine group was significantly lower than that in the control group ($P<0.05$), decreasing by 25.12% compared to the control group; dietary manganese methionine supplementation had no significant effect on serum alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities, or calcium, phosphorus, glucose, total protein, and albumin contents (P>0.05), but the serum alkaline phosphatase activity, albumin, and calcium contents in the manganese methionine supplementation groups showed an upward trend compared to the control group. In conclusion, dietary manganese methionine supplementation can improve egg quality, inhibit protein catabolism, and enhance protein utilization efficiency.

Full Text

Effects of Dietary Manganese Methionine on Performance, Egg Quality and Serum Biochemical Parameters of Laying Hens

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Abstract: This experiment was conducted to investigate the effects of dietary manganese methionine on performance, egg quality, and serum biochemical parameters of laying hens. Four hundred and eighty 53-week-old "Jinghong No. 1" commercial laying hens with similar production performance were randomly allocated to 5 groups with 6 replicates per group and 16 hens per replicate. The hens were fed a corn-soybean meal basal diet (without manganese in the 5% premix). The control group received the basal diet supplemented with 60 mg/kg manganese sulfate (as manganese), while the experimental groups received the basal diet supplemented with 20, 40, 60, or 80 mg/kg manganese methionine (as manganese), respectively. The pre-test period lasted for 1 week, and the formal test period lasted for 9 weeks. The results showed: 1) The average daily feed intake of hens in the 40 mg/kg manganese methionine group was significantly higher than that of the control group, increasing by 3.23% (P<0.05). Dietary manganese methionine had no significant effects on laying rate, average egg weight, or feed-to-egg ratio (P>0.05). 2) The Haugh unit, eggshell strength, and eggshell thickness in the 60 and 80 mg/kg manganese methionine groups were significantly higher than those in the control group (P<0.05). Specifically, the Haugh unit increased by 8.42% and 10.33% (P<0.05), eggshell strength increased by 12.13% and 17.05% (P<0.05), and eggshell thickness increased by 8.57% (P<0.05) compared with the control group. Dietary manganese methionine had no significant effects on albumen height or yolk color (P>0.05). 3) The serum uric acid content in the 80 mg/kg manganese methionine group was significantly lower than that in the control group, decreasing by 25.12% (P<0.05). Dietary manganese methionine had no significant effects on serum alkaline phosphatase, glutamic-oxaloacetic transaminase, or glutamic-pyruvic transaminase activities, nor on calcium, phosphorus, glucose, total protein, or albumin contents (P>0.05). However, the activity of alkaline phosphatase and the contents of albumin and calcium in the manganese methionine-supplemented groups showed an increasing trend compared with the control group. In conclusion, dietary manganese methionine can improve egg quality, inhibit protein catabolism, and enhance protein utilization efficiency.

Keywords: manganese methionine; laying hens; performance; egg quality; serum biochemical parameters

Introduction

Manganese (Mn) is an essential trace element with multiple biological functions in livestock and poultry nutrition. It serves as a cofactor for various enzymes and participates in multiple metabolic processes including carbohydrate and lipid metabolism [?], promotes hematopoiesis, and improves reproductive performance [?]. Manganese also plays a crucial role in skeletal development during embryonic and growth periods, and dietary manganese deficiency can lead to abnormal cartilage development, slipped tendon, and tibial chondrodystrophy [?]. Since Wilgus et al. [?] first discovered the relationship between manganese and chick skeletal development in 1937, the role of manganese in animal nutrition has received widespread attention. Xiao et al. [?] reported that dietary manganese deficiency affects eggshell quality, reducing eggshell strength, thickness, and toughness. Hossain et al. [?] found that increasing dietary manganese levels in chickens increased laying rate and egg weight. Chen et al. [?] observed that dietary manganese supplementation significantly increased serum total protein (TP) content and alkaline phosphatase (AKP) activity in broilers.

The NRC (1994) [?] recommends supplementing 60 mg/kg manganese in laying hen diets. However, since the absorption rate of manganese in chickens is only 1-3% [?] while the tolerance level can be as high as 2000 mg/kg, nutritionists have suggested increasing the manganese requirement standard [?]. Currently, manganese is primarily supplemented in diets as inorganic salts such as manganese sulfate and manganese oxide, but the low utilization rate of inorganic manganese not only causes waste but also pollutes soil and water through excretion [?]. In recent years, organic manganese products chelated or complexed with amino acids have attracted attention. These products overcome the disadvantages of inorganic manganese, offering good chemical stability, alleviating antagonism among mineral elements, and providing higher absorption efficiency and biological efficacy [?]. Therefore, this study aimed to investigate the effects of manganese methionine and its supplementation levels on laying hen performance, egg quality, and serum biochemical parameters, providing experimental evidence for guiding production and rational application of new, efficient, and environmentally friendly manganese additives.

Materials and Methods

1.1 Experimental Materials

Manganese methionine used in this experiment was purchased from Alltech Bio-Products Co., Ltd. (Tianjin, China), with a manganese content of 15%. The experiment was conducted at the San Yi Co., Ltd. breeding base in Jiande City, Zhejiang Province. The experimental animals were "Jinghong No. 1" commercial laying hens.

1.2 Experimental Design and Diets

Four hundred and eighty 53-week-old "Jinghong No. 1" commercial laying hens with similar performance were randomly divided into 5 groups with 6 replicates per group and 16 hens per replicate. The pre-test period lasted for 1 week, and the formal test period lasted for 9 weeks. Hens were fed a corn-soybean meal basal diet (without manganese in the 5% premix). The control group received the basal diet supplemented with 60 mg/kg manganese sulfate (as manganese), while the experimental groups received the basal diet supplemented with 20, 40, 60, or 80 mg/kg manganese methionine (as manganese), respectively. Each group received supplemental methionine to ensure a dietary methionine content of 0.34% across all treatments. The basal diet was formulated according to the "Feeding Standard of Chickens" (NY/T33-2004). The composition and nutrient levels of the basal diet are shown in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis), %

Note: 1) The premix provided the following per kg of diet: VA 7,500 IU, VD3 2,500 IU, VE 25 mg, VK3 2.5 mg, VB1 1.5 mg, VB2 4.5 mg, VB6 3 mg, VB12 0.02 mg, niacin 25 mg, folic acid 1.1 mg, calcium pantothenate 8 mg, biotin 0.12 mg, choline chloride 400 mg, Cu 20 mg, Fe 90 mg, Zn 90 mg, I 0.8 mg, Se 0.3 mg. 2) Nutrient levels were calculated values.

1.3 Management

The feeding trial was conducted at the San Yi Co., Ltd. breeding base in Jiande City, Zhejiang Province. A three-tier cage system was used. Feed was provided at 07:00 and 15:00 daily, with the amount adjusted according to residual feed. Hens had ad libitum access to feed and water throughout the experiment. Lighting combined natural and artificial illumination (18:00-23:00, 5 h artificial lighting at 10 lx intensity). Conventional management and immunization procedures were followed, and the chicken house was disinfected regularly.

1.4 Measurements

Performance: Daily records of egg number, egg weight, and soft-shell eggs were kept per replicate. Feed intake was recorded weekly. Laying rate (%), average egg weight (g), average daily feed intake (g/d), and feed-to-egg ratio were calculated for the entire experimental period.

Egg Quality: At the end of the experiment, 6 eggs were randomly selected from each replicate (36 eggs per group) for egg quality determination. Egg quality was measured using a Nippon Denshi DET-6000 egg quality analyzer. Measured parameters included albumen height (mm), yolk color, Haugh unit, eggshell strength (kgf/cm^2), and eggshell thickness (mm).

Serum Biochemical Parameters: At the end of the experiment, 12 hens were randomly selected from each group, fasted for 24 h, weighed, and blood samples were collected. Serum was prepared by centrifugation at 3,000 r/min for 10 min. Serum total protein, albumin (ALB), uric acid (UA), glucose (GLU), calcium (Ca), and phosphorus (P) contents, as well as alkaline phosphatase, glutamic-oxaloacetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT) activities were measured using assay kits purchased from Nanjing Jiancheng Bioengineering Institute, following the manufacturer' s instructions.

1.5 Data Processing

Experimental data were analyzed using one-way ANOVA with SPSS 17.0. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as "mean ± standard error." Differences were considered significant at $P<0.05$.

Results

2.1 Effects of Dietary Manganese Methionine on Performance of Laying Hens

As shown in Table 2 , dietary manganese methionine had no significant effects on laying rate, average egg weight, or feed-to-egg ratio ($P>0.05$). However, the average daily feed intake of hens in the 40 mg/kg manganese methionine group was significantly higher than that of the control group, increasing by 3.23% ($P<0.05$).

Table 2 Effects of dietary manganese methionine on performance of laying hens

Note: In the same row, values with different small letter superscripts indicate significant difference (P<0.05), while values with the same or no superscripts indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of Dietary Manganese Methionine on Egg Quality of Laying Hens

As shown in Table 3 , the Haugh unit, eggshell strength, and eggshell thickness in the 60 and 80 mg/kg manganese methionine groups were significantly higher than those in the control group ($P<0.05$). Specifically, the Haugh unit increased by 8.42% and 10.33% ($P<0.05$), eggshell strength increased by 12.13% and 17.05% ($P<0.05$), and eggshell thickness increased by 8.57% ($P<0.05$) compared with the control group. Dietary manganese methionine had no significant effects on albumen height or yolk color ($P>0.05$).

Table 3 Effects of dietary manganese methionine on egg quality of laying hens

2.3 Effects of Dietary Manganese Methionine on Serum Biochemical Parameters of Laying Hens

As shown in Table 4 , the serum uric acid content in the 80 mg/kg manganese methionine group was significantly lower than that in the control group, decreasing by 25.12% ($P<0.05$). Dietary manganese methionine had no significant effects on serum alkaline phosphatase, glutamic-oxaloacetic transaminase, or glutamic-pyruvic transaminase activities, nor on calcium, phosphorus, glucose, total protein, or albumin contents ($P>0.05$). However, the activity of alkaline phosphatase and the contents of albumin and calcium in the manganese methionine-supplemented groups showed an increasing trend compared with the control group.

Table 4 Effects of dietary manganese methionine on serum biochemical parameters of laying hens

Discussion

3.1 Effects of Dietary Manganese Methionine on Performance of Laying Hens

Venglovská et al. [?] reported that supplementation of 120 mg/kg manganese from sulfate, protein manganese, or glycine manganese in the basal diet had no significant effects on egg production, egg weight, feed intake, or feed conversion ratio in laying hens. Xie et al. [?] found that adding 0, 120, or 240 mg/kg manganese from sulfate or protein manganese to broiler breeder diets did not significantly affect laying rate or egg weight. Yuan et al. [?] also showed that adding 20, 40, or 60 mg/kg manganese sulfate or 20 mg/kg manganese methionine to a basal diet containing 20 mg/kg manganese had no significant effects on laying rate, egg weight, feed intake, or feed conversion ratio at various production stages. The present study found that dietary manganese methionine had no significant effects on laying rate, average egg weight, or feed-to-egg ratio, consistent with the above results, indicating that manganese supplementation level and form have no significant effects on these performance indicators. However, this study found that dietary supplementation with 40 mg/kg manganese methionine significantly increased average daily feed intake, which aligns with Yildiz et al. [?] who reported that organic manganese supplementation at 75 mg/kg significantly increased feed intake in laying hens. This suggests that manganese methionine may promote feed intake. Additionally, the present results showed an upward trend in laying rate and egg weight with manganese methionine supplementation, possibly related to increased feed intake leading to higher energy and protein intake, which promotes material deposition during egg formation. However, Zhan [?] reported that a diet containing 60 mg/kg manganese significantly improved laying rate, and Peng et al. [?] found that dietary manganese level significantly affected laying rate, egg production, broken egg rate, and feed conversion ratio. These discrepancies with our results may be attributed to differences in manganese form and level, chicken breed, and rearing environment.

3.2 Effects of Dietary Manganese Methionine on Egg Quality of Laying Hens

Egg quality measurement is important for assessing the economic value of eggs. Generally, higher albumen height and larger Haugh unit indicate better egg quality [?]. In this study, dietary manganese methionine supplementation had no significant effects on albumen height or yolk color but significantly increased the Haugh unit. This is consistent with Gheisari et al. [?], who reported that dietary supplementation with organic manganese, zinc, and copper significantly improved Haugh unit compared with equivalent doses of manganese, zinc, and copper oxides. However, Yenice et al. [?] found that the form and level of manganese, zinc, copper, and chromium had no significant effects on Haugh unit, and Yang et al. [?] reported that dietary manganese supplementation at 35 mg/kg significantly reduced yolk color. These inconsistent results may be due to differences in chicken breed, diet type, manganese supplementation method, and rearing conditions.

Eggshell strength and thickness are important indicators for evaluating eggshell quality. Improved eggshell quality can reduce economic losses during egg transport and storage. Research suggests that dietary calcium, phosphorus, and vitamin D3 sources and levels play important roles in eggshell quality, while trace elements such as zinc, manganese, and copper can improve eggshell mechanical properties by affecting calcite crystal formation and structure modification [?]. Xiao et al. [?] demonstrated that dietary supplementation with 100 mg/kg organic manganese significantly improved eggshell strength, thickness, and toughness, as well as eggshell microstructure. Venglovská et al. [?] reported that all manganese-supplemented groups (organic or inorganic) significantly increased eggshell thickness, eggshell weight, eggshell percentage, and egg shape index. Yu et al. [?] also found that all organic manganese and zinc groups had significantly higher eggshell strength and thickness than inorganic groups, with the best results observed in the group supplemented with 11 mg/kg organic zinc and 8 mg/kg organic manganese. The present study showed that the 60 and 80 mg/kg manganese methionine groups had significantly higher eggshell strength and thickness compared with the control group (60 mg/kg manganese sulfate), consistent with the above reports and suggesting that manganese methionine improves eggshell quality. The beneficial effect of manganese on eggshell quality may be related to its influence on the content of various matrix proteins involved in eggshell calcification and its promotion of glycosaminoglycan and glucuronic acid synthesis in the eggshell gland [?].

3.3 Effects of Dietary Manganese Methionine on Serum Biochemical Parameters of Laying Hens

Serum total protein and albumin contents are important indicators for evaluating protein anabolism, while uric acid content represents the degree of protein catabolism. In this study, dietary manganese methionine had no significant effects on serum total protein or albumin contents, consistent with Attia et al. [?] who reported that dietary manganese level and form had no significant effects on serum total protein, calcium, or phosphorus contents in laying hens. Other studies have suggested that amino acid manganese supplementation can significantly increase serum total protein content in broilers without significantly affecting serum uric acid content [?]. The present study demonstrated that dietary supplementation with 80 mg/kg manganese methionine significantly reduced serum uric acid content. These results indicate that dietary organic manganese can improve protein utilization efficiency, possibly because organic manganese has higher absorption efficiency than inorganic manganese. Amino acid manganese is generally believed to be absorbed intact and function directly in the body, whereas inorganic manganese enters the body in ionic form and must first bind to proteins before functioning. However, the absorption mechanism and action pathway of manganese methionine remain unclear and require further investigation.

Some studies have suggested that manganese can act as an activator of alkaline phosphatase, and dietary manganese supplementation can increase alkaline phosphatase activity [?]. However, this study found that dietary manganese methionine had no significant effect on serum alkaline phosphatase activity in laying hens. Yang et al. [?] also reported that dietary manganese or manganese-zinc mixtures had no significant effects on serum alkaline phosphatase activity. Although the present study observed an increase in alkaline phosphatase activity with manganese methionine supplementation, the effect was not significant, possibly because activation of alkaline phosphatase requires higher manganese levels. This study also found that dietary manganese methionine had no significant effects on serum calcium, phosphorus, or glucose contents, nor on glutamic-oxaloacetic transaminase or glutamic-pyruvic transaminase activities, consistent with reports by Attia et al. [?] and Wei et al. [?], suggesting that these serum biochemical parameters are not sensitive to dietary manganese level or form.

Conclusion

Dietary supplementation with 60 mg/kg manganese methionine significantly improved Haugh unit, eggshell thickness, and eggshell strength, thereby improving egg quality. When dietary manganese methionine supplementation increased to 80 mg/kg, serum uric acid content was significantly reduced, inhibiting protein catabolism and promoting protein utilization efficiency.

References

[1] Zhang Xuejun. Research progress of manganese in poultry nutrition [J]. China Feed, 2013(1): 27-30, 42.

[2] XIE J J, TIAN C H, ZHU Y W, et al. Effects of inorganic and organic manganese supplementation on gonadotropin-releasing hormone- and follicle-stimulating hormone expression reproductive performance broiler breeder hens [J]. Poultry Science, 2014, 93(4): 959-969.

[3] Zhang Lin, Li Jie, Liu Yanguo. Research progress of manganese in poultry nutrition [J]. Feed Review, 2014(1): 25-28.

[4] RICHARDS J D, ZHAO J M, HARRELL R J, et al. Trace mineral nutrition in poultry and swine [J]. Asian-Australasian Journal of Animal Sciences, 2010, 23(11): 1527-1534.

[5] WILGUS H S, NORRIS L A C, HEUSER G F. The effect of various calcium and phosphorus salts on the severity of perosis [J]. Poultry Science, 1937, 16: 232-237.

[6] XIAO J F, WU S G, ZHANG H Y, et al. Bioefficacy comparison of organic manganese with inorganic manganese for eggshell quality in Hy-Line Brown laying hens [J]. Poultry Science, 2015, 94(8): 1871-1878.

[7] HOSSAIN S M, BERTECHINI A G. Effect of varying manganese and available phosphorus levels in the diet on egg production and eggshell quality of layers [J]. Animal Feed Science and Technology, 1998, 71(3/4): 303-308.

[8] Chen Zhongjian, Lü Lin, Luo Xugang, et al. Effects of different manganese sources on growth performance, carcass performance and serum biochemical indices of broilers [J]. Chinese Journal of Animal Science, 2010, 46(13): 35-38, 71.

[9] NRC. Nutrient requirements of poultry [S]. Washington, D.C.: National Academy Press, 1994.

[10] Li Xiaoli, Lü Lin, Xie Jingjing, et al. Characteristics, influencing factors and molecular mechanisms of manganese absorption in chicken intestine [J]. Chinese Journal of Animal Nutrition, 2013, 25(3): 486-493.

[11] Qiao Fuqiang, Yao Hua, Li Hongquan, et al. Effects of organic manganese and organic chromium on growth performance, carcass traits and lipid metabolism in broilers [J]. Journal of Beijing University of Agriculture, 2007, 22(1): 32-38.

[12] Yang Bin, Cai Huiyi, Liu Guohua, et al. Evaluation of relative bioavailability of amino acid chelated manganese for broilers by slope-ratio assay [J]. Chinese Journal of Animal Nutrition, 2014, 26(8): 2110-2117.

[13] She Yongsheng, Huo Xianxian, Feng Minshan, et al. Effects of different manganese sources on growth performance and tissue manganese content of

broilers [J]. China Poultry, 2010, 32(12): 31-34.

[14] VENGLOVSKÁ K, GREŠÁKOVÁ L, PLACHÁ I, et al. Effects of feed supplementation with manganese from its different sources on performance and egg parameters of laying hens [J]. Czech Journal of Animal Science, 2014, 59(4): 147-155.

[15] Yuan Jianmin, Guo Yuming, Wu Sichao. Effect of dietary manganese level on performance of laying hens [J]. Chinese Journal of Animal Science, 2000, 36(1): 14-16.

[16] YILDIZ A O, CUFADAR Y, OLGUN O. Effects of dietary organic and inorganic manganese supplementation on performance, egg quality and bone mineralisation in laying hens [J]. Revue de Medecine Veterinaire, 2011, 162(10): 482-488.

[17] Zhan Aijun. Effect of adding different manganese levels on performance of laying hens [J]. Veterinary Pharmaceuticals & Feed Additives, 2002, 7(1): 5-6.

[18] Peng Xiuli, Deng Ganzhen, Zhao Hui. Effects of dietary calcium and manganese levels on performance and egg quality of laying hens [J]. Chinese Journal of Veterinary Science, 2002, 22(3): 311-314.

[19] Liang Xin, Yu Xiaoli. Indicators and measurement methods for egg quality evaluation [J]. Shandong Journal of Animal Science and Veterinary Medicine, 2012, 33(5): 23-24.

[20] GHEISARI A A, SANEI A, SAMIE A, et al. Effect of diets supplemented with different levels of manganese, zinc, and copper from their organic or inorganic sources on egg production and quality characteristics in laying hens [J]. Biological Trace Element Research, 2011, 142(3): 557-571.

[21] YENICE E, MIZRAK C, GÜLTEKIN M, et al. Effects of dietary organic or inorganic manganese, zinc, copper and chrome supplementation on the performance, egg quality and hatching characteristics of laying breeder hens [J]. Ankara Universitesi Veteriner Fakultesi Dergisi, 2015, 62(1): 63-68.

[22] YANG X J, ZHONG L L, AN X F, et al. Effects of diets supplemented with zinc and manganese performance related parameters laying hens [J]. Animal Science Journal, 2012, 83(6): 474-481.

[23] ŚWIĄTKIEWICZ S, ARCZEWSKA-WŁOSEK A, KRAWCZYK J, et al. Dietary factors improving eggshell quality: an updated review with special emphasis on microelements and feed additives [J]. World' s Poultry Science Journal, 2015, 71(1): 83-94.

[24] Yu Lihuai, Chen Lianmin, Ding Luoyang, et al. Effects of dietary organic zinc and manganese on eggshell quality and blood biochemical parameters of laying hens during mid-to-late laying period [J]. Chinese Journal of Animal Nutrition, 2015, 27(10): 3041-3046.

[25] Xiao Junfeng. Effects of dietary manganese source and level on eggshell quality of laying hens and its mechanism [D]. PhD Thesis. Beijing: Chinese Academy of Agricultural Sciences, 2014: 87.

[26] XIAO J F, ZHANG Y N, WU S G, et al. Manganese supplementation enhances the synthesis of glycosaminoglycan in eggshell membrane: a strategy to improve eggshell quality in laying hens [J]. Poultry Science, 2014, 93(2): 380-388.

[27] ATTIA Y A, QOTA E M, BOVERA F, et al. Effect of amount and source of manganese and/or phytase supplementation on productive and reproductive performance and some physiological traits of dual purpose cross-bred hens in the tropics [J]. British Poultry Science, 2010, 51(2): 235-245.

[28] Luo Xugang, Su Qi, Huang Junchun, et al. Effects of different dietary manganese levels on growth, leg disease incidence, and some plasma biochemical and immune parameters in broilers [J]. Chinese Journal of Animal Science, 1991(1): 12-15.

[29] Wei Maolian, Yang Weiren, Zhang Guiguo, et al. Effects of manganese supplementation levels on serum biochemical parameters, metatarsal manganese and iron contents, and apparent digestibility of manganese and iron in weaned piglets [J]. Chinese Journal of Animal Nutrition, 2015, 27(2): 551-558.

Note: Figure translations are in progress. See original paper for figures.