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Effects of Alfalfa Polysaccharide on Production Performance and Egg Quality of Laying Hens under High-Temperature Summer Conditions: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary alfalfa polysaccharide supplementation on the productive performance and egg quality of laying hens under summer heat stress conditions. A total of 540 Hy-Line Brown laying hens at 156 days of age were randomly divided into 6 groups with 6 replicates per group and 15 hens per replicate. The control group was fed a basal diet, while the treatment groups were fed experimental diets supplemented with 250, 500, 1,000, 2,000, and 4,000 mg/kg alfalfa polysaccharide in the basal diet, respectively. The experimental period lasted for 6 weeks. The results showed that, compared with the control group: 1) dietary supplementation with different levels of alfalfa polysaccharide had no significant effects on average egg weight, average daily feed intake, rate of defective eggs, or mortality and culling rate ($P>0.05$), whereas supplementation with 500, 1,000, and 4,000 mg/kg alfalfa polysaccharide significantly increased the laying rate of hens ($P<0.05$), and supplementation with 500, 1,000, 2,000, and 4,000 mg/kg alfalfa polysaccharide significantly decreased the feed-to-egg ratio ($P<0.05$); 2) dietary supplementation with different levels of alfalfa polysaccharide had no significant effects on albumen height or Haugh unit ($P>0.05$); the 250 mg/kg alfalfa polysaccharide group showed significantly improved yolk color at the ends of weeks 4 and 6 ($P<0.05$); the 500 mg/kg alfalfa polysaccharide group exhibited a significantly reduced egg shape index at the end of week 6 ($P<0.05$) and significantly improved yolk color at the ends of weeks 1, 4, and 6 ($P<0.05$); the 1,000 mg/kg alfalfa polysaccharide group showed significantly deepened shell color at the ends of weeks 4 and 6 ($P<0.05$), significantly increased shell strength and shell thickness at the ends of weeks 1, 2, and 4 ($P<0.05$), a significantly reduced egg shape index at the ends of weeks 1 and 6 ($P<0.05$), and significantly improved yolk color at the ends of weeks 1, 2, 4, and 6 ($P<0.05$); the 2,000 mg/kg alfalfa polysaccharide group exhibited significantly increased shell thickness at the ends of weeks 1 and 4 (P<0.05), a significantly reduced egg shape index at the end of week 6 (P<0.05), and significantly improved yolk color at the ends of weeks 1, 4, and 6 (P<0.05); the 4,000 mg/kg alfalfa polysaccharide group showed significantly deepened shell color and significantly increased shell strength at the end of week 4 (P<0.05), significantly increased shell thickness at the ends of weeks 1 and 4 (P<0.05), a significantly reduced egg shape index at the end of week 6 (P<0.05), and significantly improved yolk color at the ends of weeks 1, 4, and 6 (P<0.05). These results indicate that under summer heat stress conditions, dietary supplementation with appropriate levels of alfalfa polysaccharide can effectively alleviate heat stress in laying hens, improve their productive performance, and enhance egg quality, with an optimal supplementation level of 1,000 mg/kg.

Full Text

Effects of Alfalfa Polysaccharides on Performance and Egg Quality of Laying Hens under High Temperature Environment in Summer

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Abstract

This experiment was conducted to investigate the effects of dietary alfalfa polysaccharides (APS) on the performance and egg quality of laying hens under high temperature conditions in summer. A total of 540 Hy-Line Brown laying hens aged 156 days were randomly divided into 6 groups with 6 replicates per group and 15 hens per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 250, 500, 1,000, 2,000, and 4,000 mg/kg APS, respectively. The trial lasted for 6 weeks. The results showed that, compared with the control group: 1) dietary supplementation with different levels of APS had no significant effects on average egg weight, average daily feed intake, unqualified egg rate, or mortality rate (P>0.05). However, supplementation with 500, 1,000, and 4,000 mg/kg APS significantly increased egg production rate (P<0.05), and supplementation with 500, 1,000, 2,000, and 4,000 mg/kg APS significantly decreased feed-to-egg ratio (P<0.05). 2) Different levels of APS supplementation had no significant effects on albumen height or Haugh unit (P>0.05). The 250 mg/kg APS group showed significantly improved yolk color at the ends of weeks 4 and 6 (P<0.05). The 500 mg/kg APS group exhibited significantly reduced egg shape index at the end of week 6 (P<0.05) and significantly increased yolk color at the ends of weeks 1, 4, and 6 (P<0.05). The 1,000 mg/kg APS group demonstrated significantly improved eggshell color at the ends of weeks 4 and 6 (P<0.05), significantly increased eggshell strength and thickness at the ends of weeks 1, 2, and 4 (P<0.05), significantly decreased egg shape index at the ends of weeks 1 and 6 (P<0.05), and significantly increased yolk color at the ends of weeks 1, 2, 4, and 6 (P<0.05). The 2,000 mg/kg APS group showed significantly increased eggshell thickness at the ends of weeks 1 and 4 (P<0.05), significantly decreased egg shape index at the end of week 6 (P<0.05), and significantly increased yolk color at the ends of weeks 1, 4, and 6 (P<0.05). The 4,000 mg/kg APS group exhibited significantly improved eggshell color and strength at the end of week 4 (P<0.05), significantly increased eggshell thickness at the ends of weeks 1 and 4 (P<0.05), significantly decreased egg shape index at the end of week 6 (P<0.05), and significantly increased yolk color at the ends of weeks 1, 4, and 6 (P<0.05). These results indicate that appropriate APS supplementation can effectively alleviate heat stress and improve both the performance and egg quality of laying hens under summer high temperature conditions, with the optimum supplementation level being 1,000 mg/kg.

Keywords: alfalfa polysaccharides; laying hens; high temperature; performance; egg quality

Introduction

Global warming has increasingly drawn attention to the hazards of high temperature on the poultry industry in recent years. Poultry possess thick plumage but lack well-developed sweat glands, resulting in very poor heat dissipation capacity. Heat stress induced by high temperature not only leads to decreased egg production and egg weight [1], but also causes deterioration in egg quality [2], and can even result in mortality in severe cases. High temperature causes substantial economic losses to the laying hen industry and constrains its development in China. Alleviating the harm caused by high temperature to poultry can reduce production losses and generate considerable economic benefits.

Plant polysaccharides are bioactive substances widely present in plants that can be extracted and purified. They exhibit multiple biological functions including antioxidant [3-4], immunomodulatory [5], antitumor [6-7], and anti-infective activities [8]. Research on plant polysaccharides for alleviating the damage caused by high temperature environments to animals is increasing. For example, Atractylodes macrocephala polysaccharides can reduce heat stress damage in chicks by regulating cytokines and antioxidant enzyme activities [9], Lycium barbarum polysaccharides can significantly reduce apoptosis of reproductive cells in rats under heat stress [10], and alfalfa polysaccharides (APS) can significantly improve the performance and antioxidant status of heat-stressed rabbits [11]. As a bioactive plant polysaccharide, APS holds potential value for development and application in animal production. However, no studies have reported the effects of APS application in laying hens under high temperature conditions. Therefore, this experiment was conducted to investigate the effects of APS supplementation in a corn-soybean meal basal diet on the performance and egg quality of laying hens under high temperature, providing an effective method to reduce production losses caused by high temperature and offering a scientific basis for the rational application of APS in laying hen production.

Materials and Methods

1.1 Experimental Material First-cut alfalfa at the budding stage (purchased from Daqingshan Breeding Cooperative in Tumd Zuoqi, Inner Mongolia) was used as the experimental material. The alfalfa was cut into 60 cm segments, air-dried, and then subjected to water extraction, alcohol precipitation, and deproteinization to obtain crude APS extract. The APS content in the extract was determined to be 22.71% using the phenol-sulfuric acid method.

1.2 Experimental Animals and Management A single-factor completely randomized design was employed. A total of 540 healthy 156-day-old Hy-Line Brown laying hens were randomly allocated to 6 groups with 6 replicates per group and 15 hens per replicate. There were no significant differences in body weight or egg production rate among groups ($P>0.05$). The control group was fed the basal diet, while the experimental groups were fed the basal diet supplemented with 250, 500, 1,000, 2,000, and 4,000 mg/kg APS, respectively. The experiment lasted for 6 weeks. The basal diet was formulated according to the nutrient requirements for laying hens specified in NRC (1994). The composition and nutrient levels of the basal diet are presented in Table 1 .

The feeding trial was conducted during July-August 2015 (the hot season) at the Changping Experimental Base of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences. The maximum temperature inside the chicken house reached 35.0 °C, the minimum temperature was 28.1 °C, and the average temperature was 31.8 °C, with relative humidity ranging from 51% to 79%. Hens were housed in three-tier battery cages with 3 hens per cage. Feed was provided three times daily with ad libitum access to feed and water. Natural lighting was supplemented with artificial light to maintain a constant 16-hour photoperiod (controlled by a lighting program controller). Natural ventilation was used, and manure was removed every 2 days to maintain cleanliness. Flock condition was observed daily, and house temperature was recorded.

1.3 Measurement Indices 1.3.1 Performance Indices

During the experimental period, daily total egg number, egg weight, unqualified egg number, and mortality were recorded per replicate. Feed consumption was recorded weekly per replicate to calculate average daily feed intake, egg production rate, average egg weight, feed-to-egg ratio, unqualified egg rate, and mortality rate.

1.3.2 Egg Quality Indices

At the ends of weeks 1, 2, 3, 4, and 6, all eggs produced on that day were collected for egg quality determination within 8 hours. Eggshell color was measured using a QCR eggshell colorimeter (TSS, UK). Eggshell strength was determined using a Model- eggshell force gauge (Robotmation, Japan). Eggshell thickness was measured using a Model P-1 eggshell thickness gauge (Ozaki MFG, Japan). Egg shape index was determined using an FHK egg shape index gauge (FUJIHIRA INDUSTRY, Japan). Albumen height, Haugh unit, and yolk color were measured using an EMT-2500 egg quality tester (Robotmation, Japan).

1.4 Statistical Analysis Data were analyzed using the ANOVA procedure of SAS 9.3 software. Duncan' s multiple range test was used for intergroup comparisons, with significance level set at $P<0.05$. Results were expressed as "mean ± standard deviation." Egg production rate, unqualified egg rate, and mortality rate were subjected to arcsine transformation before analysis.

Results

2.1 Effects of Alfalfa Polysaccharides on Performance of Laying Hens under High Temperature As shown in Table 2 , after 6 weeks of feeding, dietary supplementation with 500, 1,000, and 4,000 mg/kg APS increased egg production rate by 7.76%, 11.48%, and 8.36%, respectively, compared with the control group ($P<0.05$). Furthermore, the 1,000 mg/kg APS group exhibited significantly higher egg production rate than the 250 and 2,000 mg/kg APS groups ($P<0.05$).

Compared with the control group, dietary supplementation with 500, 1,000, 2,000, and 4,000 mg/kg APS decreased feed-to-egg ratio by 5.91%, 8.37%, 5.42%, and 7.88%, respectively ($P<0.05$). Additionally, the feed-to-egg ratio in the 1,000 and 4,000 mg/kg APS groups was significantly lower than that in the 250 mg/kg APS group ($P<0.05$).

Dietary supplementation with different levels of APS had no significant effects on average egg weight, average daily feed intake, unqualified egg rate, or mortality rate compared with the control group ($P>0.05$).

2.2 Effects of Alfalfa Polysaccharides on Egg Quality of Laying Hens under High Temperature 2.2.1 Eggshell Color

As shown in Table 3 , dietary supplementation with 1,000 mg/kg APS significantly deepened eggshell color at the ends of weeks 4 and 6 compared with the control group ($P<0.05$). Supplementation with 4,000 mg/kg APS significantly deepened eggshell color at the end of week 4 ($P<0.05$). Among the experimental groups, eggshell color in the 1,000 and 4,000 mg/kg APS groups was significantly darker than that in the 250 mg/kg APS group at the end of week 6 ($P<0.05$).

2.2.2 Eggshell Strength

As shown in Table 4 , dietary supplementation with 1,000 mg/kg APS significantly increased eggshell strength at the ends of weeks 1, 2, and 4 compared with the control group ($P<0.05$). Supplementation with 4,000 mg/kg APS significantly increased eggshell strength at the end of week 4 ($P<0.05$). Among the experimental groups, eggshell strength in the 1,000 mg/kg APS group was significantly higher than that in the 250 mg/kg APS group at the ends of weeks 1 and 2 ($P<0.05$), and significantly higher than that in the 500 mg/kg APS group at the end of week 2 ($P<0.05$).

2.2.3 Eggshell Thickness

As shown in Table 5 , dietary supplementation with 1,000 mg/kg APS significantly increased eggshell thickness at the ends of weeks 1, 2, and 4 compared with the control group ($P<0.05$). Supplementation with 2,000 and 4,000 mg/kg APS significantly increased eggshell thickness at the ends of weeks 1 and 4 ($P<0.05$). Among the experimental groups, eggshell thickness in the 1,000 mg/kg APS group was significantly higher than that in the 250 mg/kg APS group at the ends of weeks 1 and 2 ($P<0.05$). At the end of week 1, eggshell thickness in both the 2,000 and 4,000 mg/kg APS groups was significantly higher than that in the 250 mg/kg APS group ($P<0.05$). At the ends of weeks 2 and 4, eggshell thickness in the 4,000 mg/kg APS group was significantly higher than that in the 250 and 500 mg/kg APS groups ($P<0.05$).

2.2.4 Egg Shape Index

As shown in Table 6 , dietary supplementation with 1,000 mg/kg APS significantly decreased egg shape index at the ends of weeks 1 and 6 compared with the control group ($P<0.05$). Supplementation with 500, 2,000, and 4,000 mg/kg APS significantly decreased egg shape index at the end of week 6 ($P<0.05$). Among the experimental groups, egg shape index in the 500, 1,000, 2,000, and 4,000 mg/kg APS groups was significantly lower than that in the 250 mg/kg APS group at the ends of weeks 1 and 6 ($P<0.05$). At the end of week 6, egg shape index in the 4,000 mg/kg APS group was significantly lower than that in the 500, 1,000, and 2,000 mg/kg APS groups ($P<0.05$). At the end of week 4, egg shape index in the 1,000 and 4,000 mg/kg APS groups was significantly lower than that in the 2,000 mg/kg APS group ($P<0.05$).

2.2.5 Albumen Height and Haugh Unit

As shown in Table 7 and Table 8 , there were no significant differences in albumen height or Haugh unit among all groups during the experimental period ($P>0.05$).

2.2.6 Yolk Color

As shown in Table 9 , dietary supplementation with 1,000 mg/kg APS significantly increased yolk color at the ends of weeks 1, 2, 4, and 6 compared with the control group ($P<0.05$). Supplementation with 500, 2,000, and 4,000 mg/kg APS significantly increased yolk color at the ends of weeks 1, 4, and 6 ($P<0.05$). Supplementation with 250 mg/kg APS significantly increased yolk color at the ends of weeks 4 and 6 ($P<0.05$). Among the experimental groups, yolk color in the 500, 1,000, 2,000, and 4,000 mg/kg APS groups was significantly higher than that in the 250 mg/kg APS group at the end of week 1 ($P<0.05$). At the end of week 2, yolk color in the 1,000 and 2,000 mg/kg APS groups was significantly higher than that in the 250 mg/kg APS group ($P<0.05$).

Discussion

3.1 Effects of Alfalfa Polysaccharides on Performance of Laying Hens under High Temperature The optimal temperature for laying hen production is generally considered to be 19-22 °C [12]. Heat stress may occur when temperatures exceed this range. High temperature significantly harms laying hen performance, causing declines in both egg production rate and average egg weight [13-14]. Reduced feed intake is one of the primary factors affecting performance under high temperature conditions. Song et al. [15] investigated the gene expression of appetite-regulating peptides in heat-stressed laying hens and found that heat stress upregulated the expression of ghrelin genes in the glandular stomach and jejunum. Ghrelin is a hypothalamic neuropeptide that reduces appetite, which may be a key factor underlying the decreased feed intake in heat-stressed hens. In commercial laying hen production, feed intake decreases by 1.6% for every 1 °C increase in temperature within the 21-30 °C range, and by 4.6% for every 1 °C increase within the 32-38 °C range [16]. Reduced feed intake suppresses functional ovarian activity because the pituitary gland cannot produce or release gonadotropins, which are essential for promoting follicular growth and ovulation [17]. Additionally, decreased feed intake leads to reduced numbers of large yellow follicles and small white follicles, as well as ovarian defects [18]. Small white follicles and ovarian stroma are the primary sources of estradiol [19]. Rozenboim et al. [1] and Li Yongzhu et al. [20] found that high temperature significantly decreased plasma progesterone levels in laying hens. Egg production is closely related to reproductive hormones including progesterone, follicle-stimulating hormone, luteinizing hormone, and estradiol, and high temperature disrupts the secretion of these hormones [18].

Plant polysaccharides can enhance reproductive hormone secretion in animals [21-22], and numerous studies have reported their beneficial effects on animal performance. Yang Qiuxia et al. [23] reported that dietary supplementation with different doses of Astragalus polysaccharides significantly decreased feed-to-egg ratio and improved apparent digestibility of crude protein and calcium in laying hens. Chen Qiang et al. [24] found that dietary supplementation with 800 mg/kg Astragalus polysaccharides significantly increased average body weight, average daily feed intake, and average daily gain, while significantly decreasing feed-to-gain ratio in broilers. Liang Ying et al. [25] observed that appropriate dietary supplementation with Scutellaria baicalensis polysaccharides improved broiler performance, with 200 mg/kg supplementation significantly increasing average body weight and average daily gain at 49 days of age while decreasing feed-to-gain ratio. Liu Qingxue et al. [26] reported that dietary supplementation with 500 mg/kg APS significantly increased average body weight and average daily gain while decreasing feed-to-gain ratio in broilers. However, no studies have investigated the effects of APS on laying hen performance under high temperature conditions. The present results demonstrate that under summer high temperature conditions, dietary supplementation with 500, 1,000, 2,000, and 4,000 mg/kg APS significantly decreased feed-to-egg ratio, indicating that appropriate

APS supplementation can improve feed conversion efficiency. Additionally, supplementation with 500, 1,000, and 4,000 mg/kg APS significantly increased egg production rate, although average daily feed intake increased slightly without significant differences. Since APS did not enhance egg production by increasing feed intake, it may regulate reproductive hormone levels through alternative pathways, such as reducing heat stress damage via anti-infective, antioxidant, and immunomodulatory effects, or by increasing beneficial gastrointestinal microbiota. Improved feed conversion efficiency indicates that APS can enhance the effective utilization of dietary nutrients, which represents another important reason for the increased egg production rate.

3.2 Effects of Alfalfa Polysaccharides on Egg Quality of Laying Hens under High Temperature Accurate evaluation of eggshell quality requires measurement of multiple indices, including eggshell color, strength, thickness, and shape index. Eggshell quality is influenced by various factors such as genetics, nutrition, age, and environment. Although eggshell color is not an intrinsic indicator of egg quality, some consumers prefer brown-shelled eggs over white-shelled ones. The pigments in brown eggshells mainly consist of protoporphyrin-IX, biliverdin-IX, and zinc chelates. Protoporphyrin-IX is synthesized de novo by epithelial cells of the shell gland rather than derived from senescent red blood cells, while biliverdin-IX is produced from heme degradation in aging erythrocytes [27]. These three pigments mix in different proportions to create colors ranging from purplish-blue to olive-green [28], and they are distributed throughout the eggshell, with pigment deposition occurring relatively late in the shell formation process [29]. Premature oviposition may result in lighter eggshell color [30]. Furthermore, the intensity of brown color depends on pigment content associated with the cuticle, which begins to deposit on the eggshell approximately 90 minutes before oviposition when shell deposition reaches a stable high level [28]. The present results show that dietary supplementation with 1,000 and 4,000 mg/kg APS significantly improved eggshell color, with 1,000 mg/kg being the more effective dosage.

Eggshell strength is a crucial indicator reflecting egg breakage resistance and shell compactness, and serves as the primary index for evaluating eggshell quality, playing an important role in egg storage, packaging, and transportation. Calcium content is a key factor affecting eggshell strength and is related to deposition time in the uterus. Numerous studies have demonstrated that plant polysaccharides can regulate intracellular calcium ion concentrations [31-32]. Eggshell thickness was one of the earliest indices used to evaluate eggshell quality and remains a primary indicator for assessing eggshell quality [33]. The present results indicate that under summer high temperature conditions, dietary supplementation with 1,000 and 4,000 mg/kg APS significantly improved eggshell strength and thickness, while 2,000 mg/kg APS also significantly increased eggshell thickness, with 1,000 mg/kg APS showing the best effect. Eggshell color, strength, and thickness are highly correlated; generally, eggshell strength and thickness are positively correlated with color intensity. Ingram et al. [34]

found a significant low correlation between eggshell color and thickness. Yang et al. [35] also reported significant correlations between eggshell color and strength, weight, and thickness, with correlation coefficients of -0.262, -0.255, and -0.443, respectively, indicating that darker eggshell color is associated with greater strength, weight, and thickness. These findings are consistent with the results of the present study.

The improvement in eggshell quality by APS supplementation under high temperature conditions may be attributed to two aspects: 1) APS may regulate pigment secretion processes and adjust pigment ratios to affect eggshell color, and may also regulate calcium ion concentration to increase calcium availability for shell formation, thereby enhancing eggshell strength and thickness; 2) APS may prolong the eggshell formation process, allowing more time for pigment and calcium deposition, resulting in darker eggshell color and greater strength and thickness.

Egg shape index is another indicator for evaluating eggshell quality. Specific chicken breeds have characteristic egg shape indices, which are important for packaging and transportation. Appropriate egg shape index can reduce breakage and cracked eggs and may influence consumer preference. The present results show that under summer high temperature conditions, dietary supplementation with 1,000 mg/kg APS significantly decreased egg shape index at the ends of weeks 1 and 6, and supplementation with 500, 2,000, and 4,000 mg/kg APS also significantly decreased egg shape index at the end of week 6. This indicates that APS supplementation affects egg shape index, making eggs tend to be smaller and rounder. Generally, rounder eggs have stronger shells [27], which is supported by the significantly higher eggshell strength observed in the 1,000 and 4,000 mg/kg APS groups compared with the control group in this study.

Internationally, egg freshness is evaluated using biological indices such as Haugh unit, yolk index, air cell height, albumen height, and whole egg density [36]. Haugh unit is calculated based on egg weight and albumen height; generally, greater albumen height corresponds to higher Haugh unit, thicker albumen, and better egg quality. This study used albumen height and Haugh unit to assess egg freshness and found that APS supplementation had no significant effects on these parameters under high temperature conditions. Albumen height and Haugh unit are affected by various factors including storage time and temperature, age, disease, and medication. Previous studies have shown that plant polysaccharides can improve albumen height and Haugh unit [37-39], but the present results are not consistent with these findings, possibly due to differences in polysaccharide source, supplementation level, and environmental conditions.

Yolk color intensity depends on the amount and type of carotenoids and other pigments consumed by poultry from the diet. Poultry cannot synthesize carotenoids for yolk color formation and must obtain them from dietary sources. Excessive dietary fat can destroy pigment structure and impair its coloring function. Additionally, heavy metal ions and unsaturated fatty acids in the diet can cause oxidation, which readily oxidizes lutein and diminishes its coloring capacity, resulting in lighter yolk color [40]. The present results demonstrate that under summer high temperature conditions, dietary supplementation with 250, 500, 1,000, 2,000, and 4,000 mg/kg APS all significantly improved yolk color, with 1,000 mg/kg being the most effective dosage. Since there were no significant differences in average daily feed intake among groups, APS did not enhance yolk color by increasing feed consumption. The improvement may be attributed to the antioxidant activity of APS, which prevents lutein oxidation and thereby increases pigment deposition and yolk color intensity.

Conclusions

1. Under summer high temperature conditions, dietary supplementation with 500, 1,000, and 4,000 mg/kg APS can increase egg production rate and decrease feed-to-egg ratio in laying hens, with the optimal supplementation level being 1,000 mg/kg.
2. Under summer high temperature conditions, appropriate dietary APS supplementation can significantly improve eggshell color, strength, thickness, and yolk color, while reducing egg shape index, but has no significant effects on albumen height or Haugh unit, with the optimal supplementation level being 1,000 mg/kg.
3. In summary, dietary APS supplementation can alleviate heat stress in laying hens under summer high temperature conditions, with a recommended supplementation level of 1,000 mg/kg.

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