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ORIGINAL ARTICLES

Influence of Different Levels of Astragalus Root Powder in Broiler Chick Diets on the Physiological and Biochemical Changes.

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ABSTRACT

This experiment was conducted to investigate the effects of *Astragalus membranaceus* root powder (AMRP) supplementation on growth performance, physiological and serum indices, antioxidant and immunity status, immune organ weights, serum mineral profile and carcass characteristics of broiler chicks. One hundred eighty one-week-old Cabb500 broilers were randomly divided into 4 groups with 3 replicates in each group with15 broiler chicks in each replicate. The experiment lasted for 5 weeks after start. The basal diets were supplemented with 0, 100, 200 and 300 mg AMRP /kg feed, respectively. The achieved results indicated that addition of AMRP to broiler diets had no significant effect on body weight and feed conversion but dressing weight and absolute immune organ weights were significantly increased for treated groups as compared to control group. Also, supplementation of AMRP to broiler diets had no significant impact on thermoregulation parameters except that respiration rate of group fed 300mg AMRP/kg diet (T4) which was significantly lower compared to the control group. The obtained results showed that addition of AMRP to broiler diets decreased significantly serum ALT, AST, AP, creatinine enzymes, (liver and kidney functions) and antioxidant and reduced the oxidation enzyme levels. Chicks treated with different levels of AMRP in diet showed significant increases in serum total protein, albumin, A/G ratio and immunoglobulin type G compared to untreated one, these results reflect the beneficial effect of AMRP to improve immunity status. While, values of serum total cholesterol and triglyceride for treated groups were significantly reduced compared to the control group. Treatment of broiler chicks with AMRP enhanced nutrients metabolism by increased the secretion of T3 and T4 hormones more than the control group. Moreover, incorporation of AMRP in broiler diets improved the absorption of serum minerals measured in this study. Finally, addition of AMRP is recommended to broiler diets up to 300mg/kg diet without any adverse effects.

Key words: Antioxidant enzymes, Astragalus, Biochemical, Broiler chicks, Immunoglobulin G, Physiological, Thyroid hormones.

Introduction

Poultry meat is one of the most important sources of animal protein in the world at the present. As the world population continue to increase, the need for poultry meat across the world will increase as well. Infections caused by pathogenic microorganisms such as Eimeria, Salmonella, Clostridium etc continue to threaten the poultry industry. Such infections are responsible for reduced growth rates and consequent economic losses in poultry investments. Antibiotics as growth promoters are used to treat infected chickens. Unfortunately, the long term and extensive use of antibiotics for veterinary and productivity purposes may ultimately result in selection for the survival of resistant microbial species, thereby posing a threat to both animal and human health. Consequently, some countries have restricted the use of antibiotics as growth promoters in poultry (Ohimain and Ofongo, 2012). Also, with increasing concerns about antibiotics resistance, the ban on subtherapeutic antibiotics usage in Europe and the potential for a ban in the United States, there is increasing interest in finding alternatives to antibiotics for poultry production *(*Patterson and Burkholder, 2003; Windisch *et al.,* 2008).

The increasing restriction of synthetic compounds as growth-promoting agents (such as antibiotics) in livestock production has encouraged the use of natural plant-derived compounds as alternative feed additives instead of antibiotics (Jamroz *et al.,* 2003; Hernandez *et al.,* 2004; Naidoo *et al.,* 2008). However, researchers have shown that efficacy of phytogenic feed additives in enhancing nutrients metabolism and productivity varies greatly depending on their origin and dosage, animal species, and the feeding management (Barreto *et al.,* 2008; Willis *et al.,* 2008; Tang *et al.,* 2011). Some plant polysaccharides, as complex carbohydrates, were reported as having prebiotic effects (Cummings and Macfarlane, 2002). Recently, medicinal and herbal plants are taking

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biggest importance as alternatives of antibiotics. Natural medicinal products from herbal and medicinal plants, especially polysaccharides, originating from fungi and herbs have been used as feed additives for farm animals for centuries and have shown antimicrobial activities, immune enhancement and stress reduction (Xue and Meng, 1996; Chen *et al.,* 2003; Chen, 2006).

Astragalus *membranaceus* is one of the important components of herbal medicine. It has been reported that Astragalus root contains varieties of chemical compounds including isoflavonoid, saponins, polysaccharides, g-aminobutyric acid, and various trace minerals. It was reported that Astragalus membranaceus contains a relatively high concentration of glucan (Fang, 1988). Although most of them possess varying pharmaceutical and biological activities, such as antioxidant, antimicrobial, and immune-enhancing functions (Fang, 1988; Ma *et al.,* 2002; Wang *et al.,* 2010). Polysaccharides derived from *Astragalus membranaceus* is one of these plants and has been used as immune enhancers (Guo *et al.,* 2004b; Kang *et al.,* 2010). Polysaccharides from natural sources are a class of macromolecules that can deeply affect the immune system, and therefore have the potential as immunomodulators with wide clinical applications (Tzianabos, 2000). For example, the roots of *Astragalus membranaceus* are among the most popular health-promoting herbs in many countries such China and India and used for more than 2000 years. Scientific investigations in the last 2 decades has detected much important pharmacological functions of different components of Astragalus especially its polysaccharide fractions (Cui *et al.,* 2003; Kim *et al.,* 2008). It is well documented that polysaccharides derived from *Astragalus membranaceus*, which have been used as immune enhancers (Chen *et a*l., 2003; Qiu *et al.,* 2007). Also show antibacterial (Guo *et al.,* 2004b), antiviral (Predy *et al.,* 2005), and antiparasitic activities (Guo *et al.,* 2004a; Dalloul *et al.,* 2006; Li *et al.,* 2009) in chickens.

The dry *Astragalus membranaceus* root powder (AMRP) is one of the popular additives used as a therapeutic agent in humans and livestock to treat various diseases (Cho and Leung, 2007; El-Kenawy, 2010; Cho and Chen, 2009; Zhang *et al.,* 2010), and to improve immune functions (Yang *et al.,* 2010; Wang *et al.,* 2012). Recently, there has been increasing interest in the use of AMRP as a feed additive. However, like other phytogenic feed additives, the observed effects of AMRP on animal performance and feed efficiency varied among the published reports (Chen *et al.,* 2003; Mao *et al.,* 2005; Wang *et al.,* 2010). Many factors such as those mentioned above could be attributable to the variation of the animal response to AMRP supplementation.

On the other hand, several studies reported that Astragalus has antioxidant effects. Antioxidants are beneficial to health because they eliminate or neutralize free radicals, which are end products of oxidation that harm a variety of tissues, especially the insides of arteries. It is found that compounds known to have antioxidant effects, including polysaccharides, flavonoids, saponins and triterpenes are present in significant amounts in Astragalus root. These components are strong antioxidants and may help to slow the deterioration of tissues, which are the primary cause of aging and a significant cause of many degenerative diseases such as arthritis, kidney failure and congestive heart failure (Shirataki *et al.,* 1997; Sheng *et al.,* 2005). Hong *et al.,* (1994) found that lipid peroxidation in rat heart mitochondria were inhibited by addition of *Astragalus membranaceus* extract to the rat. Supplementing a corn- and soybean meal-based laying hen diet with AMRP at levels of 5 to 10 g/kg of diet had the potential to improve the antioxidant status of laying hens either in serum and egg yolk and improve laying performance (Zuo *et al*., 2012). While, supplementation of 10g AMRP/kg diet increased average daily gain and feed conversion rate of broilers and improved antioxidant status, growth rate and feed utilization (Wang *et al.,* 2010). Zhang *et al.,* (2013) found that addition of AMRP at level of 5g/kg diet significantly increased activities of total superoxide dismutase and glutathione peroxidase, but reduced concentrations of malondialdehyde and cholesterol in the serum of broiler chicks and had no effect on growth rate, feed intake, or feed conversion rate.

The objectives of this study were to assess the effects of supplementation of AMPR on growth performance, physiological and biochemical indices, antioxidant and immunity statuses, serum metabolites and carcass characteristics of broiler chicks.

Materials and Methods

Location, Experimental Birds and Management of the Flock:

This study was conducted at Poultry Experimental Station belonging to Animal Production Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. Most of experimental analyses of this study were done in the Laboratories of Animal Health Research Institute, Agriculture Research Centre, Ministry of Agriculture, El-Dokki, Giza, Egypt. A total number of 180 broiler Cobb500 chicks one week old were used in the present study. The experiment was lasted for 5 weeks during summer and birds were housed on floor and had free access to feed and water *(ad libitum*). All experimental diets were isocaloric, isonitrogenous and were formulated to meet the requirements of the strain. Diet specifications and composition analysis are given in Table (1). All birds were reared under similar managerial and hygienic conditions. Temperature degree and humidity percentage were

recorded daily through the experiment and were ranged between 33.00°C to 36°C and 72 to 79 % as average respectively.

Experimental Design and Procedures:

At the beginning of the experiment, the birds were randomly distributed into 4 experimental groups and each group was divided into three replicates 15 birds each. Averages of body weight for the 4 experimental groups were apparently uniform. The basal broiler diet was supplemented with *Astragalus membranaceus* root powder (AMRP) to compose 4 experimental dietary groups, namely as follows:

T1 (control). T2 (100mg AMRP /kg feed).
T3 (200mg AMRP /kg feed). T4 (300mg AMRP /kg feed).

Astragalus root powder was mixed well with feed every other day to avoid decomposition. Weekly body weights of birds were recorded to the nearest (0.1g).

Carcass and immune organs test:

At the end of experiment, 24 birds were slaughtered 6 from each group for carcass and immune organs traits.

Blood Sampling:

At the end of the experimental period, blood samples withdrawn from 6 birds of each group and were taken randomly to blood analysis. Birds were fasted overnight before bleeding via jugular vein and blood was collected in unheparinzed tubes to determine the blood profiles and serum was separated and stored frozen at –20^{O} C until analyzed.

Data Collected:

1- Productive data:

a- Body weight. **b-** Body weight gain. **C-** Feed intake.
d- Feed conversion ratio. **e-** Carcass yield data.

Body weight, body weight gain, feed consumption, feed conversion ratio were calculated weekly during the experimental period.

2- Physiological and biochemical data

a- Thermoregulation measurements:

Respiration rate, skin, cloaca and feather temperatures were measured 2 times weekly till the end of experiment.

b- Serum biochemical parameters:

Alanine and aspartate amino transaminase (ALT and AST) activities were determined colorimeterically according to the method of Retiman and Francle (1957). Serum alkaline phosphatase was determined according to the modified methods of Kind and King, (1954). Serum creatinine was measured according to Husdan and Rapoport, (1968). Serum total protein was determined according to Weichselbaum, (1946). Albumin was measured according to Doumas, (1971). The globulin values were obtained by subtracting the values of albumin from the corresponding values of total proteins. Albumin/ globulin (A/G ratio) values were obtained by dividing the values of albumin on the values of globulins. Serum cholesterol and triglycerides were measured using commercially available kits from Sigma Diagnostics Company, (Taufkirchen, Germany) on an auto analyzer apparatus.

Serum calcium was measured according to Gindler and King, (1972) and serum phosphorus was measured according to Goldenberg and Fernandez, (1966). Serum potassium and sodium were measured using flame photometer according to Oser, (1979). Serum magnesium was measured according to Niel and Neely, (1956) and serum iron (Fe) was measured according to Kok and Wild, (1960). Serum selenium (Se) was analyzed according to Brown and Watkinson, (1977). Serum immunoglobulin G (IgG) concentration was measured using single radial immuno diffusion technique, as described by Fahey and Mckelvey, (1965). The method of IgG quantification involves antigen diffusing radially from a cylinder well through an agrose gel containing a

monospecific antibody. Antigen-antibody complexes are formed as a precipitin ring. The ring size increases until equilibrium. Serum Triiodothyronine (T3) and Thyroxine (T4) concentrations were analyzed by Radioimmunoassay (RIA) method using RIA kits (Amersham International Ltd., Amersham, United Kingdom). Serum total antioxidant capacity (TAC) was determined by using a kit (Antioxidant Capacity Assay Kit, Randox Chemical Co. Ann Arbor, MI, USA). Serum superoxide dismutase (SOD) was measured according to Sun *et al*., (1988). Serum lactate dehydrogenase (LDH) was measured according to Cabaud and Worblewski, (1958) while serum lipid peroxides (LPO) was determined by the amount of malondialdehyde analyzed spectrophotometrically, according to Madazli *et al.,* (1999).

Statistical analysis:

Data were subjected to analysis of variance using the General Linear Models procedure of SPSS software program package (SPSS, 2001, version 11.0). All percentages were first transformed to arcsine being analyzed to approximate normal distribution before ANOVA. Also, significant differences among means were determined by Duncan's multiple range test (Duncan, 1955) at 5% level of significance. Data were analyzed by one way method using the following model. $Y_{ij} = u + N_i + e_{ij}$ Where Y_{ij} = the observed value, u = population means, N_i = the effect of treatment, e_{ij} = the standard error.

Table 1: Compositions of experimental diets of broiler Cobb500 chicks.

Ingredients	Diets of broiler chicks		
	Starter (1-10 day)	Grower (11-22 day)	Finisher (23-42 day)
Ground yellow corn 8.8%	62.05	62.6	64.55
Soybean meal 44%	24.15	26.01	23.28
Corn gluten meal 60%	9.1	4.58	4.58
Sunflower oil	0.15	2.5	3.5
Dicalcium phosphate	2.14	2.1	2
Limestone	1.4	1.24	1.11
Premix*	0.3	0.3	0.3
Sodium Chloride (NaCl)	0.3	0.3	0.3
DL-methionine	0.12	0.17	0.17
L-lysine-HCl	0.29	0.2	0.21
Total (Kg)	100	100	100
Calculated analysis**			
Crude protein%	21.29	19.24	18.24
ME. cal/Kg feed	2986.70	3082.96	3176.00
C/P ratio	142.16	162.18	176.35
Calcium%	1.04	0.98	0.90
Available P.%	0.50	0.49	0.47
Lysine%	1.20	1.11	1.06
Methionine%	0.52	0.51	0.50
Methionine + Cystin%	0.89	0.85	0.82

[1]Composition of vitamins and minerals premix. Each 3Kg of vitamin and minerals mixture contain: Vit. A 10.000.000 IU, Vit. D_3 2.000.000 IU, Vit. E 10.000 mg, Vit. K_3 1.000 mg Vit. B_1 1.000 mg, Vit. B_2 5.000 mg, Vit. B_6 1.500 mg, Vit B_{12} 10 mg, Niacin 20.000 mg, Pantothenic acid 10.000 mg, Folic acid 1.000 mg, Biotin 50 mg, Choline chloride 500.000 mg, Copper 4.000 mg, Iodine 300 mg, Iron 30.000 mg, Manganese 60.000 mg, Zinc 50.000 mg, Cobalt 100 mg and Selenium 100 mg. **According to NRC (1994).

Results and Discussion

Productive results:

The effects of *Astragalus membranaceus* root powder (AMRP) supplementation in diet on body weight, daily gain, feed intake and feed conversion ratio of broiler chicks during the experimental period are shown in Table (2). Results showed that differences of the final body weight, daily gain, daily feed intake and feed conversion ratio among the experimental groups were insignificant, but there were noticeable increase in body weights and improvement feed conversion of treated group compared to untreated one. These results revealed that the dietary treatments had no significant effect on the mentioned parameters. However, group fed 100mg AMRP/kg feed (T2) recorded a higher numeric value of body weight, daily gain and daily feed intake followed by group fed 300mg AMRP/kg feed (T4) and group fed 200mg AMRP/kg feed (T3) compared to control group. Also, the obtained results showed that there was a little improvement but not significant in feed conversion ratio for group fed 300mg AMRP/kg feed (T3) compared to the control group. Total feed intake was significantly ($P \leq 0.05$) higher for group fed 100mg AMRP compared to other groups. The beneficial effects of AMRP on broiler performance presented in Table (2) may be attributed to the fact of prebiotics present in AMRP (polysaccharide) are able to increase digestive enzyme (intestinal protease and amylase) activities, which play a crucial role in digestion and affect energy and protein utilization as mentioned by (Xu et *al*., 2003; Yuan *et al.,*

2006; Yang *et al*., 2008). It is possible that prebiotics of AMRP, by improving the microbial ecology of the intestine, reduce passage rate of the digesta and improve the digestibility of amino acids (Biggs and Parsons, 2007). Changes in mucosal architecture, changes in gut environment and increases in villi height have been reported to be due to the supplementation of prebiotics (Yuan *et al.,* 2006; Yang *et al*., 2007; Houshmand *et al.,* 2012). Also, the β-glucan components might stimulate the gut-associated immune system by acting as a nonpathogenic microbial antigen, giving an adjuvant-like effect the importance of digestive microbial antigen stimulation on the development of lymphoid organ tissue (Pabst *et al.*, 1998; Mahdi and Abed Al-Abass, 2012). The enhancement of β-glucan can be explained in part by the improvement of intestinal function or gut health through the increase of villi height, uniformity and integrity. β-glucans have been shown to improve immune response and to block bacterial adhesion (especially enteric pathogens) to gut lining (Volman *et al*., 2008; Mahdi and Abed Al-Abass, 2012). Moreover, it has been suggested that mannanoligosaccharide (MOS), a yeast cell wall derivative, might inhibit the colonization of bacteria to the intestine by binding to bacterial mannan-binding lectin or could act as bioregulator of the intestinal microflora and reinforcing the host natural defenses, through the sanitary effect by increasing the colonization resistance and stimulation of the immune response (Shareef and Al-Dabbagh, 2009). Spring *et al*., (2000) reported that MOS acts to bind and remove pathogens from the broiler chicken intestinal tract and stimulate the immune system. Fernandez *et al*., (2002) investigated the effect of BioMos (prebiotic) on the number of microflora in chickens digestive system and showed that there was increased in numbers of *Eubacterium* spp. and *Enterococcus* spp. while the number of *Bacteroides* spp. were found to be decreased. The increased number of these bacteria probably indirectly inhibited the colonization of pathogenic bacteria by preventing their attachment to the gastrointestinal epithelial cells. In a study conducted on young turkeys fed BioMos, Bradley *et al*., (1995); Koc *et al.,* (2010) observed improvement in body weight and altered ileum morphology. In the ileum, the crypt depth was less and the numbers of goblet cells per mm of villus were increased significantly. Use of prebiotics such as fructooligosaccharide (FOS) can serve as a fiber source for certain microbial populations and enhance production of organic acids in the gut. Furthermore, use of MOS can bind to receptors on many bacterial pathogens themselves preventing their attachment to epithelial binding sites and modify intestinal commensally microorganisms (Bradley *et al*., 1995; Edens 2003; Koc *et al.,* 2010). All of these materials (prebiotics) have beneficial effects to improve productivity of broiler chicks such as body weight gain and feed conversion ratio by indirect ways as shown in the previous literature.

The effect of experimental treatments on the composition of the bird carcasses (in gram) and relative to live body weight (%) are given in Table (3). The results of some carcass variables such as live body weight, dressing weight and heart % were significantly ($P \leq 0.05$) increased in the group fed 200mg AMRP/kg feed (T3) compared to the control group. While group fed 100mg AMRP/kg feed (T2) was significantly ($P \leq 0.05$) higher in dressing % and liver % compared with the control group. In this context, AMRP had no significant effect on giblets % among all treated groups compared to the control. In general carcass yield of broilers fed different levels of AMRP was significantly higher ($P \leq 0.05$) than that of broilers fed control diet. The increased carcass yield by AMRP supplementation observed in this study is likely due to the effect of the treatment in improving muscle (breast and leg) yield, although this is not measured in this study. Wang *et al*., (2010) reported that supplementation of AMRP did not affect carcass yield or yield of both breast and leg muscles. These results are in agreements with those obtained by (Zhang *et al.,* 2013).

Physiological results:

a) Effect of AMRP levels in diet on immune organ weights:

The obtained results of immunocompetent organ weights indicate that there were significant ($P \leq 0.05$) differences in the weights of lymphoid organs between all experimental groups as shown in Table (4). There were significant ($P \leq 0.05$) increases in spleen, bursa of Fabricius, thymus and cecal tonsil absolute weights in the group fed 300mg AMRP (T4) compared with the control group. Increasing AMRP level in the broiler diet up to 300mg /kg feed resulted in an increase of the weight of these organs. The increase in the immunocopetent organ weights found in T4 may led to that T4 was significantly higher in IgG value which reflect better immunity status of broilers of group T4 compared with the control group. These results may suggest that fed broiler chicks diet contained AMRP at level of 300mg/kg feed enhanced the development of immune organs of broiler chicks, however, little work has been done on AMRP in this aspect. These results are in accordance with those obtained by (Li *et al.,* 2009). The spleen performs many functions and it is the major organ involved in immune response to some antigens, it also acts as an immunologic filter of the blood and entraps foreign materials that are antigens from the bloodstream passing through the spleen (Miller *et al.,* 1991). Furthermore, the spleen provides microenvironment, which is needed for antigens presentation and concentrating them in the white pulps where T and B cell interactions lead to the formation of antibodies. Also, the spleen and bursa are the important lymphoid organs involved in the development and differentiation of T or B lymphocytes

(Williams *et al.,* 1991; Li *et al.,* 2001). However, Ubosi *et al.,* (1985) reported that the size of the spleen of poultry species may be affected by genotype. Regarding to thymus weight, the immunological function of thymus is to provide a specific environment essential for T-cells differentiation, which essential for cell-mediated immunity and modulation of immune response (Owen, 1977). On the other hand, Makram *et al.,* (2010) reported that the size of lymphoid organs weight may not associated with higher immune response of chickens and found that the Hubbard broiler chicks had higher relative lymphoid organs weight followed by Arbor Acres ones compared to Cobb and Avian broiler ones while, the Cobb strain had a higher immune response when compared to other broiler strains Makram *et al.,* (2010). The bursa of Fabricius plays a central role in the development and maturation of the antibody-producing B-lymphocyte and humoral antibody in birds (Zhang *et al.,* 2006; Cheema *et al.,* 2007). Ubosi *et al.,* (1985) observed that a chicken line selected for high response to SRBC had a larger bursa size than the line selected for low response. Furthermore, Zhang *et al.,* (2006) showed clear association between non-MHC gen and changes in the size of lymphoid organs by using highly inbred parental and recombinant congenic chicken lines.

b) Effect of AMRP in diet on thermoregulation measurements:

Effect of AMRP supplementation on thermoregulation measurements of cloacal temperature (Tc), skin temperature (Ts), feather temperature (Tf) and respiration rate (RR) of broiler chicks are presented in Table (5). The additions of AMRP to broiler diets show no significant effects on the Tc, Ts and Tf between all groups of experiment but group fed 300mg AMRP/kg feed (T4) was numerically lower in those traits but not significantly different compared to the control group. Moreover, there was significant ($P \leq 0.05$) decrease in RR of group fed AMRP at level of 300mg/kg feed compared to the control and other treated groups. These results may suggest that AMRP had beneficial effect to reduce RR of broiler chicks fed AMRP at level of 300mg during the experiment under Egyptian summer conditions at temperature degrees ranged between 33 to 36^{O}C (M= 34.5^{o}C) and humidity percentage between 72 to 79% (M= 75.5%).These results may also reflect that broiler chicks fed 300mg of AMRP are less panting than control and other groups and may be tends to loss their heat by evaporation less than heat loss by other ways such as radiation, conduction and convection. There was no literature available or investigations have been made on the effect of AMRP on thermoregulation measurements of poultry till now. In any case, this point needs more investigations. The respiratory system is one of the major systems of the body. It has a number of very important functions including the provision of oxygen, the removal of carbon dioxide, the removal of excess heat (thermoregulation**)** and vocal communication. The respiratory system in birds is a complex one and thermoregulation is one of its functions. As this term **(thermoregulation)** indicates, it is associated with the regulation of the temperature of the bird. As it's known that fowls are homeothermic animals. However, while every attempt is made to achieve a stable body temperature, there are times when, under extreme conditions, the birds' temperature will vary up or down. When this variation is too great the bird is likely to die. The normal adult body temperature is in the range of 41-41.7^{o}C and at approximately 46^{o}C death will occur (Dingle, 1990 and 1991). Heat is lost by the bird as **sensible** heat directly to the atmosphere when the temperature gradient is sufficiently great and as **insensible** heat by the evaporation of water from the respiratory system and skin when the temperature gradient is less but relative humidity is low. At high temperature the birds increase their respiration rate to increase the amount of air passing through the respiratory system to increase the cooling by evaporation. This panting also involves the rapid movement of the upper throat tissues to increase evaporation. The movement of air around the body of the bird will assist in removing heat from the bird as sensible heat and as insensible heat (Dingle, 1990 and 1991). Anyway, the result of RR found in group fed 300mg AMRP (T4) is an interest result and require to further studies.

c) Effect of AMRP in diet on serum constituents:

1- Effect of AMRP on liver and kidney functions (ALT, AST, AP and Creatinine).

Results of ALT, AST, alkaline phosphatise (AP) and Creatinine (Creat) are given in Table (6). Serum ALT, AST and AP activities decreased significantly ($P \leq 0.05$) in treated groups as AMRP levels increased in the broiler diets compared with the control group. The significant changes in the activities of these enzymes in the serum indicate that AMRP may had useful effects for the soft tissues against tissue impairment or malfunctioning of liver which may caused by excessive stress. The reduction observed in ALT, AST and AP activities by AMRP in this study can be indicative of better function of liver and appear non-pathological metabolism of the liver and heart which reflect better health of broiler chicks subjected to this study. Mathivanan and Edwin (2012) reported that the significant decrease in the levels of biochemical marker enzymes like ALT, AST and AP in plant extract (*Andrographis paniculata)* administered broiler might be due to decreased leakage of the enzymes in liver cells. This suggests that the *Andrographis paniculata* (herbal plant) could repair the hepatic injury and/or restore the cellular permeability, thus reducing the toxic effect of liver

toxicity and preventing enzymes leakage into the blood circulation (Sivaraj *et al.,* 2011; Mathivanan and Edwin 2012). From the obtained results, it was concluded that supplementation of AMRP to broiler diet enhanced the immunoprotective and hepato protective nature of broilers. Furthermore, Vahdatpour *et al.,* (2011) noticed that AST, ALT, GGT and LDH usually appear in serum when there is damage on the liver and muscle tissues caused by excessive stress. Also, addition of Protexin®+Fermacto® (probiotic and prebiotic) to Japanese quail diet caused low AST, GGT and CPK activities in serum of male quail birds and it caused a significant decrease ($P<0.05$) in serum level of ALT in male quail birds compared to the control group. It can be concluded that Protexin® by decreasing effects of stress can be caused a lower enzyme activity and it can be a protective agent for liver and muscles against damage factors in male quails compared to other additives fed groups. Moreover, consumption of all feed additives caused to decreasing of ALT activity in male and female birds compared to the control group and they can help to health of liver and muscles as a protector agent (Vahdatpour *et al.,* 2011). Results of the present study are in agreements with those obtained by (Yalcinkaya *et al.,* 2008; Sivaraj *et al.,* 2011; Vahdatpour *et al.,* 2011; Mathivanan and Edwin 2012).

Concerning serum creatinine, there was negligible effect of AMRP on serum creatinine between groups T1, T2 and T3. While, group fed 300mg AMRP/kg feed (T4) recorded significantly ($P \leq 0.05$) lower level in serum creatinine compared to the control group, in despite of there was no significant effect in serum creatinine between the offer treated groups. AMRP had no harmful effect on kidney function and there is no kidney congestion was observed during carcass examination. Creatinine is a chemical waste molecule that is generated from muscle metabolism. The kidneys maintain the blood creatinine in a normal range. The lower values derived that no muscular wastage which might have been possibly caused by inadequacy of protein in broiler chicks (Polat *et al.,* 2011). This observation may supported by the higher body weights gained for treated groups with different levels of AMRP presented in Table (2) compared to control group.

Effect of AMRP in diet on serum antioxidant and oxidation enzyme statuses:

Results of serum superoxide dismutase (SOD), total antioxidant capacity (TAC), lipid peroxidase (LPO) and lactate dehydrogenase (LDH) are shown in Table (6). Serum superoxide dismutase (SOD) enzyme and total antioxidant capacity (TAC) levels were significantly ($P \leq 0.05$) increased as the level of AMRP increased in the broiler diets Table (6) compared to the control group. It is well established that SOD and TAC are two main indicators that reflect the antioxidant status in broiler chicks. In this connection, group fed 300mg AMRP /kg feed showed significantly ($P \leq 0.05$) that highest values in serum SOD enzyme and TAC compared to the control and other groups. On contrary, results of lipid peroxides (LPO) and lactate dehydrogenase (LDH) (oxidation) enzymes were significantly ($P \leq 0.05$) reduced as the level of AMRP in diet increased and was more pronounced in group fed 300mg AMRP/kg feed (T4). These results indicate that AMRP may works as antioxidant agent and has ability to reduce free radical production by inhibited the lipid peroxidation of cellular membrane. Feeding broiler chicks AMRP may increase SOD activity and might accelerate the rate at which SOD scavenges free radicals and it may also inhibit lipid peroxidation. Lipid peroxides are the products of chemical damage done by oxygen free radicals to the polyunsaturated fatty acids of cell membranes. Astragalus root help in minimizing free radical damage in cell membranes. The flavonoids and saponins in Astragalus can significantly inhibit membrane lipid peroxidation generated by superoxide (O_2-), hydrogen peroxide (H_2O_2), and ultraviolet rays. Astragalus has been shown to have inhibitory effects on lipid peroxidation and protein oxidative modification by copper (Chen *et al.,* 1995; Wang *et al.,* 1996; Toda and Shirataki, 1999). Researchers have now discovered that lipid peroxides play a specific physiological role in the cell. Prior to this study, scientists had already established that accumulation of lipid peroxides indicates cell stress and diseases. Lipid peroxides have further been shown to be very potent inducers of cell death (Conrad *et al.,* 2010). Enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX) have function to protect against the toxic effect of free radicals by inactivating these compounds. Superoxides are converted to hydrogen peroxide (H_2O_2) by SOD. H_2O_2, in turn, is converted to water and molecular oxygen by either catalase or GPX. Thus, SOD is the first line of defense against free radical mediated injury. It is an important indicator of antioxidation *in vivo* (Vaziri *et al.,* 2003; Sheng *et al.,* 2005; Zhang *et al.,* 2009). The main ingredients of *Astragalus membranaceus* include astragalus polysaccharides, flavonoids of Astragalus, and saponins of Astragalus, all of which have a strong effect on free radical clearance and SOD activity. Therefore, Astragalus polysaccharide can increase SOD activity and clear (scavenge) free radicals (Sheng *et al.,* 2005). The enhanced antioxidant enzyme activity and antioxidant status of broilers by AMRP is likely due to various bioactive compounds in AMRP, including 2,4-dihydroxy- 5,6-dimethoxyisoflavone, kumatakenin, betaine, polysaccharides, glucuronic acid, astraisoflavanin, saponins, and flavones (Cho and Leung, 2007; El-Kenawy, 2010; Zhang *et al.,* 2013). These compounds have been reported to possess various biological and pharmaceutical activities such as antioxidant and free radical scavenging functions (Li *et al.,* 2009; Zhang *et al.,* 2009; Wang *et al.,* 2010; Zhang *et al.,* 2013). These results agree with above authors and Sadeghi *et al.,* 2012 who observed that, plasma antioxidant capacity is increased by consumption of ginger in broiler chicks and elevation of antioxidant capacity raises the antioxidant

scavenging capacity of the body and attenuates free radical induced damage. Eventually, consumption of products rich in antioxidants as additive in broiler diets may increase physiological antioxidant defences which may decrease oxidative stress.

Effect of AMRP in diet on serum total protein, albumin, globulin, cholesterol, triglyceride, immunoglobulin G, T3 and T4 hormones:

Impact of different levels of AMRP supplementation on total serum protein (TP), albumin (Alb), globulin (Glob), A/G ratio, total cholesterol (Chol), triglyceride (Trigly), immunoglobulin type G (IgG) , T3 and T4 hormones are presented in Table (7). Results of total serum protein, total albumin and A/G ratio revealed that there were significant ($P \leq 0.05$) increase in TP, Alb and A/G ratio of treated groups compared with the control group. In contrast, there was no significant effect of AMRP supplementation in broiler diet on serum Glob. The increased contents of TP, Alb, and A/G ratio in the serum of broilers by inclusion of AMRP in the broiler diets indicated that the AMRP affected protein metabolism, which is consistent with the observation of enhanced serum antioxidant enzyme activity and increased carcass yield. The increased serum concentration of albumin as well as enhanced antioxidant status may also be indicative of enhanced immune system as the serum concentration of Alb proteins antioxidant status are regarded as the direct reference to the body immune function (Zhang *et al.,* 2013). Increment in serum protein concentration in treated groups as compared to the control group may be attributed to the hormonal regulation of protein metabolism, for example growth hormone increased the synthesis of cellular protein, glucocosteroids increased break down of most tissue proteins. The increasing of corticosterone hormone and glucocorticiods which are secreted by the adrenal cortex increased the quantity of protein in most tissues while decreased the amino acids concentration in the plasma, as well as decreased both liver protein and plasma proteins, or may be due to the decrease of thyroxin secretion, that thyroxin increases the rate of metabolism of all cells and, as a result indirectly affects protein metabolism (Guyton and Hall, 2006; Al-Daraji and Amen, 2011). In the present study it believed that increasing serum protein may reflect that the rate of anabolism was higher than the rate of catabolism in broiler chick bodies and this may the reason that treated groups had higher body weights than the control (Table 2).

Serum total cholesterol (Chol) and triglycerides (Trigly) concentrations were significantly ($P \leq 0.05$) reduced with addition of AMRP in broiler diets. The reduction in levels of Chol and Trigly in serum were increased as AMRP level increased in the broiler diet. The obtained results revealed that AMRP led to a profound reduction in intestinal cholesterol absorption and may accelerate the rate of fecal neutral sterol excretion. The mechanism that led AMRP to reduce serum Chol is still unknown. These results may suggest that the reduction in serum Chol may occurs by interfering AMRP with the intestinal cholesterol absorption and inhibit Chol absorption. The small intestines are implicated in regulating cholesterol homeostasis through affecting cholesterol absorption. An inhibition of intestinal absorption results in lower levels of circulating cholesterol. As sitosterol, which act directly at the level of intestine, lower plasma cholesterol by inhibiting intestinal fractional cholesterol absorption (Ntanios and Jones, 1999). It has been proved that the viscosity associated with dietary soluble polysaccharides interferes with cholesterol absorption by directly binding cholesterol within the intestine, interfering with the diffusion of cholesterol toward the epithelial cell surface, or reducing the capacity of micelles to incorporate cholesterol (Minekus *et al.,* 2005). The reduction of cholesterol absorption in response to Astragalus may be attributed to its viscosity, with little regulation of genes associated with cholesterol transport. Cholesterol is mainly eliminated from the body via conversion to bile acids, and the rate-limiting enzyme of the process is cyp7a-1 (Cheng *et al.,* 2011). It has been reported that dietary soluble polysaccharides decreased serum cholesterol by altering the composition of the enterohepatic bile acid pool and increasing the fecal loss of total bile acids (Fernandez, 2001; Ellegard and Andersson, 2007). Cheng *et al.,* (2011) reported that Astragalus produced a marked increase in excretion of fecal bile acids, in accordance with that the hepatic cyp7a-1 mRNA expression was significantly elevated in response to Astragalus. A further test with Western blotting also revealed that the Astragalus stimulated cyp7a-1 protein expression. The effect on bile acid metabolism would support the conclusion that Astragalus lowers blood cholesterol by increasing bile acid excretion. Mathivanan and Edwin, (2012), represented that the decreased serum cholesterol in the plant extract (*Andrographis paniculata*) might be due to increased activity of enzyme catalase involved in esterification of cholesterol in the plasma.

The obtained results show that serum IgG levels were significantly ($P \leq 0.05$) increased as the level of AMRP increased in broiler diet compared to the control group. IgG concentrations are indicators to reflect the humoral immunity situation in broiler chicks. The improvement in immunity of treated broiler chicks could be related to the inhibitory effects of AMRP on gut pathogens. Research shows that AMRP stimulates the immune system in many ways. It increases the number of stem cells in bone marrow and lymph tissue and encourages their development into active immune cells. It appears to help trigger immune cells from a “resting” state into heightened activity (Jiao *et al.,* 1999). One study showed AMRP helps to promote and maintain respiratory health. It also enhances the body’s production of immunoglobulin and stimulates macrophages. Astragalus can

help activation of T-cells and natural killer cells (Jiao *et al.,* 1999; Thorne, 2003). Also, Cho and Leung, (2007) reported that effective compounds isolated from AMRP improved body immune function *in vitro* and *in vivo*. Furthermore, Wang *et al.,* (2010), write down that AMRP contains varieties of chemical compounds that have immune-enhancing functions.

Results of serumT3 and T4 hormones are presented in Table (7) showed that AMRP had beneficial effect on the secretion of these hormones. The level of both of T3 and T4 in serum increased significantly ($P \leq 0.05$) as the level of AMRP increased in broiler chick diets compared to the control group. Also, these results may reflect that AMRP improved the rate of metabolism for treated groups more than untreated one via increase the villi height, villi length and crypt length and width. This increment in villi height and crypt length and width can increase nutrients absorption due to increase of intestinal surface area for absorption (Ribeiro *et al.,* 2007; El-Shafei *et al.,* 2010). The sole function of the thyroid is to make thyroid hormones. These hormones have an effect on nearly all tissues of the body where it increases cellular activity. The function of the thyroid therefore is to regulate the body's metabolism. It affects heart rate, cholesterol level, body weight, energy level, muscle strength, skin condition, vision, mental state and a host of other conditions. Further, both of T3 and T4 have a critical part in physiological activity. Thyroid hormones play an essential role in stimulating energy production; a deficiency of these hormones may result in extreme fatigue (Hoffmann, 2003; Mareib and Hoehn, 2007). The thyroid gland synthesizes hormones which work within the body to regulate a number of functions including metabolism and growth. When the body experiences a deficiency in thyroid secretions, the metabolism slows causing low blood pressure and fatigue. In many cases thyroid function is responsive to herbal remedies and lifestyle changes (Hoffmann, 2003; Mareib and Hoehn, 2007). These results are in agreement with those obtained by Abdel-Fattah *et al.,* (2008) who indicated that the T3 was significantly increased by addition of organic acids to broiler diets and these results pointed out superior metabolic and growth rate due to the supplementation of acidifier to broiler chick diets. Unfortunately, there was no available literature in this point and this is a first study look for the effect of AMRP on serum T3 and T4 hormones in broiler chicks.

Effect of AMRP in diet on serum minerals profile:

Results of serum minerals, calcium (Ca), phosphorus (P), sodium (Na), potassium (K), magnesium (Mg), iron (Fe) and selenium (Se) are given in Table (8). In general, the obtained results revealed that all above elements were significantly ($P \leq 0.05$) increased in serum as a level of AMRP increased in broiler diets. These are interesting results which may reflect that AMRP had enhanced effect on the digestibility of serum minerals and may improve the intestinal absorption of minerals from the gut of treated broiler chicks. These findings may attribute to that AMRP improved mineral absorption because of an osmotic effect that transfers water to the caecum, thus increasing the volume of fluid in which these minerals can be dissolved. Furthermore, as a result of fermentation, prebiotics decrease cecal pH and consequently increase concentrations of ionized minerals, a condition that favours passive diffusion (Roberfroid, 2000; Sohail *et al.,* 2011). On the other hand, Yalcınkaya et *al.,* (2012) reported that binding of the organic ligands to the chelated trace minerals in the upper gastrointestinal system minimize the mineral losses to antagonists and allowing the complex to be delivered to the absorptive epithelium of the small intestine for mineral uptake. Improvement of mineral absorption occurred by prebiotics in chicken has been reported by some researchers (Chen and Chen, 2004; Ortiz *et al*., 2009).

Table 2: Effect of different levels of AMRP in diets on final body weight (g) at 6th weeks of age, daily gain, total feed intake, daily feed intake and feed conversion during the whole of experimental period (2-6 weeks of age) of broiler chicks.

Treatment	Final body weight (g/bird)	Daily gain (g/bird. day)	Total feed intake[2]	Daily feed intake[3]	Feed conversion ratio[4]	Morta. rate %
T1	2111.67[1] ±36.34	49.78 ±1.12	4391.83[b] ±14.16	125.48[b] ±0.40	2.08 ±0.05	Nil
T2	2244.83 ±41.74	53.40 ±1.23	4602.47[a] ±29.04	131.50[a] ±0.83	2.05 ±0.03	Nil
T3	2142.33 ±54.85	50.37 ±1.54	4386.27[b] ±5.86	125.32[b] ±0.17	2.05 ±0.07	Nil
T4	2207.33 ±50.48	52.89 ±1.44	4451.33[b] ±39.85	127.18[b] ±1.14	2.02 ±0.04	Nil

[1] Least squares means ± pooled standard error.
a,b Means having different letter exponents among columns are significantly different (P≤0.05).
[2] (g/bird. 5 weeks) from 2-6 weeks of age.
[3] (g/bird. day). [4] (g feed/1g weight gain. bird) from 2-6 weeks of age.

Table 3: Effect of different levels of AMRP in diet on carcass characteristics of broiler chicks at 6th weeks of age.

Treatment	Live body weight (g)	Dressing weight (g)	Dressing %	Giblets %	Gizzard %	Liver %	Heart %
T1	2418.00[b1] ±41.99	1799.31[b] ±26.65	74.44[b] ±0.19	4.13 ±0.07	1.85[a] ±0.08	1.92[b] ±0.09	0.36[b] ±0.01
T2	2545.00[ab] ±45.55	1929.50[a] ±27.41	75.87[a] ±0.73	4.09 ±0.07	1.54[b] ±0.10	2.22[a] ±0.07	0.33[b] ±0.01
T3	2607.50[a] ±44.27	1946.57[a] ±38.18	74.63[ab] ±0.28	4.23 ±0.08	1.72[ab] ±0.01	2.02[ab] ±0.10	0.48[a] ±0.03
T4	2570.00[a] ±45.40	1928.72[a] ±27.34	75.09[ab] ±0.42	4.23 ±0.12	1.69[ab] ±0.03	2.17[ab] ±0.11	0.37[b] ±0.01

[1] Least squares means ± pooled standard error.
a,b Means having different letter exponents among columns are significantly different (P≤0.05).

Table 4: Effect of different levels of AMRP in diet on absolute immune organ weights (g) of broiler chicks at 6th weeks of age.

Treatment	Spleen weight	Bursa weight	Thymus weight	Cecal tonsil weight
T1	1.49[b1] ±0.04	1.78[b] ±0.17	6.54[c] ±0.24	0.75[a] ±0.01
T2	1.60[b] ±0.11	1.26[b] ±0.26	6.55[c] ±0.41	0.54[b] ±0.11
T3	2.04[a] ±0.09	1.85[b] ±0.11	10.14[b] ±0.28	0.74[a] ±0.02
T4	2.24[a] ±0.10	2.80[a] ±0.28	11.56[a] ±0.46	0.81[a] ±0.06

[1] Least squares means ± pooled standard error.
a,b,c Means having different letter exponents among columns are significantly different (P≤0.05).

Table 5: Effect of different levels of AMRP in diet on (thermoregulation measurements) cloacal temperature (Tc), skin temperature (Ts), feather temperature (Tf) and respiration rate (RR) of broiler chicks during the experimental period (6 weeks of age).

Treatment	Tc (°C)	Ts (°C)	Tf (°C)	RR (r.p.m)
T1	40.57[1] ±0.12	38.50 ±0.53	30.62 ±0.06	45.67[a] ±1.33
T2	40.82 ±0.20	39.38 ±0.36	30.70 ±0.36	42.50[a] ±1.78
T3	40.63 ±0.08	39.18 ±0.14	30.88 ±0.39	44.00[a] ±1.93
T4	40.50 ±0.05	38.42 ±0.06	30.57 ±0.62	37.00[b] ±1.53

[1] Least squares means ± pooled standard error.
a,b, Means having different letter exponents among columns are significantly different (P≤0.05).

Table 6: Effect of different levels of AMRP in diet on serum constituents of broiler chicks at 6th weeks of age. (ALT, AST, AP., Creat., SOD, TAC, LPO and LDH).

Treat.	ALT (GPT) Iu/ml	AST (GOT) Iu/ml	AP. u/ml	Creat. g/dl	Superoxide dismutase (SOD) u/ml	Total antioxidant capacity (TAC) mmol/L	Lipid peroxides (LPO) umol/L	Lactate Dehydrogenase (LDH) Iu/L
T1	53.67[a1] ±2.83	47.67[a] ±0.92	180.00[a] ±3.65	0.93[a] ±0.03	31.67[c] ±0.56	1.33[c] ±0.06	2.90[a] ±0.13	696.67[a] ±2.11
T2	43.33[b] ±1.05	31.67[b] ±0.56	146.33[b] ±2.01	0.89[ab] ±0.01	41.00[b] ±0.97	1.70[b] ±0.06	2.53[a] ±0.22	640.00[a] ±20.33
T3	43.33[b] ±1.12	31.33[b] ±0.56	144.33[b] ±3.39	0.90[ab] ±0.01	44.67[b] ±1.84	1.50[bc] ±0.04	2.03[b] ±0.15	574.00[b] ±20.29
T4	41.00[b] ±0.37	31.67[b] ±0.76	133.33[c] ±3.60	0.87[b] ±0.02	65.33[a] ±1.84	1.97[a] ±0.12	1.17[c] ±0.06	411.67[c] ±28.54

[1] Least squares means ± pooled standard error.
a,b,c Means having different letter exponents among columns are significantly different (P≤0.05).

Table 7: Effect of different levels of AMRP in diet on serum constituents of broiler chicks at 6th weeks of age. (Tp., Alb., Glob., A/G ratio, Total Cholest., Triglyce., IgG., T3 and T4).

Treat.	TP. g/dl	Alb. g/dl	Glob. g/dl	A/G ratio	Cholest. mg/dl	Trigly. mg/dl	IgG mg/dl	T3 ng/ml	T4 ng/ml
T1	7.77[b1] ±0.12	3.63[b] ±0.06	4.14 ±0.17	0.88[b] ±0.05	203.67[a] ±2.01	155.67[a] ±1.48	27.00[d] ±4.49	1.36[c] ±0.02	8.50[c] ±0.35
T2	7.97[ab] ±0.04	3.93[a] ±0.02	4.04 ±0.06	0.97[ab] ±0.02	164.00[b] ±4.21	135.33[b] ±1.84	44.67[c] ±3.27	1.43[b] ±0.03	9.80[b] ±0.15
T3	8.07[a] ±0.05	4.03[a] ±0.08	4.04 ±0.04	1.00[a] ±0.03	155.00[b] ±1.10	121.67[c] ±1.05	69.00[b] ±3.71	1.48[b] ±0.02	10.20[ab] ±0.26
T4	8.07[a] ±0.02	4.07[a] ±0.02	4.00 ±0.04	1.02[a] ±0.01	135.33[c] ±4.01	108.33[d] ±5.95	98.67[a] ±1.52	1.55[a] ±0.02	10.60[a] ±0.16

[1]Least squares means ± pooled standard error.
a,b,c,d Means having different letter exponents among columns are significantly different (P≤0.05).

Table 8: Effect of different levels of AMRP in diet on serum minerals profile of broiler chicks at 6th weeks of age.

Treatment	Ca mg/dl	P mg/dl	Na mEq/L	K mEq/L	Mg mg/dl	Fe ug/dl	Se ppm
T1	10.03[b1] ±0.06	5.20[b] ±0.22	137.00[c] ±0.76	3.80[d] ±0.07	3.20[b] ±0.11	134.33[c] ±2.97	0.36[b] ±0.01
T2	11.03[a] ±0.06	5.60[ab] ±0.22	138.00[c] ±0.97	4.10[c] ±0.10	3.53[ab] ±0.22	151.67[b] ±3.80	0.37[b] ±0.02
T3	11.10[a] ±0.10	5.90[a] ±0.04	141.00[b] ±0.97	4.40[b] ±0.13	3.57[ab] ±0.13	155.33[b] ±1.84	0.42[a] ±0.01
T4	11.00[a] ±0.06	5.87[a] ±0.09	144.00[a] ±0.37	5.03[a] ±0.02	3.65[a] ±0.06	195.00[a] ±3.65	0.42[a] ±0.01

[1] Least squares means ± pooled standard error.
a,b,c,d Means having different letter exponents among columns are significantly different (P≤0.05).

Conclusion:

Based on the obtained results, supplementation of AMRP to broiler diet at level of 100, 200 and 300mg/ kg of feed improved significantly serum ALT, AST, AP, creatinine enzymes, (liver and kidney functions), antioxidant status, and had beneficial effect to improve immunity status, carcass characteristics and respiration rate. Treatment broiler chicks with AMRP enhanced nutrients metabolism and improved the absorption of serum minerals measured in this study. Furthermore, these findings strongly suggested that AMRP is a promising novel natural health hypolipidemic treatment that may act by multiple mechanisms to reduce serum total cholesterol, triglyceride and oxidation enzymes. Ultimately, addition of AMRP is recommended to broiler diets up to 300mg/kg feed without any adverse effects.

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