

Effects of Lead Acetate and Probiotic on Some Physiological Parameters in Broiler Chicks

Nashat.G. Mustafa

Sinan.Th. Abdullah

*Department of Physiology
College of Veterinary Medicine
Mosul University*

(Received 31/8/2008 ; Accepted 5/1/2009)

ABSTRACT

This work was conducted to evaluate the effect of probiotic (BIOMIN)[®] on lead acetate absorption and its toxic action on certain physiological and biochemical parameters: body weight gain, Hb concentration, PCV%, serum total protein and serum lead level in chicks. One-day-old forty Ross broiler chicks were used. The probiotic was used in recommended dose (1.5g/kg diet). Lead acetate was used in two doses; full dose of 320 mg/kg diet and half dose of 160 mg/kg diet. The results show that probiotic significantly ($P \leq 0.05$) decreased serum lead level, enhanced body weight gain, while it has no direct significant effect on serum total protein, Hb concentration, and PCV%. On the other hand, lead acetate alone (in both doses) caused severe anemia, depression in the level of both Hb concentration and PCV%, while half dose (160 mg) of lead acetate caused no effect on both body weight gain and serum total protein, but death of some chicks, and decreased serum total protein were occurred with full dose of lead acetate.

Key words: probiotic, lead acetate, chicks, physiological and biochemical parameters.

(BIOMIN)[®]

:

40

(/ 320)

:

(/ 1.5)

($P \leq 0.05$)

.(/ 160)

)

(

)

(

INTRODUCTION

Lead (Pb) is considered to be one of the major environmental pollutant and has been incriminated as a cause of accidental poisoning in domestic animals and birds more than any other substances (NRC, 1984). Environmental pollution with lead is a common occurrence in cities and on their edges, significant pollution is more likely to occur near smelters or other industrial enterprises, or near major highways where pasture is contaminated by exhaust fumes of automobiles (Blood and Rodostits, 1989). However, lead absorption from intestinal tract is variable depending on animal state, presence of nutrient substances (types and amounts of these substances), duration of exposure, and chemical structure of lead derivatives, which most of lead compounds difficulty solve in water except lead acetate (Mohammad and Al-Khafaji, 2001). Commonly, lead poisoning cause anemia (both hemolytic and hemorrhagic), the mechanism of anemia caused by lead briefly occurred by two ways: first, a shortened erythrocyte lifespan and impairment of heme synthesis, lead causes increased concentration of protoporphyrin by inhibiting heme synthetase, the enzyme which combined protoporphyrin and iron to form heme. Second, lead causes inhibition of the enzyme δ -aminolevulinic acid dehydratase (ALA-D) resulting in a failure of utilization of δ -aminolevulinic acid which is excreted in increased quantities in urine (Blood and Rodostits, 1989; Murray *et al.*, 2006) and finally lead blood level measurement is one of the most important indicator to lead poisoning (Tietz, 1999).

While probiotic was defined as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal balance, it has been used as a substitute of antibiotics that is being used in considerable amounts as growth promoters in broiler products (Ahmed, 2006). However, probiotic act by different mode of action: **1.** Immune enhancement, which it has been reported (Harenaar and Spanhaak, 1994) that probiotics stimulate the immunity of chickens in two ways: **a.** flora from probiotic migrate throughout the gut wall and multiply to a limited extend or **b.** antigen released by the dead organisms are absorbed and thus stimulate the immune system. **2.** Growth stimulation : (Baidya *et al.*, 1993) stated that probiotics were the most effective growth promoter, and probiotic fed chickens had more weight gain than other groups (Lan *et al.*, 2003). **3.** Effect on feed conversion ratio: some studies showed that probiotics supplementation in feed of chickens improve the feed conversion ratio (Jagdish and Sen, 1993; Ergun *et al.*, 2000), and finally, its reported in recent years that probiotics (or some of its compounds) have detoxification action on heavy metals (as lead) (Ibrahim *et al.*, 2006), therefore our goal from the present work was to evaluate the effects of probiotic (BIOMIN)[®] and lead acetate on some physiological and biochemical parameters in broiler chicks.

MATERIALS AND METHODS

Experimental animals: One-day-old forty broiler chicks (Ross strain) were obtained from Al-Ameen company for poultry (Mosul, Iraq), chicks were put in floor cages, with suitable environmental conditions: continuous lighting, well humidity, ventilation, and temperature as recommended by NRC (1984), food and water were *ad libitum*. Experiment was done during April 2008, at the animal house unit and department of Physiology, College of Veterinary Medicine, University of Mosul.

Probiotic was purchased from local market (BIOMIN)[®] IMBO (Biomen G.T.I Gmbh company, Ember AG-Austria) and was add to the diet at dose of 1.5 g/kg diet (manufacture recommended dose).

Lead acetate used in this experiment was Lead (II) acetate -3-hydrate cryst extra pure. (Merck, Darmstadt) at a dose of 320 mg/kg diet, which documented by Vangris and Mare (1974) that this dose cause 50 % mortality (LD₅₀) in chicken. in present study lead acetate was added to broiler feed at two doses; 320 mg/kg diet and 160 mg/kg diet.

Experimental design: one-day-old forty broiler chicks were randomly divided into five groups (8 chicks per group) and fed for 28 days the following diets:

1. Group 1 (control): diet contained essential nutrients optimal to the requirement of chicks (NRC, 1984).
2. Group 2: lead acetate 160 mg/kg diet (160 ppm).
3. Group 3: lead acetate 320 mg/kg diet (320 ppm).
4. Group 4: lead acetate 160 mg/kg diet (160 ppm) + probiotic 1.5 g/kg diet.
5. Group 5: lead acetate 320 mg/kg diet (320ppm) + probiotic 1.5 g/kg diet.

Each group chicks were weighed (42 ± 6) g in the first day of experimental.

Sampling and analysis: at the end of the experiment (28 days of age) blood samples were collected from wing vein, Hb concentration (g/dl) and PCV% were determined immediately before serum separation by blood centrifugation (Shanghai instruments factory 80-2 centrifuge, China) at 3000 xg for 15 minute. The following analyses were performed:

1. Serum lead level ($\mu\text{g/dl}$): was determined according to Olsen and Jatlow (Tietz, 1999) using atomic absorption spectrophotometer (PYE UNICAM SP9 Philips).
2. Serum total protein (g/dl): determined according to Reinhold (1953) by Biuret method with spectrophotometer (PD303, Japan) at 505 nm.
3. Hb concentration (g/dl): determined according to Drabkin method (Nahas, 1951) using kit (Syrbio) with spectrophotometer (PD303, Japan) at 540 nm.
4. PCV%: determined according to Jain (1986) using heparinized capillary tube and hematocrit centrifuge (nuve NT 715, Turkey).

Statistical analysis: analysis of data were performed using analysis of variance ANOVA test by SPSS program (ver.10) to determine mean \pm S.E. Duncan's multiple range test were used to determine significant difference between groups at $P \leq 0.05$ (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Table (1) shows that serum lead level in groups 2 and 3 was significantly ($P \leq 0.05$) higher in comparison with control group (group 1), may be according to the dose level of lead that absorbed from the diet, and these levels were suggestive of toxic lead level

(Altman *et al.*, 1997), the addition of 1.5g probiotic/kg of diet was responsible for a significant ($P \leq 0.05$) reduction in the level of serum lead in both groups 4 and 5, and this may be due to the effect of probiotic in recommended dose (1.5 g/kg diet) in decreasing of serum lead level, by the action of probiotic components (special bacteria as lactobacilli) which have properties that enable them to bind toxins from food (Ibrahim *et al.*, 2006) and then preventing lead absorption from intestine, in the same time, (Halttunen *et al.*, 2007) reported that specific lactic acid bacteria have significant removal of cadmium and lead from water, and the removal was fast and metabolism-independent surface process. It has also been demonstrated that lactic acid bacteria in probiotic display various surface determinants in gastrointestinal tract, and that these are involved in their interaction with enterocytes and other epithelial cells (Chicklowski *et al.*, 2007), these determinants include passive forces, electrostatic interaction, hydrophobic forces, steric forces and specific structures as external appendages covered by lectins (Gusils *et al.*, 1999). However, the serum lead level lowering effect of probiotic was more efficient in group 4 than in group 5, in which serum lead level was reduced from 27 $\mu\text{g/dl}$ (group 2) to 14 $\mu\text{g/dl}$ (group 4) in comparison with 61 $\mu\text{g/dl}$ (group 3) to 42 $\mu\text{g/dl}$ (group 5), and this may refer to that probiotic in recommended dose has lead dose dependant lowering action. Table (1) also showed that serum protein level was unchanged in different doses when compared with control group and this result may be due to that lead acetate nor probiotic in our doses have effect on serum total protein, except in group 3 in which a significant decrease in serum protein level was observed, and this may be due to the vigorous effect of high dose of lead. From the same table (1) it is noticed that body weight was increased significantly in group 4 and 5 in comparing with control group and this refer to the effect of probiotic, which has been recorded that probiotic enhance body weight in chicks (Mohan *et al.*, 1996), while there was no significant changes in body weight in group 2 and this may be due to that lead in this dose has no effect on the body weight in comparison with the decrease in body weight gain in group 3 which may be due to the harmful effect of lead acetate in this high dose on body weight gain (Blood and Radostits, 1989). Table (2) showed that lead acetate has a significant dose dependant decreasing effect on both Hb concentration and PCV% in treated groups when compared with control, and this may be due to the deleterious effect of lead on blood causing anemia by two basic defects: a shortened erythrocyte lifespan and impairment of heme synthesis, however, lead causes increased concentration of protoporphyrin by inhibiting heme synthetase, the enzyme which combined protoporphyrin and iron to form heme (Jain, 1986), lead also causes inhibition of the enzyme- δ -aminolevulinic acid dehydratase (ALA-D), resulting in a failure of utilization of δ -aminolevulinic acid (Blood and Radostits, 1986). Finally it was obvious that probiotic in recommended dose has no improving effect on both Hb concentration and PCV% and this result concur with outcome of (Kamruzzaman *et al.* 2005) which may be due to that probiotic has no direct effect on both Hb concentration and PCV%.

From our work it could be concluded that probiotic decreased lead acetate absorption and reduced its toxic action on studied parameters in chicks.

Table 1: Effect of two doses of lead acetate with and without probiotic on serum lead level, serum total protein, and body weight (Mean \pm S.E.) *

Parameters Groups	Serum Lead level ($\mu\text{g}/\text{dl}$)	Serum total protein (g/dl)	Body weight (g)
Group 1 Control group	3.1 \pm 0.8 f	61 \pm 5.0 a	473 \pm 35 b
Group 2 Lead acetate 160 mg/kg diet	27 \pm 3.0 d	57 \pm 4.4 a	462 \pm 41 b
Group 3 Δ Lead acetate 320 mg/kg diet	61.5 \pm 4.4 a Δ	43 \pm 3.0 b Δ	405 \pm 39 c Δ
Group 4 Lead acetate 160mg/kg diet + probiotic 1.5 g/kg diet	14 \pm 2.1 e	59 \pm 3.7 a	653 \pm 28 a
Group 5 Lead acetate 320 mg/kg diet + probiotic 1.5 g/kg diet	54 \pm 3.9 b	63 \pm 4.1 a	641 \pm 37 a

* Mean \pm S.E. for 8 chicks in each group (except group 3 which contain 5 chicks).

Δ 3 of 8 chicks of group 3 were died at 3rd day of experiment.

Different letters in each column means significant difference at $P \leq 0.05$.

Table 2: Effect of two doses of lead acetate with and without probiotic on Hb concentration and PCV% (Mean \pm S.E.) *.

Parameters Groups	Hb concentration (g/dl)	PCV%
Group 1 Control group	7.2 \pm 1.3 a	34 \pm 2.4 a
Group 2 Lead acetate 160 mg/kg diet	4.8 \pm 1.1 c	27 \pm 2.8 b
Group 3 Δ Lead acetate 320 mg/kg diet	3.9 \pm 1.2 d Δ	22 \pm 3.0 c Δ
Group 4 Lead acetate 160mg/kg diet + probiotic 1.5 g/kg diet	5.0 \pm 0.9 c	28 \pm 3.7 b
Group 5 Lead acetate 320 mg/kg diet + probiotic 1.5 g/kg diet	4.2 \pm 0.8 d	21 \pm 3.1 c

* Mean \pm S.E. for 8 chicks in each group (except group 3 which contain 5 chicks).

Δ 3 of 8 chicks of group 3 were died at 3rd day of experiment.

Different letters in each column means significant difference at $P \leq 0.05$.

REFERENCES

- Ahmed, I., 2006. Effect of Probiotics on Broilers Performance. *Int. J. Poul. Sci.* 5(6): pp.593-597.
- Altman, R.B.; Clubb, S.L.; Dorrestein, G.M., and Quesenberry, K., 1997. *Avian Medicine and Surgery*. 1st Edn. Saunders Co., pp.604-607.
- Baidya, N.; Mandal, L., and Banerjee, G.C., 1993. Efficiency of Feeding Antibiotic and Probiotic in Broilers. *J. Vet. Anim. Sci.* 24: pp120-124.
- Blood, D.C., and Radostits, O.M., 1989. *Veterinary Medicine, A Textbook of the Disease of Cattle, Sheep, Pigs, Goat and Horse*. 7th Edn. Bailliere Tindal Pub., pp.1241-1250.
- Chichlowski, M.; Croom, J.; McBride, B.W.; Havenstein, G.B., and Koci, M.D., 2007. Metabolic and Physiological Impact of Probiotics or Direct-Fed-Microbials on poultry: A Brief Review of Current Knowledge. *Int. J. Poul. Sci.* 6(10): pp.694-704.
- Ergun, A.; Yalcin, S., and Sacakli, P., 2000. Usage of Probiotic and Zinc Bacteria in Broiler Rations. *Ankara Universities Veterinary Fakultesi Dergisi*, 47: pp.271-280.
- Gusils, C.; Gonzalez, S., and Oliver, G., 1999. Some Probiotic Properties of Chicken lactobacilli. *Can. J. Microbiol.* 45: pp.981-987.
- Halttunen, T.; Salminen, S., and Tahvonen, R., 2007. Rapid Removal of Lead and Cadmium from Water by Specific Lactic acid Bacteria, *Int. J. Food Microbiol.* 114(1): pp.30-35.
- Havennaar, R., and Spanhaak, S., 1994. Probiotics from an Immunological Point of View. *Curr. Opin. Biotechnol.* 5: pp.320-5.
- Ibrahim, F.; Halttunen, T.; Tahvonen, R., and Salminen, S., 2006. Probiotic Bacteria as Potential Detoxification Tools: Assessing their Heavy Metal Binding Isotherms. *Can. J. Microbiol.* 52 (9): pp.877-885.
- Jagdish, P., and Sen, A.K., 1993. Effect of Different Growth Promoters on Performance of Broilers. *Poult. Advi.* 26: pp.49-51.
- Jain, N.C., 1986. *Schalm's Veterinary Hematology*. 4th Edn. Philadelphia. Lea and Fobiger. USA.
- Kamruzzaman, S.M.; Kabir, S.M.; Rahman, M.M.; Islam, M.W., and Reza, M.A., 2005. Effect of Probiotic and Antibiotic Supplementation on Body Weight and Hemobiochemical Parameters in Broilers. *Bangl. J. Vet. Med.* 3(2): pp.100-104.
- Lan, P.T.; Binh, T.L., and Benno, Y., 2003. Impact of two Probiotics Lactobacillus Strains Feeding on Fecal Lactobacilli and Weight Gains in Chickens. *J. Gen. App. Microbiol.* 44: pp29-36.
- Mohammad, F.K., and Al-Khafaji, N.J., 2001. *Veterinary Toxicology*, 1st Edn. University of Mosul Pub., Iraq. PP.164-172 (in Arabic).
- Mohan, B.; Kadirvel, R.; Natarajan, A., and Bhaskara, M., 1996. Effect of Probiotic Supplementation on Growth, Nitrogen Utilization and Serum Cholesterol in Broilers. *Br. Poul. Sci.* 37: pp.395-401.
- Murray, R.K.; Granner, D.K., and Rodwell, V.W., 2006. *Harper s Illustrated Biochemistry*. 24th Edn. McGraw-Hill LANGE. Boston.
- NRC (National Research Council), 1984. *Nutrient Requirements of Poultry*. 8th Edn., Revised., Washington D.C., pp.11-15.

- Nahas, G.G., 1951. Spectrophotometric Determination of Hb and Oxyhemoglobin in whole Hemolyzed Blood. *Science*. pp.723-725.
- Reinhold, J.G., 1953. Total Protein Albumin and Globulin. In *Standard Methods of Clinical Chemistry*. D. Seligson, Ed. New York, Academic Press, Inc.(1): 88p.
- Steel, R.G.D., and Torrie, J.H., 1980. *Principles and Procedures of Statistics*. 2nd Edn., McGraw-Hill book Co. Inc. New York, USA.
- Tietz, N.W., 1999. *Fundamental of Clinical Chemistry*. 3rd Edn., Carl, A.B., Edward, R.A. (ed.), W.B. Saunders Co.
- Vengris, V.E., and Mare, C.J., 1974. Lead Poisoning in Chickens and the Effect of Lead on Interferon and Antibody Production. *Can. J. Comp. Med.* 38: pp.328-335.