Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Pharmacology

Effect of Dietary Supplementation of BAM-100® on Growth Performance, Haematological and Biochemical Parameters in Broiler Chickens

Taha Attia[1]; Hosny Elbanna[2]; Mohamed Zahran[1*]

(1) Department of Pharmacology, Faculty of Vet. Med., University of Sadat City.

(2) Department of Pharmacology, Faculty of Vet. Med., Cairo University.

**corresponding author: M.zahran@marvelanimalnutrition.com* *Received: 25/6/2020 Accepted: 20/7/2020*

ABSTRACT:

The efficacy of *Bacillus subtilis* & *Bacillus licheniformis* Mixture (BAM-100®, recommended dose; 100 gram / ton feed) as probiotic dietary supplementation on the performance parameters (body weight gain, food consumption, food conversion as well as mortality), the hematological profile and serum biochemical parameters in broiler chickens was investigated. A total of 40 one-day-old broiler chicks (body weight: 43.35±0.25 g) were randomly divided into two treatments, the two treatment groups were non-supplemented control group and BAM-100® supplemented group. Dietary BAM-100® supplementation significantly improved weight gain (WG), feed conversion ratio (FCR) and mortality compared to non-supplemented control treatment. WBCs, RBCs, PCV, Hb, MCV, MCH and LYM were showed a significant increase in supplemented group that fed on probiotic compared to non-supplemented control group.

Keywords: *Probiotic; BAM-100®; broiler chicks; B. subtilis; B. licheniformis.*

INTRODUCTION

Antibiotics are among the most prescribed medications worldwide. Antibiotic feed supplements have been used in commercial poultry farming for over 50 years due to their growth promoting and prophylactic properties. However, the extensive utility of antimicrobial agents has resulted in an antibiotic residue problem in poultry meat and an increase of antibiotics resistance among most of pathogenic bacteria which considered a great problem of public health and also disturbance of organism's balance that normally inhabit the gut (Deniz *et al.*,2011). This can lead to a range of symptoms especially diarrhea. As a result, natural alternatives for substituting the prohibited growth promoter antibiotics with probiotics have received much attention in the recent past .

Probiotics are organisms thought to improve the balance of organisms that inhabit the gut, counteract disturbances to microorganism balance, and reduce the risk of colonization by pathogenic bacteria (Paulina and Katarzyna, 2017). A variety of microbial species have been used as probiotics including species of *Bacillus, Bifidobacterium, Enterococcus, E. coli, Lactobacillus, Lactococcus, Streptococcus*, a variety of yeast species, and undefined mixed cultures. *Bacillus, Enterococcus*, and *Saccharomyces yeast* have been the most common organisms used in livestock (Simon *et al.*, 2001). Several probiotics used for poultry contain or consist of bacterial spores, principally of the genus *Bacillus* (Cartman and La Ragione, 2004; Hong *et al.*, 2005). Spore-based probiotics are particularly well suited for use as live microbial products as they are metabolically dormant upon administration, they may germinate in the gastrointestinal tract of chicks and function through mechanisms which require them to be metabolically active (e.g. secretion of antimicrobial Compounds and /or competition for essential nutrients) (Cartman *et al.*, 2007 & 2008). Mechanisms by which probiotics improve host animal performance include: (i) to maintain the normal intestinal micro flora by competitive exclusion and antagonism, (ii) to enhance the non-pathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, (iii) to suppress the intestinal pathogens and to enhance the digestion and utilization of nutrients (Yeo and Kim, 1997). It is reported that the major outcomes from using probiotics in livestock include improvement in growth and food efficiency (Yeo and Kim, 1997) and reduction in mortality (Kumprecht and Zobac, 1998). Consequently, the aims of the present study were to determinate the effects of dietary probiotic (B. subtilis, supplementation on performance parameters (body weight gain, food consumption, food conversion ratio as well as mortality), blood Characteristics and serum biochemical parameters in broiler chickens.

MATERIAL AND METHODS

1-Materials

Drug

Dried *Bacillus subtilis* & dried *Bacillus licheniformis* mixture (BAM-100®, recommended dose; 100 gram / ton feed, each 1gm contains 4.1x108 CFU dried Bacillus licheniformis; 3.8x108 CFU dried Bacillus Subtilis and calcium carbonate up to 1gm.(

Chickens

A total of 40 one-day-old Hubbard broiler chicks with similar body masses (43.35 ± 0.25 g) were purchased from a local commercial hatchery. Feed and water were provided ad libitum throughout the study, and all broiler chicks were fed in the same house under a relative humidity of approximately 65 %. The temperature was 33 °C in the first week and then decreased gradually to 24 °C by the third week, then maintained at 24°C to the end of the experiment. Lighting was provided 24 h/day. All birds were fed on corn and soybean diet including a starter (23% CP and ME 3000 kcal/kg feed) from 1 to 14 days, and finisher (21% CP and ME 3100 kcal/kg feed) from 15 to 42 days. Diets formulation and its chemical analysis described in table (1)

Experimental design

All 40 one-day-old broilers Hubbard chicks were randomly distributed in to two groups, each group involves twenty broiler chicks as follows:

Group (1)

All birds were fed on corn and soybean basal control diet as described previously in table (1). This group considered control negative .

Group (2)

All birds were fed on corn and soybean basal control diet as described previously in table (1) in addition BAM-100® (contains 4.1x108 CFU Dried Bacillus licheniformis; 3.8x108 CFU Dried Bacillus Subtilis and Calcium Carbonate up to 1gm) at dose 100 gram / ton feed according to the manufacturer's recommendations.

2-Methods

All birds in each group were weighed individually at hatching (0 wk) and the end of each week till the end of experiment. The amounts of added feed to each pen were recorded daily and remained feed in each pen were weighed weekly. Feed consumption was calculated weekly. Feed conversion ratio (FCR) at end of each week was calculated by dividing the weight of feed consumed in grams for each bird during a period of time by the body weight gain of the same bird in the same group at the same given time. In all trials mortality was recorded daily and weekly and reported as a cumulative percentage. Dead birds were removed daily in the morning (Alkhalf *et al.*, 2010.(

At the terminal of feeding trial, five chickens per group were picked randomly and blood samples were obtained from wing vein. The blood samples were collected into individual tubes and clotted at room temperature for 2 h. The serum was separated by centrifugation (3000×g for 10 min) then stored at −20 °C until the analysis of CBC, ALT, AST, Urea and Creatine (Azarin *et al.*, 2014 .(

Statistical Analysis :

Data were expressed as mean ± standard error and were statistically analyzed using according to (Petrie and Watson, 2013). Comparison of the mean values was performed and differences were considered statistically significant when $P \leq 0.05$.

RESULTS:

Growth performance

All birds in each two groups were weighed individually at the end of each week till the end of experiment (42 day). The amounts of consumed feed were recorded weekly. Feed conversion ratio (FCR) at the end of each week was calculated by dividing the amount of feed consumed in grams for each bird in specific treatment weekly by the body weight gain of the same bird in the same treatment at the same given time. Mortality was recorded daily and weekly. As seen in (Table 2), dietary supplementation with corn and soybean basal control diet in addition to probiotic BAM-100® from the 1st day had exhibited no significant effect on the feed consumption (FC) of broilers compared with non-supplemented control group that fed on corn and soybean basal control diet but showed improved FCR ($p \leq 0.05$) and also improved weight gain ($p \leq 0.01$) compared with non-supplemented control group that fed on corn and soybean basal control diet.

Haematological and Biochemical parameters

At the terminal of feeding trial, five chickens were picked randomly and blood samples were obtained from wing vein then CBC, ALT, AST, Uric acid and Creatine were examined. As can be seen from Table (3), dietary supplementation with corn and soybean basal control diet in addition to probiotic Baymix® grobigTM BS from the 1st day had exhibited a significant increase in WBCs, RBCs, PCV, Hb, MCV, MCH, MCHC, LYM and a significant decrease in Neutrophil, Eosinophil with no significant difference in Monocyte compared to non-supplemented control group. Serum biochemical parameters of supplemented group showed a significant decrease in ALT, AST with no significant difference in Uric acid, Creatine compared to non-supplemented control group .

Table (1): Composition and Chemical analysis of basal control diets.

Ingredient	Starter (1-14 day)	Grower-Finisher (15-42 day)
Yellow corn	555	600
Soybean meal (46% CP)	344	290
Corn gluten meal	40.0	42.0
Soybean oil	17.0	25.0
Monocalcium phosphate	15.0	14.0
Limestone	16.0	16.0
Common Salt (NaCl)	3.80	3.80
Vit. &Min. premix	3.00	3.00
Choline Chloride	1.00	1.00
DL-Methionine	2.70	2.50
L-Lysine	2.50	2.70
Total	1000 Kg	1000 Kg
Chemical Analysis	-----	-----
Crude Protein %	23.0	21.0
ME (Kcal/kg diet)	3000	3100
Fat %	4.30	5.20
Crude Fiber %	2.30	2.10
Calcium %	1.00	0.96
Phosphorus %	0.50	0.48
Lysine %	1.50	1.40
Methionine %	0.69	0.64
Meth.+Cys. %	1.00	0.99

Table (2): Comparison of growth performance of broiler supplemented with probiotic BAM-100® with non-supplemented control group.

Parameters		Control	BAM-100®
Day 0-7	FC	151.52^{a}	150.72^{a}
	AWG	132.85±2.12^{b}	137.30±1.20^{a}
	FCR	1.140±0.27^{a}	1.098±0.02^{b}
Day 7-14	FC	320.19^{a}	318.426^{b}
	AWG	279.27±1.95^{b}	284.85±2.71^{a}
	FCR	1.147±0.07^{a}	1.118±0.14^{b}
Day 14-21	FC	591.17^{a}	589.688^{a}
	AWG	430.53±2.37^{b}	437.026±2.90^{a}
	FCR	1.373±0.11^{a}	1.349±0.24^{b}
Day 21-28	FC	916.04^{a}	910.810^{b}
	AWG	540.211±3.43^{b}	550.034±1.78^{a}
	FCR	1.696±0.37^{a}	1.656±0.045^{b}
Day 28-35	FC	1255.17^{a}	1250.728^{a}
	AWG	626.067±1.67^{b}	637.09±3.72^{a}
	FCR	2.005±0.18^{a}	1.963±0.37^{b}
Day 35-42	FC	1445.23^{a}	1439.099^{b}
	AWG	659.778±2.77^{b}	671.403±2.60^{a}
	FCR	2.191±0.23^{a}	2.143±0.16^{b}

FCR: feed conversion rate, FC: feed consumption, AWG: average weight gain. $^{a-b}$ Means with different superscripts within a row were significantly different ($p \leq 0.05$).

Table (3): Comparison of Haematological and Biochemical parameters of broiler supplemented with probiotic BAM-100® with non-supplemented control group.

Parameters		**Control**	**BAM-100®**	**Unit**
Hematological Parameters	WBC	15.32 ± 1.71^{c}	15.45± 1.72^{a}	× 10^3/µl
	RBC	2.69 ± 2.04^{c}	2.91 ± 2.07^{b}	× 10^6/µl
	Hb	11.62 ± 0.15^{c}	12.35 ± 1.17^{a}	g/dl
	PCV	33.0 ± 2.17^{c}	33.37 ± 2.31^{a}	%
	MCV	116.27 ± 1.12^{b}	116.96± 1.18^{a}	fl
	MCH	39.11 ± 0.24^{c}	39.86 ± 0.23^{a}	pg
	MCHC	27.93 ± 2.51^{c}	28.79 ± 0.56^{a}	g/dl
	Neutrophil	25.40 ± 1.12^{a}	22.30± 3.14^{b}	%
	Lymphocyte	63.68 ± 1.65^{c}	66.76± 2.86^{b}	%
	Monocyte	4.15 ± 2.68^{b}	4.14 ± 1.78^{b}	%
	Eosinophil	4.30 ± 1.59^{a}	3.82 ± 3.19^{b}	%
Serum Biochemical parameters	ALT	13.33 ± 1.20^{a}	10.63 ± 2.27^{c}	u/l
	AST	233.23 ± 1.87^{a}	218.10± 1.97^{c}	u/l
	Uric acid	3.62 ± 1.92^{b}	3.71 ± 1.89^{b}	mg/dl
	Creatine	0.44 ± 0.052^{b}	0.46 ± 0.063^{b}	mg/dl

$^{a-b}$ Means with different superscripts within a row were significantly different ($p \leq 0.05$).

DISCUSSION

Probiotics are organisms thought to improve the balance of organisms that inhabit the gut, counteract disturbances to this balance, and reduce the risk of colonization by pathogenic bacteria (Paulina and Katarzyna, 2017). A variety of microbial species have been used as probiotics including species of *Bacillus, Bifido bacterium, Entero- coccus, E. coli, Lactobacillus, Lacto- coccus, Streptococcus*, a variety of yeast species, and undefined mixed cultures. *Bacillus, Enterococcus*, and *Saccharomyces* yeast have been the most common organisms used in livestock (Simon *et al.*, 2001).

Dietary supplementation with corn and soybean basal control diet in addition to probiotic BAM-100® from the 1st day at dose 100 gram / ton feed had exhibited no significant effect on the FC of broiler compared with control negative group but showed improved FCR ($p \leq 0.05$) and also improved weight gain ($p \leq 0.01$) compared with non-supplemented control group that fed on corn and soybean basal control diet. This finding is in agreement with previous reports shown that *Bacillus subtilis* & *Bacillus licheniformis* mixture have a positive effect on weight gain and FCR as in pigs (European Commission, 2000; Alexopoulos *et al.*, 2004 a,b), in fish (Azarin *et al.*, 2014) but with higher weight gain and FCR, in broiler (Cheng *et al.*, 2017; He *et al.*, 2019), shrimp (Abdollahi-Arpanahi *et al.*, 2018; Madani *et al.*, 2018) but disagree with reports of piglets (Danicke and Doll, 2010) which exhibited no significant effect on weight gain with higher feed consumption and so feed consumption ratio (FCR.(

Moreover, Dietary supplementation with corn and soybean basal control diet in addition to probiotic BAM-100® from the 1st day at dose 100 gram / ton feed in a comparison to control negative group showed a significant increase in hematological parameters as (WBCs, RBCs, Hb, PCV, MCV, MCH, MCHC) and also a significant increase in lymphocyte but lower neutrophil and eosinophil without any significant effect on monocyte. This finding is in agreement with previous reports of fish (Azarin *et al.*, 2014) but with significant decrease in WBCs and neutrophil and no effect on PCV, also agreed with reports of Persian sturgeon (Acipenser persicus) fingerlings (Darafsh *et al.*, 2018) but with significant decrease in MCV, MCH and higher neutrophil with no effect on MCHC.

At the terminal of feeding trial, blood samples were obtained from wing vein. The blood samples were collected and serum was separated by centrifugation then examined for liver (ALT, AST) and kidney function (Uric acid, Creatine), which exhibited a significant decrease in ALT, AST as reports in juvenile white shrimp (Abdollahi-Arpanahi *et al.*, 2018) and no effect on kidney function (Uric acid, Creatine).

CONCLUSION

In conclusion, results of this work clearly show that supplementation with probiotics BAM-100® significantly improved growth performance (increased weight gain and feed utilization effciency) and improve their intestinal health and gut integrity in broiler chickens.

REFERENCES

Abdelqader, A., Al-Fataftah, A. and Das, G. (2013). Effects of dietary *Bacillus subtilis* and inulin supplementation on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production. Animal Feed Science and Technology, 179: 103-111.

Abdollahi-Arpanahi, D., Soltani, E., Jafaryan, H., Soltani, M., Naderi-Samani, M., Campa-Cordova, A.I. (2018). Efficacy of two commercial and indigenous probiotics, *Bacillus subtilis* and *Bacillus licheniformis* on growth performance, immuno-physiology and resistance response of juvenile white shrimp (Litopenaeus vannamei). Aquaculture, 496: 43–49.

Alexopoulos, C., Georgoulakis, I.E., Tzivara, A., Kritas, S.K., Siochu, A. and Kyriakis, S.C. (2004). Field evaluation of the efficacy of a probiotic containing *Bacillus licheniformis and Bacillus subtilis* spores on the health status and performance of sows and their litters. J. Anim. Physiol. a. Anim. Nutr., 88: 381–392.

Alexopoulos, C., Georgoulakis, I.E., Tzivara, A., Kyriakis, C.S., Govaris, A. and Kyriakis, S.C. (2004). Field Evaluation of the Effect of a Probiotic-containing *Bacillus licheniformis and Bacillus subtilis* Spores on the Health Status, Performance, and Carcass Quality of Grower and Finisher Pigs. J. Vet. Med. A, 51: 306–312.

Alkhalf, A., Alhaj, M. and Al- homidan, I. (2010). Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens. Saudi Journal of Biological Sciences.Vol.17, Issue 3, 219-225.

Azarin, H., Aramli, M.S., Imanpour, M.R. and Rajabpour, M. (2014). Effect of a Probiotic Containing *Bacillus licheniformis and Bacillus subtilis* and Ferroin Solution on Growth Performance, Body Composition and Haematological Parameters in Kutum (Rutilus frisii kutum) Fry. Probiotics & Antimicro. Prot. 7 (1), 31–37.

Cartman, S.T. and La Ragione, R.M. (2004). Spore probiotics as animal feed supplements. In: Bacterial Spore Formers: Probiotics and Emerging Applications (Ricca, E., Henriques, A.O., Cutting, S.M., Eds.), pp.155–161.Horizon Bioscience, Norfolk, UK.

Cartman, S.T., La Ragione, R.M. and Woodward, M.J. (2007). Bacterial spore formers as probiotics for poultry. Food Sci. Tech. Bull., 4:21-30.

Cartman, S.T., La Ragione, R.M. and Woodward, M.J. (2008). *Bacillus subtilis* spores germinate in the chicken gastrointestinal tract. Appl. Environ. Microb., 74: 5254-5258.

Cheng, Y., Chen, Y., Li, X., Yang, W., Wen, C., Kang, Y., Wang, A. and Zhoua, Y. (2017). Effects of synbiotic supplementation on growth performance, carcass characteristics, meat quality and muscular antioxidant capacity and mineral contents in broilers. J. Sci. Food. Agric., 97: 3699-3705.

Danicke, S. and Doll, S. (2010). A probiotic feed additive containing spores of *Bacillus subtilis and B. licheniformis* does not prevent absorption and toxic effects of the Fusarium toxin deoxynivalenol in piglets. Food and Chemical Toxicology, 48: 152–158.

Darafsh, F., Soltani, M., Abdolhay, H.A. and Mehrejan, M.S. (2018). Efficacy of dietary supplementation of *Bacillus licheniformis and B. subtilis* probiotics and Saccharomyces cerevisiae (yeast) on the hematological, immune response, and biochemical features of Persian sturgeon (Acipenser persicus) fingerlings. Iranian Journal of Fisheries Sciences. DOI: 10.22092.

Deniz, G., Orman, A., Cetinkaya, F., Gencoglu, H., Meral, Y. and Turkmen, I.I. (2011). Effects of probiotic (*Bacillus subtilis DSM 17299*(supplementation on the caecal microflora and performance in broiler chickens. Revue Méd. Vét., 162: 11, 538-545.

European Commission (2000): Report of the Scientific Committee on animal nutrition on product BioPlus 2B® for use as feed additive. http://ec.europa.eu/food/fs/sc/scan/out49_en.pdf

He, T., Long, S., Mahfuz, S., Wu, D., Wang, X., Wei, X. and Piao, X. (2019). Effects of Probiotics as Antibiotics Substitutes on Growth Performance, Serum Biochemical Parameters, Intestinal Morphology, and Barrier Function of Broilers. Animals; 9(11):985. Doi: 10.3390.

Hong, H.A., Ducle, H. and Cutting, S.M. (2005). The use of bacterial spore formers as probiotics. FEMS Microbiol. Rev., 29, 813-835.

Kumprecht, I. and Zobac, P. (1998). The effect of probiotic preparations containing *Saccharomyces cerevisiae and Enterococcus faecium* in diets with different levels of B-vitamins on chicken broiler performance. Zivocisna Vyroba, 43: 63-70.

Madani, N.S.H., Adorian, T.J., Farsani, H.G. and Hoseinifar, S.H. (2018). The effects of dietary probiotic *Bacilli (Bacillus subtilis and Bacillus licheniformis*) on growth performance, feed efficiency, body composition and immune parameters of white leg shrimp (Litopenaeus vannamei) postlarvae. Aquaculture Research. Vol. 49(5): 1926-1933 DOI:10.1111/ are.13648 .

Melegy, T., Khaled, N.F., El-Bana. R. and Abdellatif, H. (2011): Effect of Dietary Supplementation of *Bacillus subtilis PB6* (CLOSTATTM) on Performance, Immunity, Gut Health and Carcass Traits in Broilers. Journal of American Science; 7: (12), 891-898.

Park, J.H., Yun, H.M. and Kim, I.H. (2018): The effect of dietary *Bacillus subtilis* supplementation on the growth performance, blood profile, nutrient retention, and caecal microflora in broiler chickens. Journal of Applied Animal Research, 46: 1, 868-872.

Paulina, M. and Katarzyna, S. (2017). Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Nutrients, 9 (9): 1021.

Petrie, A. and Watson, P. (2013): Statistics for veterinary and animal science. 3rd edition. Wiley-Blackwell, Hoboken, NJ, USA .

Yeo, J. and Kim, K. (1997). Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broiler chicks. Poult. Sci., 76: 381-385.