Dietary symbiotic supplementation alters the ileal histomorphology and caecal pathogen micro-organism in broiler chicks

I. Coskun[1*], M. Tad[2], G. Filik[3], A. Altop[4], A. Sahin[1], G. Erener[4], H. E. Samli[5]

[1]Department of Animal Science, [3]Department of Agricultural Biotechnology,Faculty of Agriculture, [2]Department of Histology and Embryology, Faculty of Medical Sciences, Ahi EvranUniversity, TR40001, Kirsehir, [4]Department of Animal Science,Faculty of Agriculture, OndokuzMayis University, TR55139, Samsun, [5]Department of Animal Science,Agricultural Faculty,Namık Kemal University,TR59000,Tekirdag, Turkey
*Corresponding authorEmail: isa.coskun@ahievran.edu.tr

Journal of Livestock Science (ISSN online 2277-6214) 8: 109-114
Received on 27/12/2016; Accepted on 29/3/2017

Abstract

The aim of this study was to determine the effects of dietary symbiotic supplementation on growth performance, digestive tract development, ilealhistomorphology, caecaltotal*Coliform, E coli andEnterobactericaea*counts in different chick weights. A blend of mannanoligosaccharide and *Saccharomyces cerevisiae* mixture was used as symbiotic. Two initial body weight groups (L= Light and H= Heavy chicks) and two feeds (B= basal diet and S= 0,2% symbiotic supplemented diet) were tested in 2x2 factorial experimental design. One hundred and twenty newly hatched chicks obtained from a healthy broiler parent stock aged 40 wk old. Each treatment group had 3 replicates consist of 10 chicks. Trial lasted for 21 days. Daily body weight gain (DBWG), daily feed intake (DFI), feed conversion ratio (FCR) (g feed/g gain) was recorded weekly. The DBWG, DFI, FCR and digestive tract development (heart, liver, gizzard, proventriculus, pancreas, bursa fabricius weight and gut length) were not affected by treatments. Symbiotic supplementation increased villi length. Symbiotic supplementation decreased pathogenic microorganisms (*Escherichia Coli, Coliform and Enterobactericaea*) in caeca irrespective to the DBWG of chicks. To conclude, symbiotic supplementation can be used to improve villi morphology and to suppress pathogenic microorganisms in caeca.

Key words: broiler chicks;mannanoligosaccharide;*Saccharomyces cerevisiae*

Introduction

Broiler performance depends on some factors such as chick weight, feed quality and environmental conditions such as ambient temperature and room sanitation. Some of these factors can be improved by interventions of breeders. However, chick weight are depends on parent age and their feeding status (Tona*et.al*.,2004; Alabi*et.al*., 2012). Also chick weight depends on egg weight and chick weight determines final performance of broiler. Broiler breeders lay average 45 g egg at 21 wk of age and 60 g at 32 week of age, after all egg weights increase toward the end of 72 week (Tona*et.al*., 2004). Thus small eggs mean light chicks. Final performance of light chick is lower from heavy chicks. Raju *et.al*., (1997) and Ulmer-Franco *et.al*., (2010) demonstrated that chicks hatched from small eggs had lower final body weight than those from hatched heavier eggs. Also, they reported that one day old chick weight increased with increased egg weight.

Feed additives (Probiotics, prebiotics or mixture as a symbiotic) can be used to increase body weight gain of light chicks by developing gut microbiota and histomorphological traits.As known, probiotics and prebiotics affect broiler performance. Indeed, *Saccharomycescerevisiae* and mannan oligosaccharide (MOS) have been used for competitive exclusion or manipulation of gastrointestinal microflora of poultry (Samli *et.al*., 2007; Santin*et.al*., 2001; An *et.al*., 2008; Markovicra*et.al*., 2009; Koc*et.al*., 2010). It has been reported that symbiotic increased broiler performance by decreasing pH in digesta and increasing villi length and width in duodenum, jejunum and ileum (Santin*et.al*., 2001; Markovicra*et.al*., 2009) and increasing immune response (Verduzco*et.al*., 2009). Also Yason*et.al*.,(1987);Xu *et.al*., (2003) and Sinovec*et.al*., (2005) have reported that administration of probiotics and prebiotics to broiler feed improved gut mucosal structure (increased villi length and width) has been claimed to be an indicator of increased transport area and enhanced nutrient absorption.

Although there are many studies regarding the effect of chick weight, MOS and *Saccharomyces cerevisiae* on broiler performance in the literature, there is no study in accordance with the initial chick weight and dietary symbiotic supplementation on broiler performance, ileal histomorphology and caecal *Coliform, E. coli* and*Enterobactericaea* count. The aim of this study was to test the effect of initial chick weight and dietary symbiotic supplementation on live weight gain, gastro intestinal tract development, ileal histomorphology,caecal total*Coliform, E coli* and *Enterobactericaea* count in broiler chicks.

Materials and methods

This study was conducted at the Poultry Research Unit of Ahi Evran University, Kirsehir, Turkey. The practices and procedures for this experiment were reviewed and approved by the Ahi Evran University, Animal Ethics Committee (22.01.2015/8.). One hundred twenty hatched chicks obtained from Ross 308 broiler parent stock aged 40 wk old. Then these chicks were divided into to two body weight groups' heavy and light. Two body weight groups (L: Light 43 ± 1 g and H: Heavy chicks 49 ± 1 g) and two feeds (B: Basal diet, 3.08Mcalmetabolizable energy (ME) $kg^{-1}$and 223.9 g crude protein (CP) $kg^{-1}$and S: 0,2% Symbiotic supplemented diet) were tested 2x2 factorial experimental design. Dietary groups were LB: light chick and basal diet, LS: light chick and symbiotic inclusion in diet, HB: heavy chicks and basal diet, HS: heavy chicks and symbiotic inclusion in diet. Symbiotic product provided from Global NutritechCompany (800 E. Leigh Street Richmond, VA 23219 USA) and it includes 88 g/kg MOS, $4x10^{12}$cfu/kg *Saccharomyces cerevisiae*.Each treatment group was divided into 3 replicates and each replicates include 10 chicks (5 females and 5 males). Chicks randomly transferred to battery-type four-tier cages. Each cage included then chicks or one replicate bird. Battery cages were equipped with wire mesh, dropping trays, nipple drinkers and trough feeders. All chicks were fed ad libitum the same starter diet during the period of 1-21 days (Table 1). Continuous lighting was provided during the experiment. Ambient temperature was gradually decreased from 32°C on d 7 to 28°C on d 21 and was kept constant. Body weight and feed intake were measured at 1, 7, 14 and 21 d of age. All the cages were checked for mortality twice a day and mortality was recorded as it occurred. At 21 d of age, chicks were starved for 6 h before slaughtering, and 2 birds (1 female and 1 male) per replicate were slaughtered, humanly to determine digestive tract development (inner organ weights), ileal histomorphologyand caecal *Coliform, E coli* and *Enterobactericaea*count.

Ileum samples were taken from between the Meckel's diverticulum and the ileo-ceco-colic junction and cut into 1,5 cm pieces and placed into 10% formalin for further processing. Tissues sections were placed into tissue cassettes for dehydration process and were embedded in paraffin blocks, and subsequently cut 5-µ thickness and placed on a slide. Each ilealhistomorphologic tissue sample was prepared and stained with hematoxylin and eosin solution by using standard paraffin-embedding methods. After embedding process, villi length and villus width evaluated by using an image processing and analysis system (ZEN 2012 SP2) for Zeiss Primo Star HD Light Microscope. A sample for HS group's ileal image was given in Figure 1.

Pre-weighed ileum samples (1 g) for microbiological analyses were transferred into dilution bottles. Anaerobic diluents were added to achieve a 1 to 10 (w/v) dilution. The samples were mixed with a vortex until completely suspended and dispensed using standard methods into a 1 to 10 (v/v) dilution series of tubes containing anaerobic peptone buffer. Appropriate dilutions were inoculated onto the plates. 3M Petrifilm™ (3M

Microbiology Products St. Paul MN 55114 USA) were used to determine *Escherichia Coli, Coliform and Enterobactericaea* count in caecal samples. The following the manufacturer's instructions for incubation conditions were used to enumerate microbial counts of samples: *Escherichia Coli*: at 32°C for 24 h.*Coliform*: at 35°C for 24 h.*Enterobactericaea*: at 32°C for 24 h. *Escherichia coli*, *Coliform* and *Enterobactericaea* colonies were counted and the average number of live bacteria was calculated based on per gram of original caecal contents as $\log_{10}$CFU/g.

The data were analyzed using the general linear models procedure of SPSS software (SPSS 15). Differences between groups' means were separated by Duncan's multiple range tests.

Table 1. Composition of experimental diet (%).

Ingredients	%
Maize	44.00
Soybean meal (44)	41.15
Meat and bone meal	4.00
Soybean oil	6.50
Dicalciumphosphate	2.50
L-lysine HCl	0.70
DL-methionine	0.35
Sodium chloride	0.30
Vitamin Premix*	0.25
Mineral Piremix#	0.25
Nutrient composition	
ME [kcal/kg]	3080
Crude protein	22.39
Crude fibre	2.80
Crude fat	8.50
Calcium	7.60
Available phosphorus	3.80

Premix provided per kg of diet: * Vitamin A, 12.000 IU; Vitamin D3, 2.400 IU; Vitamin E, 30 mg; Vitamin K3, 4 mg; Vitamin B1, 3 mg; Vitamin B2, 7 mg; Vitamin B6, 5 mg; Vitamin B12, 15 µg; niacin, 25 mg; # Fe, 80 mg; folic acid, 1 mg; pantothenic acid, 10 mg; biotin, 45 mg; Choline, 125000 mg; Cu, 5 mg; Mn, 80 mg; Zn, 60 mg; Se, 150 µg.

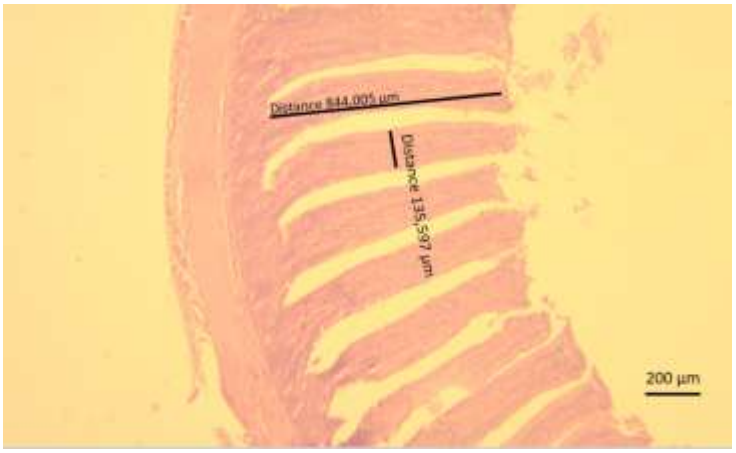

Fig 1. ileal villi length and villi width in HS group. Bar 200 µm.

Results

Although mortality happens in only LB group during the study, mortality (for the HS, HB, LS and LB groups respectively) was not affected by treatments ($P<0.05$).The effects of initial chick weight and dietary symbiotic supplementation on daily body weight gain (DBWG), daily feed intake (DFI) and feed conversion ratio (FCR) results are given in Table 2. The DBWG, DFI andFCR were not affected by dietary symbiotic supplementation and initial chick weight ($P<0.05$). The effects of initial chick weight and dietary symbiotic supplementation on heart, liver, gizzard, proventriculus, pancreas, bursa fabricius weights and gut length are

given in Table 3. Inner organ weights were not affected by dietary symbiotic supplementation and initial chick weight (P<0.05).

Table 2. The effects of initial chick weight and dietary symbiotic supplementation on daily body weight gain (DBWG), daily feed intake(DFI) and feed conversion ratio (FCR)

Chicks	L		H		P values			
Diet	B	S	B	S	SEM	CW	D	CW*D
DBWG, g	33.14	33.29	33.10	32.78	0.19	0.85	0.54	0.60
DFI, g	49.60	50.92	50.50	51.06	0.45	0.36	0.61	0.70
FCR, g feed/g gain	1.50	1.53	1.56	1.51	0.02	0.33	0.43	0.92

CW: chick weight,D: Diet, B: Basal, H: Heavy, L: Light,S: Symbiotic, SEM: Standard error of means.

Table 3. The effects of initial chick weight and dietary symbiotic supplementation on heart, liver, gizzard, proventriculus, pancreas, bursa fabricius weight (g 100 g^{-1} body weight) and gut length (cm 100 g^{-1} body weight)

Chicks	L		H		P values			
Diet	B	S	B	S	SEM	CW	D	CW*D
Heart	0.70	0.71	0.69	0.68	0.018	0.57	0.94	0.74
Liver	3.03	3.05	3.36	3.38	0.068	0.01	0.84	0.99
Gizzard	3.28	3.42	3.57	3.07	0.084	0.85	0.26	0.06
Proventriculus	0.55	0.62	0.63	0.62	0.017	0.28	0.28	0.05
Pancreas	0.35	0.37	0.35	0.32	0.011	0.28	0.93	0.25
Bursa fabricius	0.28	0.28	0.30	0.28	0.010	0.52	0.60	0.64
Gut length	21.84	21.25	21.12	21.56	0.323	0.78	0.92	0.50

CW: chick weight, D: Diet, B: Basal, H: Heavy, L: Light, S: Symbiotic, SEM: Standard error of means

Table 4. The effects of initial chick weight and dietary symbiotic supplementation on villi length, villi width, *Coliform, E. coli and Enterobactericaea* count (log_{10} cfu g^{-1}) in broiler chickens

Chicks	L		H		P values			
Diet	B	S	B	S	SEM	CW	D	CW*D
Parameters								
Villi length	610^{b}	727^{a}	587^{b}	784^{a}	19.65	0.55	0.001	0.17
Villi width	98^{bc}	112^{b}	83^{c}	130^{a}	4.08	0.77	0.001	0.02
Coliform	1.46^{b}	1.30^{a}	1.41^{b}	1.29^{a}	0.022	0.27	0.001	0.45
E Coli	1.40^{b}	1.31^{a}	1.44^{b}	1.32^{a}	0.018	0.32	0.001	0.45
Enterobactericaea	1.22^{b}	1.02^{a}	1.19^{b}	1.08^{a}	0.024	0.55	0.001	0.15

Means in the same row with the same letter are not significantly different (P > 0.05),
CW: Chick weight, D: Diet, B: Basal, H: Heavy, L: Light, S: Symbiotic, SEM: Standard error of means

The effects of initial chick weight and dietary symbiotic supplementation on villi length, villi width, caecal *Coliform, E coli and Enterobactericaea* count in broiler chickens are given in Table 4. Villi length increased with dietary symbiotic supplementation irrespective to initial chick weight (P>0.05). Villi width increased in HS group (P>0.05). Ceaecal *Coliform, E Coli* and *Enterobactericaea* count decreased with dietary symbiotic supplementation irrespective to initial chick weight (P>0.05).

Discussion

The results of the present study showed that dietary symbiotic supplementation can be used to improve villi morphology and to suppress pathogenic microorganisms in caeca of broiler chickens.An increase in daily weight gain was expected with the increase in ileal villi height and a decrease of caecal pathogenic microorganism colonization. Similarly to the results of Fritts and Waldroup (2003); Stanczuk *et.al*. (2005); Waldroup *et.al*. (2003); Corrigan *et.al*., (2011) and Chen *et.al*., (2009) daily weight gain results were not reflected by the increased ileal villilength and decreased caecal pathogenic microorganism colonization. This study was conducted in environmentally controlled room and the summer season in Kirsehir province and there were no negative environmental and dietary factors during this period of the study. Growth performance may be unaffected for these reasons. Ozturk and Yildirim, (2004) have reported that broiler performance depends on multiple factors such as genetic, environmental or different stress factors. Although there was no difference on the chick growth at 21 d of age among the groups, symbiotic supplementation improved digestive tract health. Furthermore, an increase in growth performance parameters were expected with the increase in initial chick

weight, but daily weight gain was not reflected according to the initial chick weight. The results of this study were not confirmed studies of Morrisa *et.al*., (1968); Dos Santos *et.al*., (2010); Ulmer-Franco *et.al*., (2010) and Alabi *et.al*., (2012) on initial chick weight. They have reported that chicks from light eggs had lower final body weight than those chicks from medium and large eggs. Chicks used in this study were obtained healthy 40 wk old Ross 308 broiler breeders; this may be attributed to use the 40 week old broiler breeder's eggs. Tona *et.al*.,(2001) demonstrated that egg quality is the best of 40 to 42 wk of age broiler breeders. Also, Tona *et.al*., (2004) reported that broiler breeder's age did not affect broiler performance and they concluded that growth parameters of one d old chick depend on incubating egg quality and physiological stage of breeders.

Ileal histomorphology parameters and caecal microorganism count are shown in Table 4. According to the results, initial chick weight did not affect villi length, villi width, caecal *E coli, Coliform and Enterobactericaea* count. Symbiotic supplementation increased villi length, villi width and decreased pathogenic microorganisms in caeca irrespective to the live weights of chicks.

It has been reported that symbiotic supplementation to broiler diet suppress the pathogenic bacteria in bird's digestive tract and it facilitates a barrier between the intestinal wall and the lumen of gut for the pathogenic bacteria. Symbiotic in a chick's gut increases the volatile fatty acids (VFA) production. Increased VFA levels decreased the pH in chicks gut. Lowering the pH and increased VFA create an unfavorable environment for pathogens (Samli *et.al*., 2007). Although VFA was not investigated in the present study, decreased the caecal *Coliform, E coli and Enterobactericaea* count with dietary symbiotic supplementation irrespective to initial chick weight may be an indicator of increased VFA in caecum.

Our results agree with the results of studies on using dietary symbiotic in broiler chicks, (Markovicra *et.al*., 2009; Santin *et.al*., 2001; Koc *et.al*., 2010; Brümmer *et.al*., 2010; Coskun *et. al*., 2015). These results suggested that dietary symbiotic supplementation improved the health of broiler gut although there was no improvement in broiler performance.To conclude symbiotic supplementation can be used to improve villi morphology and suppress pathogenic microorganisms in caeca (*Coliform, E coli and Enterobactericaea*) of broilers.

References

1) Alabi, O.J., Ng`ambi, J.W., Norris, D. and Mabelebele M. 2012. Effect of egg weight on hatchability and subsequent performance of potchefstroomkoekoek chicks. Asian Journal of Animal and Veterinary Advances 7: 718-725.
2) An, B.K., Cho, B.L., You, S.J., Paik, H.D., Chang, H.I., Kim, S.W., Yun, C.W. and Kang, C.W 2008. Growth performance and antibody response of broiler chicks fed yeast derived β-glucan and single-strain probiotics. Asian-Australasian Journal of Animal Sciences 21: 1027-1032.
3) Brümmer, M., Jansen van Rensburg, C. and Moran,C.A. 2010. Saccharomyces cerevisiae cell wall products: The effects on gut morphology and performance of broiler chickens. South African Journal of Animal Science 40: 14-21.
4) Chen, K. L., Kho, W.L., You, S., Yeh, H.R.H., Tang,S.W. and Hsieh C.W. 2009. Effects of *bacillus subtilis* var. natto and *saccharomyces cerevisiae* mixed fermented feed on the enhanced growth performance of broilers. Poultry Science 88: 309-315.
5) Corrigan, A., Horgan, K., Clipson,N. and Murphy, R.A. 2011. Effect of dietary supplementation with a *Saccharomyces cerevisiae* mannan oligosaccharide on the bacterial community structure of broiler cecal contents. Applied and Environmental Microbiology 77: 6653-6662.
6) Coskun, I., Tahtabicen, E., Koc, F., Okur, A.A., Yilmaz, K., Kanter, M., Aktas, C., Erboga, M and Samli, H.E. 2015. Effects of combined in ovo injection of dried whey and Enterococcus faecium on performance, ileal histomorphology, erythrocyte morphology and ileal microbiota of broiler chickens. European Poultry Science, 79 DOI: 10.1399/eps.2015.102
7) Dos Santos, T.T., Corzo, A., Kidd, M.T., McDaniel, C.D., Torres Filho, R.A. and Araújo, L.F. 2010. Influence of in ovo inoculation with various nutrients and egg size on broiler performance. Journal of Applied Poultry Research 19:1-12.
8) Fritts, C.A. and Waldroup,P.W. 2003.Evaluation of bio-mos® mannan oligosaccharide as a replacement for growth promoting antibiotics in diets for turkeys. International Journal of Poultry Science 2: 19-22.
9) Koc, F., Samli, H., Okur, A., Ozduven, M., Akyurek, H., and Senkoylu,N. 2010.Effects of *Saccharomyces cerevisiae* and/or mannan oligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. Bulgarian Journal of Agricultural Science 16: 643-650.
10) Markovicva, R., Šefera, D., Krsticvb,M. and Petrujkicva,B. 2009. Effect of different growth promoters on broiler performance and gut morphology. Archivos de MedicinaVeterinaria 41: 163-169.

11) Morrisa, R.H., Hesselsa,D.F. and Bishop,R.J. 1968. The relationship between hatching egg weight and subsequent performance of broiler chickens. British Poultry Science 9: 305-315.
12) Ozturk, E. and Yildirim, A. 2004. Prebiotics Supplementation to the Diets Broiler on Performance and Intestinal Microbiological Characteristics. Ulusal Zootekni Bilim Kongresi, Cilt 2. 152-156, ISPARTA.
13) Raju, M.V.L.N., Chawak, M.M., Praharaj, N.K., Rao, S.V.R. and Mishra, S.K. 1997. Interrelationships among egg weight, hatchability, chick weight, post-hatch performance and rearing method in broiler breeders. The Indian Journal of Animal Sciences 67:1.
14) Samli, H.E., Senkoylu, N., Koc, F., Kanter, M. and Agma,A. 2007. Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota. Archives of Animal Nutrition 61: 42-49.
15) Santin, E., Maiorka,A. and Macari,M. 2001. Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. The Journal of Applied Poultry Research 10: 236-244.
16) Sinovec, Z., Markovic,R. and Gledic,D. 2005. Influence of Bio-Mos on broilers performances and gut morphology. 15th European Symp.on Poultry Nutrition. September 25 – 29. pp.339–341. Balatonfüred,, Hungary).
17) Stanczuk, J., Zdunczyk, Z., Juskiewicz,J. and Jankowski,J. 2005. Indices of response of young turkeys to diets containing mannanoligosaccharide or inulin.Veterinarija Ir Zootechnika 31: 98-101.
18) Tona, K., Bamelis, F., Coucke, W., Bruggeman,V. and Decuypere, E. 2001. Relationship between broiler breeder's age and egg weight loss and embryonic mortality during incubation in large-scale conditions. The Journal of Applied Poultry Research 10 : 221-227.
19) Tona, K., Onagbesan, O., De Ketelaere, B., Decuypereand, E. and Bruggeman,V. 2004.Effects of age of broiler breeders and egg storage on egg quality, hatchability, chick quality, chick weight, and chick posthatch growth to forty-two days.The Journal of Applied Poultry Research. 13:10–18.
20) Ulmer-Franco, A.M., Fasenko,G.M. and O'Dea Christopher,E.E. 2010. Hatching egg characteristics, chick quality, and broiler performance at 2 breeder flock ages and from 3 egg weights.Poultry Science 89: 2735-2742.
21) Verduzco, G.G., Cuevas, A.C., Coello, C.L., González,E.Á. and Nava, G.M. 2009. Dietary supplementation of mannan-oligosaccharide enhances neonatal immune responses in chickens during natural exposure to *Eimeria* spp. Acta Veterinaria Scandinavica 51:11
22) Waldroup, P.W., Oviedo-Rondon,E.O. and Fritts,C.A. 2003.Comparison of bio-mos® and antibiotic feeding programs in broiler diets containing copper sulfate. International Journal of Poultry Science 2: 28-31.
23) Xu, Z.R., Hu, C.H., Xia, M.S., Zhan, X.A. and Wang, M.Q. 2003.Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poultry Science 82: 1030–1036.
24) Yason, C.V., Summers,B.A. and Schat,K.A. 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys.Pathology. American Journal of Veterinary Research 6: 927–938.