Journal of Animal and Veterinary Advances 8 (8): 1509-1515, 2009
ISSN: 1680-5593

Comparison Effects of Several Growth Stimulating Additives on Performance Responses and Microbial Population in Crop and Ileum of Broiler Chickens on Their 21st Day of Life

[1,2]N. Dabiri, [2]A. Ashayerizadeh, [3]O. Ashayerizadeh, [2]K.H. Mirzadeh, [2]H. Roshanfekr, [2]M. Bojarpour and [2]M.R. Ghorbani
[1]Department of Animal Science, Faculty of Agricultural, Islamic Azad University, Karaj Branch, Karaj, Iran
[2]Department of Animal Science, Ramin Agricultural and Natural Resources University, Ahvaz, Iran
[3]Department of Animal Science, Gorgan Agricultural Science and Natural Resources University, Gorgan, Iran

Abstract: This study was conducted for comparison the effect of some feed growth promoter additives on performance and gut microflora population of 21 days old broiler chickens. Based on randomized completely design, 300 days old Ross 308 broilers were distributed into 30 floor pens and reared for 21 days. The basal diet was also supplemented with Flavomycin, Primalac, Biolex-MB and mixture of Primalac plus Biolex-MB, resulting 5 dietary treatments were prepared including control group. Each dietary treatment was fed *ad-libitum* to 6 replicate group of 10 bird at the bigining of rearing period. The result of experiment indicate that diets containing growth promoters improved broilers performance. This improvement was only significant ($p<0.05$) between control group and both Biolex-MB and synbiotic treatment groups, for body weight gain and between control group and 3 bird groups fed Flavomycin, Biolex-MB and synbiotic dietary treatments for feed conversion raito. Compared with control birds group, all other treatment groups fed growth promoter diets had relatively lower total bacterial population in crop except for Primalac treatment, but this differences was significant ($p<0.05$) only between Flavomycin and control group treatments. Birds fed diets containing growth promoter had greater lactic acid bacteria populations in crop compared with control group, but this priority was significant ($p<0.05$) only for synbiotic group. A reversed trend were found for coliforms population in ileum, where all growth promoter groups, except Flavomycin, had significantly ($p<0.05$) lower population compared with control group. Additionally, such feed additives altered the pH content of crop and ileum.

Key words: Feed additive, broiler, coliform, lactic acid bacteria, probiotic, prebiotic

INTRODUCTION

The microbial flora of the Gastro Intestinal (GI) tract plays an important role in the health and optimal performance of the poultry (Yu *et al*., 1999). Pathogenic microbial flora in the small intestine competes with the host for nutrients and absorption of fat soluble vitamins (Alp *et al*., 1999). Therefore, the bird's growth is reduced and the possibility for the onset of disease is increased. The microbial species and the population of different regions of GI depend on numerous factors such as the pH, fatty acid concentration, GI tract mobility, the immune system activity, competition for nutrients and sites for adhesion, production of antibacterial compounds and the food composition (Salanitro *et al*., 1978). When controlling the microflora of the GI track, paying attention to the type and the population of microorganisms is important. The activity of beneficial flora (like *lactobacilli* and *bifidobacteria*) of the GI track against pathogenic bacteria is through competition for linking sites in mucosal cells, competition in the use of nutrients and production of preventive substances for prevention from substitution or removal of competitor bacteria. In addition, beneficial microorganisms can affect their host through correction of metabolic processes (Mc Donald *et al*., 1984). Antibiotics (such as flavomycin) that are added as growth stimulants to the poultry feed, help with the fixation of the intestinal microflora and improvement in the general performance of the bird, while protect from establishment of some pathogenic bacteria specific to the intestine. Following severe limitation or general prevention from the use of antibiotics as growth stimulants and medical agents in the poultry industry, probiotics and prebiotics were suggested as appropriate

Corresponding Author: Omid Ashayerizadeh, No. 98, 8th Alley, Fazel Street, Sardkhaneh Avenue, Shiraz, Iran

alternatives for antibiotics (Piray *et al*., 2007). But the degree of effect of such compounds and their appropriate level of use must be studied (Javed *et al*., 2002). Probiotics are live microbial feed supplements, which benefically affects the host animal by improving its intestinal balance (Fuller, 1989).

Prebiotics are nondigestible food ingeredient that benefically affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). The purpose of this study was comparing the effects of the antibiotic Flavomycin and Non-antibiotic additives containing Primalac and Biolex-MB as alternatives for the growth stimulating antibiotics on the performance and bacterial population in some part of GI tract of broiler chickens during starting period.

MATERIALS AND METHODS

Bird and diet: In this study, 300 broiler chickens of the commercial Ross 308 strain were used in a randomized completely design with 5 treatment and 6 replicates in each treatment and 10 birds/replicates and reared on the floor pens for 21 days. Before beginning this study, the dry matter, crude protein, ether extract, crude fiber and ash contents of main feed ingredients were determined in the laborabry to make sure of the presence of sufficient amounts of protein and crude fiber content of the ration (AOAC, 1984). A basal diet was formulated as control according to NRC (1994), recommendations for starter (0-21 days) period. The required amount of growth stimulating additives under study was added to the basal diet so that in addition to the control treatment, 4 dietary experimental treatments containing antibiotic Flavomycin (650 g ton^{-1}), probiotic Primalac (900 g ton^{-1}), prebiotic Biolex-MB (2000 g ton^{-1}) and Primalac plus Biolex-MB (900 and 2000 g ton^{-1} of deit), as synbiotic were prepared (Table 1). During the experiment, water and feed were given to the birds *ad-libitum* and antibiotic or coccidiostat were not offered to them. Weighing of the feed and chickens were made on a weekly basis.

Microbial culture method: On day 21, 1 bird from every cage (6 bird/treatment) was slaughtered by the cervical dislocation method. After disinfection of the abdominal surface of the carcass and areas around it, the internal organs was removed. Then about 5 cm from the length of the ileum middle part (from the Meckel's diverticulum to cacal junction) and the crop and their content and mucosa were sampled. To determine the microbial population, 1 g of crop and ileum contents was used to make serial 10 fold dilutions using buffered peptone water and then 0.1 mL of the appropriate crop and ileum dilutions were spread on MRSA plates (to detect lactic acid bacteria) and VRBA (to detect coliforms) (Izat *et al*., 1990). The culture of lactic acid and coliform bacteria was made anaerobically form. The PCA culture media was used to count the Total aerobic Bacterial Population (TBP) of the ileum and the crop (Engberg *et al*., 2000). The plates were incubated at 37.5°C for 48 h. After counting the number of colonies in each plate, the number so obtained was multiplied by the inverse of the dilution and the result was stated as the number of Colony Forming Unit (CFU) in 1 g of the sample (Downes and Lto, 2001).

Table 1: The experiment basal diets composition and calculated proximate analysis (on dry matter basis)

Ingredients	Starter (0-21 days)
Corn	58.07
Soybean meal	27.12
Cotton meal	10.00
Soybean oil	1.16
Ground limestone	1.17
DCP	1.34
Salt	0.40
Vitamin and mineral premix	0.50
Coccidiostat	-
Vitamin E	0.03
DL- methionine	0.11
L-lysine	0.10
Nutrient content	
ME (kcal kg^{-1})	2850.00
Crude protein (%)	20.48
Crude fiber (%)	4.37

Vitamin and mineral provided per kilogram of diet: Vitamin A, 360000 IU; vitamin D3, 800000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2640 mg, vitamin B3, 4000 mg; vitamin B5, 12000 mg; vitamin B6, 1200 mg; vitamin B12, 6 mg; Choline, 200000 mg, Manganese, 40000 mg, Iron, 20000 mg; Zinc, 40000 mg, copper, 4000 mg; Iodine, 400 mg; Selenium, 80 mg

pH measurment: For measuring the pH, about 1 g of the crop and ileum content of each chicken was collected and transferred into 2 mL distilled water, then the pH levels were measured using a pH meter (Izat *et al*., 1990).

Statistical analysis: All data were analyzed using the one-way Anova procedure of SAS® (SAS, 1998) for analysis of variance. Significant differences among treatments were identified at 5% level by Duncan (1955) multiple range tests.

RESULTS AND DISCUSSION

Growth performance: The effect of experimental treatments on the performance of broiler chickens are shown in Table 2. The use of the growth stimulants flavomycin, Primalac, Biolex-MB as well as the Primalac and Biolex-MB mixture (synbiotic) led into an improvement in the weight gain of birds by 104.5, 102.9,

Table 2: The main effects of treatments on performance of broiler chickens at 21 days (Mean±SE)

Treatments	Body weight gain (g)	Feed consumption (g)	Feed conversion ratio (g g^{-1})
Control	511.4±14.36^{b}	1047.7±2.12^{a}	2.05±0.053^{a}
Flavomycin	534.5±8.13^{ab}	1023.2±9.35^{a}	1.91±0.041^{b}
Primalac	526.4±6.88^{ab}	1029±9.87^{a}	1.95±0.042^{ab}
Biolex-MB	557.6±16.35^{a}	1043.3±38.07^{a}	1.87±0.019^{b}
Synbiotic	560.8±5.99^{a}	1043.4±11.78^{a}	1.86±0.026^{b}
p-values	0.020	0.865	0.009

^{a,b}Means in each column with different superscripts are significantly different ($p<0.05$)

109 and 109.6 g, respectively, as compared with the control group. Although, this differences was significant ($p<0.05$) when control group compared to Biolex-MB and synbiotic, but the others difference was no in this manner ($p>0.05$). The highest weight gain relates to the Biolex-MB and synbiotic that both group led into a 9.6% weight gain in the birds as compared with the control. Addition of the above mentioned growth stimulants had no effect on the broilers feed intake. but, all groups birds under growth promoter experimental treatments had lower Feed Conversion Raito (FCR) than control group and this improvement compared with control was significant ($p<0.05$) for all except for Primalac. In general, the positive effect of experimental additives on performance are in agreement. Mohan *et al*. (1996), Piray *et al*. (2007) and Pelicia *et al*. (2004) have observed the beneficial effects of prebiotics on the Body Weight Gain (BWG) of broiler chickens in their 1st 3 or 4 weeks of breeding. Pelicano *et al*. (2004) reported that using synbiotic could improve the BWG in days 0-21. Similar improvement in FCR was also reported by many researchers. Esteve-garcia *et al*. (1997) observed that adding flavomycin to a wheat-based ration could significantly improve the chickens FCR in all development periods (0-21 days and 22-42 days). Pelicano *et al*. (2004), Maiorka *et al*. (2001) and Santin *et al*. (2001) reported that in birds under prebiotic and probiotic nutrition, the FCR was improved significantly during the days 1-21 as compared with the control treatment. Moreover, adding synbiotic to the ration has been effective in improving the FCR (Zulkifli *et al*., 2000). The lack of effects of adding growth promoters on feed intake of birds in their early period of development in this experiment is consistent with the results reported by Celik *et al*. (2001), Pelicia *et al*. (2004), Mohan *et al*. (1996) and Gunal *et al*. (2006). Although, adverse results were reported by Celik *et al*. (1997) and Yeo and Kim (1997). In contrast to the positive effects of these additive on BWG and FCR in present study, which was consistent with the results of several other experiment, by Celik *et al*. (2001) and Willis *et al*. (2007) show that the use of such additives has no effect on the weight gain up to day 21. Similarly, the lack of significant effects of these additives on FCR were reported by Gunal *et al*. (2006) and Ceilk *et al*. (2001) for flavomycin, Piray *et al*. (2007) for probiotics, Yalcinkayal *et al*. (2008) for mannan oligosaccharids and Pelicia *et al*. (2004) for synbiotics. Variance between researchers could be related to differences in management and environmental conditions that be exist in various experiments. It's suggested that under benefit management and/or environmental conditions, the effect of such feed additives may be worthless. On the other hand, the responses of breeding bird in a warm climate condition similar to the present environmental in south west of Iran to these growth promoters may be better than an ideal condition. The reduction of harmful, but increasing the useful population of bacteria in some part of gut shown in Table 3 may confirmed this opinion. Analysis of the current experiment results shows that the synbiotic treatment has the highest mean body weight. The reason may be the synergistic effect of Primalac and Biomex-MB. Part of these positive results in present study may be assigned to the reduction in the number of pathogenic bacteria. In infections caused by pathogenic bacteria, lymphocytes collect together to destroy them and following inflammation, the thickness of the muscular layer is increased (Gunal *et al*., 2006). By removing pathogenic bacteria that can adhesion to the GI track wall, a favorable medium is provided for the use of nutrients by the birds (Fairchild *et al*., 2001). Also, the use of prebiotics, by increasing in length of the intestinal mucosa, increases the absorption areas and improves the birds growth performance (Santin *et al*., 2001).

Table 3: The main effects of treatments on gut microbial population[1] of chickens at 21 days (Mean±SE)

	Crop		Ileum	
Treatments	Total microbial population	Lactic acid bacteria	Total microbial population	Coliforms
Control	6.60±0.341^{a}	8.29±0.114^{b}	7.88±0.260^{a}	4.95±0.251^{a}
Flavomycin	5.85±0.329^{b}	8.55±0.152^{ab}	6.73±0.375^{b}	4.47±0.192^{ab}
Primalac	6.67±0.084^{a}	8.63±0.241^{ab}	7.30±0.326^{ab}	4.23±0.139^{b}
Biolex-MB	6.18±0.040^{ab}	8.58±0.110^{ab}	7.03±0.201^{b}	4.28±0.139^{b}
Synbiotic	6.35±0.083^{ab}	8.82±0.129^{a}	6.94±0.160^{b}	4.26±0.208^{b}
p-values	0.088	0.235	0.060	0.052

^{a,b}Means in each column with different superscripts are significantly different ($p<0.05$), [1]The result are mentioned as log cfu g^{-1} of crop and Ileum contents

Microbial culture: The resulss of the effect of the above mentioned additives on the population composition of the GI track microbes (the crop and the ileum) are given in Table 3. This results indicate that the birds under flavomycin treatment had the least total number of the

Table 4: The main effects of treatments on pH of crop, ileum and cecum of chickens at 21 days (Mean±SE)

Treatments	Crop	Ileum	Cecum
Control	4.85±0.138[a]	6.64±0.198[a]	5.76±0.271[a]
Flavomycin	4.27±0.091[c]	5.94±0.117[b]	5.96±0.233[a]
Primalac	4.23±0.005[c]	6.03±0.110[ab]	5.45±0.282[a]
Biolex-MB	4.76±0.230[ab]	6.35±0.268[ab]	5.64±0.233[a]
Synbiotic	4.35±0.058[bc]	6.01±0.245[b]	6.03±0.062[a]
p-values	0.018	0.104	0.428

[a,b,c]Means in each column with different superscripts are significantly different ($p<0.05$)

bacteria in the crop and this difference was significant relative to the control and Primalac treatments ($p<0.05$). The use of Primalac and Biolex-MB mixture as called synbiotic could form the highest lactic acid bacterial population in the crop, which was significant when compared to the control treatment ($p<0.05$). Analysis of the ileum microbial population showed that all growth stimulants, except primalac, led into a significant reduction in the total number of the bacteria in the ileum contents than the control treatment ($p<0.05$). Also, feeding with all growth stimulants, except flavomycin, reduced the coliform bacteria population in the ileum as compared with the control treatment ($p<0.05$). Gunal *et al*. (2006) showed that the use of flavomycin reduces the total population of the bacteria and the number of gram negative bacteria on 21st day in the ileum and the cecum as compared with the control treatment ($p<0.05$). Fairchild *et al*. (2001) and Spring *et al*. (2000) reported that the use of prebiotics in the ration reduces the total population of coliforms in the intestinal lumen. Rada *et al*. (1995) found that the use of *lacto bacillus salivari* in the chickens' ration can reduce the coliform bacteria population significantly as compared with the control treatment by reducing the intestinal pH level. Despise of this, Yang *et al*. (2007), Jin *et al*. (1998) and Ceylan *et al*. (2003) observed no change in the GI microbial flora by adding probiotics, MOS and antibiotics to the ration of broiler chickens. Flavomycin, by preventing from connection of muramyl-pentapeptide to the peptidoglycan structure, prevents from synthesis of bacterial cell wall (Huber and Nesemann, 1968). This antibiotic acts extensively against gram positive microorganisms such as *Lactobacillus* and *Bifidobacterium* and reduces the number of lactic acid producing bacteria that are dominant in the upper GI track parts (Cummings, 1995). Flavomycin acts against gram negative bacteria in a limited form, since it can not penetrate into outer membrane of this group of bacteria (Huber, 1979). Considering the above, it seems that in our experiment, the use of flavomycin by effective reduction in the total microbial population of the crop as compared with the control treatment provides a better environment for multiplication of lactic acid bacteria. Probiotic microorganisms, by production of acids (such as acetic acid and lactic acid) and other compounds that prevent from the growth of pathogenic bacteria, help the growth, multiplication and establishment of beneficial bacteria in the intestinal environment (Fuller, 1989). Adding mannose based carbohydrates for reducing the connection of the bacterial lectin to the D-mannose receivers in the GI system and prevention from formation of pathogenic bacteria is a useful one (Eshdat *et al*., 1978; Yang *et al*., 2007). Following prebiotic fermentation by lactic acid bacteria, production of lactic acid and reduction in the pH-value, multiplication of pathogenic bacteria is reduced (Line *et al*., 1995; Chung and Day, 2004). Therefore, the use of prebiotic and probiotic mixture can reduce the intestinal pH and provide a good environment for lactic acid bacterial growth and multiplication through synergism.

pH levels: The effects of experimental treatments on the pH of the crop, the ileum and the cecum of broiler chickens are provided in Table 4. This findings show that the pH in cecum of the birds was not affected ($p>0.05$) by experimental treatments. In birds that received rations containing flavomycin and synbiotic, the ileum pH was significantly reduced as compared with the control. Adding flavomycin, Primalac and synbiotic to the basic ration could reduce the crop pH significantly as compared with the control treatment ($p<0.05$). Also, the crop pH in the flavomycin and Primalac treatment was lower when compared to the prebiotic treatment ($p<0.05$). There was no significant difference among other treatments.

In our experiment, the results of pH measuring of different areas in the GI track, partly supports the microbial culture findings. Analysis of the total population of the crop bacterial and its comparison with the results of pH measurement in birds affected by flavomycin, supports the fact that thought the antibiotics reduce the total population of the bacteria, but this reduction is low for lactic acid bacteria as compared with the pathogenic bacteria (Cummings, 1995). It seems that flavomycin succeeded in reduction of the coliform population and increase in the lactic acid bacterial population by reducing the pH of the ileum and the crop. The use of Primalac and Biolex-MB mixture could, by synergism and increase in the number of lactic acid bacteria be effective in reducing the crop pH and total population of the ileum bacteria.

CONCLUSION

Based on obtaining better results for birds fed diets containing growth promoters, particularly in performance and lower microbial population in gut, it is concluded that by using non-antibiotic additives particularly mixing of both probiotic and perbiotic could obtained the advantages of antibiotic (performance) without their disadvantages.

REFERENCES

Alp, M., N. Kocabagil and R. Kahraman, 1999. Effect of dietary supplementation with organic acids and zinc bacitracin on ilea microflora, pH and performance in broiler. Turk. J. Vet. Anim. Sci., 23: 451-455. http://journals.tubitak.gov.tr/veterinary/issues/vet-99-23-5/vet-23-5-8-98069.pdf.

AOAC, 1984. Official Methods of Analysis. 14th Edn. Association of Official Analytical Chemists. The William Byrd Press. Inc. Richmond, Verginia, USA.

Celik, K., M. Denli and O. Ozturkcan, 2001. The effect of *saccharomyces cerevisiae* and Flavomycin on broiler growth performance. Pak. J. Biol. Sci., 4: 1415-1417. http://www.scialert.net/qredirect.php?doi=pjbs.2001.1415.1417&linkid=pdf.

Celik, K., S. Ozer, K. Daglioglu, F. Doran and O. Ozturkcan, 1997. The using facilities of yeast in the feed as adsorbent on mycotoxicosis. 4th Balkan Conference on Operational Research. October 20-23, Thassaloniki Greece, pp: 595-608.

Ceylan, N., Y.C. Yftcy and Z.Y. Han, 2003. The effects of some alternative feed additives for antibiotic growth promoters on the performance and gut micro flora of broiler chicks. Turk. J. Vet. Anim. Sci., 27: 727-733. http://journals.tubitak.gov.tr/veterinary/issues/vet-03-27-3/vet-27-3-33-0205-38.pdf.

Chung, C.H. and D.F. Day, 2004. Efficacy of Leuconostoc mesenteroides (ATCC 13146) isomaltooligosaccharides as a poultry prebiotic. Poult. Sci., 83: 1302-1306. http://ps.fass.org/cgi/reprint/83/8/1302?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&searchid=1&FIRSTINDEX=0&sortspec=relevance&firstpage=1302&resourcetype=HWCIT.

Cummings, T.S., 1995. The effect of probiotics and antibiotics on the intestinal microflora of poultry. In: Proceedings of the 22nd Annual Carolina Poultry Nutrition Conference. Carolina Feed Industry Association, Charlotte, pp: 88-90.

Downes, F.P. and K. Lto, 2001. Compendium of Methods for the Microbiological Analytic Examination of Foods. 4th Edn. APHA, Washington, D.C. ISBN: 978-0824700874.

Duncan, D.B., 1955. Multiple range and F-tests. Biometrics, 11: 1-42.

Engberg, R.M., M.S. Hedemann, T.D. Leser and B.B. Jensen, 2000. Effects of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poult. Sci., 79: 1311-1319. http://ps.fass.org/cgi/reprint/79/9/1311?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&searchid=1&FIRSTINDEX=0&sortspec=relevance&firstpage=1311&resourcetype=HWCIT.

Eshdat, Y., I. Ofek, Y. Yehuidit-Gan, Y. Yashouv-Gan, N. Sharon and D. Mirelmann, 1978. Isolation of a mannose-specific lectin from *E. coli* and its role in the adherence of the bacteria to epithelial cells. Biochem. Biophys. Res. Commun., 85 (4): 1551-1559. PMID: 369565.

Esteve-garcia, E., J. Brufau, A. Perez-vendrell, A. Miquel and K. Duven, 1997. Bioefficacy of enzyme preparations containing b-glucanase and xylanase activities in broiler diets based on barley or wheat, in combination with flavomycin. Poult. Sci., 76: 1728-1737. http://ps.fass.org/cgi/reprint/76/12/1728?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&volume=76&firstpage=1728&resourcetype=HWCIT.

Fairchild, A.S., J.I. Grimes, F.T. Jones, M.J. Wineland, F.W. Edens and A.E. Sefton, 2001. Effects of Hen Age, Bio-Mos and Flavomycin_on Poult Susceptibility to Oral *Escherichia coli* Challenge. Poult. Sci., 80: 562-571. http://ps.fass.org/cgi/reprint/80/5/562?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&firstpage=562&resourcetype=HWCIT.

Fuller, R., 1989. Probiotics in man and animals. J. Appl. Bacteriol., 66: 365-378. DOI: 10.1111/j.1365-2672.1989.tb05105.x. http://www3.interscience.wiley.com/cgi-bin/fulltext/120151931/PDFSTART.

Gibson, G.R. and M.B. Roberfroid, 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of probiotic. J. Nutr., 125: 1401-1412. http://jn.nutrition.org/cgi/reprint/125/6/1401?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&author1=gibson&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&volume=125&resourcetype=HWCIT.

Gunal, M., G. Yayli, O. kaya, N. Karahan and O. Sulak, 2006. The effects of Antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broiler. Int. J. Poult. Sci., 5: 149-155. http://www.pjbs.org/ijps/fin547.pdf.

Huber, G., 1979. Moenomycin and Related Phosphorus containing Antibiotics. In: Hahn, F.E. (Ed.). Antibiotics; Springer Verlag, New York, 5 (1): 135-153.

Huber, G. and G. Nesemann, 1968. Moenomycin, an inhibitor of cell wall synthesis. Biochem. Biophys. Res. Commun., 30: 7-13.

Izat, A.L., N.M. Tidwell, R.A. Thomas, M.A. Reiber, M.H. Adams, M. Colberg and P.W. Waldroup, 1990. Effects of buffered propionic acid in diets on the performance of broiler chicken and on microflora of the intestine and carcass. Poult. Sci., 69: 818-826.

Javed, M.T., M.A. Sarwar, R. Kausar and I. Ahmad, 2002. Effects of feeding different levels of formalin (37% formaldehyde) and urea on broiler health and performance. Veterinarski ARHIV., 72: 285-302. http://www.vef.hr/vetarhiv/papers/72-5/javed.pdf.

Jin, L.Z., Y.W. Ho, N. Abdullah and S. Jalaludin, 1998. Growth Performance, Intestinal Microbial Populations and Serum Cholesterol of Broilers Fed Diets Containing *Lactobacillus* Cultures. Poult. Sci., 77: 1259-1265. http://ps.fass.org/cgi/reprint/77/9/1259?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&searchid=1&FIRSTINDEX=0&sortspec=relevance&firstpage=1259&resourcetype=HWCIT.

Line, E.J., N.J. Stern, N.A. Cox and J.S. Bailey, 1995. *Saccharomyces* treatment to diminish *Campylobacter* and *Salmonella* in chickens. J. Poult. Sci., 74 (Suppl. 1): 201 (Abst.). http://www.freepatentsonline.com/6010695.pdf.

Maiorka, A., E. Santin, S.M. Sugeta, J.G. Almeida and M. Macari, 2001. Utilization of Prebiotics, Probiotics or Symbiotics in broiler chicken diets. Brazilian J. Poult. Sci., 3: 75-82. DOI: 10.1590/S1516-635X2001000100008. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-635X2001000100008&lng=en&nrm=iso&tlng=pt.

Mc Donald, I.A., R.G. Bussard, D.M. Hutchinson and L.V. Holdeman, 1984. Rutin-induced: Glucosidas activity in streptococcus feacium VGH-1 and *Streptococcus* sp. strain FRR-17 isolated from human feces: Formation of the mutagen quercetien from rutin. Appl. Environ. Microbiol., 47: 350-355. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=239673.

Mohan, B., R. Kadirvel, A. Natarajan and M. Bhaskaran, 1996. Effect of probiotics supplementation on growth, nitrogen utilization and serum cholesterol in broilers. Br. Poult. Sci., 37: 395-401. DOI: 10.1080/00071669608417870.

NRC (National Research Council), 1994. Nutrient Requirements of Poultry. 9th Edn. National Academy Press, Washington, DC.

Pelicano, E.R.L., P.A. Souza, H.B.A. Souza, F.R. Leonel, N.M.B.L. Zeola and M.M. Bioago, 2004. Productive traits of broiler chicken fed diets containing different growth promoters. Brazilian J. Poult. Sci., 6: 177-182. DOI:10.1590/S1516-635X2004000300008.http://www.scielo.br/scielo.php?pid=S1516635X2004000300008&script=sci_arttext&tlng=en.

Pelicia, K., A.A. Mendes, E.S.P.B. Saldanha, C.C. Pizzolante, S.E. Takahashi, J. Moreira, R.G. Garcia, R.R. Quinterio, I.C.L.A. Paz and C.M. Komiyama, 2004. Use of prebiotics and probiotics of bacterial and yeast origin for free-range broiler chickens. Brazilian J. Poult. Sci., 6: 163-169. DOI:10.1590/S1516-635X2004000300006.http://www.scielo.br/pdf/rbca/v6n3/a06v6n3.pdf.

Piray, A.H., H. Kermanshahi, A.M. Tahmasbi and J. Bahrampour, 2007. Effects of Cecal Cultures and *Aspergillus* Meal Prebiotic (Fermacto)on Growth Performance and Organ Weights of Broiler Chickens. Int. J. Poult. Sci., 6 (5): 340-344. http://www.pjbs.org/ijps/fin807.pdf.

Rada, V., M. Marounek, I. Rychly, D. Santruckova and K. Vorisek, 1995. Effect of *Lactobacillus salivarius* administration on microflora in the crop and caeca of broiler chickens. J. Anim. Feed Sci., 4: 161-170.

Salanitro, J.P., I.G. Blake, P.A. Muirhead, M. Maglio and R. Goodman, 1978. Bacteria isolated from the duodenum, ileum and cecum of young chicks. Applied Environ. Microbiol., 35: 782-790. http://aem.asm.org/cgi/reprint/35/4/782.

Santin, E., A. Maiorka, M. Macari, M. Grecco, J.C. Sanchez, T.M. Okada and A.M. Myasaka, 2001. Performance and intestinal mucosa development of broiler chickens fed diet containing *Sccharomyces cerevisiae* cell wall. J. Applied Poult. Res., 10: 236-244. http://japr.fass.org/cgi/reprint/10/3/236?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&searchid=1&FIRSTINDEX=0&sortspec=relevance&firstpage=236&resourcetype=HWCIT.

SAS Institute Inc, 1998. SAS/STAT User's Guide: 1998 Edn. SAS Institute Inc., Cary, North Carolina.

Spring, P., C. Wenk, K.A. Dawson and K.E. Newman, 2000. The efects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella challenged* broiler chicks. Poult. Sci., 79: 205-211. http://ps.fass.org/cgi/reprint/79/2/205?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&searchid=1&FIRSTINDEX=0&sortspec=relevance&firstpage=205&resourcetype=HWCIT.

Willis, W.L., O.S. Isikhuemhen and A. Ibrahim, 2007. Performance assessment of btroiler chickens given Mushroom Extract alone or in combination with Probiotic. Poult. Sci., 86: 1856-1860. http://ps.fass.org/cgi/reprint/86/9/1856?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&volume=86&firstpage=1856&resourcetype=HWCIT.

Yalcinkayal, H., T. Gungori, M. Bafialani and E. Erdem, 2008. Mannan Oligosaccharides (MOS) from *Saccharomyces cerevisiae* in Broilers: Effects on Performance and Blood Biochemistry. Turk. J. Vet. Anim. Sci., 32 (1): 43-48. http://journals.tubitak.gov.tr/veterinary/issues/vet-08-32-1/vet-32-1-8-0612-9.pdf.

Yang, Y., P.A. Iji, A. Kocher, L.L. Mikkelsen and M. Choct, 2007. Effects of Mannanoligosaccharide on Growth Performance, the Development of Gut Microflora and Gut Function of Broiler Chickens Raised on New Litter. J. Applied Poult. Res., 16: 280-288. http://japr.fass.org/cgi/reprint/16/2/280?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&searchid=1&FIRSTINDEX=0&sortspec=relevance&firstpage=280&resourcetype=HWCIT.

Yeo, J. and K. Kim, 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. http://ps.fass.org/cgi/reprint/76/2/381?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&searchid=1&FIRSTINDEX=0&sortspec=relevance&firstpage=381&resourcetype=HWCIT.

Yu, B., H.Y. Tsen and P.S. Chou, 1999. Caecal culture enhances performance and prevents *Salmonella* infection in broiler. J. Applied Poult. Res., 8: 195-204. http://japr.fass.org/cgi/reprint/8/2/195.pdf.

Zulkifli, I., N. Abdullah, N.M. Azrin and Y.W. Ho, 2000. Growth performance and immune response of 2 commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. Br. Poult. Sci., 41: 593-597. DOI: 10.1080/00071660020009126. http://openurl.ingenta.com/content?genre=article&issn=0007-1668&volume=41&issue=5&spage=593&epage=597.