

The Comparative Effects of Five Growth Promoters on Broiler Chickens Humoral Immunity and Performance

Afshin Zakeri and Pedram Kashefi
Poultry Section, Department of Animal Science, Faculty of Agriculture,
Islamic Azad University, Tabriz Branch, Tabriz Iran

Abstract: The effects of dietary supplementation of five growth promoters: Avilamycin (AM), Protexin (PT), Nutracid Focus (NF), Mannan oligosaccharides (MOS) and Vitacel (VC) were evaluated in broiler chicken by their effects on humoral immunity, growth performance, mortality and feed intake. Diets containing no growth promoter (control), AM (100 mg kg⁻¹), PT (100 mg kg⁻¹), NF (800 mg kg⁻¹), MOS (1000 mg kg⁻¹) and VM 6 (4000 mg kg⁻¹) were fed to broiler chicks (Cobb 500). All diets were formulated to exceed NRC nutrient requirements. Each treatment was assigned to 4 replicate floor pens containing 25 birds that were reared from 1-42 days of age. Body weights and feed consumption were recorded every week and mortality and culled birds were recorded daily. On days 9, 17 and 25 of growth (1 day before and 7, 14 days after first Newcastle B₁ vaccination), 20 birds from each treatment were chosen randomly and serum antibodies titers of them were measured against Newcastle vaccine by HI test. Dietary supplementation of growth promoters' antibiotics significantly decreased ($p < 0.05$) Feed Conversion Ratio (FCR) compared with control fed birds during total rearing period. There were no treatments effects on overall feed consumption, mortality or cull rate. Birds fed MOS and PT had superior ($p < 0.05$) serum antibodies titres at the age of 25 days old.

Key words: Protexin, vitacel, mannan oligosaccharides, nutracid focus, avilamycin, birds

INTRODUCTION

Now-a-days, one of the important goals of the poultry industry is increasing the amount of humeral immunity and producing safety meat by using safe growth promoters especially in broiler chickens. Improve and increase safety levels and the rate of production parameters of broilers important goals poultry breeding industry in the world is because the chicken with high safety level not only against various pathogenic factors and various environmental stress resistances but also in response to asked vaccination programs efficiently reacts. Now-a-days, various techniques and develop Pharmaceutical development and natural supplements to reach this goal are given. Respectively, MOS (Immunogen), Avilamycin, Protexin, Vitacel and Nutracid Focus which in fact were prebiotic, Antibiotic Growth Promoter (AGP), probiotic, natural vegetative substance and acidifier including the drug benefit that recently have entered the industry not only improves and growth but are parameters of production and increase safety level and also poultry carcasses will be no drug residue. Object of this research is study of natural growth promoters for improving productive parameters and

immune system as a suitable alternative replacement of antibiotic growth promoter in broiler chickens. The effect of growth stimulant antibiotics in different researches from point of view the immune improvement level productivity factors of broiler chickens have been tested and surveyed. Different researches have been done on the growth stimulant antibiotics such as Avilamycin, Virginamycin, Bambramycin, Lincomycin, Flavophospholipol and Bacitracine (Elwinger *et al.*, 1998; George *et al.*, 1982). The prebiotics which are from oligosaccharides groups are as one of the most important natural products in improvement and body immune level increase have been under consideration, one of the most important product of this group is MOS which different researches have been done in relation to the effects of this material on poultry immune system (Roberfroid, 2000a, b; Savage *et al.*, 1996). In the case of the prebiotics (Bailey *et al.*, 1991), in the case of the effect of fructo oligosaccharides on the replacement of salmonella in the tissue of intestine and the immunity of intestinal tissue have surveyed and the results obtained were the effectiveness of these combinations in controlling the replacement of harmful bacteria such as salmonella. On the other hand due to the increased safety level, the

success of vaccination programs, the most important issue is the industry will increase (Roberfroid, 2000a; Salminen *et al.*, 1998). Improve and increase safety broiler poultry industry one of the main goals of the whole world because the chicken with high safety level, not only against various pathogenic factors and various environmental stress resistance but in response to the program conventional vaccination efficiently respond shows (Chen *et al.*, 2005).

MATERIALS AND METHODS

In this study 600 days old Cobb 500 broiler chickens which were negative from point view of *Mycoplasma Gallisepticum* (MG), *Mycoplasma Synoviae* (MS), *Salmonella Pullorum* (SP), *Salmonella Enteritidis* (SE) were randomly assigned to 24 pens (3 m²/pen) on the day of hatch and were raised from 1-42 days of age. There were 8 replicate pens per treatment with each pen containing 25 chicks at the start of the trial. Treatments were randomly assigned to the pens within experimental treatments within the house. Pens contained used wood shavings as litter material. Standard gas heaters provided heat for the entire house. Birds were whole house brooded by maintaining an internal house temperature of 35°C for the 1st week and gradually stepped down weekly by 2.5°C until house temperature reached approximately 25°C. Ventilation was provided by natural air movement through adjusted curtain sides and air-mixing fans. One hanging tube-type feeder and drinker were placed in each pen providing *ad libitum* access during the entire experiment. Diets (Table 1) were formulated to meet all the nutrient requirements of the birds for starter (0-10 days), grower (10-24 days) and finisher (24-42 days) phases (Leeson and Summers, 1997). The 6 dietary treatments were as follows: control, a typical corn-soybean meal diet containing no growth promoter; AM, 100 mg kg⁻¹ Avilamycin; PT, 100 mg kg⁻¹ Protexin; NF, 800 mg kg⁻¹ Nutracid Focus; MOS, 1000 mg kg⁻¹ Mannanligosaccharides and VC, 4000 mg kg⁻¹ Vitacel. Dietary feed additives were added at the expense of washed builder's sand to avoid any confounding dilution effects. Individual bird body weights and feed consumption (by pen) were recorded weekly until 6 weeks of age. Mortality and culled birds were recorded as they occurred and their weights were included for the calculation of Feed Conversion Ratio (FCR). Body weight, body weight variation within pen, feed consumption and adjusted FCR were determined for each week age interval. Vaccination program for the treatments were completely similar and include at 1 day of age, vaccine H₁₂₀ bronchitis in the spray form at 10 days age, killed oily vaccine

Table 1: Dietary ingredients and chemical composition of six basic food groups (Leeson and Summers, 1997)

Ingredient (%)	Starter (0-10 days)	Grower (11-24 days)	Finisher (25-42 days)
Corn	48.90	52.90	55.45
Soybean meal	32.20	28.20	11.10
Wheat	9.40	10.20	11.10
Fish meal	2.80	1.80	1.50
Oil	2.40	3.20	4.10
DCP	1.53	1.30	1.20
NaCl	0.15	0.15	0.15
Oyster shell	1.28	1.40	1.32
Methionine	0.20	0.15	0.22
Lysine	0.07	0.06	0.07
Vitamin-mineral premix	0.60	0.60	0.60
Salinomycin	0.05	0.05	0.05
Energy kcal kg ⁻¹	2900.00	2985.00	3095.00
Crude protein	21.26	20.20	19.20
Digestible protein	16.90	16.10	15.30
Crude fiber	3.60	4.90	5.40
Methionine	0.50	0.42	0.59
Methionine+Cystine	0.91	0.82	0.99
Lysine	1.40	1.20	0.90
Available P	0.60	0.60	0.52
Calcium	1.10	0.90	0.75

Two-doubled influenza H₉N₂ and Newcastle B₁ in the injection form in the breast muscle and vaccine Newcastle B₁ in the form of eye drop; at 16 days of age, vaccine D₇₈ Gamboro in the drinking form. For investigation of the humeral immunity and its commentary at days 9, 17 and 25 of age blood samples were taken (each time 2 males and 2 females from each of the pens and serum antibodies titres of these chickens were measured against Newcastle vaccine by humeral immunity of Hemagglutination Inhibition (HI) test. The first time of blood sampling was done 1 day before vaccination B₁ Newcastle to determine the similarity of parent stock titer. The second and third time of blood sampling was done 1 and 2 week after vaccination B₁ to determine whether to be or not to be contaminated with virus field Newcastle to prove the obtained titer from each treatment. All samples of blood were justified the ratio by serologic procedure hemagglutination control test. All data were subjected to analysis of variance using the SPSS version 15 for a completely randomized block design. The pen was the experimental unit for body weight, body weight variation, feed consumption, FCR, HI titer and European Efficiency Factor. Variables having a significant global F-test value were compared using the LSD test. Differences between means were considered significant at p<0.05.

RESULTS

The effects of dietary supplementation of five growth promoters: Avilamycin (AM), Protexin (PT), Nutracid Focus (NF), Mannanligosaccharides (MOS) and Vitacel

Table 2: Mean standard deviation, means (\pm SEM) and statistical comparison titers obtained in HI tests

Age (d day ⁻¹ post-vaccination)	Treatments						p-value
	Control	MOS	AV	VC	NF	PT	
9 (day-1)	2.08 \pm 0.28	2.03 \pm 0.23	2.03 \pm 0.23	2.00 \pm 0.42	2.00 \pm 0.86	2.00 \pm 0.67	p>0.05
17 (day+7)	3.40 \pm 0.51 ^a	4.40 \pm 0.51 ^c	4.00 \pm 0.45 ^{bc}	3.50 \pm 0.62 ^{ab}	3.90 \pm 0.31 ^{abc}	4.20 \pm 0.42 ^c	p<0.05
25 (day+14)	4.00 \pm 0.89 ^a	4.90 \pm 0.31 ^b	4.10 \pm 0.31 ^a	3.60 \pm 0.65 ^a	4.00 \pm 0.47 ^a	4.70 \pm 0.48 ^b	p<0.05

^{a-c} Means with unlike superscript letters differ (p<0.05)

Table 3: Comparison of FCR in different weeks of breeding

Age/week	Treatments						p-value
	Control	MOS	AV	VC	NF	PT	
1	1.38	1.28	1.32	1.30	1.31	1.24	p>0.05
2	1.49 ^a	1.29 ^b	1.34 ^{ab}	1.38 ^{ab}	1.40 ^{ab}	1.26 ^b	p<0.05
3	1.59 ^a	1.33 ^b	1.39 ^{bc}	1.59 ^a	1.53 ^{bc}	1.32 ^b	p<0.05
4	1.69 ^a	1.50 ^a	1.59 ^a	1.63 ^a	1.59 ^a	1.48 ^b	p<0.05
5	1.89 ^a	1.63 ^b	1.71 ^{ab}	1.82 ^{ab}	1.79 ^{ab}	1.64 ^b	p<0.05
6	2.25 ^a	1.84 ^b	1.91 ^b	2.12 ^c	1.99 ^{bc}	1.85 ^b	p<0.05
Total	2.19 ^a	1.75 ^b	1.89 ^{bc}	1.99 ^d	1.93 ^c	1.79 ^{bc}	p<0.05

^{a-d} Means with unlike superscript letters differ (p<0.05)

Table 4: Comparison of average cumulative feed intake (g) in different weeks of breeding

Age/week	Treatments						p-value
	Control	MOS	AV	VC	NF	PT	
1	149	145	142	142	140	149	p>0.05
2	389	439	458	481	476	445	p>0.05
3	1092	1012	1051	1072	1059	998	p>0.05
4	1950	1852	1901	1980	1930	1889	p>0.05
5	3950	3904	3994	3800	3990	3940	p>0.05
6	4350	4159	4210	4352	4285	4280	p>0.05

Table 5: Comparison of EEF and weight gain (g) (mean \pm SD) of different groups tested at the end of the growing period (42 days)

Productive parameters	Treatments						p-value
	Control	MOS	AV	VC	NF	PT	
Weight gain	2081 \pm 190.16 ^a	2339 \pm 152.90 ^{ad}	2282 \pm 152.96 ^c	2184 \pm 94.65 ^b	2188 \pm 194.51 ^d	2392 \pm 194.51 ^d	p<0.05
EEF	308 \pm 12.00 ^a	342 \pm 18.00 ^b	315 \pm 9.00 ^a	309 \pm 15.00 ^a	335 \pm 13.00 ^{ab}	349 \pm 16.00 ^b	p<0.05

^{a-c} Means with unlike superscript letters differ (p<0.05)

(VC) on humeral Immunity (HI test) are shown in Table 2. Results showed that at day 17 and 25 of age, 7 and 14 days after vaccination with Newcastle vaccine, MOS and PT significantly (p<0.05) increased the HI titer compared to the control diet (Table 2). There were not statistically significant differences (p>0.05) between treatments for HI titer at 9 days of age, the day before vaccination with Newcastle vaccine. The effects of dietary supplementation of Avilamycin, Protexin, Nutracid Focus, Mannanoligosaccharides and Vitacel on Feed Conversion Ratio (FCR), Feed Intake (FI), European Efficiency Factor (EEF) and Body Weight Gain (BWG) broiler chicken are shown Table 3-5. No significant difference was seen between treatment groups in the first week on FCR but dietary supplementation of growth promoters significantly decreased (p<0.05) FCR in other weeks and total rearing period (Table 3). Birds were fed with MOS and Protexin had a better improvement in FCR compare with other groups. However, there were no significant differences on FI between treatments and control (Table 4). Also, dietary supplementation of growth promoters significantly increased (p<0.05) bird's weight

gain and the highest BWG was observed in broiler chickens fed diet supplemented by Protexin (Table 5). European Efficiency Factor significantly (p>0.05) affected by dietary supplementation of growth promoters and diet supplemented by MOS and Protexin improved the EEF compared to control treatment (Table 5).

DISCUSSION

Due to the use of any of these compounds means Avilamycin, Protexin, Vitacel, Nutracid focus and MOS than the control group can improve the immune response to NDVB1 vaccines and improved production factors (final weight gain, feed conversion ratio and EEF) in broilers. According to the studies done (Elwinger *et al.*, 1998; Patterson and Burkholder, 2003) it was defined that prebiotics even in controlling and halting the bacteria such as clostridium perfringens which has not any relationship with lectins sensible to mannose for connection to intestine are also effective (Hofacre *et al.*, 2003; Macfarlane and Cummings, 1999). The other mechanisms that we could mention for improvement of

immune level and intestinal micro flora are the intestinal acidity change by increasing lactic acid density in intestine and decreasing the harmful intestine bacteria activity (*Escherichia coli*, Salmonella and Clostridium) and increasing lactobacillus activity. In the case of MOS direct effects on immune level, different researches have been done (Roberfroid, 1998; Zoppi, 1998; Patterson and Burkholder, 2003) it has been proved that MOS and fructo oligosaccharides either directly or in directly cause increase in immune level. One of the direct effects of mechanisms on chickens' immune system is the increase of induction macrophage activity (as the most important cell presenting antigen in poultry). MOS induce the macrophage activity by occupying Mannose specific receptor. When one third or more of these receptors were occupied macrophages becomes more active and ready for destroying pathogenic bacteria and create more suitable immune response. Also, presenting antigens by macrophages to antibody cells producers' increases. MOS not only cause improvement in immune response but also cause equality in it. In fact, the results obtained from this study and also other studies relating to immune level increase show that MOS with direct effect on macrophage action and controlling the connection of pathogen bacteria in intestinal tissue and creating acidity environment in intestine cause immune system improvement in broiler chickens. In such a way that with increasing 0.9 log antibody ratio in response to Newcastle B₁ vaccine to control group cause immune response improvement. Also, the regulation of macrophage of intestine, the increase of feed material absorption, producing factors improvement counting for feed conversion ratio and improvement in response to vaccination. Programs are other benefits of materials according to studies. Also, according to the studies of Savage *et al.* (1996) in relation to consuming MOS in turkeys, it has been observed that ratio of IgA biliary entering intestine via bile duct and also the ratio of IgG plasma increased (Savage *et al.*, 1996). Combination of recent materials such as probiotic and prebiotic under production is as symbiotic. Composition of fructo oligosaccharides and bifidobacteria is an example of this group (Bello *et al.*, 2001). Acid fire actually is a combination of organic acid with anti-microbial and set PH of intestine, including acetic acid, citric acid, formic acid, lactic acid and their salts (organic acids but also have a Mycostate). Protexin, Vitacel, Nutracid focus and MOS the fact that a growth stimulus are natural products, any kind of drug residue would not remain in poultry meat and consuming poultry meat does not create any resistance to antibiotics in human. Regarding to June 1999 consuming

the majority of antibiotics growth stimulant in Europe were forbidden (for remaining antibiotics in consuming meat and also creating drug resistances in poultry and human), it seems that using natural combinations and also MOS which have higher function could be one of the best combinations replacing antibiotics growth stimulant. Considering this point too is necessary that combinations because of being natural from point view of living environment are completely healthy or so-called green (Lemieux *et al.*, 2003).

CONCLUSION

The results suggest that using of many of MOS (Immunogen), Protexin, Vitacel and Nutracid Focus which are natural substance and do not have any drug residual, are suitable alternative to improve growth performance and humoral immunity of broiler chicken.

ACKNOWLEDGEMENT

The researchers would like to acknowledge the Islamic Azad University, Branch Tabriz for financial support of thesis project.

REFERENCES

- Bailey, J.S., L.C. Blankenship and N.A. Cox, 1991. Effect of fructooligosaccharides on *Salmonella colonization* of the chicken intestine. *Poult. Sci.*, 70: 2433-2438.
- Bello, F.D., I. Walter and W.P. Hammes, 2001. *In vitro* study of prebiotic properties of Levan-type exopolysacch-a rides form lactobacilli and non-digestible carbohydrates using denaturing gel electrophoresis. *Syst. Applied Microbiol.*, 24: 232-237.
- Chen, Y.C., C. Nakhthong and T.C. Chen, 2005. Improvement of laying hen performance by dietary prebiotic chicory oligofructose and inulin. *Int. J. Poult. Sci.*, 4: 103-108.
- Elwinger, K., E. Berndtson, B. Engstrom, O. Fossum and L. Waldenstedt, 1998. Effect of antibiotic growth promoters and anticoccidials on growth of clostridium prefringenes in the caeca and on performance of broiler chickens. *Acta. Vet. Scand.*, 39: 433-441.
- George, B.A., C.L. Quarles and D.J. Fagerberg, 1982. Virginiamycin effects on controlling necrotic enteritis infection in chickens. *Poult. Sci.*, 61: 447-450.
- Hofacre, C.L., T. Beacom, S. Collet and G. Mathis, 2003. Using competitive exclusion, mannan-oligosaccharide and other intestinal products to control necrotic enteritis. *J. Applied Poult. Res.*, 12: 60-64.

- Leeson, S. and J.D. Summers, 1997. Commercial Poultry Nutrition. 2nd Edn., University Books, Guelph, Ontario, Canada.
- Lemieux, F.M., L.L. Southern and T.D. Binder, 2003. Effect of mannan oligosaccharides on growth performance of weanling pigs. *J. Anim. Sci.*, 81: 2482-2487.
- Macfarlane, G.T. and J.H. Cummings, 1999. Probiotics and prebiotics: Can regulating the activities of intestinal bacteria benefit health. *BMJ.*, 318: 999-1003.
- Patterson, J.A. and K.M. Burkholder, 2003. Application of prebiotics and probiotics in poultry production. *Poult. Sci.*, 82: 627-631.
- Roberfroid, M.B., 1998. Prebiotics and synbiotics: concepts and nutritional properties. *Br. J. Nutr.*, 80: S197-S202.
- Roberfroid, M.B., 2000a. Health benefits of non-digestible oligosaccharides. *Adv. Exp. Med. Biol.*, 427: 211-219.
- Roberfroid, M.B., 2000b. Prebiotics and probiotics: Are they functional foods. *Am. J. Clin. Nutr.*, 71: 1682-1687.
- Salminen, S., C. Bouley, M.C. Boutron-Ruault, J.H. Cummings and A. Franck *et al.*, 1998. Function food science and gastrointestinal physiology and function. *Br. J. Nutr.*, 80: S147-S171.
- Savage, T.F., P.F. Cotter and E.I. Zakrzewska, 1996. The effect of feeding a mannan oligosaccharide on Immunoglobulins, plasma IgG and bile IgA of Wrolstad MW male turkeys. *Poult. Sci.*, 75: 143-143.
- Zoppi, G., 1998. Probiotics, prebiotics, synbiotics and eubiotics. *Pediatr. Med. Chir.*, 20: 13-17.