ISSN 110-2047.ALEX.J.VET.SCIENCE.2007.VOL 25 NO 1

EFFECT OF FEEDING MANNAN OLIGOSACCHARIDE AS A REPLACEMENT FOR GROWTH PROMOTING ANTIBIOTICS IN BROILER RATION.

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ABSTRACT

Public concern about the increasing threats of antibiotic-resistant pathogens has forced the poultry industry to consider "biologically safer" alternatives. There is considerable evidence that mannan oligosaccharide (MOS) is among the best alternatives to antibiotic growth promotors. This study was designed to declare the effect of MOS and virginiamycin(VM) in broiler chickens. One hundred and twenty, one-day old , broiler chickens were used and allocated into 3 equal groups. Group 1 was kept as control non treated chickens. The second group was supplemented with MOS with the dose of 0.5 gram/Kg ration during the whole experimental period(7 weeks). While the third group was supplemented with VM with the dose of 1 gram/Kg ration during the whole experimental period. Three blood samples were collected from 5 birds of each group at 28,35and 42 day of age. The first blood sample was collected in test tube containing EDTA for total and differential leukocytic count. The second blood sample was collected in plastic syringe containing heparin for determination of phagocytic activity of mononuclear leukocytes while the third blood sample was collected in centrifuge tube for serum separation for determination of serum total proteins, albumin and globulin. Body weight was recorded weekly. At the end of experimental period, 5 birds from each group were sacrificed and the weight and length of intestine were recorded. The result of this study revealed that MOS supplementation elicited significant increase in total leukocytic count, lymphocytes, heterophils and monocytes while VM produced non significant effect on leukogram.MOS supplementation evoked significant increase in phagocytic activity of mononuclear leukocyte beside non significant changes in VM supplemented group. MOS supplementation evoked significant increase in serum total proteins and globulins beside non significant changes in VM supplemented group. Both feed additive (MOS&VM) produced significant decrease in weight and length of intestine in addition to significant increase in body weight. In conclusion, MOS can be a valuable tool as an antibiotic alternative to improve poultry health, immunity and performance.

INTRODUCTION

During the past 50 years, poultry industry has developed in several areas of nutrition, genetics, management and communications to maximize the efficiency of growth performance and meat yield. Today, the poultry industry must focus more attention towards addressing public concern for environmental and food safety. As many other industries, the global paradigm is shifting from an emphasis on efficiency to one of public security. Nothing demonstrates this paradigm shift more clearly than this issue concerning the use of antibiotic growth promotors. For the past 4 decades, antibiotics have been supplemented to poultry feed to improve the growth performance and protect birds from the adverse effects of pathogenic and non-pathogenic enteric microorganisms. Now, antibiotics have come under increased scrutiny by some scientists, consumers and government regulators because of the development of antibiotic-resistant human pathogenic bacteria after long use **(Ratcliff,2000)**. Many measurements have been developed to reduce the use of antibiotics as growth promotors. Enhanced biosecurity of poultry farms **(Talbante et al.,2002)**, genetic selection of poultry resistant to diseases **(Gross et al.,2002)** and vaccination to pathogenic microbes **(Williams , 2002)** have successfully protected poultry from disease loss. Competitive exclusion is also a popular strategy for preventing poultry from intestinal infectious diseases due to the effective inhibition of pathogenic bacteria **(La and Woodward, 2003)**. Mannan oligosaccharides (MOS) derived from yeast cell wall, are non digestible and can be utilized by lactic acid bacteria. MOS also bind the fimbriae of pathogenic bacteria to prevent them from attaching and colonizing the small intestine mucosa. Adhered bacteria are subsequently washed out of the small intestine with the flow of intestinal content. MOS is reported to have at least 3 probable mode of actions:

1) adsorption of pathogenic bacteria containing type-1 fimbriae with mannose sensitive lectines **(Danny,2004)** ;

2) improved gut health i.e. increased villi length, uniformity and integrity ;

3) immune modulation: it stimulates gut associated and systemic immunity by acting as a non – pathogenic microbial antigen, giving adjuvant-like effect **(Ferket et al.,2002)**. Virginiamycin (VM) was one of the most popular feed-grade antibiotic utilized within poultry industry. It controls microbial growth within the lumen of gastrointestinal tract by disrupting bacterial protein synthesis **(Parks et al.,2000)**.

The antibiotic ban necessitates the need for more studies and investigations of alternative growth promotion therapies. So, the objective of the present study was to explore the possible effects of MOS and VM on leukogram,cellular immunity, proteinogram and body weight of broiler chickens.

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MATERIALS AND METHODS

Experimental chickens

One hundred and twenty, one-day old Hubbard broiler chickens were obtained from Fat Hens Company, Tanta,Egypt.They were allocated into 3 equal groups (40 per each group). All groups were reared under good hygienic conditions and fed commercial starter and finisher rations free from mycotoxins and feed additives. All birds were vaccinated against Newcastle and Gumborou diseases
(Hitchiner B1 at 7 days of age, gumborou at 11 days, Avinew at 17 days, gumborou at 21 days and Avinew at 31 day of age).

Drugs

Myco-power: Each kilogram contains condensed molasses fermentation soluble mannose 850 grams and Brewer s dried yeast 150 gram. Brewer dried yeast is a source of mannan oligosaccharide **(White et al., 2002)**. It was produced by Probyn International Inc.,USA. It was given as feed additive by the dose of 0.5 gram/kg ration.

Stafac: Each Kg contains 20 gram virginiamycin. It was produced by Phibro Animal Health and was given by tha dose of 1 gram/Kg ration.

Experimental design

One hundred and twenty ,one day old, chickens were divided into 3equal groups. The first group was kept as control non treated group. The second group was supplemented with Myco- power (0.5g/Kg ration) during the whole experimental period (6 weeks). While the third group was supplemented with Stafac (1gram/ Kg ration) during the whole experimental period.

Blood samples

Five chickens from each group were used for collection of blood samples from wing vein at 28,35,42 day of age. Three blood samples were collected from each bird. The first blood sample was collected in test tube containing EDTA for total leukocytic count using Nutt and Herrick solution as a special diluent for chicken blood **(Harrison and Harrison,1986)**. Differential leukocytic count was performed according to **Coles,(1986)**. The second blood sample was collected in plastic syringe containing heparin by heart puncture under aseptic condition for determination of phagocytic activity of mononuclear leukocytes according to **Woldhiwet and Rowan, (1990)**. While the third blood sample was collected in centrifuge tube, left to clot for serum separation for determination of serum total proteins **(Henry,1974)**, albumin **(Doumas,1971)** and globulin **(Doumas and Biggs,1972)**.

Length and weight of intestine

At the end of the experimental period, 5 birds from each group were sacrificed and the weight and length of intestine were determined.

Body weight

Body weight of chickens of all groups were recorded weekly.

Statistical analysis

The obtained data were statistically analyzed according to **SAS,(1992)**.

RESULTS AND DISCUSSION

The use of antibiotics for growth promotion in poultry has been banned in many countries and there is a possibility that they may face similar legalization in other areas of the world **(Jones and Rickets,2003).** Whether or not the poultry industry is to blame for the emergence of antibiotic- resistant organisms, the fact that these "super bugs" are a major threat to human health in long term. The world will follow the European lead, either by governmental regulation or voluntary , to reduce or discontinue the use of antibiotics as growth promotors in poultry feed. There are a number of alternatives, including enzymes,probiotics, prebiotics(MOS) and organic acids that can be used strategically **(Mingan,2001).** Regarding to the effect of MOS on leukogram there was significant increase in total leukocytic count, lymphocytes , heterophils and monocytes besides non significant changes in esinophils and basophils. While virginiamycin supplementation produced non significant changes in total and differential leukocytic count as shown in table(1). Similar results were previously obtained by **Davis et al.,(2004)** and **Franklin et al.,(2005).** The result of this work showed significant increase in phagocytic index in MOS supplemented group at 28,35 and 42 day of age (table1). Oligosaccharides and polysaccharides act as biological modifiers and mainly affect the reticuloendothelial system and population of macrophages, lymphocytes and natural killer cells. They also known to enhance the activities of complement system as a critical component of immunity **(Fan et al.,1993).** MOS has been shown to enhance macrophage response. A variety of stimuli can activate macrophage. Phagocytosis of antigens is an initial stimulus but activity can be further enhanced by microbial cell wall products such as mannan oligosaccharide via the alternate pathway of the complement of immune system**(Spring et al.,2000).** In another explanation, oligosaccharide containing mannose have been shown to affect the immune system by stimulating liver secretion of mannose-binding protein. This protein in turn, can bind to bacteria and trigger the complement cascade of the immune system**(Newman and Newman,2001).** Indeed, MOS significantly increase and facilitate the secretion of IgA into the gut mucosa, pathogenic agent become more labile to the phagocytic action of gut associated and systemic immune cells **(Ferket et al.,2002).** Similar results regarding the effect of MOS on phagocytosis were previously recorded by **Spring et al.,(2000)** and **Davis et al.,(2004).** Regarding to the effect of VM on phagocytosis, it produced non significant change in phagocytosis. MOS supplementation elicited significant increase in serum total proteins and globulin at 28,35 and 42 day of age (table2).The increase in serum total proteins in this study was due to increased serum globulin level. The increase in serum globulin level in MOS supplemented group may be due to the increased immunoglobulin

concentration. An increase in immunoglobulin response to MOS was expected because of an ability of the immune system to react to foreign antigenic materials of microbial origin. Portions of cell wall of saccharomyces contained in MOS has been shown to elicit a powerful antigenic properties **(Neilsen et al.,1999)**.Similar results were previously recorded by **Savage et al.,**(1996),**Newman and Newman** (2001), **Cetin et al., (2005)** and **Franklin et al.,(2005).** In our study, the growth promotion effect observed by MOS and VM supplementation was associated with significant decrease in weight and length of intestine (table3). In contrast to the mode of action of most antibiotics, MOS serve as alternate attachment sites for gram negative pathogens, thereby preventing their attachment onto enterocytes and subsequent enteric infection, therefore these bacteria move away through the intestine without colonization The decrease in intestinal weight may be due to thinner muscularis layer of intestine. Similar results were recorded by **Cotter,(1997), Ferket et al., (2002)** and **Sun (2004).** The effect of VM on intestinal wall is primarily due to thinning of its wall. **Henry et al., (1987)** recorded 19% decrease in intestinal weight of broiler chickens due to dietary inclusion of VM. Antibiotics limit the microbial population and their production of toxins and byproducts in the lumen of the gut, they reduce the competition with the vital nutrients due to thinning of intestinal wall **(Catson and Leeson, 1992).** The effect of VM on intestinal weight was more attributed to a decrease in muscularis layer. The decrease in muscularis layer due to VM is reasonable because it may be associated with a reduced need for gut motility to control microbial activity**(Ferket et al., 2002).** Similar results regarding the effect of VM on intestinal weight and length were reported by **Henry et al.,(1987) , Catson and Leeson, (1992)** and **Miles et al.,(2006).**

Dietary inclusion of MOS and VM (each alone) produced significant increase in body weight at 3^{rd},4^{th},5^{th} and 6^{th} week of age as shown in table (4).MOS is reported to have at least 3 probable mode of actions by which broiler performance was improved; 1) adsorption of pathogenic bacteria; 2) improved intestinal function(increased villi height, uniformity and integrity) **(Loddi et al.,2002)** ; 3)immune modulation: it stimulates gut associated and in turn systemic immunity by acting as a non-specific microbial antigen giving an adjuvant- like effect **(Ferket et al.,2002).** In another explanation, improvement of gastrointestinal microflora can have a profound effect on the structure and function of intestinal wall. Changes in intestinal morphology such as shorter villi and deeper crypts have been associated with the presence of stressors in the gut. Shortening of the villi decrease the surface area for nutrients absorption. The crypts can be regarded as the "villi factory" and a larger crypts indicates fast tissue turnover and

high demand for new tissues . The decreased crypt depth and increased villi height due to MOS was previously recorded by **Savage et al.,(1996) and Ferket et al., (2002).** This could be the result of reduced stressors such as bacterial toxins in the digestive tract. The energy conserved by the reduced turnover rate of epithelial cells of the intestine might be utilized for lean tissue mass synthesis and might help to explain the improvement in body weight when feeding MOS.Similar results were previously recorded by **Parks et al., (2001), Waldroup et al., (2003), Danny, (2004) , Dorota et al., (2004)** and **zdunczyk et al., (2005).**

CONCLUSION

As a final thought, it could be concluded that MOS has shown promise in modulating the immune system, improving intestinal function and improving the performance of broiler chickens.

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Table (1): Total and differential leukocytic count and phagocytic index(Mean values±SE) in chickens supplemented with MOS and VM.

Age/ days	Groups	T.L.C. (10^3/UL)	Absolute differential leukocytic count					Phagocytic index
			Lymphocytes (10^3/UL)	Heterophils (10^3/UL)	Monocytes (10^3/UL)	Eosinophils (10^3/UL)	Basophils (10^3/UL)	
28 days	Group1	25.00±2.00 BC	11.93±1.08BC	6.94±0.93C	2.65±0.09AB	0.63±0.11A	2.87±0.11A	17.33±0.37C
	Group2	31.00±1.53 A	14.29±0.99AB	10.48±0.13A	2.92±0.36A	0.61±0.15A	2.80±0.46A	20.00±0.58A
	Group3	25.20±1.15 BC	11.84±0.62BC	7.70±0.59AB	2.75±0.08AB	0.69±0.10A	2.22±0.19AB	18.00±1.00C
35 days	Group1	24.33±0.67BC	12.67±0.41AB	6.31±0.16C	2.27±0.24B	0.61±0.09A	2.54±0.23AB	22.67±0.67B
	Group2	30.67±1.45A	15.16±1.01A	9.42±0.89A	2.76±0.26AB	0.62±0.12A	2.64±0.40A	28.33±0.88A
	Group3	25.00±1.53BC	12.04±1.23AB	7.27±0.81BC	2.45±0.33B	0.64±0.11A	2.79±0.44A	23.33±1.67B
42 days	Group1	22.67±0.33 C	8.20±2.30C	6.19±0.26C	2.44±0.22BC	0.65±0.08A	2.74±0.17A	21.00±0.58B
	Group2	27.33±0.88 AB	12.77±0.35A	8.53±0.50B	2.90±0.23AB	0.63±0.17A	2.77±0.06A	26.67±0.88A
	Group3	22.33±1.20 C	9.63±0.43B	6.90±0.38BC	2.46±0.16BC	0.64±0.10A	2.70±0.16AB	21.00±0.58B

Table (2): Proteinogram (Mean values ± SE) in chickens supplemented with MOS and VM.

Age/ days	Groups	Total proteins gm/dl	Albumin gm/dl	Globulin gm/dl	A/G ratio
28 days	Group1	4.80±0.05 B	2.15±0.11 A	2.65±0.11 C	0.81±0.01 AB
	Group2	5.03±0.05 A	2.20±0.10 AB	2.83±0.07 A	0.77±0.07 C
	Group3	4.81±0.08 B	2.25±0.04 AB	2.56±0.06 BC	0.87±0.02 AB
35 days	Group1	4.72±0.05 B	2.21±0.05 B	2.51±0.04 C	0.88±0.03 AB
	Group2	4.99±0.07 A	2.15±0.04 AB	2.84±0.04 A	0.75±0.01 C
	Group3	4.79±0.04 AB	2.28±0.03 B	2.51±0.05 C	0.90±0.01 AB
42 days	Group1	4.85±0.02 AB	2.25±0.02 C	2.60±0.00 B	0.86±0.03 AB
	Group2	4.98±0.02 A	2.14±0.02 C	2.85±0.04 A	0.75±0.02 C
	Group3	4.80±0.04 AB	2.23±0.03 C	2.57±0.05 B	0.86±0.02 AB

Table (3): Intestinal and cecal weight and length (Mean values ± SE) in chickens supplemented with MOS and VM.

Groups	Body Weight Kg	Small Intestine		Cecum	
		Weight/gm	Length/cm	Weight/gm	Length/cm
Group1	1603±28.13 B	103.33±7.31 A	190.67±6.67 A	10.00±1.50 A	18.00±0.02 A
Group2	1822±60.11 A	65.01±6.66 C	166.67±10.97 B	8.00±0.29 B	14.00±0.01 B
Group3	1710±43.57 A	86.33±10.48 B	173.33±3.32 B	8.25±0.88 B	13.00±0.01 B

Table (4): Body weight (Mean values ± SE) in chickens supplemented with MOS and VM.

Groups	One day old	First week	2nd week	3rd week	4th week	5th week	6th week
Group1	47.10±1.45 A	127.20±4.06 B	346.60±13.93A	660.00±17.95 B	942±27.88 B	1088±44.94 B	1603±28.13 C
Group2	47.95±0.92 A	139.00±3.09 A	373.70±11.42A	760.00±16.33A	1121±41.83 A	1462±40.63 A	1822±60.11 A
Group3	48.50±1.14 A	128.70±2.86 B	356.60±13.93A	680.00±16.23 B	1015±28.30 AB	1365±35.88 A	1710±43.57 B