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Clinicopathological and Immunological Studies on the Effect of *Saccharomyces cervisiae* Metabolitis on Broiler Chicken Performance

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Abstract: Three hundred Cubb broiler chicks, one day old were devided into two equal groups 150 birds each, group (A) considered control negative non medicated group while group (B) was treated with commercial Prebiotic preparation contains Saccharomyces cervisiae metabolitis (Celmanax®) with dose of 0.5 ml/liter for 5 successive days before lasota vaccine and till the end of the experiment (six weeks of age). Chicks of both groups were examined for both body weight gain (BWG) and feed conversion rate (FCR) together with percentage of phagocytosis, differential leukocytic count and humoral immune response to live Newcastle vaccine. The obtained results were significant ($p \leq 0.05$), there were improvement in terms of live body weight, BWG and FCR of chickens treated with prebiotic compared with control negative group, concerning celluler and humoral immune response, there was higher percentage of phagocytosis (73.2±7.0) when compaered with control negative group (32.4±4.9) at 9 days post vaccination. humoral antibody immune response against Newcastle (lasota) live vaccine was higher in prebiotic treated groups start from 7 days post vaccination (4.5) than control negative group (3.9), by 21 days post vaccination treated group containue to increase and become (6.4) and still higher than control negative groups (5.2) and by the 28 days post vaccination prebiotic treated group become (8.1) and still higher than control negative group (6.5) under similar condition, there were higher percentage of lymphocyte and haematological picture as they were improved significantly compared with control negative groups. Our study concluded that use of *Saccharomyces cervisiae* metabolites has abenificial effect on broiler performance as it improves BWG and FCR together with enhancing both cellular and humoral immune response against live vaccine (lasota Newcastle vaccination).

Key words: prebiotic · *Saccharomyces cervisiae* · FCR · broiler performance · HI test · Leucocytic count

INTRODUCTION

Saccharomyces cervisiae yeast cell wall components have been used in animal nutrition since the last decade [1, 2], their inclusion in broiler diets has resulted in improvement of animal productivity, which was attributed to physiological effect on intestinal digestive mucosa [3-5]. However, the mode of action of yeast cell wall products in improvement of broiler chicken performance is not well understood and the characteristics of yeast cell wall products have been poorly defined [6]. Morales-Lopez [7] suggested that part of the mode of action of Saccharomyces cerevisiae cell wall might be related to better maintenance of immune status in response to microbial challenge. While Spring *et al*. [8] stated that mannanoligosaccharides (MOS) present in yeast cell wall could as high – affinity ligands,with the potential benefit of offering a competitive binding site for pathogenic microorganisms Other researchers cleared that yeast cell wall components supplementation improved live weight and feed conversion rate when compared with control negative group [9], moreover it was found that this prebiotic increased humoral antibody immune response against Newcastle disease virus live vaccine at

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21and 42 days of age compared with the control negative groups [10]. Typically, commercial yeast cell wall are composed of 30 to 60% polysaccharides (15- 30% of 3-1,3/1,6-glucan and 15-30% of mannan sugar polymers), 15-30% proteins, 5-20% lipids [11, 12]. The Beta-1, 3/1, 6-glucans that present in yeast cell wall was recognized as an immune modulator substance in poultry and humans [13, 14]; thus, dietary yeast cell wall exert some benefits on the immune system of poultry intestinal mucosa [15]. From the above mentioned data our study was designated in order to evaluate the effect of commercial available Beta-glucan and manno-oligosaccharide supplemented in broiler chickens diet on performance as well as celluler and humoral immune response against live ND vaccine under present field condition.

MATERIALS AND METHODS

Material:

Experimental Birds: three hundred one day old Cubb broiler chickens, were obtained from AL-Ahram hatcheries were reared and fed commercial ration and used in this experiment.

Prebiotics (Celmanax®): Composed of Beta – glucan and manno-oligosaccharide in ratio 1:1 approximately, produced by Vi-coR® company USA

Nitro-blue Tetrazoluium (NBT) Dye: NBT dye was obtained from Sigma company-List No. 3780-34-0.

Natt and Herrick (Methyl violet 2B) Diluent: was used for counting of white blood cells.

Gimsa Stain-sigma Company: used for staining blood films for differential luckocytic count.

Bacto Latex 0.81: was obtained from Difco and used in nitro- blue tetrazolium test.

Live Vaccine Used:

- lasota Gold vaccine-Boehringer Ingelheim- Germany-Lot. No. 1307023A
- Hitchiner B1 live vaccine – FATRO – Italy – Lot. No. 406552
- Gumboro live vaccine (Bursine plus)-zoetis-USA-Lot. No. 1400328.

Methods (Experimental Design):

- A total three hundred (300) one day old broiler Cubb chicks were reared, fed on balanced commercial ration and kept under strict hygienic condition. Chicks were divided randomly in to two equal groups (A and B) 150 chicks each. Two groups received Hitchener B1 live vaccine at 6 days of age by eye drop instillation, Gumboro vaccine at 14th days of age by drinking water rout and lassota vaccine at 20 days of age by eye drop instilation. Group (B) only received Celmanax® in a dose of 0.5 ml/liter drinking water 5 days before lassota vaccine till the end of the experiments while group (A) not receive any treatment till the end of the experiments.
- Blood sampling for heterophil function and differential leuckocytic counts were carried out by collecting of 5 ml of blood on heparin from five birds from each group 3 days pre lassota vaccination and 3,6,9 days post lassota vaccine vaccination.
- Serum sample for haemagglutination inhibition test were collected 3 days pre lassota vaccination and 7,21,28 days post prebiotic treatment.
- Body weight was determined for all chicks from one day old, 4 weeks old and 6 weeks old.
- Nitro-blue tetrazolium test (NBT): was carried out according to Budny *et al.* [16].
- Total luckocytic and differential luckocytic count were carried out according to Natt and Herrick [17] and Coles [18].
- Haemagglutination inhibition (HI) test: was employed according to. Allan and Gough [19].

Statistical Analysis: statistical differences were calculated according to Student t- test with significance level at $p \leq 0.05$.

- All results were analyzed using the procedure of SAS [20].

RESULTS

The obtained results shown in Table (1) revealed a significant ($P \leq 0.05$) improvement in live body weight, body weight gain and feed conversion rate of chicks treated with Celamanx® (0.5ml/liter drinking water).

Results in Table (2) showed that, the heterophil positive formazan % in group received prebiotics were significantly higher ($P \leq 0.05$) than control group at 3,6 and 9 days post treatment.

Table 1: effect of prebiotic on live body weight (BW/gm), BWG/gm and FCR (Means ± SE)

	Initial body weight (gm)	Live body weight (gm) at 4 weeks of age	Live body weight gain (gm) at 4 weeks of age	Live body weight in (gm) at 6 weeks of age	Live body weight gain in (gm) at 6 weeks of age	Feed conversion rate at 6 weeks of age
Group (A)	42.0±3.0	980.0±12.5	938.0±10.70	1870.0±80.7	1825.0±72.3	1.87
Group (B)	42.0±3.0	1150.0±21.3	1090.0±15.7	2050.0±19.5	1996.0±32.3	1.62

Significant at ($P \leq 0.05$)

Table 2: Effect of prebiotic on the Heterophil phagocytic activity % (Mean± SE)

	Heterophil phagocytic activity %			
	3 days pre treatment	3 days post treatment	6 days post treatment	9 days post treatment
Group (A)	25.0±2.3	47.3±5.7	33.4±2.8	32.4±4.9
Group (B)	26.0±1.9	80.1±9.1	78.2±6.6	73.2±7.0

Significant at ($P \leq 0.05$)

Table 3: Leukogram of different groups all over the experimental period (Means ±SE)

	Groups	
Parameters	Group (A)	Group (B)
3 days pre treatment		
Total lukocytic count ($x10^3$/ul)	38.80±4.79	39.70±2.59
Heterophil ($x10^3$/ul)	12.70±1.46	13.00±2.95
Lymphocyte ($x10^3$/ul)	21.90±3.06	21.40±0.92
Monocyte ($x10^3$/ul)	2.82±0.78	3.10±0.96
Eosinophil ($x10^3$/ul)	0.99±0.43	1.09±0.54
Basophil ($x10^3$/ul)	0.37±0.17	0.42±0.28
3 days post treatment		
Total lukocytic count ($x10^3$/ul)	39.60±3.90	41.52±1.51
Heterophil ($x10^3$/ul)	13.30±1.30	7.56±2.24
Lymphocyte ($x10^3$/ul)	20.07±1.66	25.10±2.77
Monocyte ($x10^3$/ul)	2.50±0.50	2.38±0.51
Eosinophil ($x10^3$/ul)	0.88±0.48	1.20±0.33
Basophil ($x10^3$/ul)	0.41±0.01	0.22±0.15
6 days post treatment		
Total lukocytic count ($x10^3$/ul)	35.20±2.21	33.90±3.44
Heterophil ($x10^3$/ul)	11.79±3.15	9.53±2.54
Lymphocyte ($x10^3$/ul)	18.90±2.00	22.40±3.80
Monocyte ($x10^3$/ul)	3.67±1.56	3.99±1.95
Eosinophil ($x10^3$/ul)	0.60±0.24	0.74±0.42
Basophil ($x10^3$/ul)	0.28±0.09	0.26±0.14
9 days post treatment		
Total lukocytic count ($x10^3$/ul)	32.90±2.06	31.10±1.88
Heterophil ($x10^3$/ul)	12.69±3.32	9.05±2.55
Lymphocyte ($x10^3$/ul)	19.10±1.22	22.30±0.98
Monocyte ($x10^3$/ul)	2.31±1.20	2.92±0.62
Eosinophil ($x10^3$/ul)	0.77±0.49	0.60±0.36
Basophil ($x10^3$/ul)	0.22±0.10	0.32±0.32

Significant at ($P \leq 0.05$)

Table 4: effect of prebiotic on mean HI antibody titres against (NDV) lassota vaccine in sera of experimental chicks.

	Geometric means HI titers (log2)			
	Proier prebiotic and lassota vaccine (zero time)	7^{th} days post vaccination	21 days post vaccination	28 days post vaccination
Group (A)	2.85	3.9	5.2	6.5
Group (B)	2.9	4.5	6.40	8.1

Results in Table (3) showed that there is no significant changes in total leukocytic count of treated group all over the experimental period, while there was a significant decrease of heterophile count in prebiotic treatment group, moreover lymphocytic count of treated group were increased significantly ($P \leq 0.05$). at 3,6 and 9 days post vaccination it was found that Esinophils, Basophils and Monocyte count showed non significant difference.

Results of Haemagglutination inhibition test (HI) clarified significant increase in level of geometric means of HI titer in treated group started from day 7 post vaccination lasota vaccine when compared with control group. Similar results was found at 21 and 28 days post vaccination as shown in Table (4).

DISCUSSION

Prebiotic considered a tool for improve broiler performance in modern poultry production, one of them are yeast cell wall obtained from S. cerevisiae of the yeast extract commercially and their Beta – 1,3/1, 6-glucans-purified fractions (Celamanx®) supplement. Our results obtained in this study revealed that prebiotic improved the body weight gain and feed conversion rate of broiler chicks used in this experiments, the same conclusion was suggested byHooge [1], who mentioned that prebiotic in feed had significant effect on chickens performance. Also these results were parallel with those found by Santin *et al*. [21], also our results were matched with Sergio Gomez and Maria [22] who found that yeast cell wall extract and yeast culture improved broiler finisher weight gain, feed conversion rate and final carcass weight yield. Also our results were agreed with Afshin Zakeri and Pedram Kashefi [23] who found that MOS has a positive effect on FCR of broiler chickens and improve performance. The same results were found by Mohamed *et al.* [24] who Found that active MOS prebiotic improve growth performance including body weight and feed conversion ratio. The positive effect of prebiotics on growth performance might be attributed to induction of changes in the population and metabolic characteristic of gastrointestinal bacteria [25].

The heterophil percentage in blood from chicks treated group with prebiotic (Celmanax®) was significantly higher ($P \leq 0.05$) than the non treated group. the same conclusion was suggested by Guo *et al*. [26] and agreed with that reported by Lowery *et al*. [27], who found that supplementation of broiler chickens with prebiotic resulted in increase of phagocytic ability up to 17-23% and bacteriocidal killing.

A significant ($P \leq 0.05$) increase in lymphocytic count of chickens received prebiotic were recorded, this improvement could be explained on the base of improved bioavailability of essential nutrients [28] and increase of bacterial population enhancing vit. B synthessisand/or absorption [29].

higher antibody response against live Newcastle Disease ND vaccine was observed in prebiotic group when compared with control non prebiotic treated group. this obtained results were recorded also by Oliveira *et al.* [30] and Hassan Ghahri *et al*. [31] as they stated that use of MOS prebiotic in poultry feed improves humoral immune response against live Newcastle vaccine.therefore it could be concluded that prebiotic (Saccharomyces cervisiae metabolites) has positive impact on poultry performance including body weight gain, feed conversion rate as well as improve both cellular and humoral immune response.

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