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Behavioral and Physiological Effects of Mannan-oligosaccharide and β-glucan Prebiotic Combination on Heat Stressed Broiler Chickens

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ABSTRACT

This study was conducted to evaluate the effects of mannan-oligosaccharides (MOS) and β-glucan (BG) prebiotic (agrimos) on the behavioral and physiological parameters in heat stressed broiler chickens. One hundred sixty eight of one-day-old broiler chickens of Ross 308 strain were obtained from a local hatchery. The experiment was started at 28-day old; where birds were exposed to heat stress (HS) and were randomly allotted to four dietary treatments containing 0 (control), 0.5, 2, and 4 g/kg MOS and BG probiotic, respectively. Each treatment consisted of three replicates of 14 birds each. The results revealed that 0.5, 2 and 4 g/kg significantly increased walking, panting, wing elevation, and wing and leg but 4 g/kg agrimos only significantly increased drinking and preening, and decreased resting. Dietary inclusion of agrimos at a dose of 4 g/kg caused a significant increase in the percentage of lymphocyte, and decrease in phosphorus level. While at doses of 2 and 4 g/kg, treated chickens had a significant decrease in cortisol level together with a significant increase in hemoglobin level. Chickens fed with agrimos, regardless of dose, had significantly lower percentage of heterophils and heterophil/lymphocyte ratio. In conclusion, the current results support that MOS and BG prebiotic dietary supplementation may be considered as a protective management practice in the broiler chickens to control the negative effects of HS.

Introduction

Poultry meat and its products are the essential sources of animal proteins for human nutrition in the developed countries. Summer high ambient environmental temperature is one of the most serious problems facing poultry farmers in Egypt (Tawfeek *et al.*, 2014). Exposing broiler chickens to heat stress (HS) reduced growth rate and feed intake and disrupted intestinal microbial ecology along with high proliferation of harmful pathogens (Park *et al.*, 2013). In addition, high mortality of birds as a consequence of suppressed immunity causes further economic loss to the poultry farms (Niu *et al.*, 2009) as evident by increasing the feed cost and marketing price, and reducing the meat quality (Quinteiro-Filho *et al.*, 2010; Zhang *et al.*, 2012). From the physiological point of view, HS caused a series of physiological and metabolic changes in the broiler chickens (Lin *et al.*, 2006). These changes include increased body temperature, panting activity, respiratory alkalosis, corticosterone level, heterophil/lymphocyte ratio and heat shock protein 70 expression in tissue cells and reduced thyroid hormones, total protein and globulin levels (Zulkifli *et al.*, 2009; Imik *et al.*, 2013; Mahmoud *et al.*, 2014).

Modification of diet is one of the most preferred and practicable ways to alleviate the negative effects of high environmental temperature in the poultry. Mannan-oligosaccharides (MOS) and β-glucans (BG) are components of yeast cell walls. MOS contains proteins, glucans and phosphate radicals as well as mannose (Klis *et al.*, 2002). Recent researches had focused on the importance of MOS as functional foods to improve the intestinal microarchitecture, microbial profiles, humoral immunity and performance in the broiler and laying hens (Sohail *et al.*, 2010; Sohail *et al.*, 2012).

β- glucan (BG) is a heterogeneous group of glucose polymers. Researches work in humans and rats stated that BG reduced the harmful physiological and metabolic changes induced by oxidative stress conditions through down-regulation of c-fos and c-jun expression in the brain tissues and reactive oxygen species activities (Saluk *et al.*, 2013; Hong *et al.*, 2014). BG has recently received much attention as a dietary supplementation for promoting health in the poultry due to its beneficial effects on both the innate and adaptive immune systems (Cox *et al.*, 2010).

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Although reports concerning the significance of MOS and BG combination in the poultry are gaining popularity, still little is known about its ability to improve the health status and performance under the hot climate. Furthermore, researches are warranted to investigate in-depth its effects on the physiological and metabolic changes in the broiler chickens reared under chronic HS. This study investigated the possible effects of a specific combination of MOS and BG (agrimos) extracted from the yeast cell wall of *Saccharomyces cerevisiae* on the behavioral and physiological changes in the broiler chickens exposed to HS, aiming to evaluate the scientific and economic values of agrimos dietary supplementation as a new feeding strategy in improving poultry production during summer season as it has limited scientific bases.

Materials and methods

This study was carried out in the Laboratory animal house in the Veterinary Teaching Hospital (Faculty of Veterinary Medicine, Assiut University, Egypt) during the period from April to June 2016. All procedures were approved by the National Ethical Committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

Mannan-oligosaccharides and β-glucans combination

Agrimos was purchased from LALLEMAND SAS Co. (19 Rue des Briquetiers, 31702 Blagnac Cedex, France), distributed by Egavet Company, Egypt.

Birds and husbandry

One hundred sixty eight of one-day-old unsexed chicks of the Ross 308 strain were obtained from a local hatchery (Future Poultry, Makram Ebeid St., Nasr City, Cairo, Egypt). The birds were randomly assigned to 12 floor pens (1× 1 m per pen) in the same room at the Veterinary Teaching Hospital during April 2016. Wood shavings (5-cm depth) were used as a litter. The brooding temperature was 34°C for the first 3 days then gradually reduced by 3°C/week up to 28 day of age, thereafter, all the chickens were exposed to 32°C for 9 hours (08:00 – 17:00) daily up to 42 days. Actual pen temperatures and humidity were measured every 4 hours using wall mount thermohugrometer, which was fixed 30 cm above the litter surface. All chickens were fed diets formulated according to the requirements proposed by NRC (1994) as shown in (Table 1). The light regime was 23L: 1D. The birds had free access to feed and water during the experimental period.

Experimental design

At 28 day of age, birds were weighted individually and assigned to 12 floor pens as that each pen average body weight and weight distribution was not different. The experiment was carried out in a completely randomized design with 4 dietary treatments. In each treatment, there were three replicates of 14 birds for each. The experimental groups were as follows; treatment 1 (control) was fed with a basal diet only, and treatments 2 to 4 were fed with a basal diet supplemented with agrimos 0.5, 2, and 4 g/kg, respectively.

Behavioral observations

Fifteen birds per treatment from three independent pens at five birds per pen were randomly chosen, and were marked with stock spray on their backs (green-Sharp mark spray paint livestock marker, Cotran Corporation, USA). The birds' behaviors were observed according to the ethogram showed in Table 2, using instantaneous scanning sampling method (Alt-

Table 1. The ration formulation

Ingredient, %	Starter	Grower	Finisher
Corn	52.0	52.3	62.8
Soybean meal, 48 % CP	40.0	39.1	29.7
Soy oil	3.59	4.97	4.11
Sodium chloride	0.51	0.46	0.43
DL Methionine	0.30	0.24	0.23
L-Lysine HCL	0.13	- - -	0.07
Threonine	0.06	- - -	- - -
Limestone	1.29	1.15	1.12
Monocalcium phos	1.75	1.48	1.17
Vitamin/mineral premix[1]	0.35	0.35	0.35
Calculated analyses			
Crude protein %	23.4	22.8	19.2
Poultry ME kcal/kg	3050	3151	3200
Calcium %	0.95	0.85	0.75
Available phosphorus %	0.50	0.44	0.36
Methionine %	0.66	0.59	0.53
Methionine+ Cystine %	1.04	0.97	0.86
Lysine %	1.42	1.29	1.09
Threonine %	0.97	0.89	0.74
Na %	0.22	0.20	0.19

[1]Provided per kilogram of diet: vitamin A, 13,233 IU; vitamin D3, 6,636 IU; vitamin E, 44.1 IU; vitamin K, 4.5 mg; thiamine, 2.21 mg; riboflavin, 6.6 mg; pantothenic acid, 24.3 mg; niacin, 88.2 mg; pyridoxine, 3.31 mg; folic acid, 1.10 mg; biotin, 0.33 mg; vitamin B12, 24.8 µg; choline, 669.8 mg; iron from ferrous sulfate, 50.1 mg; copper from copper sulfate, 7.7 mg; manganese from manganese oxide, 125.1 mg; zinc from zinc oxide, 125.1 mg; iodine from ethylene diamine dihydroidide, 2.10 mg; selenium from sodium selenite, 0.30 mg.

Table 2. Behavioral ethogram

Behavioral pattern	Definition
1. Standing	Both feet are in contact with the floor but no other body part is in contact with floor.
2. Sitting	Most of the ventral region of the bird's body in contact with floor. Although no space is visible between the floor and the bird.
3. Walking	Bird is in the process of taking multiple steps.
4. Feeding	Bird's head is located inside feeder.
5. Drinking	Bird's beak is in contact with drinker.
6. Preening	Gently pecking or scratching its own feathers.
7. Panting	Bird's beak is open and respiration rate is abnormally rapid.
8. Elevated wing	A space can be seen between bird's wings and body.
9. Feather pecking	Pecking on any feathered part of a conspecific.

mann, 1974) twice daily from 08:00 to 09:00 and from 14:00 to 15:00, for three days weekly (from Monday to Wednesday) during the entire experiment (from 15 to 42 days of age). To avoid disturbing the behavioral patterns of the chickens, all observations were made from outside of the pens with a distance of 1.5 meter and by two experienced persons (individuals trained well in observing and analyzing poultry behavior based on the ethogram used and they were blind to treatment), who were familiar to the chickens from being present during all sampling times. Behaviors of all marked birds in every pen were scored every min for six min. After sampling one pen, the experimenter moved to another pen to repeat the same behavioral observation. In total, behavior was scanned 12 times daily (i.e. each pen scored six times/hours). Data were presented as the proportion of each behavioral frequency "number of scans out of the total number possible" (Mahmoud *et al*., 2015).

Physiological indicators

At 42 days old, six birds were randomly taken from each treatment, weighed and slaughtered. During the bird's exsanguinations, blood samples were collected as following: Blood smears were directly spread to determine differential leucocytic count. Three cubic centimeter of blood from each bird was collected in plain tube without anti-coagulant to determine the chemical blood parameters. The tubes were kept at the room temperature for 30 min then stored at a refrigerator for 60-90 min, and then centrifuged at 3000 rpm for 10 min and the separated serum was transferred to another Eppendorf's tube using micropipette. The sera were kept at -20ºC, until analysis using commercial kit according to the procedure outlined by manufacturer.

Hemoglobin (Hb) concentration was assayed by a colorimetric method using spectrum hemoglobin diagnostic kit (Egyptian Company for Biotechnology, Cairo, Egypt, Catalog no. 602001). 12 blood smears, from each treatment group, were prepared and stained using Giemsa stain "EDM" (Egyptian Diagnostic Media, Egypt). A total of 100 white blood cells (WBCs) were counted and heterophil/lymphocyte (H/L) ratios were calculated according to Gross and Siegel (1983) and Parga *et al*. (2001). Based on the manufacturer's instructions, total protein (TP) (Catalog no. 310001), albumin (ALB) (Catalog no. 210001), phosphorus (P) (Catalog no. 294001) and calcium (Ca) (Catalog no. 226001) were estimated by commercially available kits (Egyptian Company for Biotechnology, Cairo, Egypt), while cortisol (COR) was assessed using AssayMax cortisol ELISA kit (Immunospec Corporation, California, USA, Catalog no. EC3001-1).

Statistical analysis

The data were analyzed by one way analysis of variance using the general linear model procedures of SPSS 22.00 Software (SPSS Inc., Chicago, IL, USA). Significance was designated as $P \leq 0.05$. Means were compared by Tukey's test when a significant difference was detected.

Results

The effect of agrimos supplementation on the behavioral patterns (Table 3)

The results clarified that at 28-42 days, diet with 4 g/kg MOS and BG prebiotic significantly (*P=0.030*) decreased resting activity compared to control group. In contrast, the supplementation of diet with 0.5 or 2 g/kg MOS and BG prebiotic did not affect the resting activities in comparison to the control during the whole experimental period. The overall relationships between agrimos treatments and mobility behaviors, including standing and walking, showed that all agrimos treatments significantly (*P=0.002*) increased the walking activity of the birds in comparison to the control group. While standing was not affected (*P=0.466*). There were no treatments effects on feeding behaviors (*P=0.291*). However, drinking activity was significantly (*P=0.002*) increased in 4 g/kg group as compared to the control and other treatment groups (*P=0.008*). The percentage of panting activities in all agrimos treated birds was significantly lower (*P=0.044*) than the control group. The percentage of wing elevation activities was significantly (*P=0.012*) decreased in all doses of agrimos as compared to the control group. Only 4 g/kg agrimos significantly (*P=0.023*) increased the preening activities in comparison to the control. Although all agrimos treatments groups significantly (*P=0.020*) increased the wing and leg stretching activities, the agrimos combination did not affect dust bathing or body shaking. Agrimos did not affect on aggressive behaviors including feather (*P=0.88*) and wall pecking activities (*P=0.26*).

T*he effect of agrimos supplementation on the physiological parameters (Table 4)*

Dietary inclusion of agrimos at a dose of 4 g/kg caused a significant (*P=0.013*) increase in the percentage of lymphocyte

Table 3. The effects of different levels of MOS and BG prebiotic on the behavioral patterns of heat stressed broiler chickens

Treatment / Behavioral pattern (%)	Control	0.5 g/Kg diet	2 g/Kg diet	4 g/kg diet	P value
Resting	70.08±1.78^{a}	65.53±0.81^{ab}	64.79±1.84^{ab}	62.45±2.00^{b}	0.030
Walking	2.84±0.22^{b}	4.22±0.24^{a}	3.47±0.17^{a}	3.83±0.31^{a}	0.002
Standing	6.09±0.49	6.02±0.34	6.15±0.54	5.25±0.41	0.466
Feeding	13.10±0.39	12.33±0.69	11.82±1.15	14.24±1.18	0.291
Drinking	7.61±0.71^{b}	8.97±0.42^{ab}	9.47±0.47^{ab}	10.17±0.42^{a}	0.008
Panting	57.56±4.36^{a}	44.87±4.24^{b}	44.37±3.64^{b}	42.68±2.79^{b}	0.044
Wing elevation	2.02±0.29^{a}	1.02±0.27^{b}	1.20±0.21^{b}	0.88±0.15^{b}	0.012
Preening	2.15±0.36^{b}	3.88±0.56^{ab}	3.45±0.58^{ab}	4.47±0.62^{a}	0.023
Wing and leg stretching	1.83±0.05^{b}	2.79±0.35^{a}	2.59±0.24^{a}	2.65±0.18^{a}	0.020
Dust bathing	3.16±1.00	4.21±0.97	3.42±0.92	3.34±0.88	0.864
Body shaking	0.41±0.17	0.29±0.11	0.24±0.06	0.54±0.06	0.261
Feather pecking	0.18±0.07	0.24±0.05	0.21±0.08	0.26±0.09	0.879
Wall pecking	2.10±0.64	3.29±0.64	2.40±0.28	3.28±0.37	0.258

^{ab} Means ± SE in the same column with different letters differ significantly ($P<0.05$)

Table 4. The effects of different levels of MOS and BG prebiotic on the physiological parameters of heat stressed broiler chickens

Parameter \ Treatment	Control	0.5 g/Kg diet	2 g/Kg diet	4 g/kg diet	P value
Heterophils (%)	17.25±0.48^{a}	15.00±0.37^{b}	14.33±0.42^{b}	14.40±0.24^{b}	0.000
Lymphocytes (%)	77.00±0.41^{b}	78.00±0.93^{b}	79.83±0.54^{ab}	82.20±1.59^{a}	0.013
H/L ratio (%)	0.22±0.01^{a}	0.19±0.01^{b}	0.18±0.01^{b}	0.18±0.00^{b}	0.000
ALB (g/dl)	0.86±0.02	0.81±0.04	0.80±0.03	0.82±0.03	0.528
GLOB (g/dl)	2.96±0.18	2.9±0.09	2.50±0.24	2.71±0.19	0.279
TP (g/dl)	3.82±0.19	3.74±0.10	3.30±0.24	3.53±0.20	0.321
COR (µg/dl)	60.60±5.86^{a}	43.15±6.41^{ab}	38.53±5.91^{b}	39.58±3.07^{b}	0.034
Ca (mg/dl)	7.29±0.46	6.91±0.34	6.83±0.70	6.92±0.93	0.966
P (mg/dl)	7.48±0.01^{a}	7.45±0.03^{ab}	7.42±0.01^{ab}	7.41±0.02^{b}	0.031
Hb (g/dl)	7.80±0.08^{c}	7.23±0.05^{d}	8.80±0.07^{a}	8.27±0.11^{b}	0.000

^{ab} Means ± SE in the same column with different letters differ significantly (P<0.05).
H/L ratio, heterophil/lymphocyte ratio; ALB, albumin; GLOB, globulin; TP, total protein; COR, cortisol; Ca, calcium; P, phosphorus; Hb, hemoglobin.

relative to the control. Chickens fed with agrimos, regardless of dose, had significantly lower percentage of heterophils (*P=0.000*) and H/L ratio (*P=0.000*) as compared to the control. Agrimos supplementation did not affect serum ALB (*P=0.528*), GLOB (*P=0.279*), TP (*P=0.321*), and Ca (*P=0.966*) levels. However, chickens fed with 4 g/kg MOS and BG prebiotic had a significant (*P=0.031*) decrease in P level. While at doses of 2 or 4 g/kg, chickens fed MOS and BG prebiotic had a significant (*P=0.034*) decrease in COR level along with a significant (*P=0.000*) increase in Hb level as compared to the control diet.

Discussion

The results from the current study indicated that all agrimos treatments significantly increased the walking activity of the birds in comparison to the control group. Increased mobility activities may be due to the improvement in leg health in Agrimos fed birds. Supporting these findings, Youssef *et al.* (2011) reported that dietary supplementation of growing turkeys with 1% MOS tended to reduce the foot pad scores. The current study hypothesized that MOS and BG prebiotic may reduce the incidence of leg problems and increase walking activities due to its role in stimulating the immune system as well as its activity within the intestinal tract. This suggestion is supported by pervious published results of Ferket *et al.* (2002) who observed that dietary supplementation of MOS reduced total short chain fatty acids content of the jejunum digesta in turkeys as well as decreased the jejunum digesta pH and ammonia concentration. MOS can also modify bacterial fermentation in the intestinal tract to increase nutrient availability to the poultry (Ferket *et al.*, 2002). In addition, MOS dietary inclusion resulted in production of excreta of higher dry matter content and consequently prevents the occurrence of wet litter conditions.

In the present study, drinking activity was significantly increased in 4 g/kg group as compared to the control group. This outcome response may be attributed to the numerical increase in feed intake in this group.

In the current study, the reduction in panting rate and wing elevation activities in the heat stressed broilers fed with agrimos may reflect improvement in the oxygenation level via its effects on the hematopoietic system. This suggestion is supported by the finding of Attia *et al.* (2014) and Mousa *et al.* (2014) who recorded that MOS and BG significantly increased RBCs count and Hb values in both broiler chickens and quail. Moreover, the significant increase in water intake may benefit the birds by facilitating evaporation and this may prevent the increase in body temperature and reduce the panting behavior; as higher drinking rate helps rehydration for the body (Mahmoud *et al.*, 2015).

The results of this study showed that preening activities were increased in the birds fed with 4 g/kg agrimos, while all agrimos treatments groups significantly increased the wing and leg stretching activities. To explain the increase in preening activities, the present work built a novel suggestion based on the function of preening activities. Preening is an inducible antimicrobial behavior that can be directly affected by plumage bacterial load and composition in addition to the bird health status (Leclaire *et al.*, 2014; Chatelain *et al.*, 2016) which are both potentially affected by HS. The change in preening frequency observed in the current study may be non-exclusively resulted from the change in feather bacterial community caused by MOS and BG treatment. Whatever the mechanism underlying the differences in the preening frequency, the tendency of prebiotic to improve bird's control on its feather bacterial community may change the bacteria species and their sensitivity to preen secretions. However, this hypothesis was not examined in the present work.

The results of present study indicated that feeding 4g/kg of agrimos caused a significant increase in the percentage of lymphocytes in comparison with the control group. While, chickens fed with agrimos, regardless of dose, had significantly lower percentage of heterophils and H/L ratio as compared to the control. These findings are supported by Hosseini *et al.* (2016) who found that dietary supplementation of 5 gm of MOS/kg diet to the broilers chickens exposed to cyclic HS resulted in a significant decrease in H/L ratio. Similar results were obtained by Sadeghi *et al.* (2013) who reported that addition of 1 g/kg prebiotic-based MOS and BG to the diet of chickens challenged with *Salmonella enteritidis* induced decrease in H/L ratio, and increase in antibody titer and lymphocyte percentage. These finding may be attributed to immuno-modulatory effects of MOS and BG prebiotic.

Various stressors, including short exposure to heat (Christison and Johnson, 1972), isolation (Hashizume *et al.*, 1994), and transportation (Arave *et al.*, 1988) make stimulation of hypothalamus to release corticotrophin-releasing factor and transported to the pituitary gland, where it stimulates the production of adrenocorticotrophic hormone (ACTH). ACTH is released into the general circulatory system and is transported to the adrenal cortex. Under ACTH stimulation, the adrenal cortex increases the production and release of all of the adrenocortical hormones including cortisol and corticosterone (Daghir, 2008). HS stimulates the release of cortisol (Sohail *et al.*, 2010) which leads to immunosuppression as manifested by inhibition in the production and actions of antibodies, by its effect on lymphocyte function and leucocyte population

(Roth and Kaeberle, 1982; Munck *et al*., 1984). In the current study, dietary supplementation of Agrimos inhibited the negative effects of HS. As compared to the control, birds supplemented with 2 and 4 g/kg agrimos had a significant decrease in the cortisol level. This results agree with Sohail *et al*. (2010), who reported that the broilers supplemented with MOS in the diet under HS had a significant decrease in the concentration of cortisol. Prebiotics stimulate the growth and activity of beneficial bacteria (Upadhayay and Vishwa, 2014) like lactobacillus and bifidobacteria which have the ability to reduce the negative effect of hypothalamic pituitary adrenal axis and cortisol hormone level in the birds (Abdel-Mohsein *et al*., 2014).

The current results revealed that agrimos supplementation, regardless of doses, did not significantly affect the serum levels of Ca as compared to the control. These agree with the results of Chae *et al*. (2006) who found that BG had no effect on Ca and pH levels in the blood of the broiler chickens employed in cage and open floor housing and supplemented with three levels of BG 0%, 0.02% and 0.04%. Similarly, Yenice *et al*. (2015) stated that the blood Ca and P were not affected by the addition MOS at a dose of 0.1% in laying hens exposed to 120 ppb total aflatoxin. But this finding disagrees with the results of the current study regarding P level, which significantly decreased in the birds supplemented with 4 g/kg agrimos.

The results of the present study indicated that 2 and 4 g/kg agrimos significantly increased Hb level as compared to the control diet. The increase in Hb conentration in the current study may be attributed to increase in the count of RBCs. Similarly, Mousa *et al*. (2014) found that RBCs count were significantly increased in the quail chickens supplemented with foreign or Egyptian local commercial products containing MOS and BG. Also, Attia *et al*. (2014) reported that administration of MOS at a dose of 0.5 g/kg continuously from 0 to 35 days or intermittently in broilers increased RBCs count and Hb content.

Conclusion

Dietary supplementation of agrimos improved the behavioral patterns and physiological indicators of broilers exposed to heat stress. These findings reflected enhancement in the health status of the birds and their ability to cope with high environmental temperature.

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