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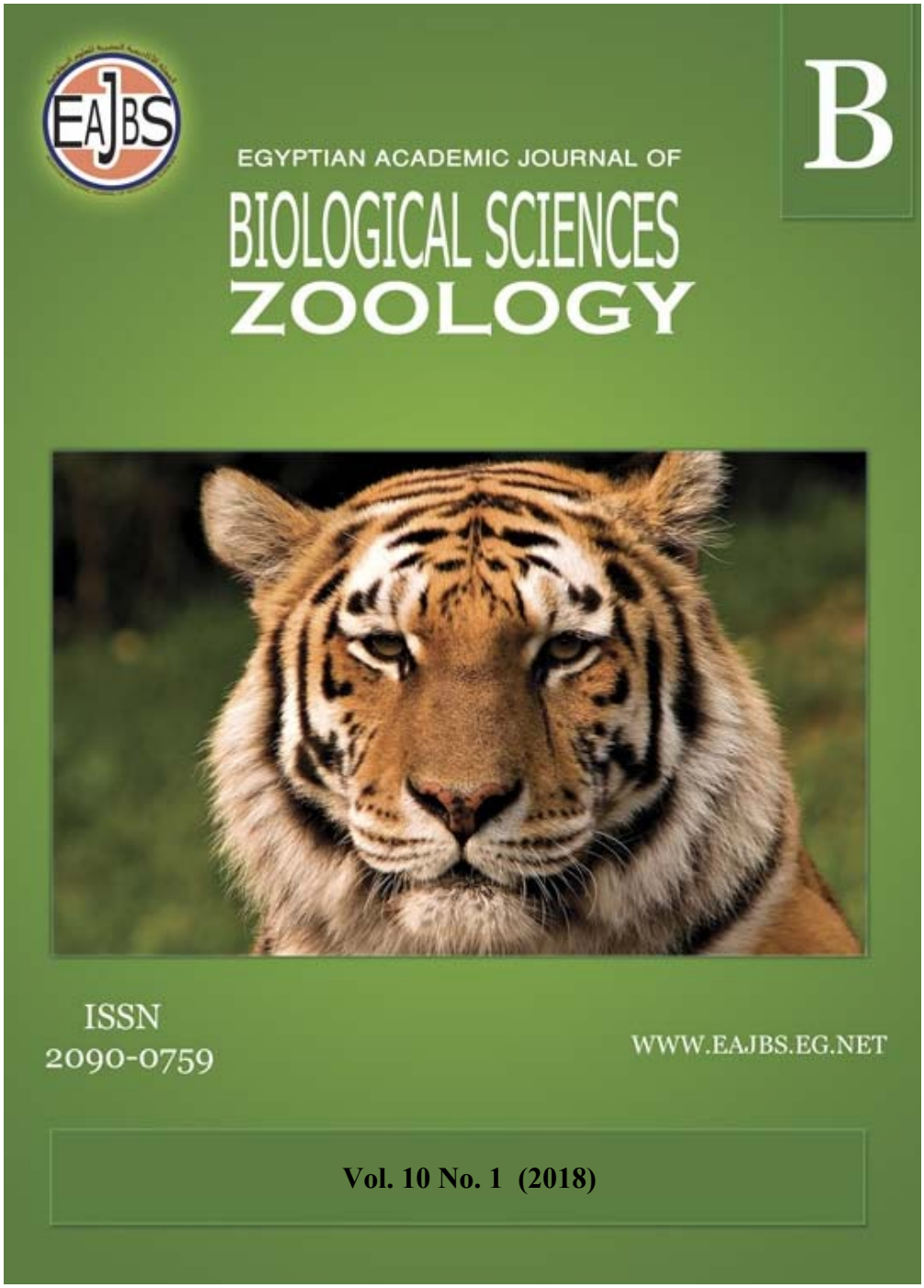

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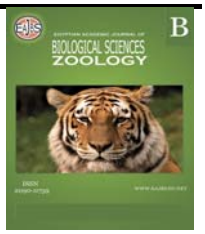

Effects of Mannan-oligosaccharide and β-Glucan Prebiotic on the Brain Oxidant/Antioxidant Balance in Broilers under Natural Egyptian Summer Conditions

Sohair M. M. Rageb[1], Elham A. Abd-Allah[2], Nasser S. Abou Khalil[3], Fatma M. Abdel-Maksoud[4], Usama T. Mahmoud[5]

1-Department of Zoology, Faculty of Science, Assiut University, Assiut, Egypt
2-Department of Zoology, Faculty of Science-New Valley, Assiut University, Assiut, Egypt
3-Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt
4-Department of Anatomy & Histology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt
5-Department of Animal Hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt
E. Mail : sohair.2008@yahoo.com

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ABSTRACT

This study was conducted to investigate the potential effects of mannan-oligosaccharide (MOS) and β-glucan (BG) prebiotic (AGRIMOS®) on the redox homeostasis, histopathology, and microglia count in the brain of heat-stressed broiler chickens. One hundred sixty eight (168) Ross one-day-old broiler chicks were obtained from local hatchery. The experiment was started at 28-day old; where birds were exposed to heat stress and were randomly allotted to four dietary treatments containing 0 (control), 0.5, 2, and 4 g/kg MOS and BG probiotic, respectively, for 14 days. Each treatment consisted of three replicates of 14 birds each (i.e., total birds/treatment count is 42 birds). The results indicated a significant decrease in catalase and superoxide dismutase activities in all MOS and β-glucan prebiotic treated groups. Supplementation with 0.5 g of prebiotic/kg diet resulted in a significant increase in glutathione levels; however, a significant decrease in superoxide radicals was found at dose of 4 g of prebiotic/kg diet. The levels of lipid peroxidation in supplemented groups exhibited a significant decrease at doses of 2 and 4 g of prebiotic/kg diet. Although there were no obvious changes in the histoarchitecture of cerebellar tissues, a significant increase in the number of microglia was evident following administration with 4 g of prebiotic/kg diet. In conclusion, supplementation of MOS and BG may be regarded as promising candidate for alleviating the undesirable effects of heat challenge on the brain of broiler chickens, nevertheless; further studies are warranted to look for other nutritional approaches.

INTRODUCTION

To satisfy the urgent needs for providing meat to consumers, the improvement of health status of broiler within hot region, especially developing countries like Egypt, is an indispensable necessity. Taken in consideration lower acclimatization of birds to hot climate and more susceptibility to heat stress secondary to intensive production operations, housing of birds in tropical and subtropical regions represents a real challenge (Lin *et al.,* 2006; Mahmoud *et al.,* 2013). High ambient temperature in Egypt during summer season is incriminated in a wide range of adverse effects including low weight gain, reduced feed intake, poor feed efficiency, and high mortality rate (Tawfeek *et al.,* 2014). Heat stress attracts much scientific and commercial attention in the poultry industry because it is responsible for a broad spectrum of disturbances including generation of reactive oxygen species that implicated in disruption of oxidant/antioxidant balance in multiple organs (El-Deep *et al.,* 2016; Felver-Gant *et al.,* 2014; Lara and Rostagno, 2013). The vital role of brain, especially the hypothalamic temperature-regulating center, in restoring thermal homeostasis and accommodate to stressful situations (Nakamura, 2011) makes it a suitable candidate for investigating the biochemical and histoarchitecture changes related to exposure to hyperthermia. Failure of the hypothalamic adaptive mechanisms to cope with increased environmental temperature results in positive feedback circuit with further increase in the body temperature, increase in intracerebral pressure, and systemic decrease in blood pressure culminating at multi-organ dysfunction (Simon, 1994). Brain is one of the most susceptible organs to oxidative stress owing to low antioxidant capacity, high metabolic load, and high content of polyunsaturated fatty acids and phospholipids (Esmekaya *et al.,* 2016; Halliwell, 2001; Song *et al.,* 2000). Neuronal degeneration and necrosis is a common finding under heat load (Mohamed *et al.,* 2015). Microglia plays a key role in scavenging of dying neurons and associated debris which is a prerequisite to ensure healthy neural circuits in the central nervous system (Noda *et al.,* 2011). The high cost, variability, and inconsistency are the major challenges stand in the front of applying management procedures to get rid of environmental heat load; therefore, application of nutritional interventions to alleviate its negative consequences is of a great point of interest (Lara and Rostagno, 2013; Rhoads *et al.,* 2013). From the nutritional point of view, prebiotics are supposed to be high ranking alternatives in the view of their promising antioxidant and cytoprotective potentials (Vahdatpour *et al.,* 2016; Zimmermann *et al.,* 2015). AGRIMOS® is a commercial prebiotic of mannan-oligosaccharide (MOS) and β-glucans (BG) derived from the cell wall of *Saccharomyces cerevisiae*. It is applied on wide range as an emerging nutritional approach in the formula feed for livestock aimed primarily at enhancement of bacterial colonization and immuno-potency (Mounsey, 2005). *Saccharomyces cerevisiae* extract and its main component MOS are beneficial supplements in counterbalancing the deleterious impacts of chronic heat stress in broiler (Haldar *et al.,* 2011; Sohail *et al.,* 2012). Suppression of lipid peroxidation and enhancement of enzymatic antioxidant activities involved in the ability of *Saccharomyces cerevisiae* extract in restoring redox balance (Uskoković *et al.,* 2013). A great deal of evidences suggested using MOS as an effective antioxidant additive by increasing activity of superoxide dismutase (SOD) and catalase (CAT), and levels of vitamin C and ferric reducing ability of plasma (Attia *et al.,* 2015; Czech *et al.,* 2006; Czech *et al.,* 2009; Ognik and Krauze, 2012). Solid experimental proof about the antioxidant potential of BG in the brain had been arisen from caecal ligation and diabetic animal models (Alp *et al.,* 2012; Şener *et al.,* 2005). BG and MOS are

well-known immunocompetent agents in poultry (Lourenço *et al.,* 2016; Rizwan *et al.,* 2016). Taking into account presence of receptors for BG and MOS on microglia mediating its immune-modulatory activity (Moran, 2004; Shah *et al.,* 2008; Tsoni and Brown, 2008), intense microglial response can be hypothesized upon dietary inclusion of the studied components providing protection against the injured effects of heat stress. However, there is no available literature up to our knowledge about its application as potential protector against adverse changes in the histo-functionality of brain exposed to the natural summer condition. Therefore, the mission of this study is to investigate the supplemental effect of MOS and BG prebiotic combination on the brain of broiler chickens reared under natural Egyptian summer stress by estimating the oxidant/antioxidant biomarkers and evaluating the neural histo-pathological changes in a hope of translating the results to industrial uses.

MATERIALS AND METHODS

Mannan-oligosaccharides and β-Glucans Combination

MOS and BG prebiotic commercial product AGRIMOS® was purchased from LALLEMAND SAS Company (19 Rue des Briquetiers, 31702 Blagnac Cedex, France), distributed by Egavet Company, Egypt.

Birds and Husbandry

One hundred sixty eight one-day-old unsexed chicks of the Ross 308 strain were obtained from a local hatchery (Future poultry, Makram Ebeid Street, Nasr City, Cairo, Egypt). The birds were randomly assigned to 12 floor pens (1×1 m per pen) in the same room at Faculty of Veterinary Medicine Hospital during April 2016. Wood shavings (5 cm depth) were used as litter. The brooding temperature was 34°C for the first 3 d then gradually reduced by 3°C/wk up to 28 d of age, thereafter, all the chickens were exposed to 32°C for 9 h (08:00 – 17:00) daily up to 42 d. Actual pen temperatures and humidity were measured every 4 hours by using wall mount thermohugrometer which was fixed 30 cm above the litter surface. The average temperature and relative humidity were 30.5±1.5°C and 40±6% during the whole period of study. All chicks were fed diets formulated according to the requirements proposed by the (NRC, 1994) as demonstrated in Table 1. A starter diet with 23.43% CP and 3,050 kcal ME/kg from day 1 to 14, grower diet with 22.81% CP and 3,150 kcal ME/kg from day 15 to 28, then finisher diet with 19.17% CP and 3,200 kcal ME/kg from day 29 to 42. The light regime was 23L: 1D. The birds had free access to feed and water during experimental period (Lin *et al.,* 2004).

Experimental Groups:

At 28 days of age, birds were weighted individually and assigned to 12 floor pens as that each pen average BW 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 3 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 (Table 1) supplemented with AGRIMOS® 0.5, 2, and 4 g/kg, respectively.

Sampling and Biochemical Measurements:

At 42 days of age, birds were euthanized immediately by cutting the jugular vein, and allowed to bleed for approximately 2 min. The brains used for the biochemical measurement and histopathological examination were collected from six broilers per group. The brains were dissected out of the skull. Half portion of diencephalon (thalamus and hypothalamus), telencephalon, mesencephalon, and

cerebellum were collected, frozen on dry ice and stored at -80°C until they were used for analyzing oxidant/antioxidant parameters, and the other portion was fixed in 10% of neutral buffered formalin to be used in the histopathological examination. To prepare 20% w/v homogenate, brain was homogenized in 5 mL (0.1 M) phosphate buffer (pH 7.4) using IKA Yellow line DI homogenizer (18 Disperser, Germany). The homogenates were centrifuged at 6000 rpm for 1 hour at 4°C, and the supernatant cytosols were kept frozen at 20°C for the subsequent biochemical assays. The total protein content in brain was determined colorimetrically using the method of (Lowry *et al.,* 1951). CAT activity was determined according to the procedure of (Luck, 1963), based on its ability to decompose hydrogen peroxide. SOD activity was determined according to its ability to inhibit the autoxidation of epinephrine at alkaline medium according to the method of (Misra and Fridovich, 1972). Glutathione (GSH) content was determined using the method of (Beutler *et al.,* 1963), while ceruloplasmin (CP) was estimated following the procedure of (Houchin, 1958). The superoxide radical (O^{-}_{2}) was determined according to (Podczasy and Wei, 1988). Lipid peroxidation (LPO) products as thiobarbaturic acid reactive substance were determined according to the method of (Ohkawa *et al.,* 1979).

Table 1. The ration formulation (NRC, 1994)

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 D_3, 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 B_{12}, 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.

Histopathological Examination:

The brains were obtained immediately after slaughtering, and fixed in 10% of neutral buffered formalin. The specimens were dehydrated in graded ethanol, cleared in xylene, embedded in paraplast, and sectioned at 3-5 µm thick sections. The sections were stained with Haematoxylin and Eosin (Harris, 1900) for demonstration of the general histological structure of the examined cerebellum, and with silver carbonate stain (Ibrahim *et al.,* 1968) for demonstration of microglia cells. The count

of microglia cells was performed using image j software (National institute of health, USA).

Statistical Analysis:

The data were analyzed by one-way ANOVA followed by Duncan post-test to compare the means when a significant difference ($p<0.05$) was detected by ANOVA. All the statistical analyses were done using SPSS program version 10 (SPSS, Richmond, VA, USA).

Ethical Statement:

All procedures and protocols were approved by the Faculty of Veterinary Medicine, Assiut University, Egypt.

RESULTS

As shown in Table (2), CAT and SOD activities significantly decreased in all experimental groups following dietary inclusion of MOS and BG prebiotic. A significant increase in GSH levels was observed following supplementation of broiler chickens with 0.5 g of prebiotic/kg diet, while there was no significant change in the CP levels of all treated groups. Administration of the prebiotic combination at doses of 4 g/kg diet succeeded in inducing significant decreases in O^{-}_{2} levels. LPO levels of supplemented groups exhibited a significant decrease at doses of 2 and 4 g/kg diet. Silver carbonate stained sections showed a gradual increase in the number of microglia cells at different agramus doses, but the number of microglia cells was significantly higher at dose of 4 g/kg diet than that in the control group.

Light microscopic examination of hematoxylin and eosin stained sections of cerebellum revealed no changes in the general structure of the cerebellar tissues (Fig. 1).

Table 2. The effects of dietary supplementation with mannan-oligosaccharide and β-glucan combination on the oxidant/antioxidant balance and number of microglia cells in the brain of chicken broilers exposed to natural summer conditions.

	Groups				
Parameters	Control	0.05%	0.2%	0.4%	*P* value
CAT activity (unite/mg protein)	0.862 ± 0.117^{a}	0.352 ± 0.089^{b}	0.461 ± 0.034^{b}	0.531 ± 0.0507^{b}	0.004
SOD activity (unite/mg protein)	2.344 ± 0.0246^{a}	1.776 ± 0.087^{b}	1.826 ± 0.153^{b}	2.016 ± 0.010^{b}	0.012
GSH level (µg/mg protein)	23.853 ± 1.339^{bc}	35.923 ± 2.259^{a}	28.681 ± 3.090^{ab}	17.725 ± 4.247^{c}	0.013
Ceruloplasmin level (µg/ml/mg protein)	156.502 ± 21.096^{a}	124.238 ± 12.506^{a}	146.470 ± 36.011^{a}	136.412 ± 16.089^{a}	0.787
O^{-}_{2} level (nmol/minute/mg protein)	0.353 ± 0.0547^{a}	0.430 ± 0.030^{a}	0.328 ± 0.024^{a}	0.171 ± 0.010^{b}	0.002
LPO level (nmol/mg protein)	0.470 ± 0.026^{a}	0.422 ± 0.019^{ab}	0.257 ± 0.016^{c}	0.388 ± 0.021^{b}	0.000
Number of microglia (215×10^{6} µm^{2})	12.5 ± 0.957^{b}	14.25 ± 0.854^{b}	13.75 ± 1.109^{b}	34.00 ± 7.616^{a}	0.000

CAT− catalase; SOD− superoxide dismutase; GSH− reduced glutathione; O^{-2} − superoxide radical; LPO − lipid peroxidation.

^{abc} − means with different superscripts in the same row are significantly different at $p<0.05$ (n = 6/group).

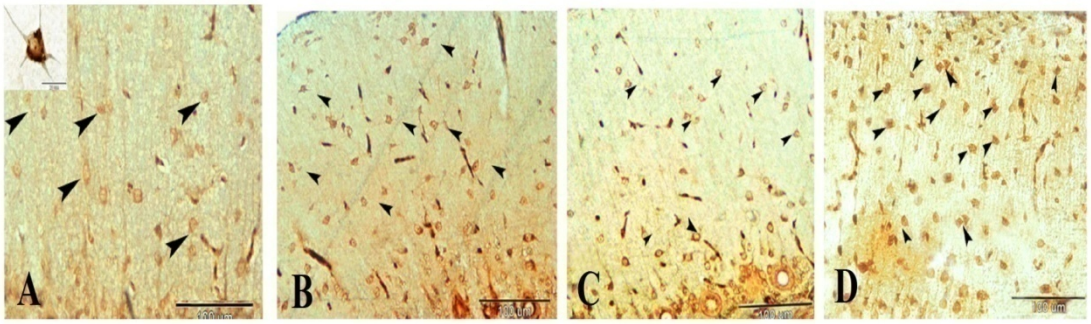

Fig.1: Distribution of micoglia cells stained with a silver method (arrow head) in the cerebellar cortex of control (A) and prebiotic treatment groups (B-D). (A): showing the normal morphology of microglia, they have spindly processes emanating from the central cell body (n = 6/group).

DISCUSSION

The major finding of this study was the ability of MOS and BG prebiotic to improve brain oxidant/antioxidant status and increase microglia count resulting in enhancement of the brain defensive mechanism against heat stress in chicken broilers. LPO is peroxidative end product of polyunsaturated fatty acids, and well-established as a valid indicator of free radical-induced oxidative stress. In our study, the significant decrease in the brain LPO following supplementation of broilers' diet with 2 and 4 g/kg prebiotic mixture is corresponding to previous reports on the antioxidant activity of MOS and BG (Haitao *et al.,* 2012; Kamel *et al.,* 2015). This finding may be attributable to the enhancement in the effectiveness of antioxidant defensive system leading to increase in resistance of neural cells to free radical attack with subsequent attenuation in the process of lipid peroxidation (Liu *et al.,* 2014). Binding free radicals to hydrogen atoms and chelating transition metal ions that act as a catalyst for free radical generation may be the main contributing aspects in the anti-peroxidative capacity of the examined prebiotics (Pan and Mei, 2010; Tsiapali *et al.,* 2001).

Marked decrease in O^{-2} in the current investigation as a result of dietary inclusion of 4 g/kg MOS and BG supplement is prominent evidence on its inhibitory effect on reactive oxygen species generation, and represents another causative factor in reduction of LPO. Broad spectrum of data had been emerged from *in vitro* experiments indicating the ability of MOS and BG in scavenging free radicals (Lei *et al.,* 2015; Maity *et al.,* 2015; Wang *et al.,* 2007). The high affinity to bind with iron, and the ability to trap oxidants and maintain mitochondrial integrity could be explanatory factors lying behind the free radical-chelating activity of BG (Faure *et al.,* 2015; Shaki and Pourahmad, 2012).

The obvious equipotent inhibitory effects of the three examined doses on CAT and SOD activities are in agreement with previous studies on BG (Aydogan *et al.,* 2013; Błaszczyk *et al.,* 2015; Ozkan *et al.,* 2010). However, literature is punctuated with controversial results regarding modulatory impacts of BG and MOS on the enzymatic antioxidant activities (Czech *et al.,* 2009; Kayali *et al.,* 2005; Lei *et al.,* 2015). Several confounding effectors are implicated in determining the antioxidant outcome response. The difference in linkage type, branching degree, molecular weight, and conformation of BG play key role in influencing the degree of antioxidant effectiveness (Kofuji *et al.,* 2012). In addition, the variability in the experimental design including dose and duration of dietary supplementation, incorporated food

vehicle, or studied model may be involved. Reactive oxygen species activate signaling pathways implicated in compensatory adaptation to oxidative stress by up-regulation of enzymatic antioxidants (Gomez-Cabrera *et al.,* 2008). Therefore, it is supposed that reduced reactive oxygen species production by supplementation of prebiotic additive in this study, as evident by decreased $O^{\cdot}_2$ and LPO levels, may result in corresponding decrease in CAT and SOD activities. These modulatory reactions may be considered as a sign of improvement in the brain redox state.

In this study, the dietary formula that contained 0.5 g/kg prebiotic combination induced a marked increase in the brain GSH levels of broiler. This finding is matched with the ability of MOS and BG to enhance glutathione redox system (Bolcal *et al.,* 2007; Pazhanivel and Balachandran, 2014; Sánchez *et al.,* 2011; Wang *et al.,* 2007). GSH, a major low molecular weight antioxidant in the cytoplasm, plays central role in improving the redox homeostatic status by sequestering free radicals and LPO and regenerating antioxidants as vitamins C and E (Carocho and Ferreira, 2013; Masella *et al.,* 2005).

The present study demonstrated that adminstration of prebiotics did not display histological alterations in the cerebellum; however, there was a marked increase in the number of the microglia cells with the highest dose. Mannose and dectin-1 receptors for MOS and BG, respectively, are expressed on microglia (Moran, 2004; Tsoni and Brown, 2008) raising the possibility of involvement of these ingredients in inducing microglial proliferation especially at the high doses. This suggestion is confirmed by *in vitro* study indicating the ability of chitooligosaccharide to inhibited apoptosis of microglial cell line N9 induced by oxidative injury of lipopolysaccharide through inhibition of reactive oxygen species generation (Zhang *et al.,* 2010). (Shah *et al.,* 2008) showed that BG activated microglia through dectin-1 signaling pathway without significant production of cytokines or chemokines.

In conclusion, the natural dietary combination of MOS and BG served as a potential candidate in enhancing oxidant/antioxidant balance in the brain of broiler chickens under natural Egyptian summer stress. Only the highest dose was enough to induce microglial proliferation in the cerebellar cortex. Further studies are highly recommended to investigate the underlying mechanistic pathways behind these findings, and explore other efficient nutritional strategies to overcome the adverse influences of heat stress especially on the brain region.

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ARABIC SUMMERY

تأثيرات مانان-أوليجوساكاريد وبيتا-جلوكان بريبيوتيك على إتزان المؤكسدات ومضادات الأكسدة في دماغ الدجاج اللاحم تحت ظروف الصيف المصرية الطبيعية

سهير محمد محمود رجب[١]، إلهام عبد الصبور عبدالله[٢]، ناصر سيد أبو خليل[٣]، فاطمة محمد عبد المقصود[٤]، أسامة طه محمد[٥]

١- قسم علم الحيوان - كلية العلوم - جامعة أسيوط
٢- قسم علم الحيوان - كلية العلوم بالوادى الجديد - جامعة أسيوط
٣- قسم الفسيولوجيا الطبية – كلية الطب – جامعة أسيوط
٤- قسم التشريح والأنسجة والأجنة - كلية الطب البيطرى - جامعة أسيوط
٥- قسم الصحة والأمراض المشتركة - كلية الطب البيطرى - جامعة أسيوط

أجريت هذه الدراسة لإكتشاف التأثيرات المحتملة للمانان أوليجوساكاريد والبيتا-جلوكان بريبايوتيك (أجراموس) على الإتزان التأكسدى والتشريح المرضي وعدد الخلايا الدبقية الصغيرة في دماغ الدجاج اللاحم المجهد بالحرارة. تم الحصول على مائة وستين من الدجاج اللاحم عمر يوم واحد من سلالة روس (١٦٨) من المفرخات المحلية. وقد بدأت التجربة عند ٢٨ يوما من العمر. بدأت التجربة عند عمر ٢٨ يوم، حيث تعرضت الطيور للإجهاد الحراري وتم تقسيمها عشوائيا لأربعة معالجات غذائية تحتوي على ٠ (المجموعة الضابطة) و٠,٥ و٢ و٤ جرام/كجم من المانان-أوليجوساكاريد والبيتا-جلوكان بريبايوتيك، على التوالي لمدة ١٤ يوما. وتألفت كل معاملة من ثلاث مكررات من ١٤ طائر لكل منها (أي أن مجموع الطيور لكل معاملة هو ٤٢ طائر). أظهرت النتائج إنخفاضا معنويا في أنشطة الكاتالاز والسوبرأكسيد ديزميتيز في جميع المجموعات المعالجة بالمانان-أوليجوساكاريد والبيتا-جلوكان بريبايوتيك. إضافة ٠,٥ جرام من البريبايوتيك لكل كجم من الوجبة أدى إلى زيادة معنوية في مستويات الجلوتاثيون، ولكن تم العثور على إنخفاض معنوى على الشوارد الفوق أكسيجينية فى جرعة ٤ جرام من البريبايوتيك لكل كجم من الوجبة. أظهرت مستويات بيروكسيد الدهون في المجموعات المغذاه إنخفاضا معنويا في جرعات ٢ و٤ جرام من البريبايوتيك لكل كجم من الوجبة. على الرغم من أنه لم يكن هناك تغييرات واضحة في البناء النسيجى للأنسجة الدماغية، كانت هناك زيادة معنوية في عدد الخلايا الدبقية الصغيرة واضحة بعد تناول ٤ جرام من البريبايوتيك لكل كجم من الوجبة. في الختام، يمكن إعتبار تناول المانان-أوليجوساكاريد والبيتا-جلوكان مرشحا واعدا للتخفيف من الآثار غير المرغوب فيها للإجهاد الحرارى على دماغ الدجاج اللاحم، ومع ذلك، هناك حاجة لمزيد من الدراسات للبحث عن سبل تغذية أخرى.