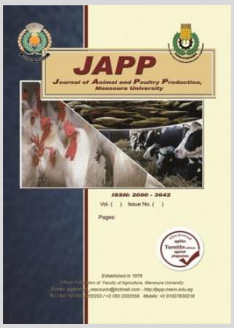
Journal of Animal and Poultry Production

Journal homepage: www.japp.mans.edu.eg
Available online at: www. jappmu.journals.ekb.eg

Determination of some Physiological and Immunological Characterisation as Dietary Biological Addition on Broiler Chicks

Tag El-Din, H. T.; Samar Rakha and K. H. El-Kholy*

Poultry Production Depart., Fac. Agric., Damietta Univ., Damietta, Egypt.



ABSTRACT

The purpose of the current study was to evaluate the effect of yeast "*Saccharomyces cerevisiae*" (SC) in comparison to sodium butyrate (SB) on some haematological and biochemical indices of broiler chickens. Therefore 270 one day old Hubbard broiler chicks were divided into 5 groups. The first group: chicks received basal ration without any treatment (control group, T1), the second group (T2): chicks treated with 0.2g SB/kg, the third group (T3): chicks treated with 0.3 g SB/kg; the fourth group (T4): chicks treated with 0.2 g SC/kg and the fifth group (T5): chicks treated with 0.3 g SC/kg. The obtained results showed that administration of SB or SC showed significant improved in blood picture. Inclusion of either SB or SC caused significant (P<0.01) increases in total antioxidant capacity, and the activities of anti-oxidant enzymes. Serum MDA level recorded a significant attenuate in SC groups when compared with SB and untreated groups. In conclusion, biological (SC and SB) additions can be used as one of the useful additive for enhancing the immunity and serum anti-oxidant enzyme activities of growing broiler chicks.

Keywords: Antioxidant enzymes; Hubbard; Immunoglobulines; Physiological; Sodium Butyrate, Yeast

INTRODUCTION

Upgrading the capacity of immunity to resist the illnesses of livestock without antibiotics would not as it were advantage the animal's health, welfare and productivity but it is additionally a vital strategy in endeavors to make strides the microbiological security of poultry products (Sejian *et al*., 2011; El-Kholy *et al*., 2018).

Most critically growth processes, especially in the 1st phase of growth, is distinguished by the generation of reactive oxygen species (ROS) due to cellular division and apoptosis (Surai *et al*., 2019). With the progressing of age, the dynamic deterioration of oxidative stress develops because of the expanded generation of free radicals (Khan *et al*., 2014). Typically since ROS are considered as the main mediators of oxygen cytotoxicity and as vital flag-bearers impulsion cell division and showing cellular signaling impacts (Buetler *et al*., 2004). Subsequently, it may be a challenging task to develop a system of ideal anti-oxidant addition to assist growing/productive birds keep up effective anti-oxidant defenses and redox adjust within the body. No doubt that, biological addition to appropriate diets could be necessary for poultry industry to achieve the best efficiency in both productivity and health (El-Kholy *et al*., 2012; Tag El-Dein *et al*., 2020). In addition, it had an inability to confer sufficient antioxidant protection against lipid peroxidation during the development stage of the chicks (Aluwong *et al*., 2013; Khan *et al*., 2014). One of the most popular probiotic products contain *Sacharomcyces spp* varieties and has long been fed to poultry, is *Saccharomyces cerevisiae* (SC) which produce from malted grains fermentation also known as "baker's yeast" (Khan *et al*., 2014). Dietary SC supplements significantly influence blood haematology and biochemical indices which could be utilized to evaluate the pathological and nutritional responses of broiler (Sun *et al*., 2020). A research by Haghighi *et al*. (2006) recommended that SC have an immune boosting effect due to improve acceptance of natural antibodies in chickens. In addition, probiotic addition has been appeared to balance the dynamics of antioxidants within body (El-Kholy *et al*., 2019). It encourages serum antioxidant enzyme activities in broiler chickens (Aluwong *et al*., 2013).

Sodium butyrate (SB) is a recently used organic acid in broiler chicken's diet for realizing optimum performance (Lan *et al*., 2020). Butyrate has the ability to adjust of the microflora in the intestine which can impact the bird's health (Candela *et al*., 2010). Also, SB is in this way known as antioxidant and antimicrobial; which together enhancing some physiological and immunological status of birds (Lan *et al*., 2020). In poultry production, organic acids have not picked up as much consideration as in swine production (Dehghani-Tafti and Jahanian, 2016).

Unfortunately, exceptionally small data is accessible concerning the impact of some-biological addition in broiler chickens. Hence, the current study was carried out to assess the impacts of dietary biological (SB or SC) addition on some physiological and immunological response of broiler chicks from 1 to 35 days of age. Our work extended also to make a comparative study between the effects of the SC as probiotic ad SB as organic acids in broiler diets.

MATERIALS AND METHODS

1. Ethics statement:

The current study protocol used in this study was approved by the Animal Care and Utilize Committee of Damietta University, Damietta, Egypt.

* **Corresponding author.**
E-mail address: khelkholy@du.edu.eg
DOI: 10.21608/jappmu.2020.108805

2. Materials:

The current study was conducted at a private-commercial poultry farm under supervision for Poultry Production Department, Faculty of Agriculture, Damietta University, Damietta Governorate, Egypt, during period extended from 26th of February 2019 till 2th of April 2019 to examine the effect of dietary addition of probiotic (*Saccharomycis cerevisiae*; "SC") and organic acids (Sodium Butyrate, "SB") on some physiological parameters of broilers.

3. Birds and Experimental design:

The 270 one day old Hubbard broiler chicks (males and females) with an initial body weight (IBW, g) of 47.43±0.16 g were supplied by commercial hatchery (El-Aml Hatching Company, Damietta). Chicks were exclusively weighed and relegated haphazardly to 5 equal experimental-groups of 54 birds each. Chicks/group were subdivided into 3 replicates of 18 birds each and reared in floor pens. All birds were kept under the same managerial conditions. Feed and water were offered *ad libitum* during the whole study period (1 to 5 weeks of age). The 1st group was fed basal diet (control; T1) while, the others fourth groups were fed the control diet with 0.2 and 0.3 g for each of SB and SC /kg diet. For each treatment group, both levels of SC and SB were added to the basal diet and subsequently mixed and stirred with a mixer. The composition and chemical calculated analysis of the basal diets are organized in Table 1.

Table 1. Composition and Calculated Analysis of Starter and Finisher Diets

Ingredients (%)	Starter (%)	Finisher (%)
Yellow corn	56.0	59.9
Soy bean meal (44%)	28.3	25.5
Corn gluten meal (60%)	10.0	08.5
Vegetable oil	01.5	02.5
DI-Calcium phosphate	01.7	01.7
Limestone	01.8	01.3
L-lysine	00.1	00.0
Salt (NaCl)	00.3	00.3
Vitamins & Minerals (Premix*)	00.3	00.3
- Total	100.0	100.0
Calculated analysis,**		
Crude protein, (CP, %)	23.06	21.10
Metabolizable energy, (Kcal/Kg)	3010	3106
Ether extract, (EE, %)	2.773	2.846
Crude fiber, (CF, %)	3.554	3.409
Calcium, (%)	1.143	0.949
Available phosphorus, (%)	0.469	0.463
Lysine, (%)	1.148	0.981
Methionine, (%)	0.55	0.52
Methionine + Cystine, (%)	0.855	0.789

***The premix at 0.30 of the diet supplies, the following per kg of the diet: A, 1000 I.U., Vit D3 2000 I.U., Vit E, 10 mg, Vit K, 1 mg, Vit B1, 5 mg, Vit B2, 5 mg, Vit B6, 1.5 mg, Vit B12, 0.01 mg, folic acid 0.35 mg, Biotin, 0.05 mg, Pantothenic acid 10 mg, Niacin 30 mg, Coline 250 mg, Fe, 30 mg, Zn, 50 mg, Cu, 4 mg and Se, 0.1 mg.**

****According to NRC, 1994.**

4. Management

The birds were housed in a very clean, well ventilated farm that already disinfectant and prepared for receiving birds for experiment. Birds were randomly housed in experimental pens (2 m×2.10 m×3 m) with stocking density 15/m^2.

The ambient temperature during brooding was 35°C±1 at one day old and gradually decreased to 25°C±1 on day 21 and then kept constant. The birds were subjected to A similar light schedule to commercial condition was used; 23 h light from one-day old until 7th day "to provide them sufficient time to discover out feed-and-water", then followed by 20h light from 8th day to the end experiment (35 days of age). Chicks were vaccinated with blend (IB Ma5+Newcastle clone,30) on day 7, and Gumboro,D78 vaccine at 14 days of age, and replicated at 21th days-age. At last, the chicks were vaccinated against Newcastle (live clone,30) at 28th days-age. The chicks were feeding on starter and finisher ration according to (NRC, 1994). Feed and water was provided *ad libtium*. The feeding trial was extended for 5 weeks of age.

5- Samplings, measurements and chemical analyses

A- Blood hematological parameters:

At 35 day of age, blood samples were gazared from 5 slaughtered birds/treatment in vial-tubes with EDTA. The haematocytometer, Cell-Dyn 3500 Hematology Unit (Abbott Laboratories, Abbott Park, IL, USA), was used to count the total white blood cells (WBC, $10^3/mm^3$); some differential of WBC's (monocytes and lymphocytes %) and blood quality parameters including red blood cells (RBC, $10^6/mm^3$), haemoglobin (Hb, g/dl), haematocrit (Ht, g/dl), mean corpuscular volume (MCV, μm^3), mean corpuscular Hb (MCH, µg), mean corpuscular Hb concentration (MCHC, g/dl) and red cell distribution width (RDW).

B- Antioxidant-enzyme activities and malondialdehyde concentration:

Serum malondialdehyde (MDA, nmol/ml) was evaluated by the methodology demonstrated by Janero. (1990), superoxide dismutase (SOD, U/L) activity was measured based on capacity of SOD to restrain the decrease of nitrobluetetrazolum superoxide (Martin *et al*., 1987). Also, serum glutathione (GSH, ng/ml) concentration, catalase (CAT, U/L) activity, and total antioxidant capacity (TAC, ng/ml) were measured through methods of (Simons and Johnson, 1978; Aebi, 1984; Koracevic *et al*., 2001, respectively).

C- Serum Immunoglobulins:

The concentrations of immunoglobulin G (Ig G, µg/ml) and immunoglobulin A (IgA, µg/ml) were evaluated in the serum by an ELISA reader (SIRIO S, Italy) with commercial test kits (Cusabio, rat immunoglobulin I & G, ELISA kit, China).

2.8. Statistical-analysis:

Data were expressed as mean±SE by one-way ANOVA with dietary treated addition as the main factor using statistical software of SPSS Ver. 25 (IBM SPSS, 2017) which used fixed model as follow: $Y_{ij}=\mu + T_i + e_{ij}$ ….where: Y_{ij} = Observation of the j^{th} rabbit in the treatment i; μ = Overall mean,; T_i = Effect of the treatments (i = 1, 2, 3, 4 & 5); e_{ij} = Random error component. Data which in percentages values were transformed to the corresponding arcsine before statistically analyzed (Warren and Gregory, 2005). A probability of $P \leq 0.05$ was required for statements of significance.

RESULTS AND DISCUSSION

1. Hematological measurements as affected by dietary biological addition:

The values obtained for all hematological parameters of broilers fed graded levels of either SC or SB in ration (Table 2) showed that Hb, Ht, WBC, RBC, MV, MCH, MCHC and RDW were within normal range (Mulatu *et al*., 2019). Hematological constituents reflect the physiological state of the animals to its internal and external environment (Cruz *et al*., 2018). The use of either SB or SC had no significant ($P>0.05$) effects on RBC for all treatment groups,

but differences between treatments were significant (P≤0.05) for Hb, Ht, WBC, lymphocytes, monocytes and MCHC. Significant increase was also noted in Hb, Ht, WBC and lymphocytes in SC groups compared to another groups and this is as in the findings of Ali *et al*. (2018) who demonstrated that the SC addition caused significantly increased in the WBC, Hb and Ht values of broiler. In contrast, the findings disagree with Djouvinov *et al*. (2005), who showed that the SC addition did not affect Hb. These differences may be credited to number and type of bacterial species present in probiotics. These indices could have contributed to the better performance of the broilers under dietary addition of SB and SC (Tag El-Dein *et al*., 2020). The SC can enhance of chick's immune system; so; it affects WBCs (Mulatu *et al*., 2019). Mohamed *et al*. (2015) detailed a positive relationship between dietary levels of SC with the haematological records like, RBC, WBC and Ht in broiler chickens. The values for MCV found in this study agree with the normal parameters considered by Cruz *et al*. (2018), who reported that the lack of change in this parameter may be related to good nutrition and the absence of challenges found in the places where the experiments are conducted. The previous studies would be clarified as the addition of either SC or SB in the basal diet might resulted in good absorption of Fe from the jejunum and better produce of vitamins B that influencing positively blood-cell forming processes as mentioned by (Mulatu *et al*., 2019).

Table 2. Blood haematology of broiler chicks as affected by dietary addition of yeast and sodium butyrate during the experimental periods

Items	Control	Sodium Butyrate(SB, g/kg)		Yeast (SC, g/kg)		Sig.
	(T1)	0.2 (T2)	0.3(T3)	0.2 (T4)	0.3 (T5)	
WBC (10^3/mm^3)	57.59^{a}±1.01	61.29^{b}±0.50	65.09^{c}±0.56	65.27^{c}±0.70	69.33^{d}±0.63	**
Lym (%)	30.51^{a}±2.97	34.23^{ab}±1.80	44.24^{bc}±4.05	41.14^{abc}±2.88	47.61^{c}±5.32	**
Mon (%)	15.14^{a}±0.65	15.62^{a}±0.65	16.27^{a}±1.69	17.40^{a}±0.84	21.06^{b}±0.65	**
RBC (10^6/mm^3)	2.61±0.06	2.74±0.08	2.62±0.03	2.62±0.09	2.87±0.09	NS
Hb (g/dl)	17.08^{a}±0.36	17.92^{a}±0.49	17.08^{a}±0.30	17.65^{a}±0.47	19.68^{b}±0.69	**
Ht (%)	28.83^{a}±0.60	30.10^{a}±1.04	29.04^{a}±0.73	29.85^{a}±1.11	34.69^{b}±1.93	**
MCV (µm^3)	106.95±4.64	109.67±1.33	110.83±2.33	113.67±1.76	118.33±2.76	NS
MCH (µg)	65.61±0.76	65.37±0.80	65.27±0.97	67.45±1.10	65.69±0.82	NS
MCHC (g/dl)	59.27^{a}±0.09	59.58^{ab}±0.59	58.89^{ab}±1.17	59.33^{ab}±0.88	55.65^{b}±1.02	*
RDW	10.38±0.36	11.19±0.53	10.68±0.34	10.88±0.27	11.80±0.58	NS

^{a,b,c,d}Means within the raw with different superscripts are significantly different Sig = significant; NS = non-significant; ** = (P ≤ 0.01); *= = (P ≤ 0.05)
WBC = White Blood Cell; RBC = Red Blood Cell; Hb = Hemoglobin; Ht = Hematocrit; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; RDW = Red blood cell distribution width

Blood serum antioxidant-enzyme activities and malondialdehyde concentration of broiler chickens as affected by dietary biological addition:

Results of serum antioxidant enzyme activities and MDA concentration of broiler chickens are appeared in Table 3. Significant (P≤0.01) differences were showed among the experimental groups in the blood serum antioxidant and MDA content. Blood TAC was significantly (P≤0.01) elevated by feeding different SB or SC diets than the control. Chicks fed diet contained 0.2 and 0.3 g/kg SC recorded a significant (P≤0.01) increment in TAC than other fed SB and the control group. The same slant was appeared in activities of SOD, GSH and CAT. The concentration of MDA recorded a significant attenuate in all treated groups when compared to the untreated one. There were significant (P≤0.01) effects occurred in MDA due to SC inclusion compared to SB and untreated groups. The percent of change were 47.5 and 55% for 0.2 and 0.3 g SC/kg, respectively than the control. Comparative findings have been showed by Al-Khalaifa *et al*. (2019) on treated broilers with dietary addition by SB and SC, respectively. Due to the rapid growth rate in new broiler line, the produce of ROS through the early life phase was natural produced (Aluwong *et al*., 2013). The ROS are considered as the major arbiters of oxygen cytotoxicity and as imperative delivery stimulating cell division and showing cellular signaling impacts (Buetler *et al*., 2004). Catalase activity, as well as the action of other antioxidant-enzymes, depends on the inclusion of antioxidants in the feed (Aluwong *et al*., 2013). The increase in TAC, SOD, GSH and CAT as well as the decrease in MDA in the present study may be credited to the sodium selenite division of the SC, which mostly upgrades antioxidative activity (Aluwong *et al*., 2013). In expansion, Kogan *et al*. (2008) recommended that SC cell wall β-glucans may have antioxidant characteristic. Also, dietary addition of SC led to significant (P≤0.01) decreased in MDA in current study may be credited to the probiotic failure to confer satisfactory antioxidant security against lipid peroxidation amid the development stage of the broiler chicks. With respect to SB, the insignificant (P>0.05) change in anti-oxidant indices by SB, including the amount of MDA, GSH, and antioxidative enzymes, its suggested that the mechanism by which butyrate enhances wound healing may not be fully due to the antioxidant stress (Song *et al*., 2017). These findings proposed that compared to other treatments, SC has articulated impact on improving the antioxidant status in broiler chicks.

Table 3. Serum antioxidants contents and MDA for broiler chicks as affected by dietary addition of yeast and sodium butyrate at 35 days of age

Items[1]	Control	Sodium Butyrate(SB, g/kg)		Yeast (SC, g/kg)		Sig.
	(T1)	0.2 (T2)	0.3 (T3)	0.2 (T4)	0.3 (T5)	
TAC (ng/ml)	0.07^{a}±0.01	0.06^{a}±0.01	0..06^{a}±0.01	0.17^{b}±0.04	0.19^{b}±0.03	**
SOD (U/L)	0.10^{a}±0.02	0.11^{a}±0.02	0.11^{a}±.02	0.20^{b}±0.02	0.22^{b}±0.03	**
GSH (ng/ml)	0.12^{a}±0.01	0.14^{a}±0.02	0.16^{ab}±0.02	0.20^{b}±0.02	0.20^{b}±0.02	**
CAT (U/L)	0.14^{a}±0.01	0.17^{a}±0.02	0.19^{a}±0.01	0.23^{b}±0.01	0.24^{b}±0.01	**
MDA (nmol/ml)	0.40^{b}±0.03	0.32^{b}±0.04	0.36^{b}±0.04	0.21^{a}±0.02	0.18^{a}±0.02	**

^{a,b,c}Means within the raw with different superscripts are significantly different Sig = significant; ** = (P ≤ 0.01)
TAC = total antioxidant capacity; SOD = superoxide dismutase, GSH = glutathione; CAT = catalase and MDA = malondialdehyde concentration

2. Serum Immunoglobulins

The serum IgA and IgG contents are demonstrated in Figure 1. On days 35, the IgA level was significantly (P≤0.01) increased in SC treated group (T5) compared to other treated groups. No significant difference was recorded in the IgA values between the control group and the SB groups (T2 and T3) and 0.2g SC/kg group (T4). On days 35, IgG levels were increased (P≤0.01) in broilers fed the SC (T4 and T5) as compared to broilers fed SB (T2 and T3) and untreated group (T1). Un-significant difference was evaluated in the IgG level between the control group (T1) and the SB addition groups (T2 and T3). The enhancements due to 0.2 and 0.3g SC/kg addition as compared to control groups were 35.6 and 47.3%, respectively. Comparative findings have been showed by Sun *et al*. (2020) and Al-Khalaifa *et al*. (2019) on treated broilers with dietary addition of SC and SB, respectively. On the opposite, Khobondo *et al*. (2015) decided that dietary probiotic addition didn't significantly affect the levels of IgM level. In addition, Mountzouris *et al*. (2010) demonstrated that SC inclusion to poultry diets had no impact on systemic humeral-immune response (IgA and IgG concentrations). The advantageous impacts of probiotics of boosting the immune system of broilers is due to enhanced acceptance of normal antibodies, as previously detailed (Haghighi *et al*., 2006). The mode of action for probiotics was demonstrated Makled (1991) via producing antibiotic substances, repressing harmful-bacteria, modifying-microbial metabolism, diminish intestine pH and impulsion the immune system. Our results also showed that the dietary addition with either SB or SC may increment the levels of serum IgA and IgG in broilers to higher levels than that in the control group. Thus, these results could be related to the role of these biological addition in enhance the immune function and reflect to better growth and health of the broilers.

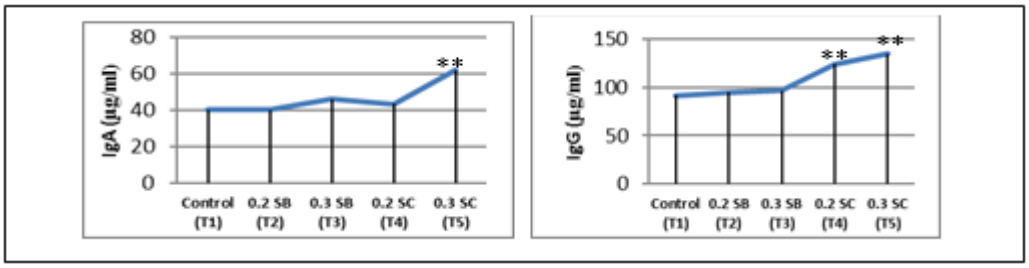


Figure 1. Effect of dietary addition of either yeast (SC) or sodium butyrate (SB) on the concentrations of immunoglobulins in broiler serum at 35 days. The asterisks (**) denotes significant differences (P≤0.01) compared with the control group, as analyzed by one-way analysis of variance (ANOVA) followed by Duncan comparison tests

CONCLUSION

It was shown that inclusion of a biological addition such as SC or SB in diet improved haematological and increased serum total antioxidant capacity and resulted in a pattern of reduced MDA concentration. Overall, this study provides evidence for the beneficial role of SC as a biological growth promoter with a potential to favor broiler chickens immune. Further research is required to assess SC and SB effects on gut microbiota indices under a variety of diet formulations combined or not with pathogenic challenge.

Competing Interests

The authors declare that they have no conflict of interest.

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تقدير بعض الخصائص الفسيولوجية والمناعية نتيجة للإضافات الغذائية البيولوجية لدجاج اللحم

تاج الدين حسن تاج الدين، سمر محمد رخا و خالد حسان الخولي*

قسم إنتاج الدواجن – كلية الزراعة – جامعة دمياط

إن الغرض من هذه الدراسة هو تقدير تأثير الخميرة في مقارنة مع بيوترات الصوديوم على بعض خصائص الدم الهيماتولوجية والبيوكيميائية في دجاج اللحم. استخدم 270 كتكوت هابرد عمر يوم وتم تقسيمهم إلى خمس مجموعات. المجموعة الأولى تم تغذيتها على علائق بدون إضافة (الكونترول)، المجموعة الثانية وفيها تم تغذية الكتاكيت على 0.2 جم بيوترات الصوديوم/كجم، المجموعة الثالثة وفيها تم معاملة الكتاكيت فيها على 0.3 بيوترات الصوديوم/كجم، المجموعة الرابعة وفيها تم معاملة الكتاكيت بـ 0.2 خميرة، أما المجموعة الخامسة فتم معاملة الكتاكيت بـ 0.3 خميرة/كجم عليقة. ومن النتائج المتحصل عليها أن المعاملة ببيوترات الصوديوم أو الخميرة أظهروا تحسن معنوي في صورة الدم مقارنة بالكونترول. كما وان تلك المعاملتين سببوا زيادة معنوية ($P<0.01$) في القدرة المضادة للأكسدة ونشاط الإنزيمات المضادة للأكسدة. كما أظهرت النتائج أيضاً انخفاض في مستوى MDA نتيجة المعاملة بالخميرة عند مقارنتها بالمعاملة بـ ببيوترات الصوديوم او الكونترول. وخلُصت الدراسة إلى ان الإضافة الغذائية البيولوجية (إما ببيوترات الصوديوم أو الخميرة) يمكن أن تُستخدم لتحسين وتحفيز الحالة المناعية ونشاط الإنزيمات المضادة للأكسدة لكتاكيت إنتاج اللحم.