Histopathological Alteration and Protein Markers Expression in Vaccinated Broilers Supplemented With *Saccharomyces cerevisiae* and Probiotic

Al-Ali. S.A.H and Hadeel B. Al-Sabaawy

1Nineveh Agriculture Directorate, Mosul, Iraq. sa2387749@gmail.com, 0000-0001-6462-8655
2Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul, Iraq. hadeelbasim2006@gmail.com, 0000-0001-8077-0854

SUBSTITUTE Antibiotics as known have strong effects on body tissue, these additive materials enhance host health condition, immunity status and increase body weight. Our study displays the effects of *Saccharomyces cerevisiae* (s. cerevisiae) and probiotic- in enhancing immune response of the host. Therefore, to explore the effects of alternative antibiotic on the histology and immunohistochemical expression on the immune organs of broilers immunized with Newcastle vaccines, sixty birds were divided randomly into 4 groups (T1-T4). T1 as a negative control, T2 immunized group with Newcastle disease virus, T3 supplemented with a mixture of *Saccharomyces cerevisiae* (3mg/kg) of body weight and probiotic (200g/100 litre of water) while T4 supplemented with a mixture of these materials and vaccinated with ND. Histological sections of thymus glands were taken at 7 & 35 days of the trial. Microscopic sections showed depletion, infiltration of inflammatory cell, hemorrhage as well as Hassal’s corpuscle appeared in different sizes in the cortex and medulla. CD68 proteins expression was severe at 35 days in contrast to 7 days that showed weak expression of these proteins’ markers in all treated groups. We concluded that administration of (S. cerevisiae) and probiotic can ameliorate the immunity.

**Keywords:** CD68, Immune stimulator, Histopathology.

Introduction

All living beings manage not only to survive but indeed thrive in potentially hostile milieu without seeming effort. The immunity system to disease depends on the existence of a complex of a highly sophisticated and inner immunity system called lymphatic system. Newcastle disease belongs to paramyxoviridae family [1], represented a serious peril on backyard chicken and poultry industry over the world and the spread of this illness may lead to severe trade restrictions on poultry products [2]. Histopathological changes following Newcastle disease virus infection includes infiltration of inflammatory cell, depletion of the lymphoid organs, degeneration and necrosis and the severity of these changes vary according to the strain, health condition of the host, immunity status and dose of the virus. *Saccharomyces cerevisiae* is one of the more probable microorganism by-products that can be utilized in ration of animals as a dietary additive [3]. Additionally, a viable substitute for feeding animals with antibiotic [4]. In chicken diets, oligosaccharides, which is generated from the outer cell wall of Saccharomyces cerevisiae can be used as a growing promoter and as alternative
to antibiotic [5]. Bread yeast is known to contain high level of mannose, non-starch polysaccharides and glucans yeast play important role in modifying the body immune function and response through interacting with a variety of immunological cell [6]. Moreover, the cell wall ingredient yeast, particularly beta glucans play a vital role to foster the immunity task through producing naturalistic antibodies in animals [7-8]. Probiotics have been classified by world health organization (WHO) as live microorganisms that, when administered in adequate amounts confer health benefits. Fibers of probiotics are conventionally known to act as substrates for probiotic synergetic bacteria, promoting their proliferation and activity, releasing of fatty acid chain and other metabolic processes in the gastrointestinal tract, providing further health benefits effects on body health condition [9]. Also, probiotics neutralize different types of enterotoxins [10], increase the activity of gastrointestinal tract–beneficial microorganism [11] and stimulate the cellular and humoral immunity [12]. Probiotics can act as anti-pathogenic material, with immunostimulant action such as stimulation of cytokines [13], development of antibody [14] and enhance expression of toll-like receptor (T-LR) [15]. The aims of the current work are to evaluate the effects of \textit{Saccharomyces cerevisiae} and probiotic on Thymus glands in broilers during immunization with Newcastle virus vaccines by using CD68 markers.

\textbf{Methods}

The experiment was performed at the animal house, Faculty of Veterinary Medicine-University of Mosul, after a period of time. The present study was approved by the committee of the ethics for the experimentation of animals at the University. All broilers were handled based on the aforementioned principles stated by EC[16] about safeguarding animals used in this paper and other scientific research purposes.

\textbf{Experimental Product}

The dose of Probiotic (Baymix® Grobegg™) used in the current work was 200g/100 litre of water based on the instruction of the manufacturers. The instant yeast (\textit{S. cerevisiae} 47.1×1010 (CFU)/g) was provided from the commercial market and used with 3mg/kilograms dose according to [17]. T1, T2 and T3 were vaccinated with (1000/10 litres of water) Lasota (Cevac NEWL) at 1,14, and 28 days of age.

\textbf{Trail design}

A total of 60 birds aged one day, weighting between 44±0.04 g were utilized for this experimental. Free access to feed and drinking water was given to the broilers. All birds were vaccinated with Newcastle disease virus at (1,14 and 28 days. Animals were divided randomly into 4 groups (T1-T4) with 15 replications for each group. T1 was a negative control, T2 positive control and immunized with ND, T3 supplemented with a mixture of these materials. T4 supplemented with a mixture of \textit{Saccharomyces cerevisiae} and probiotic and vaccinated with ND, thymus glands’ samples were taken at 7 and 35 days of the trail.

\textbf{Histopathological section:}

Formalin in a concentration of 10% was used to prepare and fix tissue samples. Fixed tissues were then routinely embedded in blocks of paraffin wax. A 4-5 µm section of Thymus glands from all broilers were stained and placed on poly-L-lysine slides for microscopic analysis using H&E stain, then these sections were assessed by Al-Sabaawy \textit{et al.} [18].

\textbf{Immunohistochemical analysis:}

IHC included the following steps dewaxing of the section in xylene, rehydration in a series of ethanol alcohol followed by washing in buffered saline and to suppress the activity of endogenous peroxidase, a solution with (3%) of hydrogen peroxides–methanol was added for 30 minutes, then blocked for 1 hour at 25 Celsius and incubated with primary Ab at 4 Celsius for 24 hours. The primary Ab utilized was CD68 rabbit monoclonal (in a dilution of1:200, kp1, China). After washing for 3 times, the sections were incubated with IgG for one hour at 36-37 Celsius and washed 3 times, a complex of an avidin–biotin was used for detection then stained with H stain for 1-hour, washed dehydration and covered by cover-slipped-slides. At last, sections were examined by a microscope and digital camera (19).

\textbf{Scoring of IHC}

Sections were examined under a microscope at higher power (400) µm to determination the immunoreaction. Positive expressions of CD68 were estimated the positive average and the staining intensity nucleus proteins expression of CD68 were recorded by three scales as a negative, mild, moderate and sever expressions [20].

\textit{Egypt. J. Vet. Sci. Vol. 54, No. 6 (2023)}
Results

Histological findings noted in various broilers Thymus glands were subjected to consideration. In T1 (negative control), predominant Thymus glands areas were visual including corte, medulla and lobule (Fig. 1). While the Thymus glands in T2 that immunized with ND showed depletion of the medulla and infiltration of inflammatory cells (Fig. 2). Meanwhile, T3 treated with a mixture of the materials and vaccinated with ND showed Hassall’s corpuscle which was more intensive in the week five in contrast to week 1 (Fig. 3). Sever infiltration of inflammatory cells was noted in T4 (Fig. 4).

IHC results

Estimations of CD68 levels in the Thymus glands of different broilers groups. Immunity organ represented by (Thymus glands) were stained with an antiCD68 markers, that appeared as brown dotes color inside the cells. Each cell in the Thymus glands of the 4 birds’ groups-T1, T2, T3 and T4 were estimated individually. As shown in T3 (Fig. 7) that showed severe positive reaction of the CD68 when confirmed to (5, 6 and 8) at week one but this marker expression was stronger in the same groups at week five (Fig. 11) when compared with the last groups (Figs. 9, 10 and 12).

Fig. 1. Histopath section, T1 group showed hemorrhage inside medulla (blue arrow) with infiltration of inflammatory cells (black arrow), H&E, 10X.

Fig. 2. Histopath section, T2 group showed depletion of medulla (black arrow) and inflammatory cells (blue arrow), H&E, 10X.

Fig. 3. Histopath section, T3 groups showed Hassall’s corpuscle (black arrow), H&E, 10X.

Fig. 4. Histopath section, T4 groups showed infiltration of inflammatory cells (black arrow), H&E, 10X.
Fig. 5. Histopath section, T1 groups, W1 showed (negative) expression of CD68, 400X.

Fig. 6. Histopath section, T2 groups W1, showed (mild) expression of CD68, 400X.

Fig. 7. Histopath. section, T3 groups W1, showed sever expression of CD68, 400X.

Fig. 8. Histopath. section, T4 groups W1, showed moderate expression of CD68, 400X.

Fig. 9. Histopath section, T1 groups W5, showed negative expression of CD68, 400X.

Fig. 10. Histopath section, T2 groups W5, showed mild expression of CD68, 400X.
Discussion

Immune stimulant material like *Saccharomyces cerevisiae* and probiotic has different beneficial impacts on human being and animals’ health condition; these agents are used as a feed supplement in farms’ livestock and broils [21]. Histological results in various broilers’ thymus glands were subjected to discernment and these lesions represented by hemorrhage, infiltration of inflammatory cells. In addition, depletion of medulla and cortex and these may be contributed to specific trail statues (length of the experiment, presence or absence of particular difficulties, stressful condition) as well as due to the difference in the components of these materials that are used as substitutes for antibiotics, live yeast containing (B-glucans and MOS) and the activity of these materials differs according to fermentation conditions, methods of cultivation and its substrates (breads, wine and beer) [22-23]. Emmanuel and his colleagues [2007] mentioned that using a complex of *Saccharomyces cerevisiae* and probiotic in diets of farm animals produced an immediate inflammatory response characterized by alteration in the expression of serum amyloid and lipopolysaccharides binding proteins. Emmanuel et al.[25] postulated this response due to lysis of pathogen by the *Saccharomyces cerevisiae* supplemented and priming of immunity.

The proportion of CD68, T cell and CD68 could be utilized to evaluate the immunity condition of the body, CD68 is a distinctive member of the class D-related scavenger receptors [26]. It is frequently serves as a macrophage indicator [27], additionally to macrophages, different immune cells can explicit this molecule like dendritic cells, basophils, mast cells and neutrophils [28] and in immunohistochemistry procedures it detected as a coarsely positive granular or a dot-like cytoplasmic staining [29]. CD68 stimulates immunity of the body, enhances B-lymphocytes to produce antibodies, improves the immunity function as well as plays an important role in proliferation and differentiation of T-lymphocytes. Expression of CD68 proteins was more stronger in the five weeks in contrast to the first week and this may be contributed to the type of alternative antibiotic, concentration, time of feeding. These results agreement with some authors[30,31] who mentioned that supplement of broilers with yeast increased the expression of CD4, CD8and CD68 in peripheral blood and in the epithelial lymphocytes, supplement of animals with these materials was useful in modulating the immune system and helped the animals against infection. Uses of *Saccharomyces cerevisiae* and probiotic enhance immune response particularly by eradicating pathogens via perforins and granenzymes. Additionally, boilers supplemented with these materials showed an increase in the expression of CD68 [32&33].

Conclusion

Over all, we concluded from our results that using of *Saccharomyces cerevisiae* and probiotic has clear effects on the immunity of the animal body and enhances its health. In addition, CD68 proteins expression was more sever in week 5 in contrast to week 1.
Acknowledgement
Non-applicable

Conflict of interest
We declare that no conflict of interest

Funding statement
Self funding.

References

Egypt. J. Vet. Sci. Vol. 54, No. 6 (2023)
