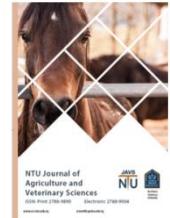




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## Effect of two types of oils on white blood cells function in broiler after Newcastle disease vaccine

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### A B S T R A C T

The Newcastle disease is an important disease problem that affects the Poultry industry because it causes large economic losses, many types of vaccines against Newcastle are widely used to provide protection. additives were used including flax seed and fish oils as food supplements and improved the immune system. This study aimed to estimate the effect of adding some essential oils (1% flax and fish oils) to the broiler diet on the white blood cell number and function after vaccination with the newcastle disease vaccine. A total and differential white blood count was done by using an automatic blood analyzer, while the phagocytic index and respiratory burst were used to detect its function. Our results show that there was an increase in total and differential white blood count in all treated groups but remained in their normal ranges in broiler, the heterophilia decreased, while lymphocytes increased with the processing of experiment time; the monocyte, eosinophilic, and basophilic percentages showed little response and reached up to 0.7, 4.1, and 0.2 %. phagocytic index and respiratory burst reveal a significant increase in all treated groups compared with the control. From our study, we conclude that the use of oils can improve total white blood counts as well as increase their ability to phagocytic and kill activity



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## Introduction

The importance of the poultry industry has risen in the last few years because it represents economic importance in the production of cheap protein [1,2]. the poultry industry sector faced many critical problems that affected its progress such as viruses or bacterial infections rather than management problems [3].

The Newcastle disease is one of the important disease problems that affect the poultry sector because it causes large economic losses in poultry farms due to a high mortality rate which reached 100% [4]. The virus that caused the disease belongs to the family of Paramyxoviridae, order Mononegavirales, genus Avula virus, which contains more than 10 Paramyxovirus serotypes that can cause broiler infection [5]. all these viruses are considered nonsegmented negative sense RNA enveloped helical viruses [6], many Newcastle infections can be caused in many farms regardless of the vaccination history [7,8]. Vaccines against Newcastle are used widely to give protection and prevent infection in many poultry farms around the world to raise the immune state of the birds [9].

Different additives are used nowadays to support the immune system of poultry and improve their responses to infection [10], many studies show that the use of essential oils additives can improve the immune system of broilers; the uses of flax seed oils and fish oils (omega-3) show many biological functions, such as improvement of broiler performance and improve the immune system as well as its effect as an antioxidant and decrease inflammation. [11,12,13,14], many researchers focus on the use of these oils in many broiler performances such as [15,16,17]; but few manuscripts focus on its effect on bird vaccine and immunity. so this study aimed to estimate the effect of adding some essential oils (flax and fish oils) to the broiler diet on white blood cell number and function.

## Materials and methods

### Study Duration and Ethical Approval:

The study extended from 5/11/2023 to 10/12/2023 all experimental animals were raised in Animals housed at the College of Veterinary Medicine/ University of Mosul. All Animal handling and all blood collection were done ethically according to procedures recommended by the Institution of Animal Care and Use Committee with certification number UM.VET.2023.068

### Animals:

the 240 one-day-old broiler birds were used in this study, clean water and feed were supplied to the animals, and all climate conditions were standardized as noted by [18].

### Vaccine :

Two types of Newcastle vaccine were used in this study first single subcutaneous killed Newcastle vaccine (MSD™, Netherland) was used to immunize the broiler at 0 days, then Live attenuation Newcastle clone 30 (MSD™, Netherland) was used to immunization of broiler at 7 days with drinking water; two booster dose was given at 14 and 21 days [19].

### Supplementary oils:

The effect of flax seed oils (al-Emad company©, Iraq) and Omega 3(Hat vet©, turkey) was used, and each of these oils was added to broiler feed at 1% concentration

### Experiment design:

The broilers were divided into eight groups (N=30) each group was treated as follow:

G1: Newcastle vaccine, no oils

G2: Newcastle vaccine, fed with 1% flax seed Oil

G3: Newcastle vaccine, fed with 1% Omega 3 Oil

G4: Newcastle vaccine, fed with 1% flax seed Oil and 1% Omega 3 Oil

G5: No vaccine, fed with 1% Omega 3 Oil

G6: No vaccine, fed with 1% flax seed Oil

G7: No vaccine, No oils were added

G8: No vaccine, fed with 1% flax seed Oil and 1% Omega 3 Oil

The blood samples were collected from the Jugular vein at 7,10,17,24 and 31 days after vaccination, blood was collected in a heparinized tube and transported quickly to the microbiology lab, College of Veterinary Medicine.

### Detection of total and differential white blood cells account :

The total and differential white cells account was done by using a blood analyzer (GeteinAnimals medical©,china).

### Phagocytic index :

the test was done according to the method proposed by [20].

### Respiratory Burst:

the test was done according to the method proposed by [21,22].

### Statistical analysis:

All results were analyzed with IBM SPSS Version 24, by using a T-test, one-way ANOVA test, and Duncan multiple ranges test to explain significant differences between groups [23].

## Results

The result of total white blood cells reveals that their number ranged between  $17.41 \times 10^9$  to  $52.9 \times 10^9$  cells which lie within the normal range of total white blood cells in chickens although there were

many fluctuations in number throughout the experiential period within groups Figure 1.

The heterophil and lymphocyte cells show opposite responses, The heterophil increased in percentage during the experimental time in all groups to reach a high percentage level on day 31 while the lymphocyte showed a lower level at the same time, Figure 2,3

Little response was shown with monocyte, eosinophilic, and basophilic percentages which reached up to 0.7, 4.1, and 0.2 % respectively in all the experimental times but remained in their normal range.

Our research shows a significant increase in phagocytic activity in all treated groups compared with the control Figure 4,5. farther than the increase in the phagocytic index are associated with a significant increase in a respiratory burst which reflected an increase killing ability of phagocytes in Figure 6,7

## Discussion

White blood cells are considered the first main line of defense against infection as well as their ability to mediate many immunological reactions leading to activation of both humoral and cellular immunity [24].

our results showed a metathetical increase in white blood cell number, but its number remains within the normal range of total white blood cells in chicken our Total white cell count results were similar to that recorded by [25,26,27] after feeding broiler with 10%, 100 % flex seed oils or using newcastle vaccine respectively. Also, The result of heterophil and lymphocyte cells showed opposite responses, The heterophil increased in percentage during the experimental time in all groups to reach a high percentage level on day 31 while the lymphocyte showed a lower level at the same time. The heterophilic result was similar to the result obtained by[26] when using 100% flex seed oils. We agree with [28] that the neutrophilia raised in numbers may result from the migration of these cells as a result of activation after using oils. While the concern of lymphocytes, the result obtained by us disagrees with the result obtained by [ 29,30,31] which reveals no effect or decrease in number after being treated with flex seed oils, omega3. we believe that the higher number recorded in lymphocytes in the first days of the experiment, may result from incomplete and ineffective immune responses caused by the Newcastle vaccine which are neutralized lastly by used oils.

Little response was shown with monocyte, eosinophilic, and basophilic percentages, but remained in their normal range which reflects little effect of used oils on these cells.

a significant increase in phagocytic activity in all treated groups compared with the control was seen in our study which comes in the same line with [32,33,34, 29]. the increase in phagocytic activity

may result from the effect of oils which alter the fatty acid cell membrane composition of phagocytic cells and increase their fluidity leading to altered cell membrane receptors and raising the phagocytic ability to adhesion and phagocytosis[ 35,36,33].

the increased phagocytic index is associated with a significant increase in a respiratory burst which reflects an increase killing ability of phagocyte cells this result comes in contrast with results reported by Res et al., 2006 when using corn oils or eicosapentaenoic acid [37], but [36] reported the similar result as we obtained. Many researchers believe that the type of fatty acid in oils affects the respiratory burst which leads to increased fluidity of cell membrane and modulates the pathway of signal transduction, gene expression, and transcription factor activation that result in enhanced membrane enzymes responsible for respiratory burst[38].

## Conclusion

we conclude that the use of oils can improve total white blood counts as well as increase their ability to phagocytic particles and increase their respiratory burst leading to improved killing activity.

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## Competing Interests

No competing interests were found.

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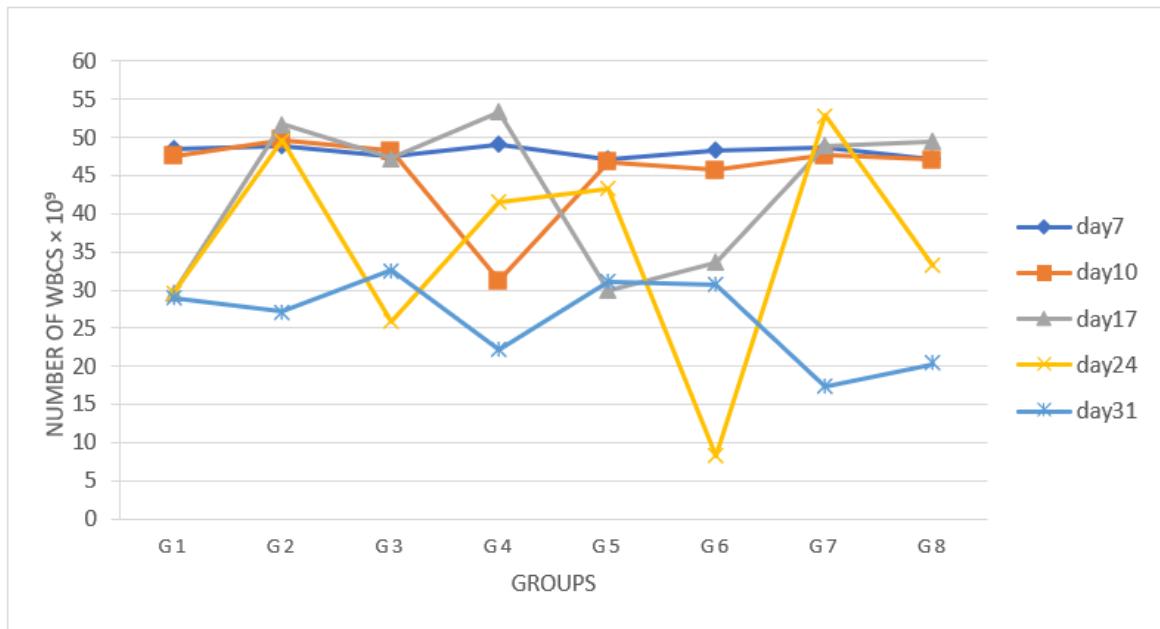
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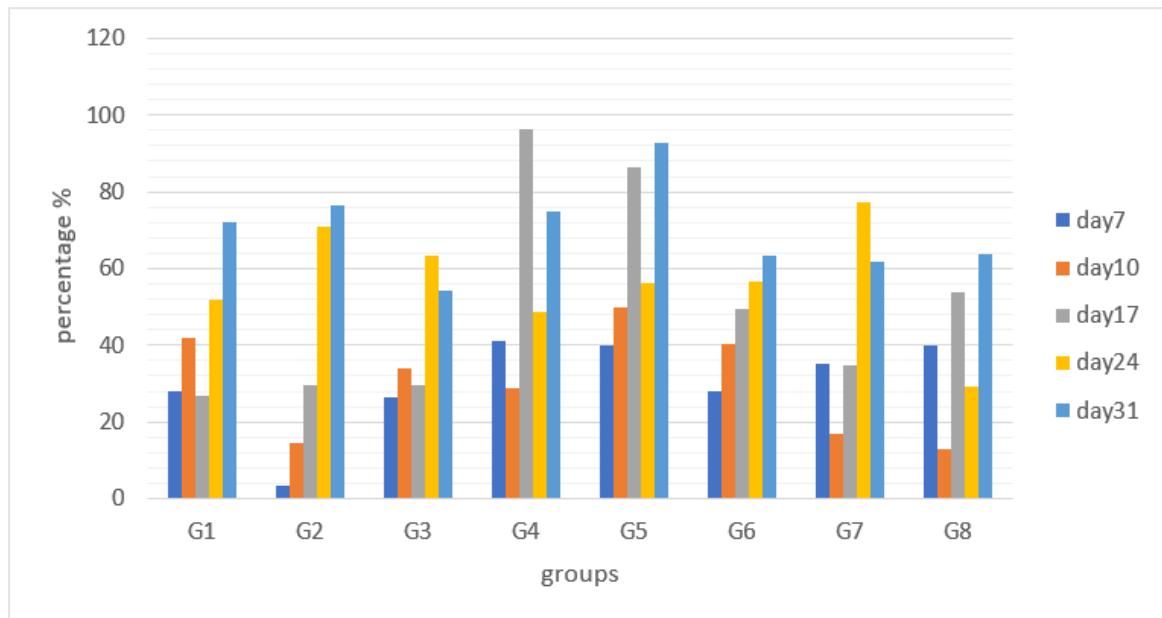
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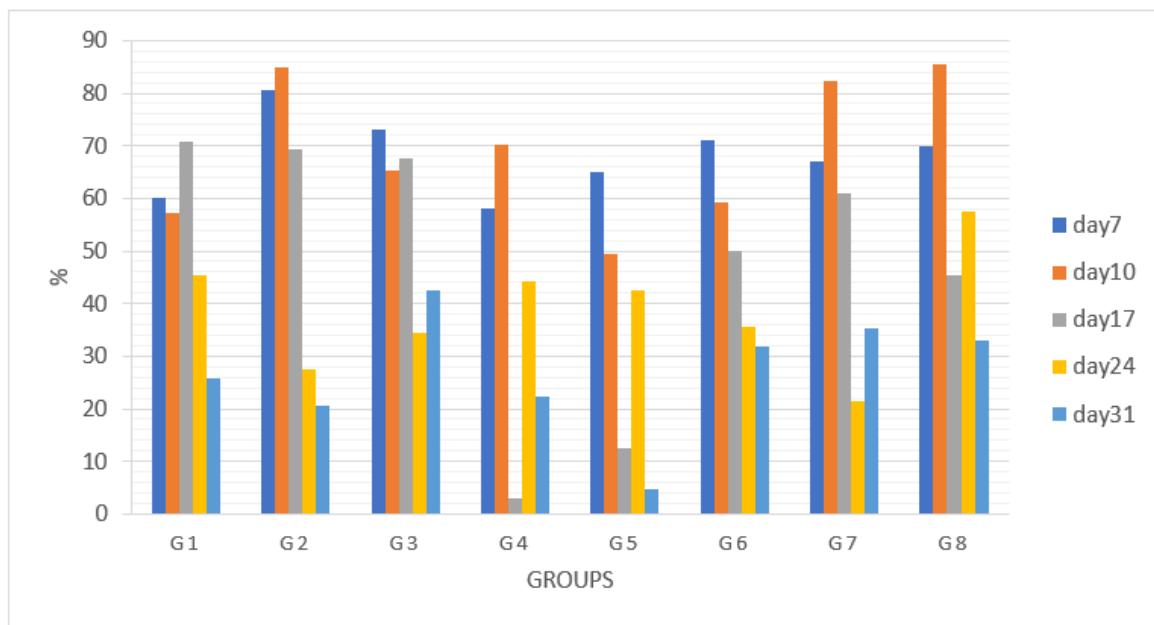
All numbers indicate mean  $\pm$  SD

**Figure 1.** Shows changes in total white blood cells account during the experimental study



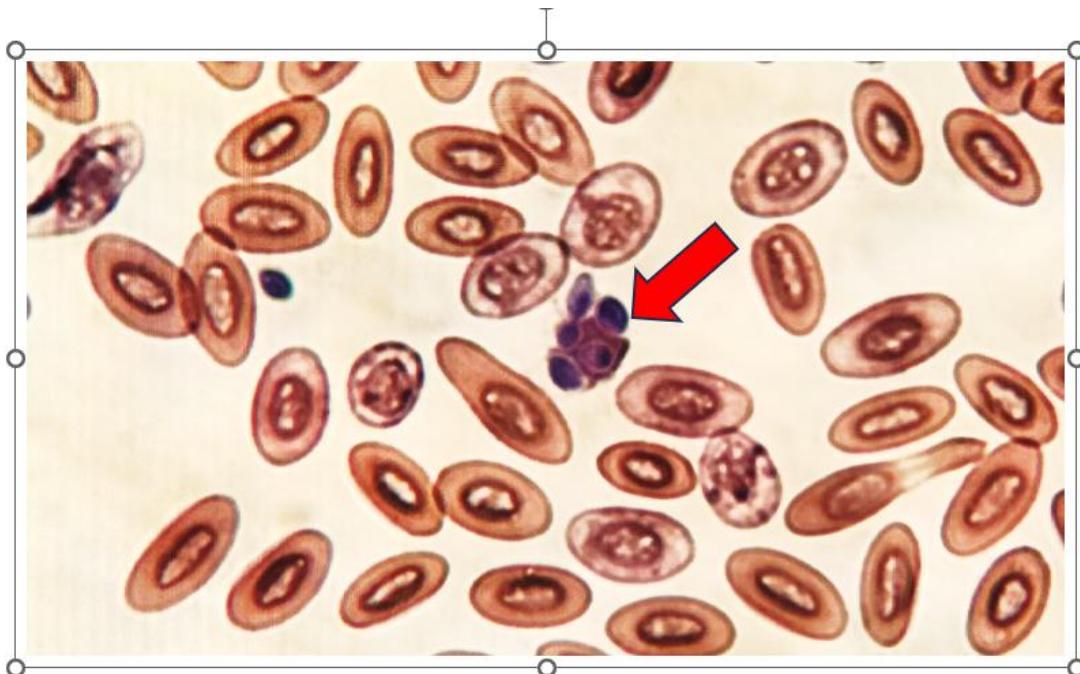
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**Figure 2.** Shows changes in Heterophila percentage during the experimental study

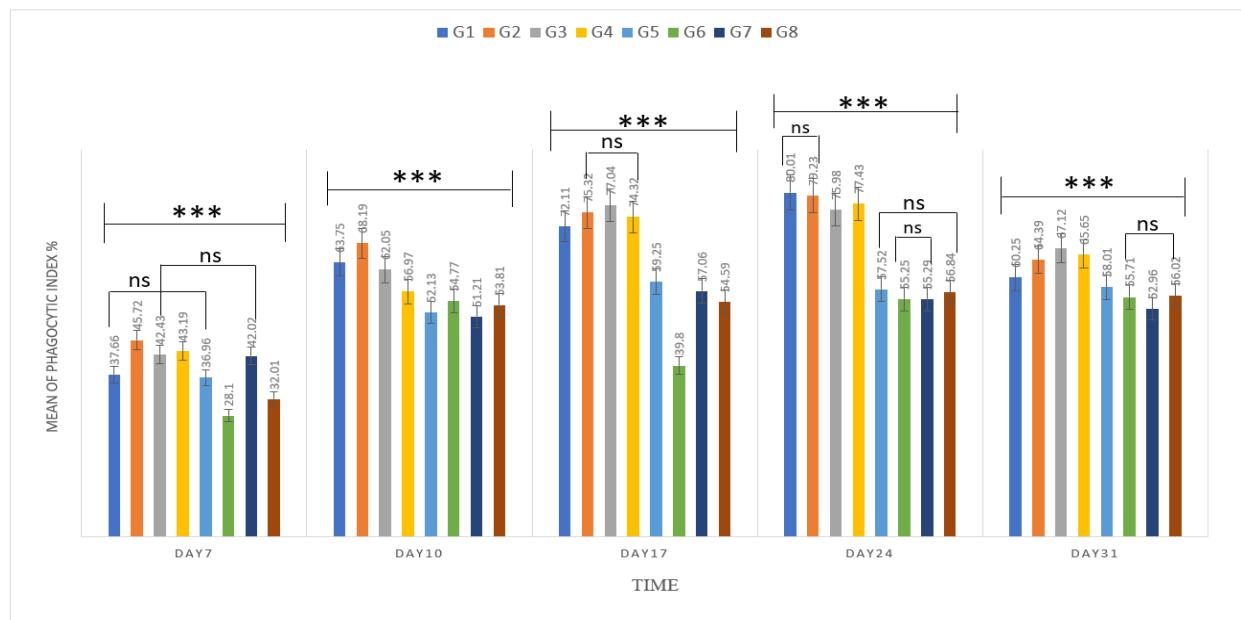


All numbers indicate mean  $\pm$  SD

**Figure 3.** Shows changes in lymphocyte percentage during the experimental study



**Figure 4.** Show the *Candida albicans* spore inside the cytoplasm of phagocytic cells ( red arrow) 100 $\times$

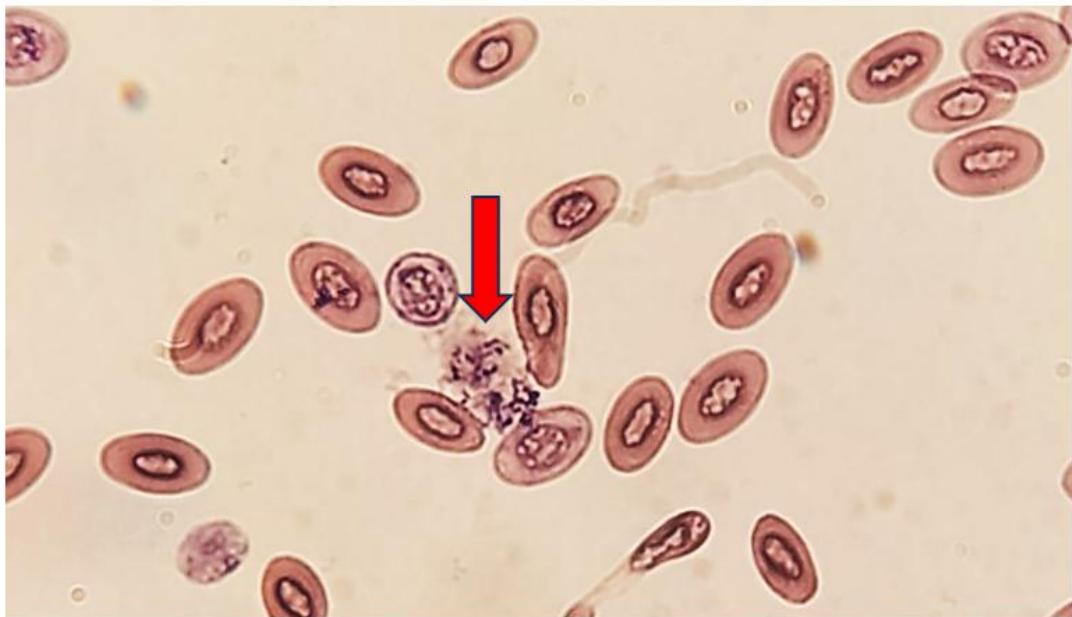


(\*) Indicate significant difference at  $P<0.05$

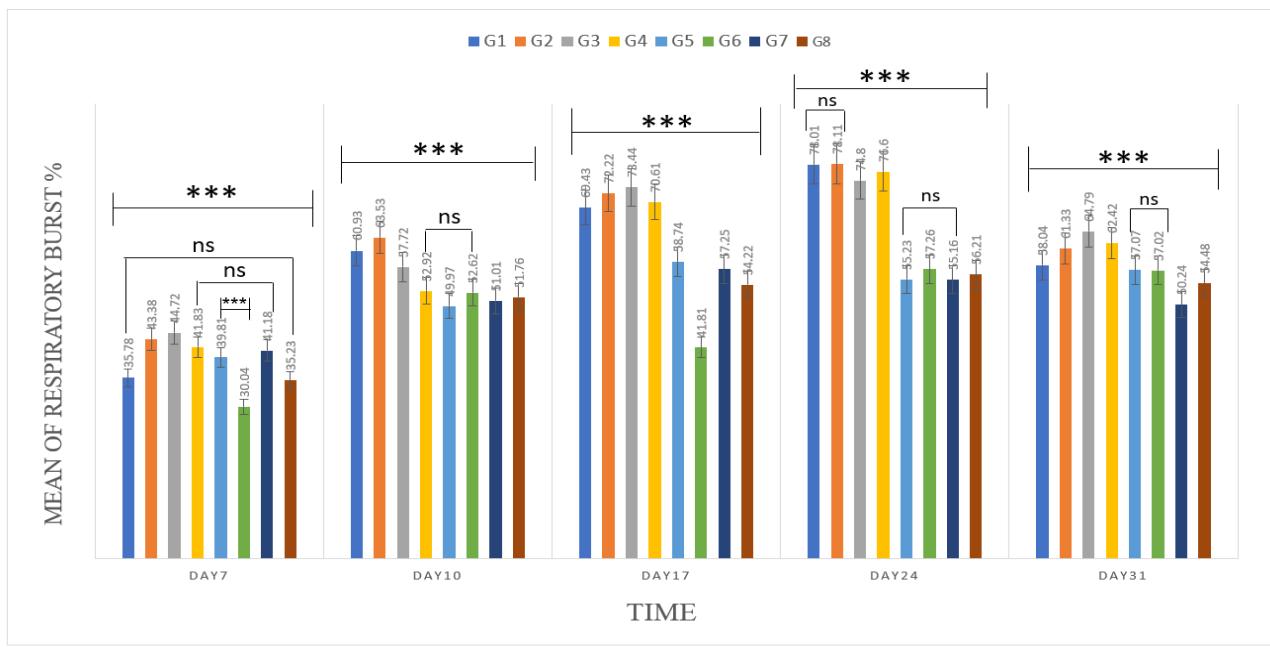
(ns) indicate no significant difference at  $P<0.05$

All numbers indicate mean  $\pm$  SD

**Figure 5.** The phagocytic index between the experimental groups



**Figure 6.** Show the formazan particles inside the cytoplasm of phagocytic cells (red arrow) 100 $\times$



(\*) Indicate significant difference at  $P<0.05$

(ns) indicate no significant difference at  $P<0.05$

All numbers indicate mean  $\pm$  SD

**Figure 7.** The respiratory burst between the experimental groups