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Impact of Spraying and Dipping Organic Acids on Hatching traits, Chick Quality, Immunity and eggshell bacterial count

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ABSTRACT

Organic Acids (BacterActive) spraying and dipping on hatching eggs were evaluated experimentally for their effect on embryonic mortality, hatchability, chick quality, immunity, and eggshell bacterial count. A total of 6300 hatching eggs of broiler breeder (Ross-308) at age 58 weeks, were collected and divided to 7 treatments as follows: (T1: General Control, T2: Dipping Water, T3: Spray Water, T4: Dipping and spraying water, T5: Dipping organic acid, T6:spray Organic Acid, T7:dipping-spray organic acid) Results indicated that using Organic acid (BacterActive) a synergetic blend of formic – propionic - lactic acids applied in a spraying manner with a concentration of 1ml/l significantly ($p \leq 0.01$) increased hatching traits, chick quality, and chicks' antibody titer against Newcastle Disease (ND) and Infectious Bronchitis (IB) also organic acid application significantly reduced embryonic mortality and eggshell total bacterial count in all treatments. while T6 showed the best result among all treatments that used organic acid and T5 showed the lowest value. among control group T3 had the best overall result while T1 shows least favorable result of all treatments. A special note to take in consideration is that dipping eggs in water T2 had least impact on embryonic death. In conclusion results showed that organic acids application via spraying, dipping, and dipping-spraying in broiler breeders leads to significant improvement of hatchability, chick quality and immune status while decreasing embryonic mortality and eggshell total bacterial count.

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INTRODUCTION

The utilization of natural occurring feed additives as substitutes for antibiotics in poultry production attracted many researchers and producers. Medicinal plants and other naturally available organic compounds consist of many pharmacologically active chemical compounds that works as antimicrobial, antioxidant, antifungal, antiviral, and anti-inflammatory substances as well as immune-modulatory properties (Sadh *et al.*, 2018). Also, herbals have received increased attention as possible antibiotic growth promoter replacements (Yazdi *et al.*, 2014). Organic acids are widely distributed in nature as they occur in animal, plant, and microbial sources. They contain one or more groups, which may be covalently linked in groups such as amides, esters, and peptides. An organic acid is a compound that has acidic properties. Carboxylic acids, whose acidity is related to their carboxylic group-cooh, are the most common organic acids (Broom, 2015). Considering the proposed mechanism of action of antibiotics and growth promoters (microbiome and immune-modulating activities), a practical alternative should possess both of these properties in addition to

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having a positive impact on feed conversion and/or growth (Kim et al., 2019). Several types of alternatives have been tested in poultry production, including organic acids, have come into existence in recent years (Gadde et al., 2017). Organic acids are being considered as one of the effective alternatives of the antibiotics in recent years because of their antimicrobial activity against wide range of pathogenic bacteria because of their ability to induce a PH reduction in the gut and these can improve nutrient utilization in poultry (Jadhao *et al.*, 2020). This fast development and use of antibiotics have resulted in many environmental issues like water, air, and soil contamination and pollution. Different types of antibiotics are being used in large amounts annually worldwide for the prevention, mitigation, and cure of diseases in poultry and livestock production (Muhammad et al., 2020). consumption of antibiotics is about 100,000 to 200,000 tons per year (Pareek et al., 2015). Because of public anxiety over antibiotic-resistant pathogens, the poultry industry started to avoid using antibiotics. The Swann committee of the European Union (EU) investigated the risk of bacterial resistance as a result of antibiotic use in poultry and livestock diets as early as late ten (Doeschate et al., 2006). The solution for these concerns is to identify replacements that have similar impacts as Antibiotic and growth promoters, such as decreasing the number of subclinical infections and their magnitude, decreasing bacteria's use of nutrients organic acid considered as one of these replacement of antibiotics (Dittoe et al., 2018).

MATERIALS AND METHODS

The study was carried out in TaqTaq poultry breeder farm and Hatchery project / Kosar company/Koya-Erbil, the trial started on 13/10/2021 to 17/11/2021., all eggs were incubated at this hatchery using all-out all-in peter-same uni-system. Bacterial culture was incubated agriculture college department of the food industry lab of Salahaddin University in the Kurdistan Region, Iraq. A total of Six thousand three hundred (6,300) eggs took from broiler breeders. A total of 6300 hatching eggs were collected from a 58wk-old Ross 308 broiler breeder flock. The collection of eggs was conducted 5 times throughout the day. Eggs that had visible fecal, litter, or egg contamination were not used for the experiment.

ORGANIC ACID PREPARATION

The organic acid solution concentrate that was chosen was (BacterActive). Which consists of a synergistic combination of organic acids (formic propionic lactic) and phytochemicals. The concentrated solution was diluted to a 1ml to 1000ml ratio by a small syringe before being used. Water was boiled to 100C to eliminate any bacterial or viral contamination from the final solution then let to be cooled down to 43 before adding concentrated Organic Acid (BacterActive).

TREATMENT PROCEDURE

Eggs were incubated for 18 days at (37.8 °C) and 55% relative humidity then taken out on the day of transportation to hatchery and set in a cleaned and disinfected room with 32°C to avoid prolonged heat-shock disposable gloves and clothes were used during the experiment. A normal sprayed water bottle and a big tub were cleaned and disinfected before being used to hold the diluted solution and water 42°C. During this transaction period, the eggs were prepared to be sprayed. Eggs were divided to 7 groups each group had 885 eggs with three replicates having 295 eggs. First group control C T1 had no treatment applied After the first group the second group T2 was sprayed with water only and then let them air dry. While third group T3 eggs were dipped in sterilized water. As for the fourth group, T4 eggs were dipped and sprayed with sterilized water. At the fifth group T5 the eggs were dipped in dilute organic acid 1ml organic acid to 100ml water ratio. Sixth group T6 was Sprayed with diluted organic acid. The final and seventh group T7 was dipped and then sprayed with diluted organic acid. After all the eggs were air dried and finished the experiment, the eggs were transferred to the hatchery for 3d at (37.25 °C) and 65% relative humidity until the end of 21 day of incubation. At the end of hatching all live and dead chicks were counted, the hatchability of set and fertile eggs, total embryonic mortality, culled chicks and chicks' weight were determined. Bacterial sampling of egg shell procedure was conducted by swabbing egg shells with clean swabs and then transferred to Xylose Lysine Deoxycholate agar (XLD). Blood samples were collected from chicks at 4 days old, each sample (4.0 ml) was collected after slaughtering 5 chicks from each replicate. The collected blood samples were centrifuged at 1000

cycles/minute for 20 min and the serum was decanted into aseptically treated vials and stored at -18°C then analysis of antibody titer of Newcastle Disease (ND) and Infection bronchitis (IB) disease measured by direct ELISA Synbiotics (Biocheck – ELX 800) at Kosar private company. All data were analyzed by using CRD (Complete Randomize Design) by SAS (SAS, 2005), significant differences among treatment means were determined by Duncan's multiple range tests at level 0.05 (Duncan, 1955).

RESULT AND DISCUSSION

Table 1 shows results of embryonic mortality percentage. Embryonic mortality overall in 15-21 days and 1-21 days was significantly ($p \leq 0.01$) lower when organic acid (BacterActive) was used as a solution compared to all control groups these findings agree with (Khan et al., 2016) and (Toosi et al., 2016). While T1 and T2 had significantly ($p \leq 0.01$) higher embryonic death rate in compare to other groups this is due to lack of protection of T1 and high moisture buildup of T2 group when dipped in water the fact that BacterActive penetrate the egg shell and aid egg nutrient digestion process had significant effect on embryonic morality. During 15-21 days period embryonic mortality was significantly lower in T6 group compared to other groups while T5, T7, T4, and T3 has significant lower mortality rate respectability compared to T2, and T1. With T1 having the highest mortality rate.

Table (1): Dipping and spraying hatching eggs with Organic acid (BacterActive) effects on Embryonic mortality

Treatments	Embryonic Death (%)	
	(15-21) days	Total (1-21) days
T1 Control	3.50±0.51 ^a	10.00±1.08 ^a
T2 dipping Water	3.33±0.35 ^a	10.00±0.92 ^a
T3 spray Water	2.00±0.21 ^b	6.67±0.47 ^b
T4 dipping-spray Water	2.00±0.25 ^b	5.34±0.45 ^{bc}
T5 dipping BacterActive	1.00±0.18 ^{bc}	5.00±0.33 ^{bc}
T6 spray BacterActive	0.33±0.09 ^c	3.33±0.26 ^c
T7 dipping-spray BacterActive	1.67±0.22 ^b	5.01±0.40 ^{bc}
S.L	**	**

a, b, c: mean within each column had the different subscript was differed significantly ($P < 0.01$). T1: Control (Non-Spraying, Non-Dipping). T2= Negative control dipping in water (18th of incubation). T3: negative control spraying in water (18th of incubation). T4: Negative control dipping and Spraying using Onley water (18th of incubation). T5: Dipping in Organic acid 1ml to 1000ml of water concentration (18th of incubation). T6: spraying with Organic acid 1ml to 1000ml of water concentration (18th of incubation). T7: Dipping and spraying with Organic acid 1ml to 1000ml of water concentration (18th of incubation). S.D.W: Sterilized distilled water - N.S: Non – Significantly.

Table (2) shows Egg dipping and spraying in Organic acid effects on the Hatching traits of broiler Chicks. Infertile eggs and fertility rate has no relation to the final results because the experiment was done on 18 days of incubation, results of each hatching of fertile eggs and hatching of total eggs were significantly ($p \leq 0.01$) increased in all groups that organic acids were applied compare with the control treatments, and highest percentage was in T6. The same results were also found by (Salahi *et al.*, 2011). The hatching of fertile eggs values indicate that T6 had the best result in terms of hatchability followed by T7 and T3 then comes T2, T4 and, T5 respectively with the lowest hatching of fertile eggs number taken by Control group T1 this variation in hatching rate is due to reduction in eggshell hardness as water applied on eggshell. higher hatching results are due to the combined effect of eggshell softness and organic acid prevention of microbial buildup as stated and agreed by (Lee *et al.*, 2015), and also E. coli fecal contents were reduced when organic acid capsules were fed to laying hens. the same lower hatching rate of Control and water dipped only treatments in comparison to organic acid-treated groups was observed by (Shafey, 2002).

Table (2): Impact Dipping and spraying hatching eggs with Organic acid (BacterActive) effects on Hatching traits

Treat-ments	Hatching traits (%)			
	Infertile eggs	Fertility	Hatching of fertile eggs	Hatching of total eggs
T1	14.75±1.78 ^a	85.25±3.11 ^c	86.46±3.51 ^c	75.41±2.78 ^c
T2	10.00±0.83 ^b	90.00±3.00 ^{ab}	92.00±3.66 ^{ab}	76.67±2.45 ^{bc}
T3	10.00±0.75 ^b	90.00±2.84 ^{ab}	90.74±2.94 ^b	81.67±2.33 ^b
T4	15.00±0.96 ^a	85.00±3.05 ^c	94.12±2.90 ^{ab}	80.00±2.65 ^b
T5	11.67±0.93 ^{ab}	88.33±2.33 ^b	94.34±3.11 ^{ab}	83.33±2.50 ^{ab}
T6	5.33±0.62 ^{bc}	94.67±2.45 ^a	96.54±3.50 ^a	88.67±2.15 ^a
T7	6.67±0.48 ^c	93.67±2.91 ^a	89.29±3.25 ^b	83.33±2.45 ^{ab}
S.L	**	**	**	**

a, b, c: mean within each column had the different subscript was differed significantly ($P < 0.01$). T1: Control (Non-Spraying, Non-Dipping). T2= Negative control dipping in water (18th of incubation). T3: negative control spraying in water (18th of incubation). T4: Negative control dipping and Spraying using Onley water (18th of incubation). T5: Dipping in Organic acid 1ml to 1000ml of water concentration (18th of incubation). T6: spraying with Organic acid 1ml to 1000ml of water concentration (18th of incubation). T7: Dipping and spraying with Organic acid 1ml to 1000ml of water concentration (18th of incubation). S.d.w: Sterilized distilled water - N.S: Non – Significantly.

Table 3 shows results of chick weight, Hight, and chick shank length Measurement Values after hatch directly the results indicate that all groups that organic acid (BacterActive) were applied had significant ($p \leq 0.05$) Higher Values than all control groups. Chick Weight was ($p \leq 0.05$) highest in T6 and T5 than all other treatments respectively followed by T7 while T3 and T4 had the same significant level with T3 having slightly higher weight with T2 with T1 having the lowest weight among all treatments respectively. It is noticed by (Khan and Iqbal, 2016) that weight gain is Increased by using organic acids, another study states this weight gain and distribution is due to lowered microbial load inside the eggs. Another study by (Adil et al., 2011) showed no significant weight gain when organic acid was used. As for chick length, all treatments that had organic acids applied showed significant ($p \leq 0.05$) results with the exception of T3 which had a significant chick length as for control groups T1 had the shortest chick length. Chick shank length was most significant in T6, T5, and T7 treatments followed by T3, T4, and T2 respectively with T1 having the lowest shank length Value.

Table (3): Impact Dipping and spraying hatching eggs with Organic acid (BacterActive) effects on Chicks Measurements

Treatments	Chick quality		
	Chick weight (g)	Chick length (cm)	Chick's shank length (cm)
T1	44.2±1.48 ^b	17.5±0.89 ^b	2.17±0.38 ^b
T2	45.1±1.40 ^b	18.4±0.73 ^{ab}	2.40±0.33 ^{ab}
T3	46.2±1.33 ^{ab}	19.2±0.85 ^a	2.46±0.29 ^{ab}
T4	45.4±1.39 ^{ab}	18.6±0.71 ^{ab}	2.45±0.33 ^{ab}
T5	47.3±1.25 ^a	19.4±0.69 ^a	2.64±0.27 ^a
T6	48.4±1.32 ^a	19.8±0.65 ^a	2.73±0.23 ^a
T7	46.5±1.40 ^{ab}	19.5±0.77 ^a	2.60±0.25 ^a
S. L	*	*	*

a, b, c: mean within each column had the different subscript was differed significantly ($P < 0.05$). T1: Control (Non-Spraying, Non-Dipping). T2= Negative control dipping in water (18th of incubation). T3: negative control spraying in water (18th of incubation). T4: Negative control dipping and Spraying using Only water (18th of incubation). T5: Dipping in Organic acid 1ml to 1000ml of water concentration (18th of incubation). T6: spray with Organic acid 1ml to 1000ml of water concentration (18th of incubation). T7: Dipping and spraying with Organic acid 1ml to 1000ml of water concentration (18th of incubation). S.d.w: Sterilized distilled water - N.S: Non – Significantly.

The results in Figure (1) illustrated antibody titer against (ND) and (IB). Results showed a positive effect of Organic Acids (BacterActive) on stimulating chicks' immunity by significantly ($p \leq 0.01$) increasing antibody titer against (ND) and (IB) in all three groups where (BacterActive) was applied had higher antibody responses with significant ($p \leq 0.01$) increased in antibody titer compared with other control groups. This was consistent with previous studies, which demonstrated that the use of organic acids enhanced specific immune responses in broilers and laying hens (Ma *et al.*, 2021). Chick antibody titer was significantly ($p \leq 0.01$) highest in T6 than in all other treatments groups that organic acid used followed by T6, T7 respectively, while T3 and T4 had the same significant level with T3 having a slightly higher response than T2 with T1 having the lowest antibody titer response among all treatments respectively. It is noticed by (Özek *et al.*, 2011) that only when organic acid is added to essential oils the antibody titer rises in broiler breeder hens which agrees with the current results. another study (Marín-Flamand *et al.*, 2013) showed that the immune response to a blend of organic acid had no significant effect on antibody titer.

Figure 2 shows that all treatments had a high TBC which is naturally due to bacterial contamination from eggs contact with soil during collection. while (BacterActive) solution had a significant influence on total bacterial count TBC compared to control groups. The groups with the lowest TBC have been observed to treatment T5 and T7 Organic acid reduced the bacterial count by 2 folds compared to the control group. Results show that as the exposure to an organic acid (BacterActive) increased in T5 (dipping) and T7 (spraying-dipping) eggshell bacterial had lower rates of development this agrees with (Fascina *et al.*, 2017) statement that using organic acid reduced the effects of heat stress and didn't allow further bacterial growth on eggshell in compare to control groups, while only spraying organic acid (BacterActive) reduced total bacterial count. Eggshell TBC was significant in T1, T2, T3, and T4. use of natural organic acid solution had a significant influence on TBC according to (Ahmed *et al.*, 2019) and (Adhikari *et al.*, 2020).

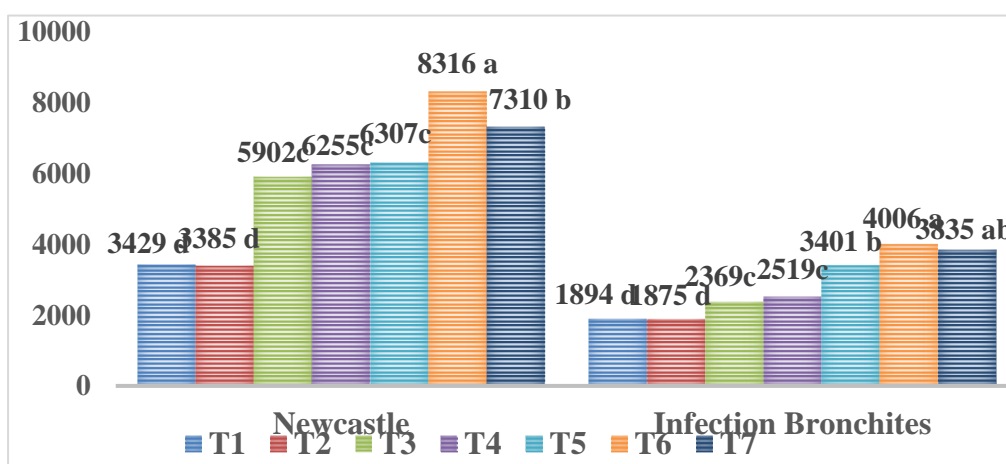


Figure (1): Impact of spraying and dipping hatching eggs with organic acid (BacterActive) in immune response

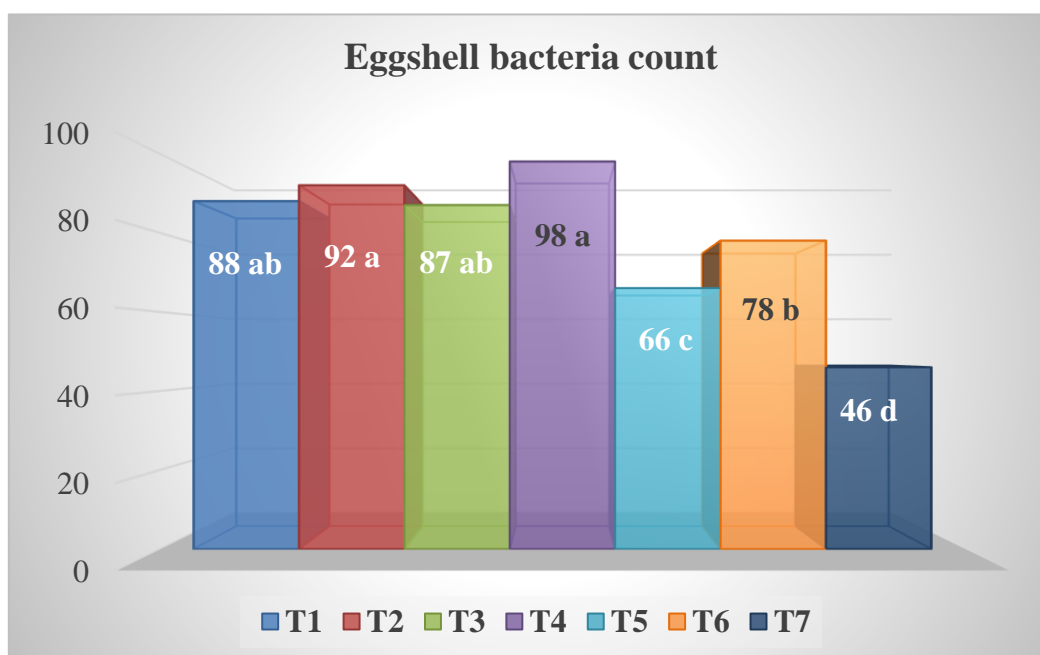


Figure (2): Impact of spraying and dipping hatching eggs with organic acid (BacterActive) in total bacterial count. total bacterial count

a, b, c: mean within each column had the different subscript was differed significantly ($p \leq 0.01$). **T1**: Control (Non-Spraying, Non-Dipping). **T2**= Negative control dipping in water (18th of incubation). **T3**: negative control spraying in water (18th of incubation). **T4**: Negative control dipping and Spraying using Only water (18th of incubation). **T5**: Dipping in Organic acid 1ml to 1000ml of water concentration (18th of incubation). **T6**: spray with Organic acid 1ml to 1000ml of water concentration (18th of incubation). **T7**: Dipping and spraying with Organic acid 1ml to 1000ml of water concentration (18th of incubation). **S.D.W**: Sterilized distilled water - **N.S**: Non – Significantly.

CONCLUSION

Using Organic acid showed significant improvement of hatching trait, chick quality, immune response, a significant reduction of embryonic deaths and total bacterial count on eggshell compared to control treatments. Using Organic as solution for spraying and dipping broiler breeders' eggs showed promising embryonic survival rate, hatching trait, chick quality, immune response and total bacterial count on eggshell in contrast to control treatments. Spraying organic gave the best results at increasing Hatching of fertile eggs, antibody titer responds to (ND) and (IB) and lowered embryonic mortality however it didn't reduce eggshell total bacterial count as the other treatments. This shows that organic acid can be used in addition to other means to increase the production and sustainability of a hatchery by reducing embryonic mortality, total bacterial count before hatch while giving better starting weigh and immune response to birds after hatch.

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تأثير رش و تغطية الأحماض العضوية (BacterActive) على صفات الفقس وجودة الافراخ والمناعة والعد البكتيري في قشر البيض

نضال عبدالغني مصطفى

قيس نوزاد عمر

كلية علوم هندسة الزراعة/ جامعة صلاح الدين- أربيل/ العراق

الخلاصة

أختبر تجريبيا تأثير رش وتغطية بيض التفقيس بالأحماض العضوية (BacterActive) في الهلاكات الجنينية وقابلية الفقس وجودة الافراخ والمناعة والعد البكتيري لقشر البيض. حيث تم جمع 6300 بيضة من أمهات التسمين (روس 308) بعمر 58 أسبوعًا ، وتم تقسيمها إلى 7 معاملات على النحو التالي (T1: السيطرة العام، T2: تغطية بالماء، T3: رش بالماء، T4: التغطية والرش بالماء، T5: تغطية بالحامض العضوي، T6: رش بالحامض العضوي، T7: تغطية و رش بالحامض العضوي) أشارت النتائج إلى أن استخدام الحامض العضوي (BacterActive) المزيج التآزري المكون من أحماض الفورميك - البروبيونيك - اللاكتيك بطريقة الرش بتركيز 1 مل / لتر ادت الى زيادة معنوية ($p \leq 0.01$) في صفات الفقس ، عدد الأجسام المضادة لافراخ ضد مرض نيوكاسل (ND) و التهاب الشعب الهوائية المعدية (IB) ، كما أدى الحامض العضوي إلى تقليل الهلاكات الجنينية والعد البكتيري لقشر البيض بشكل كبير في جميع المعاملات بينما أظهر T6 أفضل نتيجة بين جميع المعاملات التي استخدمت الحامض العضوي وكانت T5 أقل نتيجة . ولوحظ ان تغطية البيض في T2 كان له اقل تأثير على الهلاكات الجنينية، أما بالنسبة لمعاملات السيطرة فكانت T3 أفضل نتيجة بينما أظهرت T1 أقل نتيجة لجميع المعاملات ، حيث أوضحت النتائج أن تأثير الأحماض العضوية عن طريق الرش والتغطية والتغطية مع الرش في بيض التفقيس ادى إلى تحسين قابلية الفقس وجودة الافراخ و المناعة مع تقليل الهلاكات الجنينية والعد البكتيري في قشر البيض. ملاحظة خاصة يجب أخذها في الاعتبار هي أن غمس البيض في الماء T2 كان له أقل تأثير على موت الجنين.

الكلمات المفتاحية:

بكتريا اكنف (BacterActive)، التغطية، الرش، مناعة، فقس، عد بكتيري.