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# INDOOR AIR QUALITY OF COMMERCIAL BROILER FARMS AFFECTING HUMERAL IMMUNE RESPONSE AGAINST FIELD IBV VACCINE

By

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## ABSTRACT

The current work was established to assess the impact of indoor air quality (IAQ) (climate elements Ta.C, RH %, and AV m/sec. and ammonia ppm) on humeral immune response (HIR) against infectious bronchitis vaccine (IBV) in cool and non-cool seasons in broiler farms. Field investigation was carried out on available16 commercial broiler farms in which chicks are apparently healthy and some manifesting respiratory symptoms. An established questionnaire was used for recording management, biosecurity indicators and level, vaccination programs and history of respiratory symptoms during cool and non-cool seasons. On-site measuring of IAQ from different sites in farm. Random blood samples were collected for serum preparation to evaluate (HIR) using ELISA kit against IBV.

The collected data were subjected to statistical analysis.The results revealed that, both of Ta.C and AVm/sec. revealed a high significant difference in mean values between cool and non-cool seasons. RH% means value was lower in cool compared with non-cool seasons. In non-cool seasons the indoor climate elements revealed significant correlations between each other basically RH% with both Ta.C and AV m/sec.The recorded significant correlations were clarified the impact of indoor RH% and AV m/se .on broiler HIR. The mean values of AV m /sec increased in both seasons than recommended. Cool seasons showed lower Ta.C, RH% and AV m/ sec with higher ammoniaVs. non-cool seasons. A higher significant difference in means value and mean differences of Ta.C and air velocity recorded in cool seasons than non-cool ones. Decreased means value of antibodies titer against field vaccine or infection was reported in cool seasons compared with non-cool seasons.

The decreased HIR titer in cool seasons was associated with lower Ta.C, RH% and air velocity but increased ammonia Vs. non-cool seasons. A high significant difference in mean values of IBV antibodies titer was noticed between cool and non-cool seasons.

The recorded CV% and vaccination index (VI) revealed that, the lowest CV was in 3/15 of farms **(**20%) with the highest antibodies titer .The lowest VI was associated with the highest CV% and the lowest antibodies titer were recorded in 5/15 of farms (33.3%). Conclusion, seasonal impact was clarified via significant correlations between climate elements each other (RH% with both Ta.C and AV m/sec.) in non- cool seasons. In cool seasons, decreased titer was associated with lower Ta.C, RH% and AV m/sec. The lowest VI with the highest CV% and the lowest antibodies titer were recorded in 5/15 of farms (33.33%). The seasonal climate elements and indoor air ammonia impact were entangled with recorded field applied biosecurity.

**Keywords:**

Indoor air quality IAQ, climate elements, coefficient of variance CV %, seasons, humeral immune response HIR, and vaccination index VI.

## INTRODUCTION

Avian infectious bronchitis (AIB) is an acute and highly contagious viral disease of the respiratory system in chickens results in high mortality and poor weight gain in broilers **(Butcher *et al.*, 2002; Cavanagh and Naqi, 2003).** The Egyptian poultry industry in recent years has observed an increasing incidence of respiratory pathologies related to infection with infectious bronchitis virus (IBV) in vaccinated and non-vaccinated flocks that caused severe economic losses **(El-Mahdy *et al.*, 2010).** Co-infections of avian respiratory viruses including IBV may induce similar clinical signs or lesions and thus complicate diagnostic decisions, as well as complicating its control **(Nguyen *et al.*, 2013).**

The accurate estimation of risk factors associated with IB disease events is critical to the determination of the benefit–risk ratio and the most cost effective use of preventive therapies **(Tonkin *et al.*, 2003).** Climatic variation is a key threat to the poultry industry, especially for marginal poultry farmers in open-house systems. Poultry of different breeds and ages react differently to climatic variations **(Alade and Ademola, 2013).** Adequate ventilation rates provide the most effective method of controlling temperature within the hen-house. They allow for controlling the relative humidity and can play a key role in alleviating the

negative effects of high stocking density and of wet litter **(Bianchi *et al* ., 2015).** Increased detection of IBVs during cool weather could be impacted by many factors, including improper farm ventilation and litter management as well as inadequate biosecurity measures and natural air movements between farms **(Abdel-Moneim *et al.*, 2012).** The occurrence and severity of the disease is dependent on factors related to the virus, host and environment. In spite of constant efforts to control the disease by use of live and inactivated vaccines, the virus continues to cause high production losses, poor health and raises welfare concerns. In IBV infected flocks, the morbidity rate can reach 100% but the mortality rate depends on the presence ofsecondary infections, flock age,immune status,management and environmental factors**(Awad *et al.*, 2014).** Broilers exposed to 75 ppm of NH3 for 20 days experienced an increase in the production of reactive oxygen species (ROS), down regulation in the immune response proteins responsible for antigen recognition and presentation, and up-regulation in the proteins involved in muscle contraction and mucin production. It remains unknown whether NH3 impacts immunity in the context of vaccination **(Xiong *et al.*, 2016).** The detection rate of IBVs was highly influenced by sampling season. While a very low detection rate was observed during summer months, the high incidence rate of IBVs was observed during winter (reaching 100%), followed by autumn and spring. With no doubt, enhancing biosecurity measures, regular IBV monitoring and updating the vaccination schemes are crucial components to control IBV infection in commercial chickens in Egypt, and perhaps elsewhere in the region **(Setta *et al.*, 2018)**. Ammonia concentrations in the air are higher in poorly ventilated houses and appear to coincide with the elevated incidence of respiratory disease occurring during the winter months**.** Ammonia exposure was associated with an increased incidence of airsacculitis among non-vaccinated, challenged birds. Vaccinated, NH3-exposed birds were completely protected from IBV challenge. Ammonia had subtle effects on cilia morphology and function but did not affect vaccine or challenge virus replication and clearance, clinical signs, ciliostasis, tracheal histopathology scores, or immune responses **(Astona *et al*., 2019).**

IBV can be recovered routinely from the trachea and lungs, between days one and seven post infection, in considerable titers. For surveillance purposes, ELISA is the method of choice; regardless of the antigenic type of IBV involved **(Ignjatovic and Sapats 2000).** Despite the use of the IBV vaccine it is common to find IBV problems in vaccinated chickens, causing a

tremendous economic impact **(Nouri *et al.*, 2003).** The ELISA assay was a convenient method widely used to detect antibody response to IBV infection in broilers flock **(Wing *et al.*, 2002).** The ELISA assay is a convenient method for monitoring of both the immune status and virus infection in chicken flocks. **(Jahantigh *et al.*, 2013).** As a virus that goes through continuous changes. Some of the live vaccines give broad protection against most of the variants reported to be causing respiratory nephrotropic IB.There is a promising alternative of incorporating strategic strains in inactivated vaccines. Continuous active surveillances, improved management and biosecurity on farm, are all essential to suppress losses due to IBV infections **(Awad *et al.*, 2014).** It is obvious that the enzyme-linked immunosorbent assay (ELISA) is the most significant advance, especially in virus detection. Generally, the success rate depends on the genetic differences between the vaccine viruses and the field viruses **(Praveen and Narasimha 2016).**

Therefore, the current work was established to assess the impact of indoor air quality (Climate elements and ammonia) on humeral immune response against infectious bronchitis disease and vaccine in cool and non-cool seasons in field of broiler farms in Egypt.

## MATERIALS AND METHODS

**1-Establishing a structured questionnaire for** recording current information of the 16 investigated farms.

**2-Field investigation:**

The available sixteen broiler farms either had respiratory symptoms or not were visited for:

**Assessing indoor air quality (IAQ) as follow:**

**a**-Measuring indoor air climate elements (Ta.C, RH% and AVm /sec.)

**b**-Measuring indoor air ammonia ppm

**On site measures:**

**Indoor Air parameters:**

**A)** Measuring ambient temperature Ta.C from different points inside the poultry house using Indoor/ outdoor thermometer with hygrometer CLOCK (Resolution 0 - 50 °C).

**B)** Measuring relative humidity RH% from different points inside the poultry house using Indoor/ outdoor thermometer with hygrometer CLOCK (Resolution 10 - 99 RH %).

**C)** Measuring air velocity AVm/sec. from different points inside the poultry house using Mini Digital Anemometer NEDA 1604 IEC 6F22 (Resolution 0.01 - 25 m/s).

**D)** Measuring ammonia concentration ppm inside the poultry house using Ammonia Gas Meter (Resolution 0 - 100 PPM)**.**

**E)** Collecting random ten blood samples for serum separation from 16 poultry farms of 7 different governorates.

**3-Laboratory investigation**

**a- Assessing of the broiler humeral immune response (HIR) in investigated suspected farms by using Elisa kits:**

Blood samples (3-5ml) were collected from birds showing respiratory symptoms from vein by sterile syringes and poured into a clean plane tube without anticoagulant and centrifuged at 2000 rpm for 5-7 minute. The serum was separated and stored in multiple marked sterile epindorff tubes at (2-8˚C) for ELISA test **(Abed and Shayyal 2014),** for evaluation of humeral immune response (HIR) against IB to determine IB antibodies titer using (IDvet IBVS/0416 France) as follow:

- Allow all reagents to come to room temperature 21°C (± 5°C) before use.
- Homogenize all reagents by inversion or overtaxing.
- The negative and positive controls are supplied ready-to-use. Don't add dilution buffer to the control wells A1, B1, C1 and D1.
- **Samples are tested at a final dilution of 1:500 in Dilution Buffer 14 (1:50 pre-dilution followed by 1:10 dilution in the microplate).**

**1.**In pre-dilution plate set aside wells A1, B1, C1 and D1 for the control and add 5 µL of each sample to be tested + 245 µL of Dilution Buffer 14 to all wells except to control wells.

**2. In the ELISA microplate add:**

**a.**100 µL of negative control to wells A1 and B1.

**b.**100 µL of positive control to wells C1 and D1.

**c.**90 µL of Dilution Buffer 14 to as many wells as there are samples to be tested.

**d.**10 µL of pre-diluted samples as prepared above.

**3.**Cover the plate and incubate 30 min ± 3 min at 21°C (± 5°C).

**4.**Prepare the conjugate 1X by diluting the concentrated conjugate 10X to 1:10 in Dilution Buffer 3.

**5.**Empty the wells. Wash each well 3 times with approximately 300 µL of Wash Solution 1X avoid drying of the well between washes.

**6.**Add 100 µL of the Conjugate 1X to each well.

**7.**Cover the plate and incubate 30 min ± 3 min at 21°C (± 5°C).

**8.**Empty the wells.Wash each well 3 times with approximately 300 µL of Wash Solution 1X avoid drying of the well between washes.

**9.**Add 100 µL of Substrate Solution to each well.

**10.** Cover the plate and incubate 15 min ± 2 min at 21°C (± 5°C) in the dark.

**11.** Add 100 µL of Stop Solution to each well in order to stop the reaction. The Stop Solution should be added in the same order in step 9.

**12 .**Read and record the O.D at 450 nm**.**

**4-Data management and statistical analysis:**

The data concerning house indoor air quality, indoor air microbial profile, biosecurity levels, vaccination and humeral immune response titer were verified statistically by one-factor analysis of variance. The statistical analysis of data involved the determination of arithmetic means ($\bar{x}$). The significance of differences between the mean values of the investigated parameters was determined by Duncan's test. Calculations were performed using Statistic 8.0 PL software. **(Wojcik *et al*., 2010).**

## DISCUSSION

Data recorded in (Table 1) showed that, the mean values of Ta.C (22.9594 ±2.80470) in cool seasons is lower than in non-cool seasons (24.9688± 2.13458) and also AVm/sec. (.0609±.07190) was lower in cool Vs (.3794±.55854) in non-cool. A high significant difference (t -3.225, P ≤ .002) in mean values of Ta.C recorded between seasons and also AVm/sec between cool and non-cool seasons (t -3.199, P ≤ .002). A lesser significant difference

(t-1.818, P ≤ .074) was noticed in mean values (62.1875± 11.45662) of RH% between seasons. RH% mean value was lower (62.1875±11.45662) in cool seasons Vs non-cool seasons (67.0000±9.63863), despite both means value in all seasons were within recommended value (60-70%), according to **(Ross, 2010)** relative humidity of 60-70% in the house is necessary for the first three days as well, relative humidity above 70% can occasionally be reached with high stocking densities in winter time when the ventilation rate is reduced to retain heat and save energy**.** During summer, broilers may often experience discomfort due to the combined effect of high humidity and high temperature **(Meluzzi and Sirri, 2009**). Ambient humidity was the only risk factor that showed an association (inverse)

with the prevalence of IBV (p = 0.05; OR = 0.92).Within the constraints of the totally enclosed management systems that humidity had an influence on the presence of IBV, but temperature and ammonia had no effect **(Lopez, 2006)**. The recorded means value of Ta.C in both seasons (22-24C) still within those recommended by **(Holik,2009),** where he established thermoneutral zone for birds reared in the tropical regions ranges between 18- 24 for broiler. The optimum temperature for best performance ranges between 18 and 22 °C for growing broiler chickens **(EFSA, 2010).** The current mean values of AVm/sec increased in both seasons than recommended. If the temperature remains within the range of 25ºC to 30ºC, air velocity of 0.1 m/s to 0.2 m/s can be maintained, but if the temperature goes beyond that an increase in air velocity will help aid convectional cooling **(Hulzebosch,2004).**

The environment in the broiler house is a combination of physical and biological factors generating a complex dynamic system of interactions between birds, the husbandry system, temperature, and the aerial environment **(Sainsbury,2000 and Bianchi *et al*.,2015).**

Meanwhile, increased mean value of ammonia (9.67) in cool season Vs. non-cool season (8.88). Cool seasons showed lower Ta.C, AVm/sec and RH% with higher ammonia compared with non-cool seasons. In addition, a higher significant difference in means value and mean differences of Ta.C and AVm/sec. than non -cool ones. The obtained results are coincided with **(Bueno and Rossi, 2006**), where they reported that microclimatic levels of ammonia are usually influenced by a number of indoor factors such as temperature, relative humidity, ventilation system and stocking density. On contrast to **Soliman *et al.*, (2017)** as they mentioned that**,** during fall and winter, the low air temperature values induced higher relative humidity levels and as a result elevated levels of ammonia in the naturally ventilated farm compared to the environmentally controlled one. The operation of ventilation systems is a vital part to regulate sufficient air exchange to meet the air quality in poultry houses to reduce disease susceptibility and stress **(Bhuiyan *et al.*, 2021).**

Data recorded in (Table 2) revealed decreased means value of antibodies titer against field vaccine or infection **(**3847.39±2343.771) in cool seasons compared with(5272.86± 2389. 619) in non-cool ones with a high significant difference (t -3.809, P ≤ .000) in mean values between cool and non - cool seasons. It was found that low (10 ± 2$^{o}$C) and high (30 ± 2$^{o}$C) temperatures exacerbated the respiratory signs and lesions in birds infected with IBV as compared to those housed at moderate (20 +/- 2$^{o}$C) temperatures **(Lopez, 2006).** The impact

of season on detection of IBV was coincided with **Setta *et al.*, (2018)** where they mentioned the detection rate of IBVs was highly influenced by sampling season. A very low detection rate was observed during summer months, the high incidence rate of IBVs was observed during winter (reaching 100%) followed by autumn and spring. In addition to, increased detection of IBVs during cool weather could be impacted by many factors, including improper farm ventilation **(Abdel-Moneim *et al.*, 2012).**

The current decreased antibodies titer in cool seasons was associated with lower Ta.C, RH% but increased air velocity and ammonia compared with non-cool seasons, despite all values in both seasons were accepted except air movement in non-cool ones. The current results concerning impact of season on ammonia concentration with consequent health impact on birds either vaccinated or not vaccinated was previously discussed by many authors, where the occurrence of airsacculitis in NH3-stressed birds vaccinated with live attenuated infectious bronchitis virus (IBV) **(Kling and Quarles, 1974; Quarles and Caveny, 1979).**

The environment disorder as ammonia, management and biosecurity levels are responsible for the severities of the clinical signs of IBV **(Ganapathy, 2009)**. Ambient NH3 exposure (30-60ppm) was associated with a higher incidence of airsacculitis in non-vaccinated, challenged birds, despite the apparent lack of differences in the immune response between birds in the NH3-exposed and NH3 control groups. Ambient NH3 exposure from used litter had no discernible impact on the immune response to IBV vaccination **(Astona *et al.*, 2019).** High NH3 level (30 or 70 ppm) in the poultry house suppressed the immune response of broiler chickens. Neither high nor low RH (35, 60, and 85%) benefited the immune response of broilers. There was an interactive effect between NH3 and RH on the immune response of broilers.**(Wei *et al.*, 2015).**

Results recorded in (Table 3) for correlations between indoor climate elements.

During non-cool season on bird HIR low significant correlations were reported between Ta.°C & AV m/sec.in non-cool season. High significant correlation showed between RH% and AVm/sec. (.986$^{*}$, P≤.014). A significant correlation was between Ta. °C & RH% (.957$^{*}$ P≤.014) in farm2. A highly significant correlation was between Ta.°C & RH% (.991$^{**}$P≤.009) in farm 15. Significant correlations were between AV & RH% (.979$^{*}$, P ≤ .021) in farm 16, between HIR & AV (.948, P≤.052) in farm 18, between HIR & RH% (.982$^{*}$, P≤.018) in farm 19. In farms 2, 15, 16, 18 & 19 during non-cool seasons, the indoor climate elements revealed significant correlations between each other basically RH% with both Ta.C and AVm/sec.

significant correlations clarified impact of indoor RH% and AV m/sec. on broiler humeral immune response. Meanwhile, in farms 1, 3 & 17 during non-cool seasons, non-significant negative correlations were recorded within indoor climate elements and between climate elements and HIR. The correlations between climate elements each other and their correlation with HIR were shown in (Table 4).

During cool season, a high significant positive correlation was between HIR & AV (.989$^{*,}$ $P \leq .011$) in farm **7** only. The non-significant correlations were recorded between indoor climate elements in farms 8-14. Poor quality ventilation can damage the lining of the respiratory tract through initiating the immune system and can cause chronic inflammation that leads to less (15% lower IgM response) efficiency or no response to vaccination **(De Wit *et al.*, 2010 and Sharif and Ahmad, 2018).** Various risk factors involved in the failure of IB vaccination, such as various routes of application of vaccination, the interval between vaccinations, and challenge with various possible immunosuppressions of birds. A good vaccine response has been observed during the proper ventilation system, which is essential to maintain in the whole flock during vaccination except spray vaccine, fans should be off because of the uniform vaccine distribution **(Bhuiyan *et al.*, 2021)**.

The ratio between mean titer and CV percentage is critical in assessing vaccine quality, defined as the vaccine index (VI), which is calculated as follows:

$$VI = \frac{\text{Mean Titer}}{\%\ CV}$$

The VI score is estimated to provide a higher score (high mean titer, low CV percentage) in a good vaccination, and a low score with poor vaccination (low mean titer, high CV percentage). Depending on the standard parameters of different commercial kit **(Bhuiyan *et al.*, 2021).**

Data of CV% and VI recorded in (Table 5) revealed, the highest vaccination index was obtained in farms 1, 2, 3 **(**282.54), with the highest antibodies titer **(**6781), with the lowest (24) CV % in 3/15 of investigated farms (20%).

From field observations noticed and records in these farms (1, 2, and 3), were lower ammonia level was ranged 0-1 ppm, Av m/sec ranged from 0.73-1.11, and no recorded clinical respiratory signs. As well as, presence of fences with observed high restriction of people movement, presence of showering and hand washing, clothes and foot wears, footbath, apparently clean water supply, presence of incinerator for carcasses disposing (Good BSL).

Meanwhile, the lowest VI (62.02, 68.22, 79.35, 84.58, 84.58 ) were recorded in farms 15, 17, 19, 9, 10 that associated with the highest CV% (56, 54, 54, 52, 52 respectively) and the lowest antibodies titer (3473, 3684 , 4285, 4398 , 4398 respectively). The increased CV% more than 50% was reported in 5/15 (33.3%) of vaccinated farms which reflected the improper vaccination (immune-suppression). All mentioned farms with recorded higher CV % showed respiratory clinical signs as cough, sneezing, tracheal rales, gasping, and dyspnea with general improper health signs as poor appetite, conjunctivitis and depression. Farms 9, 10 were missing showering and hand washing foot bath with disinfectant, throwing wastes and carcasses in water stream, obvious presence of stray dogs and rodents and apparently unclean water supply (Bad BSL).Farms 14,17,19 recorded ammonia levels (2,20,16 ppm respectively) which were the highest values of ammonia in all investigated farms).With reference to farms 17, 19, this situation with highest levels was associated with low Av m/sec (0.03, 0.18 respectively).These farms were missing showering and hand washing, clothing and foot wears, foot bath with disinfectant, throwing carcasses in water stream, presence of stray dogs and rodents around farms (Bad BSL). Farm 14 had no fence and unclean water offered to birds. A percentage coefficient of variation (CV) that is maintained below 40% after inactivated vaccinations and less than 60% in live vaccines suggests good vaccination uniformity. Conversely, after vaccinating with high immunogenic IBV variants, CV percentage is commonly less than 45% **(Van Leerdam and Kuhne, 2017**).

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**Conflicts of Interest:**

The authors declare no conflict of interest.

## RESULTS

**Table (1):** Mean values ±SD and the significant means difference of indoor climate elements in commercial broiler farms between cool and non-cool seasons.

| Indoor climate elements | Seasons | Mean ±SD | T-Test for Equality of Means | Sig. (2-tailed) | Means Difference |
|---|---|---|---|---|---|
| Ambient temperature Ta.°C | Cool seasons | 22.9594 ± 2.80470 | -3.225 | .002 | -2.00937 |
| | Non cool seasons | 24.9688 ± 2.13458 | -3.225 | .002 | -2.00937 |
| Relative humidity RH% | Cool seasons | 62.1875 ± 11.45662 | -1.818 | .074 | -4.81250 |
| | Non cool seasons | 67.0000 ± 9.63863 | -1.818 | .074 | -4.81250 |
| Air velocity Av.m/sec. | Cool seasons | .0609 ± .07190 | -3.199 | .002 | -.31844 |
| | Non cool seasons | .3794 ± .55854 | -3.199 | .003 | -.31844 |
| Indoor Ammonia ppm | Cool | 9.67 | | | |
| | Non-cool | 8.88 | | | |

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

**Table (2):** Means ±SD and the significant means difference of humeral immune response (HIR)against IBV in commercial broiler farms between cool and non-cool seasons.

| Humeral Immune Response HIR | Seasons | Mean ± SD | T-Test for Equality of Means | Sig. (2-Tailed) | Mean Difference |
|---|---|---|---|---|---|
| Antibodies titer (ELISA) | Cool | 3847.39 ± 2343.771 | -3.809 | .000 | -1425.475 |
| | Non-cool | 5272.86 ± 2389.619 | -3.809 | .000 | -1425.475 |

**Table (3):** Correlations between indoor air climate elements and broiler humeral immune response during non-cool seasons.

| Farms | Pearson Correlation | | ELISA | Ta.C | RH% | AV. m/ sec |
|---|---|---|---|---|---|---|
| 1 | Ta.C | Pearson Correlation | .463 | 1 | .981 | .918 |
| | | Sig. (2-tailed) | .537 | | .019 | .082 |
| 2 | ELISA | Pearson Correlation | 1 | -.727 | -.939 | -.921 |
| | | Sig. (2-tailed) | | .273 | .061 | .079 |
| | RH | Pearson Correlation | -.939 | .548 | 1 | .986* |
| | | Sig. (2-tailed) | .061 | .452 | | .014 |
| 3 | Ta.C | Pearson Correlation | -.774 | 1 | .957* | -.567 |
| | | Sig. (2-tailed) | .226 | | .043 | .433 |
| | RH | Pearson Correlation | -.925 | .957* | 1 | -.438 |
| | | Sig. (2-tailed) | .075 | .043 | | .562 |
| 15 | Ta.C | Pearson Correlation | .861 | 1 | .991** | .709 |
| | | Sig. (2-tailed) | .139 | | .009 | .291 |
| 16 | ELISA | Pearson Correlation | 1 | .444 | -.485 | -.386 |
| | | Sig. (2-tailed) | | .556 | .515 | .614 |
| | AV. m/ sec | Pearson Correlation | -.386 | .634 | .979* | 1 |
| | | Sig. (2-tailed) | .614 | .366 | .021 | |
| 17 | Ta.C | Pearson Correlation | .334 | 1 | .526 | -.708 |
| | | Sig. (2-tailed) | .666 | | .474 | .292 |
| | RH % | Pearson Correlation | .307 | .526 | 1 | -.471 |
| | | Sig. (2-tailed) | .693 | .474 | | .529 |
| 18 | ELISA | Pearson Correlation | 1 | -.668 | -.627 | .948 |
| | | Sig. (2-tailed) | | .332 | .373 | .052 |
| 19 | ELISA | Pearson Correlation | 1 | .290 | .982* | -.675 |
| | | Sig. (2-tailed) | 1 | .710 | .018 | .325 |
| | AV. m/ sec | Pearson Correlation | -.675 | -.805 | -.577 | 1 |
| | | Sig. (2-tailed) | .325 | .195 | .423 | |

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

**Table (4):** Correlations between indoor air climate elements and broiler humeral immune response (ELISA titer) during cool seasons.

| Pearson Correlation | | ELISA | Ta.C | RH% | AVm /sec |
|---|---|---|---|---|---|
| ELISA titer | Pearson 7 Correlation | 1 | -.534 | -.267 | .989* |
| | Sig. (2-tailed) | | .466 | .733 | .011 |
| AVm/sec | Pearson Correlation | .989* | -.414 | -.187 | 1 |
| | Sig. (2-tailed) | .011 | .586 | .813 | |
| ELISA Titer | Pearson 8 Correlation | 1 | -.793 | .474 | .118 |
| | Sig. (2-tailed) | | .207 | .526 | .882 |
| RH% | Pearson Correlation | .474 | -.866 | 1 | -.816 |
| | Sig. (2-tailed) | .526 | .134 | | .184 |
| ELISA Titer | Pearson 9 Correlation | 1 | -.301 | .893 | .337 |
| | Sig. (2-tailed) | | .699 | .107 | .663 |
| Ta.C | Pearson Correlation | -.301 | 1 | -.651 | -.797 |
| | Sig. (2-tailed) | .699 | | .349 | .203 |
| ELISA Titer | Pearson 10 Correlation | 1 | -.357 | -.754 | .446 |
| | Sig. (2-tailed) | | .643 | .246 | .554 |
| AV m/sec | Pearson Correlation | .446 | .635 | -.478 | 1 |
| | Sig. (2-tailed) | .554 | .365 | .522 | |
| RH% | Pearson 11 Correlation | .424 | -.568 | 1 | .819 |
| | Sig. (2-tailed) | .576 | .432 | | .181 |
| AV m/ sec | Pearson Correlation | .272 | -.826 | .819 | 1 |
| | Sig. (2-tailed) | .728 | .174 | .181 | |
| ELISA | Pearson 12 Correlation | 1 | -.132 | .541 | -.789 |
| | Sig. (2-tailed) | | .868 | .459 | .211 |
| RH % | Pearson Correlation | .541 | -.881 | 1 | -.918 |
| | Sig. (2-tailed) | .459 | .119 | | .082 |
| RH % | Pearson 13 Correlation | .525 | .671 | 1 | -.225 |
| | Sig. (2-tailed) | .475 | .329 | | .775 |
| ELISA | Pearson 14 Correlation | 1 | -.782 | -.637 | .765 |
| | Sig. (2-tailed) | | .218 | .363 | .235 |
| AVm/sec | Pearson Correlation | .765 | -.238 | -.108 | 1 |
| | Sig. (2-tailed) | .235 | .762 | .892 | |

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

**Table (5):** Vaccination index (VI) in investigated broiler farms.

| Farms | C.V % | G. Mean | Percentage | Vaccination Index |
|---|---|---|---|---|
| 1 | 24 | 6781 | 100% | 282.54 |
| 2 | 24 | 6781 | | 282.54 |
| 3 | 24 | 6781 | | 282.54 |
| 7 | 36 | 3187 | | 88.53 |
| 8 | 36 | 3187 | | 88.53 |
| 9 | 52 | 4398 | | 84.58 |
| 10 | 52 | 4398 | | 84.58 |
| 11 | 37 | 3621 | | 97.86 |
| 12 | -ve | -ve | -ve | -ve |
| 13 | 47 | 4261 | 100% | 90.66 |
| 14 | 28 | 4288 | | 153.14 |
| 15 | 56 | 3473 | | 62.02 |
| 16 | 32 | 3599 | | 112.47 |
| 17 | 54 | 3684 | | 68.22 |
| 18 | 34 | 3340 | | 98.24 |
| 19 | 54 | 4285 | | 79.35 |

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