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Original article

Application of mixed design models in impact of high Ammonia concentration on Plasma Immunoglobulins and Newcastle virus vaccine titer in broilers

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ABSTRACT

High Ammonia concentration in broiler's houses contributed a reduction in performance, lowering in resistance and increased mortality. Four out of five Hubbard broiler groups (5 × 60) were subjected to 60 ppm ammonia for 2; 4; 6; 8 hours per day; respectively for 5 successive weeks. The impact of high ammonia concentration on biochemical; immunological and bacteriological status was assessed in 900 (150 sera, 150 plasma, 150 swab and 450 organ) samples collected post exposure. Data were analyzed using traditional and mixed model ANOVA considering repeated measures. Effect size estimates were quantified using Eta and partial Eta-squared. Broilers showed 10, 18, 32 and 41.2% mortalities at 2, 4, 6 and 8 hrs of exposure; respectively. A highly significant decline ($P < 0.01$) was recorded in immune organs' weight. Plasma immunoglobulins revealed a highly significant decrease ($P < 0.01$) at 8 hrs of exposure. Newcastle virus vaccine titer revealed a highly significant decrease ($P < 0.01$) in 4, 6 and 8 hrs of exposure. Effect size statistics revealed that mixed model ANOVA was advantageous in exhibiting the biological value of studied effects. The longer the period of exposure to ammonia beyond permissible limits, the larger negative influences can be detected. Management practice in broiler farms should include preventive measures to reduce ammonia concentrations as well as

researchers should keep in mind a quantitative estimate for each effect size when conducting experimental studies.

1. Introduction

Abbreviations: Total Protein (TP), Albumin (Alb), Globulin (Glob), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Urea (Ure), Creatinine (Creat), Total Bacterial Count (TBC), Total *Enterobacteriaceae* Count (TEC), Newcastle Virus Disease (ND), Average Live Body Weight (wk. LBW), Average Feed Intake (wk. FI), Weight Gain (WG) Feed Conversion Ratio (FCR), Performance Index (PI).

Ammonia concentration in broiler houses influenced by various factors, including: housing system, management practice, stocking density, season, microclimatic temperature and humidity, as well as litter conditions (Miles et al., 2004). Broilers exposed to atmospheric ammonia revealed a significant reduction in body weight; approximately 8% (Reece et al., 1981). Ammonia contributed a highly dangerous influences in poultry production, when it achieved levels as low as 10 ppm; it can negatively influence bird's health; growth and performance (Miles et al., 2004). Ammonia influences can be exaggerated at higher concentrations, as when exceeds 25 ppm; it contributed a damage in the bird's respiratory system and allow the entrance and establishment of infectious agents causing a major decline in the flock health and performance, decreased resistance and increased bird mortalities (Moore et al., 1999; Kristensen and Wathes, 2000).

Measurements of observations in experimental studies may be either repeated or independent. Repeated measures are data obtained over different time points from the same experimental units such as those pertaining to different ages, times, or even measured on the same animals but at different sites of the body. Although, designs with repeated measures data have been extensively used in animal science publications (Ma et al., 2012; Kim, 2015), many researchers become uncertain and have been confused regarding choosing the appropriate design for analyzing their data, especially, if they have repeated and independent measures in the same study. Still others analyze the repeated measures data as it was independent, and vice versa. In such cases, if the investigation was designed to study the effect of repeated and independent measures simultaneously, data should be statistically analyzed using mixed model designs. In mixed model analysis of variance (ANOVA) with two factors, the first factor (Independent measurements or treatment) will be the between-subjects, while the second factor (repeated measures or time) will be the within-subjects factor (Eyduran and Akbas, 2010; Orhan et al., 2010). Hence, this study provides the proper use of mixed models relative to traditional ANOVA with the meticulous interpretations in veterinary public health data.

Information about effect size for each effect is important when drawing interpretations and conclusions in experimental studies. Researchers relied for many years on significance tests to determine the differences between groups. These tests lead many researchers to publish a lot of articles of no practical significance, even when the null hypothesis cannot be accepted. Effect size estimates become imperative for scientific community to join the practical significance of results. Many publishers, such as; American Psychological Association (APA, 2010), Lakens (2013) and Ialongo (2016) emphasized the importance of effect sizes along with the significance tests to enhance the quality of articles. Rosnow and Rosenthal (2009) reported an inherent relationship between the effect size, significance test, and the sample size. Others reported a solid relationship between the statistical power of significance tests and the effect size estimated in applied research (Sanchez and Cervantes, 2016). The present research showed the significance of effect size estimates in recording the magnitude of each factor together with their biological contribution in the field. The aim of this study was to investigate the influence of different exposure times to high ammonia concentration (60 ppm) on performance, biochemical, bacteriological and immunological configurations and Newcastle virus vaccine titer in broilers. The size of the main effects of exposure times and age of broilers along with their interaction were quantified using traditional ANOVA versus mixed ANOVA.

2. Materials and methods

2.1. Experimental design

A total of 300 one day old Hubbard chicks were purchased; divided into five groups, each consisted of 60 chicks (six replicates of ten birds) in a separate room on deep litter system (hay). Four out of the five rooms were

supported with vaporizer (source of high ammonia concentration; 60 ppm). Rooms were supplied with partial artificial ventilation tools (supplying and suction fans) and natural ventilation tools (windows). Broilers were brooded at 35°C followed by a gradual decrease (3°C weekly) until achieving 25°C by the end of 3rd week. Artificial lights were supplied for at least 18 hours a day. Birds were given *ad libitum* access to water and a standard corn-soybean basal diet containing crude protein 21.5% (Hu and Guo, 2008). All birds were immunized during the experimental duration as showen in Table 1. The vaccination act was carried out by resuscitation of vaccines in drinking water after taking all the necessary precautions. The experiment was designed to last for about 5 weeks (38 days). Mortalities were noticed and recorded daily. Indoor temperature and relativehumidity were monitored daily during the experiment.

Table 1
Vaccination scheme of broilers during the experiment.

Age of birds / days	Vaccine administered	Disease
7th day	Live attenuated virus; IB-H120 103.5 EID50	Infectious Bronchitis
14th day	Live attenuated virus; VMG91 103.0 TCID50	Infectious Bursal Disease
18th day	live lentogenic ND virus LA SOTA 106.0 EID50	Newcastle Disease
21st day	Live attenuated virus; VMG91 103.0 TCID50	Infectious Bursal Disease
28th day	live lentogenic ND virus LA SOTA 106.0 EID50	Newcastle Disease

2.2. Ammonia exposure and measurement

Experimental groups (G1; G2; G3 and G4) were subjected to ammonia vapor (60 ppm) for a durations of 2; 4; 6; 8 hours per day; respectively. Aerial ammonia concentration was measured two successive times on a daily basis. Ammonia concentrations were measured by potentiometric titration (Ndegwa et al., 2009) using methyl red indicator and 0.05M sulfuric acid solution (APHA, 2012).

2.3. Performance indices

Average live bird body weight (wk. LBW) was measured by weighting approximately 45 birds per group per week, as well as the weekly amount consumed (wk. FI) by each bird per grams was calculated based on the bird capacity. Number of indices were calculated on the basis of wk. LBW and wk. FI, including: weekly Body Weight Gain (Bardy, 1968); weekly Feed Conversion Ratio (wk. FCR) and weekly Performance Index (Yamani et al., 1997).

2.4. Sampling

A total of 900 samples (150 sera, 150 plasma, 150 swab and 450 organ samples) were collected during the study period. Samples were collected on a weekly intervals starting from 7th day. Blood samples for serum separation were collected on a plain tubes from the five groups, kept for overnight at 4 °C; centrifuged at 3000 rpm for 20 minutes. A clear non-hemolyzed sera were divided into 2 equal parts in eppendorf tubes, stored at -20 °C until used for biochemical analysis (Coles, 1986). Whole blood samples were collected on EDTA tubes, centrifuged at 2500 rpm for 10 min, plasma was divided into 2 equal parts in eppendorf tubes, stored at -20 °C until used for immunological assay. Birds were slaughtered after blood sampling; thymus, spleen and bursa were removed and weighed, relative lymphoid organ weight was expressed as (g / kg BW). Swab samples were collected from birds' intestine on phosphate buffer saline, preserved and transferred to the laboratory for bacteriological assessment.

2.5. Biochemical analysis of sera and plasma

Sera samples were examined for the biochemical changes in some parameters as Total Protein (TP); Albumin (Alb); Alanine Aminotransferase (ALT); Aspartate Aminotransferase (AST); Urea and Creatinine (Creat) calorimetrically (Young, 2001). Newcastle disease antibody (ND) quantification in sera samples was carried-out using Hem-agglutination (HA) and HI procedures (Carbrey et al., 1974). PlasmaIgG, IgA, IgM concentrations were measured by using immunoturbidimetric assay (Wang et al., 2010).

2.6. Bacteriological examination

Swabs were prepared according to APHA, (2012). All swabs were subjected on arrival to the laboratory to tenfold serial dilution up to 10^{-8} to cover the expected range of samples contamination which could be easily

counted. Bacterial counts (Total Bacterial Count; TBC and Total *Enterobacteriaceae* Count; TEC) were applied using drop plate technique (Zelver et al., 1999; Herigstad et al., 2001). TBC was performed using standard plat count agar at 37 °C for 24 - 48 hrs. On the other hand; TEC was conducted using Eosine Methylene Blue Agar (EMB) at 37 °C for 24 - 48 hrs.

2.7. Statistical analysis

Data of this study were analyzed using two statistical methods, traditional two-way analysis of variance (ANOVA) and mixed design ANOVA. The two methods have manipulated two categorical variables that were fitted as independent factors, exposure time to ammonia and the age of broilers. In traditional two-way ANOVA, both factors were fitted as between-subjects effects along with their interaction effects. Mixed model ANOVA was used as the main statistical methodology for the current data, because of the presence of repeated measurements in the study design. In mixed models with two factors, exposure time was fitted as the between-subjects factor, whilst, age of birds was fitted as the within-subjects factor (repeated measurements). In the two methods, the main and interaction effects were tested for their statistical significance and also for their effect sizes. The statistical model was summarized as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

Where, Y_{ijk} was the measurement of any dependent variable; μ was overall mean; α_i was the fixed effect of exposure time; β_j was the fixed effect of age of birds; $(\alpha\beta)_{ij}$ was the interaction effect of exposure time by age of birds; e_{ijk} was the random error. The error term, e_{ijk} was approximately NID (0, σ^2_e), normally independently distributed with mean of 0 and variance of, σ^2_e.

The parametric assumptions of the mixed between-within subjects ANOVA such as; homogeneity of variances (Levene's test of equality of variances), homogeneity of inter-correlations (Box's M statistic) have been checked. In addition, Mauchly's test was used to check the sphericity assumption pertaining to repeated measures of the within-subjects factor.

Mauchly's test W statistic tests the null hypothesis that the error covariance matrix of the orthonomalized transformed dependent variable is proportional to an identity matrix. Hence, sphericity indicates the equality of variances of the differences between all possible combinations of repeated measurements (Lee, 2015). In cases when the assumption of sphericity was not met ($p < 0.05$), Greenhouse-Geisser corrections for degrees of freedom, and subsequently, F values were used (Keskin and Mendes, 2001). A probability level (p value) more than 0.05 ($p > 0.05$), indicated verification of sphericity. Mixed models have many advantages in repeated measures designs. For example, when the assumption of sphericity is violated, mixed models enable statisticians to fit different covariance structures, even the data have missing observations.

Effect size estimates were used in this investigation to determine contribution size of the main and interaction effects in explaining the outcomes. Eta-squared (η^2) and partial Eta-squared (η^2_p) are the preferred effect size estimates for experimental studies (Lakens, 2013). Eta-square measures the proportion of variation in Y-variable that is attributed to each of the main effects, interactions, and error term in ANOVA designs (Thompson, 2006). Eta-square for a given effect equal to the sum of squares of that effect divided by the total sum of squares as follows:

$$\eta^2 = \frac{SS_{effect}}{SS_{total}}$$

Where, SS_{effect} is the sum of squares of the effect of interest whilst SS_{total} is the total sum of squares, including all effects along with error term of ANOVA. Within the same study, Eta-square is the preferred effect size estimate. The sums of η^2 must be 100 %, provided that every effect is explained in relation total variability. However, it was difficult to compare η^2 between different studies, because SS_{total} values are varied based on the study design, and would increase if extra factors are incorporated into analysis. On the other hand, partial Eta-square was recommended to compare the effect sizes between different designs (Maxwell et al., 2008). Partial Eta-square was defined as the percentage of variance caused by a given effect in relation to that effect plus its error variance. η^2_p was calculated as follows:

$$\eta^2_p = \frac{SS_{effect}}{SS_{effect} + SS_{error}}$$

Where, SS_{effect} is the sum of squares of a given effect, SS_{error} is the sum of squares of error. That is, partial Eta-square measures the % of variability in Y-variable attributable to a given factor, excluding the other non error factors. The sum of all partial Eta-squares cannot be 100 %, due to the partitioning of denominator sum of squares. Statistical analyses were conducted using statistical package for social sciences (SPSS, 2001) and statistical analysis system (SAS Institute, Inc., 2002).

3. Results and discussion

Broilers exposed to high ammonia concentration (60 ppm) revealed mortality rates 10, 18, 32 and 41.2 % in G1, G2, G3 and G4; respectively. Newcastle virus vaccine titer as revealed in Fig. 1; despite of its natural highly significant ($P < 0.01$) curve of decline and increase in relation to age, it revealed a highly significant decrease ($P < 0.01$) at 4, 6 and 8 hours of exposure.

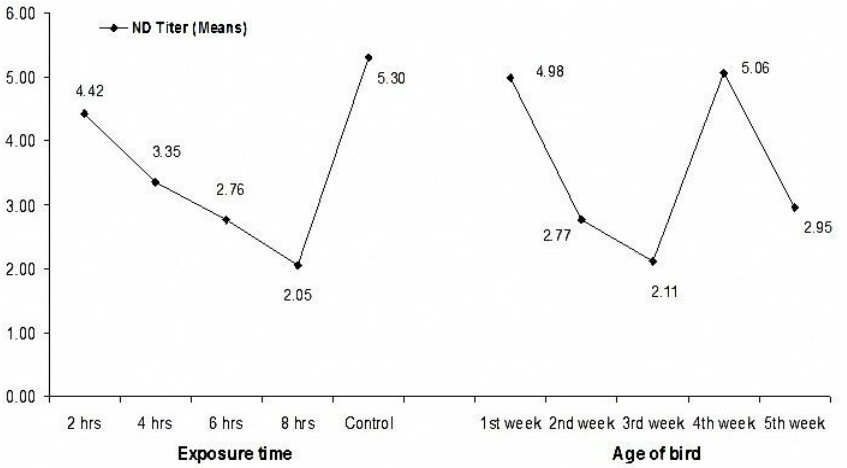

Fig. 1. Newcastle virus vaccine titer (mg / ml) at different exposure times and age (per weeks) in broilers exposed to high ammonia concentration (60 ppm).

Table 2
Main effects of different exposure times to high ammonia concentration (60 ppm) and age of broilers on performance Indices (Mean ± SE).

	Exposure times (hours per day)				
Parameters	**2 hrs**	**4 hrs**	**6 hrs**	**8 hrs**	**Control**
LBW / g	$799.59^{b} \pm 107.43$	$763.73^{c} \pm 100.5$	$665.43^{d} \pm 86.08$	$598.93^{e} \pm 78.28$	$867.18^{a} \pm 109.7$
WG / g	$340.07^{ab} \pm 34.83$	$316.03^{b} \pm 31.22$	$259.56^{c} \pm 28.2$	$235.00^{d} \pm 30.31$	$361.46^{a} \pm 34.32$
FCR %	$1.82^{b} \pm 0.07$	$1.90^{b} \pm 0.09$	$2.25^{a} \pm 0.15$	$2.42^{a} \pm 0.18$	$1.76^{b} \pm 0.07$
PI	$4.65^{a} \pm 0.677$	$4.21^{b} \pm 0.554$	$3.32^{c} \pm 0.43$	$2.97^{c} \pm 0.453$	$4.81^{a} \pm 0.628$
	Age of birds / week				
Parameters	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th Week**
LBW / g	$111.24^{e} \pm 6.17$	$292.63^{d} \pm 15.72$	$706.64^{c} \pm 7.25$	$1040.23^{b} \pm 22.1$	$1544.13^{a} \pm 44.9$
WG / g	$79.24^{e} \pm 6.17$	$181.38^{d} \pm 11.32$	$414.01^{b} \pm 12.2$	$333.59^{c} \pm 17.52$	$503.90^{a} \pm 27.93$
FCR %	$2.06^{b} \pm 0.08$	$1.83^{c} \pm 0.11$	$1.47^{d} \pm 0.07$	$2.65^{a} \pm 0.14$	$2.14^{b} \pm 0.37$
PI	$0.62^{e} \pm 0.074$	$1.84^{d} \pm 0.148$	$5.15^{b} \pm 0.78$	$4.29^{c} \pm 0.263$	$8.07^{a} \pm 0.561$

Means carrying different superscripts in the same row are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$). Means carrying the same superscripts in the same row are non-significantly different at ($P > 0.05$).

Broilers revealed in Table 2; a highly significant reduction ($P < 0.01$) in wk. LBW and WG as exposure time increased. PI showed the least calculated significant value ($P < 0.01$) in G4 (8 hrs) with non-significant differences compared to G3 (6 hrs). A highly significant increase ($P< 0.01$) in overall values of performance indices was recorded as broilers grow toward the marketing age. Although thymus, spleen and bursa weights in Table 3;

revealed a highly significant decline (P < 0.01) in both G4 (8 hrs) and G3 (6 hrs) compared to other groups and to control.

Table 3
Main effects of different exposure times to high ammonia concentration (60 ppm) and age of broilers onimmune organs'weight (Mean ± SE).

	Exposure times (hours per day)				
Parameters	**2 hrs**	**4 hrs**	**6 hrs**	**8 hrs**	**Control**
Thymus / g	2.93^{b} ± 0.15	2.74^{c} ± 0.15	2.16^{d} ± 0.11	2.09^{d} ± 0.10	3.50^{a} ± 0.23
Spleen / g	3.59^{a} ± 0.23	2.48^{c} ± 0.21	1.43^{d} ± 0.11	1.09^{e} ± 0.08	3.38^{b} ± 0.22
Bursa / g	2.19^{b} ± 0.18	1.89^{c} ± 0.19	1.45^{d} ± 0.13	0.97^{e} ± 0.07	2.67^{a} ± 0.23
	Age of birds / week				
Parameters	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**
Thymus / g	1.42^{d} ± 0.03	2.37^{c} ± 0.08	2.99^{b} ± 0.06	3.29^{a} ± 0.13	3.36^{a} ± 0.22
Spleen / g	1.01^{e} ± 0.09	1.76^{d} ± 0.17	2.60^{c} ± 0.46	3.20^{b} ± 0.23	3.39^{a} ± 0.25
Bursa / g	0.56^{e} ± 0.02	1.16^{d} ± 0.06	2.07^{c} ± 0.17	2.59^{b} ± 0.15	2.82^{a} ± 0.17

Means carrying different superscripts in the same row are significantly different at (P ≤ 0.05) or highly significantly different at (P < 0.01). Means carrying the same superscripts in the same row are non-significantly different at (P > 0.05).

Table 4
Main effects of different exposure times to high ammonia concentration (60 ppm) and age of broilers on biochemical parameters (Mean ± SE).

	Exposure times (hours per day)				
Parameters	**2 hrs**	**4 hrs**	**6 hrs**	**8 hrs**	**Control**
TP g / dl	3.61^{d} ± 0.02	4.37^{c} ± 0.02	6.37^{b} ± 0.02	9.37^{a} ± 0.02	3.09^{e} ± 0.02
Alb g / dl	2.12^{d} ± 0.05	2.84^{c} ± 0.06	5.26^{b} ± 0.06	8.65^{a} ± 0.06	1.58^{e} ± 0.05
Glob g / dl	1.49^{b} ± 0.04	1.53^{a} ± 0.04	1.12^{c} ± 0.04	0.72^{d} ± 0.04	1.52^{a} ± 0.03
ALT IU / L	25.01^{d} ± 0.11	25.58^{c} ± 0.12	27.83^{b} ± 0.32	32.88^{a} ± 0.31	24.81^{e} ± 0.11
AST IU / L	34.76^{d} ± 0.21	37.79^{c} ± 0.37	39.94^{b} ± 0.39	45.74^{a} ± 0.39	34.58^{e} ± 0.20
Urea mg / dl	88.52^{d} ± 2.14	93.52^{c} ± 2.14	103.83^{b} ± 4.83	115.02^{a} ± 2.13	83.72^{e} ± 2.07
Creat mg / dl	0.88^{d} ± 0.04	1.09^{c} ± 0.04	1.37^{b} ± 0.04	1.67^{a} ± 0.04	0.74^{e} ± 0.04
	Age of birds / week				
Parameters	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**
TP g / dl	5.15^{d} ± 0.42	5.31^{c} ± 0.41	5.39^{b} ± 0.42	5.48^{a} ± 0.41	5.49^{a} ± 0.44
Alb g / dl	3.45^{e} ± 0.47	4.06^{d} ± 0.47	4.27^{c} ± 0.47	4.31^{b} ± 0.48	4.36^{a} ± 0.48
Glob g / dl	1.69^{a} ± 0.05	1.24^{b} ± 0.06	1.12^{e} ± 0.06	1.18^{c} ± 0.06	1.14^{d} ± 0.05
ALT IU / L	26.10^{e} ± 0.50	26.45^{d} ± 0.50	26.97^{c} ± 0.51	27.85^{b} ± 0.51	28.74^{a} ± 0.78
AST IU / L	36.24^{e} ± 0.63	38.07^{d} ± 0.64	39.41^{b} ± 0.64	39.07^{c} ± 0.86	40.05^{a} ± 1.07
Urea mg / dl	82.72^{e} ± 4.51	86.64^{d} ± 4.81	95.80^{c} ± 4.67	106.92^{b} ± 4.62	112.55^{a} ± 4.82
Creat mg / dl	0.83^{e} ± 0.06	0.98^{d} ± 0.06	1.13^{c} ± 0.05	1.34^{b} ± 0.06	1.47^{a} ± 0.06

Means carrying different superscripts in the same row are significantly different at (P ≤ 0.05) or highly significantly different at (P < 0.01). Means carrying the same superscripts in the same row are non-significantly different at (P > 0.05).

Biochemical parameters in Table 4; including TP; Alb; ALT; AST; Urea and Creat revealed a highly significant deviation (P < 0.01) in both G4 (8 hrs) and G3 (6 hrs) compared to other groups and to control. On the contrary, the estimated levels of Glob revealed a highly significant decrease (P < 0.01) in G4 (8 hrs) compared to all groups. Plasma immunoglobulins (IgM, IgA and IgG) in Figures 2 and 3 confirmed the highly significant decline (P < 0.01) in G4 (8 hrs) compared to other groups.

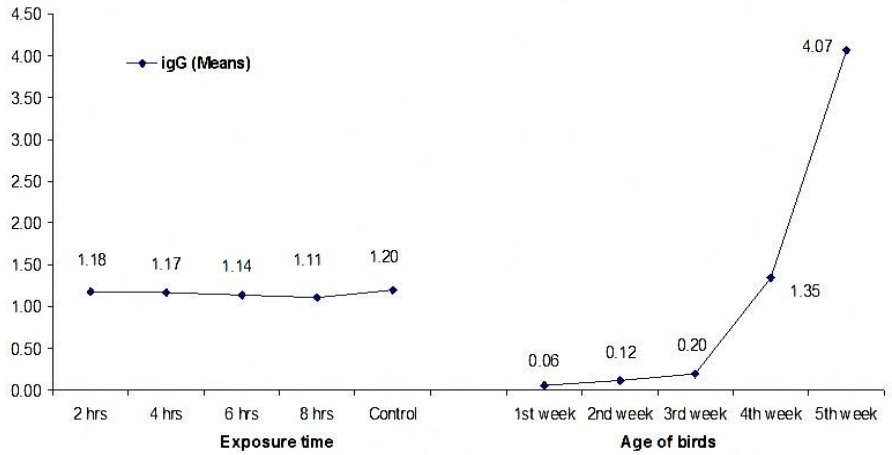

Fig. 2. Immunological changes of IgG concentrations (mg / ml) at different exposure times and age (per weeks) in broilers exposed to high ammonia concentration (60 ppm).

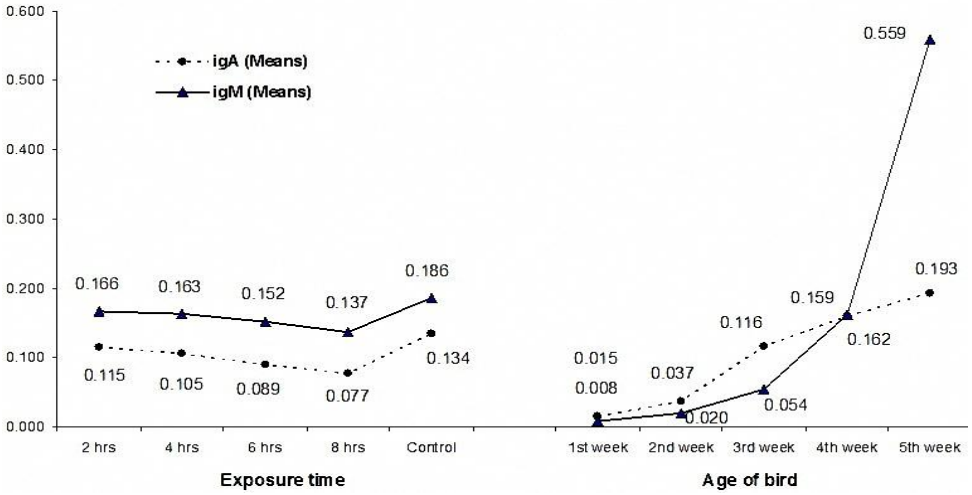

Fig. 3. Immunological changes of IgA and IgM concentrations (mg / ml) at different exposure times and age (per weeks) in broilers exposed to high ammonia concentration (60 ppm).

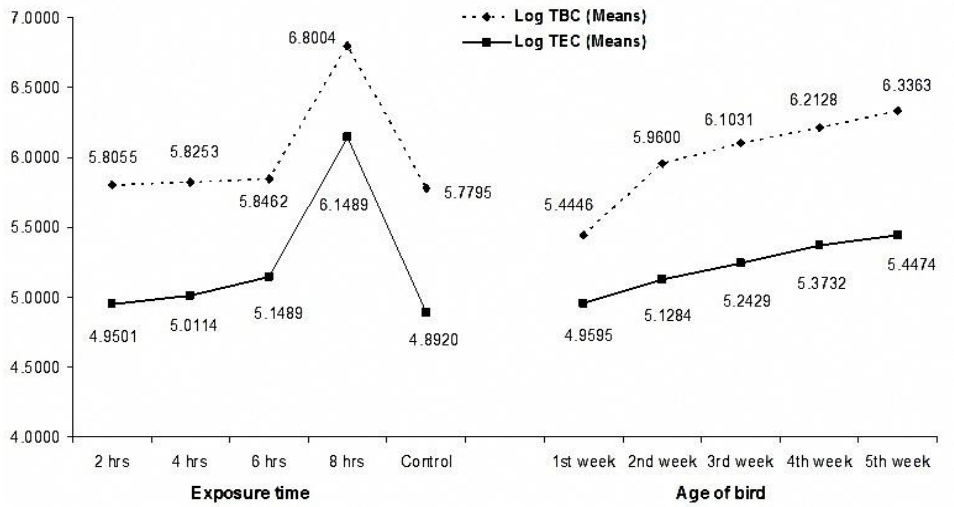

Fig. 4. Bacteriological changes in Total bacterial Count (TBC) and Total *Enterobacteriaceae* Count (TEC) (CFU / ml) at different exposure times and age (per weeks) in broilers exposed to high ammonia concentration (60 ppm).

Bacterial counts (TBC and TEC) in Fig. 4 were synchronized to some extend in showing a highly significant increase ($P < 0.01$) in G4 (8 hrs) compared to all other groups. Indicating the extent to which broilers in G4 were subjected to stress condition that contributed an elevation in bacterial counts. The presented study showed statistical data analysis using two approaches, traditional ANOVA and mixed design ANOVA. In the former, both main effects of exposure time and age of broilers were fitted as between-subjects factors, whilst in mixed design models, exposure time was the between-subjects factor and age of birds was the within-subjects factor. As large number of researchers in animal sciences analyzed their data for factorial experiments using traditional ANOVA method, such approach was used in this study relative to mixed design ANOVA.

Table 5
Comparison between the effect size estimates (partial Eta-squared) for models with all factors fitted as independent measures and mixed design models.

	Two-way classical ANOVA			Two-way mixed design ANOVA		
Parameters	Exposure time	Age	Age X exposure time	Exposure time	Age	Age X exposure time
LBW	88.8	99.6	84.4	97.1	99.7	87.7
WG	55.2	92.7	68.8	97.8	92.9	69.4
FCR	32.2	52.1	52.2	77.6	55.8	55.8
PI	50.2	92.8	76.3	94.7	93.2	77.4
TP	100	95.3	80.7	100	96.2	84.0
Alb	100	99.7	78.8	100	99.8	81.0
Glob	99.0	97.9	71.9	99.8	98.4	77.0
ALT	99.6	96.6	93.3	99.9	97.1	94.3
AST	99.8	98.0	97.3	99.9	98.5	98.0
Urea	99.0	99.1	16.9	99.8	99.2	19.5
Creat	99.9	99.7	74.7	100	99.8	78.5
IgG	20.5	99.8	2.3	60.6	99.9	2.7
IgA	83.3	98.3	33.6	95.1	98.8	40.6
IgM	52.6	99.5	22.9	83.5	99.6	27.6
ND titer	99.7	99.7	33.3	99.9	99.8	41.1
TBC	99.8	99.7	50.6	99.9	99.8	60.0
TEC	100	99.7	95.1	100	99.8	97.5
Thymus wt	92.7	96.0	88.4	98.7	96.7	90.2
Spleen wt	97.5	96.9	81.9	99.5	97.4	84.8
Bursa wt	93.8	97.0	84.5	98.5	97.6	87.5

The percentages (%) of effect size estimates, partial Eta-squared were depicted for the two designs in Table 5. The effect sizes of main effects and interaction effects were higher in mixed design ANOVA than the traditional ANOVA for all studied parameters. In mixed ANOVA, partial Eta-squared for exposure time were ≥ 60.6%. The lowest effect size of exposure time was recorded for IgG (60.6%). Effect sizes of age of bird were also ≥ 55.8%, with the lowest value for FCR. It was clear that the effect sizes of interaction between exposure time and age of bird were lower than the corresponding main effects for all dependent variables. The % of partial Eta-squared estimates for the interaction effects were ranged between 2.7% and 97%.

Table 6 showed another effect size estimate called Eta-squared. Eta-squared statistics for the main and interaction factors denoted the % of total variation in the dependent variables attributable to such factor. Unlike, partial Eta-squared, the sum of Eta-squared values should be equal one (or 100%) for the within-subjects and between-subjects effects, separately. In term of performance, biochemical, immunological and bacterial parameters, the effect size of main effects were predominant (within average close to 95%) when compared to the interaction effects. That is, the age of birds explained the highest % of within-subjects variation, while the exposure time to high ammonia levels explained the highest % of between-subjects variation. The effect size for most of interaction effects were low to medium (≤ 18.84%). The highest % of effect sizes of interaction effect were

for FCR, ALT, AST and thymus weight, which have been estimated to be 35.85%, 32.36%, 42.32% and 23.34%, respectively.

Table 6
Effect size estimates (Eta-squared) for within-subjects and between-subjects effects of exposure time (hours), age of bird (weeks), and their interactions as calculated from mixed design models.

	Within-subjects effects			Between-subjects effects	
Parameters	Main effect (Age)	Interaction effect (Age X exposure time)	Error	Main effect (Exposure time)	Error
LBW	97.40	2.28	0.32	97.05	2.95
WG	80.06	13.83	6.11	97.77	2.23
FCR	35.78	35.85	28.37	77.64	22.36
PI	75.65	18.84	5.51	94.65	5.35
TP	80.31	16.54	3.15	99.99	0.01
Alb	98.70	1.05	0.25	99.99	0.01
Glob	93.34	5.12	1.54	99.77	0.23
ALT	65.68	32.36	1.96	99.94	0.06
AST	56.79	42.32	0.89	99.95	0.05
Urea	99.03	0.19	0.78	99.84	0.16
Creat	98.93	0.82	0.25	99.98	0.02
IgG	99.85	0.00	0.15	60.61	39.39
IgA	97.91	0.84	1.25	95.24	4.76
IgM	99.42	0.16	0.42	84.44	15.56
ND titer	99.66	0.15	0.19	99.91	0.09
TBC	99.43	0.34	0.23	99.94	0.06
TEC	94.12	5.74	0.14	99.98	0.02
Thymus wt	74.11	23.34	2.55	98.71	1.29
Spleen wt	85.20	12.55	2.25	99.52	0.48
Bursa wt	83.69	14.28	2.03	98.55	1.45

Mauchly's test of sphericity revealed that the assumption of sphericity for repeated measures analysis of the within-subjects effects was violated (chi-square values were ≥ 33.43, df = 9 and P< 0.01) for all parameters, except for globulin and spleen weight (chi-square values were ≤ 16.74, df = 9 and P> 0.05). Mauchly's test W statistic tests the null hypothesis that the error covariance matrix of the orthonomalized transformed dependent variable is proportional to an identity matrix. Since the assumption of sphericity was not verified, the Greenhouse-Geisser (G-G) correction for dfs was used, because G-G sphericity epsilon estimates were < 0.75 for all dependent variables that showed the violation of sphericity. In term of statistical significance, the mixed design ANOVA showed highly significant effects of both age of bird and exposure time as main effects on all tested parameters (P< 0.01). Although, the interactive effects of both factors denoted significant effects (P < 0.05) on all parameters, the F-ratios were all small relative to those of main effects.

Exaggerated elevation of ammonia concentration in poultry farms might be attributed to mainly bad management practice, including improper control of microclimatic temperature and relative humidity, dampness of litter, improper litter treatment, and high litter moisture content. High ammonia concentration in poultry farms can contribute a serious problems including a severe reduction in livability and performance, great lowering in bird's resistance and a significant increase in bird's mortality. Miles et al. (2004) showed that broilers exposed to 25, 50 and 75 ppm microclimatic ammonia concentrations showed 2, 17 and 21% reductions in LBW; respectively. Meanwhile, Reece et al. (1980) exposed broilers to high different ammonia concentrations (50-200 ppm) during the brooding period; they revealed that WG and FCR were adversely affected in all exposed groups during brooding. Broilers were also exposed to ammonia concentrations (0-50 ppm); which are more predominant in the field; WG in broilers exposed to 25 ppm was reduced by 4% but there was no distinct influence on FCR or mortality.

The present study showed that broilers exposed to high ammonia concentration as much as 60 ppm contributed a great trend of reduction in wk. LBW and feed efficiency associated with greater mortality rates, this might be attributed to the influences of ammonia in lowering circulating immunoglobulin levels in broilers causing failure of humeral immunity; the results were supported by these of Reece et al. (1980) and Wang et al. (2010). High ammonia concentration excreted a stress factor on broilers and caused a lowered FCR and subsequently lowered PI; a state was recorded clearly in broilers exposed for 4, 6 and 8 hrs daily (Reece et al., 1981; Beker et al., 2004; Miles et al., 2004). Weights of immune organs were greatly reduced as time of exposure to high ammonia concentration was increased (Caveny et al., 1981).

Results of partial Eta-squares estimates in this study indicated that mixed design models explained high % of variability independent variables other than the ordinary ANOVA procedures. Previous investigations (Breaugh, 2003; Brown, 2008) recommended partial Eta-square as a preferred effect size estimate for comparing different factorial designs. Although, many effect size estimates were available for evaluating experimental studies, Eta-square statistics were recommended, because they are given by most of statistical packages (Ferguson, 2009; Lakens, 2013). Based on the estimates of effect sizes, especially Eta-square, it can be concluded that the main effects of exposure time to high ammonia concentration and age of broilers contribute more in explaining the variability occurred in outcome variables than did their interaction effects. That is, researchers should pay attention to main effects in practice. As in practice, breeders put in their mind all possible factors that could explain variations in an outcome, Eta-square values of this study would be important for further investigations and meta-analysis studies on broilers, because Eta-square measured variability caused by each factor, considering all other factors. For these reasons, overall results of main effects were presented for mixed design models (Tables 2, 3 and 4).

4. Conclusion

Broilers were severely and negatively affected by the used high ammonia concentration (60 ppm), as well as the longer the duration of exposure, the greater influences can be exerted on broilers. Mixed design models of ANOVA are the suitable approaches when compared with traditional ANOVA procedures to analyze data which include both independent and repeated measurements. The present study also stressed the importance of effect size estimates along with the significance tests in explaining the main and interaction effects in the empirical studies. Strict management practices in broiler farms should be considered, including various methods of litter treatment and adjusting microclimatic temperature and relative humidity for modifying the elevated concentration of ammonia that may liberate from different sources insider the farm.

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References

American Psychological Association, 2010. Publication Manual of the American Psychological Association (6th ed.). Washington: American Psychological Association.

American Public Health Association; American Water Works Association; Water Environment Federation, 2012. Standard methods for the examination of water and wastewater. American Water Work Association Publications; 22th Ed, Washington D.C.

Beker, A., Vanhooser, S.L., Swartzlander, J.H., Teeter, R.G., 2004. Atmospheric ammonia concentration effects on broiler growth and performance. J. Appl. Poult. Res., 13, 5-9.

Brady, W.L., 1968. Measurements of some poultry performance parameter. Vet. Rec., 88, 245-260.

Breaugh, J.A., 2003. Effect size estimation: Factors to consider and mistakes to avoid. J. Manag., 29, 79-97.

Brown, J.D., 2008. Statistics corner. Questions and answers about language testing statistics: Effect size and eta squared. Shiken: JALT Testing & Evaluation SIG Newsletter, 12(2), 38-43.

Carbrey, E.A., Beard, C., Cooper, R., Hansen, R.P., Pomeroy, B.S., 1974. Hemagglutination and hemagglutination inhibition tests with Newcastle disease virus-microtiter technique. 17th Ann. Proc. Am. Assoc. Vet. Lab. Diagn., 1-6.

Caveny, D.D., Quarles, C.L., Greathouse, G.A., 1981. Atmospheric ammonia and broiler cockerel performance. Poult. Sci., 60, 513-516.

Coles, E.H., 1986. Veterinary clinical pathology WB Saunders company. Philadelphia and London.

Eyduran, E., Akbas, Y., 2010. Comparison of different covariance structure used for experimental design with repeated measurement. J. Anim. Plant Sci., 20(1), 44-51.

Ferguson, C.J., 2009. An effect size primer: A guide for clinicians and researchers. Professional Psychology: Research and Practice. 40(5), 532-538.

Herigstad, B., Hamilton, M., Heersink, J., 2001. How to optimize the drop plate method for enumerating bacteria. J. Microbiol., 44(2), 121-129.

Hu, X.F., Guo, Y.M., 2008. Corticosterone administration alters small intestinal morphology and function of broiler chickens. Asian-Aust. J. Anim. Sci., 21(12), 1773-1778.

Ialongo, C., 2016. Understanding the effect size and its measures. Biochem. Med., 26(2), 150-63.

Keskin, S., Mendeş, M., 2001. Experimental designs including repeated measurement in one's levels of their factors. S.Ü. J. Agr. Facult., 15(25), 42-53.

Kim, H.Y., 2015. Statistical notes for clinical researchers: A one-way repeated measures ANOVA for data with repeated observations. Restor. Dent. Endod., 40, 91-95.

Kristensen, H.H., Wathes, C.M., 2000. Ammonia and poultry welfare: A review. World Poult. Sci. J., 56, 235-245.

Lakens, D., 2013. Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. Front. Psychol., 4(863), 1-10.

Lee, Y., 2015. What repeated measures analysis of variance really tells us? Kor. J. Anesthesiol., 68(4), 340-345.

Ma, Y., Mazumdar, M., Memtsoudis, S.G., 2012. Beyond repeated measures ANOVA: Advanced statistical methods for the analysis of longitudinal data in anesthesia research. Reg. Anesth. Pain Med., 37, 99-105.

Maxwell, S.E., Kelley, K., Rausch, J.R., 2008. Sample size planning for statistical power and accuracy in parameter estimation. Ann. Rev. Psychol., 59, 537-563.

Miles, D.M., Branton, S.L., Lott, B.D., 2004. Atmospheric ammonia is detrimental to the performance of modern commercial broilers. Poult. Sci., 83(10), 1650-1654.

Moore, P.A. Jr., Daniel, T.C., Edwards, D.R., 1999. Reducing phosphorus runoff and improving poultry production with alum. Poult. Sci., 78, 692-698.

Ndegwa, P.M., Vaddella, V.K., Hristov, A.N., Joo, H.S., 2009. Measuring concentrations of ammonia in ambient air or exhaust stream using acid traps. J. Environ. Qual., 38(2), 647-653.

Orhan, H., Eyduran, E., Akbas, Y., 2010. Defining the best covariance structure for sequential variation on live weights of Anatolian Merinos male lambs. J. Anim. Plant Sci., 20(3), 158-163.

Reece, F.N., Lott, B.D., Deaton, J.W., 1980. Ammonia in the atmosphere during brooding affects performance of broiler chickens. Poult. Sci., 59(3), 486-488.

Reece, F.N., Lott, B.D., Deaton, J.W., 1981. Low concentrations of ammonia during brooding decrease broiler weight. Poult. Sci., 60, 937-940.

Rosnow, R.L., Rosenthal, R., 2009. Effect sizes: Why, when, and how to use them. Zeitschrift Für Psychologie. J. Psychol., 217, 6-14.

Sanchez, M.E.T., Cervantès, R.J.M., 2016. Generalized eta squared for multiple comparisons on between-groups designs. Psicothema, 28(3), 340-345.

SAS Institute, Inc., 2002. SAS course notes on mixed model analysis using SAS system. SAS Institute, Inc., Cary, NC.

SPSS, Inc., 2001. SPSS for windows: Computer software. Chicago: Author.

Thompson, B., 2006. Foundations of behavioral statistics: An insight-based approach. New York, NY: Guilford.

Wang, Y.M., Meng, Q.P., Guo, Y.M., Wang, Y.Z., Wang, Z., Yao, Z.L., Shan, T.Z., 2010. Effect of atmospheric ammonia on growth performance and immunological response of broiler chickens. J. Anim. Vet. Sci., 9(22), 2802-2806.

Yamani, K.A.O., Rashwan, A.A., Magdy, M.M., 1997. Effects of copper, zinc and tafla dietary supplementation on broiler performance. Int. Conf. Anim. Poult. Rabb. Prod. Health, 457-463.

Young, D.S., 2001. Effect of drugs on clinical laboratory test, 5th edition.

Zelver, N., Hamilton, M., Pitts, B., Goeres, D., Walker, D., Sturman, P., Heersink, J., 1999. Measuring antimicrobial effects on biofilm bacteria: From laboratory to field. Meth. Enzymol., 310, 608-628.

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