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Broiler Performance, Carcass Characters and Litter Composition After Management of Fresh Litter with Two Types of Acidifier Amendments

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Abstract – **Two types of litter amendments (copper sulfate and alum) were used and their effects on broiler performance, litter coccidial count, antibody titer and litter composition were compared with a control fresh litter. The litter treatments included 3 groups as group 1, wood shaving topped with 100 g of alum /kg bedding, groups 2, wood shaving topped with 10 g of copper sulphate /kg of bedding and final group 3, as untreated wood shaving (control). Topping of fresh litter with acidifier amendments significantly (P<0.05) increased broiler's final body weights compared to chickens in the untreated litter group, However feed conversion ratio during the last week of the experiment was significantly higher (P<0.01) in chickens of control group than the two treated litter groups. Copper sulfate significantly increased dressing % and breast muscle weight than alum and untreated groups. Although alum treated litter group had the lowest coccidial count than the other treated litter with CuSo4 and control one. The results of HI testing of chicken serum revealed that chickens reared on fresh litter treated with CuSO4 had the highest titer for NDV. Alum treated group and copper sulfate treated group showed a significant increase in dry matter and total nitrogen content when compared with the untreated group. Alum treated litter had lower litter pH value than the other treated groups during all time of sampling. It was concluded that treatment of fresh litter with copper sulphate and alum improved broiler performance and litter characters.**

Keywords – **Amendments, Carcass, Litter, Performance.**

I. INTRODUCTION

Fresh litter treated with different methods to satisfactory performing its function and to reduce ammonia volatilization which adversely affects bird's performance, producer health and return. Acidifier amendments, mainly Aluminum sulfate [alum; Al2 (SO3) 4·14H2O], widely used to achieve excellent results, however, copper sulfate was not used as acidifier amendment, although its application in poultry rations with many folds over its minimum requirement and its positive effects on poultry performance without any adverse effects.

Broilers are generally reared on floor litter to absorb moisture from bird's dropping in order to keep the floor reasonably dry and to ensure comfortable conditions for birds. It also gives the birds a suitable medium on which feeding, watering and other management practices are carried out. To allow litter to carefully make these jobs it must be kept dry, clean and of acidic pH to avoid ammonia volatilization, and enhancement of many diseases like necrotic enteritis, coccidiosis and fungal infections. Different trials were used for immunization against coccidiosis in broiler chickens as a control measure [1].

Uric acid and organic nitrogen (N) in the bird excreta and spilled feed convert to ammonium (NH4+) by the microbes in the litter. Ammonium, a plant-available N form, can bind to litter and also dissolve in water. Depending on the moisture content, temperature, and acidity of the litter, a portion of the ammonium will be converted into ammonia (NH3). Ammonia production is favored by high temperature and high pH (i.e., Alkaline conditions) [2]. Ammonia (NH3) concentration in a commercial poultry house is a major airborne contaminant. Researchers suggested that 25 ppm of ammonia should not be exceeded in a poultry house [3]-[6]. Also, high concentrations of ammonia in poultry house can affect chicken by reducing growth rate, feed efficiency, decrease egg production, damage the respiratory tract through increasing tracheitis and airsacculitis and increase the susceptibility to chronic respiratory disease (CRD), *E Coli* infection and *Newcastle* disease with the incidence of kerato conjunctivitis [6], [7].

Many methods were used to keep a good litter quality that included acidic amendments which make litter PH acidic introducing unfavorable media for ammonia volatilization. Aluminum sulfate [alum; Al2 (SO3) 4·14H2O] was largely used to reduce ammonia (NH3) volatilization by converting the volatile NH3 to NH4-N, which is not volatile. [8] found that the application of alum as a top dressing for new litter (wood shaving) at a rate of 0.25kg/m2 significantly decreased the indoor ammonia concentration during 5 days (37-42) days compared to non-treated groups, also [9] reported similar results for a multi-flock litter.

Copper had received considerable attention due to its antimicrobial properties that improve performance in animals when fed over the minimum requirement [10]. Studies have shown that supplementation with various Cu sources (e.g., Cu sulfate, Cu citrate, or Cu chloride) increased growth in poultry [11]- [13] without inducing adverse effects on birds. However, almost no data are available about using copper sulfate as acidifier litter amendment.

The aim of this work was to evaluate the effects of aluminum sulfate (alum) and copper sulfate as litter acidifiers on performance traits, carcass characters, litter

composition, coccidial infestation and immunity against NDV and AI of broiler chickens during single flock rearing period.

II. Materials and Methods

Poultry and Experimental Design

A trial was performed in an experimental poultry unite located in the veterinary medicine collage, Damanhour University during 2014. One hundred and eighty (1-day-old) broiler male chicks (Arbor Acres) were obtained from a local commercial hatchery then they were randomly allocated to 12 pens to a density of 0.07 m^2/bird from one day till 5 weeks of age, with 4 replicates of 3 experimental treatments with 15 birds per experimental unit as a completely randomized design. The litter treatments included (1) wood shaving topped with 100 g of alum /kg bedding according to [14] (2) wood shaving topped with 10 g of copper sulfate /kg of bedding and (3) untreated wood shaving (control). Acidifiers were added to the superficial layer of bedding material after spreading of it on the floor at a depth of 5 cm. Light supplies continuously during the first 48 hours of life, then reduced to 23 hours/day, chicks brooded under gas brooder supplies 33^o c for the first week reduced 3^o c per week till reaches 24^o c. The chicks were fed with starter diets during first three weeks (0 to 21 days) and finisher diets during the second two weeks (21 to 35 days), ingredients of diets used during experiment are illustrated in table (1) according to [15] guidelines. Also, the vaccination program was HB1+H120 at 8 days of age; IBD at 12 days and La Sota at 18 days of age and all vaccines were applied through drinking water after following all precautions.

Broiler Performance Traits

Body weight was estimated weekly to the nearest gram from the 2nd week for the 5th week of age using a digital balance. Daily feed intake, Weight gain and Feed conversion ratio were calculated.

Carcass Characteristics

At the end of the experiment (5th week) 3 birds from each pen were slaughtered to estimate carcass and organs traits as: carcass weight, dressing percentage, breast muscle weight (included skin), thigh weight, shoulder weight and organ weights (gizzard, intestine, liver, heart and spleen) and apart from the large intestine (ileum) was used for total viable count.

Isolation and Counting of Coccidial Oocysts

Different treated litters were collected from the different groups. Freshly collected litter samples were preserved in a clean labeled plastic package at days 20th, 25th, 30th and 35th days of each treatment. They were examined for cyst count by using McMaster technique according to [16] with some modifications. The whole amount of freshly collected litter samples was washed ten sieved using distilled water, then left to be settled down. The supernatant will be discarded and only the sediment (about 2 ml) will be used in McMaster technique. All *Eimeria* spp. oocysts were counted (sporulated and non sporulated).

Haemagglutination Inhibition Test (HI)

Firstly, *Newcastle* disease virus (NDV) antigen, la Sota strain, and *Avian Influenza* virus (AIV) antigen, H5N2, were used to test serum samples collected at 35th days of age (10 samples per each group) for antibody titers against NDV and AIV in all groups. HI test was performed as described by [17]. Briefly, 50 µL of serum was serially diluted (Two fold) with normal saline in V shaped micro-titer plates up to well No 10. Fifty micro liters of 4 HA units of ND virus were added up to well No 11, which served as virus control. Twelfth well was the control for reagents (it only contains RBCs & normal saline). The plate was shaken and left for 30 min at room temperature, allowing antigen and antibody to react. After that 50 µL of 0.5% washed chicken RBCs were added to each well of the plate and left for 20 min. The HI titer was expressed as the reciprocal of the highest dilution that causes inhibition of agglutination and geometric mean titer (GMT) was calculated.

Bacterial Count

For total bacterial counting, 10 cm from intestinal samples (ileum) of each chicken group were taken and then minced separately in a bicker, then 90 ml peptone water was poured into the beaker and mixed with the samples. One ml of each mix was added to 9 ml peptone water in a test tube and tenfold serially diluted up to 10-5. These appropriate dilutions were cultured by a spread plate technique using the sterile bent glass rod on the MacConkey's agar media as a selective medium. These inoculated plates were then incubated overnight at 37^{o}C in the incubator. The bacteria of different samples were grown and formed many colonies to the MacConkey's agar. *E. Coli* was identified by the color of the colony morphology in the selective media. Then these colonies were counted, which is called Total viable count (TVC). [18].

The formula of the Total viable count (TVC) is as follows:

TVC = Mean of the colony amount of sample × 0.1ml × dilution factor.

Table 1. Ingredients and nutrient composition (% DM) of broiler starter and finisher rations

Ingredients	Starter	Finisher
Yellow corn grain	54.00	63.40
Soybean meal (44%)	31.08	24.50
Corn gluten (60%)	7.20	6.00
Vegetable oil[1]	3.97	2.65
MCP[2]	1.44	1.27
Limestone[3]	1.40	1.60
Lysine[4]	0.10	0.11
DL-Methionine[5]	0.05	0.02
Salt	0.20	0.20
Premix[6]	0.25	0.25
Total	100	100

2MCP = monoicalcium phosphate (contain 22 % P and 21 % cap). 3Limestone (contain 34% calcium). 4Lysine = lysine hydrochloride (contain 98.5% Lysine). 5DL-Methionine (Produced by Evonic Co and contain 99.5% methionine) 6The premix used was pharama mix produced by (Egypt Pharma for premix and feed additives industries), and composed of (per 3 kg) vitamin A 12000000 IU, vitamin D3 5000000 IU, vitamin E 65000 mg, vitamin K3 4000 mg, thiamin 4000 mg, riboflavin 9000 mg, pyridoxine 5000 mg, cyanocobalamine 20 mg, niacin 55000 mg, biotin 200 mg, folic acid 2000 mg, pantothenic acid 15000 mg, 6The premix used was pharama mix produced by (Egypt Pharma for premix and feed additives industries), and composed of (per 3 kg) vitamin A 12000000 IU, vitamin D3 5000000 IU, vitamin E 65000 mg, vitamin K3 4000 mg, thiamin 4000 mg, riboflavin 9000 mg, pyridoxine 5000 mg, cyanocobalamine 20 mg, niacin 55000 mg, biotin 200 mg, folic acid 2000 mg, pantothenic acid 15000 mg, Betain 400000 mg, manganese 60000 mg, zinc 1000000 mg, iron 40000 mg, copper 16000 mg, iodine 1250mg, selenium 100 mg and cobalt 100 mg. (Batch NO 07834728)

Litter Sampling and Analysis

The litter was sampled at 20th, 25th, 30th and 35th days from each poultry house. They were divided transversely into two halves, taking two litter samples per house. Subsamples from each half of the house were obtained from the full depth of the litter, from 10 random locations. Subsequently, the random litter samples were thoroughly mixed in a plastic bag, and 250 g was weighed and delivered for further processing in the laboratory. A fraction of each sample was immediately used to determine the pH with a 1 : 2 litter-to-water extract ratio by pH meter (Adwa pH meter) as described by [19]. Whereas the rest of the sample was ground to pass through a 2-mm sieve, and frozen until further analysis. Dry matter and crude nutrients: Analytical DM contents of fecal samples were determined by oven-drying at 105^{o}C for 48 h ([20] method 930.15). Ash contents of litter samples were determined by incineration at 550^{o}C overnight, ([20]; method 942.05). Nitrogen in the litter samples was determined by using the Kjeldahl method according to ([20]; method 988.05).

Statistical Analysis

All data were analyzed using the General Linear Model (GLM) procedure of [21]. Statistical differences among means as Duncan's multiple range tests were determined at $P < 0.05$.

III. Results and Discussion

Broiler Performance Traits

Topping of fresh litter with acidifier amendments either alum or copper sulfate significantly ($P<0.05$) increase final body weight (body weight at 5th weeks) of broilers compared to untreated litter group (1884.8±23.54g and 1890±25.94g vs. 1795.35±20.92g for alum, copper sulfate treated groups vs control one, respectively). However the differences between the two acidifier groups were not significant, also there were no significant effects of litter acidifier amendments neither on body weights at other ages nor on weight gains throughout the experiment (table 2). Feed conversion ratio during the last week of the experiment was significantly higher ($P<0.01$) in the control group than the two treated litter groups (3.50±0.27 vs. 2.64±0.22 and 2.55±.024 for control, alum and copper sulfate groups, respectively), therefore these are logic results as higher feed intake and lower weight gain observed in birds in the untreated litter group compared to treated groups will produce a higher feed conversion ratio (differences between the two acidifier groups were not significant table 3). The results from the experiment clearly indicate that chicks raised on fresh litter treated with acidified amendment (whatever the type of acidier) derive additional advantage in terms of better final body weight and better feed conversion, than the broilers raised on untreated fresh litter.

Similar results were recorded by [22] who recorded 4 % heavier body weight, and 3 % better feed conversion in the alum-treated houses than in the control houses due to lower ammonia levels in the early growth stage. The reason might be the synthesis of acidic medium in a litter which decreased ammonia emission and by sequence improve performance of the birds beside bacteriostatic effects of these acids. Also [23] recorded positive results to productive performance traits of broilers reared on using litter treated with acidified amendment compared to fresh untreated litter.

Carcass Characters

Copper sulfate treated litter group recorded significantly higher dressing% and breast muscle weight than untreated group (72.73±1.08% and 526.67±36.19g vs. 68.15±1.12%, and 365.97±35.19g $P<0.05$), it also recorded significantly higher breast muscle weight more than in alum treated group (526.67±36.19g vs. 394.67±36.29g $P<0.05$). The positive effect of copper sulfate on dressing percentage and breast muscle weight may be due to its antibacterial or bacteriostatic properties and litter, scratching behavior of chickens, which decreased bacterial count in letter and in birds' intestine then improve birds' carcass characters. [24] observed higher carcass weight for broilers received 188 mg of Cu/kg diet over basal requirement either as copper sulfate (cuso4) or as tribasic copper chloride (TBCC) than negative control group, also similar results were recorded by [11], [13]. All body organ weights measured during experiment did not differ significantly between different treatments (table 5).

Parasitological Examination

The parasitological examination reveals that, there was some reduction in coccidial oocyst counts in both fresh litters which treated with alum and copper sulfate than control one (table 6)

In Fig. 1 Regardless the type of litter used there was no significant difference between these litter types in the coccidial oocyst count. Fresh litter treated with alum showing high oocyst count at 20th day the reduced gradually till 30 days. While the fresh litter treated with

copper-sulfate showing high oocyst count at 20th days, then gets to be decreased at the following days.

There were few literatures attending to use fresh litters treated with alum or copper sulfate in poultry farms. [25] When used copper sulfate (100 mg/kg) as treatments for experimental *Eimeria tenella* and *E. acervulina* infections. Found that copper sulfate exerted no independent or interactive effect. [23] Found that there was a significant decrease of total oocyct count between different letters used and treated with copper sulfate and alum than fresh ones.

It was found that many researches depends on using some disinfectants mainly such as Ammonium hydroxide and Phenol [26] or using plant extracts for the controlling of avian coccidiosis and improving poultry performance worldwide [27]. The parasitological examination was revealing that the reduction in coccidial oocyst count in both amendments without significant differences from control one.

HI and Bacterial Count

The results of HI testing of chicken serum revealed that chickens reared on fresh litter treated with CUSO4 had the highest titer for NDV as 3.1 log 2 and the lowest titer for AIV as 2.3 logs 2 although this is a non protective titer for NDV, the field virus might be affected by the acidification process in the treated litter. While the chickens reared on fresh litter treated with alum had the lowest antibody titers for NDV as 2.6 log 2 and the titer for AIV was 4.1 logs 2 although the antibody titers for both viruses with higher in control non treated chicken group as shown in table (7).

The intestinal tract total viable count was significantly higher in chickens reared on fresh, non treated litter than in chickens reared on fresh litter treated with CUSo4 and alum (Table, 8 and Fig. 2) And this was a very important point in analyzing the data obtained from weight gain and carcass traits in chickens of group 2 reared on CUSo4 litter than alum treated litter and control one and this agreed with [28], [29] who recorded that the major advantages of using poultry litter treatment were derived from its ability to acidify poultry house litter and thus reducing total bacteria, *E. Coli*, and *salmonella* in the litter. Also, [30] reported that exposing the chickens to acidify litter lowers the intestinal bacterial number, especially in the ileum, it had a negative consequences for the chicken's health or performance.

Litter Sampling and Analysis

The dry matter (DM) content, total nitrogen, Ash % and pH fresh litter treated with copper sulfate, alum and without treatment at different times of the production cycle (20^{th} , 25^{th} , 30^{th} and 35^{th}) are presented in the following tables (9).

Dry matter, content, during different periods of the experiment showed significant difference between a fresh litter treated with both Alum and copper sulfate when compared with fresh litter only.

During 20^{th} of the experiment the highest DM content 65.4 %, but the lowest DM content was observed at 35^{th}. The highest DM content was observed in the alum treated group followed by copper sulfate treated group when compared with the untreated group. These results agree with [23] who found that DM content of the litters during different periods of the experiment showed significant difference between recycled litter treated with either alum or copper sulfate when compared with fresh litter only. Avoiding litter wetting is the most important step for controlling ammonia problems, as it has been reported that wet litter can lead to high ammonia levels in broiler housing [31].

The total N contents in the litter increased with time in all experimental groups. Alum treated group and copper sulfate treated group showed a significant increase in total nitrogen content when compared with the untreated group. Also Alum treated group contain 2 times than on fresh, non treated litter.

These results agree with [8]who found that alum-treated litters to have a slightly higher N concentration than the controls. The increase in total N content is explained by acidification of the litter, which, in turn, converts NH3 to NH4 1, and thus reduces gaseous losses of N [14].

Concerning ash content, it was shown that alum treated litter contained lower levels of ash when compared with fresh treated, these may be due to higher levels of Nitrogen and phosphorus content in alum treated fresh litter. Alum treatment of litter will increase the nitrogen content of the litter making it a more valuable source of fertilizer material. Also table 5 showed Alum treated litter had lower pH value when compared with other treated groups during all time of sampling. Copper sulfate treated litter showed lower pH levels during different periods of sampling when compared with fresh, untreated groups or fresh copper sulfate treated groups. These results agree with [8] who found alum lowered the litter pH during the first 4 weeks, at least. Alum resulted in a more acidic litter as it provided a source of hydrogen ions (H1), with 6 moles of protons per mole of alum. This acidic litter, in turn, was related with lower ammonia volatilization [14]. The pH of the litter increased with the amount of manure produced.

IV. CONCLUSIONS

Topping of fresh litter with acidifier amendments significantly ($P<0.05$) increased broilers final body weights compared to the untreated litter group. However feed conversion ratio during the last week of the experiment was significantly higher ($P<0.01$) in the control group than the two treated litter groups (there were no significant differences between the two types of acidifier amendments). Copper sulfate significantly higher dressing %, breast muscle weight, HI titers for NDV and lower total intestinal coliform count than alum and untreated groups. Although alum treated litter group had the lowest coccidial count than the other treated litter with CUSo4 and control one, the count in both litter amendments was not significantly different from control one, so these treatments had no effect on the coccidial oocyst count.

V. ACKNOWLEDGEMENTS

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Table 2. The effect of acidified amendment on weekly body weight (grams) and weight gain of Arbor Acres broiler chickens from 2-5 weeks of age.

Litter amendment	BW2	BW3	BW4	BW5	WG3	WG4	WG5
Alum	424.03±6.48	837.5±10.34	1295.4±18.51	1884.8±23.54 [a]	417.61±7.84	469.36±16.93	590.2±31.06
Copper sulphate	417.45±8.62	835.31±9.66	1302.18±17.65	1890.00±25.94[a]	437.65±10.52	461.44±16.1	589.25±34.37
Control	410.35±7.05	820.12±10.19	1279.65±20.7	1795.35±20.92[b]	415.68±9.79	459.84±18.83	510.86±38.24

Means within the same column under the same category carry different superscripts are significantly different. B. W= body weight, W. G= weight gain

Table 3. The effect of acidified amendment type on feed consumption and feed conversion per day from 2-5 weeks of age

Litter amendment	FI3	FI4	FI5	FCR3	FCR4	FCR5
Alum	1005.13	1087.31	1326.79	2.43±0.04	2.52±0.18	2.64±0.22 [b]
Copper sulphate	995.75	974.96	1279.88	2.32±0.06	2.51±0.17	2.55±0.24[b]
Control	960.25	1182.17	1585.90	2.30±0.06	2.75±0.2	3.50±0.27[a]

Means within the same column under the same category carry different superscripts are significantly different. FCR = feed conversion ratio. FI= weekly feed intake

Table 4. The effect of acidified amendment type on carcass and carcass cuts weight (grams) of Arbor Acres broiler chickens at 35 days.

Litter amendment	Carcass weight	Dressing %	Thigh	Shoulder	Breast muscle
Alum	1305.71±82.67	71.07±1.32[ab]	689±37.37	149.67±6.12	394.67±36.29[b]
Copper sulphate	1330.78±72.91	72.73±1.08[a]	710±37.32	147.33±6.17	526.67±36.19[a]
Control	1153.25±80.33	68.15±1.12[b]	635±35.37	139.67±6.15	365.97±35.19[b]

Means within the same column under the same category carry different superscripts are significantly different.

Table 5. The effect of acidified amendment type on body organ weight (grams) of Arbor Acres broiler chickens at 35 days

Litter amendment	Gizzard	Liver	Intestine	Heart	Spleen
Alum	41.29±2.34	61.71±3.34	116.71±7.14	12.57±0.74	2.8±0.52
Copper sulphate	38.00±2.06	53.33±2.95	106.44±6.30	12.33±0.65	1.81±0.41
Control	36.55±2.29	59.45±3.17	125.13±6.88	11.89±0.68	3.12±0.42

Means within the same column under the same category carry different superscripts are significantly different.

Table 6. Effect of litter treatments on coccidial oocysts count

Litter amendment	Coccidial count
Alum	2075±195.79
Copper sulfate	2275±190.79
Control	2375±199

Means within the same column under the same category carry different superscripts are significantly different.

Table 7. HI titers of NDV and AIV of the collected serum samples at 35 days of age

Group	HI titers Log 2 at 35 days of age	
	NDV	AI
Alum	6,7,5,4,4,5,6,7,7,6 (GM: $2^{5.7}$)	5,4,6,6,4,5,5,4,4,5 (GM: $2^{4.8}$)
Copper sulfate	4,2,2,3,4,4,3,2,4,3 (GM: $2^{3.1}$)	1,5,2,2,1,5,4,1,0,2 (GM: $2^{2.3}$)
Control	1,2,4,4,3,2,4,2,1,3 (GM: $2^{2.6}$)	4,5,5,5,5,4,4,3,3,3 (GM: $2^{4.1}$)

Means within the same column under the same category carry different superscripts are significantly different.

Table 8. Total viable count (TVC) of intestinal samples of chickens collected on the 35th day of age

Group	TVC in the intestine
Alum	86 x 10^6
Copper sulfate	46 x 10^6
Control	261 x 10^6

Table 9. Chemical composition of fresh litter with different acidified during experiment periods

Litter amendment	DM %	Total N %	Ash %	PH
Alum	68.08±0.85a	4.18±0.32a	14.75±0.46a	6.28±0.01c
Copper sulfate	62.65±0.85b	2.48±0.32b	15.8±0.46a	6.49±0.01b
Control	56.25±0.85c	1.93±0.113c	21.95±0.293b	6.65±0.023a

Means within the same column under the same category carry different superscripts are significantly different.

Table 10. Chemical composition of litter at different periods during the experiment

	DM %	Total N %	Ash %	PH
20	65.4±0.98a	2±0.37	14.77±0.54c	6.34±0.01c
25	64.53±0.98a	2.67±0.37	15.9±0.54c	6.41±0.01b
30	61.23±0.98b	3.2±0.37	17.73±0.54b	6.48±0.01a
35	60.67±0.98b	3.7±0.37	19.9±0.54a	6.52±0.01a

Means within the same column under the same category carry different superscripts are significantly different.

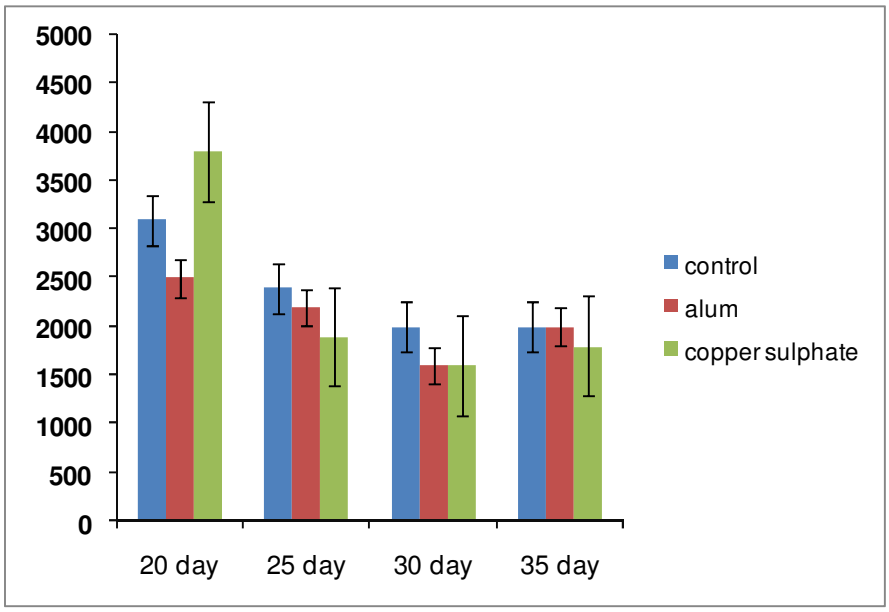

Fig. 1. The average coccidial count among different days of litter used

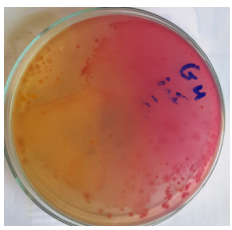

Fig. 2. Bacterial counts in the chicken's intestine of group 2 (Fresh litter CuSo4 treated)