The effect of sewage water before and after treatment on body tissues and meat quality of broiler chickens

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Abstract

This paper is a part of a multi-disciplinary research "Application of Decentralized On-Site Water Treatment System in Egypt for Use in Agriculture and Producing Safe Fish and Animal Proteins". 130 one day old broiler chicks were divided into five groups. Chickens were reared on potable water, treated sewage water, and sewage water at different concentrations (30, 70 and 100 %) and were slaughtered after 21 and 35 days of rearing. Histopathological examination of liver, kidneys, bursa of Fabricuis, spleen, thymus, crop, gizzard, proventriculus and intestine was performed. Meat samples were taken from thighs and breasts of chickens for bacteriological and sensory analysis. The histopathological examination revealed that the most affected organs in the groups drinking sewage water were the kidneys and intestine whereas lesions seen in the crop, proventriculous and gizzard were similar in the tested groups. The results of bacteriological analysis showed that all investigated bacterial counts (aerobic plate count, Enterobacteriaceae, coliforms and fecal coliforms) were higher than the limit standardized by the Egyptian standard specifications in young and old chicken meat reared on sewage water at concentrations of 70 and 100 %. The results of sensory analysis revealed that meat samples obtained from chicken reared on 70 and 100 % sewage water had flavor and overall acceptability scores lower than the acceptable level and dark meat color. In conclusion, Drinking treated sewage water had less serious impact on health and meat quality of chickens compared to the use of untreated SW in chickens.

Keywords: sewage water; water treatment; histopathology; meat quality; Johkasou system; chickens

1. Introduction

Many countries in the African and Asian continent suffer from water scarcity. The water security is being threatened by uneven water distribution, misuse of water resources and inefficient irrigation techniques. Moreover, Sewage water from industrial premises and many other areas is dumped into natural water resources untreated due to lack of water treatment plants which eventually leads to its pollution. Treating sewage water to be suitable for agricultural and rearing purposes may be one of the ways to save the drinking water and overcome the possible shortage in the future. In this regard, both Cairo and Nagoya Universities has cooperated in a strategic project aiming to localize the Japanese's JOHKASOU bioreactor (as Packaged Onsite Aerated Wastewater Treatment Plant or PAWTP) in Egypt. Johkasou de-centeralized system was developed in Japan for removing biologically oxygen demand (BOD), nitrogen and phosphorous from sewage water with consequent increase of the treatment efficiency, cost effectiveness, and reliability of cluster and individual decentralized sewage water management systems. .

Water constitutes about 55% to 75% of chicken body. They are able to survive much longer without feed than without water therefore it is essential for life **(Nesheim et al. 1979 and Scott et al. 1982).** Poultry farms may use water from municipal sources (potable for humans), wells, streams, ponds and springs. Inferior or poor water quality may depress water consumption (**Koelkebeck et al. 1999**). On the other hand, in broiler farms water quality has detrimental effects on broiler performance which negatively correlated with body weight as well as immune resistance **(Barton 1986 and Grizzle et al. 1997).**

It was demonstrated that one of the most common causes of enteritis is a biofilm in water supply pipelines and accumulation of the multiplying microorganisms located in it **(Jafari et al. 2006).** For this reason, the increased levels of intestinal bacteria are related with the increase of intestinal weight. The dietary constituents and microbial flora in intestine seem to have major effects on mucosal architecture and mucus composition (Sharma et al. 1995). The gut flora promote thickening of the intestinal mucosa which reduces its absorptive capacity. Water treatment and sanitization programs are an important control measure to minimize bacterial contamination of water systems and minimize the accumulation of biofilms **(Watkins 2006, Adetunji and Aedeji 2014).** The use of sewage water for irrigating agricultural land is a widespread practice and has a long history because of shortage of water resource and chemical fertilizers in many developing countries **(Zhang et al. 2010; Dakoure et al. 2013).** However, sewage water may carry biological hazards such as bacteria, virus and protozoa as well as chemical hazards mainly heavy metals **(Hussain et al. 2002)** that may present health risks to the human being after utilization of the products harvested after using these sewage water. Treated sewage water may be used for rearing farm animals; however, the quality of meat produced from these animals should be analyzed to make sure that this meat will not constitute health risks to the human eating this meat. The quality and safety of meat can be affected by many factors; one of them is the quality of food and water introduced to the animal during rearing.

Therefore the aim of this work is to determine the health impact and meat quality of chickens drinking treated sewage water and had no access to a clean tap water source.

2. Materials and methods:

2.1. Chickens

A total of 130, one – day old, unsexed commercial broiler chicks (Cobb 500) were purchased from a commercial farm (Badrashein, Giza, Egypt). The chicks were raised in experimental rooms in Pathology Department, Faculty of Veterinary Medicine, Cairo University. The house was an open sided poultry house with litter covered floor (wood shavings). The feed was provided *ad-libitum* throughout the experiment to all chicks which were formulated to meet or exceed the NRC (1994) requirements of broiler chicks. Chickens were vaccinated with HB1 at 7^{th} days, with H5 –ND injection at 13^{th} days old and at 27^{th} days with Colone 30.

2.2. Chemical analysis of water

Sewage water was obtained from toilets with holding tank systems and was diluted with tape water into three different concentrations (raw 100%, 70 & 30%). Moreover the non-centralized treated sewage water was obtained from the Johkasou system installed at the Faculty of Science, Cairo University which was designed to remove biochemical oxygen demand and nitrogen from sewage wastewater according to **Babcock et al. (2004).** Color, odor, pH, phosphate, hardness, chloride, ammonia, nitrite, nitrate, COD and BOD were determined in all types of water used according to the standard methods for the examination of tap water and wastewater **(APHA 1985)** (Table 1)

Table (1): water analysis of different types of water used in the experiment.

Source	sewage water (100%)	Sewage water (70%)	Sewage water (30%)	Treated sewage water	Tap Water
Appearance	Turbid	Turbid	Turbid	Clear	Clear
Colour	Yellowish	Yellowish	Yellowish	Nil	Nil
Odour	Foul	Foul	Foul	Normal	Normal
pH	6.8	6.8	7	7.1	7.9
Phosphate (ppm)	28	18	10	3	4
Hardness (ppm)	170	170	170	170	280
Chloride (ppm)	340	190	140	100	130
Ammonia (ppm)	3	2	1	0.2	0.1
Nitrite (ppm)	4	4	2	0.02	0.01
Nitrate (ppm)	10	10	7	1	0.5
COD (ppm)	65	50	35	4.5	2.5
BOD	190	120	95	10	5

2.3. Experimental Design

130 day old baby chicks Cobb 500 were assigned into 5 pens (26 birds each), in a complete randomized design. Group1 used tap water. Group 2 used treated sewage water. Group 3 used 30% sewage water. Group 4 used 70% sewage water and Group 5 used 100% sewage water. Broiler one day baby chick offered water for drinking from 0 to 35 days old with different treatment as shown in table (2). Meat and Tissue samples were collected at 21 and 35 days old for assessing the meat quality and histopathological investigation.

2.4. Histopathology

Five birds from each group were slaughtered at 21 days and 35 days. The slaughtered birds were subjected to thorough post-mortem examination and tissue specimens from liver, kidneys, bursa of Fabricuis, spleen, thymus, crop, gizzard, proventriculus and intestine were collected and preserved in 10 % neutral buffered formalin solution for histopathology and then processed by the paraffin embedding technique. Tissue sections (5 µm thick) were stained by routine H&E stain **(Bancroft et al. 1996).**

2.5. Microbiological analysis in chicken meat

Ten grams were obtained from the breast and thigh of each slaughtered bird after surface sterilization using hot spatula and homogenized 90 ml peptone water 0.1 %. After 1/10 serial dilution, 0.1 ml of diluted samples were spread on agar plates. Total viable count (TVC) was determined on Plate Count Agar (PCA Oxoid CM0463B, Hampshire, England). Plates were incubated at 35 °C for 48 h (Dale Morton, 2001). Enterobacteriaceae counts were enumerated on violet red bile glucose agar (VRBA; Difco Laboratories Inc., Detroit, Michigan, USA) incubated at 37 °C for 24 h as described by Kornacki and Johnson (2001). Coliforms were determined using Laruyl sulphate tryptose broth and the Most Probable Number technique "MPN" and Enteropathogenic Escherichia coli was isolated on Eosine Methylene Blue agar plates (Oxoid, CM 69) according to the method of Kornacki and Johnson (2001). Samples were analyzed for Salmonellae by pre enrichment on buffer peptone then enrichment on Rappaports Vassiliadis broth (Oxoid, CM 669) and plating on Xylose-Lysine Desoxycholate (XLD) agar (Oxoid,CM 469) and MacConkey agar (Oxoid,CM109)., then the positive colony were exposed to further biochemical and serological examination (Andrews et al., 2001).

2.6. Measurement of pH in chicken meat

Five grams from each of the prepared samples were homogenized with 20 mL distilled water for 10-15 sec. The pH was measured using pH meter (LovibondSenso Direct) with a probe type electrode (Senso Direct Type, 330), where 3 constant readings were obtained and the mean was recorded (**Honikel et al. 1981**).

2.7. Sensory evaluation of chicken meat

Sensory evaluation was performed in each sample (thigh) after cooking in a forced draught oven at 230 ºC for core temperature 75 ºC according to the schemes of Sumarmono and Rahardjo, (2008), Baston and Barna, (2010) and Kenawi, (2005). Five experienced panelists (from both sexes in the age range of 30 to 45 years) were chosen from the staff members of the Department of Food Hygiene and Control at Faculty of Veterinary Medicine, Cairo University, Egypt. Panelists were selected on the basis of previous experience in consuming dressed chicken. After cooking, the panelists were asked to evaluate samples in a randomized order and asked to assign a numerical value between 1 and 7 for following attributes: Color 1 (very poor) – 7 (excellent); Flavor 1 (imperceptible) -7 (extremely intense); tenderness 0 (extremely soft) - 7 (extremely tough) and juiciness 1 (extremely dry) - 7 (extremely moist). Tap water was provided between samples to cleanse the palate. At the end of evaluation of each cooked drumstick, each panelist was asked to give a score for overall acceptability from 1 (dislike very much) to 7 (like very much).

2.8. Statistical analysis

Statistical analyses were conducted for three independent trials. Microbial counts (cfu g^{-1}) were log transferred before statistical analysis. Statistical data analysis was carried out using the using SPSS 17.0 for windows (SPSS Inc, Chicago, IL, USA). Analysis of variance was performed by ANOVA procedure to compare between means of different treatment groups by the least significant difference (LSD) and significance was defined at $P<0.05$.

3. Results

3.1. Pathological findings

Directly following vaccination at day 13 of the experiment, mortalities have occurred in all experimental groups. The highest number of mortalities was recorded in groups 2 and 3 as shown in table (2).

Table (2): number of chicken mortalities in different groups at day 13 of the experiment

group	Treatment	No. of mortality
1	Tap water	2
2	Treated sewage water	2
3	30% sewage water	3
4	70 % sewage water	7
5	100% sewage water	7

The postmortem examination of the dead chickens revealed the presence of typical gout picture reported as precipitation of chalky white material on serosal surface of internal organs and in kidneys with great swelling of the latter (Figure 1). The histopathology of these organs revealed the presence of needle shaped crystals arranged in a fan shape in the serous membranes and interstitial tissue of the kidneys (Figure 2).

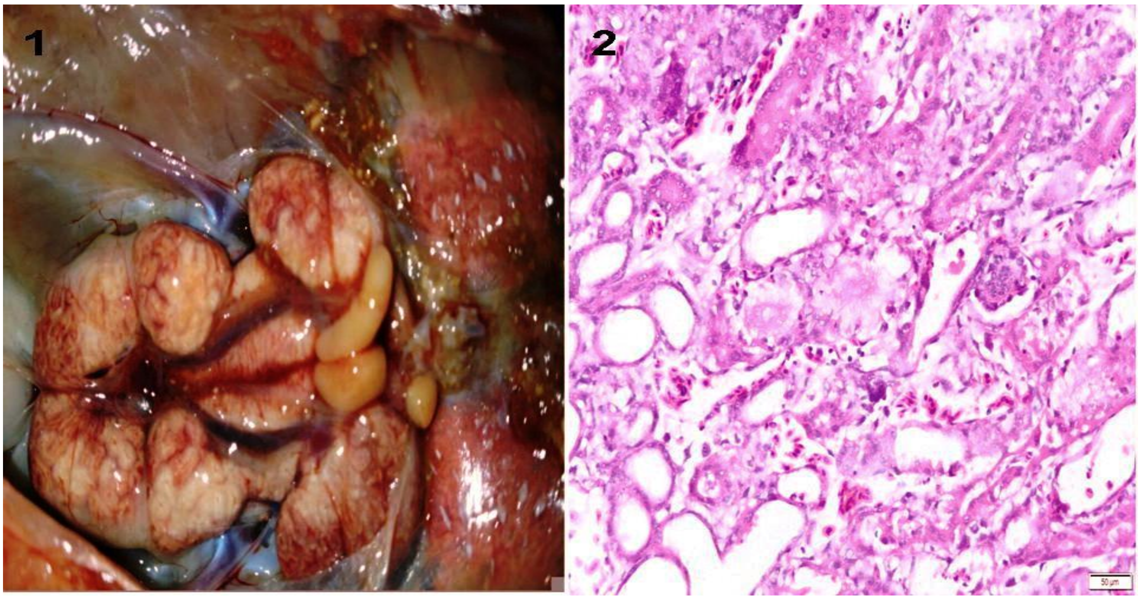
The histopathological examination of organs in the control group and the group receiving treated water were almost similar (Figure 3). However, in the group receiving treated water, there was squamous metaplasia in the lining epithelium of the proventriculus accompanied with mononuclear inflammatory cells infiltration in lamina propria and submucosa. Also there was hyperplasia in the lining epithelium of the crop and heterophilic cells infiltration and fragmentation of koilin layer.

In the group receiving different dilutions of organic water, there were mainly degeneration and necrosis in the lining epithelium of renal tubules (Figure 4), mononuclear inflammatory cells infiltration in the proventricular mucosa and hyperplasia of the lining epithelium of the intestine with mononuclear inflammatory cells infiltrations in lamina propria and submucosa. Focal hepatocytic necrosis with inflammatory cells infiltration was also demonstrated in groups receiving 30 and 70 % organic water.

The lymphoid organs (bursa of Fabricius, thymus and spleen) in the treated sewage water group exhibited a slight lymphocytic depletion (Figure 5).

In sewage water groups, there were different degrees of lymphocytic depletion with reticular cells hyperplasia in addition to prominent epithelial hyperplasia covering plicae of bursa of Fabricius (Figure 6).

Figure (1-2): dead chicken at 13 days of the experiment



Dead chicken at 13 days of the experiment, (1) swelling of the kidneys with chalky white deposits, (2) needle shaped crystals arranged in a fan shape surrounded by few inflammatory cells in the interstitial tissue of the kidneys

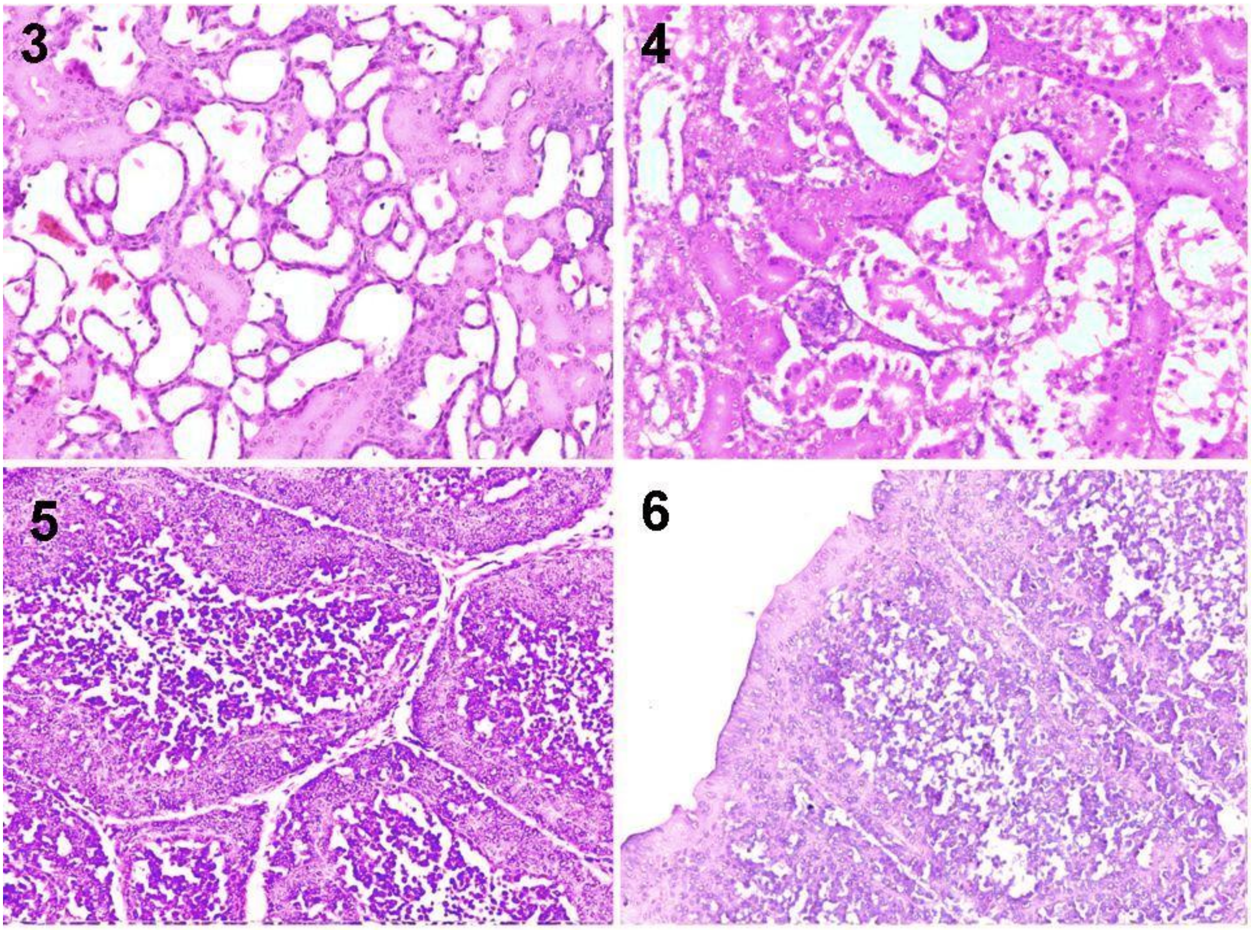


Figure 3: kidney showing slight tubular degeneration in chicken receiving treated water (**Figure 4)** compared to severe degeneration and necrosis in chicken receiving 70% sewage water. **Figure: 5** Bursa of Fabricius showing minor histopathological alterations in chicken receiving treated water whereas **figure (6)**

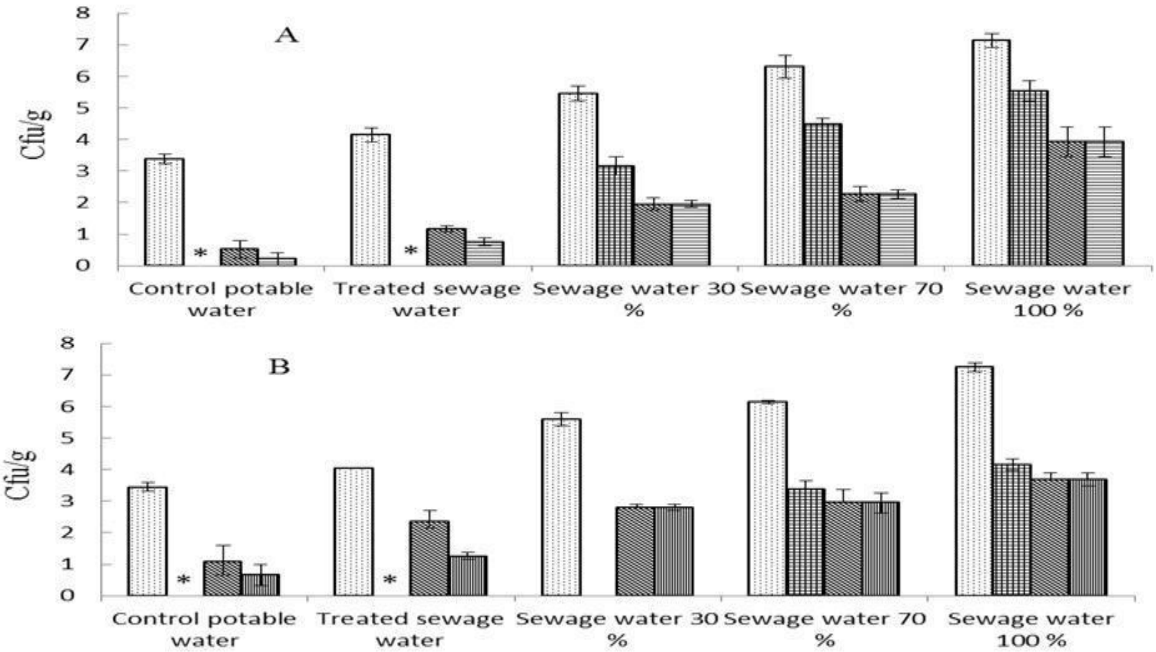
there was lymphoid depletion with reticular cells hyperplasia in chickens receiving 70% sewage water.

3.2. Bacteriological findings in chicken meat

The results revealed that the bacterial counts of meat samples obtained from chicken reared on sewage water at different concentrations were significantly (P<0.05) higher than those obtained from chicken reared on treated sewage water as well as potable water. Moreover, the bacterial counts were significantly (P<0.05) higher when the concentration of sewage water used increased from 30 % to 70 % and 100 %. The counts of aerobic plate counts (APC, log CFU/g) were increased from 3.39 ± 0.15 in meat obtained from chicken reared on potable water for 30 days to 4.14±0.23 in the meat of these chicken when reared on treated sewage water. However, the counts were increased from 3.39 ± 0.15 in meat obtained from chicken reared on potable water for 21 days to 5.45 ± 0.23, 6.30 ± 0.37and 7.14 ± 0.22 in meat obtained from chicken reared on sewage water 30 %, 70 % and 100, respectively. The counts of aerobic plate counts (APC, log cfu/g) were increased from 3.44 ± 0.14 in meat obtained from chicken reared on potable water for 35 days to 4.04 ± 0.02 in the meat of this chicken when reared on treated sewage water. However, the counts were increased from 3.44 ± 0.14 in meat obtained from chicken reared on potable water for 35 days to 5.59 ± 0.20, 6.14 ± 0.05 and 7.24 ± 0.14 in meat obtained from chicken reared on sewage water 30 %, 70 % and 100, respectively.

Enterobacteriaceae were not detected in meats obtained from chicken reared on tap water and treated sewage water, however, they were detected in sewage water and their counts were increased by increasing the concentrations of sewage water (Figure 7).

Fig 7: Bacteriological load of meat obtained from chicken broilers reared on potable water, treated sewage water and sewage water at different concentrations



A) Meat from broilers at age 21 days

B) Meat from broilers at age 35 days

***Counts under the detectable limit of standard plate count (1 log cfu/g) APC, Enterobacteriaceae counts , Coliforms (MPN), fecal coliforms (MPN/g)**

3.3. PH value of chicken meat

The results revealed that pH values of all investigated groups were significantly (P<0.05) increased by increasing concentration of untreated sewage water (Table

3). The pH values of chicken (young and old) meats reared on different concentrations of untreated sewage water were significantly (P<0.05) higher than control and treated water groups. However, the pH values of all meat samples obtained from all groups were within the normal accepted values.

Table 3: The pH values of meat obtained from chicken reared on potable tap water, treated sewage water and untreated sewage water at different concentrations

treatments	pH values*	
	21 day old chicken	35 day old chicken
Tap water	**5.32±0.03[a]**	**5.29±0.13[a]**
Treated sewage water	**5.29±0.03[a]**	**5.56±0.04[b]**
Sewage water 30 % [I]	**5.49±0.02[b]**	**5.79,[c]±0.08[c]**
Sewage water 70 % [II]	**5.04±0.06[c]**	**5.61 ±0.21[cd]**
Sewage water 100 % [III]	**5.46±0.21[b]**	**5.87±0.13[d]**

*Values are the mean of three independent trials ± SE. Values with different superscripts [a-d] within the same column are significantly different (*P<0.5*). [I] The water was prepared by mixing 30 % sewage water with 70 %tap water. [II] The water was prepared by mixing 70 % sewage water with 30 %tap water. [III] The sewage water was used without mixing with tap water.

3.4. Sensory findings of chicken meat

The sensory attributes (appearance, flavor, juiciness, tenderness and overall acceptability) of meat obtained from of chicken of 21 and 35 days old reared on potable water, treated sewage water and untreated sewage water are presented in

Tables 4 and 5. The scores of different attributes of meat obtained from chicken reared on treated sewage water were not significantly (*P>0.05*) different from those obtained from chicken reared on potable water. The sensory scores of meat obtained from chicken reared on untreated sewage water were significantly (*P<0.05)* lower than those obtained from chicken reared on potable water or treated sewage water. The sensory scores of meat obtained from chicken reared on untreated sewage increased significantly (*P<0.05)* by increasing the concentration of sewage water. The flavor and overall acceptability scores of meat obtained from chicken reared on sewage water at concentration of 100 % were lower than the acceptable limit (3.5) and were not acceptable from the sensory quality view. Moreover, the meat sample obtained from this group revealed a darker color. The meat obtained from chicken at 35 days reared on sewage water at concentrations of 70 and 100 % revealed flavor scores lower than the acceptable limit (3.5).

Table 4 : The sensory scores values (1, dislike completely to 7, like completely) of meat obtained from chicken at the 21st day reared on potable tap water, treated sewage water and untreated sewage water at different concentrations

Treatments	Sensory scores*				
	Appearance	Flavor	Juiciness	Tenderness	Overall acceptability
Tap water	**6.12±0.12[a]**	**6.04±0.18[a]**	**5.69±0.13[a]**	**5.76±0.10[a]**	**6.03±0.26[a]**
Treated sewage	**6.10±0.10[a]**	**6.03±0.27[a]**	**6.02±0.21[a]**	**5.78±0.15[a]**	**5.64±0.23[a]**

Sewage water 30 %[I]	**5.32±0.07[b]**	**5.03±0.20[b]**	**4.70±0.21[b]**	**5.36±0.16[b]**	**5.04±0.35[b]**
Sewage water 70 %[II]	**4.68±0.15[c]**	**4.05±0.24[c]**	**3.99±0.76[c]**	**4.36±0.15[c]**	**4.33±0.19[c]**
Sewage water 100 %[III]	**4.38±0.14[c]**	**3.33±0.20[d]**	**4.23±0.10[bc]**	**3.98±0.25[d]**	**3.32±0.20[d]**

*Values are the mean of three independent trials±SE. Values with different superscripts [a-d] within the same column are significantly different (*P<0.5*). [I] The water was prepared by mixing 30 % sewage water with 70 % tap water. [II] The water was prepared by mixing 70 % sewage water with 30 % tap water. [III] The sewage water was used without mixing with tap water.

Table 5: The sensory scores values (1, dislike completely to 7, like completely) of meat obtained from chicken at the 35th day reared on tap water, treated sewage water and untreated sewage water at different concentrations

Treatments	Sensory scores*				
	Appearance	Flavor	Juiciness	Tenderness	Overall
Tap water	**6.05±0.24[a]**	**6.29±0.28[a]**	**5.89±0.15[a]**	**5.68±0.16[a]**	**5.66±0.10[a]**
Treated sewage water	**5.79±0.1[a]**	**6.23±0.08[a]**	**6.04±0.43[a]**	**5.67±0.18[a]**	**5.52±0.10[a]**
Sewage water 30 %[I]	**5.36±0.15[b]**	**4.60±0.20[b]**	**5.40±0.11[b]**	**4.62±0.25[b]**	**4.61±0.17[b]**

Sewage water 70 %II	**4.33±0.22c**	**3.33±0.20c**	**4.61±0.27c**	**4.43±0.20b**	**3.23±0.11c**
Sewage water 100 %III	**3.94±0.30c**	**3.00±0.12c**	**4.33±0.20c**	**4.39±0.15b**	**3.18±0.17c**

*Values are the mean of three independent trials ± SE. Values with different superscripts a-d within the same column are significantly different (*P<0.5*). I The water was prepared by mixing 30 % sewage water with 70 % tap water. II The water was prepared by mixing 70 % sewage water with 30 %tap water. III The sewage water was used without mixing with tap water.

4. Discussion

The pathological lesions were mainly observed in the group receiving 70 % sewage water and were lesser in the group receiving 100 % sewage water. This could be due to less water consumption in the group receiving 100 % sewage water since poor water quality may decrease water consumption **(Koelkebeck et al. 1999).** The histopathological results revealed that the most affected organ in the sewage water groups was the kidneys and this agreed with **Oraby (2015)** who reported the significant increase in blood urea nitrogen and serum creatinine level in the grazed sheep drinking sewage water. He attributed that to the nephrotoxic effect of heavy metal cadmium on renal tissues. This opinion was supported by **El sharkawy et al. (2008)** who found that sheep reared on sewage polluted area had higher concentration of cadmium. The lymphoid depletion observed in the bursa of Fabricius of chickens receiving 70 % sewage water compared to minor histopathological alteration in the chickens receiving treated water emphasize on the relation between water quality and immune resistance **(Barton 1986; Grizzle et al. 1997).** The thickening of chicken gut reported in this study may be attributed to the presence of microorganisms in the contaminated water used (**Jafari et al. 2006).**

The bacteriological data obtained clarify that all of the coliforms in the chicken reared on sewage water at different concentrations were fecal in origin. Moreover, these counts of APC of meat obtained from chickens reared on sewage water were higher than the limit standardized by the Egyptian standard specifications (5 log CFU/g) **(ESS 2005).** The results of meat samples obtained from chicken that were reared on sewage water can be substantiated by the results of **Guo et al. (2014**) who observed that soils irrigated with sewage effluents for different times demonstrated higher densities of bacteria, actinomycete, and fungi.

Enterobacteriaceae were not detected in chicken meats but were detected in sewage water. These bacteria are among the various organisms, which are normal inhabitant in the intestinal tract of animals and humans and consequently contaminate the sewage water. It is well documented that the most important bacterial member is total coliforms (including Enterobacteriaceae and fecal coliforms**)** which used as indicators for sewage pollution **(Hughes and Thompson 2003).** Even though low bacterial load of meats obtained from animals reared on treated sewage water was referred to the biological treatment of sewage water via activating sludge process with aerobic bacteria by using Johkasou CE mode. These results were in good agreement with results of **Subramani and Arulalan (2012)** who recorded that the effluent obtained from a properly operating activated sludge plant is of high quality with bacteria removal is up to 90-95 percent. On the other hand, Salmonellae and Enteropathogenic E. coli were isolated from all treatments in this study. The pH value of meat is considered as one of the most important parameters for determination of meat quality. In the current study the pH values of chicken meat increased with the increase of untreated sewage water. Although the PH value of meat in all groups were still within the normal accepted limit. Sewage water contain various type of organisms, high concentration of chemicals such as ammonium, nitrate, phosphorus; carbonate and bicarbonate lead to increase its pH value **(Mara 2000; Mara and Horan 2003).** These results may be explained by reduction of both microbial and chemical content of sewage water after treatment using Johkasou CE mode (**Subramani and Arulalan 2012).** Sensory attributes are the most important factors that influence the perception of meat and meat products by consumers and manufacturers **(AMSA 1995).** Consumer acceptance of meat depends mainly on the appearance, odor, and texture of the product. The scores of different attributes of meat obtained from chicken reared on treated sewage water were not significantly (*P>0.05*) different from those obtained from chicken reared on potable water. On the other hand, the sensory scores, flavor and overall acceptability scores of meat obtained from chicken reared on untreated sewage water increased significantly (*P<0.05)* by increasing the concentration of sewage water. Moreover the meat of chicken reared on sewage water exhibited a darker color compared to control. Sewage water (30, 70, 100%) characterized by yellow color and foul odor that had direct effect on all sensory characteristics of meat specially color (dark meat) and odor (fecal odor). Dark yellow coloration of untreated sewage water may be due to presence of inorganic ions, such as iron & manganese **(Indian standard 1983)** as well as fecal pollution.

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