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Does feeding African Catfish, *Clarias gariepinus* vinegar-immersed poultry viscera affect its growth performance, hygienic status and pathogenic bacterial load?

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ABSTRACT

This study was conducted to investigate the effects of replacing commercial feed with vinegar-immersed poultry viscera on growth performance, physiological status and bacterial load of the catfish, *Clarias gariepinus*. Fish were fed on a commercial diet (T1), poultry viscera (T2) and poultry viscera immersed in commercial vinegar (T3). Catfish with an average body weight of 520g were stocked in circular fiberglass tanks each of 2 m^3 water volume for 98 days. Water quality parameters, growth performance, feed utilization, flesh composition, serum analysis and bacterial load of feed, water and fish, were measured. Ammonia content in rearing water decreased significantly in T3 compared to T2. There were not any significant differences detected ($P>0.05$) in survival and growth performance, while significant ($P\leq0.05$) differences in feed utilization and whole-body composition were detected among treatments. The quality indices of fish in terms of flesh composition were in favor of T3. A decrease in the serum's total protein and albumin and an increase in the serum's globulin and cholesterol levels were observed when fish was fed on poultry viscera only. Pathogenic bacterial load in feed, rearing water and fish intestine were at their lowest limit in T3. This study shows that *C. gariepinus* can feed on poultry viscera immersed in commercial vinegar as an alternative feedstuff source to commercial high-priced feed. The ecological impacts of treating environmentally hazardous by-products, like poultry viscera and converting it to a high value by-product are a matter of interest for both aquaculturists and environmentalists alike. More research work is needed to take advantage of the current results in a commercially applicable scale.

INTRODUCTION

African Catfish are known to be omnivorous in their food habits (Anyanwu *et al.,* 2012). They are also hardy and tolerant to a wide range of stressful environmental conditions (Nwani *et al.,* 2015). They are widely cultured in freshwater ponds because of their ease in reproduction, high growth rate, tolerance to high density culture conditions, resistance to diseases, good flesh quality and ability to accept a wide variety of feed (Nyina-wamwiza *et al.,* 2007; Khan and Abidi, 2011; Chor *et al.,* 2013).

Egyptians dating back to ancient times consider catfish one of the most favorite fish types. Many images of Catfish appeared on the walls of ancient Pharaonic temples. In 2015, the total catfish production in Egypt was 37,914 tons/year, of which 7,455 tons/year were obtained from aquaculture (GAFRD, 2015). African Catfish require about 40% CP in their diet and the best results have been achieved with crude protein value ranging from 35 to 50% for all African Catfish species (Adebayo and Quadri, 2005).

Nutrition is one of the most important factors in fish farming because it contributes to up to 50% of fish production costs (Omoruwou and Edema, 2011). Feeding is one of the major constraints facing aquaculture. The importance of fish meals in the production of animal feeds cannot be disputed, but forms the highest cost and therefore raises the price of the feed exponentially (Olaniyi and Salau, 2013). The feeding can be harmful to the fish's health and may cause a remarkable drop in water quality, reduce growth, decrease feed utilization and increase susceptibility to infection (Priestley *et al.*, 2006). Therefore, there is the need to balance between rapid fish growth and the optimum use of the supplemented feed in order to be economically viable and for the fish to be in good health (Wuraola1 and Omodara, 2014).

Many indigenously raw materials mainly poultry by-product meals, blood meals, various oil cakes, cereal by-products, leaf meals, etc… are available in most countries (Akand *et al.*, 1991). These raw materials can be used in formulating supplemental feed for rearing and culturing of different fish species (Bhadra *et al.*, 1997). Compared to plant-based proteins, poultry viscera are easily available throughout the year at a very low price. They are also high in proteins and low in carbohydrates. They have a balanced amino acids profile, are high in digestible feedstuff and lack anti-nutritional factors (Goda *et al.*, 2007; Bhaskar *et al.*, 2015). Hence, chicken viscera are considered a probable substitute for fishmeals in diets for a number of fish species (Gupta *et al.*, 2013; Bhaskar *et al.*, 2015).

Poultry by-product meals have been tested in a wide range of fish species, including common carp *Cyprinus carpio* (Paixao and Filho, 1989), grass carp *Ctenopharyngodon idella* (Tabinda and Butt, 2012), Nile Tilapia *Oreochromis niloticus* (El-Husseiny *et al.*, 2006; Metwalli, 2008; Yones and Metwalli, 2016), catfish *Pangasianodon hypophthalmus* (HAO and YU, 2003), African Catfish *Clarias gariepinus* (Abdel-Warith *et al.*, 2001), and European Seabass, *Dicentrarchus labrax* (Srour *et al.*, 2016). Worldwide, poultry viscera are being used in many catfish farms instead of commercial feed which may cause exposure to many fungal and bacterial diseases. More than 500,000 tons of poultry by-products are available yearly in Egypt (MALR, 2015). These large amounts of poultry by-product meals are causing some serious environmental problems, such as vectors for insects, vermin, bacteria, and viruses, which may result in water contamination (as a result of nutrient leaching and pathogenic microorganisms) and air pollution (noxious gases and nuisance odorants) (FAO, 2011). However, disease, which is caused by pathogenic bacteria, is a major hindrance for aquaculture production (Romero *et al.*, 2012).

Vinegar has antimicrobial properties, which makes it useful for a number of applications (Rutala *et al.* 2000; Dohar 2003). For inhibiting the growth of foodborne pathogenic microorganisms in food, consumers prefer natural preservative methods (Rauha and *et al.* 2000). To our knowledge, there hasn't been any previous data concerning the treatment of poultry by-products using vinegar. Therefore, this study aims to assess the effects of feeding catfish *C. gariepinus* poultry viscera immersed in commercial vinegar on water quality, growth performance, feed utilization, flesh composition, serum constituents and pathogenic microbial load in both water and fish, and then compare them to the effects that had resulted from those fed on untreated poultry viscera and commercial feed.

MATERIALS AND METHODS

Experimental Design

This study was conducted at the Fish Nutrition Laboratory, Baltim Research Station, National Institute of Oceanography and Fisheries (NIOF), Egypt. A total of 180 adult catfish

C. gariepinus with an average weight of 520±25 g/fish were acclimatized to experimental conditions for seven days in concrete tanks (5×10×1 m). Experimental fish were obtained from a private farm at Kafr El-Sheikh Governorate, Egypt. Nine circular fiberglass tanks and two-m^3 water volume were used for stocking the experimental catfish. The tanks were filled with de-chlorinated tap water. After acclimatization, twenty randomly selected fish were stocked in each tank for 14 weeks (98 days). The feed ration was adjusted once every 10 days, based on fish biomass. The water exchange was 20% daily. Also, the accumulated waste materials were removed every day. Fish were fed twice a day at 09:00 and 15:00 at 5% body weight for the commercial diet group and 20% of poultry viscera and poultry viscera immersed in commercial vinegar groups. The total feeds consumed in each tank were recorded and the feed consumed by each fish was calculated accordingly.

Feed Preparation

Three experimental diets were formulated as, treatment 1: (T1) fish were fed a commercial diet containing 30.5% crude protein, treatment 2 (T2): fish were fed fresh poultry viscera only, and treatment 3 (T3): fish were fed fresh poultry viscera immersed in commercial vinegar only. The experimental diets were carried out with three replicates each. Fresh poultry viscera were collected freshly from poultry shops every day. Fresh poultry viscera were cut to small pieces each of 3 cm to fit the fish feeding in T2. In T3, one-kilogram of poultry viscera was immersed in one-liter of commercial sugarcane vinegar® (5%) (weight/volume) for 1 day to avoid bacterial or fungal pathogens before being used. Chemical compositions of these diets are shown in Table (1).

Table 1: Chemical compositions of the experimental diet and poultry viscera as dry matter basis.

Ingredients	T1[1]	T2	T3
Dry matter (DM)	93.5	35.5	36.7
Crud protein (CP)	30.5	34.3	34.8
Ether extract	9.0	32.8	31.6
Crude fibre	6.2	2.2	2.1
Ash	8.5	6.0	6.0
Nitrogen free extract (NFE)[2]	45.8	24.7	25.5
Gross energy (MJ/kg DM)[3]	18.8	25.3	25.1

[1]Each kilogram of feeding pellets contains fish meal (5%), corn gluten (7%), soybean meal (30%), yellow corn (10%), rice bran (19%), wheat bran (25%), soya oil (3%), dicalcium phosphate (0.7) and premix (0.3%). Each kilogram of premix contain (mg /kg): p-amino benzoic acid (9.48), D-biotin (0.38), inositol (379.20), niacin (37.92); Ca-pantothenate (56.88), pyridoxine-HCl (11.38), riboflavin (7.58), thiamine-HCl (3.79), L-ascorbyl-2-phosphate Mg (APM) (296.00), folic acid (0.76), cyanocobalamin (0.08), menadione (3.80), vitamin A-palmitate (17.85), a-tocopherol (18.96), calciferol (1.14), K_2PO_4 (2.011), $Ca_3(PO_4)_2$ (2.736), Mg SO_4 $7H_2O$ (3.058) and NaH_2PO_4 $2H_2O$ (0.795).

[2]Nitrogen free extract (NFE) = 100 - [Ash% + lipid %+ protein %+Fiber%].

[3]GE (MJ/kg DM) = (protein content × 23.6) + (Lipid content × 39.5) + carbohydrate content × 17.2) (NRC,1993).

Water quality parameters

Water quality parameters (temperature, pH, dissolved oxygen and ammonia) were measured once a week according to the standard method described in APHA (1998).

Growth performance and feed utilization

Growth performance and feed utilization were evaluated as, gained weight (GW) = [(fw - iw)/ iw], specific growth rate (SGR% day^{-1}) =100× [ln (fw) - ln (iw)] t^{-1}, feed conversion ratio (FCR) =FI - GW, protein efficiency ratio (PER) = (GW - FI), protein productive value (PPV) =100 (PI/PF) and energy utilization (EU%) =100 (E.g./ EI). Where iw and fw= mean initial and final body weight (g), respectively, t is the duration of the experiment (98 days), FI is the total feed intake (g), PI is protein intake (g), PF is protein fed (g), EG is the energy gained (Kcal/100g) and EI is energy intake (Kcal/100 g).

Biometric parameters

At the end of the experiments, the general viscera, liver and gut were taken from each experimental fish for biometric parameters. Visceral somatic indexes (VSI), hepatosomatic index (HSI) and relative gut length (RGL) were calculated as the following equation: VSI=visceral weight (g)/fish weight (g), HSI=liver weight (g)/fish weight (g) and RG L=absolute gut length (cm)/total body length (cm). Condition factor (K %)= (FW/FL^3) × 100,

where FW=final weight (g) and FL=final length (cm). Survival rate (%) = (No. of fish at the end of the experiments/No. of fish at the start of the experiments) × 100.

Chemical analysis of diets and whole-body composition

Chemical analysis of the experimental diets and flesh composition of catfish *C. gariepinus* were performed. Fifteen fish per treatment were scaled, headed and cut from the back. The viscera of each fish were removed. The flesh was then passed rapidly through a meat chopper for several times and mixed thoroughly after each turn to estimate dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash content according to AOAC (2000).

Blood collection and biochemical measurements

The blood was collected from the caudal vessels and the blood samples were taken in dry clean centrifuge tubes. Serum was separated at 3000 rpm for 15 minutes using a centrifuge and kept in well stoppered plastic vials at -20 °C until analysis. Serum total protein and albumin were determined according to the method of Doumas (1975), urea enzymatic was measured according to Patton and Crouch (1977). Serum cholesterol was determined according to the method of Allain *et al*. (1974). The triglycerides were assessed by the Fossati three-step enzymatic reaction with a Trinder endpoint (Fossati and Prencipe, 1982). Serum lipase was determined by the enzymatic colorimetric assay according to Panteghini *et al*. (1991). Amylase was determined by the method of Winn-Deen *et al*. (1988).

Bacterial load

Preparation of samples: treatment feeds, water and fish gut were removed from fresh specimens then put in a saline solution and homogenized vigorously according to (Al-Harbi, 2010). According to (PHE, 2014), total bacterial count (TBC) was calculated as follows; 1 ml of each sample diluted in 99 ml saline then 1 ml of this dilution was cultured using pour plate technique on nutrient agar plate medium and incubated at 30°C for 18-24 hours. The counts were calculated as CFU /100 ml. Bacterial evaluation was performed by the membrane filtration technique according to ISO 9308/1(1990 b) and 7899/2 (1984). One ml of each diluted sample was filtered through 0.45µm pore with a diameter of 47 mm, and a grid of sterile cellulose membrane. For fecal streptococci, membranes were placed onto the surface of Slanetz and Bartley medium after 72 hours of incubation at 37°C. The dark red colonies were counted for the detection of Vibrio spp. The membranes were placed onto the surface of TCBS (Thiosulphate citrate bile salt sucrose) agar and incubated at 37°C for 24 hours. Large green and /or yellow colonies were considered to be Vibrio spp. Bismuth Sulphite Agar Medium and SS-agar were used in the detection and enumerations of Salmonella spp. after incubation at 35-37°C for 18-24 hours, the black colony was counted. Aeromonas Isolation Medium Base was used for the detection of Aeromonas sp. After incubation at 35-37°C for 18-24 hours, the dark green, opaque with dark center colony was counted. For the detection and counting of the Staphylococcus aureus, membranes were placed onto the surface of Mannitol Salt agar and incubated at 35°C for 18-24 hours, yellow colonies were counted. For the detection and counting of the Pseudomonas sp, membranes were placed onto the surface of Pseudomonas isolation agar blue-green colonies and observed after incubation for 40-48 hours at 35°C.

Statistical analysis

The statistical analysis of the obtained data was done by SPSS computer software package, version 20 for one-way ANOVA according to Levesque (2007). Means were statistically compared for the significance ($P \leq 0.05$) using multiple range test SPSS 20.

RESULTS AND DISCUSSION

Table 2 shows the water quality data. Non-significant differences were observed in temperature and pH among treatments during the experimental period. However, values of ammonia, nitrite and dissolved oxygen exhibited significant differences. Water temperature ranged from 20.0 to 22.5±1.0°C. Water pH was

(7.42±0.03), salinity was 0.9±0.2 ppt, NO_2-N was 0.19±0.01 for T1, while 0.26±0.04 was for T2 and 0.22±0.03 mg/L for T3.

Dissolved oxygen varied from 7.0±0.4, 6.1±0.5, 6.5±0.3 mg/L for T1, T2 and T3, respectively. Total content of ammonia exhibited higher significant ($P \leq 0.05$) values in T2 (0.516ppm) compared with moderate values in T1 (0.406ppm) and the lowest values in T3 (0.370ppm) without significant differences between T1 and T3.

Table 2: Water quality parameters during the experimental period.

Water quality	T1	T2	T3
Temperature (°C)	22.2±0.6	22.7±0.5	22.8±0.7
pH	7.363±0.01^{b}	7.497±0.01^{a}	7.40±0.02^{b}
DO_2 (ppm)	7.0 ±0.4^{a}	6.1±0.5^{b}	6.5 ±0.3^{ab}
NH_3 (ppm)	0.399±0.015^{b}	0.512±0.012^{a}	0.347±0.012^{c}
NO_2-N (ppm)	0.19 ± 0.01^{b}	0.26 ± 0.04^{a}	0.22± 0.03^{ab}

Growth performance, survival ratio and feed utilization of catfish *C. gariepinus* reared under different feeding regimes are presented in Table (3). The results indicated that there weren't any significant differences in the final body weight, gain, specific growth rate and survival rate among treatments. Data of feed utilization parameters showed significant differences among treatments ($P \leq 0.05$). The highest feed intake value was observed in T2 followed by T3 and T1, respectively. PER showed higher but non-significant differences between poultry viscera by-product treatments and commercial diet. The same trend with significant differences ($P \leq 0.05$) was observed in protein productive values where the lowest value was observed in T1 and the highest value was in T3. Better utilization of energy was detected in favor of T1, where the highest value was in T1 and the lowest value was obtained in T2.

Table 3: Growth parameters, survival and feed utilization during the experimental period.

Treatments*	T1	T2	T3
IW (g/fish)	516.7 ±12.3	530.0±9.5	535.0 ±10.6
FW (g/fish)	772.7 ± 34.9	741.0±36.4	766.3 ±14.3
GW (g/fish)	256.0 ± 34.2	211.0±31.1	231.3 ±40.1
SGR(%/day)	5.6 ± 0.13	5.4 ±0.16	5.5±0.07
Survival rate %	98.89±1.11	94.44±1.93	94.44±2.94
FI (g/fish)	576 ±67.7^{b}	1600 ± 57.7^{a}	1416 ±44.1^{a}
FCR	2.0 ±0.1^{b}	2.8 ± 0.5^{a}	2.3±0.1^{a}
PER	1.49 ±0.02	2.17 ±0.41	2.39±0.18
PPV	5.9 ±0.5^{b}	8.2 ±1.4^{b}	14.6±1.1^{a}
EU	23.9 ±2.3^{a}	10.5 ±1.4^{b}	15.0 ±1.5^{b}

*Means followed by the same letters are not significant, but different letters are significant ($P < 0.05$).

Data in Table 4 present values of hepatosomatic index (HSI), visceral-somatic index (VSI), relative gut length (RGL), condition factor (K) and pH of intestine, stomach, fish flesh and the whole body. Values of biometric parameters showed significant differences ($P \leq 0.05$) among treatments. Visceral-somatic index was significantly different ($P \leq 0.05$), where the lowest value was recorded in T2 group and the highest one was recorded in T3. The minimum values of HSI, RGL and K were recorded in T3 treatments. Results showed that there weren't any significant differences of pH values in intestine, stomach, and fish flesh, while a significant ($P \leq 0.05$) difference among treatments in the whole body was detected with the highest value in T2.

Table 4: Biometric parameters and pH values of fish at the end of the experiment.

Treatment*		T1	T2	T3
VSI		5.8 ±1.00^{b}	4.8 ±0.12^{b}	6.8 ±0.95^{a}
HSI		1.2 ±0.01^{a}	1.2 ±0.06^{a}	1.0 ±0.07^{b}
RGL		2.15 ±0.01^{b}	2.45 ±0.06^{a}	2.05 ±0.07^{b}
Condition factor (K)		0.9 ±0.04^{a}	0.9 ±0.03^{a}	0.8 ±0.05^{b}
pH	Intestine	6.52±0.48	6.75±0.45	6.59±0.51
	Stomach	6.47±0.53	6.53±0.58	6.60 ±0.60
	Flesh	6.5± 0.40	6.57±0.43	6.46±0.45
	Whole body	6.03±0.01^{b}	6.15±0.03^{a}	6.08±0.01^{ab}

*Means followed by the same letters are not significant, but different letters are significant ($P< 0.05$).

The flesh composition of the experimental fish as presented in Table 5 shows that there were significant variations ($P \leq 0.05$) in the moisture, crude protein, ether extract and ash content levels of the fish fed on different experimental diets. However, the moisture and ash were lower in T3; whereas crude protein was lower in T2 treatment. On the other hand, moisture and ash were significantly higher in T2 than in the other treatments. Crude protein value was the highest in T3 and ether extract was the highest T1 group.

Table 5: Flesh composition of fish at the end of the experiment.

Treatments*	Initial samples	T1	T2	T3
Moisture	78.31±0.45	73.95±0.45^{b}	77.6±0. 90^{a}	72.80 ±0.60^{b}
Crude protein	16.13±0.62	19.7±0.62^{b}	16.4±0.58^{c}	21.7±0.24^{a}
Ether extract	1.38±0.15	2.75±0.15^{a}	2.0 ±0.10^{b}	2.05±0.05^{b}
Ash	4.14±0.01	3.53±0.01^{b}	4.36±0.06^{a}	3.33±0.02^{c}

*Means followed by the same letters are not significant, but different letters are significant ($P< 0.05$).

Means of total protein (g dL^{-1}) and globulin (g dL^{-1}) indicated a significant difference ($P \leq 0.05$) between treatments, with the minimum values recorded for T2 and the maximum for T1 treatment (Table 6). Results of serum albumin (g dL^{-1}) and A/G indicated a significant difference ($P \leq 0.05$) between treatments, with the minimum values recorded for T3 treatment and the maximum values for T1 treatment. Means of serum cholesterol (mg dL^{-1}), triglycerides (mg dL^{-1}) and lipase (U/l) indicated that there is a significant difference ($P \leq 0.05$) between treatments. The lowest values of these parameters were observed in T2 but the highest values were observed in T3 treatment. Data analysis of serum urea (mg dL^{-1}) and lipase (U/l) indicated that the lowest values were observed in T2 and T3 treatments and the highest values were recorded for T1 treatment.

Table 6: Blood biochemical parameters at end of experimental period

Treatments*	T1	T2	T3
Total Protein (g dL^{-1})	3.9±0.18^{a}	2.5±0.12^{b}	3.5±0.12^{a}
Albumin (g dL^{-1})	1.6±0.12^{a}	1.4±0.06^{b}	1.2±0.06^{b}
Globulin (g dL^{-1})	2.3±0.06^{a}	1.9±0.06^{b}	2.3±0.07^{a}
A/G	0.7±0.04^{b}	0.8±0.01^{a}	0.5±0.01^{c}
Cholesterol (mg dL^{-1})	158.7±2.6^{b}	150.0±2.6^{c}	190.0±4.6^{a}
Triglyceride (mg dL^{-1})	108.0±1.8^{b}	88.1±1.9^{c}	255.3±5.5^{a}
Urea (mg dL^{-1})	14.3±0.88^{a}	12.0±0.58^{b}	11.0±0.58^{b}
Lipase (U/l)	7.3±0.23^{b}	7.0± 0.12^{b}	14.5±0.48^{a}
Amylase (U/l)	1.9±0.12^{a}	1.2±0.06^{b}	1.3±0.09^{b}

*Values with different superscript are significantly different ($P< 0.05$).

Table 7: Bacterial load count composition recovered from samples of treatment feed, water and intestine of *Clarias gariepinus* at end of experimental period.

Treatments*	Treatments feed			Water			Intestine of fish		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Total Bacterial Count	119	527	17	179	670	80	138	355	37
Shigella sp.	35	147	Nil	35	154	2	35	110	11
Salmonella sp.	35	90	Nil	15	45	2	Nil	Nil	Nil
Enterococcus spp.	10	180	8	Nil	188	Nil	Nil	165	Nil
Vibrio spp	2	Nil	Nil	46	89	Nil	46	4	Nil
Aeromonas sp	16	58	7	43	83	6	32	48	14
Staphylococcus aerus	9	36	Nil	12	85	Nil	Nil	17	Nil

DISCUSSION

The water quality values obtained in this study were not within the acceptable ranges for *C. gariepinus* according to the studies of Eyo and Olatunde (2000) and Olurin *et al.* (2006). However, dissolved oxygen, temperature and pH values were within the recommended levels (Moogouel et al., 2010). Oyewole et al (2008), stated that African Catfish can grow very well in culture water where DO. frequently decreases below the optimum level (5ppm). Consumption time, leaching rate, protein content and assimilation of feed are the main factors affecting the content of ammonia and nitrite in fish rearing water (Alabaster and Lloyd, 1980. Lower contents of ammonia and NO2 in T3 in the present study reflected quicker and better assimilation of poultry viscera immersed in commercial vinegar in comparison to T2. Also, low feed's attraction and leaching time in T1 reflect the moderate value of ammonia. Generally, ammonia is toxic to fish if allowed to accumulate in fish ponds. Fish cannot efficiently extract energy from feed under toxic levels. If the ammonia gets lethal levels, the fish will become lethargic and eventually fall into a coma and die (Alabaster and Lloyd, 1980; Department of Water Affairs and Forestry, 1996). Values of ammonia obtained in the current study are higher than Ajiboye et al. (2015) and lower than Moogouel et al. (2010), but still within critical limits. At a concentration of 0.19 mg NH3/l. the growth rate of channel catfish is significantly reduced. Possible sub-lethal effects in warm-water fish occur in the range of 0.3 - 0.8 mg NH3 /l. The 96-hour LC50 for African Catfish larvae (*Clarias gariepinus*) is 2.30 mg NH3 /l (Alabaster and Lloyd, 1980; Department of Water Affairs and Forestry, 1996). Data obtained in the present study for NO2 were not in the desirable range. Santhosh and Singh (2007) recommended nitrite concentration in water should not exceed 0.5 mg L-1, while OATA (2008) recommended that it should not exceed 0.2 mg L-1 in freshwater. Better values of oxygen, ammonia and nitrite were obtained in T3 rather than T2, meaning that treating poultry viscera with commercial vinegar improved environmental conditions.

There weren't any significant differences among treatments for growth performance and most feed utilization parameters. Similar results were reported by Yang *et al.* (2006) for *Carassius auratus gibelio* (Hu *et al.,* 2008), *Clarias gariepinus* (Goda *et al.,* 2007), *Clarias batrachus* (Giri *et al.,* 2010; Gupta *et al.,* 2013), grass carp *Ctenopharyngodon idella* (Tabinda and Butt, 2012), *Cirrhinus mirigala* (Tabinda *et al.,* 2013), *Pelodiscus sinensis* (Sun *et al.,* 2014) and *Lutjanus guttatus* (Hernandez *et al.,* 2014) fed on diets containing graded poultry viscera meals. In this study, poultry viscera contain nearly 35% crude protein, 32.5% ether extract and 2.2% ash, which might be a good for catfish. These results were in agreement with Nyina-Wamwiza *et al.* (2007) who obtained data. Giri *et al*. (2000) have

showed that dried chicken viscera can be incorporated in up to 30% of the diet for juveniles of *Clarias batrachus* without affecting nutrients' digestion and performance. Poultry by-product meals present a very good source of dietary protein for fish culture. However, they vary in quality between regions and countries, and some sources are deficient in one or more essential amino acids (Davies *et al.,* 1991; Nengas *et al.,* 1999; Yigit *et al.,* 2006). Results of the current study disagreed with Yamamoto *et al.* (2002) who reported that feed intake and growth performance decreased in *Clarias gariepinus* (Abdel-Wareth *et al.,* 2001; Goda *et al.,* 2007), *Clarias batracus* (Giri *et al.,* 2000; Hu *et al.,* 2008) and *Anabas testudineus* (Bhaskar *et al.,* 2015) that had been fed poultry viscera meals in their diets at high inclusion levels. .

In the present study, fish fed T2 diet had the highest FCR, which meant that they required 7.583 kg of poultry viscera to convert to 1 kg of fish meat. According to Houlihan *et al*. (2001), FCR should never go above 2 for artificial diets. However, for fresh diets, it depends on the price of feed and the total feed cost per one kg of produced fish (economic value). The results of feed utilization in our study were in agreement with Sawhney and Gandotra (2010), who reported that feed conversion ratio (FCR) can determine the efficiency of fish and the biological value of protein in feed**.** The biological value of the protein source depends on its amino acid profile (Massumotu *et al*. 1996; Sogbesan *et al.* 2006) as well as its digestion (Jabir *et al.,* 2012). Also, PER values of the present study were not significantly different. This result was in agreement with Nyina-Wamwiza *et al.* (2007). They obtained PER that was lower than the 2.7 obtained in *Clarias batrachus* that was fed a diet containing 18% of chicken viscera meal. The acceptable values of FCR and PER obtained for *C. gariepinus* fed diets T2 and T3 compared to T1, are enabled by the unconventional animal protein sources that used (poultry viscera) which are rich in essential amino acids.

The fish survival rate was not affected by the experimental diets. This result was in agreement with (NRC, 2011) as *C. gariepinus* is an omnivorous fish species which efficiently uses animal protein sources to cover energy requirements. Moreover, these results indicate that the protein quality of poultry viscera was well accepted by the fish. Survival rate during the experiment was greater than 92%, indicating that fish has grown in good experimental conditions. This result was in agreement with Oke *et al*. (2016). Also, Rawles *et al*. (2009), didn't find any significant differences in the survival rate between Hybrid Striped Bass, (*Morone chrysops* × *Morone saxatilis*) that was fed on 100% fish meal diets and after its feed was replaced by diets supplemented with poultry by-product meals at 45, 70, and 100%. The low and non-significant survival rate obtained in the current study may be related to the high content of fat that reached nearly (32%) in poultry viscera within the tested diets (T2 and T3) compared with T1 treatment.

There were significant differences among the experimental diets in VSI, HIS and GRL values. This result is in contrast with the findings of Hu *et al*. (2008), and Aydın and Gümüş (2013) and in agreement with the results obtained by Rawles *et al.* (2006), and Yang *et al.* (2006). Results of condition factors (K) revealed that treatments fed on poultry viscera kept the condition factor at a high value in T2 and T3. This result disagreed with the findings of Srour *et al.,* (2016). This may be attributed to how previous authors used dried poultry by-products, while in the present study fresh poultry viscera was used. Idodo-Umeh (2005) and Abowei and Hart (2009) reported that the length–weight relationship of fish, also known as a growth index, is an important management tool used in estimating the average weight at a given length increase. The condition factor in the intensive farms are often less than 1, while

the values of condition factors in the natural water resources are better than those in the intensive rearing tanks (Okan and Gamsiza, 2010).

The results of fish's whole-body composition have shown an increase in crude protein values and ether extract over the initial fish samples. The increase in the crude protein of all samples may have been an indicator that the poultry viscera used had a positive effect on the fish. It shows that the experimental fish effectively converted and utilized the protein from the experimental diets into their bodies' protein. These findings are in agreement with the work of Orire (2010). However, ether extract content had increased, this may have been probably caused by high fat in poultry viscera. The increased body fat content may have been due to the increased energy of poultry viscera diets, which have greater fat contents (Table 1). These results were in agreement with Goda *et al*. (2007), Giri *et al.* (2010) and Sugumaran and Radhakrishnan (2015). These authors reported that replacing fish meal with poultry viscera meal in fish diets increases the whole body's lipid content of fish. The ash content decreased in fish groups that were fed poultry viscera in the diets, and this is in agreement with the observation of Giri *et al.* (2010) and Sugumaran and Radhakrishnan (2015) in *Clarias batrachus* juveniles and African Catfish *Clarias gariepinus*, respectively.

There were significant differences in all serum biochemical parameters in the present study. Certain serum chemistry could be used to identify tissue damage (Patti and Kulkarni, 1993). The decrease in serum total protein in T2 and T3 was similar to that observed by Yadav et al. (2003) when fish were fed stem bark extract of *Croton tiglium*. However, the results reported for serum proteins are in agreement with those obtained by Malla and Bashamohiden (1995). The decrease in the serum's total protein and albumin levels may be due to their degradation and also to their possible use in metabolic purposes. The results were in agreement with those of Bradbury *et al.* (1987), who pointed that decreased protein content and albumin might also be attributed to the destruction or necrosis of cells and the consequent impairment in protein synthesis machinery. The reduction in protein observed in this study indicated that the physiological strategy used by the fish in order to manage stress was induced by feeding poultry viscera. The need for more energy in order to adapt to the changed metabolic system probably stimulates degradative processes, such as proteolysis and the utilization of degraded products for increased energy metabolism. Similar to this study, a marked decrease in serum albumin and total proteins of *O. niloticus* have been reported by El-Sayed and Saad (2007) and on *C. gariepinus* (Amin and Hashem, 2012).

The increase of cholesterol in T2 and T3 groups indicated an increase in lipid content in the blood and retardation of fat metabolism on fish. Our result is in agreement with that reported by Öner *et al*. (2008) who stated that the concentration of cholesterol, may increase due to liver and kidney failure causing the release of cholesterol into the blood of *Oreochromis niloticus*. Cholesterol is a building block for cell membranes and for sex hormones like estrogen and testosterone. About 80% of the cholesterol is produced by the liver (Hasheesh *et al.*, 2011). The results of the present study were in agreement with El-Sayed and Saad (2007) for *O. niloticus*, Borges *et al*. (2007) and *O. niloticus* Firat *et al*. (2011). Blood biochemistry parameters that had been determined also fall within the ranges reported for *Heterobranchus longifilis* and *Heteroclarias* hybrid (Okorie-Kanu and Unakalamba, 2014 a, b). Significant differences were observed in serum triglyceride of *Clarias gariepinus* that fed on the commercial diet in T1 when compared to T2 and T3. That might have been due to liver dysfunction according to Kaplan *et al*. (1988).

In the present study, serum urea levels decreased when fish were fed poultry viscera. This result is in agreement with Kefi *et al.* (2013) who recorded high levels of urea concentration in *Oreochromis andersonii.* This is likely to be a sign of stress associated with

the increase in the cortisol level. Significant differences of lipase and amylase concentrations with different directions were recorded. Increased values of serum lipase in fish may be related to the elevation of the activity of liver lipase, which mediated the mobilization of liver lipid reserves in fish.

The lowest concentration of pathogenic microbe in rearing water, diets and fish meat in T3 attributed to the antibacterial effects of vinegar. In this context, the organic acids in vinegar and mainly acetic acid pass into cell membranes of microorganisms leading to bacterial cell death (Bjornsdottir and et al. 2006; Chang and Fang 2007). The bacterial strains, temperature, pH, acid concentration and ionic strength influence the antimicrobial activity of organic acids (Cheng and et al 2003). Many organic acids are naturally found in a variety of fermented foods. When the effects of organic acids on killing of foodborne pathogenic bacteria were compared, it was reported that acetic acid was the most lethal to Escherichia coli O157: H7, followed by lactic, citric and malic acids (Ryu and et al. 1999). Different studies have reported that vinegar could be used to inhibit pathogenic bacteria on fresh fruits and vegetables (Wu and others 2000; Rhee and others 2003; Sengun and Karapinar 2004; Chang and Fang 2007). Sengun and Karapinar (2004) reported the effects of vinegar containing 4.03% acetic acid, lemon juice and a 1:1 (v/v) mixture of lemon juice and vinegar on *Salmonella typhimurium* when applied to carrots for different exposure times (0, 15, 30, and 60 minutes). While both vinegar and lemon juice demonstrated an antimicrobial effect on *S. typhimurium* at all times. The maximum reduction in *S. typhimurium* populations occurred at 60 minutes of treatment. Chang and Fang (2007) evaluated the antimicrobial effect of rice vinegar on lettuce inoculated with *E. coli* O157: H7. They noted a 3-log reduction that was caused by commercial vinegar treatment that contained 5% acetic acid for 5 minutes at 25 °C. However, less than 1 log reduction was noted using 0.5% acetic acid treatment for 5 minutes. The presence of vibrio in the intestine of T1 may be attributed to its presence in the feed, in addition to the health condition of fish that enhanced this species of bacteria.

CONCLUSION

The present study revealed that using poultry viscera immersed in commercial vinegar as a diet for catfish *Clarias gariepinus* had positive impacts on the growth, feed utilization and whole body's chemical composition. Therefore, it is recommended to use poultry viscera immersed in commercial vinegar, because it might be an effective and economic alternative feedstuff for high priced commercial feed with a slight defect in biochemical and health status of fish. At the same time, the ecological impacts of treating dangerous by-products like poultry viscera and converting them to high value by-products are a matter of interest for both aquaculturists and environmentalists alike. More research work is needed to take advantage of the current results on a commercially applicable scale.

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ARABIC SUMMARY

هل تغذية أسماك القرموط الافريقي *(Clarias gariepinus)* على أحشاء الدواجن المغمورة فى الخل تؤثر على أداءها فى النمو والحالة الصحية و الحمل البكتيرى المسبب للامراض ؟

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٢. قسم الموارد السمكية والاستزراع المائي ، كليه العلوم الزراعية البيئية ، جامعه العريش ، مصر.
٣. معمل الميكروبيولوجى ، شعبه البيئة البحرية ، المعهد القومى لعلوم البحار والمصايد ، الاسكندرية ، مصر.
٤. معمل تربيه الأسماك ، شعبة تربية الأحياء المائية - المعهد القومى لعلوم البحار والمصايد ، الاسكندرية ، مصر.

أجريت هذه الدراسة لمعرفه تأثير استبدال الأعلاف التجارية بأحشاء الدواجن التى سبق غمرها بالخل على أداء النمو والوضع الفسيولوجي والحمل البكتيري فى أسماك القرموط الافريقى (*Clarias gariepinus*)، حيث تم تغذية الأسماك علي أعلاف تجارية (T1) ، وأحشاء دواجن غير معاملة (T2) و أحشاء دواجن مغمورة فى الخل التجارى (T3)، و كان متوسط وزن السمك عند التخزين ٥٢٠ جرام. تم إجراء الدراسة فى أحواض من الفيبرجلاس سعة كل منها ٢ متر مكعب وإستمرت التجربة ٩٨ يوما، وقيست معايير جودة المياه ، وأداء النمو، والاستفادة الغذائية ، وجودة اللحم ، وتحليل الدم، والحمل البكتيرى فى الاعلاف والماء و للأسماك.

وأظهرت النتائج انخفاض محتوى الأمونيا في مياه أحواض التربية بشكل ملحوظ في المعاملة T3 مقارنه مع T2 ، كما لم يتم الكشف عن أية اختلافات معنوية ($P>0.05$) في نسبة الوفيات و أداء النمو ، في حين لوحظ وجود إختلافات معنوية كبيرة ($P\leq0.05$) في الاستفادة الغذائية و التحليل الكيماوى لجسم الاسماك بين المعاملات المختلفة، وكانت مؤشرات جودة الأسماك من حيث محتوى اللحم من العناصر الغذائية فى صالح المعاملة T3 . أيضا لوحظ وجود إنخفاض فى محتوى الدم من البروتين و الالبيومين وزيادة في الجلوبيولين و الكوليستيرول عندما تم تغذية الأسماك المختبرة على أحشاء دواجن غير معاملة بالخل . ومن النتائج الايجابية فى هذه الدراسة أن الحمل البكتيرى المرضى في العلائق المستخدمة و المياه فى أحواض التجارب وأمعاء الاسماك كان في أدني حد له في المعاملة T3 ، وتؤكد هذه الدراسة على إمكانية تغذية اسماك القراميط الافريقية على أحشاء الدواجن المعاملة بالخل التجاري كبديل للاعلاف الجافة عالية السعر ، وتعتبر معالجه الآثار البيئية للمخلفات شديدة الخطورة على البيئة مثل أحشاء الدواجن وتحويلها إلى منتجات ثانويه ذات قيمه عاليه؛ تهم كلا من العاملين فى قطاعى الاستزراع السمكى والبيئة على حد سواء ، و لتحقيق أقصى إستفادة من نتائج الدراسة الحالية وتطبيقاتها على المستوى التجاري ، فإنه يوصى بإجراء مزيد من العمل البحثي الحقلى.