

Impact of water column reduction rate at harvesting of Nile tilapia (*oreochromis niloticus*) on skin injuries, hematological and biochemical parameters and bacterial load of fish and water: a field study

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ABSTRACT

The aim of this study was to evaluate the impact of reduced water column in different periods on health and welfare of the Nile tilapia during harvesting. As a common practice for gathering and harvesting of tilapia in Egypt, the water column was reduced gradually until reach the lowest level (30 cm). The water column was reduced from 150 to 30 cm in different periods as follow; Group1 (G1, control group), at which the water column was reduced from 150 to 30 cm in 72 hour (h). Group 2 (G2), at which the water column was reduced from 150 to 30 cm in 48 h. Group 3 (G3), at which the water column was reduced from 150 to 30 cm in 24 h.

The results showed that, there was a significant ($P<0.05$) decrease in muscle total bacterial count (TBC), fish injuries, heterophil /lymphocyte (L/H) ratio, serum glucose, Lactate dehydrogenase, alkaline phosphatase and an increase in water, skin TBC, dissolved oxygen (DO), unionized ammonia (UIA), and total leucocyte count (TLC) in G3 compared to G2 and G1.

In conclusion, the process of reduced water column before gathering and harvesting needs to be carried out within 24 h to reduce injuries and bacterial load of fish. In addition, it improves the immune system and health of fish that reducing the risk of infection. Therefore, slow reduction of water column height during harvesting produces crowding stress altering health status and welfare of Nile tilapia (*Oreochromis niloticus*).

Keywords: Fish harvesting; water column; skin injuries; water quality; bacteriological parameters; fish welfare

INTRODUCTION

Fish welfare is an important issue for the industry, not just for public perception, marketing and product acceptance, but also often in terms of production efficiency, quality and quantity (Broom, 2010; Southgate and Wall, 2002). Several studies defined the welfare of fish through fisheries, aquaculture and other activities as physical, physiological and behavioural conditions facing the animal in aquaculture environment (Huntingford *et al.*, 2006; Braithwaite and Boulcott 2007).

According to FAWC (1996) and Chandroo *et al.*, (2004) in order to achieve farmed fish welfare the following freedoms must be provided; 1) sufficient and nutritionally diet, with starvation before slaughter and transport for short time. 2) adequate water quality parameters (dissolved oxygen, NH₃, pH and turbidity), flow rates, temperatures and appropriate light intensities. 3) careful procedures for avoiding

injuries, malformation, infections and diseases, through the application of best management practices during the rearing period, harvesting, good hygienic conditions and proper vaccination. 4) adequate space and appropriate facilities to express normal behaviour. 5) providing suitable living conditions by which physical and mental suffering could be avoided.

In Egypt, tilapia is reared mainly in earthen ponds in a semi extensive rearing system. Following a period of fasting, tilapias are netted and placed in ice, in water or in dry crates for transportation to the wholesale or point of slaughter. There are numerous conditions and activities used during aquaculture practices, which cause stress (chronic or acute) and promote a reduction of welfare (Conte, 2004). The harvesting process (length, intense handling, crowding during most of the catching protocols and related decrease of oxygen) could be a very traumatic time for the farmed fish, involving the onset of a stress status, which can compromise the organoleptic, merchantable and sanitary quality of the final product (Poli, 2009).

Crowding is the first stage in most gathering, harvesting and transport operations. In ponds, it is achieved by reducing the height of water column and the use of a special net or by moving partitions or trellises. Wall (2001) stated that crowding is one of the prime causes of poor welfare during harvest. Ortuno *et al.* (2001) stated that the most common problem associated with crowding is shortage of oxygen. However, even when oxygen level is maintained, crowding changes many other aspects of the fish physiology, which can be observed for days afterwards. Fish respond to crowding will differ according to species, some studies have suggested that skin damage intensifies with increasing stocking density in rainbow trout (North *et al.*, 2006) and sea bass (Person-Le Ruyet and Le Bayon, 2009), but others have reported that stocking density has no effect in Atlantic salmon (Hosfeld *et al.*, 2009).

During harvesting in fishponds, the densities of fish could be changed from few kilograms to several hundred kilograms per square meter. The vigorous swimming during crowding implies an intense use of the white muscle increasing anaerobic glycolysis with subsequent lactic acid production and lowering of muscle pH (Poli *et al.*, 2005). Additionally, under these conditions there are particular dangers to the fish of bruising, crushing, puncture and abrasion injuries from contact with other fish, contact with the net and contact through the net with other hard surfaces. So, the process of gathering and harvesting needs to be carried out unhurriedly and with great care to avoid a panic reaction in the fish, which could result in high levels of stress and mortality (Brown *et al.*, 2010). As poor crowding technique is likely to result in external injury and mortality, fish producers are trying to protect their fish from possible injurious circumstances as part of their ethical and general responsibility towards their reared animals. The main skin lesions associated with gathering and harvesting practices were skin abrasions and injuries that characterised by a cut-off of the skin layers (epidermis). In addition, it might be accompanied with detachment of scales and subcutaneous haemorrhage (Noble *et al.*, 2012). From the production outlook, injuries can lead to reduced feed intake (Dykova *et al.*, 1998), reduced growth rate (Miyashita *et al.*, 2000), increased susceptibility to bacterial infection and parasitic infestation (Turnbull *et al.*, 1996) and increased mortality rate (Cobcroft and Battaglene, 2009). In addition, injuries can reduce the market value of farmed fish and its keeping quality (Michie, 2001).

Changes in pond water parameters especially, DO, NH₃, pH, and turbidity, and bacterial load were resulted from crowding of fish (Bhatnagar and Devi, 2013) during harvesting and expectedly these changes will affect the quality of the harvested fish. In intensive systems, Ammonia is the most important parameter after oxygen that

affects fish. The un-ionized form of ammonia (UIA) is the most toxic form to aquatic life (Alabaster and Lloyd, 1980; Adams *et al.*, 2001; Abouelenien *et al.*, 2015).

The impact of the time elapsed in reduction of water column at gathering and harvesting of fish on water quality and consequently fish injuries and welfare has not been investigated previously to our knowledge. Therefore, the present work was carried out to study the effect of time elapsed in reduction of water column height during gathering and harvesting of Nile tilapia (*O. niloticus*) on fish injuries, hematological and biochemical assay and bacterial load and its consequences on fish welfare.

MATERIALS AND METHODS

Fish, rearing system and management

This study was carried out in a private fish farm at Kafr El-Sheikh governorate (N: 31, 21, 38 and E: 30, 36, and 40). The farm was composed of eight independent ponds, six for breeding and two for brooding. All ponds were irrigated from a single water source but each pond has a separate water inlet and outlet. The surface area of each breeding pond was 2 Acres and the pond dimensions were 150 x 55 x 1.5 m. The fish type was mono-sex Nile tilapia (*O. niloticus*) was treated with 17 alpha methyl testosterone with Mugil cephalos and Mugil capitos. The rearing system was a semi extensive system and the average fish density was 3 fish /m². The fish feed was pelleted sinking type, 3mm in diameter. The feeding rate ranged from 4 % from the fish biomass at the beginning of the rearing period to 1.5 % at the end of the rearing period, twice daily. Composition and proximate analyses of commercial fish feed used for Nile tilapia (*O. niloticus*) was illustrated in Table 1. The rearing period was 7 months, started at April 2014 with 20 g fingerlings.

Table 1: Composition and proximate analyses of commercial fish feed used for Nile tilapia (*Oreochromis niloticus*).

Ingredients	%
Corn	9
Rice bran	16
Wheat milling by product	10
Wheat barn	9
Soya bean pellets (47)	36
Poultry meal	5
Fish meal (60)	5
Gluten	2.5
Sun flower meal	3
Dicalcium phosphate	1.2
Salt	1
Dry fat	0.8
Soya oil	1
Vitamins and minerals	0.5
<u>Proximate analysis</u>	
Dry Matter	91
Moisture	9
Protein	30
Fat	7
Fiber	7
Ash	6.5
Digestible energy (DE)	33.5

Experimental design

During the rearing period of fish, the height of water column in the breeding ponds ranged from 130 to 150 cm and not less than 145 cm during the last month of the rearing period. As a common practice for gathering and harvesting of tilapia in Egypt, the water column was reduced gradually until reach the lowest level (30 cm). In this experiment, the water column was reduced from 150 to 30 cm in different periods as follow; Group1 (G1, control group), at which the water column was reduced from 150 to 30 cm in 72 hour (h). Group 2 (G2), at which the water column was reduced from 150 to 30 cm in 48 h. Group 3 (G3), at which the water column was reduced from 150 to 30 cm in 24 h. G1 chosen as a control group, because it is the usual practice done in fish farms in Egypt.

Sampling

Water sampling

Water sampling was done around time of fishing. Five water samples were collected from each pond by inverting 250 ml sterilized glass bottle 15 cm below the pond water surface. All samples were transferred to the lab on icebox. Analysis was initiated within 2 h of sample collection.

Fish sampling

To reduce stress, fish were rapidly netted, then anesthetized with a buffered solution of 70% benzocaine (Sigma Chemical Co., USA).

Fish injuries, detached scales and external hemorrhage

Twenty-four fish from each pond were caught from different sites of fish pond by using a net. Each fish was handled and fixed on a table. The number of injuries in head, body, dorsal fin, tail and tail fin were estimated with the help of an optical magnifier lens (x10). Presence of external hemorrhage (outer bleeding) and detached scales (few, scattered detached scales; intermediate, detached scales formed a patch less than 0.25 cm² [0.5x 0.5 cm]; sever, detached scales formed a patch equal to or more than 0.25 cm²) were estimated with the help of an optical magnifier lens (x10) and measuring board. Frequency of injuries, detached scales and outer bleeding was calculated as a percentage of total examined fish ([number of fish suffering/ total number of examined fish] x 100).

Skin sampling

Sterile cotton bud was used to take a swap from the surface of the fish immediately upon collection from the pond. The cotton bud was placed immediately in 5ml sterile peptone water in sterile plastic applicator (Rayon), labeled and kept at 4°C and transported to the laboratory.

Muscle sampling

Twelve live fish from each pond were randomly selected from the catch at each sampling time. In the laboratory each fish was rinsed with de-ionized water and the surface of the fish decontaminated by dipping it in ethyl alcohol and lightly flames. Fish were dissected according to the method described by Buras *et al.* (1987). Muscles were isolated and placed in a sterile polyethylene bag; the tissues were weighed under sterile condition. Ten grams of muscle portion of fish along with skin were homogenized for 1 min with 90 ml of physiological saline (Nacl 0.85%) in a homogenizer (Polytron®PT-MR 2100). One ml of the homogenate for skin and muscle samples was serially diluted (10⁻¹ to 10⁻⁸) as described by the study of Al-Harbi (2003).

Blood sampling

Blood was drawn from the caudal peduncle region (caudalis vein) using a 23-gauge needle of 24 fish from each pond.

Analytical methods**Bacteriological examinations**

The total bacterial count of water and skin samples was carried out according to Cruickshank *et al.* (1972). The total bacterial count of fish samples was carried out according to the method of APHA (1998).

Physico-chemical examination of water samples

Water temperature, pH and dissolved oxygen (DO) were measured in situ with (a Yellow Springs dissolved oxygen meter Model 54A). Pond water pH and temperature were determined using an Adwa AD11 and AD12 (Romania).

Total ammonia (NH₃), Electrical conductivity (EC), total dissolved solids (TDS) were measured by the standard methods described by APHA (1998).

Hematological and biochemical parameters

The blood samples were held on ice until all samples were collected. Hematological and biochemical analyses were performed within 2h of blood collection.

Hematological analysis

One ml whole blood was taken in EDTA tubes for estimation of WBC (total leucocyte count) and differential leukocytes count for calculation of H/L ratio according to the method of Svobodova *et al.* (1991).

Biochemical analysis

One ml blood was taken in vacuum tubes. Blood was centrifuged at 3000 rpm for 10 m for serum separation. Serum was stored at -40 °C for further assay of glucose, Lactate dehydrogenase (LD) and alkaline phosphatase (ALP). Assays were performed as described by Nelson *et al.* (1944), Palti *et al.* (1999) and Anderson and Siwicki (1995).

Statistical analysis

Data were tested for distribution normality, linearity and homogeneity of variance. Data were analysed using Graph Pad™ Prism 5. Results are presented as means ± SEM. Data were compared by one way ANOVA and the Tukey's Multiple Comparison Test was used as a post hoc test when appropriate. The significance level was $P < 0.05$.

RESULTS

The results in Table 2 revealed that there were significant differences ($P < 0.05$) among the analysed water parameters except for water temperature and ammonia. For unionized ammonia the highest numerical value was found in G3 (0.627ppm) and the lowest in G2 followed by G1. For pH and EC, there was no significant difference between integral water column (1.5 m) and reduced water column (0.3 m) in different periods of time (72, 48, and 24 h). While, there was no significant differences between G1 and G2 but they were significantly differed ($P < 0.05$) from G3. For TDS, there was a significant difference ($P < 0.05$) between integral water column (1.5 m) and reduced water column (0.3 m) in different groups. However, there was no significant difference between G1 and G3 but they were significantly differed from G2. The lowest concentration of NH₃ was recorded in integral water column (1.5 m) followed by G3, G2 and G1 in reduced water column (0.3 m). Additionally, there was a significant difference ($P < 0.05$) in TBC (log₁₀ cfu/ml) among water samples from different groups. Where water from G3 recorded the highest TBC (log₁₀ cfu/ml), followed by water samples from ponds have been reduced in 48 h (G2) and 72 h (G1).

respectively. While, they were significantly ($P < 0.05$) higher at TBC (\log_{10} cfu/ml) than water samples at water column 1.5 m.

Table 2: Effect of water column reduction rate at harvesting on water parameters and total bacterial count of Nile tilapia (*Oreochromis niloticus*) fish.

parameter	Integral water column (150 cm)	Time spent to reduce water column from 150 to 30 cm		
		G1 (72 h)	G2 (48 h)	G3 (24 h)
Water temp. ($^{\circ}$ C)	12.000 \pm 0.32	11.600 \pm 0.400	12.000 \pm 0.320	12.200 \pm 0.370
DO (mg/L)	6.240 \pm 0.23 ^{cd}	4.930 \pm 0.146 ^{ab}	5.340 \pm 0.133 ^b	5.860 \pm 0.187 ^c
PH	7.620 \pm 0.17 ^a	7.436 \pm 0.106 ^{ac}	7.284 \pm 0.279 ^{ac}	8.246 \pm 0.045 ^{ab}
NH ₃ (ppm)	16.240 \pm 0.56 ^b	21.840 \pm 0.90 ^a	20.720 \pm 2.10 ^{ab}	18.880 \pm 1.37 ^{ab}
UIA (ppm)	0.140 \pm 0.005 ^c	0.118 \pm 0.004 ^b	0.070 \pm 0.003 ^a	0.627 \pm 0.004 ^d
TDS (g/L)	1.473 \pm 0.007 ^c	1.558 \pm 0.005 ^b	1.618 \pm 0.019 ^a	1.532 \pm 0.004 ^b
EC (dSm ⁻¹)	3.062 \pm 0.007 ^a	3.100 \pm 0.001 ^{ab}	3.240 \pm 0.036 ^{ab}	2.868 \pm 0.108 ^{ac}
Water TBC (\log_{10} cfu/ml)	5.442 \pm 0.36 ^b	6.027 \pm 0.51 ^{ab}	6.548 \pm 0.340 ^{ab}	7.797 \pm 0.620 ^a
Skin TBC (\log_{10} cfu/g)	-----	5.663 \pm 0.350	5.574 \pm 0.340	6.206 \pm 0.240
Muscle TBC (\log_{10} cfu/g)	-----	6.674 \pm 0.290	6.542 \pm 0.340	6.450 \pm 0.380

In each row, means having different small superscripts differ significantly ($P < 0.05$)

G1, Group 1; at which the water column was reduced from 150 to 30 cm in 72h. G2, Group 2; at which the water column was reduced from 150 to 30 cm in 48 h. G3, Group 3; at which the water column was reduced from 150 to 30 cm in 24 h.

DO; dissolved oxygen, UIA; unionized Ammonia, TDS; total dissolved oxygen, EC; Electric conductivity, TBC; total bacterial count.

Results of TBC for skin and muscle samples of fish (\log_{10} cfu/g) which caught from ponds of different reduced water column times showed no significant difference at among examined samples (Table 2). However, skin samples of G3 recorded high levels of TBC (6.206 \pm 0.240), followed by 5.663 \pm 0.350 and 5.574 \pm 0.340 for G1 and G2 respectively (Table 2). Muscle samples of G3 recorded lower levels for TBC (6.450 \pm 0.380) while muscle samples of G2 (6.542 \pm 0.340) and G1 (6.674 \pm 0.290) recorded insignificant increases as compared with G3.

The results in Table 3 showed reducing the height of water column from 150 to 30 cm before harvesting has a significant ($P < 0.05$) effect on fish injury. Reducing the height of water column in G1 recorded the highest levels of injuries in head, body and dorsal fin, tail and tail fin regions. While the reduction of water column in G2 and G3 recorded the lowest levels of injuries in the same fish regions respectively. The percentage of head, body and dorsal fin, tail and tail fin injury was the highest in G1, followed by G2 and G3 respectively (Fig. 1).

Table 3: Effect of water column reduction rate at harvesting on external injuries (number) of Nile tilapia (*Oreochromis niloticus*) fish.

Fish part	Time spent to reduce water column from 150 to 30 cm		
	G1 (72 h)	G2 (48 h)	G3 (24 h)
Head	0.83 \pm 0.28 ^a	0.25 \pm 0.09 ^b	0.0
Body and dorsal fin	2.17 \pm 0.32 ^a	1.08 \pm 0.20 ^b	0.83 \pm 0.19 ^b
Tail and tail fin	0.83 \pm 0.14 ^a	0.67 \pm 0.10 ^a	0.25 \pm 0.09 ^b

In each row, means having different small superscripts differ significantly ($P < 0.05$)

G1, Group 1; at which the water column was reduced from 150 to 30 cm in 72h. G2, Group 2; at which the water column was reduced from 150 to 30 cm in 48 h. G3, Group 3; at which the water column was reduced from 150 to 30 cm in 24 h.

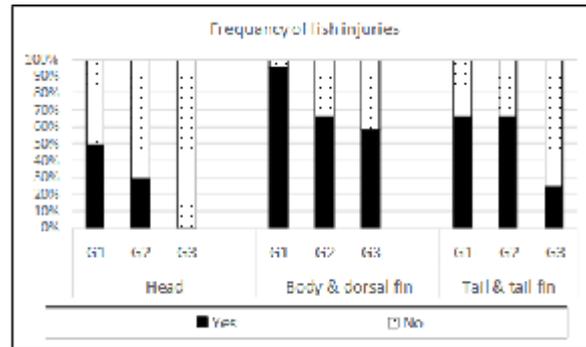


Fig.1: Effect of water column reduction rate at harvesting on frequency (Percentage) of injuries (black, yes) or absence of injuries (white, no) in head, body and dorsal fin, tail and tail fin of Nile tilapia (*Oreochromis niloticus*).

G1, Group1; at which the water column was reduced from 150 to 30 cm in 72h. **G2**, Group 2; at which the water column was reduced from 150 to 30 cm in 48 h. **G3**, Group 3; at which the water column was reduced from 150 to 30 cm in 24 h.

The percentage of fish suffering from detached scales was few detached scales (70.83, 70.83, and 83.33 %), intermediate detached scales (20.83, 20.83, and 16.67 %) and sever detached scales (8.34, 8.34, 0 %) in response to the reduction of water column height from 150 to 30 cm in 72, 48, and 24 h respectively (Fig. 2). While, the highest percentage of outer bleeding in fish was recorded in G2, followed by G1 and G3 respectively (Fig. 3).

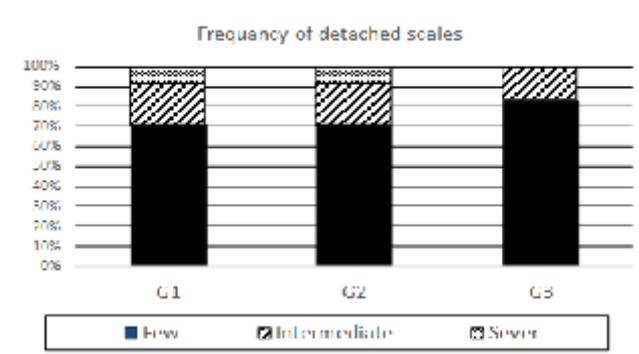


Fig. 2: Effect of water column reduction rate at harvesting on frequency (Percentage) of detached scales (black, few - bands, intermediate - dotted, sever) of Nile tilapia (*Oreochromis niloticus*).

G1, Group1; at which the water column was reduced from 150 to 30 cm in 72h. **G2**, Group 2; at which the water column was reduced from 150 to 30 cm in 48 h. **G3**, Group 3; at which the water column was reduced from 150 to 30 cm in 24 h.

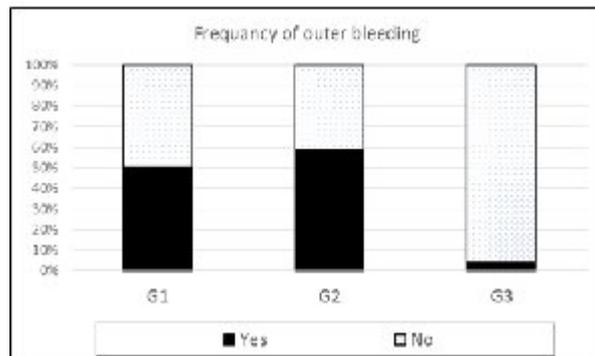


Fig. 3: Effect of water column reduction rate at harvesting on frequency (Percentage) of outer bleeding (Black, yes) or absence of outer bleeding (White, no) of Nile tilapia (*Oreochromis niloticus*).

G1, Group1; at which the water column was reduced from 150 to 30 cm in 72h. **G2**, Group 2; at which the water column was reduced from 150 to 30 cm in 48 h. **G3**, Group 3; at which the water column was reduced from 150 to 30 cm in 24 h.

The results in Table 4 showed that there was a significant difference ($P < 0.05$) in hematological and biochemical parameters estimated in fish among the experimental groups. The results of TLC showed significant higher level in G2 and G3 than level of G1. The activity of LD was significantly higher in G1 and G2 than its activity in G3. For H/L ratio and LD, there was no significant difference between G1 and G2 but they were significantly different ($P < 0.05$) from G3. In case of glucose level, there was a significant difference ($P < 0.05$) between all groups. While for ALP, there was no significant difference between G2 and G3 but they were significantly different ($P < 0.05$) from G1.

Table 4: Effect of water column reduction rate at harvesting on hematological and biochemical parameters of Nile tilapia (*Oreochromis niloticus*) fish.

parameter	Time spent to reduce water column from 150 to 30 cm		
	G1 (72 h)	G2 (48 h)	G3 (24 h)
TLC(μ l)	35,550 \pm 676.400 ^b	39,400 \pm 891.2 ^a	40,050 \pm 801.600 ^a
H/L ratio	0.060 \pm 0.004 ^a	0.051 \pm 0.003 ^a	0.024 \pm 0.002 ^b
Glucose (mg/dL)	263.600 \pm 29.180 ^a	190.200 \pm 12.840 ^b	120.000 \pm 5.740 ^c
LD (IU/L)	2578.000 \pm 118.4 ^a	3232.000 \pm 350.900 ^a	1653.000 \pm 101.000 ^b
ALP (U/L)	24.000 \pm 0.695 ^a	14.800 \pm 0.504 ^b	13.200 \pm 0.961 ^b

In each row, means having different small superscripts differ significantly ($P < 0.05$)

TLC; total leucocyte count, H/L ratio; heterophils to lymphocytes ratio; LD; Lactate dehydrogenase, ALP; Alkaline phosphatase.

G1, Group 1; at which the water column was reduced from 150 to 30 cm in 72h. G2, Group 2; at which the water column was reduced from 150 to 30 cm in 48 h. G3, Group 3; at which the water column was reduced from 150 to 30 cm in 24 h.

DISCUSSION

As far as the authors know, this is the first work taking in consideration the effects of reduced water column in different periods on health and welfare of the Nile tilapia during harvesting. As a common practice during harvesting of tilapia in Egypt, the height of fishpond water column should be reduced to its lowest level. It mainly lead to sever condition of overcrowding and an increase in fish stocking density, which in turn have a detrimental effects on fish health and welfare.

The skin of fish is considered as a natural and biological barrier encompassing in ion regulation (Esteban, 2012; Benhamed *et al.*, 2014) and providing the protection against friction that mainly originated from overcrowding (Daniel, 1981). On the other hand, skin providing protection against pollution in the aquatic environment and infectious agents (Ellis, 2001; Benhamed *et al.*, 2014). Skin injuries are direct damage or visible losses of epidermis layers, which may lead to skin discolouration, detachment of outer scales and may be hemorrhage. It not only accompanied with poor fish welfare (Huntingford *et al.*, 2006) but also causing production losses (Vagsholm and Djupvik, 1998; Ellis *et al.*, 2008). In the present study, the main skin lesions associated with the reduction of water column height during fish harvesting from 150 to 30 cm were abrasions characterised by a discontinuity of the skin layer and sometimes accompanied by detachment of outer scales and subcutaneous hemorrhage in head, body, dorsal fin, tail and tail fin. The injuries of body and dorsal fin were the highest compared to head, tail and tail fin. The overall means of fish injuries were the lowest in the group suffered from reduced the height of water column in 24 h (G3) compared to 48 (G2) and 72 h (G1). This was accompanied by very low percentage of detached scales and outer hemorrhage in G3 compared to G2 and G1. These results could be explained as follow; the reduction of

water column height in 24 h reduced the time for fish friction by contact with other fishes, nets and other hard surfaces during harvesting, consequently reducing the fish injuries and outer hemorrhage (Daniel, 1981). The reduction in the process of injury, detachment of outer scales and hemorrhage have a direct effect upon the reduction of pain sensation and nervousness as fish own free nerve cells that cover the skin surface and responsible for pain sensation (Kotrschal *et al.* 1993; Meka, 2004). These nerve cells contain special fibres that are involved in pain sensitivity. Roques *et al.* (2010) reported that there was an acute response to a painful stimulus, caudal fin clipping, in Nile tilapia (*Oreochromis niloticus*). The findings of Roques *et al.* (2010) was supported by increased Nile tilapia swimming activity up to 6 h after the damage occurred. The evidence for lower degree of surface swimming behavior observed in G3 (24 h) compared to G2 and G1 (data not shown). Therefore, intensifying skin injury, detached scales and outer hemorrhage originated from slower reduction of water column height from 150 to 30 cm (G2 and G3) at harvesting may have an unfavourable effect upon survival of fish and may affect fish quality. It may also influence upon welfare due to being a direct cause of suffering through injury to living tissue (Ellis *et al.*, 2008) and may increase susceptibility to infection.

There were significant differences among the analysed water parameters except for water temperature and ammonia. For unionized ammonia (UIA) the highest level was found in G3 and the lowest in G2 followed by G1. These values of UIA were concomitant the pond water pH in different groups (Abouelenien *et al.*, 2010; Abouelenien *et al.*, 2014; Abouelenien *et al.*, 2015). The increase in pH values with regard to accumulation of ammonia due to decomposition of nitrogenous compounds by the microbial activities (Erkan and Ozden, 2008). In the current study, the highest UIA in G3 could be due to both rapid decrease in water column (24h) and sudden increase fish activity resulted in sudden increase in nitrogenous compound concentration of pond water (Randall and Tsui, 2002). This increase in nitrogenous compound concentration together with high water temperature enhanced the microbial degradation of these compounds with production of UIA (Farag, 2012). The estimated UIA in all groups of the present study was higher than optimum concentrations, 0.05 mg/l (El-Sherif and EL-Feky, 2008).

The optimum growth of Tilapia is obtained at concentrations greater than 3 mg/l (Ross, 2000) and it is highly tolerant to low DO concentration, 0.1 mg/l (Magid and Babiker, 1975). In this field study, the measured DO concentration in all groups was lower than that obtained by Rapatsa and Moyo (2013).

Time taken for reducing water column at fishpond was found to have an obvious effect on bacterial load of pond water. As it was clear that with increasing time of reducing water column there was subsequent decrease in bacterial load of pond water which recorded highest count in G3 and G2 was higher than G1. These results may be attributed to the combined effect of UV rays (Olayemi, 1993) and sedimentation (Ali and Osman, 2012).

Microbiological evaluation of fish aims to quantify the hygienic quality of fish. Fishes are conditioned by their environment and hence it is obvious that growing and harvesting environment of fishes are polluted chemically or microbiologically (Abouelenien *et al.*, 2015; Boyd, 1984; Elsaïdy *et al.*, 2015). The concept that could explain the results of the higher TBC of skin samples of tilapia fish caught for G3 than that found for G2 and G1. The results that in agreement with Al Harbi (2003) and Elsaïdy *et al.* (2015) observed that bacterial load in fish samples was correlated with the bacterial levels in the aquatic environment. In contrast to the previous findings, muscle samples of G3 (24 h reduced water column ponds) recorded lower values for

TBC, which could be explained on the basis of reduced time for fish friction by contact with other fish, nets and other hard surfaces during harvesting.

Skin provides protection against pollution in the aquatic environment and infectious agents for fishes (Ellis, 2001). This fact is supported by lowest level of fish skin injuries observed in G3 compared to G2 and G1 of the present study. Also, these findings are in agreement with findings of Huess (1995), and Adedeji and Adetunji (2004) reporting that physical damage to fishes results in easy access of spoilage bacteria.

In the present study, fish held at high stocking density due to slow reduction in the water column height (72 and 48 h) showed a significant low level of TLC and high levels of H/L ratio, glucose, LD and ALP. This reduction of TLC and increased H/L ratio may be due to the release of cortisol that produce an immunosuppressive effect in fish (Barton and Iwama, 1991; Dunier, 1996; Palikova *et al.*, 2010; Roques *et al.*, 2012) reducing circulating lymphocytes and increasing circulating heterophils (Pickering, 1984). Furthermore, it makes reduction in the welfare and increasing the susceptibility of fish to disease (Houghton and Matthews, 1990; Ballarin *et al.*, 2004). As a secondary response of fish to the overcrowding stresses that, increase the blood glucose and make mobilisation of energy sources of fish. Higher energy mobilization due to the vigorous swimming during crowding that increase muscular activity, and denotes powerful use of white muscles and will increase blood lactate dehydrogenase production and lowering of muscle pH, due to the shifting of fish metabolism to anaerobic glycolysis (Marx *et al.*, 1997). Yada and Nakanishi, (2002) observed similar findings, chronical exposition to stressors elevated the concentration of cortisol and glucose as well as haematological changes that predisposition the fish immune system favouring the pathogen installation.

CONCLUSION

As a management procedure, the process of reduced water column before gathering and harvesting needs to be carried out within 24 h to reduce injuries and bacterial load of fish. In addition, it improves the immune system and health of fish that reducing the risk of infection. Therefore, slow reduction of water column height during harvesting produces crowding stress altering health status and welfare of Nile tilapia (*Oreochromis niloticus*). The authors recommended more investigations in management of fish water pond to improve fish welfare and product quality.

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