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RESEARCH PAPER

Humane Slaughter of Carp – A Comparison between Three Stunning Procedures

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Abstract

Fish welfare at slaughter is an area of considerable scientific and commercial importance during the last decade. The aim of this study was to evaluate the effect of three stunning procedures on stress and meat quality of common carp. The experimental fish were separated in three groups and stunned by percussion (Group PS), electricity (Group ES) or asphyxia (Group AS), followed by decapitation. Blood cortisol and glucose concentrations were measured as stress indicators, whereas drip loss was used as meat quality parameter. The lowest glucose concentration (3.86±1.26 mmol/L) and drip loss (2.02±0.42%) were observed in Group PS. On the contrary, Group AS showed the highest results (11.34±3.07 mmol/L and 3.22±0.75% for blood glucose and drip loss, respectively), whereas the electrically stunned carp took an intermediate position (6.05±1.29 mmol/L and 2.29±0.43%). We conclude that percussive stunning leads to considerable reduction of stress at slaughter and better meat quality (lower drip loss), whereas asphyxia is unacceptable method due to the strong stress reaction and increased drip loss. Electrical stunning can be an effective method for stunning carp, but requires improvements of the electrical device to eliminate the repeated current application.

Keywords: Fish welfare, stunning methods, stress, drip loss.

Introduction

Over the last decade, studies concerning fish welfare at slaughter have become a major part of the animal welfare science. This is mainly due to the need to establish adequate pre-slaughter/stunning procedures guaranteeing loss of consciousness and sensibility before death, as well as to reduce the potential commercial loss (Van de Vis *et al*., 2003).

It should be noted that "measuring" fish welfare may be extremely difficult owing to a few main reasons. First of all, there is a variety of methods (behavioral, biochemical, physiological, etc.) to assess the effect of different pre-slaughter and slaughter procedures, but none of them are optimal (Poli, 2009). On the other hand, in the scientific literature there is debate about the ability of fish to experience pain and suffering (Braithwaite and Ebbesson, 2014), as some authors (Rose *et al*., 2014) still contest the capacity of fish for mental awareness due to the absence of neocortex. The third main reason is connected with the fact that even similar fish species may exhibit considerable physiological and/or behavioral differences (Daskalova *et al*., 2016). Moreover, the current European Union (EU) legislation does not provide any specific requirements in terms of the methods for stunning and killing fish. That is why fish welfare at slaughter should continue to be widely studied in order to develop appropriate procedures for each fish species that fulfill the general requirements of Council Regulation (EC) №1099/2009, i.e. to protect animals from any avoidable pain, distress or suffering during their killing. In this line, according to Opinion of the Farm Animal Welfare Committee, all farmed fish must be stunned before killing and the stunning and slaughter of fish should be included in EU welfare legislation (FAWC, 2014).

Furthermore, the development of humane pre-slaughter and slaughter methods may result in a number of commercial benefits, such as facilitating the production process, delaying the postmortem metabolic changes and improving some meat quality parameters. Increased muscle activity and high levels of stress before and at the time of killing are closely associated with subsequent endocrine response, usually manifested by elevated levels of so-called stress hormones - catecholamines and glucocorticoids (mainly cortisol) (Barton, 2002). These physiological reactions have been reported as one of the important factors altering the normal course of postmortem

muscle metabolism, i.e. muscle pH, onset and strength of rigor mortis and changes in muscle proteins (Wilkinson *et al*., 2008; Bahuaud *et al*., 2010). All these alterations may subsequently cause impaired meat quality by affecting some quality characteristics, such as freshness, colour, texture, gaping scores, water-holding capacity (percentage of drip loss) etc. (Oliveira Filho *et al*., 2015; Sigholt *et al*., 1997; Roth *et al*., 2006). In this context, the impairment of water-holding capacity leading to higher drip loss is of great interest, as it is substantial in commercial production. Pre-slaughter stress in mammals, resulting in low pH values at early postmortem stage, seems to be one of the main causes of reduced water-holding capacity. According to some authors, water-holding capacity is highly dependent on the rate and extent of the pH decline, which in turn affects the activity of some important enzymes involved in the postmortem proteolytic and lipolytic processes (Toldrá, 2003; Huff-Lonergan and Lonergan, 2005). As reviewed by Pearce *et al*. (2011), the exact mechanism, leading to increased drip loss, involves denaturation of myofibrillar and sarcoplasmic proteins due to rapid pH decline, as well as proteolysis, loss of membrane integrity and formation of loosely bound myowater.

The objective of this study was to assess the effect of three stunning procedures on stress levels (as judged by plasma cortisol and glucose concentrations) and meat quality (as judged by percentage of drip loss) of farmed common carp (Cyprinus carpio L.).

Materials and Methods

Experimental design

A batch of 21 market-sized common carp (Cyprinus carpio L.) (average body weight 1213 ± 118 g) was purchased from a commercial fish farm, located near town of Nikolaevo, Bulgaria. The fish were separated in three experimental groups: Group PS (n = 7), Group ES (n = 7) and Group AS (n = 7), and placed in three tanks, containing 800 L tap water (water temperature 15.9 ± 0.8°C, pH 7.18 ± 0.04) and constant aeration (dissolved oxygen concentration 3.56 ± 0.12 mg/L). The carp were kept at these conditions about 24 h, and then each group was subjected to a different stunning method.

The fish in Group PS were stunned by percussion - a sharp blow on the skull with a hammer.

The carp in Group ES were stunned electrically as described by Daskalova and Pavlov (2015). For electrical stunning, a device consisting of a capacitor (condenser; low capacitance of 47 μF) and two copper plates (electrodes) was used. Each experimental fish was individually captured and transferred to a smaller plastic box, containing 10 L tap water. The electrodes of the device were immersed into the water, placed bilaterally on the skull and each carp was subjected to a current of 4.7 mA (DC) by applying high voltage (~ 300 V) for 3 s. Fish that were not stunned adequately after the first current application (as judged by the presence of positive reactions to tactile and visual stimuli), were exposed to the current for a second time with the aim to obtain an epileptic-like seizure (Robb *et al*., 2002).

The fish in Group AS were removed from the water, placed in a plastic box and subjected to asphyxia for 90 ± 15 min, which was long enough to observe torpid state without death occurs.

Immediately after stunning, 2 ml blood samples were taken from each fish (by puncturing the heart) to determine cortisol and glucose levels. Then, the experimental carp were slaughtered by decapitation. Fish, that regained consciousness after stunning, were subjected to an additional blow on the skull with a hammer before being decapitated. After removing the skin, muscle samples (~ 10 × 2 × 1 cm) were obtained from each carp to determine the drip loss after freezing followed by thawing.

Analytical methods

Blood for analyzing glucose levels was placed in tubes containing EDTA and centrifuged for 10 min at 3000 rpm-1. Plasma was separated and blood glucose was measured by automatic biochemical analyzer BS-120 (Mindray, China). For measuring cortisol levels, blood samples were placed in tubes without anticoagulant and let coagulate at room temperature (about 22°C) until serum could be separated. Cortisol level in the blood serum was estimated by Cortisol ELISA kit EIA 1887® (DRG Instruments GmbH, Germany). Results calculation was carried out by automatic ELISA reader SUNRISE® (Tecan, Austria) (Daskalova *et al*., 2014) at 450 nm.

The dissolved oxygen concentration and the water temperature were measured using the handheld meter Multi 340i/SET (WTW, Germany) by immersing the probe direct in the water sample. Water pH was estimated by Sartorius Basic Meter PB-11 (Sartorius AG, Germany).

Drip loss (%) was estimated using a method similar to that described by Roth *et al*. (2006). The obtained muscle samples were weighed (W0), individually packed in a plastic bag and put in a freezer at -18°C. After 14 days of storage, all samples were transferred to a refrigerator (at 4°C) for thawing, which took about 24 h. The defrosted muscle samples were unpacked, cleaned of the excess fluid and weighed again (W1). The percentage of drip loss was calculated using the following formula: % drip loss = [(W0 – W1) / W0] × 100.

Statistical analyses

The results were presented as Average ± SD. All quantitative parameters (except the cortisol values) were analyzed using Student's t test (Microsoft Excel 2010). Linear correlation between blood glucose values and drip loss percentage was tested by

calculating R-squared value (R2) using Microsoft Excel 2010.

Ethics

The present experiment was approved by the Ethics and Animal Welfare Committee at Trakia University, Stara Zagora.

Results

Our results showed clear differences between the experimental groups in the levels of stress indicators, as well as in the drip loss percentage.

Cortisol levels in the blood serum of each fish in each of the three experimental groups (PS, ES, AS) are shown in Table 1. The results are presented separately for each fish because of the inability to calculate averages and the presence of significant standard deviations.

The carp stunned by percussion (Group PS) showed the lowest average glucose concentration in the blood plasma as compared with the fish stunned by electricity (Group ES; $P < 0.05$) and asphyxia (Group AS; $P < 0.01$). The highest blood glucose values were observed in Group AS. These values were significantly higher ($P < 0.01$) even than those measured in the blood of the fish in Group ES. The average blood glucose levels are presented in Table 2.

Similar differences between the experimental groups were observed in the percentage of drip loss. The muscle samples obtained from the fish in Group PS showed the lowest drip loss after thawing, but this percentage was insignificantly lower ($P \geq 0.05$) as compared with the electrically stunned carp. However, the fish in Group AS exhibited significantly higher drip loss in comparison with the results observed in Group PS ($P < 0.01$) and Group ES ($P < 0.05$). The average percentage of drip loss for each experimental group is shown in Table 3.

Linear correlation analysis showed meaningful positive association between blood glucose values and drip loss percentage in Group PS ($R2 = 0.60$) and Group AS ($R2 = 0.75$), whereas in Group ES the linear correlation was weaker ($R2 < 0.50$).

Discussion

Percussive and electrical stunning were the subject of our research due to the fact that they have shown great potential in two main directions: 1 - compliance with the recommendations for humane slaughter of fish, and particularly farmed carp (EFSA, 2009); and 2 - obtaining meat with high quality (Erikson *et al*, 2012; Özogul and Özogul, 2004). Asphyxia was included in the experiment as it is still one of the commonly used methods in commercial practice (especially in Bulgaria).

To assess levels of stress after each of the three stunning treatments, blood cortisol and glucose were measured. It is well known that these two biochemical substances have been widely used as stress parameters (Fatira *et al*., 2014; Woodward and Strange, 1987). Some authors, such as Martínez-Porchas *et al*. (2009), concluded that cortisol and glucose are still important indicators of stress, as cortisol may be useful in acute stress experiments, whereas glucose concentration could be used as an auxiliary measure. In our study, blood cortisol and glucose concentrations showed observable differences in the levels of stress between the three groups. Surprising was the fact that the experimental fish in Groups PS and ES demonstrated highly varying values of serum cortisol, which led to serious difficulties in statistical processing and

Table 1. Blood cortisol values (ng/mL) in carp, subjected to percussive stunning (Group PS), electrical stunning (Group ES) and asphyxia (Group AS)

Fish №	Blood cortisol values (ng/mL)		
	Group PS	Group ES	Group AS
1	98.67	603.93	633.70
2	0.48	> 800.00	> 800.00
3	86.83	> 800.00	> 800.00
4	15.62	> 800.00	> 800.00
5	665.47	> 800.00	> 800.00
6	676.53	3.21	> 800.00
7	64.65	13.80	> 800.00

Table 2. Average blood glucose levels in carp, subjected to percussive stunning (Group PS), electrical stunning (Group ES) and asphyxia (Group AS)

Group	Average blood glucose levels (mmol/L)	Standard deviation (SD)	Standard error (SE)
Group PS	3.86^{a}	1.26	0.48
Group ES	6.05^{b}	1.29	0.49
Group AS	11.34^{c}	3.07	1.16

* Statistically significant differences ($P < 0.05$) between the experimental groups are denoted by different letters.

Table 3. Average drip loss (%) after freezing (for 14 days) and thawing muscle samples obtained from carp, subjected to percussive stunning (Group PS), electrical stunning (Group ES) and asphyxia (Group AS)

Group	Average drip loss (%)	Standard deviation (SD)	Standard error (SE)
Group PS	2.02[a]	0.42	0.16
Group ES	2.29[a]	0.43	0.16
Group AS	3.22[b]	0.75	0.28

[*] Statistically significant differences ($P < 0.05$) between the experimental groups are denoted by different letters.

inability to calculate averages. At this point, we have no good explanation for this observation as all fish within a same experimental group were transported, handled and treated identically, as well as all blood samples were obtained and tested in a same manner. Based on scientific data, it can be hypothesized that the observed strong variations in cortisol levels were a result of considerable individual neuroendocrine and behavioral differences (Bonga, 1997; Sgoifo *et al*., 1996).

However, the raw data presented in Table 1 reveals some gradation in the cortisol concentrations, determined after each of the three stunning procedures (percussive stunning < electrical stunning < asphyxia). This tendency was fully supported by the results of blood glucose analyses, showing lowest glucose levels in Group PS and highest levels in Group AS (Table 2). Similarly, Varga *et al*. (2014) reported lowest cortisol concentrations (i.e. lowest levels of stress) in common carp, stunned by percussion. All these findings indicate that percussive stunning of carp similarly to other fish species, such as sea bass (Poli *et al*., 2002), leads to considerable reduction of stress at the time of killing.

Over the last few years, electrical stunning has been proved to be one of the most effective methods for stunning fish. In line with previous studies, electricity is becoming widely used for stunning several farmed fish species by applying an electrical current into the water (Lambooij *et al*., 2008; Lambooij *et al*., 2013; Roth *et al*., 2004) or after dewatering (Erikson *et al*., 2012; Llonch *et al*., 2012; Sattari *et al*., 2010). Furthermore, the method appeared to be suitable for improving fish welfare on-board commercial fishing vessels (Lambooij *et al*., 2012). Nevertheless, it should be noted that electrical stunning can be effective method only if correctly applied. Using the same electrical settings (300 V, 47 μF) as in our study, Çağiltay *et al*. (2015) reported that carp could be handled (sampled) without causing additional stress. However, our electrically stunned fish showed significant levels of stress as judged by blood cortisol and glucose concentrations. This was probably due to the fact that some of the carp were not rendered unconscious immediately and had to be stunned more than once, meaning that using an electrical current of 4.7 mA was not completely effective for stunning commercial-sized carp.

According to our results, the fish subjected to asphyxia for about 90 min showed higher levels of stress even compared with Group ES. This finding is in line with previous studies (Bertotto *et al*., 2007; Moini *et al*, 2011), indicating that asphyxia is one of the most stressful methods for stunning/slaughter fish. Furthermore, the increased stress levels were the most probable reason for the significantly higher drip loss observed after thawing the muscle samples obtained from Group AS (Table 3). Similarly, Bjørnevik and Solbakken (2010) reported that the exposure of farmed cod (Gadus morhua) to hypoxia led to significantly higher percentage of drip loss after 4 days of storage of the fillets on ice as compared to the control group of fish. According to the authors, this finding was highly related to the lower postmortem pH measured in the stressed cod. Unfortunately, we cannot confirm that the differences observed in our study are in direct correlation with the postmortem pH levels, as the muscle pH was not determined in this trial. However, it can be suggested that the frozen storage, followed by thawing, led to most serious structural alterations in the muscle tissue of the carp subjected to asphyxia. Such alterations could be a possible cause of the reduced water-holding capacity, i.e. increased liquid loss (Olsson *et al*., 2003). Furthermore, the positive correlation found between the plasma glucose levels and drip loss suggests that there is a considerable association between these two measures. As it is well known that blood glucose concentration is highly influenced by the glucocorticoids, it can be assumed that there would be positive relation between the cortisol levels and percentage of liquid loss. Whether or not this is actually the case remains to be confirmed in additional trials.

Conclusions

In conclusion, the three stunning procedures, investigated in the present study, showed considerable differences in terms of stress response and drip loss after freezing and thawing the obtained muscle samples. Percussive stunning seems to be the optimal method for stunning commercial-sized carp, as it leads to significant reduction of stress at the time of killing (as judged by the lowest blood cortisol and glucose levels) and better water-holding capacity of the meat (lowest liquid loss). Asphyxia cannot be considered an acceptable method due to the strong stress reaction (extremely high cortisol values) and significantly increased liquid loss. Electrical stunning

is a potentially effective method for stunning carp. However, to minimize the stress response, as well as to eliminate the need of repeated current application, some improvements of the electrical device are required. The present study does not involve extensive examinations, needed to evaluate in detail the relation between fish welfare at slaughter and fish meat quality, but it could be a step for assessment of the importance of pre-slaughter stress and its effect on some quality characteristics of the obtained meat.

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