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*Original article*

# EFFECT OF TWO STUNNING METHODS ON POSTMORTEM MUSCLE PH AND MEAT QUALITY OF COMMON CARP (*CYPRINUS CARPIO* L.)

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**Summary**

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Percussive and electrical stunning are the most promising methods in terms of rendering fish unconscious prior to slaughter. The aim of this study was to determine the effect of these two stunning methods on postmortem muscle changes and meat quality of common carps. The experimental fish were stunned either by percussion (Group 1) or by electrical current application (Group 2), both followed by immediate decapitation. Muscle pH was measured at different time intervals to assess the postmortem metabolic changes, whereas meat colour, drip loss and water activity were used as quality parameters. The fish in Group 1 showed slightly accelerated postmortem muscle metabolism, higher redness (a*) and yellowness (b*) colour values and lower water activity. Percentage of drip loss after 4 days of storage at 4 °C did not differ significantly between the two groups. Based on the obtained results we concluded that both tested stunning methods were not associated with high levels of stress and significant detrimental effect on the meat quality of common carps.

**Key words**: fish stunning, meat quality, muscle pH, slaughter, stress

## INTRODUCTION

In the recent years, there has been growing scientific and commercial interest in the area of animal welfare, especially with regard to the development of humane stunning and slaughter methods. Until now, the EU legislation has not provided specific requirements in terms of the procedures for stunning and killing fish. Nevertheless, these procedures must be based on the key principle, i.e. avoiding pain and suffering (Anonymous, 2009a). This is the reason why a number of recent studies have been conducted to establish effective stunning/slaughter methods for fish (Erikson *et al.*, 2006; Lambooij *et al.*, 2010; Llonch *et al.*, 2012) and to assess their impact on fish meat quality (Kiessling *et*

*al.*, 2004; Roth *et al.*, 2007; Rahmanifarah *et al.*, 2011).

It should be noted that some of the commonly used methods for stunning and killing fish, such as $CO_2$ narcosis, asphyxia, thermal shock, live exsanguination, etc., may be too slow and aversive (Anonymous, 2004). Moreover, the inappropriate preslaughter and slaughter procedures are usually followed by extreme stress reaction and increased muscle activity at the time of killing (Rahmanifarah *et al.*, 2010; Erikson, 2011). As a result, the stressed fish may exhibit various changes in postmortem muscle metabolism, such as enhanced anaerobic glycolysis, lower initial and ultimate pH, rapid rigor mortis onset (Wilkinson *et al.*, 2008; Rahmanifarah *et al.*, 2011) and accelerated proteolytic activity (Bahuaud *et al.*, 2010). All these metabolic changes can lead to impaired meat quality, often manifested by softer texture, reduced water-holding capacity (higher drip loss), lighter colour, higher gaping scores, etc. (Robb *et al.*, 2000; Kiessling *et al.*, 2004; Bosworth *et al.*, 2007).

According to EFSA opinion (Anonymous, 2009b) further research is needed to establish proper stunning/killing procedures for common carps, with focus on the potential effectiveness of percussive and electrical stunning. This was the main reason why our study was aimed at evaluating the impact of percussive and electrical stunning, both followed by decapitation, on postmortem muscle changes and some meat quality parameters of common carps (*Cyprinus carpio* L.). Moreover, we aimed to evaluate possible negative effects of electrical stunning (using low amperage electric current) on meat quality as compared to the percussive method, which is commonly used as a control method.

## MATERIALS AND METHODS

### *Fish and sampling*

A batch of 20 market sized carps (average body weight 1482.52 ± 215.63 g) was purchased from a commercial fish farm (Nikolaevo, Bulgaria). Immediately after arrival the fish were separated in two groups: Group 1 (n=10) and Group 2 (n=10) and placed in two tanks with 800 L tap water (water temperature 17.2±0.9°C; pH 7.13±0.02) and constant aeration (dissolved oxygen concentration 3.48±0.32 $mg.L^{-1}$). The carps were kept for one week at these conditions and then, the two groups were stunned by two different methods. The experimental design was approved by the Ethics and Animal Welfare Committee at Trakia University, Stara Zagora.

The fish in Group 1 were killed by a percussive blow on the head with a 300 g hammer, followed by decapitation. The fish in Group 2 were stunned electrically using a device consisting of a capacitor (condenser) and two copper plates (electrodes), kindly provided by Dr Atanasov (Department of Animal Husbandry, Faculty of Veterinary Medicine, Stara Zagora) and used for immobilisation of fish (unpublished data). The carps were transferred to a smaller water container and the electrodes of the stunner were immersed into the water and placed bilaterally on the cranium. Each fish was subjected to an electrical current (DC) with high voltage (~ 300 V), low capacitance (47 μF) and low amperage (4.7 mA) for 3 s and then immediately decapitated. If some of the fish were not stunned adequately after the first current application, they were exposed to the current for the second time to obtain an epileptic-like seizure which, as described by Robb *et al.* (2002), is characterised by a strong muscle spasm along

the body, open mouth, stretched fins, followed by relaxation and separate muscle twitches.

Four muscle samples were obtained from each experimental fish, as followed: 1) For pH determination – a part of the dorsal muscle (12×3×2 cm); 2) For meat color – a white muscle piece (3×2×1 cm) from the upper part of the fillet; 3) For water activity ($a_w$) – 2 white muscle samples (approximately 10 g); 4) For drip loss – a part of the dorsal muscle (10×2×1 cm).

*Physicochemical analyses*

Muscle pH was measured at 0, 3, 6, 12, 24, 48 and 72 h using Consort C532 pH meter (Consort nv, Turnhout, Belgium) by inserting a penetration electrode directly into the muscle sample. Between measurements the samples were kept in a refrigerator at 4 °C.

Meat colour values (L*, a* and b*) were estimated by Lovibond SP60 spectrophotometer (X-Rite Inc., Grandville, Michigan, USA). The CIE L*a*b* color space included the following colour coordinates: lightness (L*) – ranging from 0 (black) to 100 (white); red/green coordinate (a*): +a* indicating redness and –a* indicating greenness; yellow/blue coordinate (b*): +b* indicating yellowness and –b* indicating blueness (EN 15886:2010).

For measuring water activity level, the obtained white muscle samples were cut into small pieces and put into the probe holder of HygroLab water activity analyzer (Rotronic AG, Bassersdorf, Switzerland). These analyses were performed at days 0 and 4. Between the measurements the muscle samples were wrapped in plastic bags and kept in a refrigerator at 4 °C.

Drip loss (%) was estimated as described by Roth *et al.* (2006). The obtained muscle samples were weighed ($W_0$), wrapped in aluminium foil and put in a refrigerator at 4 °C. After 4 days of storage, the samples were unwrapped, cleaned of the excess fluid and weighed again ($W_1$). The drip loss was calculated using the following formula: % drip loss = $[(W_0 – W_1) / W_0] \times 100$.

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

*Statistical analysis*

The results were presented as mean ± SD. All quantitative parameters were analysed using Student's *t* test (Microsoft Excel 2010).

## RESULTS

Results revealed clear differences in postmortem muscle metabolism and some of the tested quality parameters of the fish in the two experimental groups.

*Muscle pH*

Immediately after death the fish stunned by percussion (Group 1) had pH about 7, which decreased significantly ($P<0.001$) at hour 3 and continued to drop until the 12$^{th}$ hour when pH reached its ultimate level of 6.49±0.10. A significant increase ($P<0.001$) was observed at hour 24, followed by a decrease ($P<0.05$) at hour 48. At the last time interval (72 h) pH raised ($P<0.001$) as compared to the 48$^{th}$ hour but remained lower ($P<0.001$) as compared to the initial values (Fig. 1).

The electrically stunned carps (Group 2) had significantly lower ($P<0.01$) initial meat pH as compared to Group 1, which decreased ($P<0.001$) by the 3$^{rd}$ hour and persisted almost unchanged up to post-

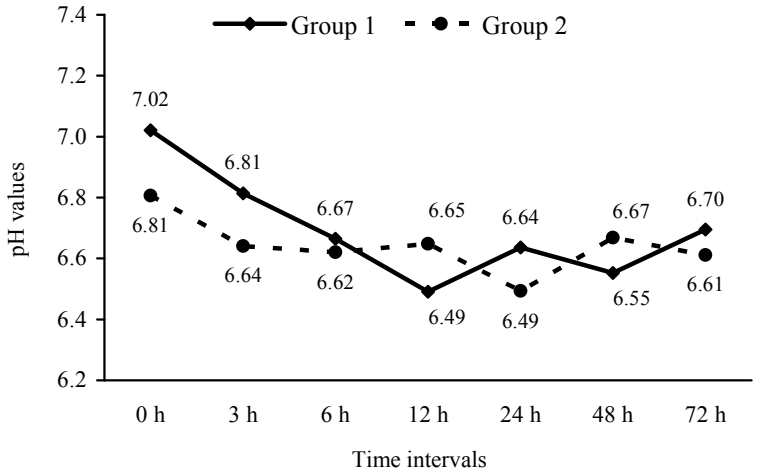


**Fig. 1.** Postmortem muscle pH of carps stunned by percussion (Group 1) or by 3 s exposure to an electrical current (Group 2), both followed by immediate decapitation. The data labels at each time point show the mean pH values.

mortem hour 12. After that, pH started to drop again and at 24 h reached its ultimate level of 6.49 ± 0.12. At hour 48 pH values increased ($P<0.01$) and remained unchanged up to the end of the study. At hour 72, meat pH of the electrically stunned carps was still lower ($P<0.01$) than the initial levels, and similar to that of fish killed by percussion (Fig. 1).

*Meat colour, drip loss and water activity*

At day 0, the lightness (L*) values did not differ significantly between the two experimental groups. At the same time, the muscle samples obtained from the carps in Group 1 showed higher redness (a*) ($P<0.001$) and yellowness (b*) ($P<0.05$) values than those from Group 2 (Table 1). Similar differences between groups were observed after 4 days of storage. Furthermore, by day 4 L* decreased considerably ($P<0.001$) in both groups as compared to the initial values and in addition, the electrically stunned carps showed lower b* values ($P<0.001$).

The drip loss percentage of the muscle samples after 4 days of storage at 4°C did not differ significantly between the two groups (Table 1).

The results from water activity analyses showed that the muscles of the fish stunned by percussion had lower $a_w$ at both days 0 ($P<0.01$) and 4 ($P<0.001$) as compared to the carps stunned by electricity. The storage of the samples for 4 days at 4 °C did not lead to any significant changes in the water activity level in either group (Table 1).

## DISCUSSION

Percussion and electricity are the two stunning methods that have proven to be effective in terms of humane killing of fish (Lambooij *et al.*, 2010; Erikson *et al.*, 2012). Whereas percussion has been used as a control method by some authors (Rahmanifarah *et al.*, 2011), electrical stunning still poses certain challenges in terms of quality (Roth *et al.*, 2003). Our aim was to

**Table 1.** Meat colour values (L*, a*, b*), drip loss (%) and water activity of carps stunned either by percussion (Group 1) or by 3 s exposure to an electrical current (Group 2), both followed by immediate decapitation. Data are presented as mean ± SD.

| | Time interval | Colour coordinates | | | Drip loss, % | Water activity |
|---|---|---|---|---|---|---|
| | | L* | a* | b* | | |
| Group 1 | day 0 | $45.64\pm1.96^{ax}$ | $3.18\pm0.30^{ax}$ | $6.03\pm0.90^{ax}$ | – | $0.946\pm0.006^{ax}$ |
| | day 4 | $44.49\pm2.02^{cy}$ | $3.12\pm0.32^{cx}$ | $5.81\pm1.19^{cx}$ | $1.68\pm0.32^{c}$ | $0.944\pm0.005^{cx}$ |
| Group 2 | day 0 | $45.15\pm0.79^{ax}$ | $2.72\pm0.20^{bx}$ | $5.12\pm0.19^{bx}$ | – | $0.960\pm0.011^{bx}$ |
| | day 4 | $43.25\pm0.80^{cy}$ | $2.73\pm0.37^{dx}$ | $4.02\pm0.41^{dy}$ | $1.46\pm0.27^{c}$ | $0.961\pm0.003^{dx}$ |

Statistically significant differences ($P<0.05$) between the groups and time intervals for each of the tested parameters are denoted by different letters (a–b indicate differences between groups at day 0; c–d indicate differences between groups at day 4; x–y indicate differences between day 0 and day 4 within the same group).

assess the differences in postmortem muscle metabolism and some meat quality parameters of carps stunned either by percussion or by applying electricity.

The fish stunned electrically showed accelerated anaerobic muscle metabolism immediately after death as this group of fish had lower initial muscle pH in comparison to the carps stunned by percussion. Similar findings were observed by Roth *et al.* (2007) in electrically stimulated turbot. Furthermore, when the fish is subjected to extremely stressful procedures before and/or at the time of slaughter, postmortem muscle pH is usually characterized by low initial values, rapid decline in time (reaching ultimate values between 3 and 9 h after death) and considerably lower ultimate levels (Wilkinson *et al.*, 2008; Rahmanifarah *et al.*, 2011). Despite the lower initial pH values, our electrically stunned fish showed slower pH decrease in time, no significant differences in the ultimate pH values and similar pH levels at the final point as compared to Group 1. Therefore, as judged by the changes in postmortem pH, we may conclude that both stunning/killing methods were not associated with unacceptable levels of stress. However, electrical stunning showed slight superiority judging by the slower and steadier course of the postmortem metabolic processes. At this point, we have no good explanation for the pH fluctuations detected in Group 1, but similar findings have been previously observed in Senegal sole and barramundi (Ribas *et al.*, 2007; Wilkinson *et al.*, 2008). These fluctuations though, were not of great importance for the conclusions made in this study, as they did not affect the interval between the initial and the ultimate pH values.

It has been found that the strong postmortem pH decline is a key factor for the development of so-called "pale, soft, exudative" (PSE) meat in turkeys, characterised by lighter colour and reduced water-holding capacity (Pietrzak *et al.*, 1997). As mentioned above, comparable changes can be observed in stressed fish. In this line, as our experimental groups of fish had very similar ultimate pH (which was not extremely low), it was not surprising that they showed no significantly different levels of drip loss and L* values. On the

other hand, the musculature of the fish from Group 1 had higher a* and higher b* values both at days 0 and 4 as compared to Group 2. Contrary to these findings, Roth *et al.* (2006) reported higher a* and b* values in Atlantic salmon (*Salmo salar*) killed by a sharp blow on the head and then exercised electrically for 2 min in comparison to salmon killed by the same method but without subsequent electrical stimulation. We may hypothesise that the reason for this discrepancy is the enhanced anaerobic muscle metabolism in the electrically stimulated salmon (earlier rigor mortis onset) which is more similar to the postmortem changes in our carps stunned by percussion (faster pH decrease).

Interestingly, the musculature of our electrically stunned carps showed significantly higher water activity levels as compared to the fish stunned by percussion. Water activity ($a_w$) expresses the vapour pressure generated by the unbound water in foods. Borba *et al.* (2013) observed no effect of the rearing system on the water activity of broiler breast muscles despite the differences found in pH and meat redness (a*). Until now, no direct relation has been discovered between stress or electrical current application and water activity of the obtained meat. As water activity is one of the most important factors affecting bacterial growth (Troller, 1971) and food stability (Mathlouthi, 2001), further research is needed to determine whether its level in our study was certainly influenced by the stunning method.

We conclude that both stunning/killing methods used in this study do not cause unacceptable levels of stress, as well as do not have any considerable adverse effect on the quality of the obtained meat. However, electrical stunning demonstrates slight superiority as judged by the slower postmortem pH decline. Therefore, electrical stunning, after some improvements of the electrical device eliminating the need of repeated current application, could be a suitable method for stunning fish in the commercial practice.

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