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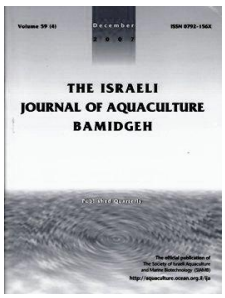

Impact of handling and pre-mortal stress on the quality of common carp (*Cyprinus carpio* L.)

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Abstract

The aim of this study was to investigate the stress in common carp (*Cyprinus carpio* L.) caused by harvesting, transport and stunning method, and the effect of the latter on meat quality.

There was a significant increase in serum cortisol concentration during harvesting and transport. Stunning method had significant impact on the blood cortisol concentration ($P<0.01$). Minimal stress was caused by the percussive stunning. It was followed by the CO_2 asphyxiation and the biggest stressor was the live chilling. Stunning method had no significant effect on the conventional meat quality. CO_2 treated group had a delayed stiffening in the *rigor mortis* development. The pH fall of CO_2 asphyxiated and live chilled groups were more effective compared to the blow on head group. Summarized, according our results percussive stunning led to the best fillet quality and this method is less objectionable from animal welfare aspects.

Introduction

Nowadays there are several stunning methods used in fish slaughter. Best known methods are percussive stunning, electrical stunning, live chilling, asphyxiation and gutting without stunning. On the one hand the fact that stress can modify the quality and value of fish flesh is widely accepted, but on the other hand there is no consensus which method is least stressful for the fishes.

Culture related to handling such as anesthesia (Altun and Danabas, 2006), transportation (Peng et al., 2012) have been widely studied. Namely handling prior to

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slaughter, different stunning methods and their effect on fish welfare (Lambooij et al, 2006, 2007, 2008, 2010) and flesh quality (Scherer et al., 2006; Nathanailides et al., 2011; Roth et al., 2009; Lefévre et al., 2008; Wilkinson et al., 2008) are well documented in salmonids and other high value species. These procedures in cyprinids nevertheless are poorly described.

Due to its fast growth rate and easy cultivation, common carp (*Cyprinus carpio*) is one of the most widely cultured species all over the world.

The purpose of our study was to model the carp trading practice and slaughtering methods, and to measure the effect of handling and slaughtering stress on the flesh quality of these fish.

Materials and Methods

Altogether 60 market sized common carps were taken from a fish farm at harvesting time (middle of November 2011). After harvesting fish were immediately transported to the Fish Laboratory of Kaposvár University in an aerated fish tank and stocked in 500 L re-circulated and aerated fish tanks.

To determine the stress level, blood samples were taken at several time-points: after harvesting and after transportation to the laboratory. Blood samples were taken from all fish after stunning and before gutting also from the tail vein with 22G needles. After withdrawal into Eppendorf tubes the blood was immediately placed on ice, left to clot, centrifuged (1500G/10 min) and the serum was stored frozen (-70 ^{o}C) until analysed.

Fishes were slaughtered by three different methods (15 fish/group). Carps of the first group underwent percussive stunning by a blow on head. Those in the second group were stunned by chilling in ice slurry. The third group was anesthetized by asphyxiation in CO_2 saturated water. Fish in each group were gutted immediately after the treatments.

10 individuals from each group of the slaughtered and gutted fish were filleted immediately and the pH value (Testo 205 pH *post mortem* 24 h) as well as their color (Minolta ChromaMeter 300, L, a*, b*) of the fillet were determined.

Fillet liquid dripping loss was determined by the method of Honikel (1998). To determine the so-called cooking loss, fillet samples (100g) were closed into sealed bags and were cooked at 75 °C for 20 min. The exudate weight, as expressed in the percentage of the initial sample weight was referred to as cooking loss. The thawing loss was determined by the same manner, i.e. samples (25g) were frozen (−20 °C) and thawed to room temperature after 2 days.

The remaining five carps from each fish group were used to record the progression of rigor in gutted fish by the following method. The fish were stored on a solid flat surface. Measurements were performed by placing the carp so that the body part behind the posterior end of the dorsal fin was hanging over the edge, unsupported. The rigor angle was calculated as $\alpha=\tan^{-1}(X/Y)$, where X: length (cm) of the horizontal leg of the right-angled triangle, and Y: length (cm) of the vertical leg of the right-angled triangle. Measurements of rigor angle were done at 3, 6, 9, 12, 24 and 48 h *post mortem*. At the same time fillet pH values were also determined.

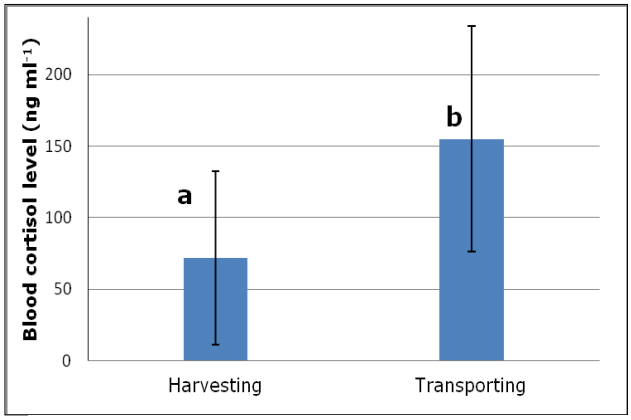
As a response to stress, the blood cortisol level increases. To follow the changes resulted by the different treatments, the blood serum cortisol concentrations were estimated by radioimmunoassay (RIA) method with Kortizol [^{125}I] RIA kit (Izotóp Intézet Ltd., Budapest, Hungary) and gamma counter (Jeney et al, 1992).

From the basic dataset outlier values (Dean and Dixon, 1951) were excluded and the remaining data were tested for normality (Shapiro-Wilk test). For the analysis of the extent of stress caused by handling and stunning methods t-test and ANOVA were used, and effect of stunning method on meat quality ANOVA (Tukey *post hoc*) was used also. In all instances SPSS 10 for Windows (1999) was used.

The experiment was approved by the Animal Experimentation Ethics Committee of the Kaposvár University, as allowed by the Somogy County Animal Health and Food Control Authority.

Results

The stress level development of carps during the harvesting and transport is shown in Fig 1. There was a significant difference ($p<0.05$) between procedures on the blood cortisol concentration.



Slaughtering method had as well a significant impact on the blood cortisol concentration (Fig 2). According to our results, minimal stress was caused by the percussive stunning. It was followed by the CO_2 asphyxiation and the biggest stressor was the live chilling.

Fig. 1. Carp's blood cortisol level during handling and storage (Significant difference between groups: a,b $P<0.05$)

The slaughtering method did not significantly affect any of the conventional meat quality parameters, and between-group differences were also not detected (Table 1). In the different characteristics concerning water holding capacity (cooking, dripping and thawing losses) no inter-group differences were found, while handling these three traits together (total moisture loss) a more expressed difference was found among the groups. The largest moisture loss was observed in the group chilled alive, and the lowest in those treated with CO_2.

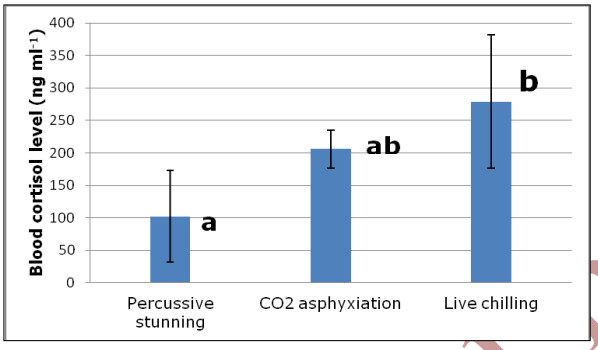


Fig. 2. Blood cortisol level of carps stunned by different methods (Significant difference between groups: a,b$P<0.01$)

Table 1 Flesh quality parameters of carps slaughtered by different methods

Parameter	*Stunning methods*			*Significance*
	Blow on head	*Live chilling*	*CO2 asphyxiation*	
Cooking loss (%)	22.74 ± 2.26	23.86 ± 3.46	22.19 ± 2.14	NS
Dripping loss (%)	2.79 ± 0.69	2.54 ± 0.21	2.75 ± 0.39	NS
Thawing loss (%)	6.07 ± 2.05	5.91 ± 1,54	6.15 ± 1.89	NS
L	44.48 ± 1.88	44.98 ± 2.09	44.08 ± 1.79	NS
a*	2.16 ± 1.76	2.38 ± 1.21	3.21 ± 1.61	NS
b*	0.38 ± 1.26	0.42 ± 0.9	0.7 ± 0.86	NS

NS: no significance

The rigor and pH development of gutted carps is shown in Fig 3. and Fig 4. The slaughter method itself did not exert a significant effect on the *rigor mortis* and pH value of the flesh. The development of rigor initiated at 6 hours *post mortem*, until this time point only a slight increase was found in the rigor declination. After the first 6 hours the process was augmented and this pace was maintained in the first 24 hours *post mortem*, when the process slowed down. The evolvement of rigor declination was highly similar in the groups stunned with head-blow and alive chilling. In the CO_2 treated fish the rigor started ca. 6 hours later and the ultimate rigor declination remained below the values reached by the other two groups.

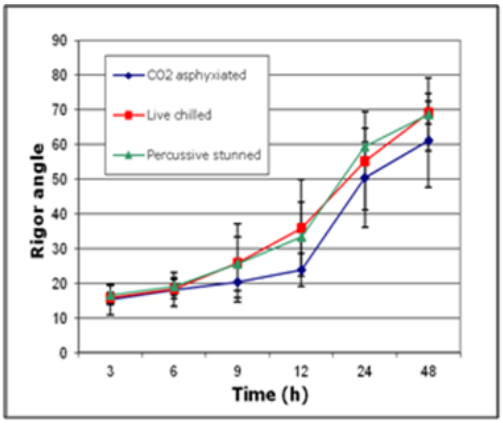


Fig 3. Rigor development of gutted carps slaughtered by different methods

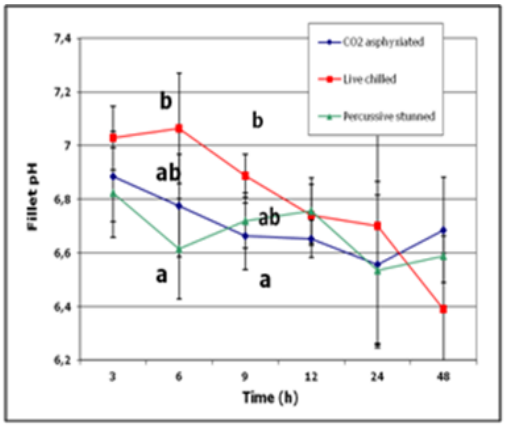


Fig. 4. pH development of gutted carps slaughtered by different methods (Significant difference between groups: a,bP<0.01)

Discussion

Higher stress was caused by the transportation, because it is a complex stressor for the live transported fishes (Harmon 2009). Likely, it is due to not only the crowding but also the hyperoxia (Lushchak et al. 2005), because the water in the transport tank was supersaturated with oxygen. Our results are supplied by the results of other authors. Dobšiková et al. (2009) described stress-related haematological changes in common carp during transportation.

According to Wilkinson et al. (2008) there was no difference in the fillet liquid dripping loss between conventional and non-stressed harvested barramundi (*Lates calcarifer*). In contrast, mild stress in seabream (*Sparus aurata*) induced lower dripping loss, as compared to the high stress stunned group (Nathanailides et al., 2011).

Flesh color was the trait providing the largest difference among the groups. While the lightness (L) of fillets was identical, the redness (a*) and the yellow (b*) color components were higher by the CO_2 treated fish, as compared to all other groups. This difference was most probably attributed to the remnant blood in the fillet. During the CO_2 asphyxiation the heart activity slows or stops completely, thus significant amount of blood is supposed to remain in the tissues. The decapitation could result minimal or nil losses during the gutting. In contrast, in the percussive stunning process and the live chilling methods, the heart contractions continues, resulting more effective bleeding compared to CO_2 asphyxiation. Furthermore, the motion of fishes slows down, but the heart rate increases (tachycardia, Lambooij et al., 2006, 2008) because of the stress, by the live chilling method. Olsen et al. (2006, 2008) published similar results in case of Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*), namely pre-mortal stress increased the rate of remnant blood (blood spotting) in the muscle tissue in both species.

The development of *rigor mortis* is closely linked to the production of lactic acid resulting from the anaerobic breakdown of glycogen, with a concomitant drop in muscle pH (Korhonen et al., 1990). *Rigor mortis* is the first *post mortem* process that has a major influence on the appearance and structure of the fish flesh (Berg et al., 1997). Processing fish in the rigor stage may result in reduced fillet yield, texture alteration and increased gaping (Einen et al., 2002). Faster emergence of rigor angle depends on the higher harvesting stress in barramundi (Wilkinson et al. 2008) and higher slaughter stress in salmon (Mørkøre et al. 2008). In our tests the correlation between *rigor mortis* development and the extent of pre-mortal stress is not so clear. Emergence of rigor is similar in the groups stunned to the highest and the lowest stress causing methods.

Carp muscle pH decreased *post mortem* (Fig 4). There are differences between groups in initial pH values. Lower initial pH value in processed fish fillet is caused by higher pre-mortal stress, as compared to non-stressed fish (Wilkinson et al. 2008; Mørkøre et al. 2008). This phenomenon is due to the enhanced stress-derived lactate level in the muscle (Lowe et al, 1993; Erikson et al., 1999). Our results are inconsistent, because it can be seen a negative correlation between stress level and initial fillet pH.

Muscle pH of CO_2 asphyxiated and live chilled fish showed stronger decrease at 24 h *post mortem*, as compared to the blow-on-head group (Fig 4). This phenomenon is in

context with the stress-related increased glycolytic activity. Immediate termination of movement is resulted by the percussive stunning, but fish responded with increased activity to the changed environment in case of live chilling and asphyxiation. In latter two cases the status of fish is dominantly hypoxic, as the oxygen demand of the increased muscle activity can not be covered in the CO_2 saturated water. Plausible the relative and absolute anoxia (besides the increased physical activity) contributes to the increase of anaerobic glycolysis, which leads to lactate-derived pH decrease in the fillet ultimately.

Summarized, significant stress was caused to fishes by harvesting and transport. From the aspect of animal welfare the most human method to stun the carp is a blow on the head. Live chilling is less recommended method in carp slaughter process, considering that the highest stress was caused by this treatment. CO_2 asphyxiation and percussive stunning led to favorable *post mortem* pH development. Considering the remnant blood in the fillet, eventually blow on the head led to the best fillet quality and this method is less objectionable from animal welfare aspects.

Acknowledgements

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