The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

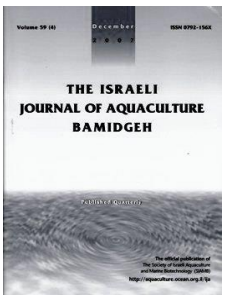
Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),
University of Hawai'i at Mānoa Library
&
University of Hawai'i at Mānoa Aquaculture Program
in association with
AquacultureHub
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

The Israeli Journal of Aquaculture - Bamidgeh, IJA_66.2014.963, 6 pages



Impact of Handling and Pre-Mortal Stress on the Flesh Quality of Common Carp (*Cyprinus carpio* L.)

Dániel Varga[1], András Szabó[1], Csaba Hancz[1], Zsigmond Jeney[2], László Ardó[2], Marcell Molnár[1], Tamás Molnár[1]*

[1] *Kaposvár University, Faculty of Animal Science, Kaposvár, Hungary*

[2] *Research Institute for Fisheries, Aquaculture and Irrigation, Szarvas, Hungary*

(Received 02.7.13, Accepted 15.5.13)

Key words: common carp, slaughter stress, flesh quality

Abstract

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

Serum cortisol concentration increased during harvesting and transport. The percussive stunning (blow on head) method caused the least stress and resulted in a significant increase in blood cortisol concentration ($P<0.01$). This method had no significant effect on conventional flesh quality. CO_2 asphyxiation which was more stressful delayed stiffening in rigor mortis development. The greatest stress was caused by live chilling. There was a decline of pH levels in the CO_2 asphyxiated and live chilled groups compared to the percussive stunned group. According to our results percussive stunning led to the best fillet quality and is the least objectionable method according to animal welfare standards.

* Corresponding author. Tel.: +36-82-505-800/292 fax: +36-82/ 320-175,
e-mail: molnart75@gmail.com

Introduction

There are several methods used worldwide in fish stunning and slaughter, the best known of these being percussive stunning, electrical stunning, live chilling, asphyxiation and gutting without stunning or after stunning or chilling. The notion that stress can modify the quality and value of fish flesh is widely accepted, but there is no consensus as to which method of stunning is least stressful for the fish.

Practices related to handling during transportation, such as use of anesthesia (Altun and Danabas, 2006; Peng et al., 2012), and handling prior to slaughter, different stunning methods, and their effect on fish welfare and flesh quality, have been widely studied in salmonids and other high value species (Lambooij, et al., 2006, 2007, 2008, 2010; Scherer et al., 2006; Nathanailides et al., 2011; Roth et al., 2009; Lefévre et al., 2008; Wilkinson et al., 2008). These procedures in cyprinids are poorly documented.

Common carp (*Cyprinus carpio* L.) is one of the most widely cultured species worldwide due to its rapid growth rate and ease of cultivation. The purpose of our study was to simulate carp trading practices and stunning methods, and measure the effect of handling, stunning, and slaughtering stress on the flesh quality of these fish.

Materials and Methods

Sixty market-sized common carp were harvested from a fish farm in mid-November 2011. The fish were harvested using conventional netting and then immediately transported for 20 minutes in an aerated plastic fish tank ($1m^3$) to the Fish Laboratory of Kaposvár University. There they were kept in 500 liter fish tanks with re-circulated and aerated water.

Using 22G needles, blood samples were drawn from the tail vein of all fish into Eppendorf tubes to determine stress level. The samples were taken after harvesting, after transportation to the laboratory, and finally after stunning. The blood samples in the tubes were immediately placed on ice, left to clot, centrifuged (1500G/10 min) and the serum was stored frozen at-70^{o}C until analysis.

Three groups of carp (15 fish/group) were stunned using three different methods: 1) percussive stunning; 2) chilling in ice slurry; 3) anesthetized by asphyxiation in CO_2 saturated water. Fish in each group were gutted immediately after the treatments. 10 fish from each group of the slaughtered and gutted fish were then filleted.

pH value was measured 24 h post mortem using a Testo 205 precision pH meter, and the color of the fillet, (L=lightness, R=redness, Y=yellowness), was determined using a Minolta ChromaMeter 300. Fillet liquid dripping loss was evaluated (Honikel, 1998). To evaluate cooking loss, fillet samples (100g each) were placed into sealed bags and 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. Thawing loss was determined in the same manner, i.e. 25 g samples were frozen (−20 °C) and thawed to room temperature after 2 days.

Rigor progression in gutted fish was measured in the remaining five carp from each group by placing the carp on a solid flat surface so that the body part behind the posterior end of the dorsal fin was hanging unsupported, over the edge. The rigor angle was calculated as $\alpha=\tan^{-1}(X/Y)$, where X=length (cm) of the horizontal base of the right-angled triangle, and Y=length (cm) of the vertical side of the right-angled triangle. Rigor angle measurements, and fillet pH values, were taken at 3, 6, 9, 12, 24 and 48 h post mortem.

Blood cortisol concentration increases as a response to stress. To track the changes resulting from the different treatments, blood serum cortisol concentrations were estimated using the radioimmunoassay (RIA) method with a Kortizol [^{125}I] RIA kit (Izotóp Intézet Ltd., Budapest, Hungary) and a 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). A t-test and ANOVA were used to analyze the stress caused by handling and stunning methods, and ANOVA (Tukey post hoc) was used to examine the effect of stunning method on flesh quality. 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 different stress levels of carp are reflected in the blood cortisol concentration during harvesting and transport (fig.1). Blood cortisol concentration varied according to different stunning methods (fig.2).

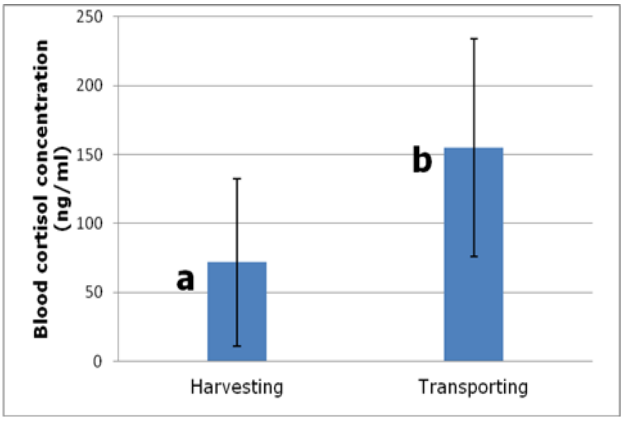


Fig.1. Carp blood cortisol concentration during harvesting and transport (Sig. diff. between groups: a,b P<0.05)

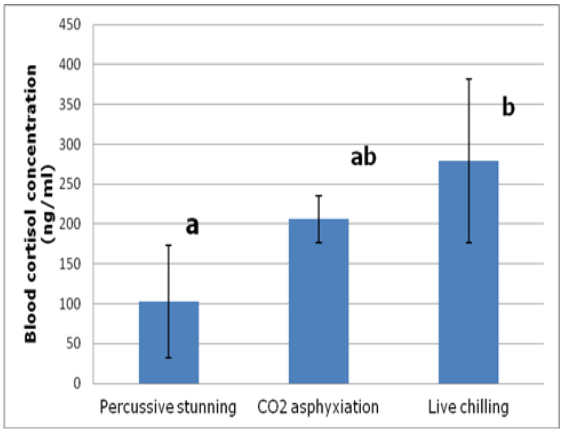


Fig. 2. Blood cortisol concentration of carp stunned by different methods (Sig. diff. between groups: a,b P<0.01)

Percussive stunning (blow on head) caused the least stress and live chilling caused the most stress. The stunning method did not significantly affect any of the conventional flesh quality parameters, and there was no significant difference between the groups. (Table 1).

Table 1. Flesh quality parameters of carp stunned 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 (lightness)	44.48 ± 1.88	44.98 ± 2.09	44.08 ± 1.79	NS
R (redness)	2.16 ± 1.76	2.38 ± 1.21	3.21 ± 1.61	NS
Y (yellowness)	0.38 ± 1.26	0.42 ± 0.9	0.7 ± 0.86	NS

NS: no significance

No inter-group differences were found in the water holding capacity characteristics (cooking, dripping, and thawing loss).

The rigor and pH change of gutted carp is shown in Figs. 3 and 4. The stunning method had no significant effect on rigor mortis but had a significant effect on pH value of the flesh. In the group stunned with a head blow, and the live chilled group, the onset of rigor was noted 6 hours post mortem; until this time only a slight increase was found in rigor declination. After the first 6 hours the process intensified up until 24 hours post mortem, after which the rate decreased. In the CO_2 treated fish the onset of rigor only began at 12 h and rigor declination remained below the values of the other two groups.

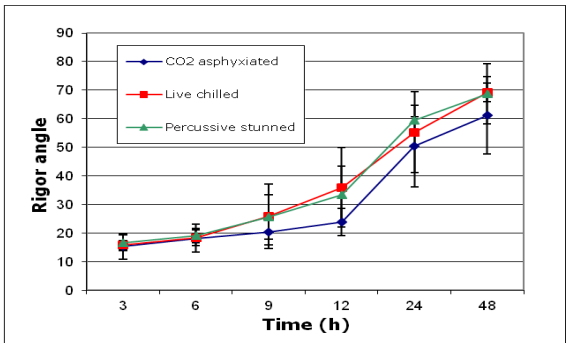


Fig. 3. Rigor development of gutted carp stunned by different methods (No significant difference)

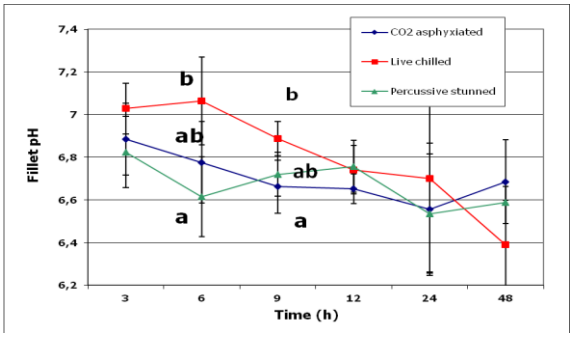


Fig. 4. pH change of gutted carp stunned by different methods (Significant difference between groups: a,b P<0.01)

Discussion

High stress resulted from transportation, a complex stressor for live fish (Harmon 2009). In addition to crowding this may also be due to hyperoxia (Lushchak et al. 2005) as the water in the tank is supersaturated with oxygen. Our results are supported by other studies which describe stress-related hematological changes in common carp during transportation (Dobšiková et al., 2009).

No difference in the fillet liquid dripping loss was found in barramundi (*Lates calcarifer)* harvested by either the conventional method using netting, or a non-stressed harvesting method combining isoeugenol sedation and netting (Wilkinson et al., 2008). In contrast dripping loss was less in mildly stressed seabream, *Sparus aurata* compared to the high stressed stunned group (Nathanailides et al., 2011).

Flesh color was the trait where the greatest difference between the groups could be seen. While the lightness (L) of fillets was identical, the red (R) and yellow (Y) color components were higher in the CO_2 treated fish, compared to the other groups. This difference may be attributed to blood in the fillet. During CO_2 asphyxiation heart activity slows or stops completely, leaving a significant amount of blood in the tissues. Decapitation may result in minimal or no loss during gutting. In the percussive stunning process and live chilling methods, the heart contractions continue, resulting in more bleeding when compared to CO_2 asphyxiation. Due to stress caused by live chilling, fish movement decreases, but the heart rate (tachycardia) increases (Lambooij et al., 2006, 2008). Pre-mortal stress also increased the rate of remnant blood (blood spotting) in the muscle tissue of Atlantic salmon, *Salmo salar* and cod, *Gadus morhua* (Olsen et al., 2006, 2008).

Rigor mortis development is closely linked to lactic acid production 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 onset of the rigor angle is related to harvesting stress in barramundi (Wilkinson et al. 2008) and greater slaughter stress in salmon (Mørkøre et al. 2008). In our tests the correlation between rigor mortis development and pre-mortal stress is unclear. Rigor development was similar in all groups regardless of the stress level of the slaughtering method used.

There are differences between groups in initial pH values. Carp muscle pH decreased post mortem (Fig 4). Lower initial pH value in processed fish fillet is caused by higher pre-mortal stress, when 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 as there is a negative correlation between stress level and initial fillet pH.

There was a greater decrease of muscle pH of CO_2 asphyxiated and live chilled fish at 24 h post mortem, compared to the percussive stunned group (Fig 4). This phenomenon is consistent with stress-related increased glycolytic activity. Percussive stunning causes immediate cessation of movement. However live chilling and asphyxiation increase fish activity as they struggle due to the anoxic conditions, since the oxygen demand of the increased muscle activity cannot be supplied in the CO_2 saturated water. The relative and absolute anoxia (apart from that induced by the increased physical activity) contributes to the increase of anaerobic glycolysis, which ultimately leads to lactate-derived lowered pH in the fillet.

Both harvesting and transport cause stress in fish. CO_2 asphyxiation and percussive stunning produced the most favorable post mortem pH results. Live chilling, which is the highest stressor is a least preferred method of carp slaughter. The percussive stunning method where remnant blood remains in the fillet, produces the best fillet quality and is considered the least objectionable method of carp slaughter according to animal welfare standards.

Acknowledgements

This study was supported by the Hungarian Scientific Research Fund (OTKA), project id. 83150, the Bolyai János research grant from the Hungarian Academy of Sciences (BO_26/11/4), and by the TÁMOP 422B project.

References

Altun T., and D. Danabas, 2006. Effects of short and long exposure to the anesthetic 2-phenoxyethanol mixed with ethyl alcohol on common carp (*Cyprinus carpio* L., 1758) fingerlings *Isr. J. Aquacult. - Bamidgeh,* 58(3):178-182.
Berg T., Erikson U. and T.S. Nordtvedt, 1997. Rigor mortis assessment of Atlantic salmon (*Salmo salar*) and effects of stress. *J. Food Sci.*, 62:439-646.
Dean R.B. and W.J. Dixon, 1951. Simplified statistics for small numbers of observations. *Analyt. Chem.*, 23:636-638.
Dobsiková R., Svobodá Z., Bláhová J., Modrá H. and J. Velisek, , 2009. The effect of transport on biochemical and haematological indices of common carp (*Cyprinus carpio* L.). *Czech J. Anim. Sci.,* 54(11):510-518.
Einen O., Guerin T., Fjaera S.O. and P.O. Skjervold, 2002. Freezing of pre-rigor fillets of Atlantic salmon. *Aquaculture*, 210:129-140.
Erikson U., Sigholt T., Rustad T., Einarsdottir I.E. and Jorgensen L., 1999. Contribution of bleeding to total handling stress during slaughter of Atlantic salmon. *Aquacult. Int*., 7:101-115.
Harmon T.S., 2009. Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. *Rev. Aquacult.*, 1:58-66.
Honikel K.O., 1998. Reference methods for the assessment of physical characteristics of meat. *Meat Science*, 49:447-457.
Jeney Z., Jeney G., and A.G. Maule, 1992. Cortisol measurement in fish. pp. 157-166. In:Stolen, J.S., Fletcher, T.C, Anderson, D.P., Kaatari, S.L. and A.F. Rowley, (eds.) *Techniques in Fish Immunol.*, SOS Publications, 43 DeNormandie Ave., Fair Haven, N. J. 07704-3303 USA.
Korhonen R.W., Lanier T.C. and Gieshbrecht F., 1990. An evaluation of simple methods for following rigor development in fish. *J. Food Sci.,* 55:30-46.
Lambooij E., Pilarczyk M., Bialowas H., Boogaart J.G.M. van den and J.W. van de Vis, 2007. Electrical and percussive stunning of the common carp (*Cyprinus carpio* L.): Neurological and behavioural assessment. *Aquacult. Eng.,* 37:171-179.
Lambooij E., Klosterboer R.J., Gerritzen M.A. and Van de Vis J.W., 2006. Assessment of electrical stunning in freshwater of African Catfish (*Clarias gariepinus*) and chilling in ice water for loss of consciousness and sensibility. *Aquaculture*, 254:388-395.
Lambooij E., Gerritzen M.A, Reimert H., Burggraaf D. and van de Vis J.W., 2008. A humane protocol for electro-stunning and killing of Nile tilapia in fresh water. *Aquaculture*, 275:88-9.
Lambooij E., Grimsbo E., van de Vis J.W., Reimert H.G.M., Nortvedt R. and Roth B., 2010. Percussion and electrical stunning of Atlantic salmon (*Salmo salar*) after dewatering and subsequent effect on brain and heart activities. *Aquaculture*, 300:107-112.
Lefévre F., Bugeon J., Aupérin B. and J. Aubin, 2008. Rearing oxygen level and slaughter stress effects on rainbow trout flesh quality. *Aquaculture*, 284:81-89.
Lowe T., Ryder J.M., Carrager J.F. and Wells R.M.G., 1993. Flesh quality in snapper, *Pagrus auratus*, affected by capture stress. *J.Food Sci.,* 58:770-773.
Lushchak V.I., Bagnyukova T.V., Husak V.V., Luzhna L.I., Lushchak O.V. and Storey K.B. 2005. Hyperoxia results in transient oxidative stress and an adaptive response by antioxidant enzymes in goldfish tissues. *Int. J. of Biochem. & Cell Biol*., 37:1670-1680.
Mørkøre T., Mazo P.I., Tahirovic V. and Einen O., 2008. Impact of starvation and handling stress on rigor development and quality of Atlantic salmon (*Salmo salar*). *Aquaculture*, 277:231-238.
Nathanailides, C., Panopoulos, P., Kakali, F., Karipoglou, C. and Lenas D., 2011. Antemortem and postmortem biochemistry drip loss and lipid oxidation of European sea bass muscle tissue. *Procedia Food Science* 1:1099-1104.
Olsen S.H., Sorensen N.K., Larsen R., Elvevoll E.O. and Nielsen H., 2008. Impact of preslaughter stress on residual blood in fillet portions of farmed Atlantic cod (*Gadus morhua*) Measured chemically and by Visible and Near-infrared spectroscopy. *Aquaculture*, 284:90-97.
Olsen S.H., Sorensen N.K., Stormo S.K. and Elvevoll E.O., 2006. Effect of slaughter methods on blood spotting and residual blood in fillets of Atlantic salmon (*Salmo salar*). *Aquaculture*, 258:462-469.

Peng S., Chen X., Shi Z., Yin F., Sun P., 2012. Survival of Juvenile Silver Pomfret, *Pampus argenteus*, Kept in Transport Conditions in Different Densities and Temperatures *Isr. J. Aquacult. - Bamidgeh,* 64.2012.678:6 pages.
Roth B., Birkeland S. and Oyarzun F., 2009. Stunning, preslaughter and filleting conditions of Atlantic salmon and subsequent effect on flesh quality on freshand smoked fillets. *Aquaculture*, 289:350-356.
SPSS for Windows, ver., 10.0., 1999. SPSS Inc. Chicago, IL. USA
Wilkinson R.J., Paton N. and Porter M.R.J., 2008. The effects of pre-harvest stress and harvest method on the stress response, rigor onset, muscle pH and drip loss in barramundi (*Lates calcarifer*). *Aquaculture*, 282:26-32.