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Effects of different slaughtering methods on rigor mortis development and flesh quality of tench (*Tinca tinca*)

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Summary

The effects of different slaughtering methods on rigor mortis and flesh quality parameters were investigated. Eighty tench were divided into four groups, whereby each group was slaughtered using a different method: percussive stunning (PS), live chilling (LC), asphyxia (CO_2), or electrical stunning (ES). Progression of rigor mortis (RM) was recorded (30 h) as well as pH, colour, cooking losses, texture, drip losses and free water after 24 h of storage at +2°C. The PS group showed a delayed onset of RM when compared to the other groups and with a later beginning of rigor resolution. The highest rigor index values were shown in the PS and CO_2 groups, and were reached at 24 and 20 h post-mortem, respectively. ES fish exhibited a significantly faster rigor onset when compared to the PS group. At 24 h, pH was not affected by the stunning methodology, nor were the colour or texture characteristics, even when a trend was observed (PS>ES>CO_2>LC) for the latter parameter. This research is evidence of how stunning procedures prior to slaughter have a significant effect on rigor mortis development, whereas no differences were found concerning quality parameters.

Introduction

According to the EU Council Directive 93/119/EC (1993), animals should be slaughtered in an appropriate manner in order to reduce pain, agitation, suffering, injury or contusions. Fish are included in this legislation and, although opinions differ in the academic world, because of a lack of tradition in considering fish as sentient animals, many references show that fish possess anatomical features that allow them to feel pain and experience fear (Chandroo et al., 2004; Ashley, 2007). The concept of pain and welfare in fish is relatively new in aquaculture and, although awareness in producers and consumers is increasing, this concept is regarded as having been insufficiently investigated (Poli, 2009).

Many authors report how pre- and post-slaughter practices and quality parameters are intrinsically linked and that the meat quality is reduced in badly-treated fish; thus commercial and economic aspects arise in addition to ethical considerations (Van de Vis et al., 2003; Acerete et al., 2009; Matos et al., 2010).

The Panel on Animal Health and Welfare was asked by the EU Commission to deliver an opinion on welfare aspects regarding the principal systems of the stunning and killing of farmed fish (EFSA, 2009). Among the critical steps that can dramatically compromise fish welfare, the methods of slaughter are of great importance (Poli, 2009). Specifically requested was also to outline recommendations with a species-specific approach, stressing that stunning/slaughtering methods should be performed quickly in order to avoid stress and pain (EFSA, 2009).

The main stunning and slaughtering methods applied in aquaculture are: electrical stunning, CO_2 stunning, asphyxia in air, ice slurry asphyxia (chilling), gill cut, and percussion (Marx et al., 1997; Robb et al., 2000; Poli et al., 2005; Rahmanifarah et al., 2011).

Several biochemical and physical parameters have been used to evaluate the quality features of fish products (Parisi et al., 2002; Kiessling et al., 2004; Scherer et al., 2006; Duran et al., 2008; Wilkinson et al., 2008). One of the first changes to occur post-mortem is the onset of rigor mortis (RM), which happens when the ATP content in the muscles decreases to below a critical level and the bond between actin and myosin becomes irreversible. Stress or inappropriate handling before slaughter leads to a large consumption of the glycogen energy reserve at the expense of post-mortem ATP regeneration. Similarly, ultimate muscle pH is due to the generation of H^+ ions associated with the lactic acid production as well as to the collapse of ATP reserves in the muscle (Poli et al., 2005; Acerete et al., 2009). These two parameters are very useful tools that reflect the pre-mortem status of reserves and stress before death (Bagni et al., 2007). Other parameters such as meat colour, texture, water retention capacity, drip losses or cooking losses are also good flesh quality indicators of pre-slaughter and slaughter practices (Matos et al., 2010).

The effects of inadequate management during pre-slaughter procedures on the final product quality have been investigated in several freshwater and marine species (Håstein et al., 2005; Poli et al., 2005; Ashley, 2007; Wilkinson et al., 2008), however, to the best of our knowledge, there are no published data on the effects of these slaughter methods on tench.

Tinca tinca (L.) is a cyprinid pond fish species widely distributed throughout the world. Furthermore, in some specific areas of Italy, the tench is considered to be a high value niche product (18€ kg^{-1}) with Protected Designation of Origin (PDO) European recognition and considered to be an interesting new species for aquaculture development (Gasco et al., 2010).

Although there are no official data on the slaughter methodologies, most tench farmed in Italy are killed by asphyxia in air, thereby suffering a prolonged period before death. This methodology is not in compliance with the requirements of animal welfare for aquaculture and, moreover, could dramatically affect the final quality of the product.

Furthermore, the Panel on Animal Health and Welfare indicated for cyprinids that, 'standard operating procedures to improve the control of the slaughter processes to prevent impaired welfare should be introduced, and validated, robust and practically feasible welfare indicators should be developed'.

In order to comply with these requests, the present study aimed to compare the effects of various slaughtering methods on rigor mortis and flesh quality parameters of tench.

Materials and methods

Animals and sampling

A total of 80 tench (*Tinca tinca*) (80.51 ± 7.92 g) were caught by gillnets, kept in a 4000-L aerated concrete tank (21 ± 1°C) for 1 week and then fasted for 24 h before killing. Twenty fish were allocated to one of the four slaughter methods: (i) live chilling (LC) by immersion of fish in chilled water (water: ice ratio = 1 : 1; T = 2–4°C) for 50 min; (ii) asphyxia by CO_2 (CO_2) by placing tench into a 10-L waterbath where CO_2 was bubbled through the water to maintain CO_2 at saturation level; (iii) percussive stunning (PS), by restraining manually and individually each of the 20 fish and stunning them by a blow to the head using a wooden club; and (iv) electrical stunning (ES), by placing simultaneously the 20 fish in a dry electro-stunner constructed according to the local health service (51V, 30 s).

Immediately after slaughter, fish were individually weighed, carefully gutted, and transferred to polystyrene boxes, covered with ice flakes and refrigerated at +2°C.

Rigor mortis (RM) index

The curvature of the tail (Bito et al., 1983) was used as a basis for the progression of the RM measurement.

After evisceration (0) and for the next 1–30 h, each fish was placed on a flat surface and the image recorded with a digital camera (Nokia D3100). Image analyses were performed using the IMAGE-PRO PLUS 5.1 software (Media Cybernetics Inc., Bethesda, MD). Measurement calibration was made to the nearest 0.01 mm. The rigor index (I_R) was then calculated by the formula $I_R = 100*(L_0 - L_t)/L_0$, where L is the vertical distance between the base of the caudal fin and the table surface, measured immediately after death (L_0) and during storage (L_t, with t = 1–30). For each group, the same five fish were used for the RM measurements, and between each measurement the fish were maintained at +2°C.

Physical parameters

Physical parameters were determined 24 h after death. Muscle pH was measured with a Crison MicropH 2001 (Crison Instruments, Barcelona, Spain) equipped with a combined electrode and an automatic temperature compensator.

Fillet colour was assessed using a Minolta CR-331C Minolta Colorimeter (Ø 25 mm measuring area, 45° circumferential illumination/0° viewing angle geometry) with the D65 illuminant and 2° standard observer. The results were expressed in terms of lightness (L*), redness (a*) and yellowness (b*) in the CIELAB colour space model (CIE, 1976). Chroma and Hue were calculated according to Boccard et al. (1981).

Cooking losses were assessed following Ramírez et al. (2004). Fillet hardness measurements were performed using a Zwick Roell® texturometer (software: TEXT EXPERT II) equipped with a 200 N load cell. One cycle compression test was done using a 10 mm diameter cylindrical probe at a constant speed of 30 mm min^{-1} until 50% total deformation. The textural attribute was measured in two locations of the epaxial muscle of each left fillet.

Drip losses (DL) were defined as the fluid lost by the fillets stored in a polystyrene bag for 24 h in a refrigerated chamber, and measured as the difference between the fillet weight before and after storage (previously dried with a paper towel), and expressed as the percentage of the weight of fillets before storage.

Water retention capacity (WHC) was determined according to the Grau and Hamm (1953) method and expressed as free water (FW).

Statistical analysis

Parameters studied were analyzed by one-way ANOVA using the slaughter method as the predictor (R. Core Development Team., 2005). In particular for the rigor mortis index, data were processed separately in the model for each time of sampling. When ANOVA was significant, differences amongst groups were evaluated with Duncan's test. Values were reported as means of group ± standard deviation of the mean. Significance was accepted as $P < 0.05$.

Results

Rigor mortis index

The different slaughtering methods had a significant ($P \leq 0.05$) impact on the RM onset (Fig. 1). At the beginning, no differences among groups on rigor status were recorded. Initial differences emerged between ES (48.18%) and others groups (28, 17.84 and 14.57% for LC, PS and CO_2, respectively) after the second hour post-mortem, and ES showing a faster rigor onset when compared to LC, PS and CO_2 fish. After 4 h, statistical differences emerged only between ES (68.28%) and PS (39.53%) groups. At 7 h after death, I_R expressed by CO_2 fish (79.88%) was significantly different from ES (67.64%) and PS (65.6%) groups, while at 8 h (CO_2 = 81.04%) this difference was only with regard to the ES (64.08%) group.

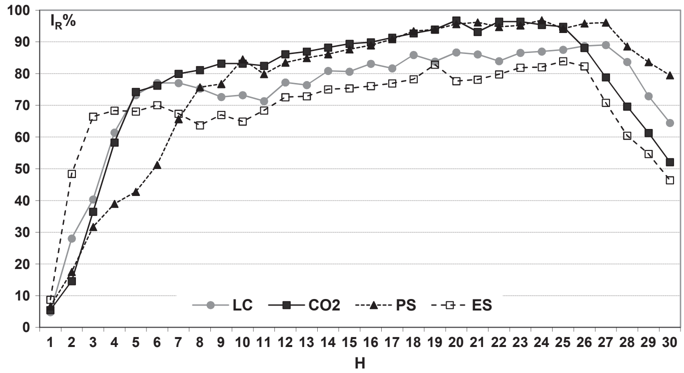

Fig. 1. Rigor index (I_r) evolution during 30 h of tench treated with different slaughter methods: live chilling (LC), CO_2, percussive stunning (PS) and electrical stunning (ES). Values reported for each sampling point (hours) are means of measurements recorded on five fish for each stunning procedure

As a general trend, the PS group showed a delayed onset of RM when compared to the other groups and a later beginning (28 h post-mortem) of rigor resolution.

The highest values of I_R obtained were shown in PS (96.84%) and CO_2 (96.80%) groups and were reached at 24 and 20 h post-mortem, respectively. From 8 to 25 h, no significant differences emerged between CO_2 and PS groups.

ES fish exhibit a significantly faster rigor onset when compared to the PS group. The value of I_R in these two groups was always statistically different except at hour 7. The maximum I_R value was reached at 25 h for the ES group (83.96%), at 27 h for the LC group (89.10%), at 22 h for the CO_2 group (96.46%), and at 24 h for the PS group (96.84%).

Physical parameters

Physical parameters at 24 h are reported in Table 1. No significant differences were notable between and among treatments.

Discussion

Rigor mortis

Similarly to several authors, this research evidenced that slaughtering procedures have a significant effect on RM development (Ashley, 2007; Poli, 2009).

Acerete et al. (2009) compared three slaughter procedures and found that the RM onset was similar for all methods and that resolution was similar between CO_2 and ice asphyxia groups that were different only at 24 h post-death.

In our research, various results are shown. During the first 12 h post-mortem, LC showed the same trend as the CO_2 group, with no significant differences in I_R values. Afterwards the trend remained similar, but from the 17 h the I_R data were significantly lower for the LC group (81.77 vs 91.35). The same trend can be noted when comparing LC and PS (90.71) fish. Scherer et al. (2005) showed that grass carp killed by electricity entered into RM earlier than ice-water slaughtered fish, as was found in this trial. Similar to Marx et al. (1997) and Morzel et al. (2003) who reported that fish stunned by PS entered in rigor later than fish killed by electricity, in our study PS tench showed a more lower rigor onset rate than the ES group as well as a higher intensity of RM. Roth et al. (2002) nevertheless did not observe these differences in *Salmo salar*. In our trial it was noted in three out of 20 cases that one percussive blow to the head was not sufficient to kill the tench, thus two blows were applied in order to be certain that the fish was dead. Nevertheless, no external or internal damages were caused.

Table 1
Physical traits 24 h post mortem of tench fillets treated with different slaughter methods: live chilling (LC), CO_2, percussive stunning (PS) and electrical stunning (ES) (mean ± SD, n = 5)

	LC	CO_2	PS	ES
pH	6.07 ± 0.14	6.08 ± 0.13	6.02 ± 0.11	6.09 ± 0.10
Colour				
L	49.80 ± 2.09	48.25 ± 2.88	51.37 ± 3.24	49.97 ± 4.49
a*	0.39 ± 1.36	0.46 ± 1.37	−0.46 ± 1.00	0.00 ± 2.14
b*	0.16 ± 1.07	−0.47 ± 1.31	−0.59 ± 1.02	−0.57 ± 1.61
Hue	32.18 ± 36.79	8.82 ± 57.70	18.45 ± 40.73	16.78 ± 52.05
Chroma	1.52 ± 0.75	1.78 ± 0.71	1.27 ± 0.92	2.26 ± 1.34
Cooking loss (%)	15.83 ± 2.26	15.39 ± 1.16	17.55 ± 2.69	17.52 ± 2.47
Texture (N)	8.59 ± 0.88	8.63 ± 3.12	10.46 ± 2.14	9.18 ± 1.38
FW (cm^2)	10.96 ± 2.98	11.68 ± 0.52	12.44 ± 1.85	10.53 ± 2.01
Drip loss (%)	5.23 ± 3.00	4.63 ± 1.09	3.09 ± 1.23	3.02 ± 0.80

Abe and Okuma (1991) reported in carp killed by electricity that a 100% I_R was observed 20 h after death, whereas in the group killed in ice-water slurry the maximum muscle contraction was recorded 24–48 h post-mortem (I_R = 92–97%). Similar values of I_R both for ES (83.96% at 24 h) and LC (89.10% at 27 h) groups were not reached for tench in the present trial, indicating a lower capacity of ATP regeneration in these groups, possibly due to high glycogen depletion.

In African catfish, Lambooij et al. (2006) reported an immediate induction of unconsciousness and insensibility using ES in water (300V). Nevertheless, the fish recovered from this procedure, thus a combination of decapitation and bleeding was suggested.

Conversely, Ashley (2007) reported that, if applied correctly, the ES methods appear to have achieved humane slaughter in several fish species and that the UK trout industry appeared to be moving towards ES as a preferred slaughter method (Lines et al., 2003). In our research, no tench recovered from ES treatment, indicating that death was immediate. Nevertheless, fish slaughtered by the ES method evidenced the characteristics of a general epileptic insult, with 67% of tench showing blood spots in the flesh. Moreover, all fish stunned by the ES procedure showed two or three burn marks on the skin, originating from the contact point between the skin and electrodes. Erikson et al. (2012) made the same type of observations by observing cod skin after dry-electrical stunning. Thus, this procedure was efficient from a killing point of view but showed negative effects on the fish appearance and fillet quality.

Hypothermia induces immobilization before unconsciousness and could be a suitable method for killing edible portion-sized sea bass for which individual stunning methods are not feasible (Parisi et al., 2002). Since immobilization does not mean lost of consciousness, some researchers found hypothermia to be highly stressful for fish (Robb et al., 2000; Van de Vis et al., 2003; Lambooij et al., 2006; Bagni et al., 2007).

In our trial, after some minutes the LC tench showed a slowing of swimming behaviour; after 15 min fish showed convulsive and rapid movements, ending only after 45 min when no opercula movements were observed. Fish were removed from the water after 50 min and none recovered. This behaviour indicated that fish were subjected to a prolonged period of stress and suffering (at least 45 min) before death. In no case may 50 min be defined as 'rapid' and compatible with the notion of welfare. Analogously, Lambooij et al. (2006) concluded that hypothermia could not be considered as an acceptable stunning method because it prolongs the period of consciousness and does not reduce the ability to feel pain. Moreover, The Farmed Animal Welfare Council (FAWC, 1996) reported that cooling of live trout on ice should be prohibited.

Scherer et al. (2006) found no differences in shelf life of grass carp slaughtered by immersion in ice and water or by electricity. Rahmanifarah et al. (2011), although reporting a slower rigor onset and resolution in carp stunned by hypothermia compared to CO_2, concluded that, in agreement with the FAWC (1996), this method should be deemed unacceptable from an animal welfare point of view because of the prolonged induction time and abnormal behaviour.

Acerete et al. (2009) compared CO_2 and ice chilling methods in sea bass and evidenced in both cases struggling movements with a longer time to death for water-iced killed fish; these authors suggested that this could indicate a higher impact on muscle quality and concluded that CO_2 showed slightly better results regarding the response to stress indicators. Considering the rigor mortis index as stress indicator, the same conclusions seem to emerge from the present study, as when in the full rigor stage the I_R values showed by the CO_2 group were higher than LC tench, even when significant differences emerged only after some hours. Nevertheless, struggling movements, erratic swimming and attempts to escape were recorded for the CO_2 tench, suggesting, as reported by several authors (Håstein et al., 2005; Ashley, 2007; Poli, 2009), that slaughtering should not be performed with CO_2 for reasons of welfare. Moreover, in our trial, RM resolution started earlier in CO_2 when compared to the LC group.

Physical parameters

It has been reported that any kind of pre-mortem stress causes high glycogen depletion, leading to an increase of lactic acid in fish muscles and that pH is affected by pre-slaughter activity (Kiessling et al., 2004).

Rahmanifarah et al. (2011) reported no significant differences in the pH value at 24 h post-mortem between CO_2 and hypothermia groups of carp, whereas statistic differences were recorded after 72 h of storage.

In our trial, pH at 24 h was not affected by the stunning methodology even when the PS group reached the lowest (but not significantly different) pH (6.02). Wedekind et al. (2003) reported pH values 24 h after death ranging from 6.40 to 6.52 for different genetic strains of tench all stunned by a blow to the head. These pH values were considered as indicators of high lactate levels in tissues. Our results were even lower (from 6.02 to 6.09) and seemed to indicate a good nutritive status of tench before harvest, good condition of storage, and no stressful handling before stunning. The lowest pH value reached by tench slaughtered by PS indicates that this method enables only a minor depletion of stored reserves (glycogen).

Acerete et al. (2009) reported no differences in pH value 24 h post-mortem between CO_2 and chilled water asphyxiate groups, whereas differences emerged when comparing these treatments with the air asphyxia group used as a reference.

Duran et al. (2008) found the lowest pH value after 24 h for carp and trout slaughtered by asphyxiation or percussion. Moreover, for carp, the pH of the percussed group 24 h post-death was lower than for the asphyxiated fish. This was justified by a faster and earlier depletion of glycogen in the asphyxiate group because of the higher muscle activity pre-mortem.

No differences emerged among groups when considering colour parameters. Nevertheless, in Table 1, notable is that SD values resulted in a dramatic variability between fillets with the same treatment that could have masked the effects of the slaughtering procedures.

Rahmanifarah et al. (2011) found that fillet colour was affected by pre-mortem stress, with carp in the hyperthermia group having lower hue values than the CO_2 group. Moreover, they stated that the biochemical effects of hypercapnea on carp blood and flesh could have caused paler fillets in the CO_2 group, even when further investigation was recommended. Contrary to these authors, in our research the lower hue value was reported in the CO_2 group and was the highest in the LC tench.

Robb et al. (2000) reported that in fish hyperactivity at slaughter (stress) causes the flesh to be significantly lighter, less red and more yellow. Fillets of tench stunned by LC were more yellow (b*) (no statistical differences) in colour than other groups. Our results for L* values are slightly higher that those reported by Wedekind et al. (2003), and in general our tench resulted in more red (a*) and yellow (b*) fillets.

Cooking losses ranged between 15.39 (CO_2) and 17.55% (PS). Similar values were reported by Wedekind et al. (2003). In our trial, the cooking losses were not significantly different among treatments even when the ES and PS groups reported higher values of this important technological parameter.

Duran et al. (2008) comparing asphyxia and PS methods in carp found that fillet textures were significantly different between slaughter methods, in agreement with the Roth et al. (2002) results on salmon. Differences were attributed to pre-mortem muscle activity: the more the activity, the more the muscle damage.

Low ultimate pH is reported to influence texture (Ginés et al., 2002), but no differences were found in our trial for muscle pH of tench at 24 h post-mortem, suggesting that these methods have no major effect on muscle structure in terms of texture analysis. These results agree with Huidobro et al. (2001) and Matos et al. (2010) but did not agree with Urbieta and Ginés (2000) on sea bream or with studies of other species (Kiessling et al., 2004; Bahuaud et al., 2010).

Regarding the texture, although the values were not significantly different among the groups, the trend observed (PS>ES>CO_2>LC) was in agreement with the findings obtained in rainbow trout by Færgemand et al. (1995) and Ambroggi et al. (1996), who found higher texture attributes in fish slaughtered with PS in comparison to ES and asphyxiation, probably as a consequence of less handling stress and muscle activity in fish of the first group.

Very little information is available on the value of free water (FW) and drip losses (DL) in tench. Wedekind et al. (2003) found values different from those of our trial, probably due to the different weights of the fish analysed (200–300 g) and to the methodologies utilised for the analysis, which were not specified in the Wedekind et al. (2003) paper.

Parameters largely affecting the commercial value and quality perception of the consumer are DL and FW, because of their important roles in the texture of the fish. Their behaviour is closely related to the pH value of flesh (Fennema, 1990). Although no significant differences were observed for the DL and FW parameters, the results highlight a clear behaviour. Except for the ES group, an inverse relationship could be deduced between the two parameters, since the PS group showed lower DL and higher FW (corresponding to a lower WHC) and vice versa, as the behaviour of LC also highlighted. The ES group was characterized by reduced loss of water from muscle, when measured in terms both of DL and FW, probably as a consequence of the higher value registered for muscle pH, although not significantly different from those of the other groups.

In turbot killed by percussion, electricity, or bleeding, Morzel et al. (2003) found higher values of WHC in fish killed by percussion than in those slaughtered by electricity, whereas in sea bream, Tejada and Huidobro (2002) did not find a clear influence of the killing method in the electricity parameter or on a cooking drip.

In conclusion, our data show that the different stunning procedures affect rigor onset and resolution in tench without significantly affecting quality parameters tested at 24 h post-mortem.

Because of the long time needed to die, neither immersion in CO_2 saturated water nor chilling while still alive meet the criteria for humane slaughter, whereas tench were immediately killed by the ES method. However, exposure to electricity seemed to be either too long or too high, with burns appearing on the skin and bloodspots on the fillet. The percussive stunning method seems to be the more appropriate methodology as it induces a slower onset of RM and has good I_R values and a later RM resolution; this procedure is considered to be humane but is highly dependent on the adequate training and working conditions of those persons performing the operation. This method is usually used for large fish, as it is otherwise too expensive and time consuming for small-sized species. Nevertheless, considering the high value of tench in a specific area, this method could be recommended. A combination of various methods together might also be suggested.

This represents the first study on tench and, considering the increasing awareness of consumers regarding the ethical and welfare aspects of aquaculture production and the high impact of these conditions on quality parameters, further investigation is needed in order to define protocols for humane and less stressful slaughter practices for this species.

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