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Methods of stunning freshwater fish: impact on meat quality and aspects of animal welfare

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Abstract Taking into account aspects of meat quality and animal welfare, three methods of stunning fish were compared: a manual technique (blow on the head, stab in the neck), one using electricity and one using CO_2. The following results were obtained using eel ($n = 72$), carp ($n = 120$) and trout ($n = 54$). From the viewpoint of animal welfare, the effects on the fish were judged. Excitation and mucus secretion as well as the time taken for the fish to be anaesthetized were recorded. With manual and electrical stunning, all fish were anaesthetized almost immediately, while using CO_2 it took 3.2 min (trout), 9.2 min (carp) and 109.7 min (eel) on average. After slaughter and after 3 and 8 days of storage on ice, the fish meat quality parameters, i.e. pH value, water-holding capacity and rigor mortis, were measured. CO_2 stunning gave rise to the lowest pH values and water-holding capacities. Rigor mortis in carp and eel advanced the most. Testing of raw and prepared fish was performed by a panel assessing organoleptic properties. In many cases, fish anaesthetized manually were ranked to be better than those in the other groups.

Key words Fish stunning · Substantial quality · Organoleptic properties

Introduction

Choosing the most suitable method for stunning prior to slaughter is one important step in assuring a good quality of fish as food. Methods of stunning that act quickly and minimize reactions in the fishes are favourable because of both practical and animal welfare reasons. Investigations into the impact of the different existing methods of stunning on meat quality and with regard to animal welfare are few [1–5]. Because of this, three techniques for anaesthezing or killing fish (blow on the heat or stab in the neck, using electricity and, finally, using CO_2) were investigated, using carp, eel and trout. The aim was to cover aspects of substantial quality, of time and of animal welfare contemporaneously.

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Materials and methods

A total of 54 trout (*Oncorhynchus mykiss*, 90–650 g), 120 carp (*Cyprinus carpio*, 150–330 g) and 72 eels (*Anguilla anguilla*, 240–440 g) were stunned and slaughtered. For the stunning of carp and trout manually, a blow on the head with a hitting stick for anglers was performed. Eels were rendered unconscious by a stab in the neck with a small kitchen knife. For anaesthesia induced by electricity and CO_2, two commercially available systems were used. Stunning induced by electricity was done with a BE 300 from Grassl, Berchtesgaden (Germany), which works by increasing DC from 0 V to 160 V in cycles of 30 s, with the fish in a 200-l basin. Stunning by CO_2 was performed with a system developed by Fischtechnik Fredelsloh, Moringen (Germany), which produces a CO_2 concentration of 2.5 g per litre of water within 15 min when running connected to a 200-l basin. Three fish were anaesthetized simultaneously when stunning with electricity or CO_2 was performed.

Before slaughter, eels and carp were kept in groups of 10–20 in 200-l tanks for 5 days. The oxygen concentration was kept within 6.6–6.7 g/l by blowing air into the tanks. Because the investigations took place from November to February, water temperatures varied from 8.6 °C to 14 °C. The pH of the water varied between 7.35 and 7.98, and electrical conductivity amounted to 535 μS/cm.

The pH and oxygen meters, the thermometer as well as the conductivity gauge used in the investigations were purchased from WTW (Weinheim, Germany). To measure the degree of rigor mortis of the fish, a rigor meter was constructed in accordance with [6] and [7]. To gather information about the water-holding capacity of the fish muscles, a system consisting of a spindle press with a set of acrylic glass planes together with exsiccated pieces of filter paper prepared according to [8] was used.

The spindle press was connected to a torque meter adjusted to 30 Nm. To prepare the fishes for the testing of organoleptic properties, standard butchery equipment (electric stove, smoking chamber) was used. The resulting data were evaluated with SPSS statistical software (SPSS, USA).

Methods. The pH was determined twice with a calibrated pH meter immediately after slaughter (pH_0), after 24 h (pH_{24}) as well as after 3 and 8 days of storage of the fishes on ice. The rigor index was calculated from the rigor values obtained straight after slaughter (R_0) and 24 h after slaughter (R_{24}) according to the formula $[(R_0 - R_{24})/R_{24}] \cdot 100$ [6, 7]. The water-holding capacity was determined immediately after slaughter as the meat/leaking water surface ratio (Q). Quantification of the surface was done with a stencil according to the German ordinance for meat hygiene [9].

To investigate the different stunning methods as far as conformity with aspects of animal welfare is concerned, increased motility and mucus secretion, as signs of excitation during anaesthesia, were noted. The persistence of swimming movements as well as the period of time until movement of gills ceased were recorded.

The fishes were tested both raw and cooked after storing them on ice for 3 and 8 days, by a trained sensory panel. The preparation of the fishes for assessment of their condition when smoked was started immediately after measuring pH and rigor values; the assessment itself was carried out 1 day after smoking. The testing team, consisting of three to eight people tasted three differently stunned fish of one species at the same time in a blind test. Special testing protocols were developed for the assessment of raw fish, involving visual evaluation of the exterior, the eyes, the gills and the condition of the abdomen. In addition, an evaluation of texture was required. For cooked fish, estimations of colour, odour, taste and texture were carried out. The assessment of smoked fish was performed according to the testing scheme developed by Deutsche Landwirtschaftsgesellschaft [10]. Testing protocols included grading [11] as well as ranking or hedonic testing [12].

Fisher's LSD test was used for the determination of significant differences concerning parametric data, χ^2-test was applied to non-parametric data. Significance was accepted for $P = 0.05$.

Results and discussion

pH value

Measuring pH values is a very common method used in the judging of meat quality, and standard methods including limits for pork and beef have been established [9]. Therefore, it seems reasonable to judge the quality of fish meat by this method too [13]. A massive decrease in pH and a low final pH is linked with a reduced water-holding capacity and a pale meat colour, described as chalky meat [14].

The changes of pH throughout the whole period of examination were similar in all types of fish. The pH of carp and eel, in particular (Figs. 1 and 2), indicate that there is an initial reduction in the average pH. The changes of pH after 24 h differed slightly in trout (Fig. 3), which could be due to the small number of measurements ($n = 7$) made at this stage. After this time, a slight recovery of the pH was noticed to occur in all fish.

Straight after slaughter all resulting mean pH values were above 6.4. According to the literature [1, 14–16] the pH of fresh fish is around 6.5. Considering this, all resulting absolute values confirmed that there was no

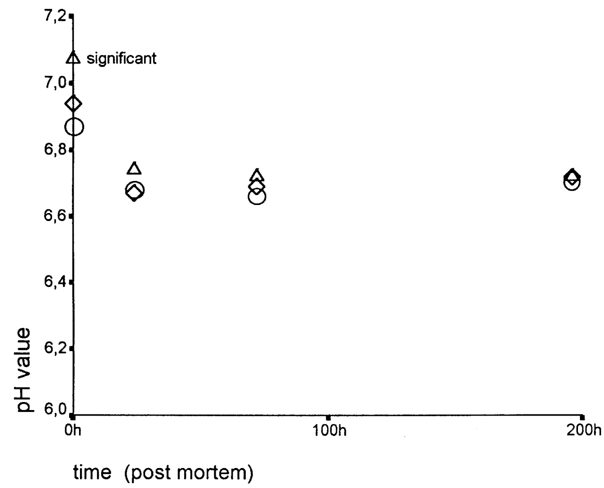

Fig. 1 pH development in carp [n(0 h) = 120; n(24 h, 72 h, 196 h) = 30]. (○ CO_2 anaesthesia, ◇ electrically induced anaesthesia, △ manual stunning)

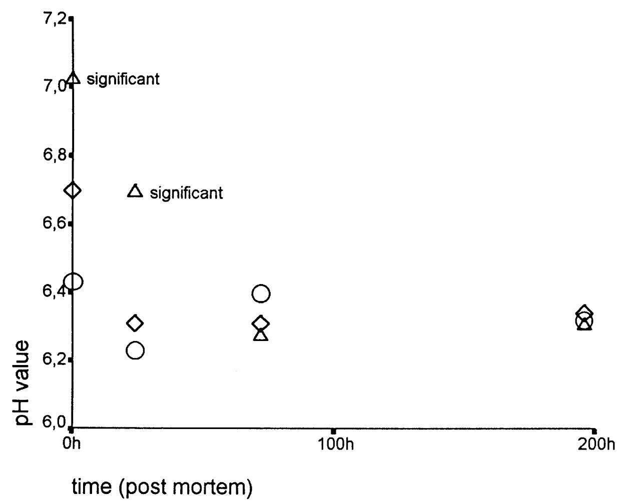

Fig. 2 pH development in eels [n(0 h) = 73; n(24 h) = 19; n(72 h) = 18; n(196 h) = 24]

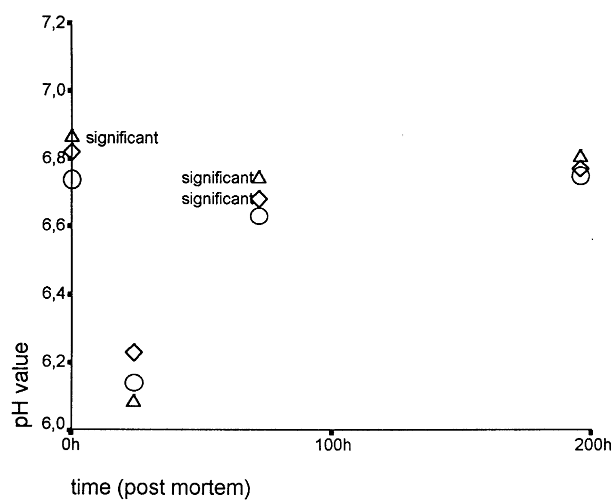

Fig. 3 pH development in trout [n(0 h) = 44; n(24 h) = 7; n(72 h) = 29; n(196 h) = 27]

reduction of pH immediately after slaughter (pH_0) as a consequence of any stunning method used.

At the same time, the pH_0 values of fish anaesthetized manually differed significantly from the values measured following electrical and CO_2 stunning. In all fish, the specimens anaesthetized with CO_2 had the lowest pH_0. One possibility for the reduction of pH during or straight after slaughter is the decomposition of glycogen followed by increasing contents of lactate. Exogenous factors such as transport, catching, confinement or anaesthesia and slaughter itself do influence meat pH [13]. The transition of H^+ from aerated water in the stunning basin to fish muscle might be responsible for the low pH values measured after anaesthesia evoked by CO_2. For pigs stunned by an atmosphere of CO_2, saturation of muscle tissue by carbonate is generally assumed [17].

Measurements made after 3 and 8 days showed that the pH varied from 6.1 to 6.7. According to a previous study [15] of perch stored on ice, any pH value within a range of from 6.1 to 6.7 does not indicate that there are changes in meat quality. The results obtained after 24 h (pH_{24}) indicate that the eels anaesthetized manually had significantly higher pH values than the others. In trout, after 3 days of storage, stunning by a blow on the head or induced by electricity resulted in significantly higher pH values in comparison to those measured following anaesthesia induced by CO_2. This fact could possibly have occurred because the initial pH values of fish anaesthetized manually or electrically were higher than those of fish anaesthetized by CO_2.

After 8 days of storage on ice, the pH values of the fish stunned by the three different methods were very similar. These results can be interpreted as being a consequence of proteolytic changes resulting in nitrogenous, basic and pH-stabilizing products of reaction [16]. The highest average pH value after 8 days was 6.8 in trout, which is just over the upper limit indicative of unchanged meat quality in perch (pH 6.7, [15]).

Water-holding capacity

The mean water-holding capacity values ($\bar{x}\ Q$, Table 1) of the fish indicate that the differences between the types of fish can be greater than those occurring as a result of the different methods of stunning of each type of fish. Despite this, the lowest water-holding capacities within one type of fish were recorded after anaesthesia induced by CO_2. The differences in water-holding capacity between the stunning methods were not statistically significant.

Rigor mortis

In trout, rigor was sometimes already present almost immediately after slaughter, leading to scattering pH_0 values. For trout, conclusions about differences between the methods of stunning could not be drawn from the resulting varied rigor indices.

Rigor values (Table 1) and rigor indices for both carp and eel indicate that anaesthesia induced by CO_2 is related to advanced rigor in these fishes. The rigor index of carp anaesthetized with CO_2 was significantly higher than the rigor indices measured following electrical or manual stunning. Rigor indices were lowest after anaesthetizing the fishes manually.

Parameters indicative of fish welfare

Increased motility and mucus secretion as well as persistent swimming and gill movements were not seen in fish anaesthetized manually. In electrical stunning,

Table 1 Water-holding capacity and rigor values (R_0, R_{24}) as well as rigor indices (*RI*) for carp, eel and trout following different methods of anaesthesia. (*n* Number of observations, $\bar{x}$ arithmetic mean, *s* standard deviation)

Method of inducing anaesthesia		Water-holding capacity (*Q*)				Rigor mortis					
		Carp	Eel	Trout		Carp			Eel		
						R_0	R_{24}	RI	R_0	R_{24}	RI
CO_2	*n*	33	15	15	*n*	10	10		6	6	
	$\bar{x}$	0.59	0.43	0.71	$\bar{x}$	13.2	5.55	56%[a]	18.28	7.40	61%
	$\bar{s}$	0.10	0.05	0.10	*s*	1.03	4.66		2.91	7.26	
Electricity	*n*	34	18	14	*n*	9	9		6	6	
	$\bar{x}$	0.60	0.51	0.75	$\bar{x}$	14.18	9.15	38%	20.31	13.40	37%
	s	0.12	0.11	0.13	*s*	2.35	5.47		4.29	6.68	
Manual	*n*	35	20	14	*n*	10	10		7	7	
	$\bar{x}$	0.62	0.50	0.78	$\bar{x}$	13.40	10.68	21%	21.01	14.92	28%
	s	0.13	0.10	0.12	*s*	1.63	4.86		1.53	6.22	

[a] Significantly different from other stunning methods

Table 2 Strength or persistence of parameters during stunning evoked by CO_2

Parameters	Strength or persistence	Carp	Eel	Trout
Increased motility	*n*	40	21	38
	Strong	55%	86%	55%
	Medium	22%	13%	34%
	None	22%	0%	10%
Mucus secretion	*n*	120	73	54
	Increased	30%	100%	10%
	Not increased	70%	0%	89%
Swimming movements	*n*	40	22	38
	$\bar{x}$	3.7 min	109.7 min	0.8 min
Movement of gills	*n*	40	Not detectable	38
	$\bar{x}$	9.2 min		3.2 min

these parameters cannot be recorded because of the immediate tonic spasms induced as soon as electricity is applied. Distinct motor activity was noticed (Table 2) to occur during anaesthesia induced by CO_2, especially in eel but also in carp and trout.

With CO_2 anaesthesia, an obvious increase of mucus secretion was noticed to occur in the eel. In this case it took 109 min, on average, until swimming movements ceased. Movement of the gills, not detectable in the eel because of anatomical reasons, persisted for 9.2 min in the carp and for 3.2 min in the trout. Following oral administration of fish anaesthetics, with some variety occurring due to the type and size of the fish and also depending on the agent, the stage of surgical anaesthesia is normally reached within 3–4 min [18]. The results indicate that stunning of eels should not be performed with CO_2 for reasons for animal welfare, and it should also be avoided in carp. We emphasize that the present investigations do not add to the knowledge and discussions concerning the ability of fish to percieve pain [3].

Table 3 Sensory investigations, results of ranking

Period, Preparation	Fish	Method of anaesthesia		
		CO_2	Electricity	Manual
3 days, raw	Carp	3	2	1
	Eel	3	1	2
	Trout	2	1	3
3 days, cooked	Carp	1	2	2
	Eel	1	3	1
	Trout	1	3	1
8 days, raw	Carp	3	1	1
	Eel	3	1	1
	Trout	2; 3	2	1
8 days, cooked	Carp	1; 3	2	1
	Eel	3	1; 2	1
	Trout	3	3	1
24 h, smoked	Carp	3	2	1
	Eel	3	1	2
	Trout	1	2	3
Rank sum	Carp	12	9	6
	Eel	13	7.5	8
	Trout	9.5	11	9
	Total	34.5	27.5	23

Investigation of organoleptic properties

With reference to the different stunning methods, the scaled assessment of organoleptic properties [11] did reveal that there were heterogeneous tendencies. Trout that had been stunned by the different methods were assigned to significantly different grades of the organoleptic property when they were tested after having been stored for 3 days and then cooked. The trout that had been stunned electrically were graded as being the least acceptable.

Ranking or hedonic testing (Table 3) [12], in contrast to scaled assessment, gave rise to more homogeneous results. Manually stunned fish were always found to be preferable. Summation of the ranks shows that, from the sensory point of view, electrical stunning of eels and CO_2-induced anaesthesia of trout seem to be as good as stabbing or hitting the fishes. Following electrically induced anaesthesia, cooked trout and eel ranked last after 3 days of storage on ice; this agrees with the results of scaled assessment. The fact that electrical stunning puts trout at a disadvantage in terms of sensory quality has been ascertained already by Bogess et al. [19], using hedonic testing.

The evaluation of cooked fish showed that comments were made concerning blood spots along the vertebral column, and red or brown discolouration of the meat from trout and carp. This was observed in 6.8% of the assessments of fish anaesthetized with electricity, in 2.9% of the assessments of fish stunned manually and in 1.4% of the evaluations of fish anaesthetized by CO_2. In addition, colour was judged to be poor in 32.8% of the assessments of fish stunned with electricity, in 20.8% when manual stunning was applied and in 15.9% when CO_2 was utilized. Following electrical stunning, the reasons for deviations in meat colour, according to the literature [15], are muscle bleeding and haematoma. Spontaneous fractures in the spinal cord or ruptures of the muscle and connected tissue, such as blood vessels, lead to these effects.

No correlation between the results of grading and ranking (hedonic testing) was detected. This fact might arise if differences are not great enough to be detected by the sensory panel. Alternatively, any mismatch in criteria for the assessment could also give rise to this lack of correlation.

In conclusion, the findings indicate that CO_2 is not appropriate for stunning carp and eel. Electrical stunning, with some improvements, could meet the requirements of meat quality and be adequate in terms of animal welfare.

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