HEAT SHOCK PROTEINS AS MARKERS FOR PRE-SLAUGHTER STRESS AND PREDICTION OF MEAT QUALITY

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Keywords: stress, slaughter, HSP70, meat quality

Introduction

It is well established that the meat quality is affected by pre-slaughter stress (Grandin , 1980), and also animal welfare is an issue of great interest and concern. The primary production sites as well as the slaughter plants are continuously improving methods and concepts for reducing the stress imposed on the meat producing animals, but the greater challenge is making these efforts visible and correlate these to meat quality. The option of differentiating the raw meat based on stress related markers at the time of salughter would optimise the use of the raw material by increasing the uniformity of the product, thus ensuring a more consistent quality of meat for consumption and different processing purposes. Various indicators of stress are expressed in the blood e.g. the hormones adrenalin and cortisol, but a hormonal response is acute and exhibit large variations over a short period of time. However, other compounds like heat shock proteins (HSP's) are also expressed in response to a number of stressors (Kilgore et al. 1998) and maintain their presence over larger periods of time, typically several hours to days (Khassaf et al. 2001). HSP's are expressed in the skeletal muscle (Thompson et al. 2003), but are also present in the blood (Fehrenbach et al. 2000a; Hunter-Lavin et al. 2004). The aim of this work was to quantify intracellular Hsp70 in the white blood cells and relate this to meat quality parameters.

Materials and Methods

Four female pigs from each of 10 litters were allocated within litter to one of 4 treatments: control without stress exposure or treadmill exercise followed by 0, 1, or 3 hours rest before slaughter. The pigs were exercised on a treadmill with stepwise increasing speed from 0.4 km/h to on average 5.2 km/h with increments of 0.4 km/h every 2 minutes (total 27 minutes, SEM=0.9). Pigs were stunned and killed by sticking where blood was collected into heparinised tubes and the temperature (insertion thermometer) and pH (pH meter with insertion glass electrode) was measured in Longissimus Dorsi (LD) and Biceps Femoris (BF). Samples for drip loss (48 h, bag method) and colour measurements (Minolta spectrophotometer after 1 h blooming) were cut after 24 h. Intracellular Hsp70 levels were determined in fixed and permeabilised white blood cells using Hsp70-FITC conjugated antibody (Assay Design, SPA810) according to the manufactures recommendations, and detected by flowcytometry using a BD FACSCanto flowcytometer. The percentage of Hsp70 positive populations and the Mean Fluorescence Intensity (MFI) of these populations were based on 30.000 events.

Results and Discussion

Exercising pigs on a treadmill significantly increased the temperature of both LD and BF muscles of pigs slaughtered immediately after the exercise by approximately 1 and 2 degrees, respectively (table 1). However, already after 1 hour of rest the temperature was back to that of the control pigs. After 24 hours, meat from pigs having rested for 1 h after the exercise had a lower pH in LD compared to the control and the lightness of LD was significantly reduced in pigs resting for 1 or 3 hours after exercise and the same tendency was observed in BF. Drip loss was increased significantly in BF from pigs slaughtered immediately after exercise, but back to that of the control when resting for 1 or 3 hours before slaughter, and also here a similar tendency was noted in LD. There was no effect of exercise on the Hsp70 present in lymphocyte and monocyte populations, but suggestion of a decrease in the granulocyte Hsp70 positive population in the exercised pigs compared to the control pigs, although this was not significant. This decrease is despite the likely increase in *de-novo* synthesis of Hsp70 that has been shown to occur in response to acute exercise in different human and animal models, not only in blood cells (Fehrenbach et al. 2000b; Clarkson et al. 2005), but also in skeletal muscle (Clarkson et al. 2005). In the present work, a little pilot study was performed where white blood cells from 2 pigs per treatment were incubated at 37°C, 40°C and 42°C respectively for 1 hour (data not shown). These results showed a tendency towards a decrease in the Hsp70 positive granulocyte population with increasing temperature, which may indicate an increased release of Hsp70 from the blood cells as reported for humans (Hunter-Lavin et al. 2004). This could have caused the decrease in intracellular Hsp70 in the exercised pigs. Hence, Hsp70 levels in serum as well as gene expression in blood cells and muscle tissue will be examined to further investigate this.

Conclusions

Exercise increased the temperature of LD and BF by 1 and 2 degrees and caused an increased drip loss in BF from pigs slaughtered immediately after exercise, which was not the case after just 1 hour of rest before slaughter. The apparent decrease in intracellular Hsp70 positive granulocytes upon exercise was no reflection of e.g. the drip loss results, and further investigations of Hsp70 serum levels and gene expression in both blood and muscle is needed in order to further pursue relations between Hsp's and meat quality (drip loss, tenderness etc.)

Table 1. The percentage of monocytes, granulocytes and lymphocytes expressing HSP70 as well as the Mean Flourescence Intensities (MFI) intensities of these populations in blood at sticking was determined together with temperature 1 minute post mortem, pH 1 min and 24 hours post mortem, drip loss % and colour (L=lightness, a*=redness) in both longissimus dorsi (LD) and biceps femoris (BF) from pigs exposed to no exercise or following exercise on a treadmill followed by 0, 1, or 3 hours of rest before slaughter (n=10 for each treatment).

			Hours rest after exercise				
Tissue	**Trait**	**No exercise**	**0**	**1**	**3**	**SEM**	**P**
LD	pH 1min	6.63	6.58	6.68	6.55	0.057	0.1644
	pH 24 h	5.60 [a]	5.54 [ab]	5.52 [b]	5.60 [a]	0.034	**0.0355**
	Temp 1 min, ºC	40.0 [b]	41.0 [c]	39.7 [ab]	39.5 [a]	0.139	**<0.0001**
	Drip loss, %	4.91	6.18	4.83	4.92	0.569	0.2340
	L, lightness	56.89 [b]	57.65 [b]	54.25 [a]	54.64 [a]	0.632	**0.0013**
	a*, redness	6.96	6.78	6.25	6.27	0.324	0.2424
BF	PH 1min	6.63	6.65	6.75	6.63	0.056	0.1549
	PH 24 h	5.65	5.63	5.61	5.71	0.045	0.1922
	Temp 1 min, ºC	40.1 [b]	41.9 [c]	39.9 [ab]	39.7 [a]	0.159	**<0.0001**
	Drip loss, %	5.39 [a]	7.52 [b]	4.19 [a]	4.15 [a]	0.675	**0.0049**
	L, lightness	50.29	50.89	48.84	49.15	0.684	0.1469
	a*, redness	12.27	12.34	12.15	12.61	0.270	0.6629
Blood	Granulocytes, HSP70, %	9.38	8.34	7.23	5.88	1.812	0.1968
	Monocytes, HSP70, %	9.97	8.01	5.59	5.87	2.078	0.2492
	Lymphocytes, HSP70, %	4.43	4.13	3.63	3.18	1.267	0.7921
	Granulocytes, MFI	361	366	311	259	41.70	0.1633
	Monocytes, MFI	305	239	221	332	57.18	0.4759
	Lymphocytes, MFI	214	212	206	218	10.70	0.7960

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