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EFFECT OF STRESS ON RABBIT MEAT QUALITY

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Abstract

The aim of the study was to examine the response of rabbits to stress stimuli using simple behavioural tests (open field test, stress-induced hyperthermia test) and to determine the effect of stress on meat quality. Rabbit welfare levels were evaluated based on physiological indicators (SIH test), baseline serum levels of cortisol, glucose and triglycerides, and behaviour of animals in the open field test. The concentration of hydrogen ions (pH), water binding and the associated colour lightness of meat were used as parameters of meat quality. Stress response, measured by the levels of glucose secretion and cortisol release was higher ($P \leq 0.01$) in fearful rabbits from group II, which is strictly related to the adaptive processes that were not optimal in this group. The estimated values of meat pH decline were evidence that glycolysis and maturation of meat took a normal course in group I, whereas the meat of rabbits from group II was classified as PSE. The change in active acidity of meat, measured 15 min and 24 h postmortem, altered L*, a* and C* colour parameters while having no effect on parameter b*, with pH_{24h} active acidity being more strongly connected with meat colour than 15 min after slaughter.

Key words: rabbits, stress, meat performance

Poultry meat has for many years been classified into normal (pH = 5.9–6.2), PSE (pH<5.7) and DFD (pH>6.4) based on measurement of pH 15 min postmortem. Pork is grouped into 6 quality classes (RFN, PFN, PSE, RSE, DFD and ASE meat) based on pH, colour and consistency. This division reflects different rates of postmortem glycolysis. According to the assumption, low pH_{15} values (PSE) will correspond to higher drip loss, lighter meat colour and a low level of water-soluble proteins. High pH_{15} values (DFD) will correspond to lower drip loss, less free water, darker colour and a high level of water-soluble proteins (Brzóska, 2001).

It has been acknowledged that the pH_{15} of good quality rabbit meat should range between 6.10 and 6.80 (Bieniek, 1997). With normal glycogen stores in muscles and uninterrupted glycosis postmortem, the concentration of hydrogen ions goes up

and pH of meat decreases. The pH of rabbit meat may be affected by changes in the rate of muscle glycogen breakdown. When the amount of muscle glycogen is low, postmortem muscle acidification does not take place. The amount of glycogen in muscles depends on antemortem rate of metabolic changes, which is affected by both the nutritive value of feeds and rabbit activity, determined by stocking density or stress. In animals more resistant to stress, pH of meat decreases more quickly. Because rabbits easily give in to stress, herds often contain animals unable to adapt to conditions created by humans. The body's response to stress depends on many factors, one of the most important being traits specific to an individual animal. The same situation may cause great stress to one animal but have no perceptible effect on another. The behaviour of animals can be evaluated using simple behavioural tests that reflect stress levels.

The aim of this study was to investigate the response of rabbits to stress stimuli using simple behavioural tests (open field test, stress-induced hyperthermia test) and to determine the effect of stress on meat quality. Rabbit welfare levels were evaluated based on physiological indicators (SIH test), baseline serum levels of cortisol, glucose and triglycerides, and behaviour of animals in the open field test. The concentration of hydrogen ions (pH), water binding and the associated colour lightness of meat were used as parameters of meat quality.

Material and methods

A total of 100 New Zealand White rabbits (58♀, 42♂) were investigated. The observations were made from weaning at 35 days to 90 days of age. All rabbits were clinically healthy and of the same age group. Animals were kept in tiered cages made of spot-welded wire mesh, with 4 animals of the same sex per cage. The same welfare levels were provided throughout the experiment.

Rabbits were fed the same complete pelleted mixture. Animals selected for the experiment were subjected to the open field test and stress-induced hyperthermia (SIH) test together with measurements of their respiratory and heart rate.

For the open field test, rabbits were tested at 60 days of age in a rectangular arena (2.0 m long × 1.4 m wide). The field was divided into 20 rectangles of equal size: 0.4 m long × 0.35 m wide. Open field walls of 1 m height were made of white plywood. A wooden start box (with a round, front-wall opening, open from above) the size of standard kindling box (50 cm long × 40 cm wide × 30 cm high) was placed in the corner of the arena. Animals were carried to the arena in wicker baskets and gently placed in the start box. The test was 5 minutes long. Rabbits were observed by the experimenter sitting near the arena. At the end of each observation, the box, floor and walls were wiped with a detergent-dampened cloth to remove scent traces.

The following rabbit behaviours were recorded during the test:

– time spent in the start box before entering the open field,

– number of movements in the box before entering the open field, with relocation of all legs counting as one movement,

– total locomotor activity in the open field (number of rectangles crossed by the front legs after leaving the start box),

– behaviour after leaving the start box, i.e. standing on hind legs, defecation, scent marking, grooming (licking, scratching), scratching open field walls, attempts at jumping out of the arena, and number of external and central fields crossed.

The SIH test was carried out at 80 days of age. It involved measuring body temperature before and after placing a rabbit for 15 min in a closed wooden box. A high or low difference between rectal temperature measured at the start and at the end of stressor, as well as the respiratory and heart rate before and after taking a rabbit out of the box were the selection criteria. Temperature was measured with an electronic thermometer (Becton, Dickinson, resolution to 0.01°C) and the result was read when the beep sounded, after temperature became stable. The respiratory rate was calculated based on the observations of chest movements, without direct contact with an animal. The heart rate was measured by gently holding the chest of a rabbit with hands.

At the end of the tests, rabbits were divided into two groups with 16 animals (8♀ and 8♂) per group. Group I consisted of rabbits whose results were within the normal range for this group of animals, and group II consisted of rabbits achieving the highest values of the analysed traits.

Blood was collected from all rabbits to determine the levels of cortisol, glucose and triglycerides. The hormone was assayed using a Spectria Cortisol RIA kit (Orion Diagnostica), glucose colorimetrically with a diagnostic kit for glucose determination (CORMAY Liquick Cor-GLUCOSE, cat. no. 2–201), and triglycerides with a diagnostic kit for determining triglyceride concentrations (CORMAY Liquick Cor-TG, cat. no. 2–253).

After 24-hour food withdrawal, animals were slaughtered at 90 days of age in an onsite slaughterhouse. Slaughter was performed in accordance with the current methods for this group of animals under the same technological conditions for all the groups. Throughout slaughter and post-slaughter processing, carcasses were individually tagged for easy identification.

Meat quality analysis was performed on hind left leg muscle (*biceps femoris*) and covered the following groups of traits:

a) measurement of pH 15 min postmortem (pH_{15}); pH after 24-hour feed withdrawal at 4°C (pH_{24h}); absolute ($\Delta pH_{abs.}$) and relative ($\Delta pH_{rel.}$) decline in pH between measurements 15 min postmortem and after 24-hour chilling,

b) determination of free water,

c) basic chemical composition (content of water, protein, fat and ash),

d) measurement of meat colour.

Measurements of meat acidity were made using a microprocessor-based CyberScan PH 10 pH/mV meter with an LCD display. The absolute decline in pH ($\Delta pH_{abs.}$) and the relative decline in pH ($\Delta pH_{rel.}$) were determined according to the method suggested by Blasco and Piles (1990), where:

$\Delta pH_{abs.} = pH_{15} - pH_{24h}$,

$\Delta pH_{rel.} = pHabs. / pH_{45}$.

Free water was determined as percentage of water in meat, which was expelled by 5-minute compression of the sample under a weight of 2 kg. Water extracted from

the meat was filtered through double-layer filter paper. The water weight to sample weight percentage was calculated from the difference in weight before and after compression, according to the method described by Grau and Hamm (1953).

Water determinations were made according to the PN-ISO 1442:2000 standard, fat content using the Soxhlet method according to PN-ISO 1444:2000, protein content by Kjeldahl analysis according to PN-75/A-04018, and total ash content according to PN-ISO 936:2000.

Meat colour was measured with a Minolta CR-310 reflectance colorimeter in the wavelength range of 400 to 700 nm with a step of 20 nm. Meat colour was measured concurrently with measurement of pH 24 h postmortem. L*, a* and b* colour parameters were determined. The a* and b* values were used to calculate psychometric chroma (C*) according to the formula $C^* = \sqrt{a^{+2} + b^{+2}}$, and hue (H*) using the formula ($H^* = \arctan \frac{b^*}{a^*}$).

The results were analysed statistically using a one-factorial ANOVA design. Significant differences between the means in groups were estimated using Duncan's multiple range test. The calculations were made with a Statistica 7.0 PL package.

Results

Table 1 presents the behaviour of animals in the open field test. Twenty-three out of 100 analysed rabbits did not leave the start box. Over 5 minutes of observation, 8 of them did not move in the box and only 3 moved more than 7 times. Among the animals that left the box, general activity in the open field varied, with most rabbits crossing 3 to 4 fields and 8 rabbits crossing more than 10 fields. Immediately after entering the open field, the animals showed characteristic behaviour (stretch attend) reflecting the conflict between exploratory drive and risk avoidance: they stretched their heads in different directions to explore the surroundings while keeping their trunk motionless. Of the different types of behaviour grooming was the most frequent, but in many cases it was forced grooming caused by novel environmental stress rather than proper grooming. Initially, animals moved along the walls of the open field and explored the central part later on. After the test, they were divided into two groups of trustful/curious rabbits (69 animals) and distrustful/fearful rabbits (31 animals).

The open field test was followed by the SIH test. In the analysed group of animals, temperature ranged from 38.55 to 39.75°C before the test, and from 38.84 to 41.01°C after the test. The respiratory rate was 51.12–75.11 before the test and 57.02–123.31 breaths/minute after the test. Among the analysed animals, pulse was 135–172 beats per minute.

The results of the SIH test were 90% consistent with the division into groups after the open field test. Trustful/curious animals showed less response to the stress stimuli and the analysed parameters were higher in distrustful/fearful animals.

Ultimately, 16 animals (8♀ and 8 ♂) with the most extreme characteristics were selected from each group for further study.

Table 1. Behaviour of animals in the open field test

Behaviour	Number
Time spent in the start box:	
5 minutes	23
4–5 minutes	10
3–4 minutes	23
2–3 minutes	12
1–2 minutes	12
0–1 minutes	20
No. of movements in the start box by animals that remained inside:	
0	8
1–3	5
4–7	7
above 7	3
No. of movements in the start box before leaving:	
0	12
1–3	35
4–7	12
above 7	18
Overall locomotor activity (no. of rectangles crossed by the front legs):	
0	7
1–2	11
3–4	23
4–5	10
6–7	9
8–9	9
above 10	8
Behaviour after leaving the start box:	
standing on hind legs	21
defecation	4
grooming	30
scratching the walls	8
jumping out of the arena	2
no reaction – free movement	12

Table 2. Serum levels of glucose, triglycerides and cortisol in rabbits

Item	Group		SE
	I	II	
Glucose (mg/dl)	139.3 A	172.9 B	7.72
Triglycerides (mg/dl)	88.2 A	143.8 B	11.88
Cortisol (nmol/l)	11.9A	15.8 B	12.3

A, B, C – values in rows with different letters differ highly significantly ($P \leq 0.01$).
a, b, c, d – values in rows with different letters differ significantly ($0.01 < P \leq 0.05$).

Blood was collected from the ear vein of all the animals to determine serum levels of glucose, triglycerides and cortisol (Table 2). The cortisol levels determined under the study conditions averaged 9.6 and 13.3 nmol/l for males from groups I and II,

respectively, and 14.2 and 18.4 nmol/l for females from groups I and II, respectively (average of 11.9 nmol/l for group I and 15.8 nmol/l for group II). Highly significant differences were found between groups I and II. Glucose and triglyceride levels were similar for males and females within groups, but differed ($P \leq 0.01$) between groups.

Animals were slaughtered at the age of 90 days. Table 3 presents the physicochemical properties of rabbit muscles. There were highly significant differences in the pH of meat analysed 15 min postmorterm (pH_{15}) and after 24-hour storage (pH_{24h}). Meat pH_{15} averaged 6.60 for group I and 6.01 for group II. After 24-hour storage, pH values dropped to 5.70 in group I and to 5.22 in group II. The average pH decline in group I was 0.90 (pH_{abs}) and 0.14 ($pH_{rel.}$). Significant differences between the groups occurred for the percentage of protein and water in meat, and highly significant differences for the percentage of free water.

Table 4 shows the colour parameters of rabbit meat. The analysis of L*, a*, b* and C* values showed that the muscles of rabbits from both groups differed in colour lightness. The highest L* value (lightness) was found for the meat of rabbits from group II. The red (a*) and yellow (b*) colour coordinates of meat were higher for group I. Highly significant differences were found between the groups for colour intensity (C*): 18.73 in group I and 13.25 in group II. Hue (H*) was similar in groups (0.91).

Table 3. Physicochemical properties of rabbit muscles

Traits	Group I	Group II	SE
pH_{15}	6.60 A	6.01 B	0.86
pH_{24h}	5.70 A	5.22 B	0.92
pH_{abs}	0.90	0.79	0.67
pH_{rel}	0.14	0.11	0.16
Water (%)	72.25 a	75.95 b	2.56
Protein (%)	23.41 a	21.78 b	2.11
Fat (%)	1.52	1.41	0.98
Ash (%)	1.15	1.17	0.56
Free water (%)	12.37 A	8.71 B	2.22

A, B, C – values in rows with different letters differ highly significantly ($P \leq 0.01$).
a, b, c, d – values in rows with different letters differ significantly ($0.01 < P \leq 0.05$).

Table 4. Mean values of colour parameters

Trait	Group I	Group II	SE
L*	47.03 A	58.57 B	1.42
a*	17.93 A	11.13 A	0.82
b*	5.34	4.80	0.21
C*	18.73 A	13.25 A	0.79
H*	0.91	0.91	0.01

A, B, C – values in rows with different letters differ highly significantly ($P \leq 0.01$).
a, b, c, d – values in rows with different letters differ significantly ($0.01 < P \leq 0.05$).

The degree of the rectilinear relationship between two measurable traits was determined based on the correlation that shows the location of the analysed experimental units in the coordinate system. Table 5 presents the correlation coefficients between colour and free water parameters and pH_{15} and pH_{24h} parameters. The results showed that L*, a*, and C* values and free water content are linearly correlated to pH_{15} and pH_{24h}. When analysing the coefficients of correlation between L*, a* and C* parameters, free water and active acidity of meat, they were found to be greater for pH_{24}, which shows that meat colour and free water content are more related to active acidity 24 h than 15 min postmortem.

Table 5. Coefficients of linear correlation for colour parameters, pH_{15} and pH_{24h}

Colour parameter	pH_{15}	pH_{24h}
L*	–0.7467 xx	–0.8362 xx
a*	0.7258 xx	0.7980 xx
b*	0.3388	0.3377
C*	0.7118 xx	0.7951 xx
Free water	0.7812 xx	0.8828 xx

xx – significant correlation at 0.001.

Discussion

The general adaptation syndrome is a model of stress described by Selye (1977). Such response to stress has three stages: alarm, resistance and exhaustion. In the first stage, the sympathetic nervous system is aroused, leading in rabbits to release of hormones (adrenalin, noradrenalin, cortisol) into the bloodstream, acceleration of the heart rate and increased blood pressure, increased muscle tone, increased hepatic secretion of sugar and fat into the blood, acceleration of respiration and inhibition of gastrointestinal motility, especially in the ileum and cecocolic segment. The resistance stage is the relative adaptation of the organism. The symptoms that appeared in the first stage disappear despite the fact that the stressor may continue. Animals vary in their individual response to such a situation. Some show increased hormone levels and overall exhaustion, while the organisms of the others are mobilized to act. Two reactions are possible: adaptation to the stressor or resistance. Rabbit herds often include animals unable to adapt to the existing conditions, which results in sudden cardiac death or loss of the mating instinct.

The rate at which new solutions are introduced into the production systems exceeds the rate at which animals are able to adapt. As a result, they are exposed to the action of stimuli to which their nervous systems are not adapted on the one hand, and they have no stimuli needed to fulfil their natural instincts on the other. Simple behavioural tests can be used to determine the response of animals to stress stimuli, which may form a criterion for selection of breeding and slaughter material on the farms.

Selection for low and high locomotor activity in the open field test helped to create two lines differing in the trait under selection. Thus, efficient selective breeding for quantitative behavioural traits is considered feasible. Studies by Kowalska and Gugołek (2007, 2009) showed that the way rabbits function in an environment is related to some production traits and meat quality.

The mean body temperature of rabbits ranges from 38.5 to 39.5°C, with the temperature of 40.5°C considered as low fever and above 41.5°C as high fever. Temperature is strictly related to the respiratory rate. Because rabbits are rather sensitive and fearful animals, their respiratory rate increases in stress situations but returns to normal within several or dozen minutes. In rabbits, the standard respiratory rate is 50–60 breaths/minute, but it may reach 150 under stress. The heart rate of a healthy rabbit ranges from 120 to 150 beats per minute. The heart rate is influenced by stress, pregnancy, high ambient temperature, walking activity or mating. In our study, we found that fearful animals assigned to group II based on the open field test respond with increased reactivity in the SIH test.

The magnitude of reaction, measured by the levels of glucose secretion and cortisol release, was higher ($P \leq 0.01$) in rabbits from group II, which is strictly related to the adaptive processes that were not optimal in this group. In rabbits, blood collection itself causes stress. This is due to the secretion of adrenalin (epinephrine), which overrides the effect of insulin, thus allowing for a rise in blood glucose level. However, both glucose and cortisol secretion levels are largely dependent on the animal's individual reaction to stress stimuli.

The quality of meat for consumption is considered in terms of where it is sold by slaughterhouses. Meat is either used as raw material for further processing into meat products or used by individual buyers. As regards pork, the first direction accounts for about 65-80% and the second for 20–35%. In Poland, only 10% of rabbit meat accounts for the first direction and 90% for the second, which means that the meat reaching the consumer should be of high quality.

Literature data give different pH values for rabbit meat of good quality. According to Bieniek (1997), quality rabbit meat has a pH_{15} value of 6.1–6.8. When pH is lower, the meat is watery and of lower processing value. The pH value measured 24 h after slaughter should range between 5.4 and 5.8. Zając (1999) reports values in the 5.7–5.9 range for pH_{24}. The pH of rabbit meat was reported to be 5.79 (Cavani et al., 2000) and 5.82 (Blasco and Piles, 1990). The pH values determined in the meat samples from group I indicate that the course of postmortem changes in acidity was correct and typical of normal meat. The pH decline values averaged 0.90 (pH_{abs}) for group I and 0.14 (pH_{rel}) for group II. These are similar to the results obtained by Łapa et al. (2008). Blasco and Piles (1990) report slightly lower values (0.77 and 0.12), which is probably due to the lower initial pH. The pH decline values estimated in the present study for group I are evidence of the normal processes of glycolysis and maturation of meat. The meat of rabbits from group II is considered PSE based on the nomenclature used for poultry meat and pork.

Stress in animals leads to a rapid breakdown of muscle glycogen. The large amounts of lactic acid generated cause rapid muscle acidification and protein denaturation while reducing the ability of muscles to bind water.

Significant differences occurred between the groups in percentage of water in meat and percentage of free water. The interaction between water and muscle cell protein structures is responsible for physical, organoleptic and technological characteristics, including tenderness, which is a highly desirable quality trait of meat and meat products (Dolatowski et al., 2004, Cygan-Szczegielniak et al., 2010). Many studies show that tenderness is positively correlated to hydration and water holding capacity of muscle protein substances (Pospiech et al., 2003; Fraga et al., 1983). Changes in meat quality have an effect on the degree of water binding and the associated colour lightness. The meat of rabbits from group II was characterized by decreased capacity to bind free water, which in this case may be due to low resistance to stress, which is a strong stimulant of postmortem changes.

The colour of muscle tissue is one of the major characteristics of consumer evaluation of meat and depends on the amount and oxidation degree of heme pigments (Feldhusen et al., 1995; Jakubowska et al., 2004). Many authors have pointed to a linear relationship between meat acidity and colour lightness (L*). An increase in meat pH decreases its colour lightness, and a decrease in pH value increases the lightness of its colour. The correlation coefficients obtained showed that the relationship between colour parameters (L*, a*, C*) and pH_{15} and pH_{24h} values is statistically significant. The change in active acidity of rabbit meat caused changes in meat lightness, redness and colour saturation, but had no effect on yellowness. For pork, a relationship was found between L*, b* and C* parameters and active acidity, although no such relationship was established for a* (Strzyżewski, 2008). Colour lightness (L*) of meat in rabbits from group I was considerably lower than that reported by Łapa et al. (2008). This parameter was reported to be 51.1 by Cavani et al. (2000) and 54.41 by Piles et al. (2000). Dal Bosco et al. (1997) found darker meat colour in the case of stressful transport (L* = 41.1) and lighter colour when transport stress was lower (L* = 60.4). Trocino et al. (2003) showed this parameter to be 55.6 after 2-hour transport and 55.0 after 8-hour transport in *biceps femoris* muscle, and 57.8 and 56.4 respectively in *longissimus lumborum* muscle. The value of this parameter is affected to a large extent by nutrition. Pogany-Simonova et al. (2010) found L* to be 48.17 when using oregano supplement in feed, and 51.07 when supplementing ginseng. Meat redness (a*) in our study was similar to the findings of Łapa et al. (2008) and much higher than that reported by Pogany-Simonova et al. (2010) and Trocino et al. (2003). Meat redness depends mainly on the relationships between myoglobin, oxymyoglobin and metmyoglobin content, and changes in individual components resulting from postmortem changes already influence meat redness after 24 hours (Dal Blasco et al., 1997).

Based on simple behavioural tests, our results made it possible to select a group of stress-susceptible animals from the herd. Stress was found to have a considerable effect on the quality of the meat obtained. It was classified as PSE based on the active acidity values and the quality traits of meat from stress-susceptible animals.

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Wpływ stresu na jakość mięsa króliczego

STRESZCZENIE

Celem prowadzonych badań było sprawdzenie za pomocą prostych testów behawioralnych (test „otwartego pola", test „SIH") reakcji królików na bodźce stresowe oraz określenie, jak stres wpływa na jakość ich mięsa. W ocenie dobrostanu przyjęto wartości wskaźników fizjologicznych (test SIH), bazowy poziom kortyzolu, glukozy i trójglicerydów w surowicy krwi oraz zachowanie zwierząt w teście „otwartego pola". Jako parametr oceny jakości mięsa przyjęto wynik pomiaru stężenia jonów wodorowych (pH), stan związania wody i powiązaną z tym jasność barwy mięsa. Wielkość reakcji mierzona poziomem wydzielonej glukozy i uwalnianego kortyzolu była wyższa ($P \leq 0,01$) dla królików z grupy II (płochliwe), co ma ścisły związek z procesami adaptacyjnymi, które nie przebiegały w tej grupie w sposób optymalny. Wartości spadku pH mięsa oszacowane w badaniach świadczyły o prawidłowym przebiegu procesu glikolizy i dojrzewania mięsa dla grupy I, podczas gdy mięso królików z grupy II zostało zaliczone do mięs z wadą PSE. Zmiana kwasowości czynnej mięsa, mierzona po 15 minutach i 24 h po uboju, powodowała zmianę parametrów barwy L*, a* i C*, natomiast nie miała wpływu na wartość parametru b*, przy czym kwasowość czynna pH_{24h} była silniej powiązana z barwą mięsa niż po 15 minutach od uboju.