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## **Garlic (*Allium sativum* L.) as an antibiotic alternative determining the hygienic quality of cow's milk from organic farms**

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Faculty of Animal Science, Warsaw University of Life Sciences – SGGW

**Abstract:** *Garlic (Allium sativum L.) as an antibiotic alternative determining the hygienic quality of cow's milk from organic farms.* The purpose of this study was to determine the efficacy of cow's diet supplementation with a garlic as an antibiotic alternative in the prevention and treatment of subclinical mastitis. The study was conducted on an organic farm located in the Natura 2000 area in the Narew river valley. The major criterions of choosing organic farm to the experiment were as follows: possession a herd of Black and White Polish Holstein-Friesian cows (numbering more than 30 cows) with diagnosed subclinical mastitis and holding a certificate for ecological production and compliance with organic farming standards. The study involved 7 cows diagnosed with subclinical mastitis, whose diet was supplemented with a garlic in a dose of 5 g/per capita/per day. The supplement was administered every day at the same time (in the evening), for 21 days individually into each animal's feeder. The hygienic status of milk samples, measured based on the somatic cells count (SCC) and colony forming units (CFU), was investigated as an indicator of the subclinical mastitis (initial level is a diagnosis). The dietary inclusion of garlic has improved each of the above-mentioned traits during supplementation, without compromising milk production and technological quality. After 21 days of supplementation, the SCC and CFU decreased by about 60%. The study needs to be repeated, taking into account the greater number of cows.

*Key words:* garlic, subclinical mastitis, somatic cell count, colony-forming unit, organic farming, cow

### INTRODUCTION

Organic farming is an alternative to the traditional farming and aims to manufacture food and products of animal origin using natural and traditional methods, excluding the use of synthetic fertilizers and plant protection agents, antibiotics, hormones, growth stimulators, and genetically modified organisms. In such an environment, all cow health interventions should be undertaken with regard to the prevention and without using allopathic medicines and antibiotics. Phytobiotics and plant extracts, homeopathic preparations, and microelements are recommended as therapeutics in morbidity prevention (Amber et al. 2018, Kuczyńska et al. 2018).

In the case of life-threatening or pain-relieving symptoms, allopathic medicines (under supervision of a veterinarian) are allowed, providing the required waiting time – doubled taking specific medicines or standing for at least 48 hours, unless otherwise specified. With the exception of vaccinations, elimination of parasites, and obligatory elimination of diseases, the animals treated with the allopathic medicines twice or more times a year, will have to be included into the conversion program for at least 6 months.

*Mastitis* is one of the main causes of decreased milk production among metabolic and reproductive disorders. Its etiological factors include about 150 species of microorganisms, the key ones including: *Streptococcus agalactiae*, *Str. uberis*, *Str. dysgalactiae*, *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium bovis*, and *Aspergillus sp.* At conventional farms, mastitis is treated with allopathic medicines in about two weeks. Subclinical mastitis usually afflicts 20–70% of the herd, showing no clinical nor milk composition changes (except for the somatic cell count). At certified organic farms, herds affected by the subclinical mastitis may be subject to the alternative treatment based on the usage of phytobiotics. Phytobiotics are plant-derived preparations obtained from herbs that contain biologically-active secondary metabolites. The form of herb application depends on the species of animal, the production group, the management system, and, potentially, on the type and form of the food. Most studies on the use of plant-derived materials in mastitis have been conducted in developing countries and examined traditional medical practices as part of a very extensive and low input production. According to Cheng et al. (2014), alternatives to antibiotics should exhibit specific properties regarding animals (non-toxicity or no side effects, easiness of elimination from the body, stability in feed and in the gastrointestinal tract) and microorganisms (the ability to kill or inhibit the growth of pathogenic bacteria, not causing resistance to bacteria or destroying intestinal microflora), and additionally should not affect the natural environment (should be easily degrada-

ble). Phytobiotics, including garlic, are valuable therapeutic products greatly appreciated by the organic agriculture as an alternative to prohibited antibiotics due to their potential medical and therapeutic applications.

Sustainable agriculture is one of the forms of organic production having the least negative impact on the natural environment. For instance products containing capsules with garlic oil (combined with cinnamaldehyde) have been shown to modulate rumen fermentation (in vitro), and thus inhibit methane production (Blanch et al. 2016). In turn, Gülzari et al. (2018) study results showed that greenhouse gases emission fell by 3.7% by reducing SCC level from 8.0 to  $0.50 \times 10^5 \text{ ml}^{-1}$ . Hashemzadeh-Cigari et al. (2014) have showed improved feed intake, performance, udder health, ruminal fermentation, and plasma metabolites in cows with moderate or high SCC in the milk due to their diet supplementation with a phytobiotics-rich herbal mixture. In turn, an experiment performed by Pasca et al. (2017) to test the inhibitory effect of phytobiotics obtained from several plant species has shown a stronger antimicrobial activity of the coupled use of the phytobiotics compared to the individual application of each plant-based products. In the literature available, not many researchers deal with the influence of garlic on mastitis prevention, especially at organic farms. Caribu et al. (2016) demonstrated that the supplementation of dairy cows diet with a garlic combined with organic minerals (Se, Cr, Zn) increased milk production from 12.9 to 20.1 kg and was ensured optimal milk production activity, as demonstrated SCC reduction in milk by

41.09% (from  $3.48 \times 10^5$  ml<sup>-1</sup> to  $2.05 \times 10^5$  ml<sup>-1</sup>). A possible threat of garlic passing into milk and dairy products was the subject of research conducted by Rossi et al. (2018) study. The garlic-like aroma, taste, and flavor of milk and cheese were significantly influenced by experimental treatments, in particular by the highest dose of garlic cloves. In addition, these authors found close exponential relationships between milk and cheese for garlic-like aroma ( $R^2 = 0.87$ ) and garlic-like flavor ( $R^2 = 0.79$ ) as a result of adding 400 g/day of garlic to the feed of lactating dairy cows. Garlic has various properties, including anti-fungal, antimicrobial, antiviral, anti-inflammatory, hepatoprotective, anti-carcinogenic, and immuno-stimulating in cow's nutrition. The above-mentioned properties of garlic are ascribed to its allicin. Allicin (diallylthiosulfinate) is a defence molecule from garlic (*Allium sativum* L.) with a broad range of biological activities. Allicin is produced upon tissue damage from the non-proteinogenic amino acid alliin (S-allylcysteine sulfoxide) in a reaction that is catalyzed by the enzyme alliinase (Borlinghaus et al. 2014). This chemical compound can also inhibit cell proliferation and induce apoptosis of tumor cells (in mammalian cell lines). Li et al. (2015) showed that a fresh garlic extract may increase the sensitivity of antibiotic-resistant strains to certain antibiotics in vitro. Similar results were obtained by Sheppard et al. (2018) and Najafi et al. (2016) where allicin-inspired pyridyl disulfides were demonstrated to be efficient agents against multidrug-resistant *Staphylococcus aureus* (MRSA). The findings reported by Gholipour et al. (2018) have demonstrated that the addi-

tion of garlic powder the feed mixture of growing calves may be an effective substitute for commonly used ionophore antibiotics, like e.g. monensin, and that it contributes to the increased nutrient digestibility, growth performance, and improvement of blood markers being indicative of their health status. A recent review by Mushtaq et al. (2018) addressing the plant treatment of bovine *mastitis*, has provided examples of the administration of numerous herbs, however it did not mention the use of garlic.

The aim of the current research was to determine the effects of a specially designed garlic on the hygienic quality of organic cows in the summer feeding season.

It was assumed that the dietary inclusion of garlic would improve the hygienic quality of milk of cows from organic production with diagnosed subclinical mastitis.

## MATERIALS AND METHODS

### Animals

The experiment was conducted at an organic farm located in the Natura 2000 area in the Narew river valley. The major criterions of choosing organic farm to the experiment were as follows: possession a herd of Black and White Polish Holstein-Friesian cows (numbering more than 30 cows) with diagnosed subclinical mastitis and holding a certificate for ecological production and compliance with organic farming standards. The study involved 7 cows diagnosed with subclinical *mastitis*.

Cows were housed in tie-stalls, bedded with softwood shavings on rubber mats

and provided free access to water. All cows grazed on the same pasture (*ad libitum*) and were fed the same concentrate (1 kg). Differences in treatment were achieved by weighing 5 g of the garlic before feeding and its administration with the feed mixture to individual cows. The chemical composition and nutritional value of feed mixtures presented Table 1.

Sciences (WULS), they were analyzed for the somatic cell count and colony forming units. Cytological quality was determined using Somacount 150. In turn, WASP equipment (Whitley Automated Spiral Plate) was used to distribute microbiological cultures on Petri dishes. The level of CFU was established using Counter mat Flash equipped with a video camera and a special application.

TABLE 1. Chemical composition (% of DM) and nutritional value of feed mixtures (g/kg)

Treatment	Crude Ash	Crude protein	Fat ether extract	Crude fiber	NDF	ADF	UFL	PDIN	PDIE
Pasture <i>ad libitum</i>	8.62	17.41	3.99	26.1	48.72	30.16	0.85	102.52	83.24
Feed mixture + concentrate (1 kg) + garlic (5 g)	2.26	13.87	2.17	6.28	38.12	24.55	1.06	74.95	97.85

DM – dry matter; NDF – Neutral-detergent fiber; ADF – Acid-detergent fiber; UFL – Feed Unit for milk production, PDIN – protein digested in the small intestine when rumen-fermentable nitrogen is limiting; PDIE – protein digested in the small intestine when rumen-fermentable energy is limiting.

The cows were fed at 6 PM, and the garlic was administered in the same proportions to the individual feed ratios at 5 g/per capita/per day.

### Measurements and analytical methods

The samples (50 ml) were collected during evening milking, placed in sterile plastic bottles, and transported at a temperature of 4°C. The hygienic status of milk samples collected during milking in the summer feeding season was determined based on the somatic cell count and colony forming units as indicators of the individuals with the subclinical *mastitis* phase. Once delivered to the Cattle Breeding Division Milk Testing Laboratory of Warsaw University of Life

The following culture media were used for the microbiological analysis of milk samples: bacteriological agar 2%; Uri-color chromogenic medium for the selection of indole (+) *E. coli*; KESC group bacteria, *Enterococcus*, *Proteus vulgaris* and *Staph. Aureus*; TBX (Tryptone Bile X-glucuronide) medium for selective proliferation of *E. coli* beta glucuronidase (+) (Bio Corp Company).

Feeding experiment was divided into two phases: initial (lasting 7 days, with an incrementally introduced dose of garlic) and final (lasting 21 days, during which each cow received supplements mixed with the concentrate (500 g). Milk samples were collected four times during the supplementation period – on day 7, 14, and 21 of supplementation,

and a control sample was collected as well (start of experience).

The obtained data were analyzed statistically using analysis of variance and Tukey post-hoc test using SPSS 23.0 software.

## RESULTS AND DISCUSSION

The reported study has shown a positive effect of the dietary inclusion of the garlic on both the cytological and microbiological quality of milk from cows with subclinical *mastitis*. A significant decrease in the number of somatic cells was observed in all samples during the supplementation period. However, SCC levels varied among individual samples collected in the following days after the experiment had been completed (Table 2). The number of colony forming units showed a downtrend from the demonstrated level of over 100 thous./ml

to the value of several tens of thousands (Table 3). In addition, a strong correlation ( $P \leq 0.01$ ) was observed between SCC and CFU with a correlation coefficient accounting for  $R^2 = 0.763$ .

The score of somatic cells reflects the status of health of the milk udder and their elevated level (over 100–200 thous./ml) indicates inflammation of this gland caused by infections. The increase in the score of somatic cells is an immune response to the presence of microbes and their metabolites. In milk from healthy udder, 75% of somatic cells are epithelial cells. In contrast, from pieces with a diseased mammary gland, their share decreases from 20 to 30% in advantage of leukocytes. In turn, the content of bacteria in milk depends on the degree of mastitis in infectious germs as well as the hygiene of milking and the conditions under which milk is stored. Pathogenic agents that cause mastitis

TABLE 2. The influence of garlic supplementation on the cytological quality of milk

Times of experiment	SCC	SE
start of experience – control samples	477.57 <sup>ab</sup>	47.94
7th day of supplementation	418.14	84.22
14th day of supplementation	237.43 <sup>a</sup>	92.98
21st day of supplementation	218.00 <sup>b</sup>	70.61

Means in the same rows marked with the same letters differ significantly at: a,  $P \leq 0.05$ ; SCC – mean of somatic cell count (thous./ml); SE – standard error;  $N = 7$  of each group.

TABLE 3. The influence of garlic supplementation on the microbiological quality of milk

Times of experiment	CFU	SE
start of experience – control samples	174.29 <sup>AB</sup>	15.28
7th day of supplementation	132.94 <sup>cd</sup>	45.49
14th day of supplementation	51.57 <sup>Ac</sup>	20.43
21st day of supplementation	41.17 <sup>Bd</sup>	10.65

Means in the same rows marked with the same letters differ significantly at: A,  $P \leq 0.01$ ; c,  $P \leq 0.05$  CFU – mean of colony forming units (thous./ml); SE – standard error;  $N = 7$  of each group.

can be divided into two groups: major and minor pathogens. The first group (major pathogens) includes *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Staphylococcus aureus*, *Actinomyces pyogenes*, *Escherichia coli* and *Mycoplasma bovis*. The other (minor pathogens) include coagulase negative staphylococci (CNS) and *Corynebacterium bovis*. Infections caused by the first group of pathogenic bacteria, including i.a. *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, and *Streptococcus dysgalactiae*, increase the SCC. Another type of udder inflammation induced by the second group of pathogenic bacteria, including i.a. *Corynebacterium bovis* and coagulase-negative staphylococci, results in a slight increase of SCC. In the present study, 10 different microorganisms were isolated from milk samples and identified. The most abundant pathogens causing subclinical mastitis were the catalase-negative bacteria: *Streptococcus*, *Enterococcus*, and *Clostridium*, that were responsible for 75–95% of the infections. *Staphylococcus aureus* was isolated from 5.2% to 25.0% of the samples, however it was not found as a single species but in a mixed flora. The inflammations caused by *E. coli* and *Klebsiella* were more likely due to the summer season characterized by high outdoor temperatures reaching 40°C.

A study conducted by Mussarat et al. (2014) on a traditional medicinal plant used in the treatment of mastitis in the Indus River region has shown the efficacy of 150 g of garlic bulbs ground and mixed with butter in 7-day recovery or an alternative dose of 1–2 glasses of garlic, resulting in a 10-day recovery,

both administered orally. A similar experiment was carried out by Amber et al. (2018) in the northwest Pakistan and has demonstrated that a dose of 100 g of a garlic mixture caused a 7-day recovery from mastitis in cows, buffalos, sheep, and goats. In turn, Gull et al. (2012) determined the inhibitory effect of *Allium sativum* and *Zingiber officinale* extracts on clinically-relevant drug-resistant pathogenic bacteria, including *S. typhi*, *Shigella*, *P. aeruginosa*, *E. coli*, *B. subtilis*, *S. aureus*, *S. epidermidis*, and *K. pneumoniae*. These authors used 6 types of extracts, each prepared from 10 g of garlic powder or ginger, dissolved in 100 ml of distilled water, ethanol or methanol, separately. The highest antibacterial activity of the plant extracts, measured as a diameter (mm) of the inhibition zone, against most of the strains (except for *E. coli* and *E. shigella*), was observed for the garlic aqueous extract.

Grzesiak et al. (2018) conducted a study to determine the effect of essential oils obtained from various herbs, including *Thymus vulgaris* L., *Origanum vulgare* L., *Origanum majorana* L., *Mentha × piperita* L., and *Allium ursinum* L. against *Prototheca zopfii* strains responsible for udder inflammation. Results of the determination of the minimum inhibitory concentration (MIC) have shown the *P. zopfii* strains to be resistant to wild garlic essential oil.

The garlic extract not only prevents the mastitis, but also improves the quality of milk. Pinilla et al. (2017) have observed that use of garlic extract in a concentration of 5% (both free and encapsulated) reduced the viable cell count of four strains of *Listeria spp.* by 4 log cycles (within 10 h). Montironi et

al. (2016) have demonstrated that both *M. verticillata* essential oil and the addition of limonene ensured the antimicrobial efficacy against *S. uberis* strains. The minimum inhibitory concentration (MIC) values determined for the essential oil were in the range from 14.3 to 114.5 mg/ml and these assayed for the limonene ranged from 3.3 to 52.5 mg/ml. The minimum bactericidal concentration (MBC) was between 114.5 and 229.0 mg/ml for *M. verticillata*, and from 3.3 to 52.5 mg/ml for limonene. Additionally Kuczyńska et al. (2015) found the highest concentration of  $\beta$ -carotene (0.450 mg/l) in milk of cows with the highest level of SCC, and believe milk SCC varies significantly with the concentration of  $\beta$ -carotene in cow's milk.

## CONCLUSIONS

Based on study results presented above, it can be concluded that supplementing diets of cows maintained at certificated farms with phytobiotics, including e.g. a garlic extract (*Allium sativum* L.), at only up to 5 g/cow/day from day 1 to day 70 since diagnosis of the subclinical mastitis may improve cows' health and hygienic quality of their milk. Study results have shown a 60% decrease in both SCC and CFU. Moreover, a tendency for the improving hygienic status of milk can be observed after discontinuation of the supplementation. The garlic extract is a completely safe phytobiological supplement and its hypothetical, accidental overdose may in the worst case cause a temporary reduction in feed intake. However, the study needs to be repeated, taking into account the greater number of cows.

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**Streszczenie:** *Czosnek* (*Allium sativum* L.) jako alternatywa dla antybiotykoterapii kształtująca jakość higieniczną mleka krowiego z gospodarstw ekologicznych. Celem pracy było sprawdzenie skuteczności działania profilaktycznego i leczniczego czosnku w przypadkach stanu subklinicznego zapalenia gruczołu mlekowego krów w chowie ekologicznym. Badania przeprowadzono w gospodarstwie ekologicznym zlokalizowanym na terenie obszaru Natura 2000 w dolinie rzeki Narew. Głównymi kryteriami wyboru gospodarstwa ekologicznego do eksperymentu były: posiadanie stada krów rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej (liczącej ponad 30 krów) ze zdiagnozowanym subklinicznym

zapaleniem gruczołu mlekowego i posiadanie certyfikatu produkcji ekologicznej i zgodności z normami rolnictwa ekologicznego. Badaniami objęto 7 krów rasy polskiej holsztyńsko-fryzyskiej (o wadze 600 kg) z wcześniej zdiagnozowanym stanem subklinicznym zapalenia gruczołu mlekowego. Eksperyment przeprowadzono w sezonie żywienia letniego, w trakcie wypasu pastwiskowego przez okres 21 dni. Czosnek zadawano w ilości 5 g/sztukę/dobę codziennie o tej samej porze przed dojem wieczornym. W próbkach mleka oceniano stan higieniczny mierzony jako liczbę komórek somatycznych (LKS) i ogólną liczbę bakterii (OLB) jako wskaźniki subklinicznej fazy *mastitis* (poziom wyjściowy tj. diagnoza). Zadawany w tym czasie czosnek w ilości 5 g/sztukę/dobę do paszy treściwej na pół godziny przed dojem przyczynił się do poprawy parametrów jakości cytologicznej i mikrobiologicznej mleka, bez uszczerbku dla produkcji mleka i jakości technologicznej. Poziom LKS i OLB uległ obniżeniu o 60% w 21. dniu suplementacji

w porównaniu do poziomu wyjściowego. Badanie wymaga powtórzenia, uwzględniając większą liczebność krów.

*Słowa kluczowe:* czosnek, subkliniczne zapalenie, liczba komórek somatycznych, jednostki tworzące kolonie, rolnictwo ekologiczne, krowa

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## Assessment of gentamicin effect on oxidoreductive balance and microstructure of trunk kidney in Prussian carp (*Carassius gibelio*)

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**Abstract:** *Assessment of gentamicin effect on oxidoreductive balance and microstructure of trunk kidney in Prussian carp (Carassius gibelio).* The aim of this study was to investigate the potential toxic effects of gentamicin on the Prussian carp (*Carassius gibelio*) by determining its effect on oxidant-antioxidant balance and by histological image analysis of trunk kidney. The fishes were injected with single standard therapeutic dose of gentamicin of 5 mg·kg<sup>-1</sup>. The samples of trunk kidney were collected in 3 days post antibiotic administration. Concentration of reduced glutathione (GSH) and activity of enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were examined. The concentration of GSH and the activity of studied enzymes did not show statistically significant differences between control and gentamicin-exposed group. The pathological changes were not observed in the trunk kidney structure. Renal tubules as well as renal corpuscles had correct structure. The obtained results indicate that a single injection of gentamicin at a dose of 5 mg·kg<sup>-1</sup> does not lead to disturbance of oxidant-antioxidant balance or histopathological changes in the trunk kidney of Prussian carp. Gentamicin administration did not change oxidoreductive

balance and trunk kidney microstructure in Prussian carp (*Carassius gibelio*).

*Key words:* antibiotic, oxidative stress, histopathology, toxicity

## INTRODUCTION

Gentamicin is an aminoglycoside antibiotic. It is a product of *Micromonospora* actinomycetes and exhibits activity against aerobic bacteria, in particular Gram negative bacilli. Gentamicin mechanism of action is typical for aminoglycosides – inhibiting bacterial protein synthesis (Bakker 1992). Gentamicin is used to treat respiratory infections in animals (Lappin et al. 2017), inflammation of hair follicles (Hillier et al. 2014), chronic foreign body associated sternal osteomyelitis (Wainberg et al. 2015). Nephrotoxicity is a primary negative effect of gentamicin on animals. The application of gentamicin causes reduction in kidney blood flow

and glomerular filtration with increase of vascular resistance. Such effects may occur without permanent morphological changes in the glomerulus and are independent from tubular damage. Main mechanism of gentamicin nephrotoxic action is tubular cytotoxicity. Tubular damage is expressed through necrosis of tubular epithelial cells and modification of function of main cellular components involved in transport of water and solutes (Randjelovic et al. 2017). Therefore, the treatment of animals with gentamicin is associated with necrosis (Edwards et al. 2007) as well as apoptosis of tubular epithelial cells (Li et al. 2009). High doses of gentamicin cause mild glomerular enlargement and changes in circular shape and density with neutrophil infiltration (Stojiljkovic et al. 2008). Despite the relatively widespread use of gentamicin in veterinary medicine, there are few studies in the scientific literature on the toxic effects of this antibiotic on animal organisms. However, Spangler et al. (1980) showed the neurotoxic effect of gentamicin in dogs. Similar effect was also reported in the case of rats (Pramila Padmini and Vijay Kumar 2012). The nephrotoxicity of gentamicin was found in fish such as Nile tilapia (*Oreochromis niloticus*) (Augusto et al. 1996) and oyster toadfish (*Opsanus tau*) (Reim-schuessel et al. 1996). It was shown that in response to gentamicin, larval zebrafish (*Danio rerio*) develop renal failure with the typical features observed in higher organisms (Hentschel et al. 2005). Gentamicin is also ototoxic to hair cells in the lateral line system of Mexican blind cave fish (*Astyanax fasciatus*) and zebrafish (*Danio rerio*)

(Van Trump et al. 2010). The use of this antibiotic in aquaculture is one of the pathways responsible for releasing it into the aquatic environment (Iliev et al. 2015). Numerous organic substances polluting the aquatic environment can cause chemical stress in fish, which is expressed, among others, as disturbance of the oxidation-reduction balance (Dorval et al. 2003, Isik and Celik 2008, Slaninova et al. 2009, Asifa and Chitra 2017). A similar effect was showed in the case of many pharmacological agents, such as ibuprofen (Bartoskova et al. 2013), captopril (Cortes-Diaz et al. 2017) and antibiotic oxytetracycline (Yonar et al. 2011). There are few studies in the literature, on the influence of pharmaceuticals on the histological structure of internal organs of fish. Reda et al. (2013) showed histopathological changes in the kidneys and liver tissue of Nile tilapia (*Oreochromis niloticus*) caused by the antibiotics: oxytetracycline and florfenicol. Studies on the effect of oxytetracycline on carp (*Cyprinus carpio*) have shown that this substance leads to histopathological changes in spleen and kidney tissues (Svobodová et al. 2006). Literature data on the toxic effects of gentamicin on the fish organism are insufficient. A better understanding of its impact on fish is important in terms of the safety of using this antibiotic in veterinary practice, as well as in terms of learning the effects of contamination of the aquatic environment with pharmaceuticals. The aim of this study was to determine the effects of gentamicin used at standard therapeutic dose on oxidative stress parameters and trunk kidney microstructure in Prussian carp (*Carassius gibelio*).

## MATERIAL AND METHODS

### Animals and experimental design

The study was approved by the II Local Ethic Commission in Krakow (permission No. 129/2018). The Prussian carp (*Carassius gibelio*) obtained from Experimental Fishery Station of University of Agriculture in Krakow (Poland) were used in this study. The fish (52 individuals) of body length 18–22 cm were divided into 2 equinumerous groups (experimental and control) and kept in 700 l plastic tanks. The water parameters were: pH  $7.23 \pm 0.08$ ;  $\text{NH}_3$   $0.01 \pm 0.01 \text{ mg}\cdot\text{l}^{-1}$ ;  $\text{NO}_2$   $0.02 \pm 0.01 \text{ mg}\cdot\text{l}^{-1}$  and total hardness  $17.5 \pm 0.84$  °n. After 1 week of acclimation to the laboratory conditions fish were injected with gentamicin (experimental group) or sterile deionized water (control group). Gentamicin (Sigma Aldrich) was administrated at a standard therapeutic dose of  $5 \text{ mg}\cdot\text{kg}^{-1}$  as a sterile solution in deionized water. Scraps of trunk kidney were collected after 3 days post administration for biochemical and histological analyses.

### Biochemical analyses

Scraps of trunk kidney were taken from 20 experimental and 20 control fish for analyses of reduced glutathione (GSH) concentration and activity of enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

Concentration GSH was determined according to Ellman (1959) method. The homogenates were prepared in an ice-cold solution containing 1.15% KCl and 0.01 M sodium phosphate buffer (pH = 7.4). The supernatants were prepared by centrifugation at 14,000 rpm for 5 min at 4°C. The GSH measure-

ments were performed at  $\lambda = 412 \text{ nm}$  using Tecan Sunrise microplate reader in deproteinized supernatant, which was due to the reaction between the thiol groups of GSH and 5,50-dithiobis-(2-nitrobenzoic acid) (DTNB).

For the analyses of enzyme activity, the tissues were homogenized in ice-cold potassium phosphate buffer (100 mM, pH = 7.0). The activities of GPx and SOD were determined using the MARCEL 330 spectrophotometer. GPx activity was determined using the procedure described by Lück (1962). The absorbance was measured at  $\lambda = 340 \text{ nm}$ . The measurements were based on the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), with the concomitant oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH).

Activity of SOD was analysed according to the method described by Kono (1978). The assay is based on the inhibiting influence of SOD on the reduction of cytochrome C by the superoxide anion, which is generated by xanthinexanthine oxidase.

Activity of CAT measurements were conducted with an electrochemical method using Clark's oxygen electrode at pH = 7.0 and 25°C according to the method described by Formicki and Starwarz (2006). The total protein concentrations were determined with Bradford's method (1976) using a microplate reader at  $\lambda = 595 \text{ nm}$ .

### Histological analyses

Scraps of trunk kidney were taken from 6 experimental and 6 control fish for histological analyses. The collected tissue was embedded in paraffin wax. Paraffin blocks of trunk kidney were cut to 6  $\mu\text{m}$

thick sections and stretched. Microtome sections were deparafinized in xylene and hydrated by passing through graded alcohols. After deparaffinization, sections were stained with Haematoxylin–Eosine and observed under light microscopy. The microstructures of the tissue were examined in the randomly selected 10 sections from each fish. The analyses were conducted using Nikon Eclipse Ci microscope.

### Statistical analyses

The Shapiro-Wilk's test was used to test the normality of distribution. The statistical verification of differences in GSH concentration as well as differences in activity of tested enzymes between control and experimental groups were performed by the Student's t-test for unpaired data. Statistical analyses were carried out at the significance level of 0.05. The analyses of basic statistics were also performed. The results were statistically analysed using procedures of the PQStat software.

## RESULTS

### Biochemical analyses

Statistical analysis indicated the normal distribution of results in case of all meas-

ured parameters, both in the experimental and the control groups. The statistical verification of GSH concentration did not show significant differences between experimental and control group. The mean values were similar in both groups (Table). Also the results of all measured enzymes activities did not show statistically significant differences between the control and the experimental groups (Table).

### Histological analyses

Histopathological changes were not observed in the trunk kidney of both control and experimental fish. The structural details of the kidney, glomeruli and renal tubules, showed normal appearance in each group of fish (Fig.).

## DISCUSSION

The discovery of antibiotics changed the treatment of infectious diseases, leading to a significant reduction in morbidity and mortality of humans and animals. The effective medication to which pathogenic microorganisms are sensitive is required for treatment of infectious diseases in fish. The widespread use of pharmaceuticals in aquaculture poses a risk of contamination of the aquatic

TABLE. Results of the analyses of basic statistics of reduced glutathione (GSH) concentration and activity of enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) for control and experimental groups

Tested parameter	Control group (mean $\pm$ SD)	Experimental group (mean $\pm$ SD)
GSH [ $\mu\text{mol}\cdot\text{mg protein}^{-1}$ ]	2.32 ( $\pm$ 1.18)	1.74 ( $\pm$ 0.74)
SOD [ $\text{U}\cdot\text{mg}^{-1}$ ]	8.06 ( $\pm$ 2.75)	9.57 ( $\pm$ 3.01)
GPx [ $\text{U}\cdot\text{mg}^{-1}$ ]	0.38 ( $\pm$ 0.23)	0.35 ( $\pm$ 0.23)
CAT [ $\text{U}\cdot\text{mg}^{-1}$ ]	12.17 ( $\pm$ 1.31)	11.98 ( $\pm$ 1.27)

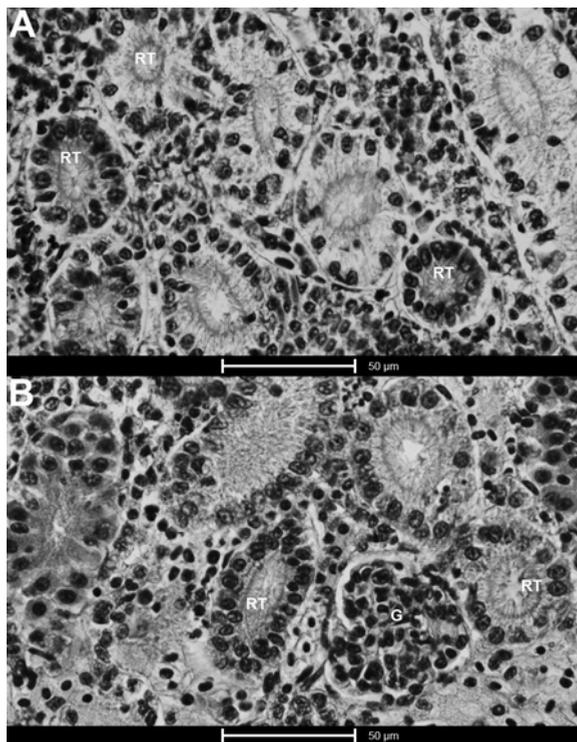


FIGURE. Normal structure of control (A) and gentamicin-treated (B) Prussian carp (*Carassius gibelio*) renal tubules; normal glomerulus of gentamicin-treated (B) fish; RT – renal tubule, G – glomerulus (HE staining, 400 ×).

environment. The high load of antibiotics has been found in sediments. The detected concentrations were enough to inhibit the growth of bacteria (Kümmerer 2008). The research conducted by Wollenberger et al. (2000) showed that antibiotics often have a low biodegradability. Another important issue is the subject of the toxicity of antibiotics to target and non-target organisms. Antibiotics can have a wide variety of damaging effects on animals including direct toxicity, adverse interactions with other drugs, tissue necrosis, impairment of the animal immune or defence mechanisms and damage to foetal or neonatal tissues

(Guardabassi and Kruse 2008). In case of fish, antibiotics may have direct effects on their organisms, including toxicity, immune suppression, alterations in normal bacterial flora of digestive tract, and on growth (Reimschuessel et al. 1996, Gelsleichter et al. 1998, Tafalla et al. 1999, Navarrete et al. 2008). One of the significant indicators of the toxicity of chemical compounds is their effect on the histological state of internal organs, including kidney. Kidney of fish receives the largest proportion of postbranchial blood, and therefore renal lesions might be a good indicator of environmental pollution (Cengiz 2006). In the present

study, histopathological changes in *Carassius gibelio* trunk kidney were not found. This may indicate lack of toxicity of a single administration of gentamycin at the dose of 5 mg·kg<sup>-1</sup> to Prussian carp. However, according to Augusto et al. (1996) exposure of Nile tilapia *Oreochromis niloticus* to gentamycin induced acute tubular necrosis that peaked in severity at 2 days following injection of 25 mg·kg<sup>-1</sup> and at 4 to 7 days following injection of 5 mg·kg<sup>-1</sup>. Necrosis following higher dose exposures was more severe than that following a low dose of tested antibiotics. Reimschuessel et al. (1996) evaluated the nephrotoxic effects of gentamycin in kidneys of toadfish (*Opsanus tau*). Gentamycin was administered at doses of 2.5, 3.5, 5, 15, and 50 mg·kg<sup>-1</sup>. The researchers noticed extensive necrosis in the proximal tubules in each fish injected with gentamycin. In 28 days after antibiotic injection, sections of kidney that were examined were essentially devoid of proximal tubules. Research conducted by Hentschel et al. (2005) demonstrated a decline in glomerular filtration rate after exposure of larval zebrafish (*Danio rerio*) to gentamycin. Gentamycin was injected into the cardiac venous sinus of zebrafish embryos and histological analysis at 96 h post fertilization showed lysosomal phospholipidosis, flattening of the brush border, accumulation of debris in the tubular lumen, as well as tubular and glomerular distention (Hentschel et al. 2005). Chemical stress may be also expressed by disturbing the oxidoreductive balance of the body. In the studies of oxidative stress in animals, the enzymatic indices, such as GPx, SOD and CAT activity, and non-enzymatic ones,

especially the concentration of GSH, are usually evaluated. CAT and GPx catalyze the transformation of hydrogen peroxide into neutral substances: water and oxygen (Fantel 1996, Andreoli 2002). SOD is a first-line antioxidant and loss of its activity undoubtedly potentiates tissue injury through increased formation of reactive oxygen species (Comhair et al. 2000). The most important non-enzymatic antioxidant is reduced glutathione (GSH), which cooperates with enzymatic antioxidants to neutralize hydrogen peroxide and protects the thiol groups of active enzyme sites (Meister 1998). It is considered that oxidative stress has a central role in gentamycin induced nephrotoxicity. However, there is no available data on whether gentamycin can cause oxidative stress in fish. Our results indicate that a single administration of gentamycin at the dose of 5 mg·kg<sup>-1</sup> did not lead to oxidation-reduction imbalance in Prussian carp (*Carassius gibelio*) trunk kidney. However, gentamycin can change the oxidation-reduction balance in other animals. In treated rats renal hydrogen peroxide generation was increased (Guidet and Shah 1989) and reduced glutathione concentrations were decreased (Ali 2002, Sener et al. 2002). The results of biochemical and histological analysis conducted by us did not show toxic effects of gentamycin in Prussian carp. Nevertheless, the results obtained by other authors do not allow to make unambiguous conclusions on the toxicity of this antibiotic to fish. Further research that will take into account the influence of factors such as fish species and the amount of antibiotic dose, which probably have an influence on possible toxic effects, is needed.

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- ślono stężenie glutationu zredukowanego (GSH) i aktywność enzymów: dysmutazy ponadtlenkowej (SOD), peroksydazy glutationowej (GPx) i katalazy (CAT) oraz wykonano preparaty histologiczne. Stężenie GSH i aktywność badanych enzymów nie różniły się istotnie pomiędzy grupą kontrolną a grupą eksponowaną na gentamycynę. Nie zaobserwowano również zmian patologicznych w nerce tułowiowej. Kanaliki nerkowe oraz ciała nerkowe odznaczały się prawidłową strukturą. Uzyskane wyniki pokazują, że jednorazowa iniekcja gentamycyny w dawce 5 mg·kg<sup>-1</sup> nie prowadzi do zaburzeń równowagi oksydoredukcyjnej ani powstania zmian histopatologicznych w nerce tułowiowej karasia srebrzystego (*Carassius gibelio*).

*Słowa kluczowe:* antybiotyk, stres oksydacyjny, histopatologia, toksyczność

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**Streszczenie.** *Ocena wpływu gentamycyny na równowagę oksydoredukcyjną i mikrostrukturę nerki tułowiowej karasia srebrzystego (Carassius gibelio).* Celem pracy było zbadanie potencjalnego toksycznego oddziaływania gentamycyny na organizm karasia srebrzystego (*Carassius gibelio*) poprzez określenie jej wpływu na równowagę oksydoredukcyjną oraz analizę histologiczną nerki tułowiowej. Do badań użyto karasi srebrzystych, którym podano jednorazowo standardową dawkę terapeutyczną gentamycyny (5 mg·kg<sup>-1</sup>). Skrawki nerki tułowiowej pobrano po 3 dniach od iniekcji antybiotyku. Następnie okre-

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## Effect of housing system on fattening and slaughter results and profitability of pig fattening

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**Abstract:** *Effect of housing system on fattening and slaughter results and profitability of pig fattening.* The study was performed on a fattener farm in a pig house with and without bedding material. In each housing system, Landrace × Duroc weaners imported from Denmark, with an initial weight of 25–30 kg, were fattened in three different seasons. Animals were fed *ad libitum* with mixed feeds prepared on the farm. The study determined mortality, daily gains, feed conversion (kg feed/kg gain), daily feed intake, hot carcass weight, and meatiness. A simplified economic calculation of fattening costs was made. Pigs kept on litter were characterized by lower daily gains (10–20%), lower daily feed intake (5–10%), less efficient feed conversion (5%), and higher lower meatiness (1–2.9% point). Taking into account the costs incurred during the fattening process and revenue from the sale of fattening pigs and natural fertilizer, keeping the pigs without bedding proved more profitable. Difference totaled 0.16 PLN per 1 kg hot carcass weight.

*Key words:* pigs, fattening, housing system, profitability

### INTRODUCTION

Poland is a significant producer of pigs in Europe. According to the Central Statistical Office (GUS 2019), the pig population in Poland in December 2018 was 11,027.7 thousand head, a decrease

of 7.4% in relation to the same period of 2017. The most common reason given for reducing the population was that live pig production is unprofitable, mainly due to an unfavourable relationship between prices of fattening pigs and prices of cereals (Pepliński 2013). Out of the 11,027.7 thousand pigs, 43.2% were fatteners. This production group is kept in two systems: with bedding material, which is more popular in less intensive production systems (Rekiel et al. 2018), and without bedding material, which is predominant in intensive pig farms (van de Weerd and Day 2009).

In the litter housing system, pigs spend less time resting, are longer active and less aggressive than in the litterless system. Litter housing also reduces the proportion of undesirable behaviours such as tail biting, ear biting, and mount attempts (Day et al. 2008, Jordan et al. 2008). Animal welfare levels increase as do the costs of production, because bedding material involves costs and higher labour inputs associated with provision of bedding material and removal of manure. When the number of animals on a farm is large, it is more problematic to obtain straw for bedding (Winnicki and Jugowar 2011). At present, bedding

may increase the risk of spreading the ASF (African swine fever) virus. Epizootic data gathered by the Veterinary Inspection shows that in Poland, the most common vectors for ASF transmission were forage, hay and straw (Pejsak and Woźniakowski 2017). These risks and costs increase the interest in housing pigs without bedding material. As reported by Fugol and Szlachta (2010), litterless housing is economically more favourable due to lower labour inputs, no bedding purchase costs, and profit from the sale of slurry as a substrate for biogas production. Litterless housing facilitates good hygiene in the pig house (Winnicki and Jugowar 2011). Lower labour inputs and lower maintenance costs provide an incentive to keep fatteners in the system without bedding material, but some pork-buying consumers are interested in housing conditions of the animals from which this meat was sourced. The question is whether obtaining 1 kg of pork from a pig kept on litter costs the same as from a pig kept without bedding.

The aim of the study was to determine the effect of housing system on performance of fattening pigs and profitability of fattening.

## MATERIAL AND METHODS

The study was conducted on a fattening pig farm in two systems: on shallow litter and without litter (full grating). Landrace × Duroc weaners imported from Denmark, with an initial weight of 25–30 kg, were investigated. The animals were of a high health status. In each housing system, pigs were fattened in three different seasons: winter, spring–summer, and summer–autumn.

In the litterless system, there were twelve pens each with 750 pigs: six pens each with 62 pigs and six pens each with 63 pigs. Each pen had an area of 65 m<sup>2</sup>.

In the litter system, there were three pens each with 300 pigs: two pens with an area of 92 m<sup>2</sup> (90 animals per pen) and one pen with an area of 125 m<sup>2</sup> for 120 pigs. Manure was spread and removed twice a week. Barley and wheat straw were used as bedding material.

The farm complied with the animal welfare requirements specified in the Regulation of the Minister of Agriculture and Rural Development of 15 February 2010 (Rozporządzenie..., 2010) and with the biosecurity requirements (Rozporządzenie..., 2018).

Two-stage fattening was based on complete diets prepared on the farm (Table 1). Pigs were fattened to around 40 kg of body weight in the first stage and from 40 kg until sold in the second stage. Animals had access to feed and water *ad libitum*. After reaching slaughter weight, the fatteners were slaughtered in meat manufacturing plants in compliance with the applicable procedures. Hot carcass weight and dressing percentage were determined. Meatiness of carcasses was evaluated using ultrasound equipment Ultra-Fom 300, and the carcasses were graded according to the EUROP classification system.

The study determined fattening duration, total weight gain, daily weight gain, feed conversion (kg feed/kg gain), dressing percentage, and meatiness. A simplified economic calculation of fattening costs was made. Manure and slurry prices were based on the prices in force on 15 March 2019; the other prices are actual prices in a given production period.

TABLE 1. Ingredients of the feed mixtures, their energy, feeding value and price

Item	Fattening period	
	I	II
Ground triticale	44.0	64.8
Ground barley	35.0	20.0
Soybean oil meal	15.0	11.0
Supplementary mixture*	4.0	2.0
Finely ground limestone	0.5	1.2
Soya oil	1.5	1.0
Energy and nutritional value of the mixture		
Energy of the mixture (kcal)	2 342	2 395
Protein (%)	17.4	15.5
Lysine (%)	1.2	1.0
Methionine + Cysteine (%)	0.7	0.6
Price (PLN/dt)	113	96

\*I – proportion in 1 kg: Ca 4.8%, Na 17.0%, P 4.0%, Lys 11.0%, Met 3.5%, Thr 5.0%, Trp 1.0%; vitamins: A 35 000 IU, E 100 mg, B<sub>2</sub> 120 mg, B<sub>12</sub> 750 mg; Fe 3 400 mg, Cu 4 000 mg, Zn 3 500 mg, I 40 mg, Se 10 mg.

\*II – proportion in 1 kg: crude protein 32.5%, Ca 6.1%, Na 11.5%, P 0.1%, Lys 18.1%, Met 4.1%, Thr 7.0%; vitamins: A 325 000 IU, E 6 000 mg, D<sub>3</sub> 65 000 IU; Fe 3250 mg, Cu 650 mg, 3900 mg, I 65 mg, Se 10.4 mg.

## RESULTS AND DISCUSSION

During whole period of observation no mortality was observed for pigs fattened in winter on litter (Table 2). In the other fattening seasons, weaners mortality was low. Mortality exceeded 1% for pigs fattened during summer–autumn in both the litter-based (1.7%) and litterless systems (1.2%). Mortality in the litter-based system was 0.66% for all three fattening seasons and similar to the observations of Kralik et al. (2013), it was slightly lower than in the litterless system.

The mean body weight of the animals at the beginning of fattening was similar in both housing systems (Table 3). The highest daily gain was achieved in the litterless system during winter (1229 g).

Compared to the spring/summer and summer/autumn fattening, weight gains during the winter period were higher by 79 g and 118 g, respectively. In the litter-based system, regardless of the fattening season, daily gains of the pigs were lower than in the litterless system by 10–20% and were approximately 1000 g. Karpiesiuk et al. (2016) observed a similar trend: pigs kept on shallow litter (in summer period) showed lower daily gains than those raised without litter. The difference in daily gains was 4%. The lower daily gains of the pigs fattened in pig houses on litter compared to those raised in the litterless system could be due to lower daily intake of concentrates (Morrison et al. 2003) and higher physical activity of the fatteners (Day et al. 2008, Jordan et

TABLE 2. Characteristics of the fattening period

Item	Housing system					
	Litter			Litterless		
	I	II	III	I	II	III
No. of animals at start of fattening	301	300	301	749	750	746
No. of animals at end of fattening	301	299	296	746	745	737
No. of dead animals	–	1	5	3	5	9
Mortality (%)	–	0.3	1.7	0.4	0.6	1.2
First day of fattening	19.12.2017	25.04.2018	19.08.2018	19.01.2018	27.03.2018	05.07.2018
Last day of fattening	22.03.2018	02.08.2018	14.12.2018	09.04.2018	25.06.2018	10.10.2018
Fattening period (season of the year)	winter	spring– –summer	summer– –autumn	winter	spring– –summer	summer– –autumn

TABLE 3. Average fattening results of experimental animals

Item	Housing system					
	Litter			Litterless		
	I	II	III	I	II	III
Initial weight (kg)	25.8	26.5	26.5	30.9	26.7	27.0
Weight of pig at slaughter (kg)	117.4	125.0	145.0	129.2	130.2	134.8
Fattening duration (days)	93	98	117	80	90	97
Total body weight gain (kg)	91.6	98.5	118.5	98.3	103.5	107.8
Daily gain (g)	986	1005	1013	1229	1150	1111
Feed intake during fattening (kg)	242.5	256.8	309.0	246.9	256.3	264.8
Daily feed intake (kg)	2.60	2.62	2.64	3.01	2.85	2.73
Feed conversion (kg feed/kg gain)	2.65	2.61	2.61	2.51	2.47	2.46

al. 2008). Different results were reported by Honeyman and Harmon (2003) in a study conducted in the summer and winter seasons. These authors found higher daily gains in the summer season in fattening pigs kept on deep litter in comparison with animal fattened in the litterless system, as well as comparable daily gains in the winter season.

Feed conversion (kg feed/kg gain) was slightly better in the litterless compared to the litter-based system (by 5%). The lower feed conversion was probably caused by the consumption of straw also, which is a fibre that reduces nutrient digestibility and absorption. In our study, feed conversion was 2.46–2.65 kg, which is considered very good compared to the

findings of Karpiesiuk and Falkowski (2008) and Taraska et al. (2016). The factors possibly responsible for the high feed conversion include well-balanced complete diets prepared on the farm, high genetic potential and high health status of the pigs.

In our study we found that meatiness of pork carcasses was high regardless of the season in which the animals were fattened. The mean meatiness of carcasses, regardless of their weight, was 57% (Table 4). We failed to confirm the relationship reported by Antosik and Koćwin-Podsiadła (2010) and Zybert et al. (2015) that carcass meatiness decreases as hot carcasses weight increases. The coefficient of correlation calculated by Antosik and Koćwin-Podsiadła (2010) suggests that an increase in hot carcass weight by 10 kg may be accompanied by a decrease in carcass meat content by around 2.8 p.p. In our study, even the carcasses heavier than 110 kg had a meatiness of 59%. A lack of differences in the meatiness of carcasses with different weights (less than 78 kg and over 92 kg) was also reported by Krzęcio et al. (2004).

In our study we observed that fattening pigs kept in the litter system were characterized by higher meatiness (by a maximum of 2.9%) compared to the pigs fattened in the litterless system (Table 4). This result could be related to the weight gains of the pigs. Bocian et al. (2015) report that the high growth rate of the pigs during fattening does not always result in favourable parameters of carcass slaughter value. Meatiness was highest (58.9%) in fatteners with the lowest daily gains (986 g), and lowest (56%) in fastest gaining pigs (1229 g). The authors determined the coefficients of phenotypic correlation between daily gains and the other fattening and slaughter traits. Although the coefficient of correlation between daily gains and meatiness was not significant, it assumed a negative value ( $R = -0.148$ ), which points to a negative relationship between these traits. That meatiness depends more on the growth rate than on the pig housing system (Gentry et al. 2002). In our study, slower growing animals with a lower daily feed intake (pigs fattened on litter system) were better muscled, which is indicative of better

TABLE 4. Average slaughter results

Item	Housing system					
	litter			litterless		
	I	II	III	I	II	III
Hot carcass weight (kg)	92.6	98.8	114.6	100.8	101.5	105.1
Dressing percentage	78.9	79.0	79.0	78.0	78.0	78.0
Meatiness (%)	58.9	58.7	58.8	56.0	57.8	57.9
Proportion of carcasses in different EUROP categories (%)						
S	24.0	30.0	32.0	8.0	32.0	37.0
E	64.0	58.0	65.0	60.0	64.5	59.0
U	12.0	12.0	3.0	29.0	3.0	3.0
R	–	–	–	3.0	0.5	1.0

utilization of dietary protein to support protein deposition in meat (Orzechowska et al. 2010). In both the litter and litterless systems, most of the carcasses (60%) were graded as class E. Meatiness was more uniform in animals raised in the litter system. The greatest differences in meatiness occurred in the group of fastest growing pigs that were kept in the litterless system in the winter period.

The profitability of fattener production depends largely on live pig procurement prices and production costs. The costs of production are primarily dependent on feed prices because feeding costs account for more than 50% of all fattening costs (Pepliński 2013, Bocian et al. 2015). Table 5 presents the production costs and simplified estimates of profitability in the two

housing systems. The production costs do not include daily handling costs (except for the costs associated with provision of bedding material and removal of manure twice a week), depreciation of buildings, and mortality costs. The production costs ranged from 500 to 576 PLN. The most important item in the structure of costs was the purchase of weaners, who presented from 39 to 53% and feed raw materials (from 42 to 56%). Animals were fattened to different slaughter weights (117.4–145.0 kg in the first and third fattening periods in the litter-based system, respectively), the weaners were purchased at various prices (211–302 PLN in the first and third fattening period in the litter-based system, respectively), but due to higher daily gains and lower feed

TABLE 5. Average results of economic analyses fattening pigs

Item	Housing system					
	litter			litterless		
	I	II	III	I	II	III
Costs (PLN/animal):						
Weaner	249.3	302.0	211.3	264.1	298.2	250.0
Feed	223.1	243.9	305.9	224.7	253.7	259.5
Veterinary services	5.0	5.0	6.0	5.5	5.5	6.0
Water, energy	5.0	6.0	6.0	6.0	6.5	7.0
Litter + labour involved	18.0	19.0	23.0	–	–	–
Total	500.4	575.9	552.2	500.3	563.9	522.5
Revenue (PLN/animal):						
Manure	20.0	22.0	30.0	–	–	–
Slurry	–	–	–	6.5	7.0	7.5
Carcass	561.3	612.6	580.0	593.7	618.1	605.4
Total	581.3	624.6	610.0	600.2	625.1	612.9
Revenue – costs (PLN/animal)	+ 80.9	+ 58.7	+ 57.8	+ 99.9	+ 61.2	+ 90.4
Cost 1 kg of body weight gain (PLN)	5.46	5.85	4.66	5.09	5.44	4.85
Profit from the sale of 1 kg of hot carcass weight (PLN)	0.87	0.59	0.50	0.99	0.60	0.86

conversion, better economic results were obtained for pigs kept in the litterless system. Difference totaled 0.16 PLN per 1 kg hot carcass weight.

## CONCLUSIONS

In summary, it is concluded that pigs kept in the litter system are characterized by lower daily gains, less efficient feed conversion and higher meatiness. Taking into account the costs incurred during the fattening process and revenue from the sale of fattening pigs and natural fertilizer, keeping the pigs without bedding proved more profitable. A potential consumer of pork obtained from pigs kept under improved welfare conditions, i.e. on litter, should pay more to compensate for the higher costs of production.

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- Streszczenie:** *Wpływ systemu utrzymania na wyniki tuczne i rzeźne oraz opłacalność tuczcu świń.* Badania przeprowadzono w gospodarstwie utrzymującym tuczniaki w chlewni bezściołowej i ściółkowej. W każdym z systemów utrzymania przeprowadzono po 3 tucze warchlaków Landrace × Duroc importowanych z Danii o początkowej masie ciała 25–30 kg. Zwierzęta żywiono *ad libitum* mieszankami przygotowanymi w gospodarstwie. W badaniach określono śmiertelność zwierząt, przyrosty dobowe, zużycie paszy na 1 kg przyrostu masy ciała, dzienne pobranie paszy, masę tuszy ciepłej oraz mięsność. Przeprowadzono uproszczoną kalkulację ekonomiczną tuczcu. Zwierzęta utrzymywane w systemie ściółkowym charakteryzowały się mniejszymi o 10–20% przyrostami dobowymi, mniejszym dobowym pobraniem paszy (5–10%), większym zużyciem paszy (5%) i większą mięsnością (1–2,9%). Uwzględniając koszty poniesione w okresie tuczcu oraz przychody ze sprzedaży tuczników i nawozu naturalnego bardziej opłacalne okazało się utrzymanie świń w systemie bezściołowym. Różnica wynosiła 0,16 złotych na 1 kg tuszy ciepłej.
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## **Weight of body, carcass and internal organs as well as paranephric fat index (KFI) as the individual condition indices of the brown hare (*Lepus europaeus*) in eastern Poland**

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**Abstract:** *Weight of body, carcass and internal organs as well as paranephric fat index (KFI) as the individual condition indices of the brown hare (Lepus europaeus) in eastern Poland.* The conducted examination on the individual condition of the brown hare was based on animals obtained by means of group hunting in the western part of the Lublin district in eastern Poland. The weight of body, carcass and internal organs as well as paranephric fat index (KFI) was estimated and compared for separate age and gender groups. The body weight of young hares at the level of just over 4.0 kg and adults amounting to 4.7 kg is higher than in other parts of Poland as well as in the Lublin region compared to previous years. High individual condition is also confirmed by the KFI index, which amounted to 3.6 and was high for this time of year. Carcass mass of acquired hares, which is labeled by means of “slaughtering capacity” (percentage ratio of meat carcass weight with internal parts to live weight of the slaughtered animal), at a level slightly exceeding 60% should be considered as average for the species. Somewhat discomposing are the results regarding the lower mass of internal organs; in particular heart and lungs. Those organs condition the possibility of significant locomotive effort, which consequently plays a very important role in emergency situations when the basic defence reaction of a hare is to escape quickly. Obtained results indicate that the hare population in the western part of the Lublin region is characterized by quite high indicators of individual quality, which should have a positive impact on survivability and repro-

ductive potential of the population. Correlation coefficients between body weight and the weight of internal organs were in most cases, except for adult hares, high and statistically significant.

*Key words:* brown hare, body weight, individual quality, KFI, internal organs

### **INTRODUCTION**

In order to assess the individual quality of wild animals, body weight is usually used. In case of males of the deer family, the weight of antlers is commonly used in such assessment. However, many scientific studies in assessing the individual condition are also based on data on the body fat stores around the internal organs. The most common measurement is the amount of adipose tissue accumulating around the kidneys, and then the ratio of paranephric fat (KFI) is calculated (Batcheler et al. 1970, Bonino and Bustos 1998, Stephenson et al. 1998, Takatsuki 2000, Majzinger 2004, Karpiński et al. 2008, Flis 2012). For the first time, this indicator was used to assess the condition of the deer population introduced in New Zealand (Riney 1955). In hares this indicator is dependent on sex, age

and season. The largest reserves, which constitute energy reserves, occur before the winter period and decrease with time, until almost exhausted in the spring period (Flux 1971, Pepin 1987, Bonino and Bustos 1998).

The mass of internal organs is used in scientific research as a rule in the field of comparative anatomy and quite often in clinical diagnostics. The size of the heart and other internal organs affects the course of physiological processes, thus also affecting the shaping of the individual condition. This is particularly important in wild animals that face the multidirectional pressures of the environments in which they live and fulfill basic life functions. Quite an important element in this respect is the mass of the myocardium, which determines the possibility of long-term locomotor activity. This is particularly important for the survival of these animals. Although hares have small home range, the only method of avoiding threats is to escape which requires considerable physical effort. Due to the features of the anatomical structure hare moves only in gallop and during the escape can reach speeds of up to 80 km/h (Pielowski 1979, Węgrzyn and Kupczyńska 1986, Bishop 1997, Barszcz et al. 2012, Nasiadka and Dziedzic 2014).

Moreover, lung mass affects quite significantly the gas exchange processes and thus the locomotor processes. Of the 4 species of mammals from the order of lagomorphs covered by the study, it was brown hare that showed the greatest adaptation of lungs and chest to significant locomotor effort (Simons 1996).

All these elements of the individual condition, interacting comprehensively

together with other factors, both endogenous and exogenous, have a significant effect on the fertility and mortality of hares. This, in turn, determines the size of the population, which has been regressing for many years. Therefore, it was justified to carry out these types of assessments, which, due to the lack of material for research, have not been carried out for many years (Dziedzic et al. 2002, Nasiadka and Dziedzic 2014, Misiorowska et al. 2014, Flis 2015).

The aim of the study was to assess the individual condition expressed by weight of body, carcass and selected internal organs, as well as the paranephric fat index (KFI) in brown hares obtained during hunting in the Lublin Upland

## MATERIAL AND METHODS

Study material consisted of 30 hares obtained by means of group hunting in 2 hunting areas of the western part of the Lublin region, eastern Poland. The hares were shot in December 2017. In terms of physico-geography, these circuits are located in the mesoregion Kotlina Chodelska and the northern part of Wzniesień Urzędowskich. This region is characterized by the fragmentation of field crops, which directly determines the considerable heterogeneity of hare living habitat. Due to the presence of fertile soils in the crop structure, in addition to typical agricultural crops, a significant proportion are orchards and perennial plantations of soft fruits. In the agricultural landscape there are numerous wastelands as well as small wooded enclaves and forest complexes. Despite the decrease compared to previous years, the hare number in this region is so high

that hunting is conducted every year, and the density ratios are among the highest in Poland (Kondracki 2000).

Age and gender of the hares was assessed directly in the field. Age was determined based on the assessment of the occurrence or disappearance of the Stroh sign (Fig. 1). Such an assessment allows the division of individuals into

two groups: young (up to 1 year old) and adult (over 1 year old) (Stroh 1931, Pielowski 1979). Sex was determined based on the appearance of secondary sexual characteristics (Fig. 2). Immediately in the field, each individual was weighed on a laboratory scale, with an accuracy of 0.1 kg. After the hunt, the hares were skinned and gutted. These

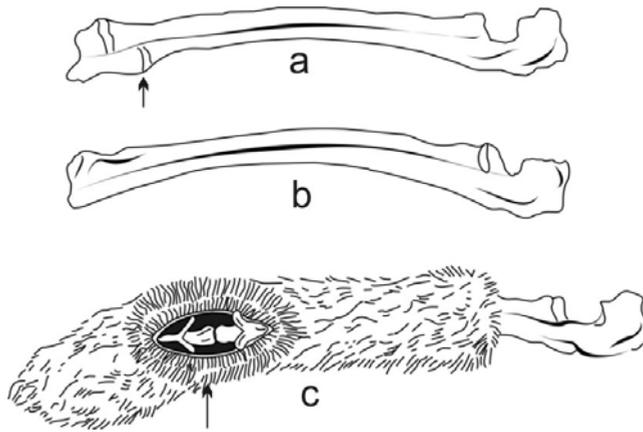


FIGURE 1. Recognition of the age of the hare based on the Stroh sign (based on Pielowski 1979)  
a – thickening of the ulna bone – young hare up to the age of about 8 months; b – ulnar bone without thickening – hare over the age of 8 months; c – view of the thickening on the ulna of the young hare after cutting the skin on the stroke

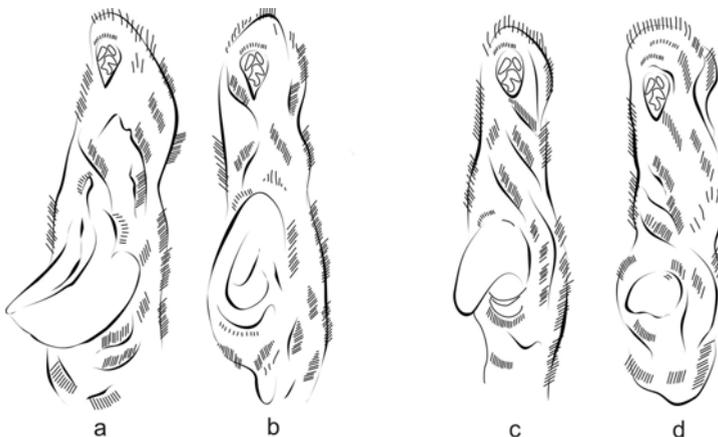


FIGURE 2. Hare sex recognition based on secondary sexual characteristics (based on Pielowski 1979)  
a – adult male; b – adult female; c – young male; d – young female

treatments were carried out in accordance with the rules of how to deal with animals after shooting (Gembarzewski and Matuszewski 2009).

Carcass was the body of the animal, without skin, internal organs, head and the lower parts of legs. During the evisceration heart, liver and lungs were dissected and weighed accurately to 1 gram. Kidneys with paranephric fat were also dissected. They were weighed together with fat and without it. Based on these measurements, the paranephric fat index (KFI) was calculated based on the formula (Bobek et al. 1984):

$$\text{KFI} = \frac{\text{mass of kidneys with fat}}{\text{weight of kidneys without fat}}$$

In order to determine the differences between the mean values of the analyzed features depending on the age and sex of hares, a two-way analysis of variance was performed. In order to verify the possible occurrence of differences between the averages, calculations were made using the Newman Keuls test in the Statistica program. Correlation coefficients between body weight and the weight of internal organs were also calculated.

## RESULTS

There was a slight variation in body weight and carcass weight between males and females in both the young and adults (Table 1). In males, males were 0.1 kg heavier and in adults the mean body weight was the same. In the case of carcass weight, young animals weighing 0.2 kg were males and 0.1 kg females in adults. There were no significant differ-

ences between the average values of these features ( $P \geq 0.05$ ). In all cases, what was predicted, both body weight and carcass weight of adult animals was higher than young and the differences were statistically significant ( $P > 0.05$ ). Also in all cases, the adults I had had heavier hearts, lungs and liver. Comparison between sexual groups indicates that in the group of younger individuals heavier hearts and livers were males, while heavier lungs were found in females. In males, males had higher heart and lung mass and 2.4 grams lower liver weight. However, the described differences in all cases did not differ statistically significantly ( $P \geq 0.05$ ).

Young males had a higher value of perirenal fat (KFI), compared to young females, however, this difference was not statistically significantly different ( $P \geq 0.05$ ), while in adults, the difference in the mean value of this attribute at the level of 0.6 was statistically significant ( $P < 0.05$ ). In turn, the value of this indicator between young and adult males did not differ significantly different ( $P \geq 0.05$ ), and between young and adult females the difference at level 1.2 was statistically significantly different ( $P < 0.05$ ).

Analysis of the relationship between body weight and the mass of internal organs indicates that in most cases they were high and statistically significant (Table 2). However, in the case of adults, the hare correlation coefficients were not statistically significant, and their value between body weight and heart weight was  $R_{xy} = 0.043$ , and between body weight and lung weight there was a negative relationship  $R_{xy} = -0.043$ .

TABLE 1. Body weight and carcass weight (kg), the mass of internal organs (g) and the values of the perirenal fat ratio (KFI) the of hares

Parameter	Young				Adult				Male <i>P</i> -values Young/ /Adult	Female <i>P</i> -values Young/ /Adult		
	male ( <i>N</i> = 7)		female ( <i>N</i> = 10)		male ( <i>N</i> = 5)		female ( <i>N</i> = 8)					
	$\bar{x}$	<i>SD</i>	$\bar{x}$	<i>SD</i>	$\bar{x}$	<i>SD</i>	$\bar{x}$	<i>SD</i>				
Body weight	4.1	0.12	4.0	0.21	0.5131	4.7	0.24	4.7	0.21	0.0503	0.0008	0.0038
Carcass weight	2.5	0.05	2.3	0.26	0.1899	2.9	0.18	3.0	0.08	0.5145	0.0018	0.0013
Heart	34.3	1.50	31.0	4.47	0.1503	42.0	2.94	39.8	2.38	0.3216	0.0064	0.0024
Lungs	31.8	2.36	32.9	3.33	0.5587	42.0	2.94	39.8	2.28	0.2526	0.0004	0.0015
Liver	83.3	2.87	80.0	7.41	0.3583	86.0	3.92	88.4	3.43	0.4949	0.4352	0.1082
Perirenal fat ratio ( <i>KFI</i> )	3.5	0.30	3.1	0.45	0.2657	3.7	0.64	4.3	0.40	0.0493	0.5683	0.0083

*P*-values – the averages difference in statistically significantly at ( $P < 0.05$ )

TABLE 2. Correlation coefficients between body weight and weight of assessed internal organs

Parameter	Sex/Age	Heart	Lungs	Liver
Body weight	male	0.685	0.750*	0.683
	female	0.933*	0.777*	0.749*
	young	0.829*	0.186	0.694*
	adult	0.043	-0.043	0.566

\* – statistically significant correlations

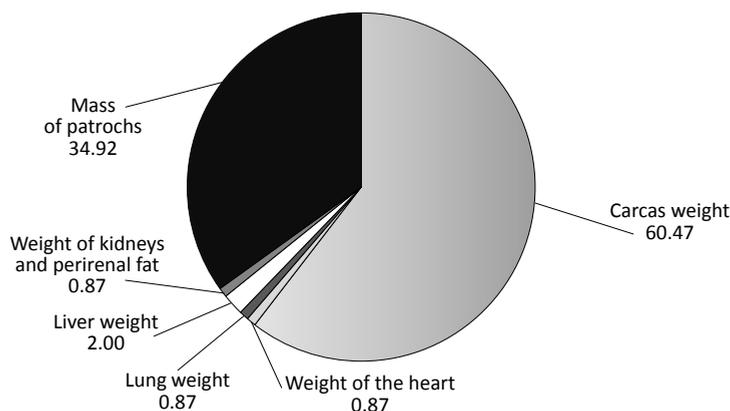


FIGURE 3. Share of individual carcass elements (%) in relation to the hare body weight

Analysis of the weight of the hare carcasses showed a low percentage in relation to body weight (Fig. 3). The low index of slaughter efficiency is conditioned by a fairly high proportion of inedible parts, i.e. skin and head, and inedible insides. The share of edible guts was small.

## DISCUSSION

Presented weighting results of hares are higher compared to the data from the same area for brown hares that were shot during the hunting season 2014/2015 (Flis 2015). The body mass of both young and adults is also higher than that of hares in the hunting districts of the Lublin Upland and Podlasie, acquired in the 1990's (Dziedzic et al. 1998). Misiorowska et al. (2014), conducting research in the areas with the highest number of hares, gave a varied weight value depending on the region and age, which was within the range of 3.42–4.32 and it was also lower than at present in the area of conducting this research. In the region of Central Pomerania,

the average hare weight gained in the years 1998–2001 was 4.13 kg (Myslek et al. 2004), hence it was lower than at present in the Lublin Upland. Obtained results also indicate a definitely higher body mass for young hares and only slightly higher in adults in relation to those obtained in eastern Poland in the years 1965–1972 (Caboń-Raczyńska 1974). The presented weight results of hares are also higher than those obtained in Croatia in the 2004/2005 hunting season and in Hungary, both in the age and gender groups (Pintur et al. 2006, Farkas et al. 2016). In turn, the percentage proportion of carcass weight to body weight in our hares (60.47%) was lower in comparison with brown hares shot in eastern Croatia, where it accounted for 64.78% (Škrivanko et al. 2008). The Croatia's hares had also a higher weight of heart, lung and liver compared to the individuals obtained in the Lublin region. In Italy, in a closed breeding, the share of hare carcass weight in the control group was 63.7% of body weight and it weighted 2.17 kg on average (Vizzarri et al. 2014).

The average value of the paranephric fat index in brown hares studied in Argentina in years 1985–1986 expressed as a percentage was around 25% and was dependent on sex, age and season (Bonino and Bustos 1998). This means that the average value of the KFI in the hare population from Lublin Upland in the month of December was higher by over 2%, and thus the results should be considered comparable. The absence of clear and statistically significant differences between the KFI value in males and females in this study confirm the results of other authors that only low and periodic differentiation of this feature happens within the sexes. These results also confirm clear differences in the rate between young and adult hares regardless of gender (Flux 1971, Farfán et al. 2004, Fernández et al. 2010).

The obtained results indicate that the hare population in the western part of the Lublin region is characterized by quite high indicators of individual quality, which should have a positive impact on the survivability and reproductive potential of the population. Such high individual condition is most likely conditioned by environmental conditions and the characteristics of agricultural production in this area. The structure of agroecosystems directly affects living conditions, and in particular the composition of the diet resulting from the nutritional requirements of this species (Pielowski 1979, Reichlin et al. 2006).

## CONCLUSIONS

1. The high individual condition of the hare, expressed as body mass and KFI index, in the area of research should

have a significant beneficial effect on the reproductive potential of the population and the survival of hares. This should contribute to the stabilization of the population in this area and even condition its growth.

2. The results regarding the lower mass of internal organs, especially of the heart and lungs, are slightly disturbing. They determine the possibility of significant locomotive effort, which in turn plays a very important role in emergency situations, where the basic defensive mechanism of the hare is to escape quickly.
3. The mass of carcasses from obtained hares, defining the slaughtering efficiency, at a level slightly exceeding 60% should be considered as an average for this species and the differences between the data from the literature result usually from the method of dressing and gutting.

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- wego (KFI), w wyodrębnionych grupach wiekowych i płciowych. Uzyskane wyniki wskazują, że masa ciała młodych zajęcy na poziomie nieco ponad 4,0 kg i dorosłych wynosząca 4,7 kg jest większa niż w innych częściach Polski, jak i w rejonie Lubelszczyzny, w porównaniu z latami wcześniejszymi. Potwierdzeniem wysokiej kondycji osobniczej jest także wartość wskaźnika KFI, który wyniósł 3,6 i był wysoki jak na tę porę roku. Masę tuszy pozyskanych zajęcy określającą wydajność rzeźną (stosunek masy tuszy oraz narządów wewnętrznych z uwzględnieniem części niejadalnych, do masy ciała pozyskanych zwierząt) na poziomie nieco przekraczającym 60%, należy uznać za przeciętną dla tego gatunku. Nieco niepokojące są wyniki odnośnie niższej masy narządów wewnętrznych zwłaszcza serca i płuc. Organy te warunkują możliwość znacznego wysiłku lokomotorycznego, co z kolei ma bardzo duże znaczenie w sytuacjach zagrożenia, gdzie podstawowym odruchem obronnym zajęcy jest szybka ucieczka. Uzyskane wyniki wskazują, iż populację zajęcy w zachodniej części Lubelszczyzny cechują dość wysokie wskaźniki jakości osobniczej, co powinno wpływać korzystnie na przeżywalność oraz potencjał rozrodczy populacji. Współczynniki korelacji pomiędzy masą ciała a masą narządów wewnętrznych były w większości przypadków, poza dorosłymi zającami, wysokie i statystycznie istotne.

*Słowa kluczowe:* zając, masa ciała, jakość osobnicza, KFI, narządy wewnętrzne

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**Streszczenie:** Masa ciała i tuszy oraz narządów wewnętrznych i wskaźnik tłuszczu okolonerkowego (KFI) jako wskaźnik kondycji osobniczej zająca szaraka (*Lepus europaeus*), we wschodniej Polsce. Badania kondycji osobniczej zajęcy przeprowadzono na podstawie zwierząt pozyskanych w drodze polowań zbiorowych w obwodach łowieckich położonych w zachodniej części Lubelszczyzny, we wschodniej Polsce. Oparto je na analizie masy ciała, tuszy oraz narządów wewnętrznych i wskaźnika tłuszczu okolonerko-



## Annual changes in hematological parameters of common carp juveniles under laboratory conditions

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**Abstract:** *Annual changes in hematological parameters of common carp juveniles under laboratory conditions.* The aim of the study was to evaluate the changes of the values of hematological parameters of carp juveniles in annual cycle under stable laboratory conditions. Some parameters showed distinct rhythms of changes, e.g. 2 peaks of hematocrit occurred in II and VIII, of hemoglobin concentration in I and VI, while erythrocyte count showed maximum in II. The largest erythrocytes were observed in VIII, and the smallest in XII. Leukocyte count showed two peaks in XII and III. Maximum lymphocyte frequency occurred in III and minimum in XI, while percentage of neutrophils showed the reverse pattern. Oxidative metabolic activity of phagocytes peaked in III, while minimum occurred in XI–XII and VI–VII. Thrombocyte count was highest in XII, and lowest in VII. The obtained results revealed that the values of hematological parameters in carp considerably changed during the year despite little alterations in environmental factors. Some of these changes, e.g. increase in oxidative activity of phagocytes in spring and increase in hemoglobin level in summer were similar to those that occur in fish under natural conditions. Another changes, such as increase in erythrocyte size or decrease in leukocyte count suggest long-term adjustment to the laboratory environment.

*Key words:* blood, erythrocytes, fish, leukocytes, season, thrombocytes

### INTRODUCTION

Hematological parameters are sensitive indicators of fish health and physiological status. However, their values may be affected by various environmental and intrinsic factors, e.g. temperature, photoperiod, water quality, fish age and phase of reproductive cycle. Therefore, determination of the reference ranges of fish blood parameters is more difficult than in homoiotherm animals (Luskova 1997). According to Leard et al. (1998), for parameters that do not considerably vary a single baseline range is appropriate, while for parameters that show distinct seasonal fluctuations it is necessary to develop seasonal or monthly reference ranges.

Most studies concerning seasonal changes in values of fish hematological parameters have been conducted under natural conditions. Their results usually show distinct patterns in seasonal changes of most indices. Hematocrit, hemoglobin concentration and erythrocyte count tend to increase in summer and to decrease in winter, while mean corpuscular volume usually shows a reverse pattern (e.g.

Morgan et al. 2008, Jeronimo et al. 2011, Golemi et al. 2013, Langer et al. 2013, Pradhan et al. 2014). However, some authors reported no significant season-related alterations in the values of red blood parameters (Abdel-Hameid 2011, Fallah et al. 2014).

Also the values of white blood indices usually show seasonal patterns. According to Bowden et al. (2007), Morgan et al. (2008), Santos et al. (2009) and Das et al. (2012), in winter immune functions decrease, while at higher temperatures immune response is enhanced. Leukocyte count is usually higher in summer compared to winter (e.g. Orun et al. 2003, Gupta et al. 2013, Kohanestani et al. 2013, Seriani et al. 2013). On the other hand Buchtikova et al. (2011) observed the lowest phagocyte respiratory burst activity in summer and the highest in winter, accompanied by the highest percentage of phagocytes in blood.

There is little information about seasonal variation in thrombocyte count in fish, and the data obtained by various authors are contradictory: increase in summer was reported by Orun et al. (2003) and Vigliano et al. (2014), Jeronimo et al. (2011), Gupta et al. (2013), while Langer et al. (2013) observed maximum thrombocyte abundance in winter. Santos et al. (2009) and Seriani et al. (2013) found no significant season-related alterations.

However, many research projects on fish are held in laboratory but information about the changes in values of hematological parameters in annual cycle under constant conditions is lacking and one could expect that in little variable environment hematological values (at least in juvenile fish that do not undergo

physiological changes related to spawning) are stable. The aim of the present study was to evaluate annual changes in hematological parameters of common carp juveniles kept under controlled laboratory conditions.

## MATERIALS AND METHODS

Six months old common carp *Cyprinus carpio* L. juveniles ( $36.7 \pm 8.0$  g) harvested from the extensive rearing pond of the Inland Fisheries Institute in Żabieniec at the beginning of October were transported to the laboratory of the Department of Animal Physiology, Siedlce University of Natural Sciences and Humanities in plastic bags with pond water filled with pure oxygen. The fish were placed in a 300 L flow-through aerated tank and acclimated to the laboratory conditions for 3 weeks prior to the beginning of the experiment. During this time water temperature was 17–18°C, pH 6.5, O<sub>2</sub> concentration 6.1–8.0 mg/l, NO<sub>2</sub><sup>-</sup> – 0.00–0.06 mg/l, NH<sub>4</sub><sup>+</sup> – 0.0 mg/l. During acclimation period the fish were fed commercial carp feed Aller Aqua Classic 4.5 mm at the rate of 1% of body mass. Water quality parameters were monitored once a week using pH meter (PRL TN 5123, Elwro, Poland), DO meter HI 9143 (Hanna Instruments, USA), and colorimetric kits Visocolor nitrite and Visocolor ammonia (Macherey-Nagel, Germany).

After acclimation period, 30 fish were transferred to another 300 l aerated flow-through tank where they were kept for 12 months at natural photoperiod. Water quality parameters were measured once a week. Water temperature was  $18 \pm 2^\circ\text{C}$ , pH 7.2–7.6, dissolved oxygen concentra-

tion  $8.5 \pm 0.3$  mg/l,  $\text{NO}_2^- < 0.1$  mg/l,  $\text{NH}_4^+ < 1$  mg/l. The fish were fed the same feed as during acclimation period every morning to satiation (only on blood sampling days fish were fed after blood collection). Every month (between the 19 and 29 day) blood was collected from 10 randomly harvested fish, always between 9 and 10 a.m. About 150  $\mu\text{l}$  of blood (4 drops) were sampled by heart puncture from each fish using heparinized chilled needles and heparinized chilled plastic Eppendorf tubes. No anesthesia was applied, the procedure lasted no longer than 30 s and the fish were returned into the tank. Hematological procedures were performed according to Svobodova et al. (1991). Hematocrit (Ht) values were measured using microhematocrit method. Erythrocyte (RBC) and leukocyte (WBC) counts were evaluated in blood diluted 100 $\times$  with Hayem solution, using microscope Nikon Eclipse E600 (under magnification 400 $\times$ ). Hemoglobin concentration (Hb) was measured using cyanmethemoglobin method (10  $\mu\text{l}$  of blood was mixed with 1 ml of Drabkin solution) with UV-Vis spectrophotometer Helios Gamma (Thermo Electron Corporation, USA) at 540 nm wavelength. Then, Hb was calculated according to the relationship between the extinction and concentrations of standard hemoglobin solutions. Other red blood cell parameters: mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the formulas:  $\text{MCV} = (\text{Ht} \times 10) / \text{RBC}$ ,  $\text{MCH} = \text{Hb} / \text{RBC}$ ,  $\text{MCHC} = (\text{Hb} \times 100) / \text{Ht}$ . Unstimulated oxidative activity of phagocytes (NBT) was measured using nitroterazolium

blue reduction method. Fifty  $\mu\text{l}$  of blood was mixed with an equal amount of 0.2% nitroterazolium blue solution and incubated for 1 hour at 28 $^\circ\text{C}$ , and then 1 ml of dimethylformamide (DMF) was added and the samples were vortexed for 5 minutes and centrifuged. Extinction of the supernatant was read at a 546 nm wavelength and concentration of formazan (a product of nitroterazolium blue reduction) was calculated according to the relationship between the extinction and concentrations of standard formazan solutions. Blood smears were also made (2 for each blood sample), stained with May-Grunwald and Giemsa solutions and fixed with Histokitt (Glaswarenfabrik Karl Hecht, Germany). In each preparation 300 erythrocytes were evaluated and percentage of erythroblasts was calculated. Both cell diameters and both nucleus diameters of 50 mature erythrocytes in each smear were measured using calibrated eyepiece reticule. Cell and nucleus perimeter areas were calculated (using the formula for ellipse area  $\Pi rl$ , where  $r$  and  $l$  are shorter and longer radii, respectively). The ratio of longer to shorter diameter was also calculated as "elongation index" and the ratio of cell to nucleus area as "nuclear index". In the smears 100 leukocytes were also viewed and classified to calculate differential leukocyte count: percentage of lymphocytes, neutrophils, monocytes, basophils and eosinophils. Thrombocytes were counted per 100 leukocytes in each smear, and then thrombocyte count (PLT) was calculated using this proportion and WBC values.

All fish survived the experiment and did not exhibit any signs of disease. Their final body mass was  $107.9 \pm 18.0$  g.

The average values of hematological parameters were calculated for each month and for statistical comparison of differences – per season (autumn: X–XII, winter: I–III, spring: IV–VI, summer: VII–IX). The results were subjected to Shapiro-Wilk's test to evaluate normality of distribution of the variables. As most of them showed normal or close-to-normal distribution, one-way ANOVA and Duncan's test were used to evaluate significance of differences (at  $p \leq 0.05$ ) using Statistica 9.1 (StatSoft, Inc., USA).

## RESULTS

Red blood cell parameters of carp juveniles reared under laboratory conditions showed distinct seasonal patterns (Table 1). Maximum hematocrit values occurred in II and VIII, both preceded by gradual increase. Seasonal statistical comparison revealed the lowest mean Ht value in autumn and the highest in winter and summer. Hemoglobin concentration also showed two peaks: in I and VI. The highest average seasonal Hb level occurred in winter and the lowest in autumn. Erythrocyte count showed one peak in II that coincided with maximum Ht level. The highest RBC occurred in winter, and the lowest in autumn. The average frequency of erythroblasts ranged from  $7.3 \pm 1.5\%$  in VIII to  $11.4 \pm 3.8\%$  in XI but the highest seasonal average value occurred in spring, while the lowest in summer. The MCV, MCH and MCHC values showed fluctuations without any distinct seasonal pattern or significant seasonal differences.

Although, the only significant differences in calculated MCV occurred between autumn and summer, direct

measurements of erythrocytes revealed significant seasonal differences in their size (Table 2). The largest erythrocytes (in terms of area) were in summer, while the smallest in autumn. Similar changes were observed in the area of erythrocyte nuclei. The changes in cell and nucleus size were related mainly to the significant increase in their long diameters. An increase in erythrocyte elongation was also observed and a decrease in area of cell occupied by the nucleus.

The changes in the values of white blood cells parameters of carp also showed seasonal patterns (Table 3). Two peaks of leukocyte count occurred: in XII and III. The highest seasonal average WBC values were observed in autumn and winter, while the lowest in summer. Differential leukocyte count also showed significant seasonal alterations although no distinct monthly patterns were observed. The highest lymphocyte frequency occurred in winter and the lowest in summer, while percentage of neutrophils showed the reverse pattern. In all seasons immature neutrophils predominated in carp blood (myelocytes and metamyelocytes) comprising from  $66.1 \pm 30.7\%$  of neutrophils (in summer) to  $83.7 \pm 30.5\%$  (in autumn), while mature cells (band and segmented) were less abundant. The maximum oxidative metabolic activity of phagocytes (NBT) occurred in III, while minimum in XI–XII and VI–VII. Seasonal average NBT values significantly differed showing the maximum in winter and minimum in summer and autumn. Thrombocyte count did not show any distinct monthly pattern, but reached the maximum in winter, while the lowest value occurred in summer.

TABLE 1. Seasonal changes in the values of red blood parameters in common carp (different letter superscripts indicate statistically significant differences, Duncan test,  $P \leq 0.05$ ,  $N = 30$ )

Season	Ht (%)	Hb (g/l)	RBC ( $10^6 \cdot \mu\text{l}^{-1}$ )	MCV (fl)	MCH (pg)	MCHC (g/l)	Erythroblasts (%)
Autumn	24.5 $\pm$ 3.7 <sup>a</sup>	54.4 $\pm$ 21.8 <sup>a</sup>	1.44 $\pm$ 0.33 <sup>a</sup>	182.9 $\pm$ 50.2 <sup>a</sup>	40.5 $\pm$ 20.5 <sup>a</sup>	221.5 $\pm$ 79.9 <sup>ab</sup>	9.6 $\pm$ 3.3 <sup>a</sup>
Winter	32.6 $\pm$ 3.7 <sup>b</sup>	82.7 $\pm$ 17.4 <sup>b</sup>	1.67 $\pm$ 0.31 <sup>b</sup>	200.3 $\pm$ 37.4 <sup>ab</sup>	51.6 $\pm$ 16.0 <sup>b</sup>	255.9 $\pm$ 57.9 <sup>b</sup>	9.5 $\pm$ 2.1 <sup>ab</sup>
Spring	29.2 $\pm$ 4.9 <sup>c</sup>	66.2 $\pm$ 23.6 <sup>c</sup>	1.52 $\pm$ 0.31 <sup>abc</sup>	198.8 $\pm$ 49.4 <sup>ab</sup>	44.5 $\pm$ 15.3 <sup>ab</sup>	222.7 $\pm$ 55.1 <sup>ac</sup>	10.5 $\pm$ 2.2 <sup>a</sup>
Summer	31.2 $\pm$ 4.7 <sup>bc</sup>	69.7 $\pm$ 23.0 <sup>c</sup>	1.47 $\pm$ 0.26 <sup>ac</sup>	217.6 $\pm$ 41.2 <sup>b</sup>	47.5 $\pm$ 14.0 <sup>ab</sup>	220.1 $\pm$ 58.2 <sup>ac</sup>	8.3 $\pm$ 1.7 <sup>b</sup>

TABLE 2. Seasonal changes in erythrocyte morphometric parameters in common carp (different letter superscripts indicate statistically significant differences, Duncan test,  $P \leq 0.05$ ,  $N = 1500$ )

Season	Long cell axis ( $\mu\text{m}$ )	Short cell axis ( $\mu\text{m}$ )	Long nucleus axis ( $\mu\text{m}$ )	Short nucleus axis ( $\mu\text{m}$ )	Cell area ( $\mu\text{m}^2$ )	Nucleus area ( $\mu\text{m}^2$ )	Elongation index	Nuclear index (%)
Autumn	9.8 $\pm$ 0.7 <sup>a</sup>	6.0 $\pm$ 0.5 <sup>a</sup>	4.3 $\pm$ 0.4 <sup>a</sup>	2.6 $\pm$ 0.1 <sup>a</sup>	45.9 $\pm$ 5.4 <sup>a</sup>	8.7 $\pm$ 0.9 <sup>a</sup>	1.65 $\pm$ 0.15 <sup>a</sup>	19 $\pm$ 2 <sup>a</sup>
Winter	10.0 $\pm$ 0.6 <sup>b</sup>	6.0 $\pm$ 0.4 <sup>a</sup>	4.4 $\pm$ 0.3 <sup>b</sup>	2.6 $\pm$ 0.1 <sup>a</sup>	47.2 $\pm$ 5.2 <sup>b</sup>	8.9 $\pm$ 0.8 <sup>b</sup>	1.68 $\pm$ 0.13 <sup>b</sup>	19 $\pm$ 2 <sup>a</sup>
Spring	10.3 $\pm$ 0.6 <sup>c</sup>	6.2 $\pm$ 0.5 <sup>b</sup>	4.4 $\pm$ 0.4 <sup>b</sup>	2.6 $\pm$ 0.1 <sup>a</sup>	50.2 $\pm$ 5.7 <sup>c</sup>	9.0 $\pm$ 0.7 <sup>c</sup>	1.66 $\pm$ 0.12 <sup>a</sup>	18 $\pm$ 2 <sup>b</sup>
Summer	10.6 $\pm$ 0.5 <sup>d</sup>	6.2 $\pm$ 0.5 <sup>b</sup>	4.5 $\pm$ 0.3 <sup>c</sup>	2.6 $\pm$ 0.0 <sup>a</sup>	51.8 $\pm$ 5.4 <sup>d</sup>	9.1 $\pm$ 0.6 <sup>d</sup>	1.71 $\pm$ 0.27 <sup>c</sup>	18 $\pm$ 3 <sup>b</sup>

TABLE 3. Seasonal changes in white blood parameters in common carp (different letter superscripts indicate statistically significant differences, Duncan test,  $P \leq 0.05$ ,  $N = 30$ )

Season	WBC ( $10^3 \cdot \mu\text{l}^{-1}$ )	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	NBT (g/l formazan)	Thrombocytes ( $10^3 \cdot \mu\text{l}^{-1}$ )
Autumn	94.8 $\pm$ 50.2 <sup>a</sup>	94.9 $\pm$ 4.6 <sup>ab</sup>	1.0 $\pm$ 1.2 <sup>a</sup>	3.6 $\pm$ 3.8 <sup>ab</sup>	0.90 $\pm$ 0.47 <sup>a</sup>	13.7 $\pm$ 9.3 <sup>ab</sup>
Winter	92.0 $\pm$ 38.8 <sup>a</sup>	96.7 $\pm$ 3.6 <sup>a</sup>	0.8 $\pm$ 1.3 <sup>a</sup>	2.4 $\pm$ 3.0 <sup>a</sup>	1.62 $\pm$ 0.63 <sup>b</sup>	15.2 $\pm$ 6.9 <sup>a</sup>
Spring	83.3 $\pm$ 36.7 <sup>a</sup>	95.0 $\pm$ 4.2 <sup>ab</sup>	1.3 $\pm$ 1.6 <sup>a</sup>	3.3 $\pm$ 2.9 <sup>ab</sup>	1.25 $\pm$ 0.60 <sup>c</sup>	13.0 $\pm$ 8.3 <sup>ab</sup>
Summer	59.8 $\pm$ 26.8 <sup>b</sup>	93.5 $\pm$ 3.9 <sup>b</sup>	1.2 $\pm$ 1.5 <sup>a</sup>	4.4 $\pm$ 2.7 <sup>b</sup>	1.06 $\pm$ 0.34 <sup>ac</sup>	9.9 $\pm$ 6.7 <sup>b</sup>

## DISCUSSION

The results of present study revealed significant seasonal changes in the values of most hematological parameters of carp under stable laboratory conditions, some of them showing distinct annual patterns. However, most values (except for slightly higher erythroblast frequency) fit within the reference ranges obtained for common carp juveniles under various environmental conditions by various authors (Witeska et al. 2016). The values of most red blood cell parameters showed minimum values in autumn and maximum in winter. Hematocrit and hemoglobin levels showed two distinct peaks (each peak Hb value took place a month earlier than Ht), while only one RBC peak occurred. The observed hematological changes were different than commonly observed under natural conditions. Most authors reported significant reduction in the values of red blood indices in winter and the increase in summer (Orun et al. 2003, Golemi et al. 2013, Seriani et al. 2013) which indicates hematological adjustment to temperature-related metabolic rate. Autumnal decreases in red blood cell parameters were observed by Santos et al. (2009) and Kohanestani et al. (2013) and they were followed by similar or lower values in winter. In the present study low values of RBC, Ht and Hb observed in autumn were followed by increase in winter which suggests that under laboratory conditions process of winter adjustment was interrupted. This was probably because water temperature did not decrease to normal winter level. Second peak of Ht and Hb observed in summer was similar as observed by other authors and was probably related to natural changes in photoperiod.

Direct measurement of erythrocytes revealed that their size and shape significantly changed: the smallest and least elongated cells with proportionally large nuclei were observed in autumn, while the largest and most elongated ones occurred in summer. Similar pattern is visible in MCV values (despite no significant differences). Size of fish erythrocytes was proved to be inversely related to metabolic rate (Maciak et al. 2011) and aerobic swimming ability (Lay and Baldwin 1999). Many authors observed the highest MCV in winter and the lowest in summer (Golemi et al. 2013, Langer et al. 2013). In our study gradual increase in erythrocyte size with time might have been related to long-term adaptation to laboratory conditions: reduced swimming activity due to limited tank space and high feed availability accompanied by stable and high level of dissolved oxygen.

Leukocyte count showed two peaks (in XII and III), and the average seasonal value of WBC gradually decreased from autumn to summer. This pattern was contrary to the data obtained under natural conditions by authors who observed WBC peak in summer (Lamkova et al. 2007, Morgan et al. 2008, Gupta et al. 2013, Kohanestani et al. 2013, Fallah et al. 2014). The NBT value reached one distinct maximum in III which coincided with one of the WBC peaks. Various authors reported different seasonal patterns of innate immune parameters in fish. Some data indicate the maximum activity of phagocytic cells in summer (Lamkova et al. 2007, Santos et al. 2009, Das et al. 2012), while another show increase in this parameter in other seasons (Morgan et al. 2008, Buchtikova et

al. 2011). Cuesta et al. (2008) reported that oxidative activity of phagocytes and other immune indices may be positively affected by melatonin and thus probably related to photoperiod.

In this study thrombocyte count showed the maximum in winter and minimum in summer, similarly as WBC and activity of phagocytes (NBT). Similarly maximum thrombocyte count was also observed by Gupta et al. (2013) and Langer et al. (2013), contrary to the findings by Orun et al. (2003) and Vigliano et al. (2014). Stosik et al. (2019) noted that thrombocytes in fish display strong defence potential which is expressed in their phagocytic activity and ability to neutralise and degrade the bacteria absorbed. There is evidence that they make up nearly a half of the population of phagocytising cells among peripheral blood phagocytes (Nagasawa et al. 2014). Kozińska et al. (1999 a, b) observed that role of thrombocytes in phagocytosis increases at low temperatures, when the activity of granulocytes and monocytes declines.

According to Bowden et al. (2007), seasonality is a complex event made up of many potential cues, among which the changes of temperature and day length are the most important. The results of present study showed significant changes in the values of most hematological parameters of common carp juveniles in annual cycle but independent of water temperature or other environmental factors most of which (except for photoperiod) were stable or varied little throughout the experimental period. Some of the changes, e.g increase in oxidative metabolic activity of phagocytes in spring and increase in hemoglobin level in summer

were similar to those that occur in fish under natural conditions which indicates the possibility of endogenous control. Therefore, the causes of these changes still are to be elucidated, with particular attention paid to photoperiod.

## CONCLUSIONS

Most of hematological values of common carp juveniles kept under stable laboratory conditions showed significant seasonal variability but they were in accordance with the reference ranges. However, variability of hematological parameters in common carp juveniles irrespective of environmental conditions makes it necessary to include reference groups sampled simultaneously with experimental groups in the laboratory experiments.

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**Streszczenie:** *Sezonowe zmiany parametrów hematologicznych u młodocianych karpia w warunkach laboratoryjnych.* Celem badań była ocena zmian wartości parametrów hematologicznych młodocianych karpia w rocznym cyklu, w stabilnych warunkach laboratoryjnych. Niektóre parametry wykazywały wyraźne rytmy zmian, np. dwa piki hematokrytu wystąpiły w II i VIII, stężenie hemoglobiny w I i VI, podczas gdy liczba erytrocytów osiągnęła maksymalny poziom w II. Największe erytrocyty zaobserwowano w VIII,

a najmniejsze w XII. Liczba leukocytów wykazała dwa piki w XII i III. Maksymalny procent limfocytów wystąpił w III, a minimalny w XI, podczas gdy odsetek neutrofilów wykazywał odwrotną prawidłowość. Aktywność metaboliczna fagocytów (NBT) osiągnęła maksimum w III, natomiast minimum wystąpiło w XI–XII i VI–VII. Liczba trombocytów była największa w XII, a najmniejsza w VII. Uzyskane wyniki wykazały, że wartości parametrów hematologicznych u karpia uległy znacznym zmianom w ciągu roku, mimo niewielkich zmian parametrów środowiskowych. Niektóre z tych zmian, np. wzrost aktywności oksydacyjnej fagocytów wiosną i wzrost poziomu hemoglobiny latem były podobne do tych, które występują u ryb w warunkach naturalnych. Inne, takie jak zwiększenie wielkości erytrocytów lub zmniejszenie liczby leukocytów sugerują długoterminowe przystosowanie do środowiska laboratoryjnego.

*Słowa kluczowe:* krew, erytrocyty, ryby, leukocyty, pora roku, trombocyty

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## Survey of consumer preferences on the bee product market. Part 1. Honey

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**Abstract:** *Survey of consumer preferences on the bee product market. Part 1. Honey.* The survey concerning the market consumer preferences of bee products was based on the internet anonymous questionnaire (517 persons). Over 95% of respondents declared honey consumption. More men than women eat honey. The quantity and frequency of honey use increases with age. As many as 70% of respondents sweeten using honey. The respondents choosing honey follow mainly the taste, individual properties of type of honeys, availability, price and appearance. Respondents the most willingly consume honey coming from Poland, and more than half of the respondents buy honey directly from a beekeeper. The most preferred are multi-flower, lime, acacia, buckwheat, honeydew and rapeseed honeys. A definitely higher percentage of respondents working in the sectors related to agriculture ate buckwheat, heather and dandelion honeys.

*Key words:* consumer preferences, honey

### INTRODUCTION

Honey, bee pollen, bee bread, bee glue, royal jelly, bee venom, and beeswax are bee products which are more often used in the human therapy (Giza 2004, Narkiewicz-Jodko 2014). It is connected to their antibacterial, cytostatic, and anti-inflammatory effect (Hołderna-Kędzia and Kędzia 2013, Hołderna-Kędzia and

Kędzia 2014, Premratanachai and Chanchao 2014). Their administration reduces the side effects of chemotherapy (Salles et al. 2014) and radiotherapy (Hołderna Kędzia and Kędzia 2015) and eliminates the problem of malnutrition during illness and convalescence (Koszowska et al. 2013). Consumption of bee products increases the body's immunity (Fratini et al. 2016).

The aim of the work was to examine consumer preferences on the bee products market. Evaluation of the knowledge of the properties of bee products and a declared willingness of their use by the respondents.

### MATERIAL AND METHODS

The research was carried out in 2018. The diagnostic poll methods was used in this work. The survey concerning the market consumer preferences of bee products was based on the Internet anonymous questionnaire. The questions concerned the respondent's living area, sex and age as well as preferences of bee products on the market. Questions concerning the bee products were divided on single and multiple choice questions. The survey was completed by 517 persons.

Among respondents 63% derived from Mazovian voivodship ( $N = 326$ ). The share of respondents from other voivodships was small and ranged from 2 to 4%. More than half of the respondents lived in big cities (100–500 thous. inhabitants) and people from cities with less than 100 thous. inhabitants were 21%. Rural areas were inhabited by 27% respondents.

There were 69% of women ( $N = 358$ ) and 31% men ( $N = 159$ ). Among respondents, the highest number of answers was given by people at the age of 18–24 (49%,  $N = 253$ ). At the age of 25–34, there were 26% of the respondents ( $N = 134$ ) and in the group from 35 to 44 years 11% ( $N = 57$ ). Definitely fewer answers were obtained from older people. In the age group above 44 years ( $N = 73$ ) there were 14% of respondents.

Most of the respondents obtained university diplomas (61%,  $N = 315$ ), 181 people completed grammar school (35%), and 21 respondents (4%) primary ones. Mostly the education or profession of the surveyed people was not related to agriculture (61%).

In the case of quantitative variables, the distribution of the variable is put in the tables containing measures of central tendency and dispersion. The significance of the correlation between variables of a nominal nature has been tested using the independence  $\chi^2$  test.

Correlations between rank and quantitative variables were checked using the Spearman's rank correlation coefficient ( $RS$ ). In the statistical analysis the significance level  $P = 0.05$  was assumed. The analyses were done using the SPSS Statistics 24.0.0 programme and the MS Office 2016.

## RESULTS AND DISCUSSION

Only 0.8% of the surveyed group people were allergic to bee products. Similar results were reported by Karakay (1999), who noticed the occurrence of allergy to honey in 0.7% of patients.

The honey consumption was declared by 95.7% of respondents. The frequency of honey consumption is shown in Figure 1. The obtained results are similar to those showed by Žak (2017) and higher than those reported by Gontarz et al. (2016) and Kowalczyk et al. (2017). Comparing the consumption of honey depending on the place of residence, it was found that when number of inhabitants was increasing the frequency of honey consumption decreased ( $RS = -0.087$ ,  $P = 0.049$ ). However, Gontarz et al. (2016), who conducted a survey among students, stated that honey was more often consumed by those who lived in a city than in the countryside. These differences may have resulted by the smaller number and the age of respondents.

Almost all men (98.1%) have consumed honey, and 94.7% among women. The difference in honey consumption depending on sex has been statistically significant ( $\chi^2 = 3.158$ ,  $P = 0.050$ ). The significant relationship between the age of respondents and the frequency of honey intake was found (Table).

Elder respondents declared honey consumption more often than young people ( $P < 0.001$ ). Along with the respondents' increase in the frequency of honey consumption the percentage of people using honey instead of sugar increased ( $P \leq 0.001$ ). Žak (2017) reports that 83% of Poles over 65 years of age consume 500 g of honey in a month.

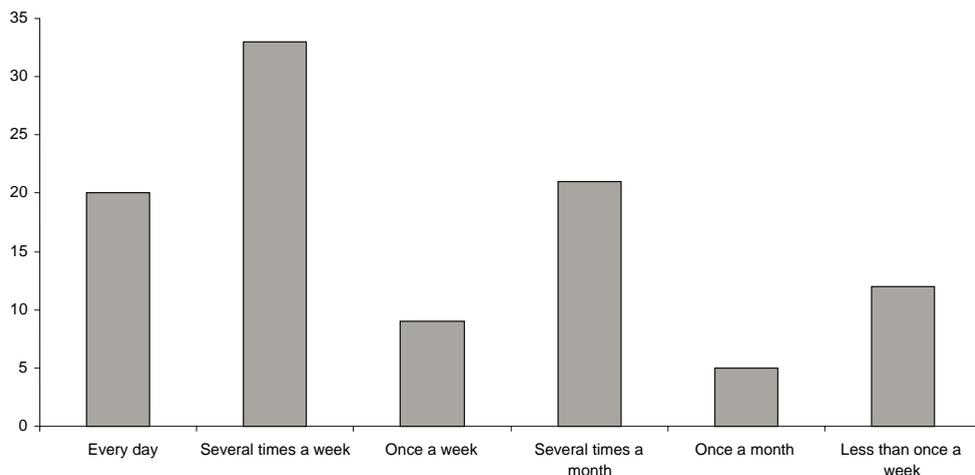


FIGURE 1. The frequency of honey consumption (%)

TABLE. The relationship between frequency of honey consumption and age

Question	Answer	Number and percentage	Age		
			18–24 years old	25–44 years old	above 44 years old
How often do you eat honey?	every day / several times a week	number	102	108	48
		horizontal (%)	39.5	41.9	18.6
		vertical (%)	43.2	57.1	71.6
	once a week / several times a month	number	78	55	12
		horizontal (%)	53.8	37.9	8.3
		vertical (%)	33.1	29.1	17.9
	once a month / less than once a month	number	56	26	7
		horizontal (%)	62.9	29.2	7.9
		vertical (%)	23.7	13.8	10.4
$RS = 0.204; P < 0.001$					

Analysing the consumer preferences of honey it was found that the respondents most often choice was multi-flower (84%) and lime (72%) honeys. Acacia (40%), buckwheat (40%), honeydew (39%) and rapeseed (31%) honeys were less readily bought (Fig. 2). Other authors confirm that multi-flower and lime honeys are the most popular among

Polish consumers (Giemza 2004, Kowalczyk et al. 2017). Żak (2017) reported that young consumers preferred bright honey, and older ones with a dark colour.

Education or work related to agriculture influenced the individual selection of types of honey ( $P \leq 0.05$ ). Definitely higher percentage of respondents whose

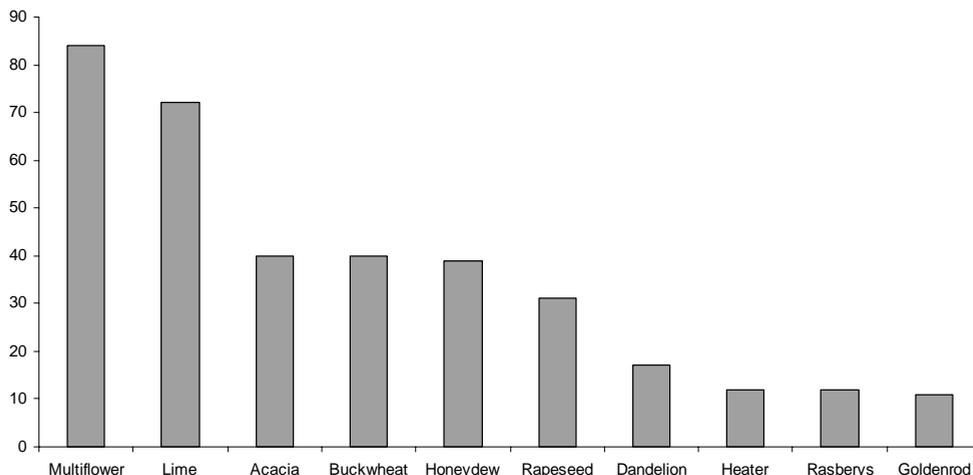


FIGURE 2. The type of honey chosen by consumers (%)

work have been related to agriculture have preferred buckwheat, heather, rape, acacia and dandelion honey.

When buying honey, the respondents pointed out: taste (72%), individual properties of types of honey (37%), availability (31%), price (31%) and appearance (29%). Among another factors determining the purchase of honey, the origin was most often mentioned. More than 58% of respondents was buying honey directly from a beekeeper, 24% on the market and 17% in the supermarket. A significantly smaller number of consumers purchased honey in beekeeping shops (7%) and with so-called “healthy food shops” (8%). Only 1.2% of respondents bought honey online. As many as 21% of respondents used honey from their own apiary. Żak (2017) reports that Poles over the age of 36 most often buy honey in the bazaar (22%) and directly from beekeepers (13%). According to Bratkowski et al. (2008) the respondents most often buy honey from a beekeeper (84.8%), valuing direct contact with the producer. How-

ever, according to studies by Kowalczyk et al. (2017) 32% of people buy honey in the bazaar and 27.4% of people in the apiary, and these are people over 45 years of age. About 20% of young Poles declare purchasing honey online (Żak 2017).

Poles definitely preferred domestic honey (89%). Only 9% of respondents chose honey from EU and non-EU Member States, while 9.8% of respondents did not pay any attention to the origin of honey.

## CONCLUSIONS

Honey is a widely used bee product, over 95% of respondents declared honey consumption.

More men than women eat honey. The quantity and frequency of honey use increases with age.

With the increase of honey frequency consumption the percentage of people using honey instead of sugar increases. As many as 70% of respondents sweeten using honey.

The respondents choosing honey follow mainly the taste, individual properties of type of honeys, availability, price and appearance.

Respondents the most willingly consume honey coming from Poland, and more than half of the respondents buy honey directly from a beekeeper.

The most preferred are multi-flower, lime, acacia, buckwheat, honeydew and rapeseed honeys.

A definitely higher percentage of respondents working in the sectors related to agriculture ate buckwheat, heather, rapeseed, acacia, and dandelion honeys. The most consumers prefer Polish honey bought in an apiary.

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**Streszczenie:** *Badanie preferencji konsumenckich na rynku produktów pszczelich, Część 1. Miód.* Celem pracy było zbadanie preferencji konsumenckich na rynku produktów pszczelich na podstawie 517 ankiet internetowych. Ponad 95% respondentów, a wśród nich więcej mężczyzn niż kobiet zadeklarowało konsumpcję miodu. Ilość i częstotliwość włączania miodu do swojej diety wzrastała wraz z wiekiem. Ponad 70% ankietowanych zastępowała cukier miodem. Wybierając

miód, większość osób kierowała się kolejno jego: smakiem, właściwościami, dostępnością, ceną i wyglądem. Konsumenci preferowali polskie miody, a połowa z nich kupowała miód bezpośrednio od pszczelarza. Największą popularnością cieszył się kolejno miód: wielokwiatowy, lipowy, akacjowy, rzepakowy oraz spadziowy. Zdecydowanie więcej respondentów pracujących w sektorach związanych z rolnictwem konsumowało miód gryczany, wrzosowy i z mniszka lekarskiego.

*Słowa kluczowe:* preferencje konsumenckie, miód

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## Influence of energy level reduction in young bulls diet on meat quality

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**Abstract:** *Influence of energy level reduction in young bulls diet on meat quality.* The influence of energy level reduction in bulls' diet at the end of the fattening period was evaluated. The bulls were divided into 2 groups, a control group (K) and a study group (D) in which the energy level limit was 80% of the maintenance requirement. The diet was altered during the last 60 days of the fattening period, however, it did not affect the level of protein, mineral compounds and vitamins intake as their balancing was in line with the animals' needs. The fattening bulls were slaughtered at the age of 25 months. The aim of the study was to analyse the impact of reduced energy levels on the meat traits which proved its culinary usefulness and nutritional value. An assumption was made that reducing energy level in the bulls' diet at the end of the fattening period would not significantly deteriorate the said indicators. D group animals had a smaller weight gain, an average of 35.1 kg. This effect, with full coverage of the protein demand, triggered energy reserves from internal fat. However, the deterioration of the quality of carcasses has not been confirmed. It was found a higher percentage of carcasses in EUROP classes better. The *Longissimus lumborum* muscles had a lower content of dry matter and fat in ( $P < 0.05$ ). There were no significant changes in the colour (L) and acidity of the meat. There were, however differences in thermal loss. The meat of group K had significantly higher results ( $P < 0.05$ ). From a practical point of view, these changes should be considered minor. What is important is the fact

that there was no significant effect on the characteristics of the most frequently subject to consumer evaluation. This applies to colour (L) and marbling, which were at an acceptable level. Not without significance is the lack of deterioration in nutritional quality, for evaluation the participation of protein and fat was assumed. Meat of group D had a comparable protein content and lower fat content. It was shown that the used solution did not worsen the culinary traits of the meat; the crispness and surface of the roast beef.

*Key words:* fattening, cattle, energy level, quality, beef

### INTRODUCTION

The beginning of the 21st century witnessed an increase in the production of beef both in Poland and abroad. The data published by the GUS (2016) showed an increase in beef production from 599 to 918 thous. tons in 2005 and 2015 respectively.

The increased beef production more and more frequently calls for the search for solutions to raise the efficiency of fattening, at the same time maintaining high quality of the meat. Studies indicate that, among environmental factors, the greatest impact on meat quality is

exerted by animal nutrition, followed by the maintenance system and age.

Recent research has shown multiple opportunities to improve the culinary properties of beef by employing proper animal nutrition (Kuczaj 2010, Maciaszek and Strzetelski 2006). The energy value of the ration applied throughout the entire fattening period is deemed to constitute a highly significant factor in the nutrition of fattening cattle (Jurczak 2004, Kuczaj 2010).

Also, the growing awareness of consumers who are starting to appreciate meat of animals fed less intensively as well as from farms where balanced feeding systems are applied, is an important aspect of the search for solutions aimed at increasing the effectiveness of beef production. Decreasing the amount of energy that is indispensable to produce a kilogram of beef contributes to lowering the so-called carbon footprint (Rotz et al. 2010, IPCC 2013). Worldwide trends of evolving consumer demands and minimising the impact of production on the environment make the search for new beef production solutions still very much valid.

The purpose of the present study was to determine the impact of lowered energy levels of the feed ration in the final phase of the fattening of young bulls on the quality of meat.

## MATERIAL AND METHODS

### Material and feeding

The study group consisted of 26 bulls – crossbred hybrids produced by mating Polish Holstein-Friesian cows with Limousine bulls. The age at slaughter was 25 months.

After the rearing period the young bulls were kept in confinement, provided with fodder twice a day, and had constant access to water by means of automatic water tanks and mineral licks. The nutrition of animals in individual groups was as follows:

- control group (K – 13 bulls) – the bulls intended for slaughter were fed with a ration that was balanced in terms of protein and energy;
- study group (D – 13 bulls) – in the last 60 days before slaughter, the bulls' energy consumption level was reduced to 80% of the maintenance requirement.

The nutritional basis was maize silage and grass silage (*Lolium perenne* L., *Medicago sativa* L. ssp. *Sativa*; harvested during the flowering). The ration of roughage was supplemented with concentrated fodder (barley, triticale, post-extraction rape meal, soybean). In the case of the study group, the reduction of energy level in the final stage of fattening was obtained by decreasing the amount of cereal and maize silage in the ration, with simultaneous increase in the amount of high protein fodder (mainly soy meal) and fodder containing high levels of rumen protected proteins. The animals were fed ad libitum; twice daily (morning and afternoon). The diets were formulated according to the maintenance requirement of animals specified by French National Institute for Agricultural Research (INRA) feeding standards with the use of INRAration Software for Ruminant Diet Calculation, version 2.03 (DJ Group, Krakow, Poland), drawing on the earlier chemical analysis of feeds and calculating their nutritional value. Maintenance requirement was predicted

by the equation: protein (BTJ) =  $3,25 \text{ g} \times (\text{MC})^{0,75}$ ; energy (EN) =  $88 \text{ kcal} \times (\text{MC})^{0,75}$ .

### Study methods

The analyses were performed using samples of *M. Longissimus lumborum* (*MLL*) taken from the carcasses of both bull groups. In order to diagnose the typical post-mortem transformations, pH level was measured after 45 minutes (pH<sub>45</sub>) as well as following 24 and 48 hours (pH<sub>24</sub> and pH<sub>48</sub>) after slaughter. The acidity measurement was carried out in the raw meat by means of a glass electrode meter, using pH-meter. After 24-hour storage at 4°C, the *MLL* sample was subject to visual assessment with respect to marbling, and contours of the roast beef area were set and evaluated by planimetry. Meat marbling was assessed using a subjective 5-point scale (1 point – no visible presence of fat, 5 points – rich and even distribution of fat).

Meat sections from the *MLL* muscle were collected, packed in PE foil and stored under refrigeration conditions at a temperature of  $4.0 \pm 0.5^\circ\text{C}$ . The following parameters were assessed seven days after slaughter:

- basic chemical composition (water content by drying method according to PN-ISO 1442: 2000P, general protein content by Kjeldahl method according to PN-A-04018: 1975, fat content by Soxhlet method according to PN-ISO 1444: 2000);
- meat colour was evaluated with the Minolta CR-310 Chroma Meter, by Joo et al. method (2000). Standardisation of the apparatus was carried out using the Minolta CR 310 model plate

with the value  $Y = 92.80$ ,  $x = 0.3175$  and  $y = 0.3333$ . The colour parameters were described in the CIE Lab system (CIE, 1986), using D65 illuminant and a standard 2° observer;

- tenderness of heated meat (internal temperature + 70°C) using ISTRON 3342 apparatus with Warner-Bratzler blade ( $N/\text{cm}^2$  – measurement taken from  $1 \times 1 \text{ cm}$  samples),
- thermal loss, as a percentage loss of meat weight while heating in tightly sealed foil bags in a water bath until the internal temperature of 75°C is achieved;
- water holding capacity (WHC) using the Grau and Hamm method (1957), infiltrations were evaluated by planimetry, and the result was converted into a loss of water from the sample (%).

### Statistical analysis

The obtained results were subject to mathematical analysis using one-way analysis of variance ( $P < 0.05$ ) with the Statistica 12.5 programme. The results were shown in the form of the mean ( $x$ ) and standard deviation ( $SD$ ).

## RESULTS AND DISCUSSION

Carcasses of animals from group D were more often classified as better EUROP classes. This is evidenced by the proportions of carcasses belonging to the extreme EUROP grades found after slaughter. Group of animals with reduced a (60 days before slaughter) supply of energy to 80% of living needs, had less fatness of carcasses and 38.3% less amount of internal fat and 38.3% lower amount of internal fat. This may

explain an average of about 35.1 kg less mass of slaughter (Table 1). In the *M. longissimus lumborum* (MLL) sampled in the study group (D), pH45 amounted to an average of 6.71, compared to the level of 6.69 in the control group (K) (Table 2). Twenty four hours after slaughter the obtained pH value was 5.74 and 5.70 in the study and control group respectively. The reduction of energy level in the final phase of the fattening period did not have any influence on the pH level

evaluated 48 hours post mortem. The obtained pH48 values in the MLL were similar in both groups (5.62 and 5.67).

As far as acidity was concerned, the results of the own research were consistent with the outcomes of the research conducted by Domaradzki et al. (2011), which did not show the effect of refrigeration storage on pH values. In view thereof, the studied decrease in energy supply in the final phase of fattening did not affect the glycogen levels, whose

TABLE 1. Characteristics body mass of the bulls in the subsequent stages of the experiment and selected carcass quality indicators

Specification	Feeding group				P-value
	D		K		
	X	SD	X	SD	
	(N = 13)		(N = 13)		
The weight:					
– start of fattening (kg)	323.48	32.83	329.41	29.72	0.829
– before changing the ration (kg)	492.21	48.25	487.29	45.59	0.621
– mass of slaughter (kg)	511.65	42.25	541.79	51.92	0.358
Internal fat* (kg)	9.78	2.22	13.53	2.98	0.042
Carcass conformation score** : U (%)	40.0		20.0		0.045
O (%)	13.3		33.3		
Carcass fatness score** : 1 (%)	38.5		15.4		0.038
4 (%)	23.1		53.8		

\*types of internal fat: kidney fat, caul fat, gut fat; \*\*extreme EUROP class obtained during the evaluation

TABLE 2. The effect of nutrition on the formation of pH in *M. longissimus lumborum*

pH after slaughter	Feeding group				P-value
	D		K		
	X	SD	X	SD	
	(N = 13)		(N = 13)		
45'	6.71	0.11	6.69	0.07	0.592
24h	5.74	0.12	5.70	0.13	0.632
48h	5.62	0.08	5.67	0.09	0.572

breakdown is usually responsible for meat acidity after slaughter (Immonen et al. 2000, Mlynek et al. 2012). The results of  $\text{pH}_{48}$  found in our own research corresponded to the values of this indicator observed by Florek et al. (2007) as well as Daszkiewicz et al. (2009). These studies showed that in the case of culinary use of beef, the pH value after 48 hours from slaughter should amount to 5.8. The final pH values obtained in our study did not indicate any changes suggestive of deviations in meat quality.

The results pertaining to the content of basic chemical components discussed in Table 3 showed that the *MLL* of the young bulls from group D exhibited a lower content of dry matter, compared to the meat of bulls from group K, by an average level of 0.8% ( $P \leq 0.05$ ). This is undoubtedly due to the lower fat content, by 0.73% ( $P < 0.05$ ), which was seen in the meat of the young bulls from group D (0.98% compared to 1.71% in group K). The use of energy from fat reserves in animals from group D, not

only decreased the amount of internal fat (Table 1). It reduced the intramuscular fat content, but also to a small extent on the loss of muscle protein (Table 3). However, if the difference in protein content of the *MLL* not confirmed the statistically significant ( $P < 0.325$ ). In view of the pro-health and dietary value of meat, the fat content obtained in group D should be deemed to be beneficial. Grębowiec (2015) showed the significance of fat content as a factor that consumers bear in mind when choosing meat to buy. The researcher proved that the meat with more than 2.5% fat content is considered unattractive. However, too small proportion of fat may turn out to be insufficient to ensure satisfactory meat juiciness or aroma (Keane and Allen 1998).

In comparison with the outcomes of the present study, the results obtained by Chmielnik et al. (2005) showed a slightly lower share of dry matter and protein and at the same time a higher fat content in *M. longissimus dorsi*. A similar tendency was observed by Wajda et al. (2014),

TABLE 3. The formation of selected traits and colour of *M. longissimus lumborum*

Specification	Feeding group				P-value
	D		K		
	X	SD	X	SD	
	(N = 13)		(N = 13)		
Traits:					
– dry matter (%)	24.21	1.08	25.01	1.11	0.048
– protein (%)	22.95	0.91	23.02	0.97	0.325
– fat (%)	0.98	0.22	1.71	0.48	0.015
Colour:					
L	38.54	1.91	38.48	1.99	0.859
a	18.39	1.09	19.91	1.23	0.012
b	4.16	2.65	4.26	2.79	0.722

however, it should be added that the study animals were fed with a balanced ration. The influence of feeding intensity on beef quality traits was demonstrated, among others, by Vestergaard et al. (2000) and Młynek et al. (2014). Domaradzki et al. (2016) proved that intensive fattening caused fatter carcass, whereas elevating the energy value of the ration at the end of the fattening period contributed to increased meat marbling.

The obtained 4 points results (Table 4) showed that the energy limit in the final phase of fattening (group D) favoured lower accumulation of fat in *MLL* and decreased marbling. The difference in marbling found in the study and control groups amounted to an average of 0.34 points ( $P < 0.05$ ). Similar findings related to the correlation between fat content and meat marbling were also obtained in studies of Dasiewicz et al. (2002). In this respect, the results of our research were consistent with the tendency observed by Młynek (2011), with the average marbling value of 2. The studies conducted by Póltorak et al. (2013) showed that obtaining the content of fat in *m. longissimus dorsi* at the level

of 1.1–1.3%, with fat content in the carcass of 2 to 3%, allowed obtaining the optimal degree of meat marbling. These findings are extremely significant since intramuscular fat constitutes an important factor affecting the taste and juiciness of culinary meat (Domaradzki et al. 2016), and its excessive amount may hinder the nutritional value of meat. In spite of the effect of limited energy level on meat marbling shown in Table 4, the obtained small values of the said assessment seemed to have little influence on consumer evaluation. Although, the difference (0.34 points) found was confirmed statistically ( $P < 0.05$ ), in practice its significance may be of minor importance on the consumer assessment. The performed analysis of the indicators for meat colour assessment (Table 3) did not show any influence of the group on colour clarity (L), which was however observed in the case of yellow colour intensity (a) ( $P < 0.05$ ). Our own findings were partly consistent with the results of the study conducted by Młynek (2011) which showed the effect of feeding intensity on the clarity of the colour of bulls' meat. The lack of such differences may prove

TABLE 4. Effect of feeding on the formation of selected parameters of the quality of *M. longissimus lumborum*

Traits	Feeding group				P-value
	D		K		
	X	SD	X	SD	
	(N = 13)		(N = 13)		
Roast beef area (cm <sup>2</sup> )	101.28	12.27	108.02	17.07	0.089
Tenderness (N/cm <sup>2</sup> )	56.28	16.05	57.79	16.98	0.215
Thermal loss (%)	26.97	3.12	30.18	2.19	0.034
WHC (%)	32.87	3.58	34.61	2.98	0.196
Marbling (pts)	1.89	0.63	2.23	0.48	0.028

the reduction of the energy level in the final period of fattening to be a favourable solution due to lower consumption of high energy fodder. Similar results were obtained by Wajda et al. (2014), where feeding intensity exerted a slight influence on the colour of bulls meat.

The data contained in Table 4 showed that *MLL* specimens from group D showed smaller weight loss, with an average of 3.21% ( $P < 0.05$ ). This constituted valuable information most of all in terms of the culinary importance of this trait and consumer assessment. Similar thermal loss values, at an average level of 30.01%, were obtained by Domaradzki et al. (2011), whereas significantly higher values, at a level of 34.32%, were demonstrated by Chavez et al. (2012). However, the study conducted by Chmielnik et al. (2005) did not confirm the observed trends since the meat of animals whose dietary energy levels were decreased at the end of the fattening period showed a higher loss during heating.

Another characteristic trait of *MLL* that ought to be considered as significant in technological terms was WHC. However, it should be noted that the applied nutritional model did not have a significant influence on WHC values since the obtained difference was 1.74% and was not confirmed statistically. Nevertheless, the meat obtained from group D bulls showed a higher value of this indicator. In practice, this may have a significant effect on the efficiency of meat processing and storage. Similar findings were obtained by Daszkiewicz et al. (2005) where this was also connected with a higher loss of meat juice, thus a more intense loss of water-soluble ingredi-

ents. The authors of the said study also demonstrated the resulting worsening of sensory properties. Lower WHC values of the meat of Polish Holstein-Friesian bulls, at an average level of 30.00%, were observed by Litwińczuk et al. (2006). However, in this case the trait in question showed significant variability, which may be proved by the obtained value of  $SD = 7.59$ .

As far as the commercial value of carcasses is concerned, an important indicator of the assessment was the share of valuable parts of the carcass, including roast beef. According to Wajda et al. (2014), morphological analysis and the weight of primal cut may constitute a good basis for carcass pricing and beef quality. In the present study, a correlation between bulls' diet and the cross-section area of roast beef was not established. This finding should be regarded as positive since, in practical terms, the costs of fattening can be lowered with no reduction of the quality of this trait.

In the case of beef, the traits affecting its tenderness are of great importance. According to Kołczak (2008) and Młynek et al. (2014), tenderness is one of the most significant features of culinary assessment. Meat tenderness is, among others, conditional upon animal nutrition (Domaradzki et al. 2010) and muscle microstructure (Młynek et al. 2014). The research conducted by Młynek (2014) involving the *biceps brachii* muscle showed that this was primarily connected with the area of muscle fibres. In the said studies the cutting value was lower by an average of 12.4 N/cm<sup>2</sup> in the case of muscles of the bulls which were subject to more intense feeding, with lower values of the area of muscle fibres. The

results contained in Table 4 didn't show any significant influence of animal nutrition on meat tenderness. The difference between the study group and the control group was 1.51 N/cm<sup>2</sup>, on average.

## CONCLUSIONS

Reduction of energy level in the feeding ration during the last 60 days of fattening of young bulls decreased the fat content in meat, thereby leading to a decreased concentration of dry matter as well as slightly lower marbling and thermal loss. It is of key importance that no significant influence on the acidity and clarity of the colour of the meat, or on the protein content, tenderness and the area of roast beef was observed. The obtained results suggest that the reduction of the energy level at the end of the fattening period may be used in the production of culinary beef.

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**Streszczenie:** *Wpływ obniżenia poziomu energii w dawce pokarmowej buhajów na jakość mięsa.* Badano wpływ wprowadzenia pod koniec opasu, okresu żywienia z obniżonym poziomem energii w dawce pokarmowej. W doświadczeniu wydzielono grupę kontrolną (K) oraz grupę, w której ograniczenie poziomu energii wynosiło 80% zapotrzebowania bytowego (D). Zmianę diety stosowano w ostatnich 60 dniach opasu i nie obejmowała ona poziomu białka, związków mineralnych i witamin. Ich bilansowanie było zgodne z zapotrzebowaniem zwierząt. Opasy ubijano w wieku 25 miesięcy. Celem badań było przeanalizowanie wpływu ograniczenia poziomu energii na cechy mięsa, świadczące o jego przydatności kulinarnej i jakości żywieniowej. Założono hipotezę, że ograniczenie podaży energii w diecie pod koniec opasu, nie wpłynie znacząco na pogorszenie analizowanych wyróżników jakościowych. Opasy z grupy D uzyskały mniejszy przyrost masy ciała, przeciętnie o 35,1 kg. Efekt ten, przy pełnym pokryciu potrzeb białkowych, powodował uruchomienie rezerw energii z tłuszczu wewnętrznego. Nie stwierdzono jednak pogorszenia się jakości tusz. Uzyskano większy odsetek tusz zaliczanych do lepszych klas EUROP. Mięśnie *M. longissimus lumborum* miały mniejszą zawartość suchej masy i tłuszczu w ( $P < 0.05$ ). Nie odnotowano zasadniczych zmian jasności barwy (L) oraz kwasowości mięsa. Odnotowano natomiast różnice dotyczące wycieku cieplnego, który w mięsie grupy K okazał się istotnie większy ( $P < 0.05$ ). Jednak z praktycznego punktu widzenia, zmiany te na-

leży uznać za niewielkie. Istotny jest fakt, że nie odnotowano znaczącego wpływu na cechy podlegające najczęściej ocenie konsumenckiej. Mianowicie barwy i marmurkowości, które kształtowały się na akceptowalnym poziomie. Nie bez znaczenia pozostaje brak pogorszenia się jakości żywieniowej, za której wyznaczniki przyjęto udział białka i tłuszczu. Bowiem mięso buhajów z grupy D miało porównywalną zawartość białka oraz mniejszą zawartość tłuszczu. Stwierdzono również, że zastosowane rozwiązanie nie pogorszyło cech kulinarnych mięsa – kruchości i powierzchni rostbefu. Można uznać, że przyjęte działanie może być z pozytywnym efektem wykorzystywane do produkcji kulinarnego mięsa wołowego.

*Słowa kluczowe:* opas, bydło, poziom energii, jakość, wołowina

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## **The effect of the brewing and milling by-products containing in the lambs diet on body weight growth, slaughter value and meat quality**

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**Abstract:** *The effect of the brewing and milling by-products containing in the lambs diet on body weight growth, slaughter value and meat quality.* The aim of the study was to evaluate the growth rate, slaughter value and meat quality characteristics of lambs fed of fresh brewer's grain and by-product of the milling industry. Research was conducted on 45 Polish merino ram lambs, which were divided into three feeding groups and fattened to their slaughter weight of 40 kg ( $\pm 2.0$  kg). Lambs in control group were fed meadow hay, oat meal and steamed potatoes. In one of experimental group (WBG group) 35% of wet brewer's grain was added to the feed. The second experimental group (BPM group) received 44% of by-product of the milling industry in the form of crushed grains of various cereal species. The obtained results indicated that the by-products of the brewing and milling industry used in nutrition on fattened lambs did not negatively affect the body weight growth and slaughter value. The application of these components also did not affect the quality of meat in respect to its chemical composition and physical characteristics. The brewer's grain share in the ration increased the content important for human health monounsaturated and polyunsaturated fatty acids and the isomer C18:2 *cis*9, *trans*11 (CLA).

**Key words:** lambs, by-products, slaughter value, meat quality

### INTRODUCTION

The high quality and health benefits of lamb meat, which are increasingly perceived by consumers are a challenge for producers of slaughter lambs. Maintaining this niche type of meat on the market requires both a reduction in the production costs without reducing its quality. In order to increase the efficiency of fattening, producers of lamb meat are looking for less expensive fodder, because expenses related to animal nutrition may amount to 60–70% of the total costs incurred (Korman 2001). The one of the cheapest feeds that can be used in animal nutrition are the by-products of the agri-food industry. However, the condition for use of these feeds is a short transport and ensuring the appropriate value, especially microbiological (Ben-Hamed 2011).

The by-products of distillers, millers, sugar and oil industry are successfully used in nutrition not only ruminant but also monogastric animals (Hetherington and Krebs 2002, Aguilera-Soto et al.

2008, Essien and Udotong 2008, Homm et al. 2008, Grzeškowiak et al. 2010, Borys et al. 2013, Borzuta et al. 2014, Peña and Posadas 2016). However, there is concern if the use of such components will not reduce the slaughter value and does not negatively affect the quality of meat. Many studies have confirmed the positive effect of both fresh and dried brewers, providing high quality protein (21–33%), on the increase of body weight gain and the quality of lamb meat (Anigbogu 2003, Mussatto et al. 2006, Aguilera-Soto et al. 2007). Similarly, the lack of a decrease in the growth rate and a beneficial effect on the content of bioactive components such as polyunsaturated n-3, C18:2 *cis*9, *trans*11 (CLA) and the ratio n-6 / n-3 were registered using rapeseed and sunflower cake in the diet (Grzeškowiak et al. 2010).

In sheep nutrition, the by-products from the milling industry are also commonly used. Wheat bran is the most popular. Their availability and low cost can affect fattening economics, especially in a more extensive way and to higher weight standards (Borys 2011). A very valuable but not very popular by-product of the milling industry is bread. It contains about 70% easily digestible starch and about 13% of protein. This product belongs to the energetic feed material and according to Afzalzadeh et al. (2007) should not exceed a 25% of the feed ration. Although, Hetherington and Krebs (2002) using a 50% share of this product in the diet of fattened merino lambs obtained daily gain comparable to the control group.

The dynamic development of bio-fuel production has created the possibility of using by-products in the form the

distillers grain solubles (DGS) in animal nutrition. In sheep nutrition, a corn pulp is particularly useful, which can replace soybean meal. It has a significant amount of protein and fat, although a small dry matter content (Borys 2011). Nevertheless, studies conducted on lambs showed that even a large proportion of distillers in concentrate, up to 60%, had no negative effect on fattening characteristics and carcass quality (Estrada-Angulo et al. 2008, Van-Emon et al. 2008).

The aim of the present study was to determine the effect of the use of fresh brewer's grain and by-product of the milling industry in the form of crushed grains of various cereal species in the diet of fattened lambs on the growth rate, slaughter value and quality characteristics of the meat.

## MATERIAL AND METHODS

The research was carried out on 45 ram lambs of polish merino breed. The weight-balanced lambs were divided into 3 feeding groups (15 animals in each): control group, experimental with the proportion of brewers' grain in the diet (WBG group), experimental with the by-product of the milling industry (BPM group). After 14-days adaptation period, the lambs were fattened to achieve slaughter weight of 40 kg ( $\pm 2.0$ kg).

Ethical approval for the experiment was obtained from the Local Ethics Commission in Warsaw. The animals were maintained under uniform environmental conditions with constant zootechnical and veterinary supervision. The lambs were fed in groups according to the standards for fattened lambs up to a body weight of 30–40 kg (Osikowski

et al. 1998). In the control group lambs received meadow hay 28%, oat meal 32%, steamed potatoes 39% and mineral mixture 1%. In the experimental WBG group a 35% share of fresh brewer's grain (WBG) was used in the ration and 18% meadow hay, 22% oat meal, 24% of steamed potatoes and 1% mineral mixture. In BPM group, oat meal was replaced by a by-product of the milling industry in the form of crushed grains of various cereal species 44%, meadow hay accounted for 25%, steamed potatoes 30%, and mineral mixture 1%. The chemical composition and nutritional value of fodder are presented in Table 1. The animals were fed twice a day, and they had constant access to water.

In order to analyze the growth rate of lambs, the control weighing was carried out: at the beginning of fattening after adaptation period, than every 14 days and on the day of slaughter. On this basis the daily gain (g/day) and total body weight gain (kg) for fattening period for each group were estimated.

After reaching assumed body weight lambs were slaughtered in a slaughterhouse according to accepted procedures.

The slaughter value evaluation of carcasses was carried out after 24 h cooling at 4°C in the hanging position.

The carcasses were weighed and the hind leg was measured according to method given by Niżnikowski (1988). Then, the carcass was divided into two halves. The right half-carcasses were cut into joints, which were weighed and the percentage of each in half-carcass was calculated.

The measurements of width (cm), depth (cm) and area (cm<sup>2</sup>) of *longissimus dorsi* muscle (LD) as well as fat thickness over LD muscle (mm) were also performed.

From the lumbar region of each carcass the LD muscle was sampled in order to determine the quality traits of the meat. Than the samples were vacuum packed and stored at -22°C (about 30 days) until chemical analysis.

The meat pH was measured 24 h after slaughter using Elmetron CP-411 pH-meter with dagger electrode calibrated at 4.0, 7.0 and 9.0 pH values.

The expressed juice was determined according to Grau and Hamm (1953) method. The meat color was measured

TABLE 1. The chemical composition and nutritional value of feeds used in lambs fattening

Specification	Grass hay	Oat meal	Steam potatoes	WBG	BPM
Dry matter g·kg <sup>-1</sup>	870.4	873.5	192.8	260.9	851.0
Crude protein g·kg <sup>-1</sup> DM	129.8	97.0	115.0	255.0	130.2
Ether extract g·kg <sup>-1</sup> DM	22.0	34.0	3.0	59.0	18.9
Crude fiber g·kg <sup>-1</sup> DM	317.0	120.0	37.0	147.0	54.6
Ash g·kg <sup>-1</sup> DM	36.5	27.4	52.9	37.2	27.9
EN (MJ/1kg DM)	3.72	6.82	8.87	4.75	7.81

EN – net energy; MJ – megajoule; DM – dry matter; WBG – wet brewer's grain; BPM – by-products of the milling industry.

24 h after slaughter on the LD muscle surface by Konica-Minolta CR-410 device specifying lightness –  $L^*$ , redness –  $a^*$  and yellowness –  $b^*$ .

The chemical composition and nutritional value of lambs fodder were analyzed according to AOAC standard methods (1990). The basic chemical composition of LD muscle was determined by analyzing the contents of moisture, crude protein, intramuscular fat and collagen using a spectrometric technique with near-infrared transmission (NIR) method (PN-A-82109). The meat samples (200 g) were homogenized in an Elektrolux DITO K35 processor. Afterwards unified samples were placed in a measuring cell of FoodScan analyzer. The device uses the near-infrared transmission method within 850–1050 nm range and is fitted with ANN calibration developed using a model of artificial neural networks. The analysis is performed by indicating in the computer program the number of measurements in the sample, and then the program automatically calculates the average and presents the result.

The LD muscle samples were analyzed for fatty acids contents. The lipids from the muscle were extracted according to Folch et al. (1957). Saponification of fat made in 0.5 M KOH in methanol and esterification in 10%  $\text{BF}_3$  in methanol. The fatty acid methyl esters extracted in the hexane.

Fatty acid profile of lipids was performed by gas-chromatograph analysis using Agilent Technologies GC 6890 N instrument equipped with capillary column BPx70 (length 60 m, internal diameter 0.22 mm, film thickness 0.25  $\mu\text{m}$ ). Operation conditions were:

helium gas (41 psi); a FID detector at 240°C. The temperature programme was: 3 min at 130°C, an increase to 235°C by 2°C/min; 4 min at 235°C.

The fatty acids were identified via reference material BCR 163 (Beef/Pig Fat Blend). The isomer linoleic acid (CLA) was determined by standard *cis9*, *trans11* octadecadienoic acid-Larodon AB, Sweden.

Statistical analyses of the data obtained was performed using the SPSS 23.0 packet software (2016), based on a linear model that included the effect of treatment group. In analysis of slaughter value the life body weight at slaughter was included to the model as a covariate. All effects were tested against residual middle-squares to determine the level of significance.

The results are presented as the means of least squares for each trait (*LSM*) and standard deviation (*SD*).

## RESULTS AND DISCUSSION

Analysis of the development of body weight showed no differences between the lambs from the control group, the group fed with WBG and the group in which the by-product of the BPM mill industry was used, both in terms of growth rate and fattening time (Table 2). The fattening in all groups lasted 76 days, and the lambs in this period reached almost the same weight. Similarly, Aguilera-Soto et al. (2008) did not register differences in the growth rate between the control group fed without WBG and experimental groups using different levels of supplementation at Rambouillet  $\times$  Pelibuey ewes, although the growth rate during 90 days of fattening was higher (216 g)

compared to the present study. In a study conducted by Hetherington and Krebs (2002) the application in Merino ewes diet 25 and 50% share of waste bakery also had no effect on lowering daily gains. In turn, an increase in the growth rate of lambs fed with the participation of dried brewers' grain (DBG) expressed in daily gains and final body weight was obtained by Anigbogu (2003) and Moges et al. (2008). Better results of final body weight and faster growth of heifers fed by WBG were also obtained by Homm et al. (2008).

The use of WBG and BPM by-products in the diet of tested lambs did not affect the linear measurements made on the hind leg and *longissimus dorsi* muscle (Table 3). Slightly higher value of

the hind leg tightness index and a larger area of LD muscle were registered in the BPM group in comparison to other groups and a slightly higher fat thickness measured over LD in the WBG group, but the differences were not statistically significant. The better muscling parameters of the lumbar part both in width and depth of LD muscle was reported by Radzik-Rant et al. (2018) in previous studies conducted on lowland lambs fed with fresh brewers' grain. In turn, Homm et al. (2008) on the carcasses heifers recorded a reduction in the area of the *longissimus* muscle and increased fatness along with an increase in the level of WBG in the diet.

The results of the analysis of slaughter traits and the share of cuts of the

TABLE 2. The fattening performance of lambs from examined groups

Item	Control group		WBG group		BPM group	
	LSM	SD	LSM	SD	LSM	SD
Initial body weight (kg)	27.76	3.32	28.06	3.61	27.88	3.13
Final body weight (kg)	39.36	2.90	39.84	3.80	39.70	3.96
Fattening period (days)	76.50	7.23	76.50	7.06	76.50	7.21
Average daily gain (g/day)	155.34	23.03	152.63	40.95	156.25	47.96
Total body weight gain (kg)	11.81	1.75	11.60	3.11	11.88	3.65

TABLE 3. The hind leg and LD muscle measurements traits

Item	Control group		WBG group		BPM group	
	LSM	SD	LSM	SD	LSM	SD
Hind leg length (cm)	23.33	1.18	23.40	1.06	23.17	1.50
Hind leg perimeter (cm)	38.63	1.94	38.93	2.24	39.37	1.48
Hind leg tightness index (%)	166.05	12.96	166.61	11.02	170.66	13.60
Fat thickness over LD muscle (mm)	1.14	0.33	1.19	0.36	1.15	0.41
Width of LD muscle (cm)	6.02	0.43	5.97	0.35	5.90	0.38
Depth of LD muscle (cm)	2.49	0.34	2.55	0.42	2.54	0.34
LD muscle area (cm <sup>2</sup> )	13.86	2.03	13.89	2.20	13.99	1.97

Hind leg tightness index = (hind leg perimeter/hind leg length) × 100%

tested lamb carcasses are presented in Table 4. The dressing percentage in all groups was at a similar level, although the highest value exceeding 42% was achieved by lambs from the BPM group. The slaughter analysis also did not show differences in relation to the weight and share of individual cuts in lamb carcasses from the control group and experimental groups, except for the rack and hind legs. The content of the rack in the carcass from WBG group was smaller ( $P \leq 0.01$ ) in comparison to the BPM group, and the percentage of hind leg in

this group was greater ( $P \leq 0.01$ ) than in the control group and also higher than in the BPM group, although not statistically confirmed (Table 4). The share of valuable cuts remained at the same level in all groups. Thus, it can be concluded that used in fattening by-products of the distillery and milling industry did not have negative impact on obtaining the most desirable commercially parts of carcasses.

The pH of the meat, expressed juice and meat color showed no significant differences between the analyzed groups

TABLE 4. Slaughter and carcass traits

Item	Control group		WBG group		BPM group		
	<i>LSM</i>	<i>SD</i>	<i>LSM</i>	<i>SD</i>	<i>LSM</i>	<i>SD</i>	
Cold carcass weight (kg)	16.88	1.22	16.83	1.55	17.19	1.29	
Cold dressing yield (%)	41.70	2.60	41.62	3.24	42.18	2.65	
Kidney with fat	(kg)	0.16	0.04	0.14	0.03	0.16	0.03
	(%)	1.89	0.38	1.67	0.38	1.90	0.34
Neck	(kg)	0.71	0.17	0.71	0.13	0.75	0.13
	(%)	8.48	2.03	8.65	1.02	8.66	1.68
Middle neck	(kg)	0.57	0.07	0.53	0.08	0.56	0.05
	(%)	6.76	0.53	6.33	0.81	6.49	0.50
Rib and flank	(kg)	1.35	0.14	1.34	0.12	1.38	0.13
	(%)	16.15	0.75	16.05	0.67	16.07	1.17
Shoulder	(kg)	1.34	0.09	1.32	0.15	1.34	0.10
	(%)	15.70	0.62	15.74	0.60	15.63	0.61
Rack	(kg)	0.50	0.06	0.47	0.08	0.52	0.07
	(%)	5.88 <sup>B</sup>	0.41	5.54 <sup>A</sup>	0.59	6.06 <sup>A</sup>	0.53
Loin	(kg)	0.60	0.11	0.58	0.09	0.64	0.11
	(%)	7.10	1.02	6.91	0.63	7.42	0.85
Hind leg	(kg)	2.31	0.18	2.40	0.24	2.41	0.20
	(%)	27.52 <sup>A</sup>	1.31	28.67 <sup>A</sup>	1.00	28.08 <sup>B</sup>	1.08
Valuable cuts	(kg)	3.40	0.30	3.45	0.39	3.57	0.35
	(%)	40.51	1.34	41.13	1.36	41.55	1.56

Within a row, means denoted with same letters are statistically different a, b  $P \leq 0.05$ ; A, B  $P \leq 0.01$ .

of lambs. The pH value of the meat was insignificantly lower in the control group. The meat from lambs fed with WBG was characterized by poorer expressed juice and was slightly lighter in color, but the differences were not confirmed statistically (Table 5). Likewise, no difference in the value of the  $L^*$  parameter in beef meat derived from animals fed with different amounts of both fresh and dry distillery grains was registered by Roeber et al. (2005). The differences in the research of these authors concerned the  $a^*$  and  $b^*$  parameters, which were not observed in present studies.

Analysis of the chemical composition of the meat of the studied groups of lambs showed no differences in the content of the tested parameters (Table 6). The content of protein, collagen and water was very similar in all groups, only the share of intramuscular

fat was higher in meat of lambs from the WBG group, especially when compared to the BPM, but the differences were not confirmed statistically (Table 6). Similarly, Shand et al. (1998) did not observe differences in the content of basic ingredients in beef meat between the control group and a group of steers in which WBG diet was used. In other studies, the 15% share of maize “Dried Distillers grain with Solubles” DDGS in the concentrate significantly affected the increase in intramuscular fat content (Borzuta et al. 2014). In turn, the use of bakery waste in the diet, regardless of its level, did not change the content of this ingredient in sheep meat of Zandi breed (Afzalzadeh et al. 2007). Although, higher intramuscular fat content may have a positive effect on meat tenderness and its culinary usefulness, most consumers are looking for lean meat.

TABLE 5. The meat quality traits

Item	Control group		WBG group		BPM group	
	<i>LSM</i>	<i>SD</i>	<i>LSM</i>	<i>SD</i>	<i>LSM</i>	<i>SD</i>
pH <sub>24h</sub>	5.72	0.50	5.80	0.57	5.89	0.31
Expressed juice (cm <sup>2</sup> /g)	11.17	2.84	12.56	4.34	11.03	2.58
Meat color						
Lightness – $L^*$	34.83	2.76	36.30	3.18	35.82	3.64
Redness – $a^*$	17.23	2.69	17.41	2.70	16.15	2.17
Yelowness – $b^*$	1.35	0.99	1.38	1.08	1.35	0.81

TABLE 6. The chemical composition of meat

Item	Control group		WBG group		BPM group	
	<i>LSM</i>	<i>SD</i>	<i>LSM</i>	<i>SD</i>	<i>LSM</i>	<i>SD</i>
Protein (%)	20.25	0.33	20.31	0.53	20.74	0.94
Collagen (%)	1.31	0.11	1.35	0.16	1.39	0.29
Fat (%)	4.64	1.26	5.06	1.84	4.00	1.14
Moisture (%)	74.53	1.02	74.09	1.37	74.68	0.97

The profile of fatty acids in intramuscular fat was the most favorable in lambs carcasses from the WBG diet group (Table 7). The meat of these animals contained a higher ( $P \leq 0.01$ ) amount of MUFA compared to the BPM group and a higher ( $P \leq 0.05$ ) PUFA also relative to the control group. In the intramuscular fat of lambs from the experimental group fed with the by-products of the

TABLE 7. The fatty acid composition in meat (mg/100 g fat)

The fatty acid	Control group		WBG group		BPM group	
	<i>LSM</i>	<i>SD</i>	<i>LSM</i>	<i>SD</i>	<i>LSM</i>	<i>SD</i>
C10:0	0.10	0.02	0.11	0.03	0.11	0.02
C14:0	2.00	0.31	2.11	0.48	2.14	0.39
C15:0	0.42	0.05	0.48	0.08	0.43	0.12
C16:0	23.43	0.92	23.51	1.53	23.36	1.43
C17:0	1.64 <sup>A</sup>	0.22	2.11 <sup>AB</sup>	0.20	1.51 <sup>B</sup>	0.60
C18:0	17.83 <sup>a</sup>	1.69	17.63 <sup>ab</sup>	2.70	19.32 <sup>b</sup>	1.64
C20:0	0.09	0.02	0.10	0.04	0.10	0.03
C14:1	0.06	0.01	0.06	0.01	0.06	0.01
C16:1	2.11	0.18	2.10	0.30	2.13	0.31
C17:1	0.77 <sup>A</sup>	0.17	1.01 <sup>AB</sup>	0.16	0.66 <sup>B</sup>	0.25
C18:1n9	38.18 <sup>a</sup>	1.95	39.37 <sup>B</sup>	2.04	36.53 <sup>Ba</sup>	2.92
C18:1n7	1.15	0.12	1.29	0.20	1.03	0.18
C20:1	0.10	0.02	0.09	0.03	0.11	0.02
C18:2n6	3.91 <sup>A</sup>	0.65	4.09 <sup>AB</sup>	1.08	3.18 <sup>B</sup>	0.78
C18:2 <i>cis</i> 9, <i>trans</i> 11 (CLA)	0.34 <sup>ab</sup>	0.04	0.38 <sup>ac</sup>	0.06	0.31 <sup>bc</sup>	0.04
C18:3n3	1.81 <sup>a</sup>	0.04	1.86 <sup>B</sup>	0.08	1.77 <sup>Ba</sup>	0.07
C20:3n6	0.08	0.02	0.08	0.03	0.08	0.03
C20:4n6	0.82	0.25	0.85	0.33	0.84	0.25
C20:5n3	0.06	0.01	0.05	0.02	0.05	0.02
C22:5n3	0.10	0.03	0.11	0.04	0.11	0.03
C22:6n3	0.14	0.01	0.14	0.02	0.15	0.01
Σ SFA	45.61 <sup>a</sup>	1.70	46.15 <sup>b</sup>	2.31	47.14 <sup>ab</sup>	2.43
Σ MUFA	46.50 <sup>A</sup>	1.78	46.72 <sup>B</sup>	2.54	44.66 <sup>AB</sup>	2.17
Σ PUFA	7.39 <sup>ab</sup>	0.88	7.68 <sup>ac</sup>	1.44	6.67 <sup>bc</sup>	0.99
Σ n-6	4.81	0.84	5.01	1.38	4.10	0.96
Σ n-3	2.11	0.05	2.16	0.10	2.08	0.08
n-6/n-3	2.27	0.38	2.31	0.52	1.96	0.42
PUFA/SFA	0.16	0.02	0.17	0.03	0.14	0.02

Within a row, means denoted with same letters are statistically different a–c  $P \leq 0.05$ ; A–C  $P \leq 0.01$ .

milling industry the highest content of SFA ( $P \leq 0.05$ ) was registered. It should be noted, that in the muscle tissue of these lambs a significantly larger share ( $P \leq 0.05$ ) in the group of saturated acids concerned only stearic acid, whose impact on human health is not defined as negative, on the contrary, its bioactivity is compared to bioactivity of polyunsaturated acids (Tholstrup et al. 1994). The higher content of monounsaturated fatty acids in the group of WBG lambs was determined by the higher ( $P \leq 0.01$ ) content of oleic acid C18:1 *cis*9, and in the group of polyunsaturated acids the higher ( $P \leq 0.01$ ) content of C18:2 *cis*9, 12 and C18:3 *cis*9,12,15 (Table 7). In contrast to these studies, the lower PUFA content and the higher SFA have been determined by Shand et al. (1998) in intramuscular fat of steers fattened with the participation of WBG. Other authors investigate the impact of the use of by-products in the form of maize DGS or sunflower cake on the quality of meat of fattened lambs also noted a significant increase in the content of PUFA and MUFA. However, in the study of these authors, the diet was enriched with flax seeds (Borzuta et al. 2014). Similarly, Dierks et al. (2017), using in the diet DDGS and WDGS also obtained a significant increase in PUFA but in cooked meat. On the other hand, Afsalzadeh et al. (2007) stated that using the by-products of the baking industry did not affect the level of C16:0 content which is one of the main acids from the SFA group, but increased the linoleic acid content in the intramuscular fat of Zandi sheep.

In meat tissue of lambs from the WBG group, except C18:3 *cis*9,12,15

and C18:2 *cis*9,12, a higher ( $P \leq 0.05$ ) content of C18:2 *cis*9, *trans*11 (CLA) isomer compared to the BPM and control group have been recorded (Table 7). This isomer, determined to as rumen acid, prevents atherosclerosis, osteoporosis, stimulates the immune system and also has anticancer properties (Collomb et al. 2006). Higher C18:2 *cis*9, *trans*11 content was also recorded in intramuscular fat of lambs from lowland sheep fed with fresh brewer's grain (Radzik-Rant et al. 2018). The growth of CLA was not recorded by Borzuta et al. (2014) under the influence of the use of DDGS in fattening.

## CONCLUSION

On the basis of the conducted research, it can be concluded that the by-products of the brewing and milling industry used in fattening lambs did not negatively affect the development of body weight and slaughter value. These products also did not worsen the quality of meat in respect of chemical composition and physical characteristics.

The by-product used in fattening lambs like wet brewer's grain, had a positive effect on the health benefits of meat. The brewer's grain participation in the diet increased the content important for human health monounsaturated and polyunsaturated fatty acids and the isomer C18:2 *cis*9, *trans*11 (CLA).

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- Streszczenie:** Wpływ zastosowania ubocznych produktów przemysłu browarnianego i młynarskiego w diecie jagniąt na wzrost masy ciała, wartość rzeźną i jakość mięsa. Celem badań była ocena tempa wzrostu, wartości rzeźnej i cech jakościowych mięsa jagniąt żywionych z udziałem świeżego młóta browarnianego i produktu ubocznego przemysłu młynarskiego. Badania prowadzono na 45 jagniętach merynosa polskiego, które podzielono na trzy grupy żywieniowe i tuczono do masy ciała 40 kg ( $\pm 2,0$  kg). Jagnięta z grupy kontrolnej były żywione sianem łąkowym, śrutą owsianą i parowanymi ziemniakami. W jednej grupie doświadczalnej (grupa WBG) zastosowano 35% dodatek świeżego młóta browarnianego. Druga grupa doświadczalna (BPM) była żywiona z 44% udziałem produktu ubocznego przemysłu młynarskiego w postaci pokruszonych i uszkodzonych ziaren różnych gatunków zbóż. Uzyskane wyniki wykazały, że produkty uboczne przemysłu browarnianego i młynarskiego wykorzystywane w tuczu jagniąt nie miały negatywnego wpływu na rozwój masy ciała i wartość rzeźną. Zastosowanie tych komponentów nie wpłynęło również na skład chemiczny i cechy fizyczne mięsa. Udział młóta browarnianego w dawce pokarmowej spowodował wzrost ważnych dla zdrowia człowieka jedno- i wielonienasyconych kwasów tłuszczowych oraz izomeru C18:2 *cis*9, *trans*11 (CLA).
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## The production system of high quality pork products – an example

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**Abstract:** *The production system of high quality pork products – an example.* The aim of the article was to present the Spanish production model of dried, ripening hams – Jamon Ibérico. Many years of Spaniards' experience in extensively fattened Iberian pigs, using the potential of pastureland areas called dehesa and refining the technology of ham production in a slow drying process makes the obtained product (Jamon Ibérico) characterized by very good quality (confirmed by scientific research) and palatability. The established law regulations of this kind of production in royal decrees issued in 2007 and then in 2014 introduced a number of regulations to improve and stabilize the quality of the final product. Because of the care for the safety of genetic resources of Iberian pigs, regulations on the origin of animals, their treatment and breeding methods have been tightened. In addition, the labeling conditions for regional products have been defined. The aim of these new labeling conditions is to ensure the possibility of identifying the breeding system and the way of feeding Iberian pigs by placing appropriate markings on the labels of finished products. The above mentioned actions are an example of the Spanish concern for the interests of both consumers and producers of Iberian pork products. With the dynamically operating promotion system, dried ham Jamon Ibérico is known not only in Spain but throughout the whole Europe.

**Key words:** Ibérico pigs, fattening, regional product, law regulations

## INTRODUCTION

In recent years, an intensive growth of pig population has been observed in Spain. According to Eurostat (2017) from 2014 to 2017 pig population increased more than 3 million head, reaching nearly 30 million head. During that time, the number of slaughtered pigs also increased and exceeded 50 million heads in 2017 (Fig. 1).

In Spain, pig production is conducted in two directions. The first one is focused on intensive fattening of highly productive breeds and/or lines based on the latest technologies. It is also the main reason of increasing professionalisation, export and production in this country. The second direction is extensive production with use of the native Ibérico breed, in order to obtain a high-quality product – Iberian ham (Jamón Ibérico). Although, only 11% consists extensive production in Spanish pig production, the final product is worldwide known and appreciated. The products from native Ibérico breed are good example of close cooperation with scientists, producers and public institutions. Ibérico is the common name of several native pig breeds in Spain.

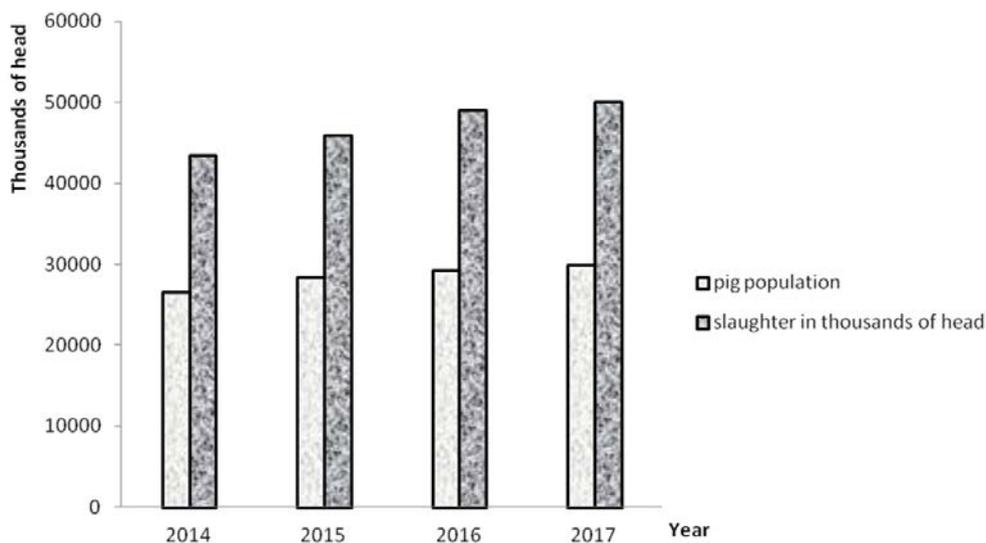


FIGURE 1. Pig population and number of pigs slaughtered in Spain in 2014–2017

Source: Eurostat data (2017).

According to FAO (<http://dad.fao.org/> – for 2016) the largest number of animals were kept in the Ibérico breed (Iberian black) – 560 591 animals, and then in varieties of Ibérico: Retinto (red) – 287 735 animals, Entrepelado (red and black) – 38 428 pigs, Torbiscal (red) – 4 619 pigs, Negro Lampiño (black with spots around a snout) – 3 461 pigs, Manchada de Jabugo (spotted) – 123. In 1997, two additional breeds were bred: Dorado Gaditano and Mamellado, but at present its number is unknown. The supervision of breeding herds and certification of animals is carried out by the Spanish Association of Iberian Pig Breeders (*Asociación Española de Criadores de Cerdo Ibérico* – AECERIBER). Ibérico pigs (Fig. 2) have primitive type, are characterized by relatively small size with long snout and legs. The withers high usually reached 90 cm in boars, with body weight to 140 kg, while sows

75 cm – withers high and 120 kg body weight. Moreover, their growth is slow, from 360 g/day (Ibérico) to 700 g/day (Torbiscal). Those traditional Spanish breeds, are raised free-range in the dehesa – mountains area with the oak forests and grasslands. The area of oak forest occurs only in the south of the Iberian Peninsula, in 0.7 million hectares in Portugal and around 2.3 million hectares in Spain (System report...). Most of these areas occur in Andalusia (approximately 1.2 million hectares) in the provinces of Córdoba, Huelva, Seville, Jaén, Cádiz, Granada, Malaga and Almería (Dehesas...). The dehesa is a grassland featuring in herbaceous plants and from the heath family and species in the genus *Quercus* (oak). Depending of the region the different oak trees occur: evergreen oak trees (*Quercus ilex*), cork oaks (*Quercus suber*) or yellow oaks (*Quercus lusitanica*) (Lopez-Bote 1998) (Fig. 3).



FIGURE 2. Ibérico fatteners

Source: photo by M. Szyndler-Nędza



FIGURE 3. Oak acorn

Source: photo by J. Nowicki

In dehesa may also occur trees such as: beech, pine and chestnuts. This unique ecosystem was created by removing Mediterranean forests and planting oaks. Oak trees were planted in an orderly manner to maximize productivity by balancing the amount of light for grass and water in the soil. Dehesa is the habitat of protected animals such as eagle, lynx, for many birds (60 species), over 20 species of

mammals and many reptiles and amphibians. Although, it is primarily use for grazing cattle, sheep and goats and to produce acorns for pigs. Iberian pigs are fed on acorns and grass from end of October (the maturation of oak acorns) until January (Olea and San Miguel-Ayanz 2016). To reduce damages (caused by rooting) to the tree layer and forest undergrowth, pigs have nose rings (Fig. 4).



FIGURE 4. Iberico pig with nose ring  
Source: photo by M. Szyndler-Nędza

The final stage, where pigs are feed on acorns and pasture have the greatest impact on quality and taste of meat, which have been confirmed by numerous studies (Fernández et al. 2007, Gandemer 2009, Pugliese and Sirtori 2012).

## FACTORS AFFECTING THE QUALITY OF MEAT AND ITS PRODUCTS

### IMF and bred

The organoleptic properties of raw meat are mainly associated with intramuscular fat (IMF). The minimum acceptable fat level should range between 2.0 and 2.5% (Affentranger et al. 1996). IMF affects the quality traits of raw-ripened ham such as: color, brightness and shear force. These features decrease with the increases of IMF. Moreover, IMF level prevents water losses during meat processing (cooking, grilling etc.) (Tyra

et al. 2013) and plays important role in the sensory evaluation. Due to the presence of intramuscular triacylglycerols, which are a good solvent for most aromatic compounds, the ham (made from raw meat with high IMF) is characterized by an intense fat aroma (Gandemer 2002, 2009). The content of IMF depends on bred, sex and varies with pig' growth. Fernández et al. (2007) showed wide range of IMF in longissimus muscles from 3.27 to 29.21% in Ibérico pigs.

Native breeds have a great predisposition to the deposition of one unsaturated fatty acid from the MUFA group (oleic acid) in comparison to selected/ improved breeds, which have higher amount of saturated fatty acids (SFA) (Pugliese and Sirtori 2012). The high level of unsaturated fatty acid in local breeds may be a consequence of different lipids synthesis and may also affect the length of fattening. Additionally, the ability to deposit unsaturated fatty acids increase with age (Edwards 2005). However, those differences can be overcome by mating pure-breed Ibérico with Duroc pigs (Carrapiso et al. 2003). In Spain, crossing native sows with Duroc boars is commonly used to improve the production parameters of offspring without affecting their resistance and IMF. Both traits are important for processing sector, for example level of marbling is a recognized criterion for the quality of dry hams (Edwards 2005).

### Fattening stage

The numerous studies showed that quality of pork is affected by duration of fattening period but not by housing system (Pugliese and Sirtori 2012). Jonsall et al. (2001) and Lopez-Bote et al. (2008) did

not found differences between pigs of the same age, housed in different systems: free range and indoor, in tenderness of meat, while minor differences in juiciness and color were found. The research results suggested that activity on pasture is not sufficient to impact meat quality features. Meat from fatteners (slaughter at 88 kg, 487 days), housed free range is characterized by darker color and higher shear force in comparison to raw meat obtained from fatteners (100 kg, 448 days) housed indoor (Pugliese et al. 2004). The color of meat is related to the myoglobin concentration in the muscles, which increases with animals' age (Mayoral et al (1999). According to Janicki and Buzala (2013) hardness and tenderness of meat is influenced by content of collagen in the muscles, while breed, feeding, muscles activity, age at slaughter and time of maturation and castration affected the content of collagen.

### **Feeding**

The production of Iberian pigs is deeply bound to the grasslands and forests. Thus, the animals are grazed on acorns and pasture which is one of the main differences between commercial feed. As was mentioned above, fat composition is related to genotype and type of feeding system as well (Gandemer 2002). It has been shown, that fatty acid profile in Iberian pigs' ham and bacon have higher level of unsaturated fatty acids, especially MUFA (oleic acid) and lower SFA (palmitic and stearic acids) than ham obtained from commercial breed (reared inside) (Andrés et al. 2001, Cava et al. 2000, Diaz et al. 1996). Moreover, pigs' diet based on pasture and enriched with chestnuts increase the PUFA level in fat

(Pugliese et al. 2005). It is due the fact, that chestnuts have higher concentrations of polyunsaturated fatty acids than acorns (Lopez-Bote 1998). Nutrition also affects the fatty acid profile in ripened products, especially when pigs are reared outdoor on pasture or forest, with access to acorns and grass (Pérez-Palacios et al. 2010).

Green fodder and acorns are good source of vitamin E ( $\alpha$ -i  $\gamma$ -tocopherol) which is known of its anti-oxidant properties. In pigs, vitamin E is accumulated primarily in fat and then in meat. Rey et al. (2006) confirmed the increased of  $\gamma$ -tocopherol in the tenderloin and bacon obtained from pigs reared in extensively system, in comparison to pork obtained from animals reared indoor and feed with commercial feed.

## **PRODUCTION SYSTEM**

### **Breeding**

The breeding of Iberian pigs' has a long tradition, as well as a many years of experience handed from one generation to the next. Thus, the quality and unique taste of the products are results of knowledge, hard work and proper breeding management.

Ibérico sows have from 6 to 10 piglets per litter, and two litters per year. Piglets are separated from their mothers when they are 43 days of age. Ibérico sows are mostly mated with Duroc boars or Ibérico  $\times$  Duroc boars to obtain better fattening parameters from offspring. However, the best quality products are obtained from pure Ibérico fatteners but they are characterized by the highest price. Piglets of both sexes are castrated in young age, to avoid unplanned pregnancy in females (during fattening period in dehesa), and

boar taint in meat. The fattening period in the dehesa starts in parallel with production of acorns, usually from October until December. During this time, average daily gains are small so the first stage of fattening period is slow and should be finished at (minimum) 12 month of age and 90 kg body weight. Additionally, pigs have access to both pasture and shelter, where they are fed 2 kg of feed per day. The feed consists: cereals (wheat, barley) and field peas (Fig. 5). Then, pigs are released on dehesa and fed with acorns (Bellota system) – this is the last stage of fattening. Due to the fact, that one pig can eat ten kilograms of acorns a day, the required space for one pig is 1 hectare of dehesa. Crossbred pigs (50% Ibérico) are released onto the dehesa at the age 12 months and 115 kg of body weight, while pure breed pigs (100% Ibérico) at age 14–15 months and 90–100 kg. The fattening period ends when pigs reach a body weight over 150 kg (approximately 60 day at dehesa for crossbred fatteners, and 6 months for pure Ibérico pigs). It is worth to mention, only 5% of

pure breed fatteners are kept so long in the dehesa. Slaughter is carried out in slaughterhouses in accordance to regulation and care of stress-free slaughter to obtain better quality products.

Fatteners which could not be kept in the pasture (dehesa), are reared outdoor in fenced area with access to feed with cereals and legumes (de Cebo de campo system, mainly for crossbred pigs) or indoor with access to fenced pasture (de Cebo system, only for crossbred pigs).

### Processing

Typical Spanish products are produced in a slow, natural drying and curing processes. The first stage of production is multi-day maturing of meat at 4°C, then hams are covered with salt, which is replaced every week. After three weeks, the hams are rinsed in water to remove salt from the surface. The next stage is drying in increasing temperature (controlled through ventilation) for about 2 weeks, which causes “sweating” (dissemination of fat through the muscle fibers) – ([www.jamon.com](http://www.jamon.com)). According



FIGURE 5. Feed for Ibérico pigs

Source: photo by M. Szyndler-Nędza



FIGURE 6. Maturation of ham  
Source: photo by M. Szyndler-Nędza



FIGURE 7. Final product – Ibérico ham and bacon  
Source: photo by M. Szyndler-Nędza

to Andrés et al. (2004) increase in temperature during the drying phase reduced rancidity in the products. The last stage of production is maturing and drying at a constant temperature for a minimum of 2 years up to 4 years (Fig. 6). Thus, the entire production cycle from piglet to a final, highest quality product in which the aroma of the acorn is perceptible lasts a maximum of 6 years (Fig. 7).

#### THE RELATIONSHIP OF BEHAVIOUR, WELFARE AND MEAT QUALITY WITH PROMOTION OF PORK FROM PIGS FATTENED OUTDOORS

It is estimated, that over half of commercial world pig farming is highly intensive. The rearing and fattening pigs take place mainly indoors and is also known as industrial pig production or “factory”

farming. Pigs in most countries are kept in barren pens at high stocking densities (Compassion in World Farming). Some alternative solutions to intensive farming systems have been developed to overcome welfare concerns associated with “factory farming”. Most of them are based on traditional farming systems, that were commonly used before industrialisation, which took place in the 1950s. Some farms use modern technology to achieve better welfare (Compassion in World Farming), but in fact only outdoor systems for pigs provide full expression of natural behaviour comparable to the behaviour of the wild boar (Stolba and Wood-Gush 1989).

Outdoor pig production offers animals increased environmental diversity and behavioural freedom but imposes challenges for breed adaptation, management control, biosecurity (what is especially

important nowadays in case of ASF), and environmental protection. Each of these issues has potential implications for the real as well as perceived quality of the product (Edwards 2005). Especially the shaping proper consumer perception is the chance for farmers to develop outdoor housing and make it profitable.

In most conventional Northern European production systems, only adult and suckling animals are at pasture. However, in traditional Mediterranean systems (like in Spain) and in organic production systems, meat animals may be maintained outdoors throughout their lives (Edwards 2005). Not only Spain is a centre of outdoor pig production. As shown by Edwards (2005), outdoor pig production systems come in many forms. In some European countries, particularly UK and France, there are significant numbers of outdoor herds contributing to conventional pig meat supplies. In the UK, it has been estimated that approximately 30% of the national sow breeding herds are housed outdoors.

The major difference between pigs produced outdoors and indoors is the exposure to plentiful fresh air and greater extremes of climate. Such circumstances may influence both primary and secondary product quality attributes (Edwards 2005). The outdoor environment clearly has potential for both positive and negative effects on pig health and welfare (Edwards and Zanella 1996). As shown by Olczak et al. (2015) weather conditions (temperature, humidity, solar radiation, air pressure, wind strength, wind direction and precipitation) have a significant impact on the behaviour of farm animals. Pigs have developed a wide range of thermoregulatory behav-

iours that are particular for this species. Together with the increasing temperature, the activity decreases but the wallowing in wet surfaces increases highly. In addition to this, rooting and wallowing are highly affected by temperature and humidity. The most important is that outdoor enables the expression of above mentioned behaviours, at all. The lack of possibility to express these behaviours in most of the indoor housings leads to development of stereotypes and tail and ear biting (Nowicki et al. 2015). Interestingly, low temperatures do not increase the use of shelter, if not in combination with wind or precipitation (Olczak et al. 2015). Furthermore, wild pigs build stronger nests in severe conditions. Also, resting behaviour and reproduction may be disturbed by high temperature (Olczak et al. 2015) which is typical in Spain.

Studies on the relationship between housing system for pigs and meat quality characteristics were carried out, but their results are not homogenous (EFSA 2005). Enfalt et al. (1993) observed greater leakage of water and the lighter colour of meat of more active pigs (the daily activity in outdoor is about 70–80% of time, while in indoor only 20% (Stolba and Wood-Gush 1989). In contrast, in studies of Gentry et al. (2002) pigs housed in open outdoor system had better quality characteristics of meat: meat was darker and redder, and more fragile. There are still no clear data about daily behavioural profile of pigs housed outdoors and its effects on meat quality. Nowicki et al. (2014) found no statistical differences in physico-chemical parameters of loin from fatteners housed indoors and in outside run enclosures,

but better sensory quality of cooked meat originated from fatteners housed on the enclosures was found by the 6 person team with a proven pre-sensory sensitivity. Similar results were obtained by other authors as showed in Edwards review (2005). According to this paper (2005) the majority of studies reported no difference in juiciness or tenderness. Some of the studies reported reduced juiciness in outdoor. A significant number, however, have reported reduced muscle pH and/or increased drip loss, suggesting greater susceptibility of outdoor pigs to pre-slaughter stress, probably because the animals are not used to be kept in small areas which are common during transport and in the abattoir.

On the other hand, the outdoor housing has positive influence on the animal welfare especially due to the wide range of environmental enrichment on the pasture (Edwards 2005) and possibility of free movement (Stolba and Wood-Gush 1989). There is no doubt that in the environment poor in stimuli, strongly motivated propensity to foraging and exploration is directed to the other pigs in the pen (Lyons et al. 1995, Kelly et al. 2000) and its equipment (Lyons et al. 1995). This can lead to aggression and cannibalism (Beattie et al. 1995). In such conditions, abnormal behaviours happen more often (van de Weerd et al. 2005, Scott et al. 2006). The reduction of the frequency of agonistic behaviour in the enriched environment, reflects the lesser need to reciprocate persistent provocations from other pigs (Beattie et al. 1995, Nowicki and Klocek 2012, Nowicki et al. 2015).

As shown in the Edwards (2005) review, since early enrichment has been

shown to modify the physiological response to stressors, and since stress physiology can have a very significant effect on meat quality (as either PSE or DFD muscle depending on the nature and duration of stress response), enrichment has the potential to exert a major influence on final product quality (Warriss 1994). However, research results have been equivocal. It has been suggested that pigs from more enriched environments are less stress susceptible and might therefore deal more adequately with transport and pre-slaughter handling. Barton-Gade and Blaabjerg (1989) observed that pigs reared outdoors were calmer and more easily handled at the abattoir than intensively-reared pigs, although muscle pH values were lower for these pigs. In another study, outdoor pigs showed a decrease in ultimate pH and in water retention capacity, suggesting that these pigs experienced greater pre-slaughter stress (Gandemer et al. 1990). Similarly, in more controlled experiments, environmental enrichment has sometimes resulted in reduced pig meat tenderness (Beattie et al. 1993),

As shown by Nowicki et al. (2014) members of the taste panels when aware of the origin, rated free range pork as more valuable than meat from indoor pigs. Consumers' perceptions of a production system are therefore highly likely influences their perception of the quality of product produced from outdoor system (Edwards 2005). This phenomenon is especially highly relevant to marketing of pork from outdoor systems because, consumers perceive outdoor systems to be more humane, environmental friendly, traditional and sustainable (Edwards 2005).

In the Mediterranean systems, outdoor pigs are important for management of the forest heritage. The dehesa is a man-made ecosystem and, when abandoned, it rapidly becomes unproductive, due either to shrub growth, which limits the grazing and provokes risk of fire, or to disappearance of trees and desertification. Pig production in silvopastoral systems, even if the pasturage of animals is limited to the finishing period, is therefore of major significance in the conservation of landscape in Mediterranean areas (Edwards 2005). So the environmental protection, important for modern consumers is ensured. Finally, consumers often perceive that meat produced in outdoor systems is more nutritious and safer, with lower use of feed additives and antibiotics (Edwards 2005).

The next important issue is the conviction of consumers that animals are able to express the natural behaviours. Public opinion is more and more interested in animal welfare and even in animal rights. Foraging is the basic behaviour in wild boar and this behaviour did not disappear in domestic pigs. Natural selection favoured those wild boars who foraged and rooted most and the behaviour is now fixed, or hard-wired, also in the modern, domestic pig. This means that all pigs are still highly-motivated to explore their surroundings and forage for food (Beattie and O'Connell 2002, Studnitz et al. 2007). It is the reason why only outdoor housing can fulfil behavioural requirements of pigs. The forage feeding may indeed increase some health promoting aspects of pig meat (Nilzen et al. 2001, Hogberg et al. 2002). This type of feeding is especially used in Spain on the dehesa pastures where pigs during

autumn and winter are able to look for and eat acorns and herbs. Chemotherapeutic medication use is also lower in outdoor herds, and they are not permitted as standard treatments in organic production systems. However, the reduced control of biosecurity in outdoor systems, and the threat of zoonotic infection from wildlife, may mean that microbiological quality of the carcass is actually poorer in some instance (Edwards 2005).

Outdoor housing of original Polish breeds of pigs together with the promotion of meat originated from these system and shaping the perception of consumers through the marketing procedures may be the way to develop smaller farms, producing high quality meat. It seems to be possible when the argumentation presented above is used. But one important factor is necessary – strict cooperation of farmers, marketing specialists and scientists to achieve this goal. The good example is just Spain where not only tradition guarantees the success of dried hams (Jamon Ibérico) and other pork products, but the consumers' conviction about animal friendly and environmental friendly farming systems is crucial.

## LAW REGULATIONS

As a consequence of the above production, dried Iberian ham is classified in several quality categories according to the conditions of breeding and rearing animals. Originally these categories were included in Royal Decree 1469/2007 (BOE-A-2007-19073) approving the quality standards of Iberian pork, ham, shoulders and pork loin. This document drew attention to the fact that it should be possible to compile existing texts on

the processes of producing these regional products of pork origin. At the same time, the scope of its application has been extended to the products derived from the cutting of carcasses, sold as fresh meat. The introduction of uniform regulations, especially in the area of labelling, was an example of concern for the interest of consumers and producers of Iberian pork products (including Jamon Ibérico). The introduction of strict legal regulations was also necessary due to the need to protect and maintain forest pastures at an appropriate level, which are closely related to pig production. According to the decree, this production should be carried out in a sustainable way and not endanger the extremely delicate pasture ecosystems (dehesa). As a result of the provisions of this regulation, the control mechanisms were strengthened by strengthening the provisions on the so-called Independent Control Units. A special office has also been set up to monitor, harmonize and develop everything related to the quality standard of Iberian pork products. In 2014, however, a new royal decree 4/2014 (BOE-A-2014-318) was published. It currently approves the quality standards for meat, ham, shoulder ham and pork loin produced in Spain.

The necessity of issuing a new regulation was related to the fact that some imperfections in the production process were visible, such as regress in keeping Ibérico pigs breed pure after several years of functioning of quality standards based on previous regulations. What is even more important, the difficulties associated with the acceptance and familiarity of products by consumers as a result of excessive diversity of labels and descriptions on labels that could lead

to confusion for the consumer were also detected. It was found that the use of many product labels was inappropriate and misled consumers. The consumer was not able to distinguish pork products of different categories effectively. It was therefore concluded that this type of unfair competition between producers should be stopped. It was also proved that there were cases of unauthorized use of trademarks, logos, images, symbols and descriptions that referred to aspects related to products whose trade name did not correspond to their actual origin and quality. This caused huge confusion among consumers who believed that they were purchasing a product associated with dehesa, meanwhile, in fact, they were buying a product from an animal that was never in the ecosystem. An interesting fact is that between 2005–2008, the Spaniards supported their production of dried hams by importing meat (ham) from Polish domestic breed pigs. An authorized person from the Spanish side chose heavy fatteners (minimum 150 kg) of Puławska and Złotnicka Spotted pigs, which were slaughtered and their elements (hams) were exported to Spain. The export took place through the Constar Starachowice (nowadays Animex).

In the new Royal Decree (BOE-A-2014-318; 2014) all deficiencies related to the 2007 legislation were improved. The quality standards are nowadays strictly attributed to the labelling conditions that aim to facilitate the differentiation of Iberian pork labels. Current legislation also takes into account the general requirements for labelling and provision of food information in the EU. It was also found that there is a need to improve the racial purity of animals placed on the

market according to the quality standard, because the use of non-registered sires in the 'Iberian Swine Race' in crossbreeds with other breeds can be dangerous to the genetic resources represented by the Iberian race. Regarding the production models, the need to change the farming and animal nutrition conditions in each of them was noted, as well as the need to reduce the acceptable stocking density in the dehesa system in order to avoid possible deterioration of the ecosystem quality. For acorn-fed animals, the newly introduced legislation is expected to reinforce control at the stage of using the pasture by individual identification of each animal. Controls of forest areas are also necessary to ensure that animal nutrition is based on the use of acorns and is a factor guaranteeing the improvement of the dehesa ecosystem. In case of the animals fed on feed, it was considered necessary to lay down implementing rules. These provisions should apply to both the treatment of animals and farming methods, all aimed at improving the quality of products.

Another novelty in comparison to the previous regulations is law focused on the improvement of the traceability of products by the requirement of placing seals on products that must enable identification of the breeding system and the way of feeding pigs.

In addition, stricter and clearer requirements were set for product labelling to improve the quality of information received by consumers. It was considered important to inform the consumer about the percentage share of the Iberian pig from which the purchased product comes from. In addition, restrictions have been imposed on the use of certain

descriptions, logos, images, drawings, acronyms, brands or emblems on labels that may mislead the consumer in relation to the product purchased, in particular to avoid confusion between products in which the raw material is based on pigs housed on the pastures, and swine foodstuffs derived from pigs fed with compound feed.

In this legal act from 2014, a number of concepts related to pig production as well as the processing and preservation of high-quality pork products have been strictly defined. The most important definitions include:

- Dehesa – in other words, meadow, pasture, or geographical area in which agro-forestry and land-use techniques are used, based mainly on the presence of animals on the grassland and forests, where mainly oaks occur. In this area, human activity is necessary to ensure protection and durability of these oaks. The presence of at least 10 trees in the area of 1 hectare is required.
- “Montanera” – is an animal diet based on the use of acorns and grazing resources of dehesa in Spain and Portugal.

#### **Designation by type of the product:**

- Designation by type of the product:
  - In the case of processed products, these are: ham, shoulder, pork or “emborrado pork” or sirloin.
  - In the case of products obtained from cutting a fresh carcass, the markings of meat elements (cuts) originating from cutting shall be used in accordance with accepted market names.
- Descriptive indications depending on the diet of pigs

- “De Bellota”, or “acorn”: in relation to products from animals slaughtered immediately after finishing the fattening period, based on acorns, grass and other natural resources of the meadow, without the use of additional nutrition.

In case of products marked as “Bellota”, the annex to the mentioned decree (2014), the stocking density of animals staying on dehesa according to the percentage of the area covered by trees should be as presented in the Table 1.

On the other hand, in the case of products originating from animals whose feeding and proceedings to achieve the slaughtering weight, does not include the above mentioned point, the following descriptive markings are used:

- De Cebo de Campo – in free translation, nutrition in the field – used in the case of products from animals that during fattening in the pasture used the resources of this pasture, but were also fed with feed consisting mainly of cereals and legumes.
- De Cebo – in the case of animals fed with feed, consisting mainly of cereals and legumes, which are kept in intensive systems (with a small range).

- Designations according to the breed or crossing pattern

- 100% Ibérico – 100% Iberian breed – in the case of products derived from animals of 100% genetic purity of the Iberian race, whose parents also represent 100% racial purity and are registered in the appropriate breeding book.

- Ibérico – in the case of animal products with at least 50% of the Ibérico bred, whose parents fulfil the following conditions:

- For products labelled “75% Ibérico” from animals with 75% Ibérico, mothers must be pure-bred Ibérico sows, registered in the studbook, while fathers must come from the crossbreed of the Ibérico pure-bred sire and Duroc’s pure-bred father.

- For products designated “50% Ibérico” from animals, Ibérico pure-bred sows are used, while fathers represent pure Duroc breed. Both must be registered in the appropriate genealogy lineage of the breed.

Determining the origin of parents is carried out using the so-called “racial

TABLE 1. The stocking density of animals staying on “La dehesa” according to the percentage of the area covered by trees

Percentage share of the area covered with trees	Maximum allowable stocking density (animals/ha)
to 10	0.25
to 15	0.42
to 20	0.58
to 25	0.75
to 30	0.92
to 35	1.08
Larger than 35	1.25

certificates”, issued by an appropriate officially recognized association to manage the so-called book of origin. Verification of the breeding factor of animals intended for slaughter to obtain Iberian products is carried out by a control body accredited by a Spanish national accreditation organization. In the label of pig product it is mandatory to give the percentages of Iberian pigs.

### **Labelling**

Product labels, in accordance with the decree, must comply with the general applicable provisions on the labelling of food products. It is forbidden to use the trade name which is incomplete, adding to it terms other than the conditions specified in art. 3 of the Decree (2014). According to these regulations (BOE-A-2014-318; 2014) signs forming the trade name must be in a visible place and in each case, in the same field of view as the trade brand, in the same font size of the same size, thickness and colour.

In addition to the trade name, the products included in the discussed standard with the exception of fresh meat, must acquire the following mandatory information on the label:

In the case of products originating from crossbreds, the percentage of the Iberian animal from which the product originates is indicated by the expression “% Iberian breed”. This indication must appear very close to the product name, using a font size of at least 75% of the product name and not less than the minimum size required in Article 13, paragraph. 2 of Regulation (EU) No 1169/2011. The label must also include the term “certified by” followed by the name of the independent control body or its acronym. This expres-

sion should be placed on the label next to the trade name. Currently, contrary to the regulation of the previous decree of 2007 (Royal Decree 1469/2007; BOE-A-2007-19073), the use of the terms “Recebo” (fed with acorns and fed with compound feed) and “Ibérico puro” (pure Iberian) is also prohibited.

Products labelled “De bellota” must come from animals whose fattening is based on specific conditions, including: the introduction of animals into pastures and should take place between October 1 and December 15, which means the period of slaughter between December 15 and March 31.

The most important minimum conditions that animals must meet primarily relate to the body weight, which at the beginning of the fattening phase with the use of acorns must be ranged between 92 and 115 kg, with the minimum slaughter age – 14 months.

For consumers, however, the most important is the ability to easily identify product categories. Therefore, in contrast to the decree of 2007 (Royal Decree 1469/2007; BOE-A-2007-19073), the latest legislation Royal Decree 2014 (BOE-A-2014-318; 2014) introduces the colourful labels, where each colour is assigned to an appropriate category of hams depending on the way pigs are raised. The following colours are used:

- Black – means feeding pigs during the period of being on dehesa only with acorns, the breed of pigs is 100% Ibérico (De Bellota, 100% Ibérico).
- Red – means feeding with acorns, pigs are crossbreds with the Ibérico breed (De bellota, Ibérico)
- Green – means feeding with commercial feed mixtures, however, animals

can use its resources in the pasture, the pigs are crossbreds with the Ibérico breed (De Cebo de Campo, Ibérico).

- White – colour means animals fed with commercial feed mixtures, which are kept in buildings with access to small runs, pigs are crossbreds with the Ibérico breed (De Cebo, Ibérico).

Due to the fact that the process of ham ripening lasts for several years, transitional periods have been planned, according to which the processor holding products in development after the entry into force of this standard, but whose marketing is planned after March 1, 2014, while stocks last, may label products in accordance with the provisions of royal decree 1469/2007, or move to labelling them with new trade names in accordance with the 2014 standard.

## SUMMARY

Years of experience in the extensive Iberian pig production on pastureland areas and excellent ham production technology (Jamon Ibérico) led to fame and recognition of Spanish products around the world. Moreover, production technology is strengthened by law regulations (Royal decrees 2007 and 2014), which protect and stabilize the quality of final products. Iberian pigs, as a genetic recourse, are also under regulations concerning on the pigs' origin, treatment and breeding. Additionally, the labelling of regional products have been defined for easy identification of the breeding and feeding system of Iberian pigs. All the above actions are an good example of state's concern for the interest of both,

consumers and producers, which also positively influence on promotion of dried ham (Jamon Ibérico) in many places around the world.

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**Streszczenie:** *Przykład systemu produkcji wysokojakościowych produktów z wieprzowiny.* Celem artykułu było przedstawienie hiszpańskiego modelu produkcji szynek suszonych Jamon Ibérico. Wieloletnie doświadczenie Hiszpanów w ekstenywnym tuczu świń iberyjskich z wykorzystaniem potencjału obszarów pastwiskowo-leśnych zwanych dehesa oraz dopracowanie technologii produkcji szynek w powolnym procesie suszenia sprawia, że otrzymany produkt (Jamon Ibérico) charakteryzuje się bardzo dobrą jakością (potwierdzoną badaniami naukowymi) i smakowością (niespotykanym w innych produktach aromacie mięsa). Ustanowione regulacje prawne tej produkcji w dekretach królewskich wydanych w 2007 roku, a następnie w 2014 roku wprowadziły wiele przepisów mających na celu poprawę i stabilizację jakości produktu końcowego. Dbając o bezpieczeństwo zasobów genetycznych świń rasy iberyjskiej, zastrzono przepisy dotyczące pochodzenia zwierząt, sposobu ich traktowania oraz metod hodowli. Ponadto określono warunki etykietowania produktów regionalnych, które umożliwiają identyfikację systemu chowu i sposobu żywienia świń

iberyjskich poprzez umieszczanie odpowiednich oznaczeń na etykietach gotowych produktów. Powyższe działania są przykładem troski Hiszpanii o interes zarówno konsumentów, jak i producentów wieprzowych produktów iberyjskich. Przy jednocześnie prężnie działającym systemie promocji szynka suszona Jamon Ibérico znana jest nie tylko w Hiszpanii, ale i w całej Europie.

*Słowa kluczowe:* świnie Ibérico, tucz, produkt regionalny, regulacje prawne

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