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GLUTAMINE AS A FEED SUPPLEMENT FOR PIGLETS: A REVIEW*

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Abstract

Weaning is a crucial moment in a piglet's life. It is characterized by a generally low nutrient intake and adverse changes in the small intestinal mucosa. Proper feeding is therefore necessary to ensure normal development of the gastrointestinal tract. One substance that could provide intestinal epithelial cells with necessary energy is the amino acid glutamine. It improves epithelium structure and accelerates the growth of intestinal villi in which nutrients are absorbed, thus improving feed utilization and growth performance in piglets. The effect of glutamine on intestinal microflora also improves animal health. In addition to liver and kidneys, small intestine is the main site of glutamine metabolism, which leads to the synthesis of purine and pyrimidine nucleotides and of the important antioxidant glutathione. Glutamine is also a precursor for the synthesis of proline and arginine, the components of body proteins. Glutamine downregulates the expression of genes responsible for oxidative stress and immune activation, and increases the expression of genes that are necessary for cell growth and removal of oxidants. Due to these properties, glutamine is considered an essential amino acid in diets for weaned piglets.

Key words: L-glutamine, piglet rearing, digestive tract

Because several years ago the European Union banned the use of antibiotics as growth promoters in livestock feeds, some replacers have to be found, especially for young animals (Anadón, 2006). The digestive tract and the immune system of piglets is not fully developed, which makes them particularly sensitive to adverse environmental impacts (Bailey et al., 2005). Because growing animals have a high requirement for protein and energy, proper development and function of the gastrointestinal tract is crucial. In piglets, the weight of the gastrointestinal tract increases threefold

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(from 2% to 6% of body weight) between birth and second week after weaning (Burrin and Stoll, 2003). The nutrient requirement of the gastrointestinal tract tissues is met directly from digested feed or from blood circulation (Burrin et al., 2000).

After weaning piglets have to adapt to new stressful conditions, which is associated with reduced feed consumption, temporary malnutrition and growth retardation (Lallés et al., 2004). Weaned piglets only after three days take in energy in amounts necessary for maintenance and after 8 to 14 days this amount reaches the level consumed before weaning (Le Dividich and Séve, 2000). This is due to villous atrophy, especially in the proximal part of the small intestine, caused either by an increased rate of cell loss or a reduced rate of cell renewal (Pluske et al., 1997). On the fifth day after weaning, villous height at most sites along the small intestine is approximately at 50% of the initial values found at weaning (Hampson, 1986). As a consequence, absorption of water and electrolytes from the small intestine is also temporarily lowered (Nabuurs et al., 1996). Simultaneously with the change of feed, secretion of carbohydrases is changing: activity of lactase decreases and that of maltase and saccharase increases (Le Dividich and Séve, 2000).

Weaning is associated with significant changes in amino acid metabolism; in enterocytes, the metabolism of glutamine, arginine and citrulline is more intensive than before weaning (Wang et al., 2008). This leads to the production of compounds such as glutathione, which are important for adaptation, regeneration and preservation of intestinal tissues under conditions of stress (Reeds et al., 1997). After weaning synthesis of protein increases in the intestine while decreasing in muscles (Séve et al., 1986), which protects the intestine against protein deficiency.

Many studies suggest that after weaning feed consumption, body weight gains and intestinal villi height are correlated. A decisive factor is the low feed consumption that accompanies weaning (Pluske et al., 1997), which is the cause of changes in intestinal structure and function. Thus, improving feed intake by feeding piglets with milk products (e.g. skim milk powder or whey), which are readily consumed by piglets and are a good source of highly digestible protein and energy, should improve the structure and function of intestinal epithelium (Thacker, 1999; Lallés et al., 2004). Another way of preventing intestinal atrophy linked to weaning can be to supplement piglet feed with glutamine or glutamate (Ewtushick et al., 2000). A previous experiment (Newsholme et al., 2003) has demonstrated that glutamine is "conditionally essential" during weaning and also under conditions of stress such as injury or infection. Apart from the positive effect on intestinal cell regeneration, glutamine stimulates lymphocyte regeneration (Matés et al., 2002) and macrophage activity (Newsholme, 2001) without affecting the normal intestine structure.

The effect of glutamine on structure of the small intestine mucosa and piglet performance with and without a microbial challenge

One of the ways of keeping piglets supplied with glutamine can be the addition of this amino acid to sow's feed and thus enriching sow's milk. Such an experiment was performed by Kitt et al. (2004), who used 16 pregnant sows allocated to two treatments: control fed corn soybean meal diet and experimental diet in which part

of corn was replaced by 2.5% of glutamine. Sows received the experimental diet from the day of parturition. One hundred and twenty-eight piglets (64 barrows and 64 gilts) were weaned on day 21 of lactation and within sow treatments assigned to one of two nursery treatments. One received saline (0.90%) injection and the second group received injection of *Escherichia coli* lipopolysaccharide (LPS). After weaning all piglets were fed the standard starter diet. On days 3 and 7 four pigs per treatment were slaughtered and samples of duodenum and jejunum were taken to measure small intestine characteristics. No difference in body weight loss between sows fed control or experimental diet was observed but sows fed supplemental glutamine tended to have increased plasma glutamine concentration, which suggests that glutamine was not metabolized in sow intestinal epithelium cells. Sows fed glutamine had greater glutamine milk concentration compared to control sows by 46% on day 7 and 265% on day 21. In spite of these differences, piglets' plasma glutamine concentration in both groups did not differ significantly. There was also no difference in piglet body mass.

E. coli endotoxin (LPS) significantly reduced piglet body weight gains and feed intake. It also reduced duodenum villus height by 22%, but progeny of sows receiving glutamine had 12% greater villus height on day 3. On day 7 villus height was similar in both groups. Piglets originating from control sows had 10% greater small intestine length and 12% greater empty weight on day 7 compared to those from sows fed glutamine.

Contrary to expectations, piglets from both the challenged and unchallenged group that received milk with glutamine had lower weight gains. They also consumed less feed and had shorter small intestine. It is possible that greater glutamine intake during suckling increased plasma glutamine level, which can be a signal for the intestine to decrease glutamine catabolism, which in turn decreased intestinal growth. These data suggest that dietary glutamine supplementation increases sow milk glutamine concentration, but does not positively influence progeny growth performance during lactation or immediately after weaning.

Wu et al. (1996) fed piglets weaned on the 21st day of age with corn and soybean meal based diet for 14 days. The diet was supplemented with 0, 0.2, 0.6 or 1% of glutamine. They found that glutamine was not subjected to measurable acid hydrolysis in the stomach and upper part of duodenum and was substantially available to the small intestine for metabolic utilization. One percent of glutamine supplement increased the concentration of glutamine in duodenal digesta fluid eightfold. On day 7 postweaning the villous height in jejunum significantly decreased in control piglets but it was maintained at the preweaning value in piglets fed with 1% of glutamine. In both control and glutamine-supplemented piglets villous height in jejunum was greater on day 14 postweaning compared with preweaning piglets. Thus, when acid hydrolysis in the stomach is avoided and a relatively small glutamine supplement (1%) is available for metabolic utilization in the small intestine, jejunal atrophy in weaned pigs is prevented.

Similar diets were used in a three-week experiment on piglets weaned on the 28th day of age by Hsu et al. (2010). Piglets were not cannulated and 1% or 2% supplements of glutamine replaced the corresponding amount of corn starch. Apart

from changes in intestinal epithelium, structure and growth performance, absorption activity of the small intestine was measured. For this purpose piglets were fasted and then fed by gavage 10% xylose solution. Concentration of xylose in blood was estimated before and 1 hour after feeding of xylose. The glutamine dietary supplement improved the villous height of duodenum and jejunum when compared to the control but there was no difference between 1 and 2% of glutamine supplement. The integrity of intestinal morphology was better in the glutamine supplemented groups and in some pigs from the control group damaged villi with erosion of surface epithelium were detected. Glutamine supplementation also increased plasma net xylose absorptive concentration. In spite of these positive changes, no significant differences in the average daily gain and feed intake were observed regardless of the treatment group.

Similar results were obtained by Domeneghini et al. (2004). They performed an experiment weaning piglets kept in controlled environmental conditions and fed different diets for 28 days. Control group received soybean meal and corn diet. The second group was fed the same diet supplemented with 0.5% of glutamine, the third group was fed control diet with supplement of nucleotide (there is no additional data except the manufacturer), and the fourth group received both these supplements. Piglets were slaughtered and the distal ileum and liver were examined histologically. In all cases the microscopic anatomy of the liver was normal. Feeding glutamine and/or nucleotides resulted in an increase in villous height and crypt depth. The differences between control on one side and glutamine and nucleotides groups on the other side were most pronounced. The percentages of mucosal macrophages and intraepithelial lymphocytes were also greater in the experimental groups than in the control group. On the other hand, these positive changes had no effect on piglet growth, thus it is possible that the dosages used in this study were efficient in affecting and improving structural aspects of the ileum but not so effective on growth performance.

These results were confirmed in a later experiment in which only glutamine was used (Domeneghini et al., 2006). The changes in morphofunctional characteristic of piglet ileal mucosa were similar to those found in the previous experiment. Results of both these experiments corroborate the nutraceutical role of glutamine as a trophic agent for mucosal repair and improvement of barrier function.

According to Zhao et al. (2009), addition of 1% of glutamine to diets for weaning piglets maintains integrity of morphological features of the intestinal mucosa, improves intestinal microflora and reduces pathogenic bacteria. Compared with control, lactobacilli content in digesta increase when glutamine diet is used.

Results of described experiments show that supplementation of piglet feed with glutamine has a beneficial effect on the structure of intestinal mucosa and potentially makes piglet intestine less vulnerable to infection.

Changes in intestinal epithelium structure and in rearing indices are usually estimated during the same experiments. This was the case with the study by Ewtushick et al. (2000), in which glutamine supplementation improved both epithelial structure and feed consumption of early weaned piglets. Similar results were obtained when arginine was used.

Domeneghini et al. (2004) moved 21-day-old piglets from the farm to a university laboratory, which, according to the authors, was likely to markedly stress the

animals, which in turn may have caused slowed growth in the early days of weaning. During the first 7 days after weaning piglets receiving 0.5% of glutamine grew faster than the control ones but later this difference was compensated and at the end of the experiment (28 days of age) piglet body weights did not differ significantly.

A much higher dose of glutamine (4%) was given to piglets weaned on the 21st day of age by Johnson et al. (2006). The experiment lasted two weeks and glutamine did not affect feed intake, weight gain or final body weight. Piglet performance was not the main focus of this experiment which aimed at identifying natural ingredients that would improve the health of pigs and which could be used instead of antibiotics as growth promoters. Results confirmed the importance of the weaning period for immune development (Johnson et al., 2003). It was found that feeding glutamine had beneficial effects on neutrophil lysozomal acivity, peripheral immune cell maturation and antigen exposure in the gut.

In an experiment performed in the National Research Institute of Animal Production in Balice, 2% of glutamine introduced to piglet feed from 7 to 84 days of life significantly improved daily weight gains by 8.7%. Piglets fed glutamine also have better feed efficiency (data not published).

The improvement in performance of piglets weaned on the 21st day of age which received soybean diet with 1% of glutamine was found also by Zou et al. (2006). During 20 days of the experiment piglets receiving glutamine were healthier. Cases of diarrhoea were rare and less serious in this group. During the first 10 days of the experiment piglets receiving glutamine consumed 12% of feed less per kg of body weight gain than the control ones. During the next 10 days there were no differences in feed consumption but piglets fed glutamine grew faster by 28%. In both cases differences were statistically significant.

Thus it seems that although glutamine has beneficial effects on small intestine mucosal structure and nutrient absorption, these positive changes not always improve piglet body weight gains.

Metabolism of glutamine and related amino acids in the digestive tract

Amino acids of the arginine group, i.e. arginine, glutamine, proline, asparagine, ornithine and citrulline, can turn one into another in metabolic transformations in majority of mammals, including pigs (Wu et al., 2007). The main sites of glutamine metabolism are small intestine, kidneys and liver and the process is regulated by cortisol. As feed proteins are usually rich in these amino acids, except for ornithine and citrulline, for a long time nutritionists were not interested in their utilization. The significance of glutamine for cell reproduction and growth was found in *in vitro* experiments by Ehrensvard et al. (1949) and Eagle (1955). Presently it is known that glutamine is so important for physiological processes that it is present in 10- to 100-fold excess of any other amino acid in tissue culture and cannot be replaced by glutamic acid (Newsholme et al., 2003). In the body glutamine is the most abundant free amino acid (Labow and Souba, 2000).

It is also known that apart from being an energy source for intestinal epithelium cells, glutamine is a precursor of neurotransmitters and other important molecules and it is essential for cell proliferation and immune functions (Haynes et al., 2009).

A large number of cells and tissues utilize glutamine at high rates. These are tissues of kidney, liver, cells of immune system and pancreatic ß-cells. It should be mentioned that the first product of glutamine metabolism in most cells is glutamate, which is produced by the activity of glutaminase, an enzyme associated with mitochondria and found in the body at high concentration (Newsholme et al., 2003). Recently it has been also shown that glutamine is able to regulate gene expression (Wang et al., 2008).

Much of the available glutamine (55–70%) is extensively metabolized in the intestine and oxidation to carbon dioxide is its major metabolic fate (Burrin and Stoll, 2009). Carbon atoms which remain after oxidation are used to produce other amino acids, including citrulline, ornithine, arginine and proline, which are released into the blood circulation (Windmueller and Spaeth, 1975). Glutamine is a major energy source for piglet enterocytes (Wu et al., 1995) and it is an essential precursor for the synthesis of purine and pyrimidine nucleotides that are crucial for proliferation of cells (Curi et al., 2005). As a substrate of glutamate, glutamine plays a role in the synthesis of glutathione, the most abundant small-molecular antioxidant in the small intestine (Wu et al., 2004).

Glutamine has been shown to be an antiapoptotic agent in the intestine (Brasse-Lagnel et al., 2010). Various mechanisms proposed to explain the protective effects of glutamine against apoptosis were reviewed by Ban and Kozar (2010). Glutamine can increase the expression of ornithine decarboxylase to promote intestinal restitution and prevent cytokine-induced apoptosis in intestinal epithelial cells via the pyrimidine pathway. More directly, glutamine can upregulate the expression of anti-apoptotic proteins and downregulate the expression of proapoptotic proteins (Chang et al., 2002). The protective effect of glutamine on intestinal mucosa may also be related to the induction of cytoprotective proteins, such as the heat shock protein family (Ropeleski et al., 2005).

Wu and Knabe (1994) quantified free and protein-bound amino acids in sow's colostrum and milk between days 1 and 28 of lactation. It turned out that concentration of free glutamine in milk increased progressively with advancing lactation, and reached the highest value at the end of the test. Concentration of arginine was substantially lower than that of glutamine. The lactating sow's mammary gland takes up large amounts of arginine and branched-chain amino acids – Leu, Ile and Val (Trottier et al., 1997) – and actively degrades them to form proline and glutamine (O'Quinn et al., 2002). Thanks to this mechanism, sow's milk is rich in glutamine and proline but not in arginine. Glutamine taken up by piglets in milk is converted into citrulline in mitochondria of enterocytes, and the subsequent conversion of citrulline into arginine takes place in the cytosol (Dillon et al., 1999). This indicates that glutamine plays an important role in intestinal synthesis of citrulline and that abundance of glutamine in sow's milk is of nutritional and physiological significance for compensating for low concentration of arginine in neonatal piglet diet (Wu et al., 2007).

According to Wu and Knabe (1995), sow's milk provides at most 40% of arginine requirements of the 7-day-old piglets. Deficiency of this amino acid is one of the main factors limiting piglet growth (Kim et al., 2004). The rate of synthesis of citrulline from glutamine in enterocytes of pre-weaning, 14- to 21-day-old piglets

is low but it increases 10- to 20-fold in post-weaning pigs (29–58 day of age), thus providing sufficient amount of this amino acid for endogenous arginine synthesis. Arginine is therefore an essential amino acid for suckling piglets but not for older pigs (Easter et al., 1974; Wu et al., 1994).

Results of the artificial rearing system indicate that the biological potential for neonatal piglet growth is about 400 g per day, i.e. more than that for sow-reared piglets, and that suckling piglets start to exhibit submaximal growth from the 8th day after birth (Boyd et al., 1995). This reduction in growth occurs around the time when intestinal synthesis of citrulline and arginine is markedly reduced (Wu and Knabe, 1995). According to Wu and Knabe, synthesis of citrulline and arginine from glutamine decreases by 70–73% in 7-day-old suckling piglets in comparison with newborn ones. Wu et al. (2007) found that dietary supplementation with 0.2 and 0.4% of arginine for 7- to 21-day-old milk-fed pigs (artificially reared on a liquid-milk feeding system) dose-dependently enhanced plasma arginine concentrations (30% and 61%) and increased weight gain by 28% and 66%.

Production of proline from glutamine or directly from arginine is another way of glutamine metabolism in the small intestine epithelium. This synthesis is important from the viewpoint of nutrition because it is probably the main source of proline contained in body proteins (Jones, 1985). This reaction occurs in older animals only and thus proline is an essential amino acid for young piglets weighing about 2.5 kg but not for older ones weighing about 13.5 kg (Mertz et al., 1952).

A more accurate insight into the mechanism of glutamine activity can be obtained by examination of its effect on expression of genes crucial for metabolism and function of intestines. Such an examination was conducted in two experiments by Wang et al. (2008) who used the microarray technique. In the first experiment they obtained small intestine from 28-day-old piglets weaned at the 21st day of age and from age-matched suckling piglets. In the second experiment other piglets from weaning at the 21st to the 28th day of age received a diet supplemented with 1% of glutamine or isonitrogenous alanine as a control. Daily body weight gain was reduced by 47% in weaned piglets compared with age-matched suckling pigs. Glutamine supplement increased weight gains by 19% compared with the control group. Early weaning resulted in increased (52–346%) expression of genes related to oxidative stress and immune activation but decreased (35–77%) expression of genes related to macronutrient metabolism and cell proliferation in the gut. Dietary glutamine supplementation increased intestinal expression of genes that are necessary for cell growth and removal of oxidants, while reducing expression of genes that promote oxidative stress and immune activation.

Concentrations of reduced glutathione (GSH) in jejunal tissue were 25% lower in weaned than in sow-reared piglets and weaning increased jejunal concentration of oxidized glutathione (GSSG). As a result, the GSSG:GSH ratio (an indicator of oxidative stress) was 59% greater in weaned than in sow-reared piglets. Dietary glutamine lowered this ratio by 38%.

In conclusion Wang et al. (2008) state that results of the microarray analysis reveal that early weaning resulted in increased expression of genes that promote oxidative stress and immune activation but decreased expression of genes related to

nutrient utilization and cell proliferation in the piglet small intestine. Dietary supplementation of glutamine to weanling piglets enhanced expression of genes that prevent oxidative stress, improve antibacterial activity, enhance nutrient absorption, and stimulate cell growth.

Zhong et al. (2011) conducted an experiment to determine the effects of oral glutamine supplementation on growth performance, intestinal morphology, and expression of heat shock protein (Hsp) 70 in piglets weaned at 21 days of age. Piglets received 0 or 1 g of oral glutamine per kg of body weight every 12 hours. Average daily gain and feed intake were greater in piglets supplemented with glutamine. The weights of their jejunum and ileum were also greater while the incidence of diarrhoea was 24% lower than that in control piglets. The villus height in the jejunum and the ileum was also greater in piglets receiving glutamine. The expression of *hsp70* mRNA and Hsp70 proteins in the duodenum and jejunum was greater in piglets supplemented with glutamine but this supplementation had no effect on the expression of *hsp70* mRNA and Hsp70 proteins in the ileum. These results indicate that glutamine supplementation may be beneficial for intestinal health and development and may thus mitigate diarrhoea and improve growth performance. The protective mechanisms of glutamine in the intestine may be associated with the increase in Hsp70 expression.

As can be seen from this short review, glutamine, which for a long time was regarded as a non essential amino acid from the viewpoint of nutrition, is involved in a number of metabolic processes especially important for a correct morpho-functional development and assessment of small intestine in young animals.

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Glutamina jako dodatek do paszy dla prosiąt: przegląd

STRESZCZENIE

Odsadzenie jest decydującym momentem w życiu prosiąt. Zmniejsza się wówczas spożycie paszy i zachodzą niekorzystne zmiany w nabłonku jelita cienkiego. W tym okresie niezbędne jest odpowiednie żywienie zapewniające prawidłowy rozwój przewodu pokarmowego. Jedną z substancji mogących dostarczyć komórkom nabłonka jelitowego niezbędnej energii jest aminokwas glutamina. Poprawia ona strukturę nabłonka stymulując wzrost kosmków będących miejscem absorpcji składników odżywczych, co poprawia wykorzystanie paszy i wskaźniki produkcyjne. Glutamina poprawia również zdrowotność prosiąt poprzez korzystne zmiany w mikroflorze jelitowej. Jelito cienkie jest, oprócz wątroby i nerek, głównym miejscem przemian metabolicznych glutaminy. Prowadzą one do syntezy nukleotydów purynowych i pirymidynowych, a także ważnego przeciwutleniacza – glutationu. Glutamina jest także prekursorem dla syntezy proliny i argininy wchodzących w skład białek organizmu. Wpływa na obniżenie ekspresji m.in. genów odpowiedzialnych za stres oksydacyjny, a zwiększa ekspresję genów niezbędnych do wzrostu komórek. Dzięki tym właściwościom glutaminę można uznać za aminokwas niezbędny w żywieniu odsadzonych prosiąt.

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THE USEFULNESS OF PREBIOTICS AND PROBIOTICS IN MODERN POULTRY NUTRITION: A REVIEW*

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Abstract

A probiotic is a culture of live microorganisms that can manipulate and maintain a beneficial microflora in the gut. Prebiotics are nondigestible feed ingredients that can positively affect the animal organism by stimulating the activity and growth of beneficial native bacteria in the gastrointestinal tract and eliminate the pathogenic ones. Some studies have shown their beneficial effects when they have been used separately or simultaneously in the form of synbiotics, to obtain enhanced mutual effect. These supplements were proposed with success as alternatives to antibiotic growth-promoting feed additives but further studies are needed to better understand their mode of action and effects. This review article presents growing interest in using these antibiotic alternatives, the potential mechanism of their action in the live organism, and discusses some recent data on the effects of these supplements in poultry nutrition.

Key words: probiotic, prebiotic, synbiotic, poultry, laying hens, broilers

Besides quality attributes, special attention in recent years has been paid by the consumers to safety of animal products. Considering some evidence that the use of antibiotic growth promoters (AGP) may cause pathogen resistance (Phillips et al., 2004), the application of antibiotics as animal growth enhancers had already been prohibited in the European Union since 2006. Today's animal farming, especially the poultry industry have been greatly intensified with respect to both large number of animals and modern feeding systems. Concerns about the losses in animal performance and thus sustainability of production and its profitability coupled with this ban have led to an increase in research on the alternative supplements to AGP and strategies for food-producing animals.

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To date a number of products, including essential oils and plant extracts, spices, organic acids, probiotics and prebiotics have been recognized and proposed as antibiotic alternatives in farm animal nutrition. Although most of them have generated attention, extensive studies are primarily focused on prebiotics and probiotics. Probiotic means "for/in favour of life". This term was introduced into the literature by Lilly and Stillwell (1965). It contrasts with the term antibiotic, which means "killing life".

Today, the most accepted definition states that probiotics are mono or mixed cultures of live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002).

Unlike probiotics, prebiotics are not microorganisms – they are a sort of nourishment source for existing flora, allowing the natural colony of gut to grow naturally and replicate. Prebiotics were defined as non-digestible food (feed) ingredients that beneficially affect the host by selectively stimulating the growth and/or activities of one or a limited number of bacteria in the gut, thereby improving host health (Gibson and Roberfroid, 1995). However, more recent definitions stated that a prebiotic is a selectively fermented ingredient that allows specific changes, in both the composition and activity in the gastrointestinal microbiota which confers benefits to the host (FAO/WHO, 2002). The common point of these definitions is that prebiotics are characterized by a selective effect on the microbiota, and thus can improve the host health. Prebiotics include mainly oligosaccharides, sugar molecules of three to six chains, and soluble fibre. These carbohydrates are found naturally in fruit and vegetables (Charalampopolus and Rastall, 2009).

Nutritional supplements combining probiotics and prebiotics are referred to as synbiotics, which are a combination of "a probiotic and a prebiotic that beneficially affects the host by improving the survival and establishment of live microbial dietary supplements in the gastrointestinal tract" (Trachoo et al., 2008). The main importance of this form of synergism is that a probiotic alone, i.e. without a source of nourishment which can be represented by a prebiotic, cannot survive well in the digestive system (Bhupinder and Saloni, 2010). Synbiotics are gaining popularity and scientific credibility as functional food (feed) supplements at nutritional and therapeutic levels. It is believed that they can ensure a high level of viable probiotic cells once ingested (Trachoo et al., 2008). Some studies have shown the importance and benefits of this kind of synergy between probiotics and prebiotics and the effectiveness in helping young animals to achieve better growth performance (Patterson and Burkholder, 2003).

Aims of the use of probiotics, prebiotics and synbiotics as feed supplements

Pathogenic bacteria control

Many studies have focused on the ability of probiotic additives to reduce and control pathogenic bacteria. Probiotics can provide antimicrobial substances that may be effective at the same level as antibiotics, especially in stress conditions, high temperature and abnormal intestinal pH. According to Charalampopolus and Rastall (2009) probiotics show high efficiency in reducing colonization of *Salmonella* and *Campylobacter*. Moreover, they can modulate immunological response

and suppress inflammatory immune reactions in the intestinal walls preventing tissue damage (Ferreira et al., 2011).

Prebiotics containing xylose, fructose, galactose, mannose and glucose, earned much attention and appear to be particularly promising (Gibson and Roberfroid, 1995; Patterson and Burkholder, 2003). Some of them have proved the protection against *Salmonella* (Charalampopolus and Rastall, 2009) by providing binding sites for pathogenic bacteria flushing out of the digestive tract. Spring et al. (2000) screened some bacterial strains for their ability to agglutinate mannanoligosaccharides in yeast cell preparations (*Saccharomyces cerevisiae*). Five of seven strains of *E. coli* and 7 of 10 strains of *Salmonella typhimurium* and *Salmonella enteritidis* agglutinated MOS and *S. cerevisiae* cells.

Improved health and production performance

Many beneficial effects of probiotics were suggested, such as improved immune system, modification of gut microbiota, reduced inflammatory reactions, decreased ammonia and urea excretion, lower serum cholesterol, and improved mineral adsorption; on the other hand probiotics may have an indirect positive impact on performance parameters and production profitability (Ferreira et al., 2011).

A number of studies have shown improvements in growth performance, decreased mortality and morbidity or increased resistance to colonization by pathogens associated with feeding prebiotics. Numerical improvements in performance may be economically important on large-scale production farms (Patterson and Burkholder, 2003).

Reduced antibiotic use in animal agriculture

Pro-, pre- and synbiotics have been studied for their potential to replace antibiotics, as the latter contribute to the acquisition of resistance in the bacterial flora of livestock. The desired effects on animals were represented by maintaining high growth performance, particularly in poultry and swine, or diminution of methane production by ruminants (Charalampopolus and Rastall, 2009). The use of probiotics and prebiotics for long-term consumption and prophylactic approaches is much more safe as they do not cause side effects, such as antibiotic associated diarrhoea, sensitivity to UV radiation or liver damage, and do not stimulate antimicrobial resistance genes and allergic inflammatory response (Ferket, 2003; Lee and Salminen, 2009). In spite of promising results in preliminary studies, further research is needed to create an overall management strategy to match the performance efficacy comparable to that of antibiotics (Patterson and Burkholder, 2003).

Probiotics

Probiotic is a culture of living microorganisms that are used as functional ingredients to manipulate and maintain good health by controlling gut microflora and increasing digestive enzyme activity. Probiotics were defined by Fuller (1992) as "live microbial food supplements which beneficially affect the host either directly or indirectly by improving its intestinal microbial balance".

The probiotic microorganisms can have an effect only by surviving the digestive conditions, including bile acids, and these must facilitate their colonization of the

gastrointestinal tract without any harm to the host. However, only certain strains of microorganisms have these properties. Most probiotic active cultures are members of two bacterial genera: *Lactobacillus* and *Bifidobacterium*, or belong to yeasts, especially *Saccharomyces* (Charalampopolus and Rastall, 2009).

Characteristics of ideal probiotics and their mode of actions

Probiotics display several important ways of action, an antagonistic action towards pathogen bacteria by modification of gut pH, direct antimicrobial effect by secretion of products which inhibit their development, such as bacteriocins, organic acids and hydrogen peroxide, production of short chain fatty acids (SCFA) in the intestine, regulation of the immune system of the host, normalization of gut microbiota, and different metabolic effects (Vamanu and Vamanu, 2010; Ferreira et al., 2011). Another mode of action is competitive exclusion which represents colonization ability and adhering competition in the intestinal mucous membranes to prevent adhesion and invasion of pathogens and, which is a key performance parameter, inhibition of their colonization and replacement of already adhered ones; competing for available nutrient substances and growth factors (Patterson and Burkholder, 2003). A detailed list of ideal probiotics characteristics and their beneficial effects are presented in Table 1.

Table 1. Characteristics of ideal probiotics and their desirable properties (adapted from Simmering and Blaut, 2001 after Patterson and Burkholder, 2003)

Probiotics	
Properties	Positive influences
Belong to host origin	Change intestinal microbiota
Non pathogenic	Induce immune system
Resist processing and storage	Decrease inflammatory reactions
Endure gastric acid and bile	Prevent pathogen proliferation
Epithelium or mucus attaching capability	Enhance animal performance
Persist in the intestinal tract	Reduce carcass contamination
Produce repressive compounds	Decrease ammonia and urea excretion
Immune response regulation	
Modify microbial activities	

Most used probiotic genera

A variety of microbial species have been used as probiotics with nearly 20 known species, which beneficially affect the host by improving its intestinal microbial balance (Bhupinder and Saloni, 2010). Various types of probiotic bacteria include species of *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, other bacteria and a kind of yeast species, and indefinable mixed cultures. *Lactobacillus* and *Bifidobacterium* species have been used above all in humans, whereas species of *Bacillus*, *Enterococcus*, and *Saccharomyces* yeast have been the most predominant organisms used in livestock (Ferreira et al., 2011). However, recently, enhanced performance was noticed when feeding *Lactobacillus* to livestock (Vicente et al., 2007; Vicente et al., 2008).

These bacteria species are often grouped according to their common metabolic, morphological and physiological characteristics rather than a phylogenetic class. This entails bacteria known as lactic acid bacteria (LAB). LAB includes species of *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus* and *Leuconostoc* genera. Recent taxonomic adjustments have proposed several new genera and the rest of the group is now represented by the following: *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Globicatella*, *Lactococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella*. *Lactobacilli*, *Carnobacteria* and some *Weissella* are rods while the rest of genera are cocci (Jin et al., 2009). Classification of LAB genera was based on morphology, mode of glucose fermentation, growth at certain temperatures, and range of sugar utilization. These bacteria are gram-positive, nonsporulating, non-respiring cocci or rods, which do, through fermentation of carbohydrates, produce lactic acid as their major end product (Kolida and Gibson, 2011). Practically, the major part of the antimicrobial activity demonstrated by probiotics can be assigned to the production of lactic acid (Makras et al., 2006). The remaining unlisted probiotic microorganisms belong to the non-lactic acid bacteria like *Bifidobacterium* species or *Saccharomyces* yeast.

Prebiotics

Most used prebiotics

Various types of oligosaccharides, including inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), soya-oligosaccharides (SOS), xylo-oligosaccharides (XOS), pyrodextrins, isomalto-oligosaccharides (IMO) and lactulose, are commonly considered as prebiotics. But the majority of studies completed to date point to inulin, FOS and GOS (Macfarlane et al., 2008). An array of prebiotics exists and the most reported in a large number of studies and with the most consistent evidence accumulated for the effects of prebiotics have been non digestible oligosaccharides (NDOs), which results from various origin and chemical properties (Chen and Chen, 2004; Macfarlane et al., 2008; Zduńczyk et al., 2011). The regulatory regimes for NDOs have been under active review in many countries in recent years. Fructooligosaccharides, and the polyfructan inulin, galactooligosaccharides, lactulose and polydextose are recognized as the established prebiotics, whereas lactosucrose, xylo-, isomalto-, and soybean-oligosaccharides are categorized as emerging prebiotics (Piva, 1998). To date, only three oligosaccharides: fructans (inulin and fructooligosaccharides), galactooligosaccharides and lactulose have accomplished prebiotic status in the European Union (Kolida and Gibson, 2011).

Animal feeding and *in vitro* trials data showing possible bifidogenic effects have been indicated for gluco- and galactomannan oligosaccharides, alpha-glucooligosaccharides, pectic-oligosaccharides, gentiooligosaccharides, and oligosaccharides from agarose (Cao et al., 2005; Macfarlane et al., 2008; Zduńczyk et al., 2011). Chicory root inulin-derived fructooligosaccharides, xylooligosaccharides and wheat bran-derived arabinoxylooligosaccharides (AXOS) were shown to have ample applications (Xu et al., 2003). Lactulose, mannitol, maltodextrin, raffinose, and sorbitol are also prebiotics with established health benefits (Vamanu and Vamanu, 2010).

In animals, prebiotics have a long history. Many ancient farmers guided animals to specific pastures in order to obtain the desired prebiotic from the pasture (Charalampopolus and Rastall, 2009). In recent years prebiotics have been commonly utilized in *in vivo* feeding experiments with a range of companion and livestock animals, such as poultry, cattle, pigs and horses, to investigate the effects on gut microflora, immunomodulation of the host, suppressive effects on the enteric and systemic infections by pathogens, nutrient digestibility, performance indices, quality of products of animal origin and general welfare of animals (Charalampopolus and Rastall, 2009). Gibson and Roberfroid (1995) originally classified prebiotics, as defined previously; this definition, however, was based on prebiotics use in humans and their use in animals and especially in poultry and ruminants may be more complicated.

Characteristics of ideal prebiotics and their mode of actions

Prebiotics beneficially interact with animal's physiology by selectively stimulating favourable microbiota in the intestinal system. This may have valuable effects in reducing the incidence of enteric pathogens. However, from the available published evidence, the exact mechanism involved in prebiotics to reduce pathogenic infections is still unclear. Competitive exclusion of pathogens by increasing numbers of microbiota that are associated with a healthy host can produce a variety of bacteriocins that have a detrimental effect on the pathogen by promotion of macrophages, stimulation of antibody production, and antitumour effects (Vamanu and Vamanu, 2010).

Contrary to these microbiota-dependent theories, it may be that prebiotics are able to directly affect the pathogen or host in a microbiota-independent manner. It has been suggested that the principal mechanisms of prebiotics is immunomodulation, that includes selective growth of lactic acid-producing bacteria, resulting in an increased concentration of SCFA like acetate, propionate, and especially butyrate which is the preferred energy source of colonocytes and stimulates gut integrity. High fermentation activity and high concentration of the SCFA is correlated with a lower pH, which is associated with a suppression of pathogens and increased solubility of certain nutrients (Józefiak et al., 2004). This increase of SCFA with immune cells, direct contact in the digestive tract and the change in mucin production contribute to lower incidence of bacteria moving across the gut barrier (Lee and Salminen, 2009). This phenomenon may inhibit some pathogenic bacteria and reduce colonization of some species like *Salmonella* and *Campylobacter* (Charalampopolus and Rastall, 2009).

Other beneficial effects of probiotic addition can be reflected in increased intestinal enzymes secretion, diminution of ammonia and phenol products, and promotion of resistance to pathogenic bacteria proliferation in the gut (Yusrizal and Chen, 2003 b). The advantage of prebiotics compared to probiotics is that they promote growth of useful bacteria which are ubiquitous in the host organism and are capable to survive in all environmental conditions.

Fructooligosaccharides selectively promote the growth of beneficial bacteria by acting as a source of nutrients. FOSs can be found in a variety of foods, including

onions, garlic, banana and asparagus (Charalampopolus and Rastall, 2009). FOS is neither hydrolyzed nor absorbed in the upper gastrointestinal tract and acts as a food source for host-beneficial bacteria, which competitively excludes pathogenic bacteria.

Mannanoligosaccharides provide alternate binding sites for pathogenic bacteria. These are typically derived from yeast (*Saccharomyces cerevisiae*) outer cell wall components. According to Ferket (2003) supplementary dietary MOS improves animal resistance to enteric disease and promotes growth by six different means: (1) restricts colonization of enteric pathogens by inhibiting bacterial adhesion to gut lining; (2) improves the brush border mucin barrier; (3) changes microflora fermentation to favour nutrient availability for the host; (4) improves immunity; (5) enhances the unity of the gut lining; and (6) brings down enterocyte turnover rate. In Table 2 are presented the most important characteristics of prebiotics and their positive effects on animal organism.

Table 2. Characteristics of ideal prebiotics and their desirable properties (adapted from Simmering and Blaut, 2001 after Patterson and Burkholder, 2003)

Prebiotics	
Properties	Positive influences
Not hydrolyzed or absorbed	Higher SCFA production
Selectively stimulate growth of one or a limited number of beneficial bacteria	Better biomass and stool bulking
Beneficially modify the intestinal microbiota activities	Enhanced vitamin B synthesis
Positively modulate host defence system	Positively affected mineral absorption
	Cancer prevention
	Decrease in blood cholesterol level
	Diminution in ammonia and urea excretion
	Lower excreta content of skatole, indole, phenol, etc

Action of prebiotics in animal organism

The principal effect of prebiotics is to stimulate the resident microbiota of host to proliferate, to stop harmful bacteria, and to share health benefits to the host. The supplementation of poultry and pig diets with oligosaccharides is generally associated with stimulation of microbiota proliferation. Specifically, in pigs, GOS has been associated with increasing the numbers of bifidobacteria in both *in vivo* and *in vitro* evaluations (Charalampopolus and Rastall, 2009). Prebiotic oligosaccharides supplementation of the diets, such as FOS, may have an effect on improvements in the gut microbial population, including a reduction in *Salmonella* colonization. This suggests that supplemental dietary FOS may be a viable option in both *Salmonella* control and antibiotic free programmes.

MOS have been used to modulate the resident gut flora of the host and subsequently reduce the incidence of pathogen colonization by binding and eliminating pathogens from the intestinal tract and stimulation of the immune system (Spring et al., 2000; Fernandez et al., 2002). Concerning the prebiotic use to reduce pathogens

in livestock, the majority of studies have been performed in poultry, with positively influenced growth and improved host intestinal health (Spring et al., 2000). According to Macfarlane et al. (2008), supplementation with GOS increases the growth of certain gastrointestinal bacteria, especially the LAB, bifidobacteria, and/or their fermentation products. Fermentation products such as SCFA increase after prebiotic supplementation as a result of oligosaccharide fermentation by resident microbiota. SCFA production is an important physiological process of colonic microorganisms and may be useful in improving gastrointestinal health by reducing the occurrence of diarrhoea through modulating the microbiota (Macfarlane et al., 2008).

Macfarlane et al. (2008) reviewed the importance of the colonic microbiota for improvement of host's immune system, and underlined that lactobacilli and bifidobacteria have been linked to an increase of sIgA levels and phagocyte number. *In vitro* and *in vivo* studies have been conducted to show the relation between prebiotics and immune system (Fernandez et al., 2002). Apparently, prebiotics have not only an immune-stimulatory effect on the host but can act as adjuvants to boost vaccine induced immune responses.

Other prebiotic effects have been investigated for the effect on lipid metabolism, mineral absorption and reduction of fatty acid synthesis (Macfarlane et al., 2008).

Use of lactose derived from whey as a prebiotic

Whey products are consumed in varying amounts by humans and a wide variety of animal species, including swine, young ruminants (calves), dogs and cats, poultry and aquaculture. Lactose (disaccharide sugar formed from galactose and glucose) derived from whey products is highly functional and growing interest in its prebiotic effects is currently observed (Szczurek, 2008). Purified natural lactose or whey powder, a product with high (70–80%) lactose content, are obtained in a series of steps of liquid whey processing with membrane filtration technology (Bednarski, 2001). Unlike mammals, birds lack the enzyme lactase (β-galactosidase) required to digest lactose.

Studies with above whey products in poultry diets reviewed by Szczurek (2008) have shown some inhibiting effects of lactose on *Salmonella* and other pathogenic bacteria in the digestive tract of broiler chickens by production of SCFA and lactic acid from lactose as a substrate for host bacteria enzymes, with deep reduction in cecal pH. Whey lactose may also act in favour of enhancing immunity, improving survival rates, and stimulating growth of beneficial intestinal bacteria (Majewska et al., 2009). However, data from some experiments have shown that the high lactose inclusion in the diet may decrease performance of birds grown to market age and cause incidence of diarrhoea (Kermanshahi and Rostami, 2006). Thus, there is a need for further comparative studies with broilers to determine the optimum dietary level of lactose and/or dried whey with high lactose content.

Synbiotics

Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a synergistic form. The principal reason for using a synbiotic is the conception that a probiotic, without its prebiotic substrate does not survive well in the diges-

tive system. Without the necessary source of nutrients for the probiotic, it will have a more important intolerance for oxygen, low pH, and temperature. As prebiotics furnish better conditions for probiotics to expand, the colonies of these "good" bacteria are maintained. Studies have shown that by using the benefits of both prebiotics and probiotics, the number of desirable bacteria in the digestive system increases, and as a result the positive effects on health status can be observed. Such positive influences of synbiotics are obtained in two ways: (1) by improving the viability of probiotics and (2) by delivering specific health benefits (Bhupinder and Saloni, 2010).

In the last few years, studies on synbiotics have started to appear, with the main role on applications against diseases (Kolida and Gibson, 2011). The intake of a synbiotic leads to a regulation of the gut metabolic activities with a maintenance of the gut biostructure. Particularly, the significant increase of SCFA, carbon disulfide, ketones, and methyl acetate indicated possible health promoting effects of the synbiotic feed supplements.

Use of probiotics, prebiotics and synbiotics in poultry nutrition

Due to the ban in Europe many alternatives to AGP have been evaluated in poultry production with mixed results. Most of the experiments conducted with pro-, pre- and synbiotics have focused on improving the microbial health, performance, and decreasing carcass contamination of young meat birds.

Reported effects in broiler chickens

Broiler chicks display very fast growth rates, attaining more than 60 times their hatching weight at 6 weeks of age. This high growth rhythm is reached thanks to genetic selection, improved housing techniques, sanitary and veterinary care, and extremely balanced high-energy diets.

The effect of probiotics on broilers and layer hens were thoroughly reviewed by Fuller (1992), and more recently by Kabir et al. (2004) and Kabir (2009), who confirmed the existence of some effects. However, after a number of years, there is still insufficient evidence regarding the efficacy of probiotics in poultry other than for the competitive exclusion of pathogens. Many of the studies conducted today still remain poorly designed, with either limited number of animals or insufficient statistical analysis. However, there seems to be a unique point of view about the efficiency of probiotics in poultry when they are kept under suboptimal conditions (Fuller, 1992).

One of the most successful probiotic bacteria used in poultry are *Bacillus subtilis* (Lee and Salminen, 2009). Apart from improving the growth performance, *B. subtilis* is also efficient in inhibiting the growth of pathogens in the digestive tract of chickens, which can lead to a considerable economic loss. Dietary supplementation with *B. subtilis* could improve also the performance, body weight, and immune response. Lee et al. (2010) investigated the effect of *Bacillus* on *Eimeria maxima* infection in broiler chickens, and found that *Bacillus subtilis* reduced the clinical signs of experimental avian coccidiosis and increased various parameters of immunity in broiler chickens. A *Lactobacillus* probiotic was shown to increase the quantity of lactic acid producing bacteria and decrease the gut lesion score of broilers infected

with coccidiosis, and *Salmonella* (Vicente et al., 2008). In the experiment of Brzóska et al. (2012) the beneficial effect of *Lactobacillus* Spp. or *Lactococcus lactis* bacteria on chicken liveability was observed, but the probiotics used had no effect on body weight gain and feed conversion.

Prebiotic research on poultry has been performed since 1990 and, as a result, a large database of research is accessible in this area. Prebiotics in broiler diets have been shown to increase lactobacilli counts in the gastrointestinal tract (Xu et al., 2003; Yusrizal and Chen, 2003 b; Baurhoo et al., 2007). Also, increased bifidobacteria and decreased clostridia have been reported in some studies that investigated the microbial effects of prebiotic supplementation (Sims et al., 2004; Cao et al., 2005). Some authors reported decreased *Salmonella* and coliforms (Fernandez et al., 2002; Spring et al., 2000), while others observed a decrease in *E. coli* (Xu et al., 2003; Zduńczyk et al., 2005; Baurhoo et al., 2007). Some other pathogenic bacteria like streptococci, staphylococci, bacilli, and yeast, have also been reported to decrease with prebiotic supplementation (Samarasinghe et al., 2003; Cao et al., 2005).

Prebiotic supplementation of poultry diets modifies fermentation profiles. Increased butyrate concentrations were reported (Zduńczyk et al., 2004; Yang et al., 2008). Supplementation with FOS decreases cecal indole and phenol concentrations (Cao et al., 2005). Total SCFA and lactic acid concentrations more often increased with intestinal pH decrease when prebiotics were supplemented (Yang et al., 2008; Zduńczyk et al., 2005). Regarding intestinal morphology, increased intestinal villus height was reported when prebiotics were included in the broiler diet (Xu et al., 2003; Baurhoo et al., 2007). Other changes of intestinal characteristics have been observed, including increased gut length (Yusrizal and Chen, 2003 a). Some authors studied also the effect of prebiotics on mineral utilization and bone quality in broilers, but the results were not positive (Świątkiewicz and Arczewska-Włosek, 2011).

Performance parameters in broilers have been evaluated with prebiotic supplementation. Body weight was reported to increase in the majority of studies (Yusrizal and Chen, 2003 a; Sims et al., 2004; Zduńczyk et al., 2005). In parallel, body weight gain, feed conversion and carcass weight were improved (Samarasinghe et al., 2003; Xu et al., 2003; Yusrizal and Chen, 2003 a; Sims et al., 2004; Józefiak et al., 2008; Yang et al., 2008). Feed intake and feed:gain ratios (F:G) generally decreased with supplementation of fructans and MOS (Baurhoo et al., 2007; Samarasinghe et al., 2003; Xu et al., 2003; Yusrizal and Chen, 2003 a). Also, MOS and inulin supplementation increased carcass weight and abdominal fat weight (Samarasinghe et al., 2003; Yusrizal and Chen, 2003 a).

Synbiotics are relatively recent among additives used in poultry nutrition. Studies have suggested that performance can be further enhanced when using both prebiotics and probiotics. Investigations demonstrated that synbiotics are much more efficient when used in combinations than singly (Ušćebrka et al., 2005). As an example, Fukata et al. (1999) found in broilers that a probiotic and FOS each reduced intestinal *Salmonella enteritidis* colonization when used singly, but their combination was more effective.

Reported effects in laying hens

Some studies have shown that pro- and prebiotics would enhance the performance of egg laying birds by using them during stress periods, at the first three to four weeks of life, and immediately prior to and after the move from pullet house to layer house (Radu-Rusu et al., 2010; Zarei et al., 2011). It has been suggested that the continuous feeding of a probiotic will improve layer performance, feed cost/dozen eggs and feed cost/kg eggs compared to the control and control plus antibiotic treatments.

Zarei et al. (2011) fed laying hens on diets supplemented with some commercial pro-, pre- and synbiotics, and reported increased egg mass and weight, and egg shell weight and thickness.

Chen et al. (2005 b) demonstrated some changes in digestive system, mainly by an elongation of both small and large intestine in laying hens receiving FOS supplementation. As a consequence, increased egg production and improved feed efficiency were observed. Furthermore, FOS supplementation increased egg shell strength by skeletal and plasma calcium levels augmentation (Chen and Chen, 2004) and reduced yolk cholesterol concentrations without affecting yolk weight (Chen et al., 2005 a). Significantly decreased concentration of yolk cholesterol was also reported when inulin was added to the diet for layers (Shang et al., 2010). The results of some studies with hens have also shown that such prebiotic fructans as inulin or oligofructose may positively affect mineral utilization and in this way, improve eggshell and bone quality (Świątkiewicz et al., 2010 a, b; Świątkiewicz and Arczewska-Włosek, 2012).

As mentioned previously, prebiotics may have some protective effects against *Salmonella*, and it would be a viable option for maintaining a healthy microbial population in fasted egg layers by using prebiotics or even a synbiotic combination, especially during stress, because the number of bifidobacteria and lactobacilli populations in these periods would decrease in the gut of stressed birds.

Reported effects in turkeys

Some of probiotic effects on turkeys can be summarized in a significant increase of body weight gain, improved feed efficiency and decreased gut colonization by pathogens (Vicente et al., 2007). Improved physiological and health status with prebiotics has also been reported in turkeys. As mentioned before, Zduńczyk et al. (2005) demonstrated that a FOS inclusion in the feed led to decreasing cecal pH and increasing cecal production of SCFA, especially butyrate.

MOS are of particular interest to the turkey industry, because taking into account their rapid growth rate and longer growth period, turkeys are relatively sensitive to gut colonization by harmful bacteria species. Much of the research on MOS has focused on their ability to improve the overall performance of poults like increasing weight gain and reduce the incidence and severity of coccidiosis and necrotic enteritis in turkeys. Research has shown reduced clostridia levels in MOS fed turkeys (Ferket et al., 2003). MOS may be an alternative component to an antibiotic free management in broilers and turkeys as *Clostridium perfringens* is generally associated with nectrotic enteritis.

Vicente et al. (2007) evaluated the effect of a lactobacillus culture as a probiotic jointly with dietary lactose as a prebiotic in turkey poults challenged with *Salmonella enteridis*. An improvement of body weight and feed conversion ratio in challenged birds was observed while no differences in unchallenged poults were noted. However, Buteikis et al. (2008) found that the addition of dietary lactose or a compound of lactose with probiotic negatively alter the growth rate of turkeys, but had a positive effect on mortality of turkeys. This suggested that dietary lactose with appropriate probiotic organisms may act differently on poult performance.

Conditions and prospects for the use of these dietary supplements in poultry

Live microorganisms, together with enzymes and feed additives of biological origin were added to the list of feed additives regulated by the European Union in the 1980s due to the emerging market trends.

The term "probiotics" has been declined on the grounds of being too generic. In 2002, under the framework of establishing the European Food Safety Authority, a new draft regulation would group microorganisms as "zootechnical additives," defined as agents producing beneficial effect on gut microflora. This proposal was adopted in 2003, when the European Commission passed a new regulation (EC) No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition.

The microorganisms included in probiotic preparations should be generally recognized as safe (GRAS) to be used in Europe. This status can be achieved by a positive safety evaluation by qualified, independent experts, with respect to the target species. It should be resistant to bile, stimulate immune system, have reduced intestinal permeability, produce lactic acid, and be able to survive in both acidic environment of the stomach and alkaline environment of the duodenum. It is very important that a probiotic remain viable during processing and transit through the gastrointestinal tract (Simmering and Blaut, 2001). These ranges of test evaluations may vary from one country to another and are often very complicated and difficult, which can limit the number and the approval of these kinds of products, especially in the case of multispecies or symbiotic combinations which may have better advantages compared with the single use, but because of the rigorous regulations, feed additives available on the market contain generally only one, or exceptionally two strains (Applegate et al., 2010).

These additives must satisfy several criteria with regards to their identity, characteristics, and conditions for use of the additive; their safety of use in animals, humans, and environment such as the lack of pathogenicity and production of antibiotics and antibiotic resistance; and their efficacy on animals or categories of the target animal species such as improved zootechnical performance, reduction of morbidity and mortality. It is not easy to select and introduce the optimal quantities at the optimal conditions in an adequate way.

Authorization of feed additives in Europe is granted by The European Food Safety Authority (EFSA), which evaluates the data delivered on safety, efficacy, and toxicology of the feed additive. Once the commission is satisfied with the data, it prepares a draft regulation to grant authorization, following the procedure involv-

ing Member States within the Standing Committee on the Food Chain and Animal Health–Animal Nutrition. Authorizations are given for specific animal species, particular conditions of use and for 10-year periods. These are important procedures to ensure safety of probiotics and prebiotics used as feed additives that eventually contribute to their efficacy. Approved feed additives are published in the Community Register of Feed Additives (EFSA, 2005; Lee and Salminen, 2009).

Conclusion

Probiotics, prebiotics and synbiotics are gaining importance because they seem to exert their nutritional benefits in various animal species and their concept is more and more comprehensible. Poultry studies have demonstrated clear benefits in performance and health status of birds. They affect the intestinal microbiota and immune system to decrease colonization by pathogens in some conditions.

As with growth promoting antibiotics, environment and stress may have an impact on the efficiency of prebiotics and probiotics. These feed supplements can present immense potential as alternatives for antibiotics to totally eliminate antibiotic use, because they do not cause microbial resistance. The beneficial consequences of these supplements may generally translate into improved health and performance parameters. More studies are now focusing on their symbiotic effects that apparently can be more efficient than a separate use.

This represents an important perspective of applied biotechnological research that must be better understood to reveal more mechanisms through which they could positively impact poultry, but these will depend largely on the regulatory developments in the area that can bring new products for poultry and livestock, which are currently limited because of time and generated expenses of safety testing.

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MOHAMED NABIL ALLOUI, WITOLD SZCZUREK, SYLWESTER ŚWIĄTKIEWICZ

Przydatność prebiotyków i probiotyków w nowoczesnym żywieniu drobiu – przegląd

STRESZCZENIE

Probiotyki to kultury żywych mikroorganizmów, które modyfikują i utrzymują korzystną mikroflorę przewodu pokarmowego. Prebiotyki to niestrawne składniki paszy korzystnie wpływające na organizmy zwierząt poprzez stymulowanie aktywności i wzrostu korzystnych bakterii naturalnie występujących w przewodzie pokarmowym i eliminowanie bakterii patogennych. Niektóre badania wykazały ich korzystne efekty przy stosowaniu pojedynczym lub równocześnie w postaci synbiotyków, w celu nasilenia ich wzajemnego działania. Dodatki te z powodzeniem stosowano jako alternatywę dla antybiotykowych stymulatorów wzrostu w paszy, jednak konieczne są dalsze badania w celu lepszego zrozumienia sposobu ich działania i skutków. Niniejszy artykuł przeglądowy prezentuje rosnące zainteresowanie użyciem tych zamienników antybiotyków i potencjalny mechanizm ich działania w żywych organizmach, omawia także najnowsze dane dotyczące wpływu tych dodatków w żywieniu drobiu.

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ANALYSIS OF THE POSSIBILITY OF IMPROVING THE INDICATORS OF PORK QUALITY THROUGH SELECTION WITH PARTICULAR CONSIDERATION OF INTRAMUSCULAR FAT (IMF) CONTENT*

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Abstract

The aim of the study was to estimate coefficients of heritability for intramuscular fat (IMF) content and other fattening, slaughter and meat quality traits of the pig breeds raised in Poland. In addition, genetic correlations were estimated between IMF content and a group of fattening, slaughter and meat quality traits, which enables this parameter to be included in the BLUP estimation of breeding value. The experiment used Polish Landrace (PL), Polish Large White (PLW), Puławska, Hampshire, Duroc, Pietrain and line 990 animals. A total of 4430 gilts of these breeds, tested at Pig Performance Testing Stations (SKURTCh), were investigated. Heritability of IMF was at intermediate level for the two most common breeds raised in Poland (h^2 = .318 for PLW, h^2 = .291 for PL). In the group of meat quality traits, high heritability was noted for meat colour lightness (L*) measured by Minolta (from h^2 = .453 to h^2 = .572). No relationships were found between IMF level and indicators of fattening performance. The highest value observed in this group of traits concerned the genetic relationship with daily feed intake (r_G = .227) for the entire group of animals. For the PLW and PL breeds, these relationships were with feed conversion (kg/kg gain) (r_G = .151 and r_G = .167, respectively). One of the higher relationships observed were genetic correlations with water holding capacity (above r_G = -.3) and, for the PLW and PL breeds, with meat redness (a*), which amounted to r_G = .155 and r_G = .143, respectively.

Key words: pigs, heritability, genetic correlations, intramuscular fat, performance, meat quality

The current overproduction of meat, notably pork, makes its quality particularly important. Faced with a wide range of products to choose from, conscious consumers attach increasing importance to the quality of meat. This consumer attitude will have to be addressed by the first link of the animal production chain, namely breeders. Their task will be to produce breeding material with proper parameters, which

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will be passed on to the lower tiers of the production chain. To make this feasible, breeders should be provided with a system of information on the quality of breeding material being produced. The list of factors that determine this quality is long (Wood et al., 2008), from aspects of microbiological evaluation to animal housing system. However, the main determinant of several traits associated with sensory perceptions of consumers is the level of intramuscular fat (IMF). This fat has positive effects on meat texture parameters such as tenderness, palatability and juiciness, and reduces cooking and grilling loss. In higher amounts it positively affects the water holding capacity of meat and carries taste (Enser, 2004). When analysing IMF level in the domestic population of pigs (Tyra and Żak, 2010) and its unfavourable downward trend, as well as the lack of significant relationships between IMF level and basic fattening and slaughter indicators, it seems appropriate to include this parameter in the estimation of breeding value using BLUP. Positive results in this area were obtained by Schwab et al. (2006). When using standard selection we cannot improve this parameter (there is no objective method to evaluate this parameter on live animals), and selection based on intermediate indicators cannot be applied in practice (Tyra and Żak, 2012). This parameter can be introduced to BLUP evaluation using relevant results obtained from Polish Pig Performance Testing Stations (SKURTCh). The implementation of this task is conditional on subjecting a greater number of pedigree herds to station testing. Another precondition is to identify genetic parameters (coefficients of heritability) for IMF level in different breeds raised in Poland and to determine the association between IMF level, other indicators currently used in the testing, as well as other traits of economic and breeding importance. In the case of IMF level, these parameters are unknown for the domestic population of pigs. Therefore, the aim of the present study is to estimate the coefficients of heritability for breeds recorded in herd books. The study also attempts to estimate genetic correlations between IMF level and a group of fattening, slaughter and meat quality traits, which will enable this parameter to be included in the estimation of breeding value using BLUP.

Material and methods

Animals, performance test and collection of fattening and slaughter data

The experiment used purebred Polish Landrace (PL), Polish Large White (PLW), Puławska, Hampshire, Duroc, Pietrain and line 990 gilts. Animals were tested at the Pig Performance Testing Stations (SKURTCh) in Chorzelów, Mełno, Pawłowice and Rossocha. A total of 4430 gilts (1240 PLW, 2083 PL, 104 Puławska, 35 Hampshire, 152 Duroc, 208 Pietrain and 608 line 990) were investigated. Throughout the test, animals were maintained and fed individually at the testing stations according to the feeding programme (Różycki and Tyra, 2010).

The performance test started when the animals reached 30 kg and finished when they reached a final weight of 100 kg. Animals were slaughtered at a final weight

of 100 kg on average. Stunning with high-voltage electric tongs was followed by exsanguination. After 24-h chilling at 4°C, half-carcasses were measured. On the right half-carcass, backfat thickness was measured with a caliper to the nearest 0.1 cm, at five locations: at the thickest point over the shoulder, on the back above the joint between the last thoracic and first lumbar vertebrae, at three points above the loin – above the rostral edge (loin I), above the middle (loin II) and above the caudal edge (loin III) of gluteal muscle section. These measurements were used to calculate average backfat thickness from 5 measurements, which was accounted for when characterizing the test material. Next, the half-carcass was dissected into different cuts. All the dissected components, including the weight of ham without backfat and skin, were weighed. The contour of the *longissimus* muscle section was traced using plastic film on loin at the intersection of the last thoracic vertebra and the first lumbar vertebra, on the cephalic plane. Height and width of the *longissimus dorsi* muscle was measured on this contour and loin eye area was planimetered. The cuts obtained (loin and ham) were dissected into tissues to estimate carcass meat percentage, based on a regression equation calculated according to the testing station method (Różycki and Tyra, 2010).

Meat quality measurements

The following indicators of meat quality were studied: intramuscular fat (IMF) content of loin, loin colour (L*a*b*), water holding capacity, and meat pH 45 min and 24 h postmortem. The IMF content of meat was determined as raw meat using Soxhlet extraction with fat solvents (Soxtherm 406, Gerhardt). The modified Soxhlet method used in this apparatus allows for very accurate measurement while considerably shortening the extraction time. A sample for analysis was taken from the middle part of the *longissimus* muscle section behind the last rib. Colour was measured in loin muscle off the midline over the last rib. This parameter was measured on loin surface at 24 h postmortem using a Minolta CR 310. Meat pH was measured twice (45 min and 24 h postmortem) using a pH meter equipped with an insertion glass electrode (Matthäus). pH_{45} was measured in loin muscle off the midline over the last rib, and pH_{24} on *longissimus dorsi* muscle cross-section at three locations along cross-section length.

Statistical analysis

Statistical analysis was performed by analysis of variance using the GLM procedure of the SAS program (1989). The statistical model used in the calculations was as follows:

$$Y_{ijk} = \mu + d_i + f_j + \alpha(x_{ij}) + e_{ijk}$$

where:

Y_{ijk} – ijkth observation,
μ – overall mean,
d_i – effect of ith breed,
f_j – effect of jth sire,
$\alpha(x_{ij})$ – covariance on right half-carcass weight (for slaughter traits and IMF),
e_{ijk} – random error.

Differences between means for individual breeds were tested at the 5% and 1% level by Duncan's multiple range test.

The coefficients of heritability (h^2) for performance test traits were estimated from the sire component. Genetic parameters were estimated by GEN3 software using variance and covariance analysis in a hierarchical design according to the formula:

$$Y_{ijkl} = m + a_i + s_{ij} + d_{ijk} + e_{ijkl}$$

where:

Y_{ijkl} – ijklth observation,
m – population mean,
a_i – fixed effect of HYS,
s_{ij} – random effect of jth sire,
d_{ijk} – random effect of kth dam,
e_{ijkl} – random error.

Heritability (h^2 – heritability coefficient) is defined as expected share of phenotypic differences in each trait caused by differences in additive gene value of animals. Calculation of heritability (h^2) according to the method of intra-class correlation between half-siblings (sisters) of boar sires was done using the following formula:

$$h^2 = 4 \frac{\sigma^2_{IZ}}{\sigma^2_{IZ} + \sigma^2_{UN}}$$

where:

h^2 – heritability,
σ^2_{IZ} – variance between groups (sires),
σ^2_{UN} – variance within groups (sires).

Genetic correlations show the association between the additive gene effects that influence two traits whereas phenotypic correlations represent correlation between the result of measuring two traits on individual animal, calculated according to the following formula:

$$r_{Pxy/Gxy} = \frac{Cov_{Pxy/Gxy}}{\sqrt{\sigma^2_{Px/Gx} \times \sigma^2_{Py/Gy}}}$$

where:

$r_{Pxy/Gxy}$ – coefficient of phenotypic or genetic correlation between traits x and y,
$Cov_{Pxy/Gxy}$ – phenotypic or genetic covariance between traits x and y,
$\sigma^2_{Px/Gx}$ – phenotypic or genetic variance of trait x,
$\sigma^2_{Py/Gy}$ – phenotypic or genetic variance of trait y.

Results

Because the experimental animals originated from the testing stations (SKURTCh) in which the entire population of breeding animals is evaluated, the test material was represented by all the breeds raised in Poland, namely PLW, PL, Puławska, Hampshire, Duroc, Pietrain and line 990. In addition, the proportion of individual breeds representing the test material was commensurate with the breed structure of pedigree breeding. For Puławska, Duroc, Hampshire, Pietrain and line 990 animals, genetic parameters were not estimated due to their inadequate number. For this reason, standard errors mostly exceeded the values estimated for these parameters, in the case of both the coefficients of heritability and the genetic correlations. Accordingly, the tables present the results for PLW and PL breeds and, in the column, for all the breeds (including those mentioned above). Table 1 shows basic statistical characteristics for selected slaughter, fattening and meat quality traits, which are of great breeding and economic importance. In terms of fattening and slaughter traits, both maternal breeds (PLW and PL) are characterized by similar parameters except for mean backfat thickness from 5 measurements and loin eye area, for which PL animals obtained more favourable results ($P<0.05$). Meanwhile, in terms of meat quality PLW animals had a slight advantage over the PL breed ($P<0.05$) for IMF content of *longissimus dorsi* muscle, its pH measured 45 min postmortem, and water holding capacity.

Table 2 contains the coefficients of heritability (h^2) and their errors ($\pm se$) for different traits investigated. In the case of slaughter traits, the highest heritability was observed for carcass muscling traits (carcass meat content, loin eye area, meat weight of primal cuts). This ranking of the coefficients of heritability was observed for both the PLW and PL breeds and for the entire group comprising all the breeds. A relatively high heritability was found for weight of ham without backfat and skin (PLW and PL breeds) and for weight of loin (PL breed only). As regards fattening traits, the highest heritability was characteristic of lifetime daily gain (from $h^2 = .414$ to $h^2 = .512$) and station test daily gain (from $h^2 = .351$ to $h^2 = .374$), and the lowest values were observed for feed conversion (kg/kg gain) (from $h^2 = .124$ to $h^2 = .177$). Heritability of the trait of most interest to us, namely IMF, was at intermediate level for the two most common breeds raised in Poland ($h^2 = .318$ for PLW, $h^2 = .291$ for PL). In this group of traits, i.e. meat quality traits, high heritability was observed for meat colour lightness (L*) measured by Minolta (from $h^2 = .453$ to $h^2 = .572$).

Table 1. Characteristics of the test animals in terms of slaughter, fattening and meat quality traits

Traits	Total				PLW				PL			
	n	x	s	v	n	x	s	v	n	x	s	v
Slaughter:												
weight of loin – whole cut (kg)	4430	7.80	± .62	7.97	1240	7.79	± .59	7.61	2083	7.75	± .61	7.99
weight of skin and loin backfat (kg)	4430	1.57	± .44	27.9	1240	1.57	± .45	28.4	2083	1.56	± .45	28.5
weight of loin without backfat and skin (kg)	4430	6.22	± .56	9.09	1240	6.23	± .56	9.00	2083	6.18	± .55	8.90
weight of ham without backfat and skin (kg)	4430	8.91	± .69	7.81	1240	8.86	± .65	7.38	2083	8.79	± .63	7.21
mean backfat thickness from 5 measurements (cm)	4430	1.55	± .38	24.8	1240	1.52 a	± .37	24.6	2083	1.47 a	± .35	23.7
loin eye height (cm)	4430	10.2	± .76	7.43	1240	9.99	± .69	6.95	2083	10.2	± .69	6.83
loin eye width (cm)	4430	6.98	± .63	9.02	1240	6.96	± .64	9.16	2083	6.93	± .61	8.87
loin eye area (cm^2)	4430	53.8	± 6.86	12.7	1240	52.7 a	± 6.35	12.0	2083	53.4 a	± 6.47	12.1
carcass meat content (%)	4430	59.3	± 3.55	5.99	1240	58.7	± 3.23	5.51	2083	58.9	± 3.22	5.46
weight of meat of primal cuts (kg)	4430	23.5	± 1.71	7.27	1240	23.3	± 1.61	6.89	2083	23.3	± 1.57	6.76
Fattening:												
slaughter age (days)	4430	172	± 27.8	16.6	1240	171	± 26.8	15.5	2083	166	± 23.7	14.2
length of fattening period (days)	4430	83.6	± 11.6	13.9	1240	84.1	± 12.9	15.3	2083	82.9	± 11.8	14.2
daily gain, 30 to 100 kg (g)	4430	890	± 120	13.5	1240	894	± 130	14.6	2083	895	± 122	13.6
lifetime daily gain (g)	4430	606	± 87.3	14.4	1240	607	± 84.5	13.9	2083	623	± 84.8	13.6
feed conversion (kg/kg gain)	4430	2.83	± .37	13.3	1240	2.81	± .36	13.0	2083	2.83	± .38	13.6
daily feed intake (kg)	4430	2.50	± .38	15.1	1240	2.49	± .41	16.7	2083	2.52	± .39	15.6
Meat quality:												
IMF (%)	4430	1.83	± .69	37.5	1240	1.84 a	± .69	27.4	2083	1.77 a	± .66	37.4
water holding capacity (%)	3994	34.9	± 7.29	20.9	1133	35.1 a	± 6.29	17.9	1854	35.9 a	± 7.72	21.5
meat colour – L*	3186	54.9	± 3.07	5.59	983	54.8	± 2.75	5.02	1342	55.1	± 2.81	5.09
meat colour – a*	3186	15.0	± 1.50	10.0	983	14.9	± 1.45	9.73	1342	14.9	± 1.39	9.32
meat colour – b*	3186	4.05	± 1.66	40.9	983	3.77	± 1.51	40.0	1342	3.85	± 1.58	41.0
pH_{45}	2628	6.38	± .36	5.64	665	6.41 a	± .37	5.77	1289	6.36 a	± .34	5.34
pH_{24}	2628	5.61	± .17	3.03	665	5.59	± .23	4.11	1289	5.62	± .16	2.84

Values with the same superscripts show significant differences between genotypes (A, B... = P< 0.01; a, b... = P<0.05).

Table 2. Coefficients of heritability and their errors for analysed slaughter, fattening and meat quality traits

Traits	Total		PLW		PL	
	h^2	± se	h^2	± se	h^2	± se
Slaughter:						
weight of loin – whole cut (kg)	.200	± .011	.311	± .022	.414	± .024
weight of skin and loin backfat (kg)	.317	± .010	.255	± .021	.286	± .030
weight of loin without backfat and skin (kg)	.136	± .013	.298	± .021	.289	± .017
weight of ham without backfat and skin (kg)	.311	± .009	.435	± .022	.503	± .024
mean backfat thickness from 5 measurements (cm)	.327	± .012	.208	± .026	.179	± .029
loin eye height (cm)	.136	± .013	.294	± .021	.319	± .019
loin eye width (cm)	.187	± .009	.310	± .024	.418	± .020
loin eye area (cm^2)	.417	± .010	.419	± .024	.503	± .027
carcass meat content (%)	.448	± .012	.564	± .025	.614	± .026
weight of meat of primal cuts (kg)	.404	± .012	.410	± .023	.407	± .024
Fattening:						
slaughter age (days)	.168	± .027	.213	± .028	.177	± .028
length of fattening period (days)	.169	± .010	.218	± .023	.179	± .024
daily gain, 30 to 100 kg (g)	.358	± .012	.351	± .026	.374	± .024
lifetime daily gain (g)	.490	± .016	.414	± .035	.512	± .026
feed conversion (kg/kg gain)	.128	± .010	.124	± .030	.177	± .021
daily feed intake (kg)	.214	± .011	.262	± .036	.201	± .027
Meat quality:						
IMF (%)	.281	± .011	.318	± .057	.291	± .039
water holding capacity (%)	.443	± .014	.396	± .072	.372	± .024
meat colour – L*	.572	± .017	.483	± .052	.453	± .025
meat colour – a*	.546	± .016	.435	± .036	.381	± .017
meat colour – b*	.306	± .012	.299	± .045	.272	± .019
pH_{45}	.197	± .029	.251	± .091	.215	± .021
pH_{24}	.191	± .021	.263	± .095	.272	± .014

Table 3 shows both genetic and phenotypic correlations between IMF level in the *longissimus dorsi* muscle and the other slaughter, fattening and meat quality traits. In the group of slaughter traits, most genetic correlations, and especially phenotypic correlations, are low. For this group of traits, the highest relationships (genetic correlations) were observed between IMF level and weight of loin (whole cut) and loin eye area. No relationships were found between IMF level and indicators of fattening performance. For this group of traits, the highest relationships (undesirable from a breeding perspective) were observed between IMF level and loin weight (whole cut) and loin eye area, and these were genetic relationships. The highest but favourable for breeding (improved IMF content) were the relationships with mean backfat thickness from 5 measurements. No relationships were found between the level of this fat and the indicators of fattening performance. The highest value in this group was observed for the genetic relationship with daily feed intake (r_G = .227) for the whole group of animals. For the PLW and PL breeds, these relationships were with feed conversion per kg gain (r_G = .151 and r_G = .167, respectively). The phenotypic correlations in this group of traits were below r_P<.1. One of the higher relationships observed in our study were genetic correlations with water holding capacity (above r_G = -.3) and, for the PLW and PL breeds, with meat redness (a*), which were r_G = .155 and r_G = .143, respectively.

Table 3. Coefficients of genetic (r_G) and phenotypic (r_P) correlations between the level of intramuscular fat (IMF) in *longissimus dorsi* muscle and selected slaughter, fattening and meat quality traits

Traits	Total			PLW			PL		
	r_G	± se	r_P	r_G	± se	r_P	r_G	± se	r_P
Slaughter:									
weight of loin – whole cut (kg)	-.135	± .013	-.009	-.138	± .015	-.021	-.191	± .009	-.006
weight of skin and loin backfat (kg)	.131	± .010	.058	.104	± .015	.026	.133	± .008	.086
weight of loin without backfat and skin (kg)	-.147	± .015	-.057	-.077	± .011	-.043	-.030	± .011	-.063
weight of ham without backfat and skin (kg)	-.181	± .060	-.069	-.156	± .016	-.035	-.186	± .009	-.108
mean backfat thickness from 5 measurements (cm)	.151	± .011	.037	.154	± .017	.006	.182	± .009	.020
loin eye height (cm)	-.016	± .015	-.058	-.013	± .014	-.016	-.010	± .011	-.064
loin eye width (cm)	-.086	± .013	-.062	-.015	± .015	-.047	-.012	± .010	-.059
loin eye area (cm^2)	-.207	± .011	-.090	-.180	± .014	-.060	-.167	± .009	-.085
carcass meat content (%)	-.095	± .012	-.084	-.103	± .018	-.027	-.091	± .009	-.149
weight of meat of primal cuts (kg)	-.011	± .011	-.078	-.041	± .015	-.038	-.035	± .010	-.107
Fattening:									
slaughter age (days)	.096	± .008	.012	.102	± .017	-.061	.079	± .010	.011
length of fattening period (days)	.221	± .014	.015	.127	± .017	.009	.149	± .007	.015
daily gain, 30 to 100 kg (g)	-.085	± .012	-.037	-.142	± .015	-.026	-.117	± .009	-.017
lifetime daily gain (g)	-.081	± .010	-.016	-.139	± .013	-.013	-.129	± .011	-.007
feed conversion (kg/kg gain)	-.209	± .013	.016	-.151	± .012	.080	-.167	± .004	.039
daily feed intake (kg)	.227	± .016	-.020	.016	± .013	.022	.115	± .011	.017
Meat quality:									
water holding capacity (%)	-.335	± .009	-.067	-.323	± .015	-.048	-.357	± .007	-.016
meat colour – L*	.099	± .013	-.024	.076	± .011	-.037	.072	± .006	-.028
meat colour – a*	.132	± .007	.028	.155	± .012	.110	.143	± .008	.038
meat colour – b*	-.127	± .011	-.097	-.095	± .017	-.131	-.132	± .012	-.143
pH_{45}	-.113	± .008	-.015	-.104	± .025	-.051	-.108	± .027	-.138
pH_{24}	-.110	± .007	-.083	-.103	± .026	-.017	-.114	± .028	-.126

Discussion

The unfavourable trend for a decreasing IMF level, which has been observed in the Polish pedigree population, concerns mainly the PLW and PL breeds. In these breeds, IMF level decreased over 6 years from 1.88% to 1.76% (Tyra and Orzechowska, 2006; Tyra and Żak, 2010). An even greater difference was noted for the Puławska breed (2.43% and 2.17%, respectively). According to the latest data, IMF level in the Duroc and Puławska breeds is 2.23% and 2.17%, respectively (Tyra and Żak, 2010). The lowest level of this fat is observed in the Pietrain breed (1.68%), which receives considerable use as a sire component in commercial production. Measures should therefore be taken to stop this process. However, the process of IMF deposition in muscle tissue is not completely understood and attempts to find genetic markers associated with this process produced inconclusive and unsatisfactory results (Gerbens, 2001; Li et al., 2010; Schwab et al., 2009 a; Tyra and Ropka-Molik, 2011).

The coefficients of heritability obtained for IMF in the PLW and PL breeds (0.3) are not high but do not differ significantly from the findings of other authors (Suzuki et al., 2005 – 0.39; Cai et al., 2008 – 0.28; Schwab et al., 2009 a – 0.38; van Wijk et al., 2005 – 0.31; Newcom et al., 2005 a – from 0.42 to 0.57). Analysis of the current breeding programme for the national pedigree population of pigs, which is oriented towards improvement of fattening and slaughter traits (growth rate, meatiness and fatness of carcasses) offers hope that also IMF level will see some progress. With systematic breeding work, only slightly higher coefficients of heritability for these traits (Table 2) resulted in considerable progress in this respect over the last decade. It will be possible to obtain a satisfactory IMF level in the national pedigree population when, following the example of other EU countries, this indicator will be included in the selection index as part of the national breeding programme, or breeding value will be estimated for this indicator using BLUP. In Austria, Germany, Switzerland, Denmark or France (Österreichische Schweineprüfanstalt, 2007) meat quality traits have a weighting of up to 30%, a considerable proportion of which (over 50%) is given to IMF level. To incorporate this parameter into the BLUP method, it was necessary to identify the basic genetic parameters (heritability, genetic correlations with other traits included in the BLUP analysis) for the population being improved. In the case of the Polish pig population, these parameters were unknown.

At present, there is no objective and inexpensive method to determine IMF directly on live animals. Attempts to determine this parameter based on analysis of ultrasound images are associated with large errors (Morlein et al., 2005), while the use of computer tomography or magnetic resonance imaging, despite their high accuracy, is limited due to high costs and lack of equipment portability (Goodpaster et al., 2004). In this case, it might be well to use indirect selection, but the results obtained in this respect are not promising (Table 3). Because no significant genetic or phenotypic correlations were found between IMF level and the indicators that might be determined on a live animal, the use of intermediate traits for this purpose will fail to produce expected results in the form of increased IMF level in a relatively short period of time. The only positive result of this analysis is the low correlation

between IMF level and the overall level of carcass fatness. It was believed for many years that external fat thickness is strongly correlated with the level of intramuscular fat. Our results concur with the findings of Suzuki et al. (2006) and Newcom et al. (2005 a), who also reject the view about the strong correlation between subcutaneous and intramuscular fat because they obtained low coefficients of genetic correlation between backfat thickness and IMF content (from r_G = -0.03 to r_G = 0.29). The lack of a relationship between backfat thickness and IMF level was also shown by Ville et al. (1997), who also found that the two traits are highly variable, as observed in our study (Table 1). This possibly indicates that these two fatness traits may be determined by other groups of genes (Tyra and Ropka-Molik, 2011). This information is valuable for breeders because, to a certain extent, it enables the amount of external (subcutaneous) fat to be decreased without reducing the amount of intramuscular fat, which means that selection can be made in these two directions at the same time.

Despite the unfavourable results of the analyses, it is possible to achieve progress in IMF level, as evidenced by the results of Schwab et al. (2006) and Newcom et al. (2005 b), who made considerable progress in this trait over several generations using BLUP estimation of breeding value. Similarly, Schwab et al. (2009 b) achieved a considerable increase in IMF level in the herd being improved using the BLUP method with support from the results of molecular research (FABP3). Thus, the use of this method in the national breeding programme together with genetic markers, despite the fact that they do not produce very high results in combination with BLUP, may translate into a real increase in this parameter. Another possibility is the application of BLUP and station test results, which can be used to monitor about 20% of the pedigree boar population. By obtaining IMF data of these boars from the testing station and using BLUP pedigree relationships, we can estimate breeding value in terms of this trait for a considerable proportion of pedigree animals, which may translate into improvement of this trait. The current focus is on searching for relationships between histological structure of muscles and the degree of their fatness. Our study suggests that such relationships (one of the higher relationships observed between IMF and loin eye area – Table 3) exist. However, considerable breakthrough in this respect will be obtained by understanding the genetic mechanisms of IMF accumulation in muscle tissue, by developing genetic markers that determine this trait (based on the knowledge obtained), and by identifying genetic factors that determine the histological composition of muscles.

The heritabilities obtained for IMF content of the *longissimus dorsi* muscle, which are at the same level as other fattening and slaughter traits used in the current breeding programme, allow a conclusion that it is feasible to improve the national pedigree population in this respect. An unfavourable result from our study is that IMF level is unrelated to most parameters analysed, which means that this trait cannot be improved through indirect selection. The lack of a relationship between the analysed traits, in particular between IMF and carcass fatness, is an important information for breeders, allowing them, to a certain extent, to improve the national pig population in both directions at the same time (improvement of IMF level and reduction of external fat level). However, to stop the unfavourable downward trend for the trait of interest, the national breeding programme based on the BLUP method

should estimate IMF level as an independent breeding value or as a component of total breeding value by using station test data.

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Analiza możliwości poprawy wskaźników jakości wieprzowiny na drodze selekcji ze szczególnym uwzględnieniem zawartości tłuszczu śródmięśniowego (IMF)

STRESZCZENIE

Celem pracy było oszacowanie współczynników odziedziczalności dla zawartości tłuszczu śródmięśniowego (IMF) i innych cech tucznych, rzeźnych i jakości mięsa dla ras hodowanych w kraju. Ponadto oszacowano korelacje genetyczne pomiędzy tym wskaźnikiem (IMF) a grupą cech tucznych, rzeźnych i jakości mięsa, co daje możliwość uwzględnienia tego parametru w systemie oceny wartości hodowlanej metody BLUP. Badania prowadzone były na zwierzętach ras wbp, pbz, Puławskiej, Hampshire, Duroc, Pietrain i linii 990. Łącznie w badaniach uwzględniono 4430 loszek wymienionych ras, które ukończyły ocenę w Stacjach Kontroli Użytkowości Rzeźnej Trzody Chlewnej (SKURTCh). Odziedziczalność IMF była na średnim poziomie dla obu najliczniej hodowanych w kraju ras (dla wbp h^2 = .318, dla pbz h^2 = .291), natomiast w grupie cech jakości mięsa wysoką odziedziczalność obserwowano dla jasności barwy mięsa (L*) mierzonej aparatem Minolta (od h^2 = .453 do h^2 = .572). Nie stwierdzono zależności pomiędzy poziomem tłuszczu śródmięśniowego a wskaźnikami dotyczącymi użytkowości tucznej. Najwyższa wartość jaką obserwowano w tej grupie cech dotyczyła genetycznej zależności z dziennym pobraniem paszy (r_G = .227) dla całej grupy zwierząt, natomiast dla ras wbp i pbz były to zależności z wykorzystaniem paszy na kilogram przyrostu (odpowiednio r_G = .151 i r_G = .167). Jedną z wyższych zależności jaką obserwowano były korelacje genetyczne z wodochłonnością (przekraczały poziom r_G = -.3) oraz w przypadku ras wbp i pbz z odcieniem czerwonym barwy mięsa (a*) i wynosiły odpowiednio r_G = .155 i r_G = .143.

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MORPHOMETRIC CHARACTERISTICS OF THE REPRODUCTIVE SYSTEM IN POLISH LARGE WHITE AND POLISH LANDRACE GILTS AT 100 KG BODY WEIGHT*

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Abstract

The study involved an analysis of the developmental stage of reproductive organs collected at slaughter from 160 gilts (Polish Large White (PLW), n = 80; Polish Landrace (PL), n = 80) at 100 kg body weight. Due to a large variation in slaughter age (140–190 days), three groups of animals were set up: A (less than 160 days), B (160 to 180 days) and C (more than 180 days). PL gilts reached their slaughter weight earlier than PLW gilts (P≤0.05). Uterine weight increased with the age of animals but due to high variability and large deviations from the mean value, statistically significant differences were demonstrated only between gilt groups A and B for both breeds together (120.57 g vs. 148.83 g; P≤0.05). Larger differences related to the age of the gilts were found for cervical length between the groups compared (P≤0.01). The total length of the right and left uterine horns showed a significant increase with age in PLW gilts (P≤0.05). The ratio between uterine weight without ligament and the length of uterine horns (g/cm) was significantly higher in group B than in group A in gilts of both breeds together (P≤0.05), which might indicate thickening of the uterine walls. Uterine capacity was significantly higher in older animals yet due to a large variability of this trait, no significant differences between the groups were shown. The length and diameter of oviducts, the weight of each ovary, their sum and dimensions did not reveal any consistent changes associated with the age or breed of pigs. However, the size of the ovaries determined volumetrically and reported as the volume of ovaries in gilts of both breeds was significantly larger in group B compared with C (P≤0.01). No significant differences related to the studied traits were stated between PLW and PL prepubertal gilts. However, the effect of age on morphometric development of the reproductive system was more pronounced in PLW than in PL gilts.

Key words: pig, gilts, age, reproductive tract, morphometric measures

The attainment of sexual maturity in gilts is preceded by a complete anatomical and physiological development of their reproductive system. The development of the reproductive system in gilts begins as early as foetal life, continues in the postnatal

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period and is connected with morphogenesis and cell differentiation (Bartol et al., 1993; 1999). Progressive growth of the reproductive system is possibly allometric and is not directly proportional to the increase in body weight (Ji et al., 2005; Szostak, 2010; Wu and Dziuk, 1995). Determining the actual stage of development of the reproductive system and the relationship between individual elements of its anatomy at a fixed body weight of 100 kg will allow for deeper and better understanding of the mechanisms regulating litter size in pigs. The main determinants of litter size include ovulation rate, embryo survivability, uterine capacity and losses before weaning. Uterine capacity is commonly defined as the ability of the uterus to deliver the nutrients necessary to maintain the foetuses and provide them with ample space (Bazer et al., 1969; Foxcroft et al., 2009; Vallet, 2000; Vallet and Freking, 2005). The possibility of collection and morphometric evaluation of reproductive organs concurrently with a slaughter assessment of gilts conducted at testing stations is highly conducive to obtaining valuable information about the stage of development and formation of the organs.

The purpose of this study was to assess the relationship between morphometric characteristics of the reproductive system in Polish Large White and Polish Landrace gilts before the onset of sexual maturity in order to predict their potential fertility.

Material and methods

The study was conducted at the Slaughter Pig Testing Station (SKURTCh) in Mełno in the years 2009–2010. It covered 160 gilts, of which 80 were Polish Large White (PLW) and 80 Polish Landrace (PL) animals. The selection of experimental gilts and the feeding and management conditions were consistent with the methodology developed for the Slaughter Pig Testing Station (Różycki and Tyra, 2010).

Complete reproductive systems of gilts collected directly after slaughter served as material for morphometric evaluation. After the dissection the vaginal length was measured and the uterus was separated, including the cervix, oviducts and ovaries. The weight of the uterus with the broad ligament was then determined and after its separation the uterine weight was measured without the ligament. Measurements of the cervical length, the length of the uterine horns (right and left), the length of the oviducts (left and right) and the diameter of the oviducts were taken. Length measurements were performed using a measure with an accuracy of up to 0.5 cm, whereas the diameter was determined with an electronic caliper with an accuracy of up to 0.01 mm.

In order to obtain a more comprehensive description of the uterus in the analysed gilts, the uterine weight to length ratio (g/cm) was calculated.

The analysis of uterine capacity was based on the method applied to determine the capacity of cavernous organs developed by Kwaśnicki (1951) and modified by Brudnicki et al. (2001). This method was revised once more for the purpose of uterine capacity measurement at the Department of Pig Breeding of the University of Technology and Life Sciences in Bydgoszcz. The uterus was completely immersed

in a clear vessel with physiological solution and then weighed. The immersed uterus was filled with solution through a funnel placed in the cervical canal previously cleared with a trocar designed for small ruminants. Filling up was continued until an internal pressure of a 10 cm water column inside the organ was reached, i.e. until the liquid appeared in the cervix stabilized 10 cm above the level of solution in the vessel. Then the whole vessel was weighed again on laboratory balance with an accuracy of up to 1 g. The uterine capacity was calculated from the difference between the weight of the container filled with physiological solution and the weight of the uterus before and after filling up, and then provided in cm^3 (weight g/1.042 g/cm^3). Composition of the physiological solution was 0.9% NaCl and its density 1.042 g/cm^3. The uterus was immersed and filled with physiological solution on account of further histological examinations of the organ.

The ovaries obtained were subjected to detailed analysis involving determination of their weight and the length, height and width measurements, taken using electronic calliper with an accuracy of up to 0.01 cm. Ovarian volume (cm^3) was determined by immersing the ovaries in a calibrated measuring cylinder containing a predetermined volume of physiological solution.

Age at slaughter was the criterion for allocating the gilts of both breeds to three groups: A (less than 160 days), B (160 to 180 days) and C (more than 180 days).

The results were analysed statistically. Both the arithmetic mean (x) and standard deviation (s) were calculated. A two-way analysis of variance (ANOVA/MANOVA) was performed allowing for the breed and age of the gilts at slaughter. The significance of differences between the analysed groups was estimated using the Duncan test. For calculations the STATISTICA 8 PL (2008) computer software was used.

Results

The study focused on the assessment of a number of properties of the reproductive system in PLW and PL gilts after reaching a fixed body weight of 100 kg. Slaughter performed at a steady body weight resulted in significant differences in the age of the animals, ranging from 140 to 190 days. In order to estimate the age-related differences the gilts were divided into three groups: A – less than 160 days, B – 160 to 180 days, and C – more than 180 days of age. Group B was the largest group of PLW animals, while the youngest group A was also the largest group of PL gilts, which indicates a faster growth of this breed.

Table 1 contains data on the age of the gilts and the characteristics of the uterus. Upon achieving 100 kg body weight, PL gilts were slightly younger than PLW animals ($P \leq 0.05$). The weight of the uterus with the ligament was generally similar for all gilts of both breeds and showed a large deviation from the mean. However, the weight of the uterus measured without the ligament was significantly greater in older gilts of both breeds ($P \leq 0.05$). Similarly, the length of the cervix in gilts of both breeds combined was significantly greater in older than in younger animals (PLW gilts $P \leq 0.01$, PL gilts $P \leq 0.05$).

Table 1. The age of gilts at slaughter and the weight and size of the uterus

Characteristic	Breed	Age at slaughter (days)			Average
		A ≤160	B 160–180	C >180	
Size (n)	PLW	22	39	19	80
	PL	38	28	14	80
Total		60	67	33	160
Age at slaughter (days)	PLW	147.8 A±9.42	169.23 B± 5.48	193.63 C±13.06	169.10 x ±18.69
	PL	148.71 A±10.35	168.78 B± 7.24	189.50 C±4.55	162.87 y±17.49
Av.		148.33 A±9.95	169.04 B± 6.23	191.88 C ±10.42	165.99 ±18.31
Uterine weight incl. ligament (g)	PLW	132.09±44.29	160.59±80.51	160.50±77.46	152.73 ±71.90
	PL	142.11±56.85	175.25± 89.65	164.21±54.67	157.57 ±70.62
Av.		138.43±52.43	166.72± 84.10	162.07±67.77	155.15 ±71.08
Uterine weight excl. ligament (g)	PLW	112.61±49.39	142.72±76.74	138.44±65.77	133.42 ±68.09
	PL	125.17±52.51	157.36± 84.66	145.00±50.34	139.91 ±66.14
Av.		120.57 a±51.33	148.83 b±79.85	141.22±58.93	136.66 ±66.99
Cervical length (cm)	PLW	10.91 A±1.69	12.36 B± 2.17	13.05 B±1.31	12.12±2.01
	PL	11.58 a±1.82	11.83± 2.41	13.07 b±2.46	11.93±2.20
Av.		11.33 Aa±1.79	12.14 b± 2.27	13.06 Ba±1.85	12.03±2.10
Length of the right horn (cm)	PLW	44.04±13.30	47.04± 7.87	49.28±12.99	46.75±10.91
	PL	45.79±9.07	47.03± 7.78	48.31±10.06	46.67±8.76
Av.		45.15±10.74	47.04± 7.77	48.87±11.67	46.71±9.86
Length of the left horn (cm)	PLW	45.11±13.08	49.87± 8.20	52.94±14.02	49.29±11.44
	PL	48.84±9.03	49.20± 7.54	51.42±9.70	49.42±8.60
Av.		47.47 a±10.74	49.59±7.88	52.30 b±12.22	49.35±10.09
Length of horns (R + L) (cm)	PLW	89.16 a±24.39	96.91±15.62	102.22 b ±26.39	96.04±21.37
	PL	94.63±17.51	96.23±14.55	99.73±19.24	96.08±16.74
Av.		92.62 a±20.28	96.63±15.07	101.16 b ±23.31	96.06±19.14
Weight/length (g/cm)	PLW	1.27±0.46	1.45±0.62	1.33±0.40	1.38±0.53
	PL	1.30±0.41	1.60±0.67	1.44±0.39	1.43±0.52
Av.		1.29 a±0.43	1.51 b±0.64	1.38±0.39	1.40±0.53
Uterine capacity (cm^3)	PLW	117.35±58.00	145.10±60.64	149.37±88.63	138.48 ±68.02
	PL	141.50±54.00	158.89±77.64	171.29±73.37	152.80 ±66.68
Av.		132.64±56.25	150.87±68.04	158.67±82.02	145.64 ±67.53

in rows A, B, C – P≤0.01; a, b – P≤0.05.
in columns x, y – P≤0.05.

Table 2. Characteristics of the oviducts and the ovaries

Characteristic	Breed	Age at slaughter, days			Average
		A ≤160	B 160–180	C >180	
Length of the right oviduct (cm)	PLW	17.07 X,A± 5.55	19.33 B± 2.86	18.72 C±2.93	18.56 X±3.88
	PL	19.84 Y±3.27	20.40±4.58	20.00±2.54	20.06 Y±3.65
Av.		18.82±4.42	19.78±3.69	19.26±2.81	19.31±3.83
Length of the left oviduct (cm)	PLW	18.23 x,a ±5.49	20.70 b±2.74	20.28±2.90	19.92±3.83
	PL	20.31 y±2.84	20.78±3.76	19.23±2.39	20.29±3.14
Av.		19.55±4.10	20.74±3.18	19.84±2.71	20.11±3.49
Length of oviducts, (R + L) (cm)	PLW	35.29±5.52	40.04±5.38	39.00±5.14	38.49±7.44
	PL	40.16±5.83	41.18±8.15	39.23±4.37	40.35±6.50
Av.		38.37±8.29	40.52±6.64	39.10±4.75	39.42±7.02
Average oviduct diameter (mm)	PLW	2.51±0.82	2.32±0.62	2.61±0.87	2.44±0.74
	PL	2.55±0.75	2.38±0.71	2.54±0.25	2.49±0.67
Av.		2.53±0.77	2.35±0.65	2.58±0.67	2.47±0.71
Weight of the right ovary (g)	PLW	3.50±1.16	3.35±1.19	3.21±0.77	3.36±1.09
	PL	3.54±0.98	3.27±0.90	3.10±1.01	3.37±0.96
Av.		3.53±1.04	3.32±1.07	3.16±0.86	3.36±1.02
Weight of the left ovary (g)	PLW	3.66±1.27	3.90±1.42	3.76±0.87	3.80±1.26
	PL	3.96±1.25	3.50±0.91	3.56±0.84	3.73±1.09
Av.		3.85±1.26	3.73±1.24	3.67±0.85	3.77±1.17
Weight of ovaries (R + L) (g)	PLW	7.17±2.37	7.25 ±2.52	6.97±1.56	7.16±2.26
	PL	7.50±2.11	6.77±1,56	6.66±1.69	7.10±1.88
Av.		7.38±2.20	7.05±2.17	6.84±1.60	7.13±2.08
Average ovarian height (R + L) (mm)	PLW	12.22±3.22	12.29±2.88	12.82±1.86	12.40±2.76
	PL	12.34±2.00	11.36±1.57	11.93±1.64	11.92±1.83
Av.		12.29±2.49	11.91±2.45	12.44±1.80	12.16±2.35
Average ovarian length (R + L) (mm)	PLW	24.22±5.72	24.37±5.11	25.33±2.59	24.56±4.79
	PL	25.65±2.47	24.94±2.53	24.97±2.49	25.28±2.49
Av.		25.13±3.99	24.60±4.21	25.18±2.51	24.92±3.82
Average ovarian width (R + L) (mm)	PLW	17.70±4.90	18.21±3,91	19.01±2.44	18.26±3.91
	PL	18.55±2.66	17.94±1.98	17.64±1.39	18.18±2.26
Av.		18.24±3.63	18.10±3.23	18.43±2.15	18.22±3.19
Volume of the right ovary (cm^3)	PLW	2.20±0.95	2.82 A±1.31	1.92 B±0.73	2.44±1.16
	PL	2.62±1.07	2.78±1.07	2.31±1.17	2.62±1.08
Av.		2.47±1.04	2.80 A±1.21	2.08 B±0.94	2.53±1.12
Volume of the left ovary (cm^3)	PLW	2.52±1.07	3.09 a±1.52	2.19 b±0.91	2.72±1.32
	PL	2.96±1.29	2.88±1.25	2.61±0.90	2.87±1.21
Av.		2.80±1.22	3.00 A±1.40	2.37 B±0.92	2.80±1.26
Total ovarian volume (P + L) (cm^3)	PLW	4.73±1.95	5.91 a±2.72	4.11 b±1.59	5.16±2.39
	PL	5.58±2.26	5.66±2.24	4.92±1.89	5.49±2.18
Av.		5.27±2.17	5.80 A±2.51	4.45 B±1.74	5,32±2.29

in rows A, B, C – P≤0.01, a, b – P≤0.05.
in columns X, Y – P≤0.01, x, y – P≤0.05.

For further uterine characterization of the analysed gilts the ratio between the weight of the uterus without the ligament and the length of its horns (weight/length) was calculated. This trait was differentiated by the age of the animals. The increase in the proportion of uterine weight to the length of its horns may indicate thickening of the uterine walls occurring in gilts aged 160–180 days compared with younger animals ($P \leq 0.05$). The size of the uterus filled up with physiological solution was also evaluated. Thus measured capacity was clearly larger in older gilts, which was particularly noticeable in the PL animals. These differences, however, were not statistically confirmed.

The characteristics of the oviducts and ovaries are presented in Table 2. The length of the oviducts considered separately for the right and the left ovary showed significant differences only between the youngest gilts of both breeds (group A). The length of the right oviduct in PLW gilts was significantly lower than in PL gilts ($P \leq 0.01$) and this was also the case for the length of the left oviducts ($P \leq 0.01$). These relationships did not recur in the assessment of the total length of both oviducts.

The weight of the ovary, its dimensions (height, length and width) and volume were also determined in the course of our study. The weight values for the right and left ovary, as well as their dimensions, were similar in all gilts, regardless of the breed or age. Such data as the height of the ovaries, their length and width were not significantly different in terms of breed or age of the gilts. Volume, on the other hand, showed significant age-related changes. Particularly marked changes in the ovarian size were demonstrated by PLW gilts. The largest ovarian volume (right, left and total) was noted in group B gilts aged 160–180 days compared with gilts older than 180 days ($P \leq 0.01$).

Discussion

Much of the literature data indicates the critical importance of the size of the uterus and in particular the length of its horns for the potential formation of a large litter. Uterine development begins prenatally and continues after birth. According to Bartol et al. (1993), the transformation of the uterine wall in terms of construction and histological structure occurs between birth and the 120th day of age. Further developmental changes are associated with the proliferation of uterine glands, formation of endometrial folds and myometrial growth, while appropriate development of these properties ensures higher survivability of embryos and foetuses during pregnancy (Bartol et al., 1993). Determining the weight and size of the uterus may serve as a reliable indicator of the degree of uterine development in gilts.

The degree of development of the uterus evaluated by its weight (without the ligament) was significantly diversified in this study by the age of the animals. Animals of both breeds together, less than 160 days old, had significantly lower weight of this organ compared to older animals in group B ($P \leq 0.05$).

The length of the uterine horns may exhibit large variability between individual animals (Chen and Dziuk, 1993). There is also no rule affecting the total length

of the right or left horn of the uterus. Larger total length may be characteristic of a potential ability of the uterus to provide nutrients for a higher number of foetuses (Chen and Dziuk, 1993; Vallet, 2000; Wu and Dziuk, 1989; Wu et al., 1987). There is little research evidence that would clearly demonstrate the correlation between the length of the uterus before and after the attainment of sexual maturity and during pregnancy (Christenson et al., 1987; Wu and Dziuk, 1988). Subsequent studies, however, consider the length of uterus determined in sexually immature gilts to be a good predictor of its further development and potential size of the litter (Vallet, 2000; Wu and Dziuk, 1995).

As this study demonstrated, the length of both uterine horns increased significantly with age in PLW gilts ($P \leq 0.05$). Our calculation of the ratio of uterine weight to the length of its horns in gilts of both breeds together showed that it was the highest at the age of approximately 170 days. This might indicate uterine wall thickening occurring in that period. This result is consistent with the opinion expressed by Bartol et al. (1993) that the purpose of the changes in development of the uterus after the 120th day of age is to ensure favourable conditions for embryo implantation.

Uterine capacity seems to characterize the possibility of accommodating foetuses during pregnancy and ensuring higher prenatal foetal survivability. The mortality of embryos and foetuses during early pregnancy is relatively high and ranges from 17% to 34% compared with the number of corpora lutea (Wu and Dziuk, 1989; Wu et al., 1987). Increased density of embryos in the uterus is conducive to higher mortality rate (Vallet, 2000; Wu and Dziuk, 1995). The uterine capacity determined in the course of our study in sexually immature gilts was slightly higher in older animals, primarily in PL gilts.

There is no evidence in the literature to suggest a significant role of the length of the oviducts in the evaluation of potential fertility of gilts. A considerably greater average length of the oviducts than that reported in this paper was demonstrated by Klocek (1997) in sows at 30 days of pregnancy. The average diameter of both oviducts was very similar in all gilts, regardless of their age or breed.

The role of the ovaries in the production of efficient to fertile ova cells is very important and is characterized by the ovulation rate determining to a large extent the reproductive potential of sows. Ovarian development is initiated in the prenatal period and undergoes various stages of ovarian follicle formation during the growth of animals (Bolamba et al., 1994; Dufour et al., 1985; Klocek, 1997; Klocek et al., 2006; Pejsak, 1984). The follicular growth increases with age and upcoming sexual maturity, as demonstrated in morphological (Dyck and Swierstra, 1983) and hormonal (Lutz et al., 1984) studies. Detailed microscopic examination of ovaries in growing Yorkshire and Hampshire gilts at the age of 105, 140 and 170 days demonstrated large variations in the diameter and size of ovarian follicles (Dufour et al., 1985).

The results of studies carried out by the authors cited above showed dynamic changes in ovarian morphology between 160 and 180 days of age before the attainment of sexual maturity by the gilts. It can be therefore assumed that the highest ovarian volumes demonstrated during the course of our study in gilts of both breeds at that particular age may reflect the intensification of hormonal changes at that time of a pulse and fluctuating nature, regulating the functions of ovarian follicles.

In conclusion it should be pointed out that no significant differences associated with the breed of gilts were observed in the reproductive system morphology. Nonetheless, the age of animals had some effect on certain characteristics. The youngest gilts of both breeds demonstrated a significantly lower weight of the uterus, uterine horn length and cervical length compared to the older gilts of the same body weight. A clear tendency towards higher capacity of the uterus, particularly in the PL breed, was observed in older gilts. Changes in the size of the ovaries did not show a linear relationship with the age of the animals. Significantly greater capacity of the ovaries was demonstrated by gilts of both breeds aged 160-180 days. No significant differences related to the studied traits were stated between PLW and PL prepubertal gilts.

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Charakterystyka morfometryczna układu rozrodczego loszek wielkiej białej polskiej i polskiej białej zwisłouchej przy masie ciała 100 kg

STRESZCZENIE

W niniejszej pracy oceniano u 160 loszek (wbp n = 80; pbz n = 80) stan rozwoju narządów rodnych pobranych przy uboju przy masie ciała 100 kg. Ze względu na duże zróżnicowanie wieku przy uboju (140–190 dni) utworzono trzy grupy zwierząt: A (poniżej 160 dni), B (od 160 do 180 dni) i C (powyżej 180 dni). Nieco wcześniej osiągały masę ubojową loszki rasy pbz niż rasy wbp ($P \leq 0.05$). Masa macicy zwiększała się wraz z wiekiem zwierząt, jednak ze względu na dużą zmienność i dużą wartość odchylenia od średniej, statystycznie istotne różnice wykazano tylko między grupą loszek A i B obu ras łącznie (120.57g wobec 148.83 g; $P \leq 0.05$). Większe różnice związane z wiekiem loszek wykazano dla długości szyjki macicy między porównywanymi grupami ($P \leq 0.01$). Długość prawego i lewego rogu macicy podana łącznie wykazała istotny wzrost wraz z wiekiem zwierząt u loszek rasy wbp ($P \leq 0.05$). Proporcja masy macicy bez więzadła do długości rogów (g/cm) była istotnie większa w grupie B, niż w grupie A u loszek obu ras łącznie ($P \leq 0.05$), co może wskazywać na pogrubienie ścian macicy. Pojemność macicy była wyraźnie większa u zwierząt starszych, lecz ze względu na dużą zmienność tej cechy nie wykazano istotności różnic między grupami.

Długość i średnica jajowodów, masa poszczególnych jajników, ich suma i wymiary nie wykazały systematycznych zmian związanych z wiekiem i rasą świń. Jednak wielkość jajników określona metodą wolumetryczną i podana jako objętość jajników u loszek obu ras była istotnie większa w grupie B w porównaniu z C ($P \leq 0.01$).

Nie wykazano istotnych różnic związanych z rasą w morfometrycznej budowie układu rozrodczego niedojrzałych płciowo loszek wbp i pbz. Jednak wpływ wieku na badane cechy był bardziej wyraźny u loszek rasy wbp niż pbz.

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CIRCADIAN ACTIVITY OF DAIRY EWES KEPT INDOORS*

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Abstract

The aim of this study was to characterize the activity of Polish Milk Sheep 05 ewes housed in the indoor system. One group of 28 animals was observed. The observations lasted 227 hours and were conducted during day and night. The activity of each ewe was recorded every 10 minutes. The observed sheep rested for most of the day. Their activity increased at dawn, during milking (and feeding) and after green forage was provided in the afternoon. The time of feeding was mostly dependent on human activities. Rumination always occurred after the feed was offered. Sheep mainly slept lying down after milking and at night. REM (Rapid Eye Movement) sleep was observed. There were statistically significant ($P \leq 0.05$) differences in individual forms of behaviour (moving, standing, lying, feeding and ruminating) between different times of the day. In conclusion, the activity pattern of the indoor-housed ewes resembled that of pastured ruminants, but it was also strongly influenced by farm staff (milking, feeding, etc.).

Key words: daily activity, behaviour, ruminant, feeding pattern

The behaviour of sheep (*Ovis aries*) is strongly related to the fact that they are ruminants and are gregarious by nature (Hulet et al., 1975). The circadian activity of sheep, especially grazed ones, is determined by the environmental factors. Piccione et al. (2010, 2011) claimed that locomotor activity of sheep during the day is influenced by food availability and light/dark cycle. The activity can also change periodically, for example depending on seasons. Arnold (1962) found that grazing usually began at dawn and ended at dusk. In the spring and summer the grazing reached two peaks, while in the autumn and winter, it reached only one. The proportion of grazing between 6:00 a.m. and 6:00 p.m. changed depending on the season and it was the highest in spring and early summer. Dudzinski and Arnold (1979) stated that the time of starting and ending major morning and afternoon grazing periods and the time spent grazing on particular days depended on the time of dawn or dusk,

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air temperature, humidity and the time spent grazing the previous night. Piccione et al. (2008 b) observed that in goats there was one peak (in the middle of the day) of the activity rhythm. The same authors also noticed that the daily rhythm of activity displayed seasonal variations.

Previous research of daily activities in sheep was mostly concerned with pasture management and grazing of sheep along with goats. Animut and Goetsch (2008) noticed significant discrepancies in measurements of distance travelled depending on the environmental conditions and measurement methods. Stevenson et al. (2004) observed the behaviour of feral Soay sheep on the island of Hirta in St. Kilda archipelago (north-western shore of Scotland) and found that the time spent for sexual activity, feeding and moving was different depending on the season. Second half of November was characterized by a distinct increase in sexual activity and moving with a simultaneous decrease in the time spent feeding compared to the summer and winter periods.

Behaviour of domestic animals is affected not only by daily and annual changes in the environment (light, air temperature, humidity, etc.), but also by smaller living space and greater stocking density in indoor systems, and by management practices. The behavioural observations of sheep kept indoors showed that the duration of specific activities varied according to the type of sheepfold (Nowicki and Zwolińska-Bartczak, 1983). The activity of sheep kept indoors (milking, feeding, etc.) was mainly controlled by humans. Piccione et al. (2008 a), who investigated general activity in animals kept under artificial conditions (12:12 light:dark cycle) without any social contact, found that sheep and goats were mostly diurnal animals.

Hutchins et al. (2003) stated that sheep and goats were domesticated earlier than other farm animals. Sheep breeding gives many benefits to humans by providing meat, milk and wool, among others. Among the many breeds and varieties found all over the world are Polish Milk Sheep 05.

Polish Milk Sheep 05 were raised in the experimental farm in Złotniki, belonging to the Poznań University of Life Sciences. The genotype of this variety is based on the East Friesian (13/16) and Polish Merino sheep (3/16) (Annexe…, 1999). East Friesian sheep are a unique breed in that they rather do not tend to join other individuals and seek contact with humans (Schwintzer, 1983). However, this does not mean that they cannot be kept in a group of 40 other animals with proper space allowance and access to feed and water (Gräser-Herrmann and Sambraus, 2001). The purpose of this study was to characterize the activity of Polish Milk Sheep 05 ewes housed in the indoor system.

Material and methods

The observations were conducted on the farm in Złotniki near Poznań, Poland. The observed group contained 28 non-pregnant ewes of the Polish Milk Sheep 05 aged between 2 and 7 years (mean±SD: 3.71±1.46). Sheep were milked at 6:00 a.m. once a day in a side-by-side milking parlour. They received green forage and

hay in the afternoon (2:00 p.m.) and supplementary forage during milking. All feeds were provided *ad libitum*. The animals were housed together in a sheepfold. The total space available for the observed group was 175 m^2 (6.25 m^2 per sheep), which represents a low density compared to usual farming conditions (0.8 m^2 per sheep) (Regulation…, 2010). All sheep were individually spray marked with numbers.

The observations were conducted in summer between 27 July and 9 September. Instantaneous scan sampling was used (Altmann, 1974) and the activity of each animal was recorded every 10 minutes. Animals were observed for a total of 227 hours. All observations were made by the same person. The behavioural categories observed were ruminating, drinking, feeding, self-grooming, sleeping, social behaviour, moving, and vocalization, but not all of these categories were taken into account.

The day was divided into three eight-hour blocks: morning (5:30 a.m. – 1:20 p.m.), afternoon (1:30 p.m. – 9:20 p.m.) and night (9:30 p.m. – 5:20 a.m.). The observations in each block were made for 10 days. Because some hours were missed in every block, 227 h of observations were made. Preliminary observations revealed that sudden turning on of the light at night caused ewes to awake and consume feed. The increased activity persisted for an hour, after which sheep went to sleep again. As a result, five days before night observations the animals were accustomed to the light. Light was turned on right after sunset, left for the night and turned off during the morning milking about 6:00 a.m., when it was bright outside. During the night observations, with lights turned on early enough, the ewes did not awaken as described previously. Four light bulbs were used to illuminate the pen (3 × 60W and 1 × 75W).

In order to show the general proportions of each activity during the day, we calculated the mean for each one, where 100% was the number of all observations of any activity. The results were analysed in two ways: general activity analysis and finer behavioural analysis.

For the calculations of statistical differences in all cases, the G test was used at the significance level of $P \leq 0.05$. We checked if standing, lying, moving, ruminating, feeding and sleeping are dependent on time of day (morning, afternoon, evening) and analysed behaviours in standing and lying positions. To check if ruminating, feeding and sleeping are correlated, Spearman's correlation test was used.

Results

The proportion of moving, standing and lying during the day differed significantly (moving $G = 84.92$, standing $G = 308.75$, lying $G = 560.04$; $P \leq 0.05$). In general, an increased proportion of standing posture was observed during the feeding of green forage (afternoon) and during milking (morning) (Fig. 1). During the remaining hours, the ewes were mostly lying. A seven-fold increase in records presenting moving (morning), in relation to the average, was the result of animals' movement into and outside the milking parlour (Fig. 1). Ewes were becoming active also in the

evening, when the temperature outside was lower. In the total number of records, 50.5% accounted for standing posture, 44.8% for lying posture and 4.7% for moving.

The analysis of lying and standing postures is presented in Table 1. Between 5:30 and 7:20 a.m. the lying posture was not analysed, because it was the time before and after milking with a significant decrease in the proportion of lying posture in general (Fig. 1).

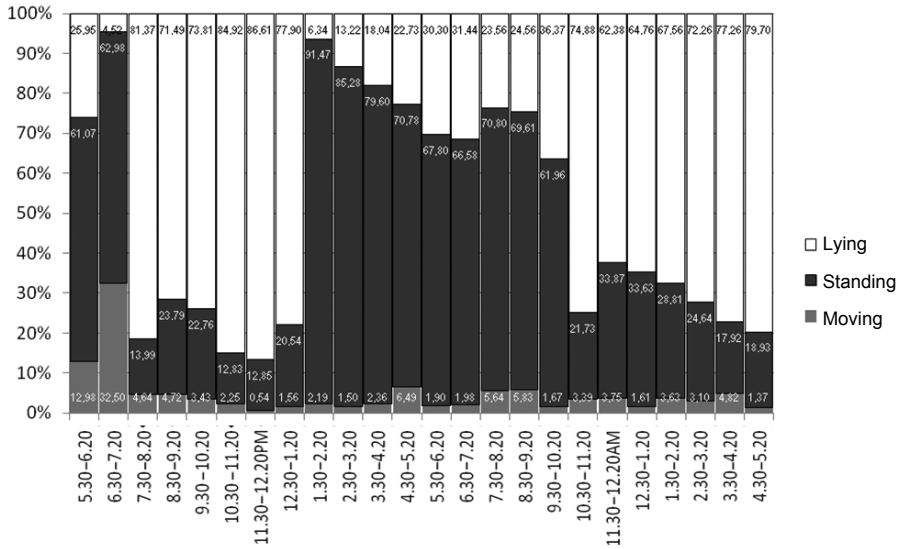

Figure 1. Percentage of basic activities during consecutive hours. Group means in total observations

Table 1. Percentage of specific behaviours in standing and lying posture

	Ruminating	Feeding and drinking	Idling	Sleep	Total
Standing	30.51	38.11	29.64	1.74	100.00
Lying	35.48	0.00	13.55	50.97	100.00

Figure 2 presents the schedule of ruminating and feeding during the day. There was a significant negative Spearman correlation between feeding and sleeping (–0.82; P≤0.05). The correlation between feeding and ruminating was also negative (–0.50; P≤0.05) On the other hand the correlation between ruminating and sleeping was positive (0.47; P≤0.05). The increase of feeding was related to the decrease of ruminating and vice versa. The difference in ruminating frequency, dependent on the time of day, was statistically significant (feeding G = 154.05; ruminating G = 343.72; P≤0.05). After receiving the supplementary forage during milking (5.20–7.20 a.m.) sheep were ruminating. About 2:00 p.m., after green forage was

offered, sudden feeding activity was noticed, which gradually decreased in favour of ruminating. Sheep were ruminating mostly lying (61.78%). There was no record of eating in lying position.

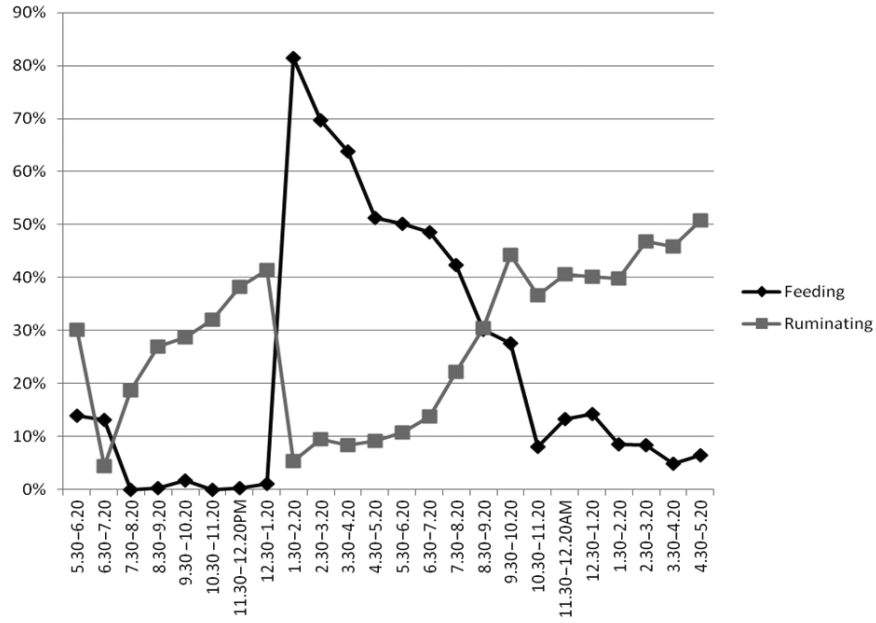

Figure 2. Feeding and ruminating during consecutive hours. Group means in total observations

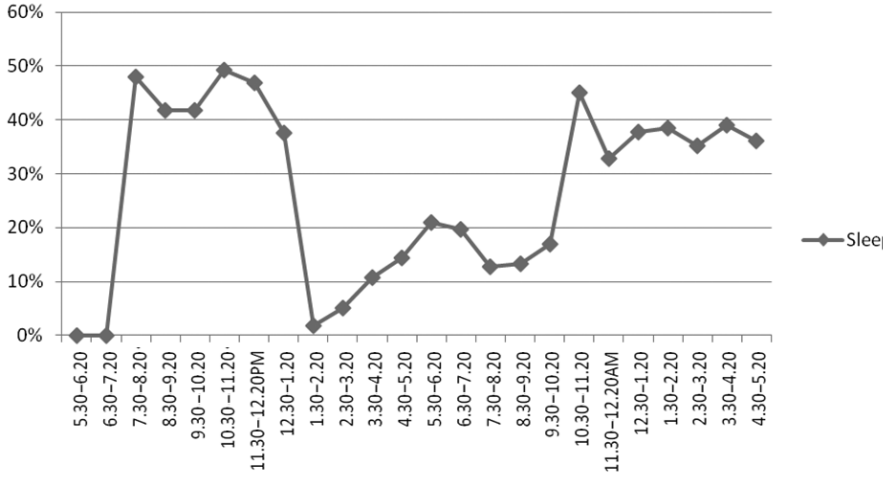

Figure 3. The proportion of sleep in consecutive hours. Group means in total observations

The locomotor activity of sheep was increasing during the time of eating alfalfa and at dusk. On the other hand, until noon and at night, more than one-thirds of

records showed sleep (Fig. 3). In total, sleep represented 29.34% of records. Sheep were sleeping after milking (7:20 a.m.) until the green forage application (2:00 p.m.), when the sudden movement began. Next, the sleep percentage was growing until dusk, with a small decrease about 7:30 p.m. Ewes slept mostly lying (97.36% of the observations). The difference in the proportion of sleep, dependent on the time of day, was statistically significant ($G = 471.39$; $P \leq 0.05$).

Discussion

The largest proportion of moving was noticed during the time of milking (5:30–7:20 a.m.). Lying posture was observed mainly after milking (7:30 a.m.) until green forage application (2:00 p.m.) and from about 10:00 p.m. until morning milking. Standing posture was observed mainly during eating of green fodder. Nearly 80% of records showed rest, which comprised the following behaviours: lying idle, lying and ruminating, standing idle, ruminating in standing posture, and sleeping in standing and lying posture. According to Chudoba et al. (2000), sheep breeds differ from each other by the amount of time spent resting; for example, Suffolk sheep kept on pasture for 24 hours spent 56.3% of this time resting. The difference in resting time between our own observations and those made by Chudoba et al. (2000) may relate to the sheep farming system, because sheep on the pasture are forced to look for their food actively, while sheep kept indoors have their food in one place and do not have to look for it. What is more, Rutter (2002) claimed that sheep typically spend 8 hours grazing, whereas goats spend 11 hours 'browsing'. According to the author goats are more inclined to browse for food (leaves, bushes, etc.) even when grass is bountiful. Cows kept in a loose barn spent 36.32% of the day standing, 49.44% lying and 14.24% feeding (Neja and Bogucki, 2005). The observed Polish Milk Sheep 05 spent more time feeding (23.29% of the day). Sztych and Wilczak (2005) reported that goats spent 57–69.5% of the day lying, which is much more than observed for sheep 05 (44.8%). Moreover, these authors observed increased activity at feeding time, which is in agreement with our observations.

The group of observed ewes had two feeding times: in the morning and afternoon. This was due to the fact that supplementary forage was provided in the morning and green forage in the afternoon. For comparison, sheep kept on pasture all the time are also grazing periodically but with four cycles per day (Hughes and Reid, 1951; Nowicki and Zwolińska-Bartczak, 1983): at dusk, in the early morning, before noon, and from noon till dusk (the longest cycle). Neja and Bogucki (2005), who observed tethered cows, confirmed Jezierski's argument (1987) that with rationed feeding, the daily rhythm of feed intake depended mostly on the time of forage feeding. Where cows were kept freely and had access to the fodder all the time, most of them ingested feed within 1.5 h of milking. According to Fraser (1980), grazing activity of sheep occurs only during the day and begins at sunrise. Overall time of grazing on a pasture is about 10 hours, with 4–7 grazing periods. Periods of the most intensive grazing in the summer are in early morning and from afternoon until dusk (Fraser, 1980). These

observations are consistent with the times when sheep 05 received green fodder and hay, but feeding time was controlled by humans in our study.

Sheep ruminate several times per day, mainly during the night hours (Nowicki and Zwolińska-Bartczak, 1983), and during grazing the proportion of rumination is much smaller. We observed a similar rumination and feeding pattern in ewes 05, namely increased frequency of rumination after the supplementary forage was provided, and a similarly high proportion from about 9:00 p.m. to 5:00 a.m. (Fig. 2). Also Sztych and Wilczak (2005) reported that goats ruminated mainly at night. Fraser (1980) stated that sheep ruminate with irregular gaps at night and during the day. Research conducted on a herd of ewes by Borys et al. (1990) showed that sheep kept in sheepfold spent 34.7% of their time ruminating, two-thirds of which was in lying posture and one-third in standing posture. Similar results were obtained during our observations, with a lower proportion of rumination (28.13% of records) and similar proportions of lying (61.78%) and standing (38.22%) postures.

According to Fraser (1980) sheep are active for 16 hours and sleep for about 4.5 h per day. In the observed group, one-fourth of the records indicate sleep, mostly lying. Piccione et al. (2007, 2008a) reported that sheep and goat activity is mostly diurnal. Our results are consistent with their findings: in the observed group, a strong decline in activity was observed after milking and at night. Hobson (1994) listed 3 repeated sleep phases: waking, non-REM (non-rapid eye movement) and paradoxical sleep phase or the REM phase (rapid eye movement). In our study, sleeping sheep happened to bleat and move their limbs, which indicated the latter sleep phase.

To conclude, the activity of the observed ewes resembled the pattern observed in pastured ruminants, but was also strongly influenced by farm staff (milking, feeding).

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Aktywność dobowa maciorek mlecznych utrzymywanych w chowie alkierzowym

STRESZCZENIE

Celem niniejszej pracy było scharakteryzowanie aktywności maciorek owcy mlecznej 05 utrzymywanych w chowie alkierzowym. Przeprowadzono 227 h obserwacji (obejmujących dzień i noc) dwudziestu ośmiu osobników tworzących jedną grupę. Aktywność każdej owcy była notowana co 10 min. Obserwowane maciorki odpoczywały przez większą część doby. Ich aktywność wzrastała o świcie, podczas doju (i podawania paszy) oraz po popołudniowym podaniu zielonki. Pory pobierania pokarmu zależały głównie od aktywności człowieka. Przeżuwanie występowało zawsze po podaniu paszy. Owce spały głównie w pozycji leżącej, po doju i nocą. Zaobserwowano fazę snu REM (Rapid Eye Movement). Części doby różniły się istotnie ($P \leq 0.05$) pod względem występowania poszczególnych form zachowań (chodzenia, stania, leżenia, pobierania pokarmu i przeżuwania). Wzorzec aktywności obserwowanych maciorek przypominał wzorzec aktywności wypasanych przeżuwaczy, jednak silnie wpływała nań również obsługa gospodarstwa (karmienie, dój, itp.).

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EFFECT OF INULIN AND GARLIC SUPPLEMENTATION IN PIG DIETS

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Abstract

The aim of the study was to determine the effect of supplementing inulin and inulin with garlic extract to pig diets on performance, carcass traits, blood metabolic profile and fatty acid composition of *longissimus* muscle. The experiment was carried out on 48 crossbred [(PL × PLW) × Duroc] fattening pigs with an initial body weight of 30.0 ± 0.5 kg, which were allocated to 3 groups: I (control), II (3% inulin) and III (3% inulin + 500 ml garlic extract added to 1000 l of drinking water). The pigs whose diets were supplemented with inulin or inulin and garlic achieved significantly (P≤0.05) higher daily weight gains compared to control. Supplemental inulin and water extract of garlic significantly (P≤0.05) lowered cholesterol content in blood and *longissimus* muscle. The highest level of omega-3 and omega-6 fatty acids was established in the *longissimus* muscle from pigs in group III.

Key words: pig, inulin, garlic, blood, fatty acids

Implementation of the ban on antibiotic growth promoters in animal feeds has prompted the search and more intensive studies on the application of alternative biologically active substances that improve the overall efficiency of animals and, importantly, are safe for humans, animals and the environment (Windisch et al., 2008). The alternative growth promoters including herbs, prebiotics and/or eubiotics deserve special attention (Grela et al., 2011).

Extensive research has been carried out on the use of fresh, dried or lyophilized forms of garlic (*Allium sativum* L.) and its preparations as essential oils or water extracts for supplementation of animal diets (Holden and McKean, 2000; Grela and Klebaniuk, 2007; Yan et al., 2011). Garlic contains active substances such as alliin, which is converted into another compound allicin, that have strong bactericidal and bacteriostatic properties and are very effective against some strains of bacterial pathogens in the digestive system (Amagase et al., 2001; Tatara et al., 2005). Where-

as other compounds from the cysteine derived group, i.e. S-allyl cysteine (SAC), S-ethyl cysteine (SEC) and S-propyl cysteine (SPC) are likely to enhance the immunomodulatory and immune changes in the body (Kandil et al., 1987; Isaacsohn et al., 1998). Garlic additives lower cholesterol content in hepatocytes (Konjufca et al., 1997), reduce formation and secretion of VLDL proteins as well as inducing health-promoting changes in the dietary fatty acid profile in humans (Yan et al., 1992).

A positive impact of the prebiotic inulin on monogastric animals was indicated by some authors (Loh et al., 2006; Kjos et al., 2010) who highlighted the stimulation of *Bifidobacteria* and *Lactobacillus* development, limitation of enterobacteria population growth, beneficial influence on the intestinal immune system and modulation of lipid metabolism (Crittenden and Planyne, 1996). These feed additives affect gastrointestinal flora and the body metabolism and thus can improve productive performance of animals, their body condition as well as modifying blood metabolic profile.

The objective of the present study was to assess the effect of inulin or inulin and garlic water extract inclusion in pig diets on overall efficiency and carcass quality, some parameters of blood metabolic profile and fatty acid composition in *longissimus* muscle.

Material and methods

The experiment was carried out on 48 crossbred [(PL × PLW) × Duroc] growing pigs with an initial body weight of 30.0±0.5 kg, which were allocated to 3 equal groups (Table 1) and kept in a group of 4 animals per pen. The fattening pigs were fed standard grower (30–70 kg) and finisher diets (71–110 kg). The diets comprised ground grain (wheat and barley), soybean meal, soybean oil and mineral feeds (salt, monocalcium phosphate and ground limestone), and standard mineral-vitamin premix for pigs. The feeds were balanced for metabolizable energy, protein, amino acids, minerals and vitamins (Grela et al., 2009). In groups II and III, wheat starch was replaced with inulin extracted from chicory roots (Orafti HPX, 100% inulin). Group III received extra 500 ml of water garlic extract added to 1000 l of drinking water. The garlic preparation was standardized for allicin content (5000 mg in 1 l). The animals had free access to feeders that allowed *ad libitum* consumption and to drinkers. The hygienic conditions, that is temperature, relative humidity and cooling were the same in all the groups. The content of basic feed nutrients was determined with standard methods (AOAC, 2005) and metabolizable energy was calculated according to the equation of Kirchgessner and Roth (1983).

Table 1. Experimental design

Trait	Groups		
	I control	II inulin	III inulin and garlic
Inulin additive (g kg^{-1} feed)	0	30	30
Aqueous extract of garlic (ml 1000 l^{-1} water)	0	0	500

Blood for analyses was collected three times from the jugular vein of 8 pigs at about 40, 70 and 100 kg body weight. Blood serum was obtained by centrifugation of whole blood at 3000 rpm for 10 min. at 4°C. Blood serum was used to assay the level of lipid metabolism parameters: triacylglycerol, total cholesterol and HDL (high-density lipoprotein) cholesterol fraction. The level of LDL (low-density lipoprotein) cholesterol was estimated by the formula of Friedewald et al. (1972). These parameters were analysed in the blood serum with colorimetric methods using Biomaxima monotests, a Metrolab biochemistry analyser and a Cary 50 spectrophotometer.

The pigs were slaughtered at about 110 kg body weight. Carcasses were assessed according to the SKURTCh (Pig Testing Station) methods (Różycki and Tyra, 2010). The samples of *longissimus* muscle for analyses were collected between the last thoracic and the first lumbar vertebra. Fat extraction process from muscle tissue was carried out by the method of Folch et al. (1957), after which fat was examined for cholesterol content according to Rhee et al. (1982) procedure, whereas fatty acid profile was evaluated with gas chromatography on a Varian CP-3800. The operating conditions for chromatographic separation were as follows: capillary column CP WAX 52CB 0.25 mm × 60 m, carrier gas – helium, flow rate 1.4 ml min^{-1}, column temperature 120ºC gradually increasing by 2ºC min^{-1} up to 210ºC, determination time – 127 min, feeder temperature – 160ºC, detector temperature – 160ºC, other gases – hydrogen and oxygen.

The results were subjected to analysis of variance (ANOVA) to provide mean values for the groups and standard error of the mean, while significance of differences for the mean values of the studied traits was determined with Duncan's test using Statistica package.

Results

The crude protein and metabolizable energy content of pig feeds in the growing and finishing periods (Table 2) was close to that calculated before the experiment began. Productive performance parameters for fattening pigs fed the inulin- and garlic-supplemented diet are summarized in Table 3. It is worth noting the reduced time of pig fattening when either inulin or inulin with garlic water extract were added. There were observed significantly higher ($P \leq 0.05$) daily weight gains throughout the fattening period in animals from groups II and III. Markedly better results were found in group III during the first stage of fattening and in groups II and III (supplemented with inulin and inulin with garlic extract, respectively) during the final stage. The maximum efficiency of feed conversion was established in group III and the differences between the groups were confirmed statistically.

In each group, the values of the analysed biochemical parameters (Table 4) fell within the normal reference range (Kuleta et al., 1993; Friendship and Henry, 1996; Winnicka, 2004). The blood lipid profile of pigs was modified as a result of the diet supplementation. Significantly ($P \leq 0.05$) lower LDL cholesterol levels compared to control were found in the blood serum of animals receiving the inulin and garlic additive. The dietary inclusion of inulin alone (group II) only tended to reduce total

cholesterol compared to the control; however, the differences were not significant. In animals from group III, the HDL fraction (55.98%) was significantly higher than in the other groups. Likewise, the pigs receiving 3% dietary inulin (group II) had higher HDL cholesterol (49.07%) compared to group I (35.74 %).

Table 2. Nutritional value of the diets

Item	I Control	II Inulin	III Inulin + garlic
Grower			
Dry matter	876.3	877.1	877.2
Crude protein	180.3	179.8	179.6
Ether extract (g)	24.3	24.2	24.2
Crude fibre (g)	37.8	37.4	37.5
Crude ash (g)	43.8	43.7	43.7
N-free extract (g)	590.1	592.0	592.2
Metabolizable energy (MJ)	12.52	12.51	12.51
Finisher			
Dry matter	887.1	885.9	886.2
Crude protein	161.3	162.4	161.2
Ether extract (g)	24.9	24.8	24.8
Crude fibre (g)	39.3	39.1	39.2
Crude ash (g)	40.3	40.2	40.2
N-free extract (g)	621.3	619.4	620.8
Metabolizable energy (MJ)	12.93	12.91	12.91

Table 3. Fattening days, daily gains, feed intake and FCR

Item	Fattening period	Groups			P value	SEM
		I	II	III		
Fattening days	Grower	53 A	51 A	47 B	0.008	0.15
	Finisher	52 a	46 b	48 ab	0.033	0.26
	$\bar{x}$	105 a	97 b	95 b	0.019	0.14
Average daily gains (g)	Grower	763 A	774 A	862 B	0.007	5.52
	Finisher	781 a	885 c	858 b	0.039	5.67
	$\bar{x}$	772 a	826 b	860 c	0.021	4.47
Feed intake (kg)	Grower	2.22 a	2.41 b	2.31 ab	0.041	0.02
	Finisher	3.02 a	3.31 b	3.05 a	0.033	0.04
	$\bar{x}$	2.62 a	2.86 b	2.68 a	0.045	0.03
Feed conversion ratio (kg kg^{-1})	Grower	2.91 b	3.11 c	2.68 a	0.038	0.05
	Finisher	3.87 c	3.74 b	3.55 a	0.043	0.08
	$\bar{x}$	3.39 b	3.46 b	3.12 a	0.037	0.05

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

Fattening pigs from each group were slaughtered at 110±1.5 kg body weight (Table 5). Significantly higher indices of carcass meatiness, including ham percentage and loin eye area were determined in group III, in which pigs received the inulin and garlic extract additive in drinking water. Animals in this group also showed lowest backfat thickness ($P \leq 0.05$) and liver weight that was about 34% higher compared to groups I and II.

Table 4. Triacylglycerol and cholesterol content in blood plasma

Item	BW (kg)	Groups			P value	SEM
		I	II	III		
Total cholesterol (mmol l^{-1})	40	2.25 b	2.18 ab	2.03 a	0.039	0.034
	70	2.09	2.07	2.01	0.174	0.037
	100	2.13	2.09	2.11	0.269	0.033
	$\bar{x}$	2.17 b	2.11 ab	2.05 a	0.046	0.032
Triacylglycerols (mmol l^{-1})	40	0.22	0.26	0.21	0.138	0.010
	70	0.34 b	0.28 ab	0.25 a	0.031	0.012
	100	0.35 a	0.29 ab	0.27 a	0.043	0.010
	$\bar{x}$	0.30	0.28	0.24	0.058	0.011
HDL cholesterol (mmol l^{-1})	40	0.83 a	1.08 b	1.11 b	0.011	0.026
	70	0.76 a	1.03 b	1.02 b	0.012	0.039
	100	0.72 A	1.01 B	1.06 B	0.010	0.037
	$\bar{x}$	0.77 a	1.04 b	1.06 b	0.011	0.029
LDL cholesterol (mmol l^{-1})	40	1.33 C	0.99 B	0.82 A	0.009	0.032
	70	1.17 b	0.91 a	0.88 a	0.012	0.039
	100	1.25 B	0.95 A	0.93 A	0.007	0.037
	$\bar{x}$	1.25 B	0.95 A	0.88 A	0.004	0.035
% HDL cholesterol	40	36.87 a	49.41 b	54.95 c	0.013	5.234
	70	36.47 A	49.70 B	50.74 B	0.006	5.602
	100	33.87 A	48.09 B	50.25 B	0.004	5.857
	$\bar{x}$	35.74 A	49.07 B	51.98 B	0.004	4.985
Total/HDL cholesterol	40	2.71 c	2.02 b	1.82 a	0.012	0.108
	70	2.74 B	2.01 A	1.97 A	0.004	0.125
	100	2.95 B	2.08 A	1.99 A	0.003	0.127
	$\bar{x}$	2.80 B	2.04 A	1.93 A	0.003	0.132

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

Table 5. Carcass quality traits

Item	Groups			P value	SEM
	I	II	III		
Body weight at slaughter (kg)	110.6	110.7	111.2	0.367	0.142
Cold dressing yield (%)	77.6	77.1	77.2	0.538	0.036
Meat of ham (%)	79.9 a	80.3 a	81.4 b	0.046	0.121
Loin eye area (cm^2)	55.6 a	56.2 ab	58.9 b	0.032	0.167
Meatiness of carcass (%)	52.4 a	52.3 a	54.6 b	0.033	0.183
Backfat thickness:					
– shoulder (mm)	34.3 b	32.8 ab	29.8 a	0.043	0.218
– midback (mm)	23.0 b	21.8 ab	19.7 a	0.027	0.211
– rump (3 measurements) (mm)	15.3	15.4	14.9	0.121	0.072
– average from 5 measurements (mm)	20.6 b	20.2 b	18.8 a	0.027	0.213
Weight of liver (kg)	1.48 a	1.49 a	1.99 b	0.043	0.024

a, b, c – mean values in rows with different letters differ significantly ($P \leq 0.05$).

Table 6. Fatty acid composition (% of total FA) and cholesterol content (mg g^{-1}) in *longissimus* muscle

Fatty acids (%)	Groups			P value	SEM
	I	II	III		
∑ SFA	42.12 b	41.39 ab	39.83 a	0.042	0.41
∑ MUFA	54.77 a	55.48 ab	56.32 b	0.043	0.52
∑ PUFA	2.84 a	2.91 a	3.46 b	0.034	0.11
∑ PUFA omega-3	0.22 a	0.25 ab	0.39 b	0.031	0.07
∑ PUFA omega-6	2.47 a	2.63 ab	3.01 b	0.027	0.15
Total cholesterol (mg g^{-1} tissue)	64.5 b	63.6 b	58.9 a	0.038	0.92

a, b – mean values in rows with different letters differ significantly ($P \leq 0.05$).

Table 6 presents the fatty acid profile and cholesterol content in the *longissimus* muscle. Group III was found to demonstrate a significantly ($P \leq 0.05$) lower percentage of saturated fatty acids and higher percentage of unsaturated acids, especially omega-3 and omega-6 as against the control. Importantly, fat from the *longissimus* muscle obtained from this group had a notably reduced cholesterol content.

Discussion

Numerous Polish and foreign studies address the problem of beneficial impact of garlic or inulin incorporation into fattener diets. The effectiveness of these dietary supplements depends on dose size and form as well as their source. A study by Kjos et al. (2009) on pigs clearly shows that the effect of polysaccharide additive varies according to the dose. Weight gains in the first and second stage of fattening were found to increase noticeably along with dietary inulin increasing from 3% to 6%. Other studies highlight a positive impact of garlic on productive performance (Grela and Klebaniuk, 2007; Yan et al., 2011). The beneficial impact is attributed to bacteriostatic compounds of garlic, mainly allicin and cysteine derivatives (Kandil et al., 1987; Isaacsohn et al., 1998; Amagase et al., 2001). The noteworthy performance results obtained in the present study in group III support the appropriateness of the combined application of inulin and garlic as an eubiotic (Grela et al., 2011). The combined use of inulin and garlic extract (group III) in the finisher period decreased weight gains of pigs compared to those receiving inulin alone. Therefore it seems that the garlic extract additive can be recommended mainly in the growing fattening period.

Physiologically relevant hematological parameters are important markers of animal health and homeostasis. Supplementation of the diet with 3% inulin contributed to increased HDL cholesterol in the blood serum of fatteners. However, Pedersen et al. (1997) did not find any considerable effect of inulin addition on the discussed blood parameters. Meanwhile, Yasuda et al. (2006) reported that growing pigs responded positively to a 4% inulin supplement with elevated hemoglobin levels. A number of authors (Qureshi et al., 1987; Yeh and Yeh, 1994; Yeh and Liu, 2001; Durak et al., 2004) have stressed the important and well-proven health benefits of

garlic, e.g. its antiatherosclerotic activity. According to Yan et al. (2011) and Chen et al. (2008), dietary garlic supplement boosts the immune system and improves the overall efficiency of pigs. Likewise, the nutritional value of pork for human consumption was generally more favourable (Paschma and Wawrzyński, 2007). The investigations of Omojola et al. (2009) indicated that increasing the garlic level has lowered total cholesterol. Its concentration in the control group was 135.3 mg 100 g^{-1} as compared to 133.3, 66.7 and 44.4 mg 100 g^{-1} in the respective groups with 0.5%, 1% and 1.5% garlic supplementation. In the present study, the significantly lower ($P \leq 0.05$) cholesterol level determined in the *longissimus* muscle of garlic-supplemented pigs may be attributed to saponins and sulfur compounds found in garlic that affect lipid and cholesterol biosynthesis (Konjufca et al., 1997; Omojola et al., 2009). The increased share of omega-3 polyunsaturated fatty acids could partly result from a high content (over 60%) of C18:2 in garlic oil or from an indirect effect of some garlic components on fatty acid metabolism (Kamanna and Chandrasekhara, 1980; Dieumonu et al., 2012).

The significant increase (by about 34%) of liver weight, observed in group III fatteners may be attributed to the biologically active garlic components. A similar increase in liver weight (by 35%) in 8-week-old piglets receiving an aged garlic extract was reported by Tatara et al. (2005).

In conclusion, the incorporation of the prebiotic inulin into fattener diets together with water garlic extract given in drinking water is recommended in the growing period. The benefits include better productive performance, correct blood metabolic profile and potential effect on the composition of fatty acids, which is so important for pork consumers.

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Efektywność dodatku inuliny i czosnku w żywieniu tuczników

STRESZCZENIE

Celem pracy była ocena wpływu dodatku inuliny oraz inuliny i wodnego wyciągu z czosnku w żywieniu tuczników na efekty produkcyjne, wskaźniki metaboliczne krwi oraz profil kwasów tłuszczowych mięśnia *longissimus*. Doświadczenie przeprowadzono na 48 tucznikach mieszańcach rasy (pbz × wbp) × Duroc o masie początkowej 30±0,5 kg, podzielonych na 3 grupy: I (kontrolna), II (3% inuliny) oraz III (3% inuliny + 500 ml wodnego wyciągu z czosnku w 1000 l wody). Tuczniki otrzymujące dodatek inuliny lub inuliny i czosnku osiągnęły wyższe dobowe przyrosty masy ciała (P≤0,05) w porównaniu do grupy kontrolnej. Dodatek inuliny i wyciągu wodnego czosnku wpłynął istotnie (P≤0,05) na obniżenie zawartości cholesterolu we krwi oraz w mięśniu *longissimus*. Zawartość niezbędnych kwasów tłuszczowych omega-3 i omega-6 w mięśniu *longissimus* była najwyższa u tuczników z grupy III.

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THE EFFECT OF DIFFERENT FORMS OF SUNFLOWER OIL AND PROTEIN SOURCES IN THE DIET ON PANCREATIC JUICE SECRETION AND PANCREATIC ENZYMES ACTIVITY IN SHEEP*

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Abstract

The aim of the study was to determine the effect of various forms of sunflower oil in the diet with protein degraded at different rates in the rumen on pancreatic juice secretion and activity. The experiment was conducted on 24 adult Corriedale rams weighing about 40 ± 1.5 kg, catheterized in the pancreatic and bile ducts and fistulated in the duodenum. The animals were fed diets consisting of meadow hay, potato starch, different degradable protein (casein or maize gluten, a source of zein) and different forms of sunflower oil (calcium salts, seeds and oil). It was stated that addition of various forms of fat to the diet did not significantly influence the secretion of pancreatic juice, regardless of the source of protein. However, sunflower seeds and oil used in the diet had a significant effect on bile secretion, protein content, proteolytic activity of trypsin and plasma lipid indices. No significant differences were observed in the lipolytic activity of the pancreatic juice, although lipase activity was higher when zein was used as the main protein source. It was concluded that dietary addition of certain combinations of protected or unprotected sunflower oil and different degradable protein may improve pancreatic activity and probably affect plasma lipid indices in sheep.

Key words: sheep, sunflower oil, zein, casein, secretion of pancreatic juice, pancreatic enzymes

High-yielding ruminants require high energy and protein concentrations in the diet. To fulfil these needs, various sources of them are used including oil or full-fat seeds and proteins, the ruminal degradability of which varies significantly. The composition of a diet can influence microbial activity in the rumen and the composition of digesta flowing to the stomach and duodenum (Kowalski, 1997).

The proteolytic activity of the pancreas and its capacity for proteolytic adaptation depending on protein source and concentration in the diet is less investigated in ruminants than in monogastric animals. However, the composition of protein flowing

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to the duodenum is more stable due to rumen fermentation, in which the main protein flowing to the small intestine is microbial. In animals with well-developed forestomach, feed intake and digesta flowing into the duodenum are also less diet dependent than in monogastric animals, because of constant inflow of digesta (Hvelplund and Madsen, 1985; McAllan et al., 1988).

However, it is thought that an increase in lipolytic activity of the pancreas is associated with increased fat concentration in the diet, and thus also in the small intestine. In ruminants, most of fat flowing to the duodenum is in the form of saturated fatty acids, mainly palmitic and stearic fatty acids. The protection of vegetable oils against hydrogenation in the rumen can significantly influence changes in composition of fat flowing to the duodenum, in particular the concentration of triglycerides (Bauman and Griinari, 2003).

In the literature there are only a few studies about dietary compounds affecting pancreatic juice secretion and pancreatic enzyme activity. Some results of these experiments are inconsistent. It is not clear if the differences between nutrient digestion in the small intestine are caused by the differences in the extent of secretion and activity of pancreatic juice enzymes. Khorasani et al. (1990), when supplementing diets with soybean and rapeseed meal, which are known for their different digestion rates, did not observe any significant differences in trypsin and chymotrypsin activity in pancreatic juice, as well as in its secretion. However, opposite findings were noted after casein infusion to the duodenum (Ben-Ghedalia et al., 1982). In some experiments carried out on sheep, rapeseed oil in comparison with protected oil in the form of salts was found to have an effect on pancreatic juice and bile secretion (Rawa et al., 2007). However, Johnson et al. (1974) noted a decrease in secretion of pancreatic juice and lipase activity during saffron or coconut oil infusion to the duodenum.

The effect of the diet with different forms of sunflower oil, protected or unprotected against bacterial hydrolysis in the rumen on intestinal digestion has received less study. Knowledge of the correlations between amount or forms of fat, different digestion rates of dietary protein flowing to the small intestine and also the activity of pancreatic enzymes could show ways of improving nutrient digestion by ruminants. Different degradable protein (casein or zein) and different forms of sunflower oil with a high concentration of linoleic acid may change the content of duodenal digesta, which probably affects the secretory activity of the pancreas.

The aim of the study was to determine the influence of different forms of sunflower oil (SFO) and protein sources in the diets for sheep on pancreatic juice secretion and activity of pancreatic enzymes. Sheep were used as a model for ruminants.

Material and methods

The experiment was carried out on 24 adult Corriedale rams weighing about 40 ± 1.5 kg, fitted with catheters in the pancreatic and bile ducts (Kato et al., 1999; Pierzynowski, 1983). The animals were also fistulated with modified duodenal can-

nulas, which allowed pancreatic and biliary juice to return to the duodenum after the samples were taken (Rawa et al., 2008). The procedures were accepted by the Local Ethics Committee for animal experiments.

The animals were fed twice a day at 8 a.m. and 4 p.m. with two isoprotein diets (17% of crude protein in DM) consisting of meadow hay (600 g), potato starch (500 g), casein (140 g) – which contained 120 g of easily degradable protein in the rumen, or maize gluten (200 g) containing 120 g of zein, which is slowly degraded in the rumen. Moreover, diets were enriched with unprotected and protected forms of fat against bacterial hydrolysis in the rumen – sunflower oil (5% in DM) or its forms in amounts which allowed reaching 5% oil in DM, i.e. calcium salts of sunflower fatty acids oil or whole dehulled sunflower seeds. The control diet was not supplemented with fat. For animals from the groups which received no sunflower seeds in the diet, the protein level was compensated by sunflower oil meal addition. Total protein level in the diet met lamb requirements according to IZ-INRA standards (1993). The level of energy content varies because of oil supplementation.

After a two-week adaptation and feeding period the animals were moved to metabolism cages, where the pancreatic juice was collected for 8 hours during three days. The pancreatic juice and bile were collected into beakers placed in a cold bath after feeding time, which were weighed after every 30 minutes. After 5% of pancreatic juice was collected, taken and cooled, the remainder was mixed with bile and pumped back into the duodenum by a peristaltic pump.

After the end of collection, the animals returned to the rearing pens and a new diet with another protein source was given. The same procedure was repeated with all forms of fat and different sources of protein (Table 1).

Table 1. Experimental design

Protein in basal diet		Form of fat added to the basal diet (C)
Casein	Zein	
C	C	Without fat addition (control)
Ca-S	Ca-S	Calcium salts of sunflower oil
S	S	Sunflower seeds
O	O	Sunflower oil

The blood plasma collected from the jugular vein 2 h after feeding was analysed for total protein, lipase and cholesterol. The experimental samples of lipolytic and proteolytic enzymes were stored at –80°C. The protein content was analysed by the method of Lowry et al. (1951), trypsin and chymotrypsin activity according to Hummell (1959), and lipase by SIGMA Diagnostics LIPASE-PS, Procedure No. 805-B. The total protein level in blood plasma was analysed using the method described by Lowry et al. (1951), and lipase activity and cholesterol concentration using a VITROS DT 60 II analyser. The results obtained were subjected to one-way analysis of variance for every experimental factor (forms of fat or different degradable protein) with Tukey test (Statgraphics Plus 7.0). Significance was declared at $P \leq 0.05$ and $P \leq 0.01$.

Results

Different forms of sunflower oil in the diet did not significantly influence the pancreatic juice secretion, but the impact of protein on this index was noted (Table 2). The secretion of pancreatic and pancreatico-biliary juice in sheep fed diets with sunflower seeds (S) and maize gluten, containing protein with low rate of ruminal degradation (zein), was significantly higher in comparison to the animals receiving casein (P≤0.05).

However, a substantial decrease was observed in pancreatic and pancreatico-biliary juice secretion in group O receiving dietary zein (P≤0.05).

Bile secretion was affected partly by both experimental factors. In the group of animals receiving sunflower oil with dietary casein, an increase in bile and pancreatico-biliary juice secretion was noted in comparison to groups C and Ca-S (P≤0.01). A similar situation was observed for sheep fed diets with sunflower seeds and casein (P≤0.05). The source of protein strongly affected bile secretion in group S supplemented with zein, where a significant increase was observed (P≤0.05).

Table 2. Secretion of pancreatic juice, bile and pancreatico-biliary juice (ml/h)

Groups	Casein	Zein	SEM, for the row
Secretion of pancreatic juice			
C	16.7	17.5	0.90 NS
Ca-S	16.8	17.5	0.54 NS
S	15.0	17.9	0.63*
O	17.5	15.0	0.52*
SEM, for the column	0.42 NS	0.47 NS	
Secretion of bile			
C	61.2 Aa	67.2 a	3.42 NS
Ca-S	60.1 Aa	68.2 a	3.46 NS
S	69.8 ABa	88.4 b	3.47*
O	89.3 Bb	73.2 ab	3.97 NS
SEM, for the column	2.69 0.01	2.77 0.05	
Secretion of pancreatico-biliary juice			
C	77.9 A	84.7 a	3.53 NS
Ca-S	76.9 A	85.7 a	3.70 NS
S	83.8 A	106.3 b	4.16*
O	106.8 B	88.1 a	4.12*
SEM, for the column	2.77 0.01	3.02 0.05	

C – control, Ca-S – calcium salts of sunflower oil, S – sunflower seeds, O – sunflower oil.
SEM, for the row * – P≤0.05; NS – non-significant.
SEM, for the column a, b – P≤0.05; A,B – P≤0.01.

The protein content in pancreatic juice differed for both experimental factors (Table 3). The addition of sunflower oil to the diet increased protein content in pancreatic juice, when zein was used as protein (P≤0.05). In turn, the lowest concentration of protein was observed after full-fat seeds were supplemented (P≤0.05). The opposite results were stated for animals receiving casein in the diet (P≤0.01).

Moreover, casein increased the protein content of pancreatic juice in all feeding groups except for the O group (P≤0.01).

Table 3. Protein content in pancreatic juice

Groups	Protein content in pancreatic juice (mg/ml)		SEM, for the row
	Casein	Zein	
C	79.6 AB	71.7 ab	3.57 NS
Ca-S	90.4 B	79.0 a	4.62 NS
S	89.9 B	61.5 b	3.69**
O	67.2 A	81.3 a	3.44*
SEM, for the column	2.70 0.01	2.73 0.05	-

C – control, Ca-S – calcium salts of sunflower oil, S – sunflower seeds, O – sunflower oil.
SEM, for the row * P≤0.05; ** P≤0.01; NS – non-significant.
SEM, for the column a, b – P≤0.05; A,B – P≤0.01.

Table 4. Trypsin, chymotrypsin and lipase activity in pancreatic juice

Groups	Casein	Zein	SEM, for the row
Trypsin activity (U/ml)			
C	70.3 ABa	65.3 AB	3.31 NS
Ca-S	89.1 Bb	90.5 C	3.65 NS
S	72.5 ABa	52.3 A	3.44**
O	58.4 Aa	76.9 BC	3.11*
SEM, for the column	2.39 0.01	2.50 0.01	-
Chymotrypsin activity (U/ml)			
C	19.7 Aa	29.0 AB	1.09**
Ca-S	41.2 Cc	35.5 B	2.08 NS
S	34.5 BCd	23.4 A	1.77**
O	26.6 ABb	45.8 C	1.91**
SEM, for the column	1.16 0.01	1.28 0.01	-
Lipase activity (U/L)			
C	837	948	36.9 NS
Ca-S	841	935	45.6 NS
S	869	932	35.6 NS
O	910	938	40.8 NS
SEM, for the column	28.6 NS	28.4 NS	-

C – control, Ca-S – calcium salts of sunflower oil, S – sunflower seeds, O – sunflower oil.
SEM, for the row * P≤0.05; ** P≤0.01; NS – non-significant.
SEM, for the column a, b – P≤0.05; A,B – P≤0.01.

It was noted that source of protein and forms of fat used in the diet had an influence on proteolytic activity in pancreatic juice (Table 4).

Taking into account the forms of fat, the highest trypsin activity was observed in Ca-S groups, irrespective of protein source (P≤0.01). However, the lowest concentration for this enzyme was stated in animals which received combinations of sunflower oil and casein, and sunflower seeds and zein, respectively (P≤0.01).

On the other hand, when we consider the source of protein added to the diet, the highest trypsin activity was found in group Ca-S. However, significant differences were observed for S and O groups between casein and zein groups.

Similar to trypsin activity, the diet with casein and calcium salts of sunflower oil offered to the animals contributed to the highest activity of chymotrypsin in comparison to groups C and O (P≤0.01). However, the lowest concentrations of this enzyme were obtained in group C (P≤0.01).

Furthermore, where zein and sunflower seeds were added to the diet, the values of chymotrypsin were significantly reduced, while sunflower oil addition improved this parameter. Source of protein significantly affected chymotrypsin activity in all oil groups except for zein and oil supplementation.

Both experimental factors did not significantly influence lipase activity in pancreatic juice, although there was increased lipolytic activity in animals receiving dietary zein, regardless of the form of fat used.

In lambs fed diets with sunflower oil, a significant increase in plasma lipase activity in comparison to other experimental groups was observed (P≤0.05) (Table 5). The addition of calcium salts of sunflower oil with zein substantially decreased total protein concentration in blood plasma when compared to other experimental groups (P≤0.01). No significant influence of different forms of fat on total cholesterol level was stated, although elevated total cholesterol concentrations as a result of sunflower oil and sunflower seeds supplementation were observed for both protein sources.

Table 5. Lipid and protein indices in blood plasma of sheep fed protein of different origin and different forms of SFO

Indices	Total protein (g/L)	Lipase (U/L)	Total cholesterol (mmol/L)
Control group – casein	80	164 b	0.31
+ calcium salts of sunflower oil	78	181 b	0.29
+ sunflower seeds	78	154 b	0.37
+ sunflower oil	79	264 a	0.47
Control group – zein	81 A	197 b	0.39
+ calcium salts of sunflower oil	76 B	221 b	0.38
+ sunflower seeds	82 A	198 b	0.48
+ sunflower oil	81 A	308 a	0.46

a, b – values in columns with different letters differ significantly (P≤0.05); A, B – as above for P≤0.01.

Discussion

Knowing the action of the pancreas on specific diets may show the possibilities of improving digestion in ruminants. We suppose that supplementing protein with different rates of degradation (casein or zein) and different forms of sunflower oil, which are not a natural feed for ruminants, will modify the secretory activity of the pancreas, leading to better utilization of fatty acids in the carcass. The addition of unprotected forms of fat to sheep diets may also increase plasma concentrations of lipase and total cholesterol.

The literature reveals enormous variation in the amount of pancreatic juice secretion in sheep and in other species. These discrepancies can also be caused by pancreatico-biliary secretion and its relations, or by pancreatic juice alone.

In the present study the total pancreatic juice and bile secretion ranged from 76.9 to 106.8 ml/h depending on the diet. However, Johnson et al. (1974) reported significantly lower secretion values (from 57.5 to 69.5 ml/h). On the other hand, Kowalik et al. (2001) noted increased secretion of biliary-pancreatic juice (from about 83 to 174 ml/h) in sheep fed diets with starch. Therefore, differences in the amount of juice obtained during collection depend mainly on animal breed, feeding (nutrients), body weight, as well as method of catheter insertion (Harmon, 1992; Kato et al., 1999; Kowalik et al., 2001; Żebrowska et al., 2001; Šileikienė et al., 2004).

The results of our research showed that the use of easily degradable protein in the rumen (casein) and different forms of sunflower oil had no significant effect on pancreatic juice secretion. The decrease in pancreatic juice secretion in rams fed diets with casein and sunflower seeds and with zein and sunflower oil is due to different rates of energy and protein utilization in the diet. Diets supplemented with casein and sunflower seeds reduced the energy utilization from seeds, which probably flowed to rumen less degraded. Thus, the energy found in seeds could be available only in the small intestine. On the other hand, in the diet with zein, the energy supplied with sunflower oil was not utilized in the rumen, and, what is more, zein as a protein resistant to rumen degradation was available only in the small intestine. Richards et al. (2003), who postruminally infused casein and corn starch into the abomasum of steers, did not observe any changes in pancreatic juice secretion. The amounts of casein used in the diet (0, 60, 120, or 180 g/d) also did not affect trypsin and chymotrypsin concentration. Furthermore, Wang and Taniguchi (1998) did not show any differences in pancreatic juice secretion after casein addition to the diet, compared to the unsupplemented group. Brannon (1990) observed that changes in the proteolytic activity of pancreas were associated with changes in the amount and content of protein flowing to the small intestine. Żebrowska et al. (2001) confirmed these results when feeding sheep with 200 g of total protein in dry matter, which increased the secretion of pancreatico-biliary juice (1398 g) and chymotrypsin activity, when compared to animals receiving smaller amounts of protein (130 g; 1160 g) ($P<0.01$). When the dietary protein content was lower, endogenous nitrogen was observed to decrease from 3.4 to 2.7 g N/24 h in secreted juice ($P\leq0.05$). However, the activity of trypsin and amino acids content in pancreatico-biliary juice were similar for all diets.

In the present experiment it was observed that protein concentration in pancreatic juice was significantly higher in animals receiving casein and sunflower seeds in comparison to the group receiving zein. Protein readily degraded in the rumen and partially protected fat may affect microbial protein synthesis by utilizing products of protein degradation. Furthermore, this process was not interfered by unsaturated fatty acids, because sunflower seeds did not directly act on rumen microflora, in comparison to natural oil. The only exception were sheep fed oil-supplemented diets in which zein, as a protein resistant to rumen degradation, caused a marked increase in protein content of juice. However, when abomasally infusing coconut oil with similar fatty acid profile to sunflower oil, Johnson et al. (1974) did not observe any significant effect of these fats on either the protein content of pancreatic juice or its lipolytic activity.

In the present study trypsin and chymotrypsin activity was modified by different protein source and form of fat added to the diet, which supports our research hypothesis. Some literature data concerning the proteolytic enzyme activity of the pancreas suggests that, depending on protein concentration in the diet, this activity can be modified by protein rate and products of protein digestion in the small intestine. In ruminants, most protein flowing to the intestine after digestion is of microbial origin. Because the concentration of microorganisms protein is constant, it cannot affect the proteolytic enzyme activity (Harmon, 1992). However, when slowly degraded protein is fed to animals, the amount of "feed" protein flowing to the duodenum can be greater, although Richards et al. (1998) noted no significant effects of casein on the trypsin activity. Also Swanson et al. (2002) did not observe any changes in the proteolytic activity between control group and animals receiving casein infusion to the abomasum. Khorasani et al. (1990) did not show any differences in trypsin activity when animals were fed diets with various sources of protein from soybean and canola meal with the exception of rumen. In turn, Swanson et al. (2008) observed a positive influence of crude protein in the diet on trypsin activity as well as noting that gradually increasing crude protein concentration from 8.5 to 14.5% could increase proteolytic trypsin activity. However, Richards et al. (2003) did not observe any differences in trypsin and chymotrypsin activity when feeding different casein levels to cows.

On the other hand, the strong effect of fat added to the diet on pancreatic proteolytic activity in our experiment can be supported with the manner of trypsin and chymotrypsin activation. Linoleic acid, which is the main compound of sunflower oil, takes part in activation of these enzymes. This acid can be converted to arachidonic acid (AA), which is known as one of eicosanoid precursors. Intestinal juice contains enterokinase, which is necessary for activation of trypsinogen to trypsin, which in turn activates chymotrypsinogen. Thus, such a process is necessary for a proteolytic activation of pancreatic juice. A physiological correlation between fatty acid profile in the diet and proteolytic enzyme activity was stated in several studies (Jelińska 2005; Sommer et al., 2002).

In the present study, the lipolytic activity of pancreatic juice was not strongly dependent on diet composition, probably due to differences within groups. Our findings disagree with the study assumptions. Nonetheless, the increase in bile secretion contributed to a numerical increase in lipase activity in the group receiving dietary zein. This upward trend could depend on the specific interaction between lipase and biliary salts (Konturek et al., 2004; Maldonado-Valderrama et al., 2011). Furthermore, the increase in lipase activity in the group supplemented with casein and sunflower oil could also be considered to result from the highest bile secretion. These observations are in agreement with Arienti et al. (1974), who suggested that bile, functioning as a buffer, might have a major influence on lipase protection from inactivation caused by duodenal digesta. Wang and Taniguchi (1998) observed a significant influence of casein on lipase activity, and suggested that increasing protein content in the abomasum could affect pancreatic lipase and amylase secretion. However, when examining pancreatic secretion in lambs fed different diets, Pierzynowski (1986) noted very high stability of pancreatic lipase activity. The activity of this

enzyme increased by a factor of two when fasted animals were fed a diet containing easily degradable compounds.

Unprotected vegetable oils may influence physiological blood indices (Huard et al., 1998). Already in 1978, Nestle et al. suggested that supplementing sunflower oil to ruminant diet increased plasma cholesterol concentrations from about 1.9 to 2.5 mmol/L. Moreover, elevated levels of this index were also observed by supplementing sunflower seeds (Zhang et al., 2006; Borowiec et al., 2008). In the present study, feeding sheep diets with zein and protected form of fat significantly reduced the plasma concentration of total protein due to slow degradation of zein in the rumen, where less ammonia was absorbed. According to the research hypothesis, sunflower oil addition to the diet significantly increased lipase activity, probably because of a greater absorption of medium-chained fatty acids in the rumen and free fatty acids in the small intestine in the blood. Likewise, the increased contribution of unprotected forms of fat (sunflower oil and sunflower seeds) to the diet for ruminants may increase the total cholesterol level. However, the reference values were not exceeded, which can be explained by the natural ability of healthy organisms to regulate the ratio of cholesterol intake to cholesterol synthesis (Wong et al., 1993). Sunflower oil contains a large proportion of unsaturated fatty acids, which are known as cholesterol suppressors. Therefore, the increasing concentration of cholesterol in blood plasma after fat supplementation is derived from specific digestion processes of fats in the rumen, where unsaturated fatty acids undergo biohydrogenation by bacteria, thereby increasing the saturated fatty acid pool.

To summarize, feeding animals diets with proper combinations of protected or unprotected forms of sunflower oil and otherwise digested protein can modify mainly proteolytic (and to a lesser extent lipolytic) activities of pancreas and probably affect plasma lipid indices in sheep. Further experiments should be conducted to evaluate more precisely the impact of diet composition on ileal digestibility of nutrients.

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Wpływ formy oleju słonecznikowego oraz źródła białka w dawce na sekrecję soku trzustkowego oraz aktywność enzymów trzustkowych u owiec

STRESZCZENIE

Celem podjętych badań było określenie wpływu różnych form oleju słonecznikowego podawanych z białkiem o różnym tempie degradacji w żwaczu na sekrecję i aktywność soku trzustkowego. Doświadczenie przeprowadzono na 24 dorosłych tryczkach Corriedale o masie ciała około 40 kg z założonymi kateterami do przewodu trzustkowego i żółciowego oraz dwunastnicy. Zwierzęta żywiono sianem łąkowym i skrobią ziemniaczaną z dodatkiem kazeiny (szybki rozkład w żwaczu) lub glutenu kukurydzianego, jako źródła zeiny (wolno rozkładanej w żwaczu) oraz różnymi formami oleju słonecznikowego (sole wapniowe kwasów tłuszczowych, nasiona i olej). Wykazano, że rodzaj tłuszczu w diecie nie wpłynął znacząco na sekrecję soku trzustkowego, natomiast obecność różnych form tłuszczu (nasiona słonecznika lub olej słonecznikowy) istotnie wpłynęła na sekrecję żółci, zawartość białka w soku, aktywność proteolityczną trypsyny oraz wskaźniki lipidowe w osoczu. Nie zaobserwowano istotnych różnic w aktywności lipolitycznej soku trzustkowego, natomiast aktywność lipazy była wyższa przy zastosowaniu zeiny jako źródła białka.

Dodatek chronionych lub niechronionych form oleju słonecznikowego oraz białka o różnym stopniu rozkładu w żwaczu do diety owiec może kształtować aktywność trzustki i prawdopodobnie wpływać na wskaźniki lipidowe w osoczu. Jednak potrzeba dalszych badań by dokładniej określić wpływ składu diety na jelitową strawność składników pokarmowych.

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EFFECT OF DIETARY ACIDIFIER ON GROWTH, MORTALITY, POST-SLAUGHTER PARAMETERS AND MEAT COMPOSITION OF BROILER CHICKENS*

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Abstract

An experiment with 608 broiler chickens was conducted to investigate the effect of dietary acidifier level on body weight, feed consumption and conversion, mortality, dressing percentage, postmortem carcass traits, tissue composition of breast and leg muscles, and plasma chemical parameters. Feeding the acidifier to chickens at 3, 6 and 9 g/kg of the diet reduced the pH of starter and grower diets from 6.90 to 5.89, and from 6.28 to 5.73, respectively. Compared to the control group, dietary acidification significantly increased body weight of chickens by 6.2, 8.2 and 8.2% at 21 days of age, and by 2.7, 3.6 and 3.7% at 42 days of age, respectively (P<0.01). Mortality decreased from 2.58% in the control group to 0.00–0.59% in the experimental groups (P<0.01). Acidification of the diets increased EEI-index from 327 (control group) to 348 points in the experimental group supplemented with 9% (9 g/kg) acidifier, but had no significant effect on feed consumption and feed conversion ratio among treatments. The relative weight of breast and leg muscles, gizzard, liver and carcass depot fat was not affected by dietary treatments. Breast muscles represented 27.7% (control group) and 27.9% (experimental groups) of the carcass weight. Leg muscles made up 21.5% and 20.7% of the carcass weight, respectively. There were no significant differences in chemical composition of breast and leg muscles, including dry matter, protein and fat content. No significant differences between the control and experimental chickens were noted for determined blood plasma constituents, glucose, total protein, triglycerides, total cholesterol and high density lipoprotein. The results suggested that organic acid acidifier used in this experiment at the rates of 3 to 9 g/kg diet has a growth enhancing and mortality reducing effect in broiler chickens, with no significant influence on carcass yield, proportion of individual carcass parts and blood plasma constituents. It seems that the amount of 6 g of the applied acidifier per kilogram of feed may be recommended as the optimum dietary level if protein in the diet does not exceed 200–230 g crude protein per kilogram of diet.

Key words: broiler chicken, dietary acidifier, growth performance, mortality, carcass indices, meat composition, blood plasma parameters

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The characteristics of low-molecular-weight organic acids and their antibacterial activity were extensively discussed by Cherrington et al. (1991). Some of these acids are used as food additives, in dairy, vegetable and meat products. Short-chain organic acids, such as lactic, acetic, propionic and butyric are generated as end-products of anaerobic fermentation in conserved feeds, and also in the digestive tract of animals and humans. They are added to food and feed as preserving and anti-moulding agents (Dixon and Hamilton, 1981). The importance of these acids in livestock nutrition increased considerably in response to the ban on in-feed antibiotics. Formic, sorbic and propionic acids, applied in different combinations in feed mixtures or diets for animals, are used against *Salmonella* Spp. and *Clostridium perfringens* (Thomson and Hinton, 1997; Bassan et al., 2008; Mikkelsen et al., 2009). Feed additives containing low-molecular-weight organic acids are referred to as acidifiers. The solid-form acidifiers contain organic acids, organic acid salts or their blends, usually based on carriers, which do not react chemically with the active ingredient. They are used in poultry nutrition for the purpose of maintaining the pH of digesta at a level preventing the growth of pathogenic bacteria. They also show bactericidal activity against pathogenic intestinal microflora. Most often, pathogenic bacteria begin to develop in the digestive tract when the lumen pH of the small intestine and caecum exceeds 5.8–6.0, and that of large intestine exceeds 6.2 (Garcia et al., 2007; Paul et al., 2007; Mikulski et al., 2008). The multiplication of pathogenic microorganisms in the intestine may result in the inflammation of intestinal mucosa or necrosis of intestinal epithelium. These processes, accompanied by increased secretion of intestinal fluids, lead to diarrhoea (Mikkelsen et al., 2009). Diarrhoea and the associated dehydration causes birds to stop eating and may lead to their death in a very short period of time.

Acidifying additives can be used in feed or incorporated in drinking water, which improves its quality. Low-molecular-weight organic acids, particularly propionic acid, have also a strong inhibitory effect on the growth of mould fungi. They suppress the growth of pathogenic microflora in feeds produced in feed meal plants. Cereals used for feed production may contribute pathogenic bacteria and mould fungi, which may synthesize harmful mould mycotoxins under poor storage conditions.

The amount of acidifier recommended for inclusion in poultry diets depends on several factors, mainly on alkalizing effects of feed ingredients and mineral supplement such as calcium sources. Under production conditions, the ban on in-feed antibiotics may result in considerable mortality rates, especially during the first 21 days of rearing the birds. According to modern farming standards, chicken mortality rates must not exceed 4%. Excessive mortality may be due to the strong alkaline effect of high protein content of diets for young birds, when the digestive tract and its secretory capacity are not fully developed. Some studies found that the efficiency of acidifier fed to chickens increases in the presence of probiotic lactic acid bacteria and a prebiotic (Kalavathy et al., 2003; Jamroz et al., 2004; Brzóska et al., 2005, 2007; Brzóska and Stecka, 2007; Mountzouris et al., 2007). Furthermore, previous research studies have also shown that acidifiers and probiotics fed to young animals are more effective when used concurrently with prebiotics (Patterson and Burkholder, 2003; Brzóska et al., 2007).

An important issue is to optimize the dietary level of the acidifier for broiler chickens. Recommendations seem not precise enough. Insufficient amounts of the acidifier may inadequately acidify the digesta, while excessive amounts may inhibit the secretion of digestive enzymes, lowering the degree of nutrient hydrolysis, and nutrient absorption processes. There is also an economic aspect to optimizing the acidifier level. The use of mineral acid salts in acidifier formulas slows down their action, by prolonging acidification of digesta during passage through the digestive tract. The presence of acids is responsible for their rapid hydrolysis in the feed and has antibacterial and antifungal action.

The aim of the study was to determine the effect of the dietary level of acidifier containing butyric and propionic acids and salts of formic and butyric acids as the main active components, on broiler performance, mortality, carcass parameters, chemical composition of breast and leg muscles, and the level of main plasma metabolites.

Material and methods

The experiment procedures were approved by the Local Ethic Committee for Animal Experimentation. A total of 608 unsexed, day-old Ross 308 broiler chickens were randomly divided into 4 groups, with 4 replicates of 38 birds. Birds in each replicate were kept in pens covered with deciduous wood shavings at a density of 18 birds/m^2 and with approximately 33 kg of live birds/m^2 at the end of rearing. Indoor temperature, humidity and air exchange were in accordance with hygiene standards for young birds. The birds were vaccinated against Gumboro disease at day 5 and against fowl plague at day 12 of age. Vitazol (Biowet Drwalew, Poland), a vitamin supplement, was administered to chickens at several-day intervals throughout the experiment. All chickens received *ad libitum* starter type diets (1–21 days) followed by grower type diets (22–42 days). The diets were composed of ground maize, wheat grain and soybean meal as the main ingredients (Table 1). Water was provided in spot drinkers during the 3 weeks and in trough-type drinkers during weeks 4 to 6. A commercial Acidomix AFG (Novus) acidifier was used, which contained 20.7% butyric acid (E236), 17.5% ammonium propionate (E295), 12.5% propionic acid (E280), and 4.2% ammonium propionate (E284). The acidifier were applied in both diet types at the levels of 3, 6 and 9 g per kg (experimental group), and the control group was fed without Acidomix addition.

Dietary nutrients and feed acidity were determined by chemical analysis (AOAC, 1990). Amino acid composition of the feedingstuffs was determined using an automatic HPLC analyser after acid hydrolysis, and methionine following perchloric acid oxidation. Feed consumption and mortality were recorded throughout the study and the feed conversion ratios were subsequently calculated.

On day 43, ten birds (5 males and 5 females), 40 animals in total, were selected from each group and killed by decapitation after stunning. During slaughter, blood samples were collected into heparinized tubes and centrifuged. Fresh plasma was

used to analyse glucose content. Plasma was frozen and stored until analysis for total protein, triglycerides, total cholesterol and high-density lipoproteins (HDL). Blood components were analysed using Cormay Diagnostic kits. Measurements were performed on a Beckman DU 640 spectrophotometer. After slaughter, carcasses were defeathered and eviscerated. The weight of 40 bird carcasses, gizzard, liver, feet, omental fat and abdominal fat from the posterior part of the body cavity was determined. Both types of fat are referred to as depot fat. Carcasses were chilled at 5°C for 24 h. The next day, carcasses were dissected according to the method described by Zgłobica and Różycka (1972). Breast and leg muscles, depot fat, skin and leg bones were weighed. The weight of individual carcass parts was related to total carcass weight and expressed as percent. Samples of breast and of leg muscles from the right carcass part were collected, ground and frozen at –18°C to analyse dry matter and basic nutrients. After thawing, muscle samples were analysed for dry matter, crude protein, crude fat and crude ash. The analyses were performed by standard methods (AOAC, 1990).

Table 1. Components and nutritive value of the diets

Item	Diet	
	Starter (1–21 days)	Grower (22–42 days)
Feed ingredients (%)		
Maize	34.00	30.00
Wheat	26.10	34.10
Soybean meal	32.50	28.50
Rapeseed oil	4.00	4.00
Dicalcium phosphate	1.70	1.70
Ground limestone	0.60	0.60
NaCl	0.35	0.35
L-lysine HCl (78%)	0.11	0.11
DL-methionine (99%)	0.14	0.14
Mineral-vitamin premix[1) 2)]	0.50	0.50
Nutrients in 1 kg of dry matter:		
Crude protein (g)	229.9	205.6
Lysine (g)	12.7	10.9
Methionine+Cysteine (g)	5.2	5.7
Crude fat (g)	27.7	24.5
Crude fibre (g)	50.5	63.1
Calcium (g)	8.8	83
Phosphorus (g)	4.2	4.1
Metabolizable energy (MJ)	12.44	12.23

[1)] Supplied per kg of starter diet: vit. A – 13 5000 IU; vit. D_3 – 3 600 IU; mg: vit. E – 45; vit. B_1 – 3.25; vit. B_2 – 7.5; vit. B_6 – 5; vit. B_{12} – 0.0325; vit. K_3 – 3; biotin – 0.15; nicotinic acid – 45; calcium pantothenate – 15; folic acid – 1.5; choline chloride – 100; Mn – 100; Cu – 1.75; Fe – 76.5; Se – 0.275; I – 1; Zn – 75; Co – 0.4; Endox (antioxidant) – 125; Sincox (coccidiostat) – 1 g; calcium – 0.679 g.

[2)] Supplied per kg of starter diet: vit. A – 12 000 IU; vit. D_3 – 3 250 IU; mg: vit. E – 40; vit. B_1 – 2; vit. B_2 – 7.25; vit. B_6 – 4.25; vit. B_{12} – 0.03; vit. K_3 – 2.25; biotin – 0.1; nicotinic acid – 40; calcium pantothenate – 12; folic acid – 1.0; choline chloride – 450; Mn – 100; Cu – 1.75; Fe – 76.5; Se – 0.275; I – 1; Zn – 75; Co – 0.4; Endox (antioxidant) – 125; Sincox (coccidiostat) – 1 g; calcium – 0.79 g.

The data were subjected to analysis of variance (ANOVA) using SAS/STAT® ver. 5.1 (SAS, 1994–2001). Mean values for the groups were compared using Duncan's multiple range test at the 1% and 5% level of probability.

Results

The components and nutritive value of the feeds are presented in Table 1. Adding the acidifier to chicken feeds reduced the pH of starter diet from 6.90 to 5.89, and that of the grower diet from 6.28 to 5.73. Supplementing diets with the increasing amounts of acidifier (from 3 to 9 g/kg) significantly increased body weight of chickens at 21 and 42 days of age ($P \leq 0.01$) compared to the control birds (Table 2). Mortality decreased significantly, with significant differences in relation to the control group ($P \leq 0.01$). Feed consumption and conversion remained unchanged. Feeding the acidifier significantly increased carcass weight at 43 days of the experiment ($P < 0.01$; Table 3). Significant differences were found in dressing percentage, which was the highest with the acidifier supplemented at 3 g/kg and the lowest at 9 g/kg, but did not differ from the control value. There were no statistically significant differences in the weight of individual carcass parts, including breast and leg muscles, in gizzard and liver weight, and in the amount of depot fat. The proportion of individual carcass parts dissected (muscles, depot fat, skin, leg bones, feet) and the proportion of organs (gizzard, liver) were similar across dietary treatment. There were no significant differences in chemical composition, including dry matter, protein, fat and ash content of breast and leg muscles. No significant differences were also obtained for blood plasma parameters, including glucose, total protein, triglycerides, total cholesterol and HDL cholesterol (Table 4).

Table 2. Feed pH and growth performance of broiler chickens

Item	Control	Acidifier level (g/kg diet)			SEM
		3	6	9	
Feed pH					
– Starter	6.90	6.42	6.10	5.89	
– Grower	6.28	5.97	5.81	5.73	
Body weight, 21 days (g)	597 aA	634 bB	646 bB	646 bB	4
Body weight, 42 days (g)	2394 aA	2459 bAB	2480 bB	2483 bB	11
Mortality (%)	2.58 bB	0.00 aA	0.00 aA	0.59 aA	0.78
Feed intake (kg/bird/42 days)	4.06	4.30	4.23	4.20	0.42
Feed conversion (g/kg BWG)	1.70	1.75	1.71	1.69	0.12
European Efficiency Index (points)	327	335	345	348	41

a, b – values in rows with different letters differ significantly ($P < 0.05$).
A, B – values in rows with different letters differ significantly ($P < 0.01$).
SEM – standard error of the mean.
BWG – body weight gain.

Table 3. Post-slaughter characteristics of broiler chickens

Item	Control	Acidifier level (g/kg of diet)			SEM
		3	6	9	
Slaughter weight (g)	2492	2588	2668	2604	43
Carcass weight (g)	1768 aA	1899 bcBC	1946 cC	1834 bB	32
Dressing percentage	70.95 ab	73.38 b	72.94 ab	70.43 a	0.42
Absolute weight (g)					
breast muscles	490.5	538.1	550.6	498.4	10.1
leg muscles	379.6	399.4	404.1	374.2	7.5
gizzard	28.0	28.0	29.0	27.9	0.4
liver	57.1	53.6	55.8	59.5	1.4
depot fat	37.3	41.0	42.5	34.5	1.4
skin	101.1	105.9	111.1	97.8	2.3
leg bones	99.3	98.4	102.7	104.4	2.4
feet	84.1	86.8	89.2	91.0	2.3
Relative proportion (% of carcass weight)					
breast muscles	27.7	28.1	28.3	27.2	0.3
leg muscles	21.5	21.0	20.8	20.4	0.2
gizzard	1.6	1.5	1.5	1.5	0.4
liver	3.2	2.9	2.9	3.2	0.1
depot fat	2.1	2.2	2.2	1.9	1.4
skin	5.7	5.6	5.7	5.3	0.1
leg bones	5.6	5.2	5.3	5.7	0.1
feet	4.8	4.6	4.6	5.0	0.1

For abbreviations see Table 2.

Table 4. Chemical composition of breast and leg muscles and blood plasma parameters

Item	Control	Acidifier level (g/kg of diet)			SEM
		3	6	9	
Breast muscle:					
dry matter (%)	25.28	25.61	25.66	26.07	0.09
crude protein (% DM)	23.58	23.56	23.75	24.27	0.07
ether extract (% DM)	1.05	1.00	1.14	0.88	0.03
ash (%DM)	1.18	1.16	1.15	1.17	0.01
Leg muscle:					
dry matter (%)	26.11	25.49	25.81	26.03	0.11
crude protein (%DM)	19.56	19.51	19.57	19.67	0.07
ether extract (%DM)	5.65	5.14	5.34	5.47	0.13
ash (%DM)	1.10	1.11	1.09	1.09	0.00
Plasma parameters (mg/dl):					
glucose	261.3	262.3	249.7	276.3	6.7
total protein	3.78	5.58	3.79	3.60	0.06
triglycerides	43.12	35.08	29.59	35.43	1.81
total cholesterol	134.8	129.0	132.1	123.2	2.1
high density lipoproteins	99.3	94.1	99.0	89.5	1.7
HDL/TC×100	73.7	73.0	74.9	72.7	-

For abbreviations see Table 2.

Discussion

The ban on the use of in-feed antibiotics in farm animals, including poultry, has prompted a search for feed additives that control gut microbial status. In addition to the respiratory tract, the digestive tract of birds runs the greatest risk of being infected with pathogenic microorganisms. Although the digestive tract of the newly hatched bird is sterile, its contact with feed and water leads to rapid implantation of gastric mucosa by lactic acid bacteria, including *Lactobacillus* Spp., but infection with pathogens is also possible. *Salmonella* spp., *Clostridium*, *Campylobacter* and *Shigella* spp. bacteria represent a health risk to poultry as they may induce diarrhoea in young birds, slow the rate of growth and impair feed intake, and thus cause deaths. Pathogenic food poisoning is a threat to consumers of poultry meat and eggs (Simon et al., 2001). It may occur in abattoirs and poultry processing plants that do not conform to hygiene standards. It has been shown that the addition of organic acids, e.g. lactic acid to drinking water protects chicks from *Campylobacter* colonization (Chaveerach et al., 2004; Byrd et al., 2009).

It is more common to add organic acids to the chicken feed, which eliminates pathogenic bacteria, including *Salmonella* Spp., from the digestive tract (Hinton and Linton, 1988; Rouse et al., 1988; McHan and Shotts, 1992). The usefulness of short-chain monocarboxylic fatty acids and their derivatives, unsaturated, hydrogenated, phenolic and polycarboxylic acids was investigated with poultry feeds (Cherrington et al., 1991). Short-chain fatty acids, e.g. acetic, propionic and butyric, are produced in millimolar quantities in the animal and human digestive tract with its resident microflora. They are anaerobic lactic acid bacteria (Gram-positive *Lactobacillus* Spp. and *Enterococcus* Spp.) which ferment glucose in the intestinal mucosa to acids. Organic fatty acids, in particular propionic and formic ones, are known to be feed additives that inhibit the growth of moulds in wet feed materials (Dixon and Hamilton, 1981). These acids are used in different combinations as agents against *Salmonella* Spp. and *Escherichia* coli (Thompson and Hinton, 1997; Ricke, 2003; Paul et al., 2007). The production of organic acids in the digestive tract of poultry is stimulated by the feeding of probiotic bacteria (Kalavathy et al., 2003; Brzóska et al., 2005; Brzóska, 2007; Brzóska and Stecka, 2007; Award et al., 2009).

Another gastrointestinal disease found in broiler flocks is necrotic enteritis (Van Der Sluis, 2000). It is difficult to detect in its subclinical form and is detrimental in economic terms. The main causative agent is *Clostridium perfringens*, which is found in small intestine and liver (Gholamiandehkordi et al., 2007). It is conjectured that acidifiers limit the incidence of necrotic enteritis and the associated losses (Mikkelsen et al., 2009).

The results of this study show that the dietary level of 0.3, 0.6 or 0.9% acidifier, increases the growth rate of chickens during both the first 21 days of age and over the entire 42-day period. In the experimental groups, the live weight gains of the chickens fed with increasing amounts of the acidifier added were 65, 86 and 89 g/bird higher than the control value, which in relative terms corresponded to an increase of 2.7 to 3.7%. Better body weight gain was accompanied by increased feed consumption (by 0.24, 0.17 and 0.14 kg/bird, respectively), although no significant

differences were found in feed conversion ratio (1.71 kg/kg at average). These data may be indicative of the superior conversion of the amino acids and energy from the acidifier supplemented diets and of the superior conversion of energy into broiler tissues.

The gradual increase in acidifier amounts in the experimental diets prevented mortality in chickens. The addition of 3 and 6 g/kg of diet completely eliminated deaths, bringing mortality equal to zero. This suggests that the acidifier protected the chickens from intestinal infections and gastrointestinal disorders, which are a common cause of mortality. The highest dose of the acidifier (9 g/kg) slightly increased chicken mortality to 0.59%, which was 4-fold lower than the value obtained for the control group, and 6.6-fold lower than acceptable standards for mortality in large-scale operations. In the present experiment, in terms of stocking density and body weight per unit floor area, the housing conditions were similar to the conditions used in large-scale production. The degree of diet acidification had no effect on carcass evaluation parameters, which is in agreement with the results of an earlier study (Young et al., 2001). In a study on male turkeys Mikulski et al. (2008) evaluated the physiological and growth effects of organic acids, organic acids with essential oils or herbal extracts added to diets, and found a significant decrease in pH of the crop contents, but no effect on the pH of caecal digesta. All supplements significantly increased the body weights of turkeys at the age of 84 days, with no influence on carcass traits.

The Acidomix acidifier used in the current experiment contained ammonium formate, propionic acid and ammonium propionate. Such composition resulted in the hydrolysis and dissociation of both salts into formic and propionic acids in the aqueous environment of the intestine during the digesta passage through the intestinal tract. Considering the slow hydrolysis of salts in the digestive tract, it can be assumed that the acidifying effect persisted into the final section of the digestive tract, which is the least acidic and most vulnerable to growth of pathogenic bacteria. In a study with broilers, Czerwiński et al. (2010) investigated the effect of dietary pea inclusion in the presence of organic acids or probiotic, composition of fumaric acid, calcium formate, calcium propionate and potassium sorbate. Organic acid supplementation slightly increased the *Lactobacillus/Enterococcus* counts, but total bacterial counts in caecal contents were not affected. Świątkiewicz et al. (2010) investigated the effect of prebiotic and organic acid on eggshell quality of laying hens. They concluded that acidifiers can lower the pH of the diet and beneficially influence egg shell quality.

The mode of action of low-molecular-weight organic acids on pathogenic bacteria has been elucidated by several authors. Ricke (2003) described a mechanism in which organic acids penetrate the lipid membrane of the bacterial cell and once incorporated into the neutral pH of the cell cytoplasm dissociate into anions and protons (Eklund, 1983; Salmond et al., 1984; Cherrington et al., 1990, 1991). Export of excess protons requires consumption of cellular adenosine triphosphate (ATP). This may result in a depletion of cellular energy, and thus in cell death. It has also been speculated that organic acids interfere with cytoplasmic membrane structure and intercellular transport as a result of changes in electrical gradients across cell

membrane, which may also be lethal to pathogenic bacterial cells (Russell, 1992; Axe and Bailey, 1995).

Of special importance was the presence of butyric acid in the evaluated acidifier. Butyric acid and its salts, including ammonium isobutyrate, are known to be factors that regenerate intestinal epithelium of young suckling animals, resulting in regeneration of intestinal villi, which constitute the main absorptive area of the digestive tract (Jang et al., 2008; Puyalto and Locatelli, 2008). Some authors showed that dietary supplementation with a blend of organic acids containing lactic, formic, citric, phosphoric and butyric acids had no effect on growth rate but increased gizzard weight and the length of intestinal epithelium in broilers (Jang et al., 2008). However, Mahdavi and Torki (2009) found no direct effect of feeding chickens with butyric acid (2 or 3 g/kg feed) on weight gains, feed intake and conversion, and bird mortality. The experimental factor had also no effect on the weight of individual carcass cuts, but a significant effect of increasing small intestinal length was stated. The results cited above indicate that the increase in chickens' body weight in response to acidifying additives does not occur in every situation. It is of economic importance that the acidifiers significantly reduced chicken mortality in all studies mentioned above. In our study, both the higher weight gain than in the control group and a similar feed conversion rate may suggest that the experimental factor contributed to better utilization of dietary protein, amino acids and metabolizable energy. However, this issue requires more in-depth nutritional and physiological research. Based on the studies with organic and inorganic acidifiers, Viola and Vieira (2007) suggested that acidifiers are as efficient as antibiotics in maintaining the performance and morphology of the small intestines of broiler chickens.

In conclusion, due to the high protein contents, in addition to minerals, conventional broiler diets may have a highly alkalizing effect. Because the digestive tract of chickens is not ready to counteract the effects of alkaline digesta, the administration of acidifiers and additives that reduce digesta pH seem the most important factor regulating the status of intestinal microflora. Our study with Acidomix AFG acidifier showed that the most advantageous dietary level of this feed additive ranges from 3 to 9 g/kg, with 6 g/kg regarded as the optimum dose.

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FRANCISZEK BRZÓSKA, BOGDAN ŚLIWIŃSKI, OLGA MICHALIK-RUTKOWSKA

Wpływ zakwaszacza diety na masę ciała, śmiertelność, wydajność rzeźną i skład mięsa kurcząt rzeźnych

STRESZCZENIE

W doświadczeniu wykonanym na 608 kurczętach rzeźnych Ross 308 badano wpływ zakwaszacza diety na masę ciała, spożycie i wykorzystanie paszy, śmiertelność ptaków, wydajność rzeźną, cechy poubojowe tuszek, skład chemiczny mięśni piersiowych i nóg oraz wskaźniki chemiczne osocza krwi. Podawanie zakwaszacza kurczętom w ilości 0, 3, 6 i 9 g/kg diety obniżyło odczyn (pH) diety na pierwszy okres chowu (1–21 dni) z 6,90 do 5,89, a drugi okres chowu (22–42 dni) z 6,28 do 5,73. W porównaniu z grupą kontrolną, nie otrzymującą zakwaszacza, istotnie wzrosła masa ciała kurcząt 42. dnia życia, odpowiednio o 2,3; 3,6 i 3,7% ($P<0,01$). Śmiertelność zmalała z 2,58% w grupie kontrolnej do 0,00–0,59% w grupach doświadczalnych ($P<0,01$). Zakwaszenie diety nie miało istotnego wpływu na spożycie i wykorzystanie paszy. Nie stwierdzono istotnych różnic w masie mięśni piersiowych i mięśni nóg, a także masie żołądka, wątroby i tłuszczu zapasowego ptaków. Mięśnie piersiowe miały 21,6% w grupie kontrolnej i 21,3% masy tuszki w grupie doświadczalnej. Zakwaszenie diety zwiększyło wartość indeksu EEI z 327 (grupa kontrolna) do 348 (grupa doświadczalna), przy 9 g/kg zakwaszacza. Nie stwierdzono istotnych różnic w składzie chemicznym mięśni piersiowych i mięśni nóg, w tym zawartości suchej masy, białka ogólnego, tłuszczu surowego i popiołu. Zawartość białka w mięśniach piersiowych wynosiła 23,58% w grupie kontrolnej i 23,86% średnio w grupach doświadczalnych ($P\geq0,01$). Zawartość białka w mięśniach nóg wynosiła odpowiednio 19,56% i 19,58% ($P\geq0,01$). Nie stwierdzono istotnych różnic we wskaźnikach osocza krwi. Wnioskowano, że zakwaszacz zawierający kwas propionowy i sole kwasu mrówkowego oraz masłowego poprawia efektywność produkcji kurcząt rzeźnych istotnie obniżając straty powodowane zakażeniami bakteryjnymi przewodu pokarmowego. Użycie zakwaszacza od 3 do 6 g/kg diety istotnie zwiększa masę ciała i tuszek kurcząt nie powodując istotnych różnic w masie i proporcjach poszczególnych partii tuszek. Optymalny poziom preparatu of Acidomix AFG w diecie dla kurcząt zawierającej 206–230 g białka ogólnego wynosił 6 g/kg.

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FEEDING CORN DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS) AND ITS EFFECT ON EGG QUALITY AND PERFORMANCE OF LAYING HENS*

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Abstract

The purpose of this experiment was to determine the effect of corn DDGS as a feed ingredient on egg quality and performance of laying hens. The experiment was conducted in three feeding groups of 100 hens each (10 replicates of 10 layers). ISA Brown laying hens were administered a feed mixture containing 15% (E1) or 20% (E2) corn DDGS for 18 weeks. The hens from the control group (C) received a standard diet based on soybean meal as the main protein source only. Laying performance, average egg weight, average daily feed intake and feed conversion ratio were recorded over the study period. Egg quality traits (egg weight, thick albumen quality, yolk colour, yolk content, shell content and shell thickness) were evaluated twice: at the start and at the end of the experiment. On both dates, all daily laid eggs from each group were analysed, i.e. 90, 93 and 92 eggs from groups C, E1 and E2, respectively at 31 weeks, and 92, 94 and 81 eggs, respectively at 48 weeks of age. Compared to the other groups, the hens from group E2 (20% DDGS) were characterized by a slight – though statistically significant (P≤0.01) – decrease in laying performance and by a higher FCR value. The content of DDGS in the feed mixture had no significant effect on mean egg weight nor on daily feed intake. At the end of the experiment, the eggs laid by the hens from group E2 were characterized by significantly poorer (P≤0.01) albumen and shell quality. Yolk colour in both experimental groups was significantly darker (P≤0.01) than in the C group. The 15% addition of corn distillers dried grains with solubles to feed mixtures for commercial flocks of laying hens is advisable. At corn DDGS addition exceeding 15%, a slight decrease in production results and deterioration in selected parameters of egg quality shall be expected.

Key words: corn DDGS, laying hens, egg production, egg quality

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According to the Main Statistical Office (GUS, 2011), the structure of corn utilization in Poland has been changing since 2000, i.e. the area of croplands for silage has been decreasing proportionally to the increasing area of corn croplands for grain. In the year 2002, corn was cultivated on over 318.000 ha, whereas in 2010 – on 342.000 ha of arable lands. One of the factors that influence a change in these proportions is the increasing production of biofuels. Compared to potatoes and rye, corn grain is characterized by the highest yield of ethanol production (Stecka, 2003). According to Shurson and Noll (2005), 100 kg of corn grain enable producing: 36 l of ethanol, 32 kg of distillers dried grains with solubles (DDGS), and 32 kg of CO_2. These authors expected that in the year 2005, the production of DDGS only in the USA would exceed 7 millions of tonnes, whereas within a few successive years it would be estimated to reach 10–14 millions of tones. The distillers dried grain with solubles is an industrial by-product, the quantity of which is estimated to increase in the future. In order to meet environment protection standards, the DDGS requires management. The developed production technology of DDGS allows achieving a product with parameters similar to those of the feed concentrate, that may be easily stored and transported.

As presented by Cozannet et al. (2010), the chemical analysis of corn DDGS demonstrated that, compared to corn grain, it was characterized by a threefold higher content of total nitrogen and fat, and by a very low content of starch that is utilized in the fermentation process, which results in a low level of metabolizable energy. The same authors emphasize that the nutritive values of this product may vary depending on grain quality and processing method.

The objective of this study was to investigate possibilities of applying corn distillers dried grains with solubles (DDGS), a by-product of ethanol production, as a substitute for extracted soybean meal in the feeding of laying hens and to determine the effect of corn DGGS as a feed constituent on quality of eggs and performance of laying hens.

Material and methods

The study was conducted on 300 ISA Brown laying hens aged 31 weeks at the onset of the experiment. The study spanned 18 weeks (31–48 weeks of age). From the beginning of the laying period until the peak of production hens were fed a standard laying diet (Table 1). Before and during the experiment the layers were kept in the same conditions in cages of 10 hens arranged in a three-tier battery. Lighting programme and temperature in the building were according to the ISA Brown Management Guide (www.hendrix-genetics.com, 2008 b).

The experiment was conducted in three feeding groups of 100 hens each (10 replicates of 10 layers): E1 and E2 (experimental) and C (control). Both experimental groups were fed a feed mixture containing 15% (E1) or 20% (E2) of corn DDGS as a replacement for part of soybean meal. The hens from the control group (C) were given a standard laying diet based on soybean meal as the main protein source only. The composition of feed mixtures and their nutritional value are presented in Table 1.

Table 1. Composition (%) and nutritional value of feed used before and in the experiment

Ingredients	Content in diet (%)			
	all groups before the experiment	group C	group E1	group E2
Corn	45.00	45.00	20.00	20.00
Wheat	16.60	16.60	36.40	33.60
Wheat bran	6.40	6.40	7.00	7.00
Soybean meal	21.60	21.60	11.40	9.20
Corn DDGS			15.00	20.00
Soybean oil	0.60	0.60	0.60	0.60
Dicalcium phosphate	0.90	0.90	0.42	0.30
$CaCO_3$	7.76	7.76	8.00	8.06
Lysine			0.16	0.22
Methionine	0.12	0.12	0.10	0.10
Sodium bicarbonate	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.20	0.20
Enzyme (PX446-012% Layer Phyzyme 500)	0.12	0.12	0.12	0.12
Mineral-vitamin premix	0.50	0.50	0.50	0.50
Nutritional value				
ME MJ/kg	11.31	11.31	11.32	11.30
Total protein (%)	17.00	17.00	16.96	16.96
Crude fibre (%)	2.73	2.73	3.46	3.71
Total Ca (%)	3.28	3.28	3.26	3.26
P available (%)	0.54	0.54	0.51	0.50
Na	0.16	0.16	0.18	0.20
Linoleic acid	1.52	1.52	1.97	2.24
Methionine	0.38	0.38	0.37	0.38
Methionine + cystine	0.70	0.70	0.71	0.71
Lysine	0.84	0.84	0.79	0.80

Feed formulas have been prepared by the Feed Mill in Reguły based on its raw materials accepted as the standard feed. The experimental feed formulas were developed taking into account results of the proximate analysis of corn DDGS (Table 2). The chemical composition was determined according to AOAC methods (AOAC, 2005).

Table 2. Nutritional value of corn DDGS (data from feed analysis)

Nutritional value	Content (%)
Dry matter	90.21
Total protein	25.84
Crude fat	14.85
Crude fibre	8.37
Crude ash	5.27
EM MJ/kg *	14.811

*calculated by method of Smulikowska (Smulikowska and Rutkowski, 2005).

All birds were individually weighed just before the start and at the end of the experiment. Each successive week, the daily egg production, daily egg weight and weekly feed intake were noted in each group. Based on this data, calculations were performed every week for: laying performance (% of lay), average egg weight (g), egg production per hen per day (g), average daily feed intake (g per hen), and feed conversion ratio (FCR – kg of feed per kg of egg weight).

At the start and at the end of the experiment all daily laid eggs from each group were collected. Eggs were subjected to standard quality control with the use of EQM system, version 1.0. Parameters estimated in fresh eggs on both dates were: egg weight (g), thick albumen quality (Haugh units), yolk colour (RCF), yolk content in egg (%), and shell thickness (mm). In order to determine dry shell content in egg (%), the shells were cleaned and dried for 24 hours at a temperature of 110ºC. In total, 90, 93 and 92 eggs were analysed from groups C, E1 and E2, respectively at 31 weeks, and 92, 94 and 81 eggs, respectively at 48 weeks.

The data for each trait were analysed by one-way analysis of variance calculated by the least squares method, separately for different factors: group and age (SPSS 14.0, GLM procedure). Results in tables are presented as least square means (LSM) with the standard error of the mean (SEM).

Results

Over the experimental period, no death cases were reported in any of the groups examined. Both at the onset and at the end of the experiment, the mean body weight of hens was similar in all groups, and the significance of differences between the groups was not statistically confirmed (Table 3). As expected, in all groups, the body weight of hens increased during the period of the experiment. In both experimental groups receiving DDGS, body weight gains were identical and negligibly higher than in the control group.

Table 3. Hens body weight (kg) at the start (BW-31) and at the end (BW-48) of experiment

Group	BW-31 (kg)		BW-48 (kg)		Gain (g)
	LSM	SEM	LSM	SEM	
C	1.68 A	0.013	1.84 A	0.016	+ 160
E1	1.67 A	0.013	1.85 A	0.016	+ 180
E2	1.69 A	0.013	1.87 A	0.016	+ 180

A – Values in columns with the same letter – non significant differences.

At the beginning, the administration of DDGS to feed mixtures for hens did not result in a rapid decrease of laying performance in both experimental groups, and the mean values of this parameter noted in all groups were similar to the standard value (www.hendrix-genetics.com, 2008 a). Nevertheless, in group E2 fed the mixture with 20% DDGS a successive decrease was observed in laying performance. This group was also characterized by the least unvaried values of this parameter

(Figure 1). The laying intensity (%) in this group was significantly lower (P≤0.01) than in groups C and E1 (Table 4). In group E1 (15% DDGS), the laying performance was slightly higher than in group C, but the difference was insignificant.

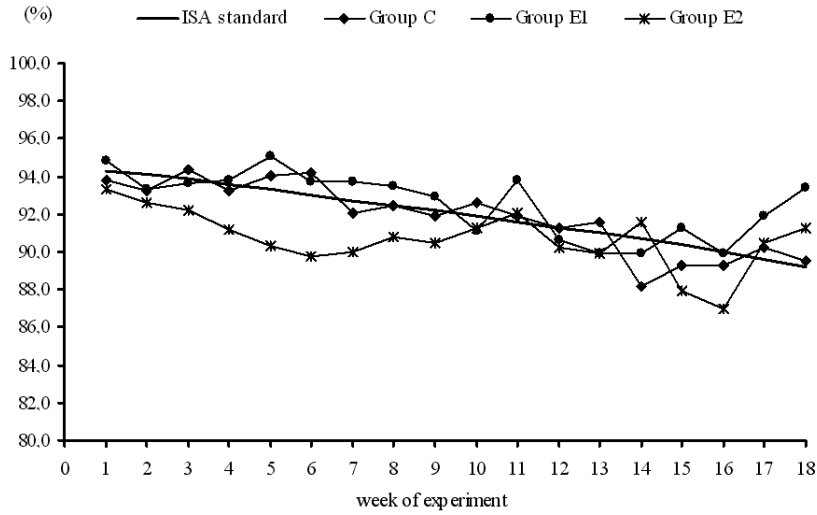

Figure 1. ISA Brown laying production (%) over the experimental period

Table 4. Production results during the period of 18 weeks

Parameters	Group C		Group E1		Group E2	
	LSM	SEM	LSM	SEM	LSM	SEM
Laying performance (%)	91.9 A	0.32	92.5 A	0.31	90.7 B	0.31
Mean egg weight (g)	60.3 A	0.11	60.0 A	0.11	60.2 A	0.11
Feed intake (g/hen/day)	114.1 A	0.05	113.9 A	0.05	113.8 A	0.05
Feed conversion (kg/kg egg mass)	2.07 A	0.01	2.05 AB	0.01	2.09 B	0.01

A, B – Values in rows with different letters differ significantly at P≤0.01.

The mean egg weight in all groups was at a similar level, and the minute differences were not confirmed statistically (Table 4). The mean daily feed intake in the experimental groups was almost identical as in the control group. However, owing to the lower laying performance in group E2, the FCR per kg of egg weight was significantly higher (P≤0.01) in this group than in the other groups (Table 4).

Results of standard qualitative assessment of eggs were presented in Table 5. Over the 18-week experimental period, egg weight was observed to increase significantly (P≤0.01) in all groups (Table 5) but to a different extent in particular groups, i.e. in group C the increase was the highest and reached +6.49 g, whereas in groups E1 and E2 it reached +4.47 g and +3.84 g, respectively.

Table 5. Egg quality parameters analysed on two dates: at the start of experiment (Date I) and at the end of experiment (Date II)

Trait	Group	Date I			Date II			Differences between dates P values
		n	LSM	SEM	n	LSM	SEM	
Egg weight (g)	C	90	54.30 A	0.39	92	60.79 a	0.44	0.000
	E1	93	54.80 A	0.39	94	59.27 b	0.43	0.000
	E2	92	56.08 B	0.39	81	59.92 ab	0.47	0.000
Thick albumen quality (HU)	C	90	89.83 A	0.65	92	83.81 A	0.76	0.000
	E1	93	86.56 B	0.64	94	83.62 A	0.75	0.002
	E2	92	88.46 A	0.64	81	78.07 B	0.81	0.000
Yolk colour (RCF)	C	90	8.03 A	0.12	92	7.40 A	0.11	0.000
	E1	93	7.85 A	0.12	94	8.02 B	0.11	0.315
	E2	92	8.09 A	0.12	81	7.82 B	0.12	0.101
Yolk content (%)	C	90	24.25 a	0.16	92	26.83 A	0.19	0.000
	E1	93	24.72 b	0.15	94	27.38 B	0.18	0.000
	E2	92	24.08 a	0.15	81	27.69 B	0.20	0.000
Shell content (%)	C	90	9.94 A	0.08	92	10.01 a	0.09	0.490
	E1	93	10.07 A	0.08	94	10.04 a	0.09	0.831
	E2	92	9.81 A	0.08	81	9.73 b	0.10	0.512
Shell thickness (mm)	C	90	0.352 A	0.003	92	0.370 a	0.003	0.000
	E1	93	0.361 A	0.003	94	0.372 a	0.003	0.009
	E2	92	0.358 A	0.003	81	0.360 b	0.004	0.668

A, B – Values in columns with different letters differ significantly at P≤0.01.
a, b – Values in columns with different letters differ significantly at P≤0.05.

Throughout the study period, thick albumen quality expressed in Haugh units (HU) decreased in all groups (Table 5). The greatest decline in albumen quality, by as many as 10.39 HU, was determined in group E2 (20% DDGS), followed by group C (by –6.02 HU). In group E1 (15% DDGS), albumen quality deterioration was minimal (–2.94 HU).

At the start of the experiment, there were no differences in yolk colour between groups (Table 5). After 18 weeks of feeding hens with corn DDGS, differences appeared between the control group and experimental groups in which yolks were significantly darker (P≤0.01). In turn, yolk content of egg increased significantly in all groups in the study period (Table 5). The greatest increase in this parameter was noted in group E2, and the least one in group C. At the end of the experiment, its value in the control group was significantly different (P≤0.01) from the values recorded in the experimental groups.

Egg shell quality was assessed with two traits: shell content of egg and shell thickness. Based on the values collated in Table 5, it may be concluded that the

quality of shells was good irrespective of analytical period and diet administered to hens. At the beginning of the experiment, no differences in both analysed traits were noted between the groups. In the course of the study, no significant differences were either observed in shell content of egg. In turn, shell thickness increased, though not always significantly, in all groups, whilst in groups C and E1 it was significantly better than at the onset of the experiment. A comparison of values of this trait demonstrates that the improvement in shell quality varied in particular groups, i.e. shell thickness increased by 0.018 mm in group C, by 0.011 mm in group E1, and by 0.002 mm in group E2. Along with an increasing content of DGGS in the feed mixture, the improvement in shell quality was less tangible. Considering both traits of egg shell quality, the values noted in group E2 differed significantly ($P \leq 0.01$) compared to the other groups.

Discussion

Based on the results obtained (Table 3), it may be concluded that the distillers dried grains with solubles introduced to feed mixtures for laying hens had no negative effect on their body weight gain. The positive effect of corn DDGS on body weight values of young hens during rearing was reported by Masa'deh (2011), but only in the birds aged 14–16 weeks. In a study on mature laying hens in the production period (24–76 weeks), Masa'deh and Scheideler (2008), Masa'deh et al. (2008, 2011) did not demonstrate any significant effect of corn DDGS on their body weight. The body weight values and body weight gains of laying hens from the groups receiving feed mixtures with various contents of DDGS were very alike, though lesser gains were observed in the birds fed the mixture with 20–25% of DDGS.

The production results obtained in the study (Table 4) do not differ significantly from findings reported in a previous study by Niemiec et al. (2012) addressing wheat DDGS. The authors did not find it to affect laying performance. Also other authors (Roberts et al., 2007; Masa'deh and Scheideler, 2008; Masa'deh et al., 2008; Green et al., 2010; Masa'deh, 2011) who investigated effects of corn DDGS addition to feed mixtures for laying hens, demonstrated that it had no effect on laying performance, even at 20–30% of the feed mixture. As reported by Green et al. (2010), already 50% addition of DDGS to the feed mixture was observed to significantly diminish the performance. These authors speculated on methionine deficiency as the reason of the reduced laying performance upon corn DGGS administration to birds. Likewise in a research by Gazalah et al. (2011), substituting corn with 40–60% of DDGS had a significant effect on laying performance decline. In contrast, improved laying performance was observed by Jung et al. (2008 a, 2008 b), but only after the application of 3% and 12% of DDGS in feed mixtures. According to Loar et al. (2010), 16% of DDGS in a feed mixture assured the highest laying production, compared with the control groups and groups with different DDGS addition.

The lack of significant differences in the mean egg weight (Table 4) is consistent with results reported by Roberts et al. (2007), Loar at al. (2010) and Ghazalah et al.

(2011). In contrast, it disagrees with the findings of Masa'deh et al. (2008a, 2008b, 2011), Pescatore et al. (2010) and Masa'deh (2011), who showed a tangible tendency for a decreasing egg weight along with an increasing content of DDGS in the feed mixture. A lack of this dependency was observed by Masa'deh et al. (2008b, 2011) and Masa'deh (2011) already in the second laying phase, i.e. after the 46th week of hen life.

The small and insignificant decrease in feed intake noted in the experimental groups (Table 4) is consistent with observations made by other authors. In studies by Roberts et al. (2007), Loar et al. (2010), Masa'deh and Scheideler (2008), Masa'deh et al. (2008, 2011) and Masa'deh (2011), the daily feed intake was alike in all groups irrespective of DDGS content in the diet. In contrast, Pescatore et al. (2010) demonstrated a significant decrease in feed intake. In a research by Green et al. (2010), a significant decrease in feed intake was observed already upon 50% DDGS addition to feed mixtures. Equally strong response was noted by Ghazalah et al. (2011) at DDGS additions of 40% and 60%. As a consequence, they also obtained a significant decrease in FCR, despite a significant decline in laying performance. Contrarily, Roberts et al. (2007), Pescatore et al. (2010) and Jung et al. (2008 a, 2008 b) did not confirm the effect of DDGS content in the diet on the FCR value. In turn, in the present study, as a consequence of lower laying performance in group E2 (20% DDGS) and with the lack of significant differences in the mean egg weight and feed intake, the FCR value was observed to increase significantly ($P \leq 0.01$) (Table 4).

When analysing changes in the value of the evaluated egg quality traits, consideration shall be given to the age of laying hens which affects egg quality, irrespective of the experimental factor. Typical physiological changes due to the age of a flock include an increase in egg weight, deterioration of thick albumen quality and deterioration of shell quality. In contrast, yolk colour is completely independent of hen age (Sauveur, 1988).

As expected, with the advancing age of the laying hens, the physiological increase in egg weight was observed in all groups, with the increase being lesser along with an increasing DDGS ration in the feed mixture (Table 5). At the start of the experiment, the egg weight in group E2 was significantly the highest, but after 18 weeks it did not differ statistically from the values noted in the other groups. Perhaps this was due to a higher content of corn DDGS in the feed mixture, which would be in accordance with a tendency observed by Masa'deh and Scheideler (2008), Masa'deh et al. (2008, 2011), Pescatore et al. (2010) and Masa'deh (2011).

The changes noted in thick albumen quality in the study period do not indicate the effect of the nutritional factor on values of this trait (Table 5). Likewise, such an effect was not demonstrated in experiments conducted by Lumpkins et al. (2005), Masa'deh and Scheideler (2008), Masa'deh et al. (2008, 2011) and Ghazalah et al. (2011). The results obtained did not confirm earlier findings of Niemiec et al. (2012) from a study on the use of corn DDGS in laying hen nutrition, which demonstrated a positive effect of DDGS on thick albumen density. The increase in Haugh units upon administration of DGGS was also noted by Loar et al. (2010) and Pescatore et al. (2010). Also Sauveur (1988) claimed that, when added to a feed mixture, the products of grain fermentation had a beneficial effect on thick albumen density. This

author ascribes this effect to the likely influence of an increased quantity of microelements.

The reported increase in yolk content of egg (Table 5) is due to the age of the flock (Sauveur, 1988). Pescatore et al. (2010) demonstrated that the increasing DDGS content in the feed mixture was accompanied by a decreased weight of yolk. This was, however, not confirmed in our study, where in the group with 20% addition of DDGS the increase in yolk content was the greatest.

Yolk colour is a trait that depends on the content and type of pigments in a feed (Nys, 1999). Roberson et al. (2005), Roberts et al. (2007), Loar et al. (2010), Pescatore et al. (2010), Masa'deh and Scheideler (2008) and Masa'deh et al. (2008, 2011) demonstrated a positive effect of dietary corn DDGS on yolk colour. Such an effect was, in turn, not observed by Lumpkins et al. (2005), Jung et al. (2008 a, 2008 b) and Ghazalah et al. (2011). It may be speculated that slightly more intensive colour of yolk in groups E1 and E2 at the end of the experiment (Table 5) resulted from a significant content of yellow pigments in the corn DDGS. Although statistically significant ($P \leq 0.01$), the difference could not be perceptible to a consumer. So negligible differences could be due to the higher content of corn grain in the control diet (45%) than in experimental diets (20%). But DGGS are a more concentrated raw material than pure grain and contain three times higher nutrient levels (except starch) (Świątkiewicz and Koreleski, 2008). Thus we could expect that the total content of xanthophylls in E1 and E2 feeds was higher than in C feed. On the other hand – according to Nys (1999) – an increase in colour intensity by each point on the RCF scale requires the administration of increasing amounts of pigment in the diet.

With the advancing age of laying hens, the quality of egg shells usually deteriorates (Sauveur, 1988). In the reported experiment, no shell quality deterioration occurred; on the contrary, shell thickness was observed to increase (Table 5). Earlier investigations (Roberson et al., 2005; Loar et al., 2010; Koreleski et al., 2011; Krawczyk et al., 2012) did not demonstrate the effect of DDGS on the quality of egg shells; only Pescatore et al. (2010) reported a lower weight and content of shell and lower shell resistance to crushing in the 4th week of administration of the DDGS-containing feed mixture, whereas Ghazalah et al. (2011) showed a decrease of shell thickness along with an increasing DDGS ration in the diet.

Based on the results obtained in the study, it may be concluded that the introduction of corn distillers dried grains with solubles to feed mixtures for commercial laying hens is advisable. With corn DDGS addition exceeding 15%, a slight decrease in production results and deterioration in selected parameters of egg quality shall be expected. Yet, in the total costs of flock maintenance this may be compensated for by a reduced cost of the feed mixture.

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Zastosowanie DDGS z kukurydzy w żywieniu kur niosek i jego wpływ na jakość jaj i wyniki produkcyjne

STRESZCZENIE

Celem doświadczenia było określenie wpływu kukurydzianego DGGS (distillers dried grains with solubles) zastosowanego jako zamiennik śruty sojowej w paszy dla niosek na jakość jaj i wydajność nieśną kur. Kury nioski ISA Brown (podzielone na 3 grupy po 100 szt.: 10 powtórzeń po 10 kur w każdej) przez 18 tygodni były żywione mieszanką zawierającą 15% (grupa E1) i 20% (grupa E2) DDGS z kukurydzy. Kury z grupy kontrolnej (C) otrzymywały typową paszę bez udziału DDGS, w której główne źródło białka stanowiła poekstrakcyjna śruta sojowa. W okresie doświadczenia kontrolowano wydajność nieśną (% nieśności), średnią masę jaja (g), średnie dzienne spożycie paszy (g/szt./dzień) oraz wykorzystanie paszy (kg/1 kg jaj). Jakość jaj: masa jaja (g), jakość białka gęstego (jH), kolor żółtka (RFC), udział żółtka w jaju (%), udział skorupy w jaju (%) i grubość skorupy (mm) oceniano dwukrotnie – przed rozpoczęciem i na koniec doświadczenia. W obydwu terminach analizie poddano cały dzienny zbiór jaj z każdej grupy, tj. 90, 93 i 92 jaja odpowiednio z grupy C, E1, E2 w wieku 31 tygodni oraz 92, 94 i 81 jaj w 48. tygodniu, odpowiednio z tych samych grup.

W okresie objętym doświadczeniem w żadnej z grup nie odnotowano padnięć. Nie stwierdzono wpływu żywienia DDGS na masę ciała niosek. W grupie E2 (20% DDGS) stwierdzono, w porównaniu do pozostałych grup, niewielkie obniżenie nieśności i wyższą wartość FCR. Odnośnie obu cech różnice potwierdzono statystycznie ($P \leq 0,01$). Nie stwierdzono istotnego wpływu udziału DDGS w paszy na średnią masę jaja i dzienne spożycie paszy. Jaja pochodzące od kur z grupy E2 charakteryzowała pod koniec doświadczenia istotnie gorsza ($P \leq 0,01$) jakość białka i istotnie gorsza jakość skorupy ($P \leq 0,01$). Kolor żółtka w obu grupach doświadczalnych był istotnie ciemniejszy ($P \leq 0,01$) w porównaniu do grupy C. Na podstawie przedstawionych wyników można uznać, że wprowadzenie DGGS z kukurydzy w ilości 15% do mieszanek przeznaczonych dla stad towarowych kur nieśnych jest zasadne. Po przekroczeniu 15% udziału DDGS należy liczyć się z możliwością niewielkiego obniżenia wskaźników produkcyjnych i pogorszenia niektórych cech jakości jaj.

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INFLUENCE OF WIND ON AIR MOVEMENT IN A FREE-STALL BARN DURING THE SUMMER PERIOD*

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Abstract

Use of natural ventilation in the barn should lead to optimal microclimatic conditions over the entire space. In the summer, especially during hot weather, higher air velocity cools cows, which helps to avoid heat stress. The paper presents the results of studies on the evolution of air movement in a modernized free-stall barn of the Fermbet type with the natural ventilation system during the summer period. Based on measurements of velocity and direction of air flow (inside and outside the barn) and observations of smoke indicator, the movement of air masses in different parts of the barn was identified. Significant variations of air flow at different levels of the barn were found. These differences deviate from the accepted patterns of natural ventilation, which can be found in the literature. The range of a draught and stagnant air along with the conditions in which they are built was determined. On this basis, recommendations regarding the location of barns on the plots and the improvement of ventilation in summer were made.

Key words: summer, wind, ventilation, dairy cows, free-stall barn

Dairy cow welfare and milk productivity are largely influenced by barn microclimate which depends on outside climate conditions. High production cows need significant amounts of fresh air (Albright and Timmons, 1984; Zähner et al., 2004). Insufficient amount of oxygen slows down metabolic processes, which in turn affects milk production. Inefficient ventilation systems in barns are quite often a cause of mammary gland infections or skin diseases (Cook et al., 2005). For example, Holstein-Friesian cows sweat out up to 30 litres of water to the air in the summer. High air humidity may decrease animal welfare and additionally such conditions are conducive to bacteria development. Moreover, insufficient air exchange ratio inside the barn results in the increased concentration of noxious gases produced as a result of animal waste decomposition.

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Natural ventilation systems inside barns should be designed in such a way as to obtain optimal microclimatic conditions throughout the entire area and at the level which would not be harmful to animals. Natural ventilation is the most popular ventilation system used in free-stall barns. Air movement is mainly driven by thermal displacement, wind and difference between the level of inlet and outlet openings (Reppo et al., 2004).

According to Romaniuk et al. (2005), air flow velocity inside a barn should remain within the range 0.2–0.5 $m \cdot s^{-1}$ depending on season of the year. When air velocity inside a building exceeds recommended values, cows may suffer from overcooling, which influences animal welfare and milk production levels. However in the summer season, especially during heat waves, increased air velocity and cooling play a positive role because they reduce the risk of thermal stress (Armstrong, 1994; Lautner and Miller, 2003).

Material and methods

The aim of the paper is to define the influence of wind energy and direction on air movement inside a free-stall barn in the course of two summer months. Based on measurements of air velocity and direction inside and outside the building as well as observations of smoke produced by smoke generators, the authors have determined air flow directions in particular sections of the cow barn. It was also possible to mark out areas where air stood still or where draughts occurred.

Research object

Measurements of air velocity and direction were conducted inside a cow barn (Fermbet construction system) adapted as a free-stall barn for 176 cows. The building of 1580 m^2 usable floor area is located in the village of Kobylany, the Małopolska region. It is oriented along the east-west axis. From the south, the building is extended with some additional social rooms for employees and milking parlour with the holding area. It is a typical building constructed from pre-fabricated reinforced concrete with a double-pitched roof (gradient 45%). The building is equipped with a natural gravitational ventilation system with an adjustable curtain wall on longitudinal walls and outlet openings in the form of ridge vents.

Measurement methods and the distribution of measurement points

To carry out the measurements, 18 measurement points were identified across the barn area, 6 in each of the manure alleys (alley I, alley II, and alley III) (Fig. 1). Instantaneous measurements of air flow velocity were conducted in selected points at the level of 1 meter above the floor. This was done with the help of Windmaster 2 anemometers. Moreover, relative instantaneous humidity and air temperature were measured with a thermo hygrometer HD 8901 provided by Delta OHM. At the same time, weather conditions outside the building were recorded (wind velocity and direction, temperature and relative air humidity). The measurements were made daily throughout June and July 2010, at 11 am and 2 pm.

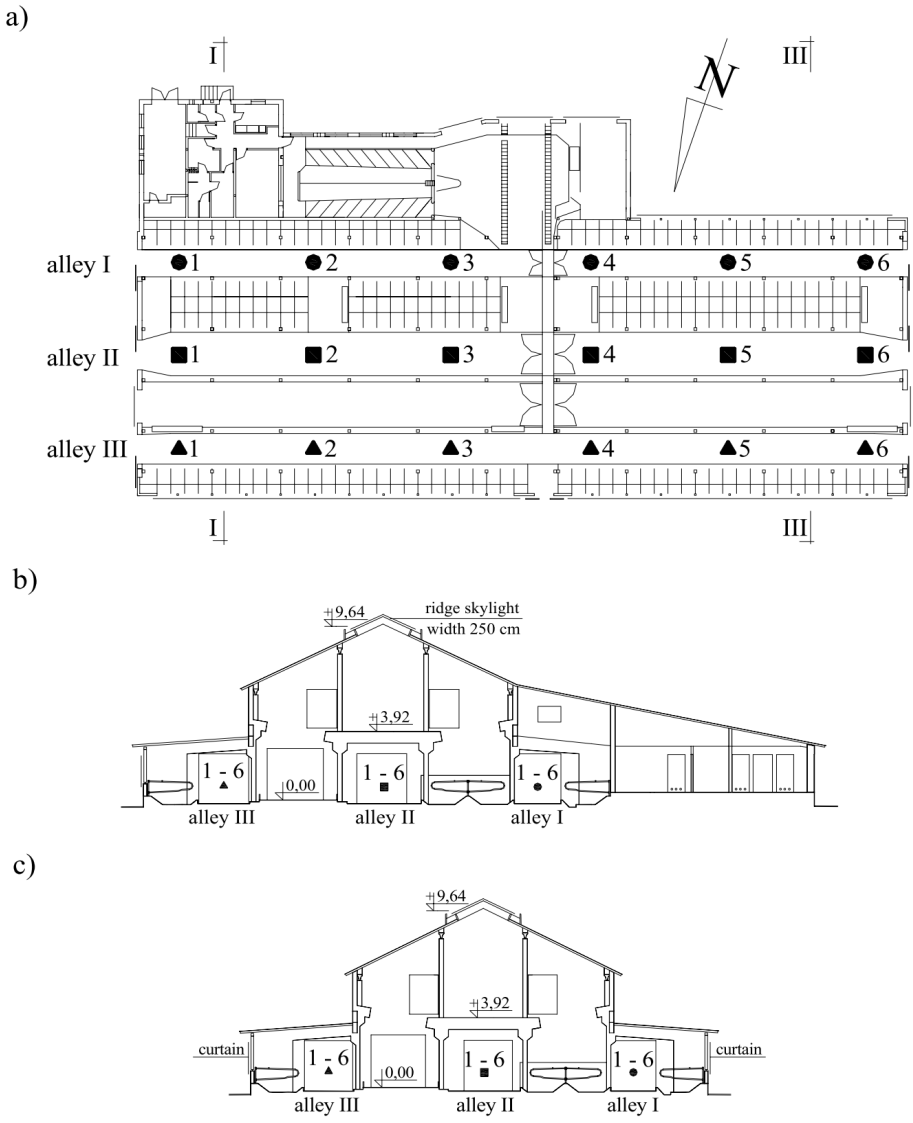

Fig. 1. Distribution of measurement points for wind velocity, temperature and relative air humidity (1–6) in the Kobylany barn
a – projection, b – cross-section I–I, c – cross-section III–III

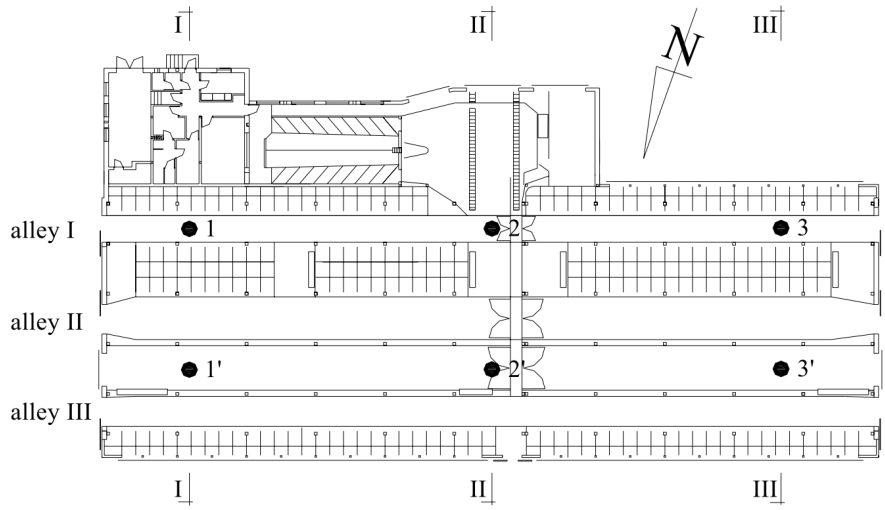

Fig. 2. Location of smoke generators (1–3; 1'–3') during the experiment in three observation cross-sections I–I, II–II, III–III

Observations of air flow in the barn were possible thanks to the use of smoke from smoke generators. The movement of that smoke was photographed and recorded. Smoke generators were positioned in three significant observation cross-sections (Fig. 2). During the measurements and observations of air movement, curtains in the longitudinal walls were completely deserted and all the gates were opened.

Results

The analysis of air velocity measurements in the selected points was carried out by calculating average values of all results obtained, separately for winds blowing perpendicularly and parallel to longitudinal building axis. In June and July 2010, wind velocity was between 1 and 2.5 $m \cdot s^{-1}$. Outside air temperature remained in the range 20.3–25.9°C, with relative humidity of 70–73%. Indoor air temperature was at the level between 21.4 and 24.4°C, and relative humidity did not exceed 70%.

When the wind blew perpendicularly to longitudinal walls of the building, air movement velocity in the manure alley I, where the lying areas are directly adjacent to the wall, was lower than the value recommended for the summer season. Moreover, the recommended air flow velocity (V_{opt} = 0.5 $m \cdot s^{-1}$) was significantly exceeded in measurement points at all manure alleys at the western wall. A similar situation occurred in the lying area in the eastern side of the barn, in alley 2, where air movement velocity at the points 1 and 2 equalled on average 1 $m \cdot s^{-1}$ and 0.6 $m \cdot s^{-1}$ respectively (Fig. 3). Optimum air flow velocities for dairy cattle were observed in the central part of the barn (measurement points 3 and 4), around double boxes between manure alleys no. 1 and 2.

The measurement results obtained were confirmed by observations of smoke movement in particular sections of the barn (Fig. 4).

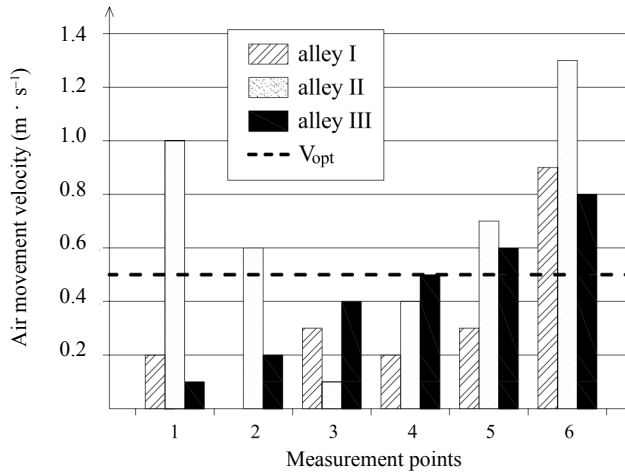

Fig. 3. Average air movement velocities measured in June and July 2010 at 11 am and 2 pm in scraper alleys when winds blew perpendicularly to the barn wall

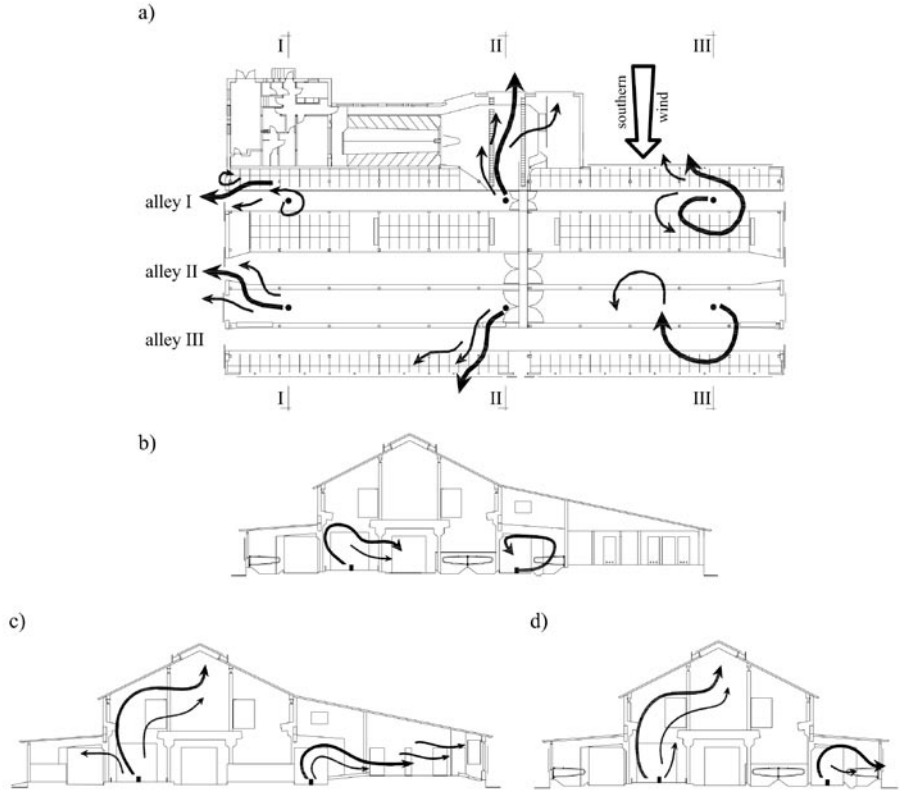

Fig. 4. Smoke movement during the experiment with smoke generators in the barn on 30 June 2011 at 12 am; air temperature equalled 25.9ºC, southern wind (perpendicular to the barn):
a – horizontal projection, b – cross-section I–I, c – cross-section II–II, d – cross-section III–III

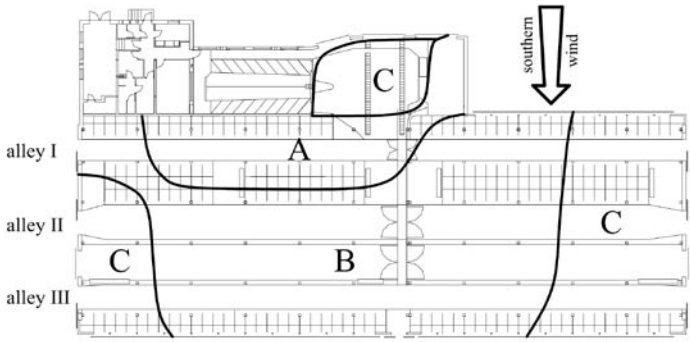

Fig. 5. An overview of the cow barn with the three zones: A – standstills, B – optimal air velocities, C – draughts; observed when wind blew perpendicularly to the building

Based on measurements and observations of air movement, the authors have divided the building into zones with different air velocity. Most convenient conditions were observed in the central part of the barn (zone B). Taking into consideration the temperature in the barn on that day (23.2°C), we can say that the zone C and the draughts that occurred in this zone (velocity 0.5–1.4 $m \cdot s^{-1}$) protected the animals from overheating. The zone A, nearby the milking parlour and adjacent areas (Fig. 5), was characterized by standstills.

In the case of western wind blowing parallel to the building axis, air movement velocity in windward manure alleys decreased gradually. The highest velocity (1 $m \cdot s^{-1}$) was recorded along longitudinal walls in the western part of the barn and around the gate leading to the feeding alley. In this part of the barn, the cows were most severely exposed to draughts, and the fresh air entered mainly through the open gate. In the central part of the barn, air velocity remained at the level which could be considered as unsatisfactory. The lowest velocities were recorded in this part of the scraper alley where the lying area is connected to the extension room and in the northern part, mostly along the longitudinal wall (Fig. 6).

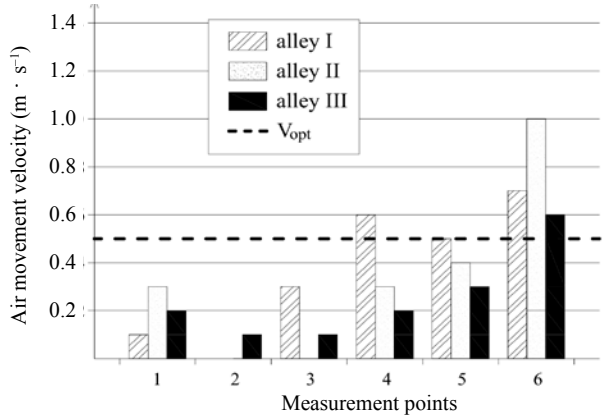

Fig. 6. Average air movement velocities measured in June and July 2010 at 11 am and 2 pm in scraper alleys when winds blew parallel to the barn wall

Smoke movement observations were also carried out for wind blowing parallel to the barn. In the case of west-east winds (parallel), the air mainly entered the building through the open gate in gable walls of the barn. Openings in longitudinal walls served rather as air outlet openings (Fig. 7). The eastern part of the barn suffered from poorest ventilation (cross-section I–I). The air entered the barn through inlet openings in longitudinal walls with lowered curtain walls and left the building through the open gate in the eastern part. In the central part of the barn (cross-section II–II), smoke movement during the experiment was disturbed the most. Contrary to the situation in the eastern part of the barn, the air left the building through outlet ridge vents only in the central part of the barn. In the western part of the barn, the movement of air was disturbed by the open gate, which resulted in draughts.

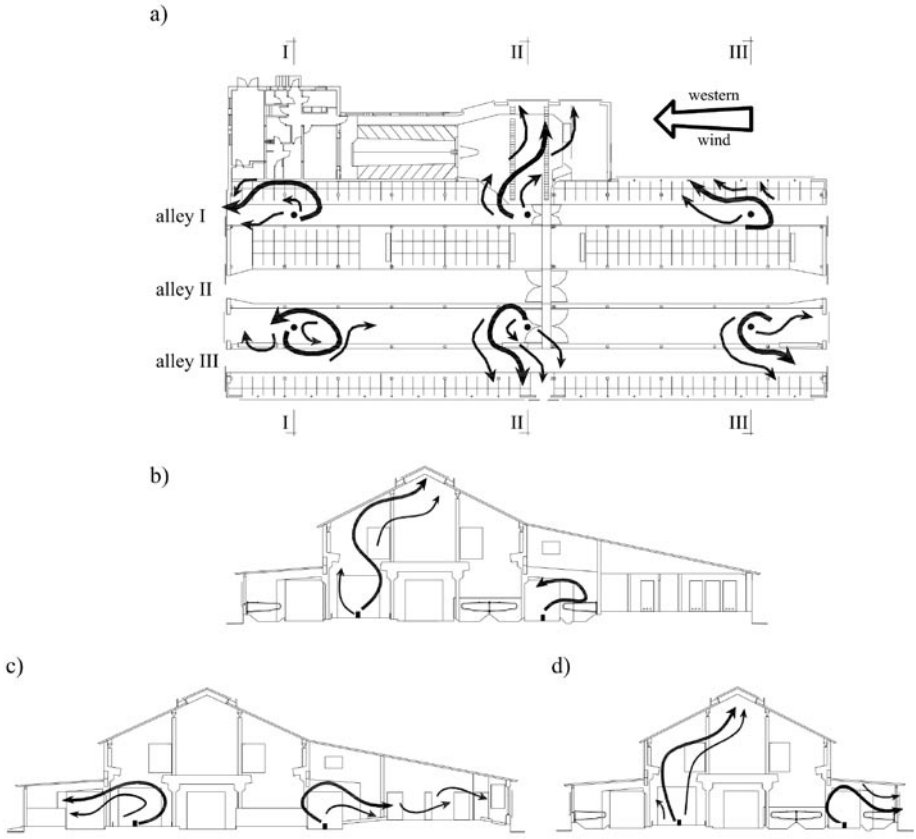

Fig. 7. Smoke movement during the experiment with smoke generators in the barn on 9 July 2011 at 12 am; air temperature equalled 19.9°C, western wind (parallel to the barn):
a – horizontal projection, b – cross-section I–I, c – cross-section II–II, d – cross-section III–III

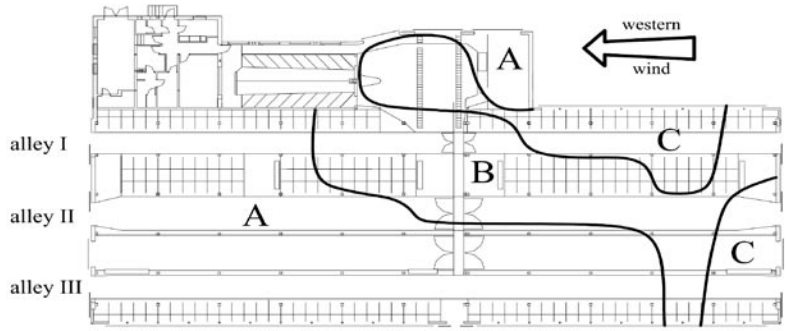

Fig. 8. A projection of the cow barn with the three zones: A – standstills, B – optimal air velocities, C – draughts; observed when wind blew perpendicularly to the building

The air was distributed the best way in the lying area with double boxes between alleys 1 and 2 in the western part of the barn and the perpendicular alley leading to the holding area. In the central and eastern part of the barn, air movement was not satisfactory and air velocity was near 0.0 $m \cdot s^{-1}$ (Fig. 8).

Discussion

Cattle are able to adapt well to changeable temperature conditions, particularly if they are bred in free-stall barns. The temperatures between –10 to 20°C do not exert much influence on milk production when relative air humidity remains at the level of 50–70% (Lautner and Miller, 2003; Romaniuk at al., 2005; Jaśkowski et al., 2005).

Animals experience heat stress when the temperature in the barn increases above 20°C and when air humidity is at an unsatisfactory level. The cows begin to move slowly, they eat less and breathe out heavily to get rid of the heat. If the temperature rises from 20°C to 30°C, a cow eats approximately 1.5 kg less fodder and produces 3 to 5 litres of milk less. As a result, the animal's physiological parameters go down (Spiers et al., 2004).

Air flow velocity in barns should not exceed 0.2 $m \cdot s^{-1}$ during the winter, and 0.5 $m \cdot s^{-1}$ during the summer if the heat exchange ratio remains within the range of 350 to 400 $m^3 \cdot animal^{-1} \cdot h^{-1}$ (Solan and Jóźwik, 2009). Draughts are not a positive phenomenon because they result in sudden cooling of the body, which leads to increased vulnerability to diseases. However in special cases, this principle may not be followed. In such situations, air movement velocity is increased in order to lower the temperature inside the barn, especially during hot days. According to Wathes et al. (1983), summer winds of 6–7 $m \cdot s^{-1}$ are not detrimental to cow welfare, and the cooling effect is already observed at 1–2.5 $m \cdot s^{-1}$. The challenges appear when high inside air temperatures are accompanied by complete air standstill. In such conditions, cow fertility may be negatively influenced (Ray et al., 1992). Standstills in the investigated barn mainly depended on wind direction. It could be assumed that

wide barns with large manure and feeding alleys will be more easy to ventilate. The investigated barn contradicted these assumptions.

Proper air flow directions inside a free-stall barn in the summer period should develop as in Figure 9 (Chastain, 2000). However, this is a purely theoretical scheme as it assumes that the wind will blow perpendicularly to the barn. In fact, the influence of wind on air movement inside building and gravitational ventilation efficiency depends on local conditions, such as relief configuration, surrounding buildings and how the building is located. Also, the building itself and the positioning of milking parlour, holding area and other rooms influence the way air is distributed at different areas of the building (Herbut, 2010). In the investigated barn this type of air movement configuration (Fig. 9) was observed in some parts of the building and changed depending on the dominating wind direction. Scarce literature on cattle breeding, cattle welfare, barn ventilation and building construction takes into consideration the influence of weather conditions on air movement in barns (Brouk et al., 2001; Teye et al., 2008). In the case of free-stall barns this factor is very important.

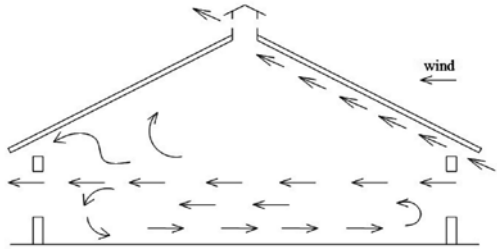

Fig. 9. Proper air movement directions in the summer with open side curtains and wind blowing perpendicularly to the building (Chastain, 2000)

In the case of free-stall barns with natural ventilation, the power and direction of wind largely influences the performance of ventilation systems. When the gates situated in gable walls were open, the situation inside the investigated barn was very unfavourable, because the air moved uncontrollably in both gable zones. The air entering the building through openings in curtain walls was blown out through these gates instead of moving in accordance with the diagram in Fig. 9. Optimal conditions of air movement, which were created in the central part of the barn when the wind blew perpendicularly to the walls, confirm recommendations that can be found in many other works (Romaniuk et al., 2005). They define most favourable conditions of air exchange in free-stall barns when inlet openings are located opposite each other and perpendicularly to most common wind directions.

It could be also agreed at this point that Palmer's recommendations (2005) are correct. He suggests that it is necessary to position barns in such a way that their longitudinal axis runs north-south, which would improve the performance of natural ventilation supported by wind velocity. Unfortunately, in the investigated barn longitudinal axis of the east-west direction was not consistent with the directions of predominant winds. That is confirmed by Lorenz (2005), who says that Poland is dominated by western winds, which are the strongest in winter period. Results

of wind velocity and directions obtained in the course of this research in Kobylany were in line with velocity values and wind directions determined by multiannual studies carried out in the nearest meteorological station in Balice (Ośródka et al., 2010). According to this study, wind velocity in the area ranges between 0 and 3.5 $m \cdot s^{-1}$ and western winds are most frequent. In Kobylany, the average velocity was 0–2.5 $m \cdot s^{-1}$, which was probably caused by local winds, relief configuration and the existing building development, which most significantly influence air movement inside the barn.

It would be possible to improve air movement conditions in the eastern part of the barn by installing devices supporting natural ventilation, for example mixing fans hung at the ceiling. The application of these solutions would improve air quality in the barn in the summer season and also improve cow welfare and their productivity.

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PIOTR HERBUT, SABINA ANGRECKA, GRZEGORZ NAWALANY

Wpływ wiatru na ruch powietrza w oborze wolnostanowiskowej w okresie letnim

STRESZCZENIE

Użytkowanie wentylacji naturalnej w oborze sprowadza się do osiągnięcia optymalnych warunków mikroklimatycznych na całej przestrzeni użytkowej hali. W lecie, zwłaszcza podczas upałów większa prędkość ruchu powietrza ochładza krowy i umożliwia uniknięcie zjawiska stresu cieplnego.

W pracy przedstawiono wyniki badań nad kształtowaniem się ruchu powietrza w zmodernizowanej oborze wolnostanowiskowej typu „Fermbet" z systemem wentylacji naturalnej podczas lata. Na podstawie pomiarów prędkości i kierunku ruchu powietrza wewnątrz i na zewnątrz obory oraz obserwacji dymów wskaźnikowych dokonano rozpoznania przemieszczania się mas powietrza w poszczególnych jej częściach.

Stwierdzono duże zróżnicowanie kształtowania się ruchu powietrza w różnych płaszczyznach obory odbiegające od przyjmowanych literaturowo schematów wentylacji naturalnej. Wyznaczono strefy występowania przeciągów, zastoisk powietrza oraz warunków podczas których powstały. Na tej podstawie sformułowano zalecenia dotyczące sytuowania obór na działkach oraz poprawy działania wentylacji w lecie.

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THE EFFECT OF CARCASS CONFORMATION CLASS (EUROP SYSTEM) ON THE SLAUGHTER QUALITY OF YOUNG CROSSBRED BEEF BULLS AND HOLSTEIN-FRIESIANS*

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Abstract

The objective of this study was to determine the effect of genotype and carcass conformation class on the slaughter quality of 200 young bulls, including 108 crossbred beef bulls and 92 Holstein-Friesians (HF), aged 21–22 months, selected in the lairage. The lean meat content was estimated and body measurements were taken before slaughter. After slaughter, the carcasses were graded according to the EUROP system, and carcass quality parameters were determined. Intramuscular fat was extracted from samples of *m. longissimus dorsi*, and the fatty acid profile of extracted fat was determined by gas chromatography. 61.11% carcasses of crossbred beef bulls were graded in the conformation class R, and 56.53% carcasses of Holstein-Friesians were classified as O. The majority of carcasses belonged to fat class 2, which was not consistent with intramuscular fat content. Within the same conformation classes, crossbred beef bulls were characterized by higher slaughter quality than Holstein-Friesian bulls. Meat from hybrid beef bulls had a higher (by 0.42% on average) content of fat with a more desirable composition. Since the population size of beef cattle will probably not increase in the nearest future, efforts should be continued to optimize the production of high-quality beef from dairy cattle herds.

Key words: beef bulls, slaughter quality, EUROP classification, fatty acids.

The quality of bovine carcasses is influenced by the selection of genetic material, production technology as well as the terms of slaughter animal purchase (Litwińczuk et al., 2006; Wajda et al., 2003). Only an objective evaluation can encourage produc-

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ers to raise the quality of their livestock. In an attempt to harmonize Poland's laws with EU legislation, the EUROP grid method of carcass classification was introduced in large meat processing plants. Countries that have introduced this popular classification system are the leading suppliers of high quality meat in Europe (Daszkiewicz et al., 2003; Florek, 2000).

Global improvements in quality of life raise consumer expectations toward food, including beef. The quality of beef is determined mostly by the sex, diet, slaughter age and genotype of animals (Smith et al., 2009). Intramuscular fat content significantly affects the sensory attributes of meat. A certain percentage of dark, firm, dry (DFD) meat, characterized by a low intramuscular fat content, a dark color and inconsistent acidification, was noted in young Black-and-White dual-purpose bulls (Nogalski, 2002). Until recently, bovine fat was generally regarded as an unhealthy carcass component due to relatively high levels of saturated fatty acids and cholesterol that increase the risk of ischemic heart disease (Wood et al., 2008). However, only some of the saturated fatty acids contribute to hypercholesterolemia, thrombogenesis (C 14:0 and C 16:0), increased risk of thrombosis and ischemic heart disease (C 18:0), whereas some monounsaturated fatty acids (e.g. oleic acid) have beneficial health effects (Florek et al., 2007). Recent studies have demonstrated that the fat of ruminants may offer certain health benefits (Clapham et al., 2005; Jump et al., 2006). Conjugated linoleic acid (CLA) is an intermediate product of the conversion of linolenic acid to stearic acid, produced through the action of anaerobic rumen bacteria. This natural substance is an antioxidant which inhibits the development of neoplastic diseases and stimulates the immune system (Clapham et al., 2005). A high ratio of *n-3* to *n-6* polyunsaturated fatty acids (PUFAs) (10–15:1 vs. the optimal ratio of 2–4:1) significantly increases the risk of lifestyle diseases (Breslow, 2006). Excess quantities of *n-6* fatty acids may block *n-3* fatty acids, thus contributing to insulin-dependent diabetes and hypercholesterolemia (Jump et al., 2006).

Prime quality beef is produced from beef breeds that account for less than 1% of Poland's entire cattle population. For this reason, beef production in Poland is based mainly on dairy cattle herds. The carcasses of this cattle are characterized by poorer slaughter and quality parameters (lower carcass dressing percentage and degree of conformation) compared to single-purpose beef cattle or crossbred cattle (Litwińczuk et al., 2012; Młynek and Guliński, 2007; Oprządek et al., 2007). Due to the fact that Black-and-White dual-purpose cattle have been crossbred with Holstein-Friesian dairy cattle, hybrid beef bulls should be used on a larger scale to increase beef production and improve beef quality (Bartoň et al., 2005; Voříšková et al., 2002; Wajda et al., 2004). The population of crossbred beef cattle can be expanded by inseminating dairy cows and heifers of lower genetic value with the semen of beef bulls. Research results show that that commercial crossing not only contributes to higher growth rates and better feed conversion ratios in the offspring, but it also improves carcass conformation and meat quality (Bartoň et al., 2005; Nogalski and Kijak, 2001; Nogalski, 2002; Voříšková et al., 2002). In Poland, the slaughter value of different cattle breeds and crossbreds has been investigated extensively, but few studies focus on the hybrids resulting from the crosses of purebred dairy cattle with beef cattle.

The objective of this study was to determine the effect of genotype and carcass conformation class on the slaughter quality of young crossbred beef bulls, aged 21–22 months, raised in a semi-intensive production system, the most common beef production regime in Central Europe.

Material and methods

The experimental material comprised 200 young bulls, including 108 crossbred beef bulls (Holstein-Friesian cows crossed with bulls of the Limousin, Hereford or Simmental breeds) and 92 Holstein-Friesians. Young bulls of known origin, aged 21–22 months, were selected between 4 January 2011 and 30 March 2011 in an abattoir in north-eastern Poland. The animals were raised in a semi-intensive production system, and they were fed grass haylage, maize silage and concentrate. The bulls were transported to the lairage 15–20 hours prior to slaughter, and they were kept in individual boxes equipped with drinkers.

Live body measurements

The body weight of animals was determined immediately before slaughter, and lean content was estimated on a scale of 1 (low lean content) to 10 (very high lean content). The following live body measurements were performed: chest girth, height at sacrum, trunk length (from withers to the point of intersection with the line connecting coxal tubers with the spine) and width at hips.

Carcass measurements

The experimental bulls were sacrificed in accordance with industrial standards. Half-carcasses were weighed within an accuracy of 0.5 kg and evaluated based on EUROP system criteria by a trained grader. The right half-carcass was measured to determine: half-carcass length (from the first thoracic vertebra to pubic symphysis), front half-carcass width (at the level of the 5th rib) and leg circumference (at ¾ leg length, measured from the hock joint). Carcasses were chilled for 48 h (at a temperature of around 2ºC), and the pH of *m. gluteobiceps* was measured using the Testo 205 pH-meter with a penetration tip. Right half-carcasses were divided into primal cuts which were weighed to determine their percentage in the half-carcass. Samples of *m. longissimus dorsi* were collected from the loin (between the 11th and 13th thoracic vertebrae) to determine intramuscular fat content and the fatty acid profile. Intramuscular fat was extracted from samples of ground meat using the Büchi B-811 device according to Polish Standard PN-ISO 1444-2000. The concentrations of 33 fatty acids were determined by gas chromatography, in the Varian CP 3800 chromatograph equipped with a split/splitless injector and a flame-ionization detector (FID). 1 µl ester samples were placed on a capillary column with a length of 100 m and an internal diameter of 0.25 mm, with the CP-sil88 stationary phase. Helium was used as the carrier gas, injector temperature was 260ºC, and the total time of a single analysis was 68 min. The percentage of fatty acids was calculated using Galaxie software.

Statistical analysis

The results were analysed with the use of Statistica 9.0 software (Statsoft, 2009). The effect of bull genotype on conformation class and fat class was evaluated by the $\chi 2$ test:

$$\chi 2 = \sum [\frac{(fi - Fi)^2}{Fi}],$$

where:

fi – observed distribution,

Fi – expected distribution.

In the analysed population, R and O were the predominant classes, and a significantly ($P \leq 0.01$) higher number of carcasses of crossbred beef bulls (61.11%) than Holstein-Friesian carcasses were classified as conformation class R (Table 1). As regards conformation class O, the bull genotype had the reverse effect. None of the analysed carcasses were assigned to conformation class E. The bull genotype had no significant effect on carcass fat classes (Table 2), and the majority of carcasses were graded as fat class 2. Very few carcasses were categorized into conformation classes U and P, therefore only the carcasses graded as conformation class R are analysed in further parts of the study.

Table 1. Beef carcass conformation classes according to the EUROP system

Conformation class	Crossbred beef bulls		Holstein-Friesians		Statistical significance
	head	%	head	%	
U	10	9.26			
R	66	61.11	31	33.69	xx
O	30	27.78	52	56.53	xx
P	2	1.85	9	9.78	NS
Total	108	100	92	100	

xx – $P \leq 0.01$; NS – no significance.

Table 2. Beef carcass fat classes according to the EUROP system

Fat class	Crossbred beef bulls		Holstein-Friesians		Statistical significance
	head	%	head	%	
1	34	31.49	29	31.52	NS
2	73	67.58	63	68.48	NS
3	1	0.93			
Total	108	100	92	100	

NS – no significance.

The effect of bull genotype (crossbred beef bulls and Holstein-Friesians) and conformation classes on live body measurements, carcass characteristics and the fatty acid profile was evaluated by least squares analysis of variance using the following model:

$$Y_{ijklm} = \mu + A_i + B_j + (AB)_{ij} + e_{ij}$$

where:

Y_{ij} – value of analysed parameters,
μ – population mean,
A_i – effect of the ith bull genotype (1, 2),
B_j – effect of the jth carcass conformation class (1, 2),
$(AB)_{ij}$ – genotype × conformation class interaction,
e_{ij} – random error.

Results

Young crossbred beef bulls whose carcasses were categorized into conformation class R were characterized by insignificantly higher values of height at sacrum and lower values of trunk length in comparison with dairy Holstein-Friesians (Table 3). Width at hips was significantly (P≤0.05) smaller and chest girth was larger in crossbred beef bulls, which testifies to a higher lean meat content of their carcasses, as demonstrated by the results of live body measurements. Holstein-Friesian bulls with carcasses in conformation class O were taller, and their chest girth was larger in comparison with crossbred beef bulls. The differences in the remaining body measurements were similar to those noted in conformation class R.

Table 3. Selected live body measurements of bulls representing different genotypes and carcass conformation classes

Traits	Statistical measures	Conformation class R		Conformation class O		Interaction
		crossbred beef bulls	Holstein--Friesians	crossbred beef bulls	Holstein--Friesians	
Number	N	66	31	30	52	
Height at sacrum (cm)	x	137.6	136.5	136.0	137.5	
	sd	4.97	6.92	7.41	6.52	
Chest girth (cm)	x	202.5	201.2	197.6	198.3	
	sd	10.44	15.28	11.74	8.96	
Width at hips (cm)	x	48.7 x	49.8 x	46.9	48.0	
	sd	2.63	2.73	4.79	2.37	
Trunk length (cm)	x	98.1	99.4	96.5	97.8	
	sd	6.12	5.53	7.88	5.82	
Live estimation muscle content (points)	x	7.4	6.9	5.4	5.0	
	sd	1.08	0.98	1.96	1.43	

Within carcass conformation classes x – P≤0.05.

Regardless of breed, class R bulls were heavier than class O bulls (Table 4). Holstein-Friesian bulls were characterized by high body weight gains, and their body weight exceeded 600 kg already at the age of 21–22 months. The average difference in carcass dressing percentage in conformation class R was significant (P≤0.05), reaching +1.40% to the advantage of crossbred beef bulls. The difference between

crossbred beef bulls whose carcasses were assigned to conformation classes R and O was 3.44%. There was a significant (P≤0.05) interaction between genotype and conformation class with respect to carcass dressing percentage. The expected differences in carcass dressing percentage between crossbred beef bulls and Holstein-Friesians were noted only in animals whose carcasses were graded as conformation class R. Genotype and conformation class were weakly correlated with fat scores in the carcass classification process under the EUROP scheme. Chilled carcasses were characterized by optimal levels of muscle acidification (pH 5.52–5.56). No significant differences were noted in half-carcass measurements within the analysed conformation classes. In comparison with Holstein-Friesians, the carcasses of crossbred beef bulls in class R were characterized by significantly larger leg circumference, which points to superior muscular conformation of the latter. The cross-sectional area of *m. longissimus dorsi* was significantly (P≤0.01) larger in crossbred beef bulls. The mean difference was 11.3 cm^2 in conformation class R, and 10.7 cm^2 in class O.

Table 4. Carcass characteristics of bulls representing different genotypes and carcass conformation classes

Traits	Statistical measures	Conformation class R		Conformation class O		Interaction
		crossbred beef bulls	Holstein--Friesians	crossbred beef bulls	Holstein--Friesians	
Body weight before slaughter (kg)	x	637.3	632.7	575.5	605.3	-
	sd	81.09	110.48	98.24	66.61	
Dressing percentage	x	57.85x	56.44x	54.41	54.28	x
	sd	2.37	2.24	2.04	2.21	
Fat class according to the EUROP system	x	1.70	1.82	1.68	1.67	-
	sd	0.39	0.36	0.37	0.32	
pH of cold carcass	x	5.52	5.56	5.54	5.55	-
	sd	0.18	0.07	0.07	0.17	
Leg circumference (cm)	x	117.9 x	114.4 x	116.0	116.2	-
	sd	4.88	6.08	5.33	3.50	
Half-carcass length (cm)	x	143.9	141.7	146.9	148.8	-
	sd	6.30	4.27	10.37	5.32	
Half-carcass width (cm)	x	74.7	73.2	75.3	75.6	-
	sd	3.59	3.36	5.22	4.80	
Rib eye area (cm^2)	x	92. 5xx	81.2 xx	84.8 xx	74.1 xx	-
	sd	13.97	15.60	14.82	14.56	

Within carcass conformation classes x – P≤0.05; xx – P<0.01.

Half-carcass weight was significantly (P≤0.05) affected by the interaction between genotype and conformation class (Table 5). The above can be attributed to differences in weight at slaughter and carcass dressing percentage. The slaughter quality of cattle is determined by the proportion of cuts with the highest market value in the total carcass weight. A higher proportion of premium retail cuts in carcass hindquarters was reported in crossbreed beef bulls in comparison with Holstein-Friesians, and in class R carcasses in comparison with class O carcasses. Regardless of conformation class, the carcasses of crossbred beef bulls were characterized by

a higher proportion of sirloin than Holstein-Friesians. The percentage of loin, another high-quality retail cut, was also higher in crossbred beef cattle. Within conformation class R, a significantly (P≤0.05) higher proportion of topside was observed in crossbred beef bulls, whereas in conformation class O, the carcasses of crossbred beef bulls had a significantly higher percentage of bavette in comparison with the carcasses of Holstein-Friesians.

Table 5. Percentage of retail cuts in the carcasses of bulls representing different genotypes and carcass conformation classes

Traits	Statistical measures	Conformation class R		Conformation class O		Interaction
		crossbred beef bulls	Holstein--Friesians	crossbred beef bulls	Holstein--Friesians	
Half-carcass weight (kg)	x	174.3	168.4	148.5	153.9	x
	sd	25.76	25.56	32.66	17.47	
Percentage in half-carcass:						
sirloin	x	2.23	2.14	2.17	2.02	-
	sd	0.35	0.35	0.25	0.46	
tenderloin	x	1.22	1.15	1.14	1.08	-
	sd	0.20	0.09	0.18	0.20	
topside	x	5.80 x	5.02 x	5.48	5.25	-
	sd	0.69	1.19	0.69	0.56	
silverside	x	4.01	4.05	3.35	4.13	-
	sd	1.06	0.54	1.01	0.65	
thick flank	x	3.82	3.90	3.89	3.70	-
	sd	0.55	1.23	0.73	0.35	
bavette	x	1.67	1.62	1.63 x	1.42 x	-
	sd	0.29	0.22	0.33	0.27	
rump	x	3.60	3.50	3.55	3.18	-
	sd	1.06	0.67	0.91	0.42	

Within carcass conformation classes x – P≤0.05.

The *m. longissimus dorsi* of class O carcasses contained more intramuscular fat, compared with class R carcasses (Table 6). With regard to genotype, significantly higher levels (P≤0.05) of intramuscular fat were noted in the *m. longissimus dorsi* of crossbred beef bulls, compared with Holstein-Friesians, in both conformation classes. Carcass classification to fat classes in line with the EUROP system showed no differences between the analysed genotypes, thus suggesting a similar intramuscular fat content.

Intramuscular fat extracted from the *m. longissimus dorsi* of crossbred beef bulls contained from 49.22% to 51.07% saturated fatty acids (SFAs), subject to conformation class and genotype (Table 6). The level of biologically active acids is also an important consideration. Within conformation class R, significantly (P≤0.05) higher levels of CLA (main isomer of conjugated linoleic acid cis-9, trans-11) were found in the meat of crossbred beef bulls than in Holstein-Friesians. In conformation class R, intramuscular fat had a significantly (P≤0.05) higher proportion of total PUFAs in crossbred beef bulls (4.22%) than in Holstein-Friesians (3.51%). In conformation

class O, the proportion of PUFAs was insignificantly higher (0.13%) in Holstein-Friesian bulls. In our study, genotype and carcass conformation class did not affect the *n-6/n-3* PUFA ratio which was determined in the range of 4.14 (crossbred beef bulls, conformation class O) to 4.52 (crossbred beef bulls, conformation class R).

Table 6. Fatty acid profile of the *longissimus dorsi* muscle (% of the total fatty acid pool)

Fatty acids	Statistical measures	Conformation class R		Conformation class O		Interaction
		crossbred beef bulls	Holstein--Friesians	crossbred beef bulls	Holstein--Friesians	
Intramuscular fat content (%)	x	1.43 x	0.97 x	1.67 x	1.28 x	-
	sd	0.80	0.58	0.88	0.67	
C 14:0	x	2.66	2.88	2.83	2.65	x
	sd	0.59	0.76	0.82	0.51	
C 16:0	x	26.38	27.75	28.21	25.64	-
	sd	5.25	1.80	2.63	6.84	
C 18:0	x	18.11	17.12	17.13	18.28	-
	sd	4.90	4.24	3.89	4.66	
C 18:1	x	1.28	1.07	1.08	1.03	-
	sd	0.84	0.49	0.43	0.33	
C 18:1	x	37.80	38.00	37.03	38.60	-
	sd	4.38	3.62	6.10	4.39	
C 18:2	x	2.44	2.13	2.27	2.36	-
	sd	0.55	0.47	0.48	0.67	
C 18:3	x	0.61	0.53	0.59	0.57	-
	sd	0.29	0.14	0.16	0.15	
CLA *(Cis 9, Trans 11)*	x	0.27 x	0.21 x	0.24	0.22	-
	sd	0.10	0.05	0.06	0.06	
C 20:4	x	0.33	0.21	0.30	0.36	-
	sd	0.23	0.10	0.17	0.31	
C 20:5 EPA	x	0.08	0.04	0.06	0.07	-
	sd	0.09	0.03	0.05	0.05	
C 22:5 DPA	x	0.18	0.10	0.18	0.18	-
	sd	0.14	0.05	0.10	0.10	
SFA	x	49.74	50.15	51.07	49.22	-
	sd	4.94	4.36	6.83	5.50	
UFA	x	50.30	49.91	48.93	50.76	-
	sd	4.95	4.26	6.83	5.48	
MUFA	x	46.09	46.40	45.01	46.71	-
	sd	4.80	4.53	6.42	5.24	
PUFA	x	4.22 x	3.50 x	3.92	4.05	-
	sd	1.23	0.58	0.83	1.16	
n-6	x	2.77	2.35	2.57	2.72	-
	sd	0.75	0.49	0.63	0.93	
n-3	x	0.70	0.57	0.65	0.64	-
	sd	0.34	0.14	0.19	0.18	
n6/n3	x	4.52	4.34	4.14	4.37	-
	sd	1.59	1.33	1.29	1.31	

Within carcass conformation classes x – $P \leq 0.05$.

Discussion

It was shown that the genotype did not affect the body size of slaughter cattle. The exception was width at hips, which was higher in class R dairy bulls. Leg circumference and rib eye area were higher in hybrids, which testifies to their higher slaughter value. In the EUROP system, the price a farmer receives is calculated by multiplying the carcass weight by the classification price for a particular category of animal. Carcass dressing percentage is, therefore, an important consideration for beef producers (Wajda et al., 2003). In this experiment, the carcass dressing percentage of Holstein-Friesian bulls was 2–3% higher than that noted in a previous study of Black-and-White bulls (Nogalski and Kijak, 2001). The observed difference could have resulted from higher slaughter weight of Holstein-Friesians (which is positively correlated with dressing percentage), in comparison with Black-and-White bulls (Wajda et al., 2003). According to Pfuhl et al. (2007), greater fat deposition in Holstein-Friesians can be attributed to the breed's ability to accumulate energy reserves for the first stage of lactation. Chilled carcasses were characterized by optimal levels of muscle acidification. This study did not validate the results of previous experiments where many Black-and-White carcasses showed low levels of acidification (Nogalski, 2002). Sirloin is the most valuable cut of the beef carcass (Wajda et al., 2003). Intramuscular fat enhances the flavor and aroma of meat. Its optimal content varies subject to carcass muscle, and it contributes to tenderness and juiciness of beef (Kołczak, 2008). Carcass classification to fat classes in line with the EUROP system showed no differences between the analysed genotypes, thus suggesting a similar intramuscular fat content. However, chemical analyses revealed higher intramuscular fat concentrations in the meat of crossbred beef bulls, indicating that carcass fatness grading in the EUROP system is not consistent with intramuscular fat content. Similar observations were made by Węglarz (2010).

A high proportion of SFAs and a low proportion of PUFAs in meat fat results from the hydrogenation of dietary fat by the ruminal microflora (De Smet et al., 2000). For optimal results, beef producers should decrease the concentrations of SFAs in fat and/or increase the content of PUFAs, in particular *n-3* fatty acids (Kołczak, 2008). In studies analysing the composition of intramuscular fat in *m. longissimus dorsi* in various cattle breeds, Lengyel et al., (2003) and Florek et al., (2007) reported 47.9% and 44.72% SFAs, respectively, in young Holstein-Friesian bulls, whereas Węglarz (2010) noted 37.43% SFAs in Black-and-White bulls. Our results do not corroborate the findings of De Smet et al. (2000), who demonstrated that an increase in bovine carcass fatness is accompanied by an increase in the concentrations of SFAs and MUFAs and a decrease in PUFA content. A low proportion of PUFAs in the fatty acid profile could be related to the age of bulls at slaughter (21–22 months). Lengyel et al. (2003) noted that the PUFA content of intramuscular fat in *m. longissimus dorsi* decreases with age, reaching 25.5% at 7 months, 18.4% at 14 months and 13.6% at 19 months. The *n-6/n-3* fatty acid ratio recommended by the FAO and WHO is around 5.0 (Kołczak, 2008). In our study, the *n-6/n-3* PUFA ratio offered even greater health benefits.

It is concluded that:

1. Based on the EUROP carcass classification system, the majority of carcasses of crossbred beef bulls were classified into conformation class R, and the carcasses of Holstein-Friesians were classified into class O. Most carcasses were assigned to fat class 2, and fat scores were not consistent with intramuscular fat content.

2. Within the same conformation classes, crossbred beef bulls were characterized by higher slaughter quality than Holstein-Friesian bulls. Meat from crossbred beef bulls had a higher content of fat with a more desirable fatty acid profile (a higher proportion of functional fatty acids).

3. The growth rate of beef cattle population in Poland is slow, and it is unlikely to increase rapidly in the near future, which is why efforts should be made to optimize the production of high-quality beef from dairy cattle herds. This will provide additional income to farmers and increase the consumption of beef.

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Porównanie wartości rzeźnej buhajków mieszańców mięsnych i holsztyno-fryzów w zależności od klasy uformowania w systemie EUROP

STRESZCZENIE

Badano wpływ genotypu i klasy uformowania tuszy na wartość rzeźną 200 buhajków. W magazynie żywca wybrano sztuki w wieku 21–22 miesięcy, z czego 108 były to mieszańce mięsne a 92 holsztyno-fryzy (ho). Przed ubojem oceniano ich umięśnienie i wykonano pomiary zoometryczne ciała. Po uboju sklasyfikowane tusze według systemu EUROP poddano szczegółowej ocenie wartości rzeźnej. Z próbki mięśnia najdłuższego grzbietu wyekstrahowano tłuszcz śródmięśniowy, w którym metodą chromatografii gazowej określono udział kwasów tłuszczowych. W klasyfikacji EUROP tusze buhajków mieszańców mięsnych w 61,11% uzyskały klasę uformowania R, a tusze holsztyno-fryzów w 56,53% oceniano jako O. W ocenie otłuszczenia dominowała klasa 2. i ocena otłuszczenia nie była zbieżna z zawartością tłuszczu śródmięśniowego. W obrębie jednakowych klas uformowania, mieszańce mięsne charakteryzowały się wyższą wartością rzeźną, w porównaniu z holsztyno-fryzami. Ponadto mięso ich zawierało średnio o 0,42% więcej tłuszczu o korzystniejszym składzie procentowym. Wobec braku realnych perspektyw na szybkie zwiększenie się wielkość populacji bydła ras mięsnych, należy prowadzić badania w kierunku opracowania metod optymalnej produkcji wołowiny o podwyższonej jakości w oparciu o stada mleczne.

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OESOPHAGOSTOMINAE (NEMATODA: CHABERTIIDAE) OF SUIDS FROM SOUTHERN POLAND*

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Abstract

Until recently, the genus *Oesophagostomum* was the only Oesophagostominae occurring commonly in both domestic and wild suids of Europe. A few years ago, an alien oesophagostomin nematode *Bourgelatia diducta* was recorded in the wild boar population from southern Poland, and Vietnamese potbellied pig was blamed for introduction of this Far Eastern parasite. Apart from wild boars kept in captivity for meat production purposes, Vietnamese potbellied pigs can be raised in extensive, organic, or especially agrotourism farms, which constitutes an infection hazard to domestic pigs. The aim of the research was to determine and compare species composition of Oesophagostominae in wild boars from the natural environment, and in domestic pigs from extensively managed farms, located in the area where *B. diducta* was previously noted for the first time. A postmortem examination of the large intestines of 25 wild boars and 20 domestic pigs, each from different smallholdings, was conducted in the autumn and winter season of 2010–2011. *Oesophagostomum dentatum* with coexisting *O. quadrispinulatum* were ascertained in swine, whereas the sole *Bourgelatia diducta* was recorded in wild boars. All the parasites occurred commonly in their hosts, with the prevalence of 80, 50 and 32% for *O. dentatum*, *O. quadrispinulatum* and *B. diducta*, respectively. Mean number of worms was many-fold higher in pigs, reaching 181 (range 1 to 2500) specimens in individual host, versus 3 (1–6) parasites in wild boars. A presumable influence of the alien nematode species on the European wild boar population as well as the potential for further spread of the parasite are elucidated.

Key words: wild boar, domestic pig, *Oesophagostomum* spp., *Bourgelatia diducta*

The European wild boar (*Sus scrofa scrofa*) and the domestic pig (*Sus scrofa* f. *domestica*) constitute the same animal species and are the hosts of shared parasites. In the world, two species of Oesophagostominae subfamily are prevailing and occur concurrently in pigs and wild boars, namely *Oesophagostomum dentatum* (Rudolphi,

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1803) and *O. quadrispinulatum* (Marcone, 1901) (Poelvoorde, 1978; Barutzki et al., 1991; Urquhart et al., 1996). Some other species are observed in suids of America (*O. brevicaudum* Schwartz et Alicata, 1930) and Asia (*O. watanabei* Yamaguti, 1961) (Stewart et al., 1996; Sato et al., 2008). Two additional named species, the American *O. georgianum* (Schwartz et Alicata, 1930) and the European *O. granatensis* (Herrera, 1958), are most probably morphovariants of *O. dentatum* (Poelvoorde, 1978; Cutillas et al., 1999).

Apart from oesophagostomins of *Oesophagostomum* genus, *Bourgelatia diducta* (Railliet, Henry et Bauche, 1919) is commonly observed in tropic and subtropic regions of Southeast Asia (India, Indochina, Java, Thailand) and Oceania (Papua New Guinea, Solomon Islands) (de Jesus and Waramontri, 1961; Soulsby, 1968; Talbot, 1972; Martin and Epstein, 1999). The species was also noticed in pigs of China (Fan-yao and Yi-chiang, 1965) and wild boars of Japan (Yamaguti, 1954; Sato et al., 2008), in the regions with such a climate.

In Poland, the infection of suids with *Oesophagostomum* sp. is well documented in studies by many authors. However, by means of coproscopic examinations usually carried out, the species of nodular worms could not be distinguished on the basis of eggs appearance. Postmortem, the presence of *O. dentatum* has been proven by Tarczyński (1956, 1961) in domestic pigs and wild boars, and by Gadomska (1981) in boars. As regards *O. quadrispinulatum*, it was noted for the first time in 2006, in pigs from the southern region of Poland (Nosal et al., 2007), which was probably the result of breeding material import. In wild boar of the area, *O. quadrispinulatum* was absent (Nosal, 2010), though its coexistence with *O. dentatum* has been observed in wild populations of neighbouring countries, e.g. in Germany (Barutzki et al., 1991). It was mentioned elsewhere (Nosal, 2010) that apart from *Metastrongylus asymmetricus* – a lungworm typical of Asiatic wild boars – the other alien nematode species, *Bourgelatia diducta* appeared unexpectedly in European wild boar inhabiting the area presented herein. As a source of infection with Far Eastern parasites, Vietnamese potbellied pig (*Sus scrofa vittatus*) was blamed (Nosal, 2010), following its escape into the wildlife from one of the farms in the area examined. Vietnamese potbellied pigs are sometimes raised in Poland in extensive and organic farms, and especially in agrotourism farms, thus posing an infection threat to domestic pig herds. In addition, wild boars are raised in enclosures situated in agricultural environments. The current research was aimed to reveal the present species structure of Oesophagostominae community settled in large intestine of suids from this part of Europe, and to compare wild boar populations to domestic pig herds for the similarity of oesophagostomins.

Material and methods

The study was conducted from November 2010 to February 2011, and all the examined animals originated from the area situated in the proximity of Kraków, Małopolska province. This region of southern Poland is characterized by mostly

traditional or organic farm management, and arable lands border woodlands or even primeval forests.

The guts of animals derived from 25 hunted wild boars (for which the data on the site of origin, age and sex were collected), as well as from 20 fattening pigs (6–7 months of age) – each of different small farm origin – slaughtered at a local abattoir. The large intestines were uncoiled, divided into three sections: I – caecum and the first 20% of the total length of colon; II – next 20–60% part of large intestine; III – the last 60–100% of its length, and from each section 30% of its contents was processed according to Rospestorff and Nansen (1998). Gathered Oesophagostominae specimens were differentiated (either all the worms, or 250 from the more intensively affected individuals), following the descriptions given by Haupt (1966) and Poelvoorde (1978), or on the basis of Lichtenfels (1980), Yamaguti (1954), and Fan-yao and Yi-chiang (1965), as regards *Bourgelatia diducta*. Measurements and photographs were made using Motic Images Plus 2.0 program, under 100 and 400× magnification. Quantitative Parasitology 3.0 (Rózsa et al., 2000) was used to reveal associations between helminth infections and wild boar site of origin (arable land vs. primeval forest), age group (juveniles under 1 year vs. adults) or sex.

Results

Eight of the 25 examined wild boars were infected (prevalence of 32%) with sole *Bourgelatia diducta* species identified (Fig. 1, Table 1); the mean intensity of infection equalled 3 parasites, ranging from 1 to 6 specimens in an individual host. The animals proved to be infected irrespective of their age, sex and habitat. Altogether, 21 nematodes were gathered, including 6 males and 15 females (sex ratio 1:2.5). Only one egg could be seen in the vagina of a female worm, resembling very much those produced by *Oesophagostomum* spp. (Table 1). All worms, apart from one male collected from section II of the large intestine, lived in caecum.

Table 1. Morphometric data of the examined Oesophagostominae species

Feature	Mean ± SD (range) of measured features (μm)		
	Bourgelatia diducta n = 10*	*Oespohagostomum dentatum* n = 10	*Oesophagostomum quadrispinulatum* n = 10
Distance from vulva to anus in females	471.67 ± 35.98 (407.1÷ 504.9)	360.74 ± 34.56 (313.1 ÷ 400.4)	469.96 ± 30.64 (429.1 ÷ 515.2)
Length of tail in females	424.51 ± 55.83 (327.2 ÷ 511.7)	315.72 ± 29.39 (285.2 ÷ 359.7)	481.76 ± 49.78 (395.5 ÷ 503.0)
Eggs in vagina:			
– length	65.5	76.33 ± 2.57 (73.4 ÷ 78.2)	63.65 ± 3.18 (60.9 ÷ 68.0)
– width	33.5	44.83 ± 4.02 (41.5 ÷ 49.3)	33.42 ± 3.90 (31.4 ÷ 39.0)
Length of spicules in males	1197.36 ± 101.06 (1077.7 ÷ 1321.2)	1029.42 ± 56.20 (982.2 ÷ 1105.9)	840.58 ± 13.39 (822.2 ÷ 858.4)

* In the case of *B. diducta* males, all the 6 collected specimens were measured.

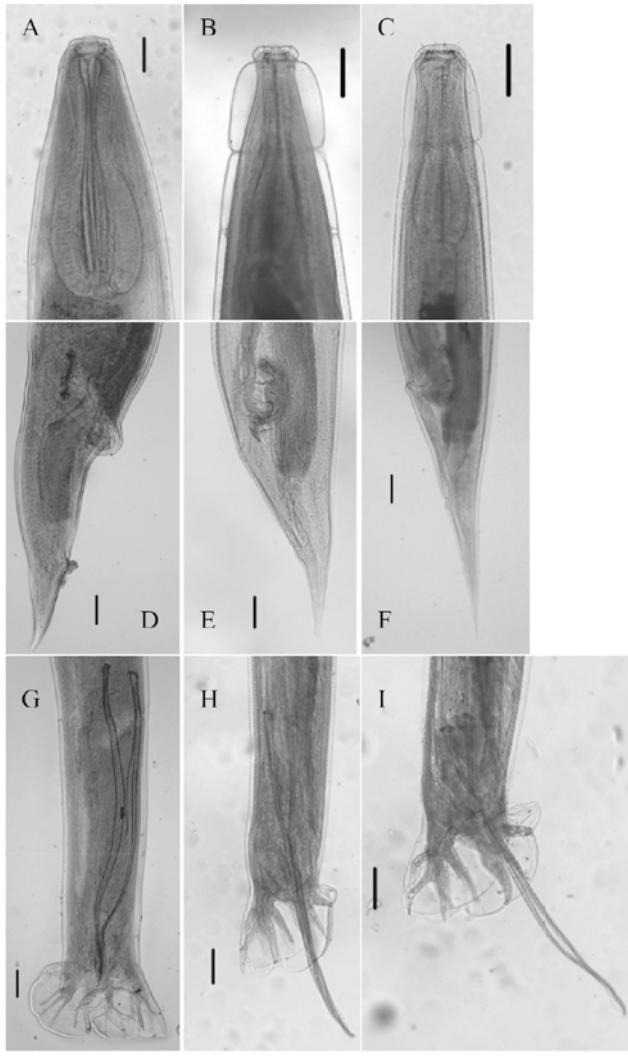

Fig. 1. Morphological features characteristic of *Bourgelatia diducta* (A, D, G), *Oesophagostomum dentatum* (B, E, H) and *Oesophagostomum quadrispinulatum* (C, F, I) (scale bar = 100 μm). (A, B, C) Lateral view of the anterior end of body and oesophageal region. (D, E, F) Lateral view of the posterior end of a female adult showing the position of vulva and anus. (G, H, I) Lateral view of the posterior end of a male adult showing spiculae, and copulatory bursa with distinctive arrangement of rays, branches and twigs.

Out of the 20 fattening pigs investigated, 18 harboured *Oesophagostomum* spp. (prevalence of 90%), and the mean intensity of infection reached 181 (1–2500) worms. Among infected swine, concurrent infection of *O. dentatum* and *O. quadrispinulatum* was observed in 7 animals, whereas pure *O. dentatum* or *O. quadrispinulatum* infection in 9 or 2 pigs, respectively. Within 893 nodular worms differentiated (Fig. 1, Table 1), predominating *O. dentatum* constituted 78.2%. This species

occupied mainly section II (48.7% of specimens identified) or section III of the large intestine (39.4%), whereas *O. quadrispinulatum* was found primarily in sections I (66.2% of worms) and II (31.3%). The male to female ratio was 1:1.2 for *O. quadrispinulatum*, and 1:1.7 for *O. dentatum*.

Discussion

In the present study, Oesophagostominae of domestic swine were confirmed to be typical of the host and region (Tarczyński, 1956; Nosal, 2010). Nematodes of *Oesophagostomum* genus are called "nodular worms" since their infective L3 larvae tend to become encapsulated – by excessive reactive inflammation – deep in the intestinal mucosa of the sensitized host. After moulting to fourth stage, the larvae may remain arrested within the nodules for several months until emerging and developing to adults in the lumen. Clinical signs of oesophagostomosis are usually associated with host reactions to the larval stages in the gut wall, thus the acute disease occurs during the prepatent period (Urquhart et al., 1996). Nodule formation may cause catarrhal enteritis, spoils sausage casings, and interferes with feed efficiency and maximum growth of young swine. Pregnant sows show inappetence, become very thin, and after farrowing milk production is reduced, which affects litter performance and influences the growth and meat quality of fatteners (Romaniuk et al., 1981; Stewart and Hale, 1988; Urquhart et al., 1996; Theodoropoulos et al., 2004; Knecht et al., 2012).

From the two common species of nodular worms, *Oesophagostomum quadrispinulatum* is generally found more proximally, especially prevailing in the caecum of large intestine, and causes more severe damage and larger nodules in caecal and colonic mucosa than *O. dentatum* (Christensen et al., 1997). It is uncertain whether this could be attributed to a higher degree of host tissue reaction against *O. quadrispinulatum* (Christensen et al., 1997). Its prepatent period is longer and varies between 18 and 42 days, compared to *O. dentatum* with the interval of 18–28 days (Várady et al., 1996). *O. quadrispinulatum* is also more difficult to remove, which may be related to the pharmacokinetic properties of anthelmintics along the large intestine, or denotes natural tolerance of this species to the drugs (Várady et al., 1996). When presenting Oesophagostominae community, Christensen et al. (1997) reported about the negative influence of *O. quadrispinulatum* on *O. dentatum* establishment, location and distribution in the gut, and fecundity, but at the same time emphasized that a variety of interactions may exist between the two closely located helminths.

In contrast to *Oesophagostomum* spp., the biology of *Bourgelatia diducta*, alien to Europe, as well as its pathogenicity, remain unrecognized (Soulsby, 1968; Yadav and Tandon, 1993), and merely Hanzhong and Yiqiang (1987) report that the early stages of the nematode follows the development of other oesophagostomins, with third stage infective larvae reaching maturity after 39–42 days of prepatent period. Thus, it is not known what actually might happen when the close-related parasite enters the gut already inhabited, and whether incoming infective larvae can establish,

causing the pre-existing worm species to be displaced or expelled. Further, it is not certain whether or not *Bourgelatia diducta* can influence the healthiness of European wild boar more than nodular worms of *Oesophagostomum* spp. already do. It is interesting that in the previous work (Nosal, 2010) *B. diducta* was observed together with *Oesophagostomum dentatum* in wild boars originating from arable lands of the region characterized. These species coexist in the native range of *Bourgelatia*, which was noted in some available publications (Talbot, 1972). Therefore, it cannot be said that the present investigation provides evidence that one Oesophagostominae species begins to displace the other within their community. Nevertheless, *Bourgelatia* occupies a privileged anterior location in the large intestine of infected hosts, similarly to *O. quadrispinulatum*, and it is important to recall that – as defined by Pence et al. (1988) – the nematode occurred already commonly in the studied population as the sole Oesophagostominae species.

Sus scrofa is considered to be an invader (ISSG Database). For ecologically invasive species, the presence of their own parasites sometimes seems to be essential in competition with native, closely related fauna. Since *Bourgelatia* comes to prevail within the oesophagostomins of wild boar population, it speaks also in favour of the nematode as a new alien invasive species. Fernandez-de-Mera et al. (2003) caution that wild boar translocations constitute an important source of foreign disease introduction, and it might be substantial to mention here that, e.g. in ruminants, the more pathogenic species of *Oesophagostomum* occur in the subtropics and tropics (Urquhart et al., 1996).

Pathogenicity of any parasitosis is conditioned by the infection level. Pence et al. (1988) state that *Oesophagostomum* species, with their direct life cycle, may require a certain host population density in order to maintain worm transmission potentials, thus reduced wild boar population could explain even the apparent loss of nematodes which occur at low prevalences. The lack of *Oesophagostomum* was reported in autochthonous wild boars from Spain (Fernandez-de-Mera et al., 2003), or the isolated wild boar population close to the northern border of its habitat area (Järvis et al., 2007). However, the population of wild boars inhabiting Poland rapidly increases nowadays (Kamieniarz and Panek, 2008). They live also in breeding conditions, where a higher level of *Oesophagostomum* infection is observed as compared with animals from the wildlife (Gadomska, 1981; Popiołek et al., 2010). In swine production, following stock density, nodular worms of *Oesophagostomum* spp. are the most common parasites, and in relation to wild boars from nature, optimal abundance is many-fold higher there (Tarczyński, 1961; Fudalewicz-Niemczyk and Nowosad, 1988; Knecht et al., 2011). An adequate increase in the level of infection with new, foreign nematode species in farm-reared game animals, or swine production, could be therefore very harmful.

If completely different Oesophagostominae fauna of wild and domestic suids, despite the same area occupied, testify to the impossibility of direct parasite transmission between wild populations and domestic pigs, still oesophagostomins may be distributed to swine herds by a wide variety of biological mechanisms (flies, cockroaches, earthworms, rats) (Jacobs et al., 1971), which pose some threat of *Bourgelatia* transmission to domestic animals. In organic, and especially agrotourism farms

holding various species of animals, the risk of the alien parasite introduction may therefore be high.

Whether or not *Bourgelatia* is significant, and occurs in the wildlife or household conditions of other European countries, it should be considered henceforth. Accordingly, postmortem oesophagostomin species identification should be essential in any parasitological research or monitoring programme conducted on suid populations or herds in the area.

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Oesophagostominae (Nematoda: Chabertiidae) u świniowatych z Polski południowej

STRESZCZENIE

Do niedawna rodzaj *Oesophagostomum* stanowił jedyne nicienie z Oesophagostominae pasożytujące powszechnie zarówno u domowych, jak i dzikich europejskich świniowatych. Kilka lat temu w popu-

lacji dzików z południowej Polski stwierdzono obcego nicienia *Bourgelatia diducta* należącego do tej podrodziny, a za wprowadzenie pasożyta pochodzącego z Dalekiego Wschodu zostały obwinione wietnamskie świnie zwisłobrzuche. Oprócz dzików chowanych w celu produkcji mięsa, w ekstensywnym lub ekologicznym chowie oraz w gospodarstwach agroturystycznych utrzymywane mogą być też świnie wietnamskie, co stanowi zagrożenie zarażeniem świń domowych. Celem przeprowadzonych badań było określenie i porównanie składu gatunkowego Oesophagostominae u dzików z przyrody oraz świń domowych z małych gospodarstw rolnych zlokalizowanych na terenie, gdzie poprzednio po raz pierwszy odnotowano występowanie *B. diducta*.

W okresie jesienno-zimowym 2010–2011 przebadano sekcyjnie 25 dzików i 20 świń domowych pochodzących z różnych gospodarstw. W jelicie grubym świń został stwierdzony *Oesophagostomum dentatum* ze współistniejącym *O. quadrispinulatum*, podczas gdy u dzików odnotowano jedynie zarażenie *Bourgelatia diducta*. Wszystkie pasożyty występowały powszechnie u swych żywicieli, z ekstensywnością zarażenia odpowiednio na poziomie 80, 50 i 32% dla *O. dentatum*, *O. quadrispinulatum* i *B. diducta*. Średnia intensywność zarażenia była wielokrotnie wyższa u świń, osiągając 181 (od 1 do 2500) pasożytów u pojedynczych żywicieli, w porównaniu do 3 (1–6) pasożytów spotykanych u dzików. W pracy przedstawiono możliwy wpływ obcego nicienia na populacje dzika europejskiego oraz potencjalne możliwości dalszego rozprzestrzeniania się pasożyta.

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VALIDATION OF A METHOD FOR DETERMINING CHOLESTEROL IN EGG YOLKS*

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Abstract

The aim of the study was to validate a gas chromatographic method for determining cholesterol in egg yolks according to the EN ISO/IEC 17025 standard. Of the two methods, with and without internal standard, the former was characterized by lower uncertainty, with a repeatability of 4% and within-laboratory reproducibility of 6%. The method's uncertainty (n = 2, P≤0.05), which included sample preparation errors and chromatographic measurement errors, was 10.6%. Mean recovery was 99.9% and limit of quantification was 0.16 mg/g. The coefficient of variation for repeatability, which is calculated during routine analyses, should not exceed the 8% limit of repeatability. The method is reliable, as confirmed by the results of validation, and the procedure is relatively rapid and simple.

Key words: cholesterol, egg yolk, validation, uncertainty, gas chromatography

The present study reflects the Central Laboratory's consistent quality policy to extend the range of analyses for animal-derived products such as egg yolks, meat and others. This research translates into better quality and safety of foodstuffs as well as health protection of the consumers of animal raw materials and products. The development (in accordance with EN ISO/IEC 17025 standard, 2005) and implementation of modern chromatographic procedures for cholesterol determination is associated with studies on the quality of eggs from conservation breeds (Cywa-Benko et al., 2000) and will help to extend the range of studies conducted at the National Research Institute of Animal Production on the nutritional value of products of animal origin (Barowicz and Pietras, 1999; Pietras et al., 2002; Brzóska, 2004 a, b; Połtowicz and Wężyk, 2005; Świątkiewicz and Koreleski, 2006).

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The role of cholesterol in metabolic processes is significant and well known. Cholesterol is mainly used for synthesis of steroid hormones and bile acids, which play a major role in digestion of fats. It is also crucial in building cell walls and semipermeable membranes. Although the presence of cholesterol is required for normal functioning of human (and animal) organisms, its excess due to genetic and dietary factors is harmful and, in extreme cases, may be hazardous to health and life. Cholesterol exists in two major forms: that transported in blood as low-density lipoproteins (known as "bad" cholesterol), and that carried by high-density lipoproteins (known as "good" cholesterol). Attention has recently been drawn to the fact that oxidized forms of cholesterol (oxysterols) rather than its pure forms are more harmful. It is beyond question, however, that all forms of cholesterol, including the harmful ones, are reflected in the level of total cholesterol, the quantitative determination of which in both blood (using rapid diagnostic monitoring methods) and foods consumed by humans is very important and necessary. This has prompted the Central Laboratory to develop a method for determining cholesterol in egg yolks, among others.

Cholesterol was determined in many products using different procedures. Colorimetric (Rhee et al., 1982; Korzeniowski et al., 1992) and enzymatic-colorimetric methods (Hwang et al., 2003; Hanczakowski et al., 2004) that were once popular were replaced with liquid and gas chromatography methods that are most common and accurate today. In addition to the analytical technique employed to determine cholesterol, the mode of sample preparation is important. The most common methods used are based on preliminary clean-up of the sample by solid phase extraction columns (Russo et al., 2005) or saponification of the sample and extraction with organic solvent (Hwang et al., 2003). In addition, the procedure of sample preparation for analysis may, but need not, include the stage of pre-column derivatization with silylation reagent. The use of such reagent allows reducing the limit of quantification, but makes the procedure slightly more complicated and increases the cost of analysis. Among other methods the simple method using direct hydrolysis, extraction and HPLC cholesterol determination in materials of animal origin can also be used (Czauderna et al., 2009). In this study, derivatization was not used but the sample components were saponified and extracted with organic solvent.

In recent years, increasing emphasis has been placed, and rightly so, on the quality of analytical techniques, which should be appropriately tested and characterized for suitability in research. Such procedure is conducted in compliance with the principles of Good Laboratory Practice (GLP, 2003) and in accordance with the requirements of the accreditation standard EN ISO/IEC 17025 (2005). The present study made use of modern gas chromatography technique, and the developed methods were characterized for such parameters as repeatability, within-laboratory reproducibility, limit of repeatability, limit of quantification (LOQ), calibration curve parameters, uncertainty, and recovery (Arendarski, 2003; Dobecki, 2004; Gąsior and Pieszka, 2006; Gąsior et al., 2009; Gąsior and Szczypuła, 2010).

The aim of the study was to validate a gas chromatographic method for determining cholesterol in egg yolks according to the EN ISO/IEC 17025 standard. The developed method should be reliable and relatively simple and fast.

Material and methods

Cholesterol was determined by gas chromatography using FID detector with a column with 5% phenyl, 95% dimethylpolysiloxane phase, after saponification and hexane extraction.

Reagents and equipment

The following reagents (of at least pure for analysis grade) were used: double-distilled water, n-hexane (Merck, Darmstadt, Germany), KOH (POCH, Gliwice, Poland), NaCl (POCH, Gliwice, Poland), ethanol 96% (Chempur, Piekary Śląskie, Poland), cholesterol (5-Cholesten-3β-ol, Sigma-Aldrich, St. Louis, USA), 5α-cholestane (>97%, Sigma-Aldrich, St. Louis, USA), stigmasterol (95%, Sigma-Aldrich, St. Louis, USA). These reagents were used to make aqueous solutions of KOH (60 g/100 ml) and NaCl (1 g/100 ml) and standard hexane solutions of cholesterol (4 mg/ml, basic standard solution) and internal standards (IS): 5 α-cholestane (2 mg/ml) or stigmasterol (2 mg/ml). Solutions were evaporated dry under nitrogen.

In addition to basic laboratory equipment, use was made of a water bath, freeze-drier (Christ Beta, Germany), gas chromatograph (GC 2010, Shimadzu, Japan) with a flame-ionization detector and AOC-5000 autosampler. A GCMS-QP2010 Plus mass detector (Shimadzu, Japan) was used for preliminary identification of cholesterol.

Determination procedure

Fresh, freeze-dried or frozen (below –12ºC) yolks were collected for the analyses. The material was mixed and a representative sample was taken for analysis. To the tube with the sample (approx. 0.2–0.3 g of fresh or frozen yolk, or 0.17 g of freeze-dried yolk, weighed to the nearest 0.0001 g) was added 4 ml of ethyl alcohol followed by 0.5 ml of KOH solution (60 g/100 ml), after which the tube was tightly closed and thoroughly shaken. After hot maceration (75±3ºC, 1 h), cooling and salting out of the sample using 4 ml of NaCl solution (1 g/100 ml), cholesterol was double extracted in 8 ml of hexane by vortexing, and the extracts were combined in a glass vial and dry evaporated (40±3ºC) under inert gas (nitrogen). After adding each batch of hexane, 10 minutes were waited before phase separation (upper hexane layer). To the evaporation residue was added 1.5 ml of hexane and 0.5 ml of IS (total of V=2 ml), to eliminate most crucial errors of solvent evaporation before sample injection, and monitor a chromatographic accuracy. After thorough mixing, the sample solution was transferred to a chromatography vial and injected onto a chromatographic column. If necessary, the sample was diluted (f = 1 in the present study).

Five intermediate standard solutions were prepared by making consecutive solutions of the basic standard solution (4 mg/ml in hexane) to obtain solutions with concentrations of 4, 2, 1, 0.5 and 0.25 (mg/ml). Calibration standard solutions (3, 1.5, 0.75, 0.375 and 0.1875 mg/ml) were obtained by adding 0.5 ml hexane to 1.5 ml of each intermediate standard solution and injecting onto the chromatographic column. The above solutions were used for plotting the calibration curve.

Calculations

The amount of cholesterol CH (mg/g) was calculated using formula 1:

$$CH = \frac{c}{m} \times V \times \frac{100}{R} \times f \quad (1)$$

where:

c – concentration established from the calibration curve equation (mg/ml),
V (2 ml) – volume of hexane added to the sample after evaporation,
m – weight of sample (g),
R – recovery (%),
f – dilution factor.

The value of concentration c was calculated from the second-degree polynomial calibration curve showing relationship between cholesterol concentration and peak area.

Chromatographic analysis

Chromatographic separation was performed on a Zebron ZB-5 column (30 m × 0.25 mm, 0.50 µm, Phenomenex, Torrance, USA) housed in an oven at 265ºC. Carrier gas (helium) flow rate was 1.7 ml/min, sample volume was 5 µl. Injector and FID detector temperature was 300ºC, split ratio was 1:25, analysis time was 30 minutes.

Validation

Repeatability and within-laboratory reproducibility tests were performed with 33 samples based on a total of 224 analyses (78 analyses of fresh yolk, 72 analyses of frozen yolk, and 74 analyses of freeze-dried yolk). The analyses, for determination of repeatability and within-laboratory reproducibility, were performed with internal standard (16 analyses for repeatability and reproducibility each) and without internal standard (160 and 32 analyses for repeatability and the reproducibility, respectively). Percent repeatability was defined as being not less than the pooled coefficient of variation (CV_{ln}) for single determinations performed with the same method, using identical material, in the same laboratory, by the same laboratory assistant and during the same time period. Percent within-laboratory reproducibility was defined as being not less than the pooled coefficient of variation for single determinations performed using the same method and identical material, in the same laboratory, by two laboratory assistants at different times. CV_{ln} for l samples analysed in n replications was calculated from formula 2, where CV_{n2} is the coefficient of variation for determination of a given sample in duplicate (n = 2):

$$CV_{ln} = \sqrt{\frac{\sum_{l} CV_{n2}^{2}}{l}} \quad (2)$$

Double the coefficient of variation for repeatability was accepted as the criterion for repetition of the determinations (limit of repeatability). The recovery was determined with two methods, using a total of 87 analyses: the standard addition method (addition of 4 mg cholesterol, 11 analyses), the standard being added prior to the saponification, and by comparing the results of analyses performed by the Central Laboratory (76 analyses) with the reference values. These values were determined based on two reference materials (freeze-dried yolks) analysed by another accredited laboratory, with uncertainty of 11.3%. The limit of quantification was determined based on the calibration curve. The working range of the calibration curve was also determined.

The main components of method uncertainty (expressed in relative form, %) were determined, such as uncertainty of within-laboratory reproducibility (*u1%*), uncertainty of recovery (*u2%*), uncertainty of purchased standard purity (*u3%*) and uncertainty associated with lack of trueness of pipettes (*u4%*) and flasks (*u5%*). Before combining, uncertainties were expressed as standard uncertainties u_i% (68% confidence level, P≤0.32). The combined standard uncertainty of the u_c% method was calculated based on the law of propagation of uncertainty from formula 3:

$$u_c\% = \sqrt{u1\%^2 + u2\%^2 + u3\%^2 + u4\%^2 + u5\%^2} \tag{3}$$

The standard uncertainty of within-laboratory reproducibility (*u1*%), which comprises most errors, including sample preparation errors, was defined as within-laboratory reproducibility % (Rep%) divided by the root of n analyses of a given sample (formula 4):

$$u1\% = \frac{\text{Re}\,p\%}{\sqrt{n}} \tag{4}$$

The standard uncertainty of recovery was calculated as a coefficient of variation for the arithmetic mean of recovery values determined during the validation. The standard uncertainties concerning standard purity and the flasks and pipettes used (as regards lack of trueness but not precision) were calculated based on relative (%) values of limiting errors a_i. For flasks and pipettes, a_i values were estimated based on the calibration procedure accepted in the laboratory. For standard purity, ai values were estimated based on the manufacturer's specifications. Assuming a symmetric rectangular distribution of the values measured around the nominal value, u_i% uncertainties are calculated using the formula $u_i\% = a_i/\sqrt{3}$ (Ellison et al., 2000). The uncertainty factor associated with the lack of trueness of the pipettes and flasks was calculated by combining the individual components in accordance with the law of propagation. Method uncertainty U_c% (95% confidence level, P≤0.05) was computed by including the coverage factor $k = 2$ ($U_c\% = k \times u_c\%$) (Ellison et al., 2000). The u_c% and U_c% uncertainties were determined for n = 2 and n = 1.

The storage life of standard solutions and samples and the content of impurities in the blank sample (without weighing the material) was determined.

The validation (but not routine analyses) also included the identification of cholesterol in the analysed solutions using a mass spectrometer.

Results

The repeatability and within-laboratory reproducibility values for the analyses with and without standard differed significantly, but were similar independently of material analysed (fresh, freeze-dried or frozen). These values are presented, together with the limit of quantification and standard uncertainty of within-laboratory reproducibility in Table 1. The recovery, determined by adding a known standard amount to yolk samples was 95.1%, and the recovery for two reference freeze-dried yolks was 102.2%. The mean recovery was practically equal to 100% (99.9%). LOQ, which corresponds to cholesterol concentration that can be reliably measured within specified limits, calculated from the calibration curve, was 0.16 mg/g. The relationship between cholesterol content and peak area was described with a second-degree polynomial curve characterized by the determination coefficient r^2 not lower than 0.99. The working range of the calibration curve ranged from 0.25 mg/ml to 4 mg/ml. The uncertainty budget, which includes all the significant factors of uncertainty, combined standard uncertainty and combined expanded uncertainty (both values for n = 2 and n = 1) is grouped in Table 2. A sample chromatogram of freeze-dried yolk analysis using both internal standards is presented in Figure 1.

Table 1. Validation parameters of the method for determining egg yolk cholesterol

Method	Repeatability (%)	Repeatability limit (%)	Within-laboratory reproducibility (%)	Standard uncertainty of within-laboratory reproducibility (u1%), n=2/n=1*, (%)
With Internal Standard (5α -cholestane)	4.0	8.0	6.0	4.2/6.0
Without Internal Standard	6.0	12.0	14.0	9.9/14.0

* n – number of analyses of one sample.

Table 2. Standard uncertainty budget, combined standard uncertainty u_c% (68% confidence level) and combined expanded uncertainty U_c% (95% confidence level, k = 2)

Method	u1% * n=2/n=1	u2% *	u3% *	u4% *	u5% *	u_c % n=2/n=1	U_c % (k=2), n=2/n=1
With IS (5α -cholestane)	4.2/6.0	2.5	0.6	2.0	0.5	5.3/6.8	10.6/13.6
Without IS	9.9/14.0	2.5	0.6	2.0	0.5	10.4/14.4	20.8/28.8

* For explanations, see Validation in Material and Methods section.

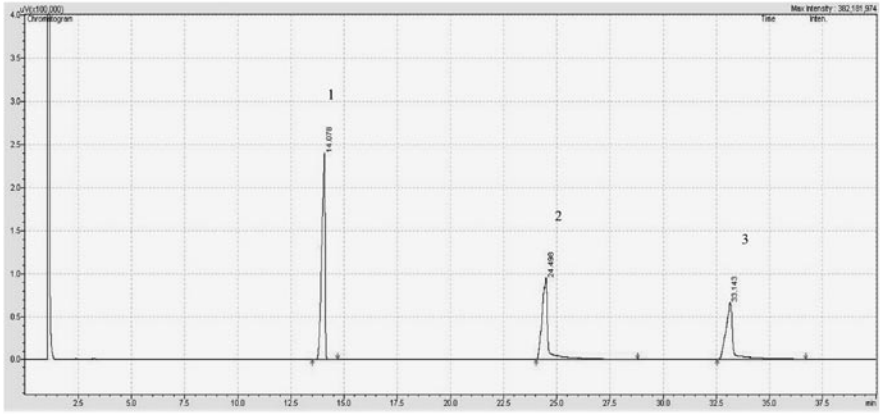

Figure 1. Sample chromatogram of freeze-dried yolk analysis. Peaks 1, 2 and 3 represent 5α–cholestane, cholesterol and stigmasterol, respectively

Discussion

The method used for determining egg yolk cholesterol does not include derivatization, which simplifies the analysis and reduces the costs. The use of sample preparation technique involving saponification and extraction instead of solid phase extraction is also beneficial and adequately cleans up the sample before chromatographic analysis. For many years, this inexpensive method of sample preparation has been used with success at the Central Laboratory of the National Research Institute of Animal Production for analysing animal and plant samples.

The present results show clear differences in uncertainty values (Table 2) depending on the sample preparation procedure: with or without the internal standard. The first method is characterized by about twice as low within-laboratory reproducibility uncertainty, which also had a similar effect on extended uncertainty (95% confidence level). Therefore, this method is recommended for routine analyses. Two internal standards were used in the study: 5α-cholestane and stigmasterol. They had similar effects on the above validation parameters (data not presented) and both can be successfully used for determining cholesterol, although 5α-cholestane is preferred for practical reasons (smaller retention time, sharper peak).

The method described and validated in the present paper is repeatable and reproducible. This is confirmed by the Horrat value H = 1.60 falling within the accepted values (0.5–2), calculated for the expected repeatability RSDr% = 2.50 (CIPAC 3807, Korol et al., 2011), according to the equation H = Repeatability % / RSDr% (4/2.50). RSDr% was calculated from the equation $0.67 \times 2C^{-0.1505}$, for the concentration C = 0.016 (the average cholesterol content in the samples was 16.0 mg/g, the content range was from about 6 mg/g to 34 mg/g). No reagent-derived impurities were found (blank sample). The working range of the calibration curve is rather narrow but enables obtaining more reliable results.

The lower limit values of analytes that can be determined, have been named and defined differently in various publications. As an example, Russo et al. (2005) estimated this value (0.8 mg/g) as 'effective' accompanied by a small standard deviation. While the LOQ values based on multifold standard deviation of blank sample (usually 10 × SD) are lower, and depending on analytical method and sample preparation, may range from 0.003 to 0.016 mg/g (Stroher et al., 2012), and even reach 88 ng/g (Mazalli et al., 2006), with the lower values for the GC methods with pre-column derivatization vs HPLC methods. In this paper the LOQ value of cholesterol determination (0.16 mg/g) concerns a weighed sample of 3 g and corresponds to the lowest point of the calibration curve, and this 'effective' value is associated with the accepted repeatability of the method. We can see that the cholesterol level, possible to determine is low enough, and the method presented here is very good for the determination of cholesterol content in cholesterol-rich yolks. This high content is good for method reliability because when even weighed amounts of test material are low, the concentrations of sample solutions obtained roughly correspond to the middle part of the calibration curve. What is more, the reliability of the method was confirmed by mass spectrometer.

The recovery obtained in this study was high and practically equal to 100%. Therefore, when cholesterol content is determined in egg yolks there is no need to adjust the raw data for recovery (R), but a general formula (1) that accounts for this parameter was proposed for the calculations.

The main factors of uncertainty that essentially determine uncertainty of the method are uncertainty of within-laboratory reproducibility, uncertainty of recovery and standard purity, and uncertainty associated with the lack of trueness (i.e. bias defined as the difference between the actual and nominal value) of pipettes and measuring flasks (trueness being defined in the ISO/IEC Guide (2007) and described in Hauck et al. (2008)). These components can be regarded as separate uncertainty factors that form the uncertainty budget. Other components, such as those related to weighing precision and precision of the pipettes and measuring flasks are not included in the uncertainty budget as separate factors (Gąsior et al., 2009). This is because they had been automatically accounted for in within-laboratory reproducibility, which is already found in this budget. This is consistent with the remark of Ellison et al. (2000) that double calculation of the uncertainty components should be avoided. It must be added that uncertainty of the calibration curve was not listed in the uncertainty budget either, because a separate curve was plotted for each series of analyses, and that meant that the associated errors were already included in the reproducibility. The uncertainty of within-laboratory reproducibility includes most sample preparation and chromatographic measurement errors. However, it is essential that these errors are automatically included in the uncertainty only when the results calculated from two replicates concern cholesterol determinations in two parallel weighed samples. If the sample was not weighed in duplicate and the solution for chromatographic analysis was analysed twice, then the uncertainty of within-laboratory reproducibility would be understated and would only include the chromatography assay error (Gąsior et al., 2005; Gąsior et al., 2007). These factors are the most important and, according to the Gaussian propagation law, they contribute the most to method uncertainty in the case

of analyses performed in one laboratory. Together with the result that is the mean of the measurements, method uncertainty ($P \leq 0.05$) is of practical significance during the interpretation of the result. It determines the tolerance interval in which the actual result should be determined with 95% probability. Uncertainty should be monitored during the analysis of every sample by checking, under repeatability conditions, the coefficient of variation for individual determinations, which should not exceed a specified limit of repeatability. It should be added that method uncertainty can be reduced by increasing the number of analyses performed on one sample ($n \geq 2$).

The observations made during validation of the method provided a basis for determining the storage life of the solutions. The standard solutions, which were stored in tightly closed flasks in a refrigerator (+2°C to +8°C) were stable for at least 2 months. For solutions of the samples stored in closed chromatography vials, the safe storage life was determined to be 2 days. Longer storage is possible but increases the risk that the solvent will evaporate and cholesterol concentration will change due to possible seal (septum) leaks in the chromatography vial. It is much better to store samples dry following evaporation under inert gas. In such a state, the samples can be stored at low temperature (+2°C to +8°C) for at least 1 month.

In conclusion, a sample preparation method and a chromatographic method for determining cholesterol in egg yolks was developed and validated according to the EN ISO/IEC 17025 standard and the principles of Good Laboratory Practice. The internal standard method was chosen as the preferred method for routine analyses due to its lower uncertainty. The validated method is relatively fast, simple and reliable, as confirmed by the validation results. Besides, the method used does not include the pre-column derivatization which can undoubtedly be regarded as a great advantage. The present study and its results form a significant part of the quality system implemented in 2004 at the Central Laboratory. The testing procedure, developed according to this system, will be used in routine analyses performed for the purposes of the National Research Institute of Animal Production and external entities.

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