The assessment of microbiological purity of selected components of animal feeds and mixtures which underwent thermal processing

Ocena czystości mikrobiologicznej wybranych komponentów pasz i mieszanek poddanych obróbce termicznej

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Abstract

Microorganisms which contaminate animal feeds pose a threat not only to animals but also indirectly to humans through their consumption of products of animal origin. The aim of the present study was to assess microbiological cleanness of selected resources and ready-made feed mixtures before and after thermal processing. The results indicated that the most bacteriologically contaminated resources were oats (*Avena sativa*), wheat middlings, wheat (*Triticum vulgare*), and poultry feed mixture KDKA F35%. The least contaminated were maize (*Zea*) and Prowit – feed mixture for livestock. The examined feed resources were contaminated with moulds, among which dominated: *Aspergillus* and *Penicillium*. The findings of bacteriological and mycological contamination assure instead of allow stating that thermal processing limits microbiological contamination of animal feeds. In order to protect the health of animals as well as consumers it seems advisable to seek new methods of thermal processing in the production of animal feeds to provide their high quality and safety.

Keywords: animal feeds, bacteria, microbiological purity, moulds, thermal processing

Streszczenie

Mikroorganizmy zanieczyszczające pasze stanowią zagrożenia zarówno dla zwierząt, ale także pośrednio dla ludzi poprzez spożywanie produktów pochodzenia

zwierzęcego. Celem niniejszej pracy była ocena czystości mikrobiologicznej wybranych surowców oraz gotowych mieszanek paszowych przed oraz po obróbce termicznej. Z przeprowadzonych badań wynika, że najbardziej zanieczyszczonymi bakteriologicznie surowcami był owies, śruta pszenna, pszenica oraz mieszanka paszowa dla drobiu KDKA F35%. Najmniej zanieczyszczeń posiadała kukurydza oraz mieszanka dla trzody Prowit. Badane surowce paszowe zanieczyszczone pleśniami, wśród których dominowały rodzaje: *Aspergillus* i *Penicillium*. Uzyskane wyniki badań zanieczyszczenia bakteriologicznego i mykologicznego pozwalają stwierdzić iż proces obróbki termicznej ogranicza zanieczyszczenie mikrobiologiczne pasz. W celu ochrony zdrowia zwierząt oraz konsumentów wydaje się być wskazane poszukiwanie nowych metod obróbki termicznej w produkcji pasz, aby zapewniać im wysoką jakość i bezpieczeństwo.

Słowa kluczowe: bakterie, czystość mikrobiologiczna, obróbka termiczna, pasze, pleśnie

Streszczenie szczegółowe

Wśród mikroorganizmów zanieczyszczających pasze wyróżnia się przeważnie grzyby pleśniowe. Głównym źródłem pleśni w paszach są surowce pochodzenia roślinnego, a przede wszystkim zboża. Ilość pleśni w materiale uzależniona jest od wilgotności paszy, temperatury, dostępności substancji odżywczych, tlenu czy pH podłoża. Obecność pleśni niesie ze sobą ryzyko zakażeń grzybiczych, alergii, a przede wszystkim chorób wywołanych działaniem mykotoksyn (metabolitów wtórnych pleśni, głównie z rodzajów: *Penicillium, Aspergillus, Fusarium*). Mykotoksyny są niezwykle niebezpieczne ze względu na ich działania: kancerogenne, mutagenne, teratogenne, estrogenne. U zwierząt powodują pogorszenie stanu zdrowia, a poprzez skażoną żywność pochodzenia zwierzęcego mogą wywoływać choroby u ludzi.

W niniejszej pracy oceniono czystość mikrobiologiczną wybranych składników pasz (jęczmień, owies, pszenica, kukurydza, śruta słonecznikowa, śruta rzepakowa, śruta sojowa, śruta pszenna) oraz gotowych mieszanek paszowych: Prowit (pasza dla trzody) oraz KDKA F35% (pasza dla drobiu - „Finisher”). Każdy z surowców został poddany obróbce termicznej po czym przeprowadzono badanie czystości mikrobiologicznej, dla określenia efektu zastosowanego procesu. Materiał kontrolny stanowiły surowce i mieszanki nie wystawione na działanie temperatury. Na początku oznaczono ogólną liczbę tlenowych bakterii mezofilnych. W kolejnym etapie badań wykonano ocenę składu ilościowego grzybów pleśniowych występujących w pobranych próbkach, a na podstawie cech makro- i mikroskopowych dokonano analizy ich składu jakościowego.

Najbardziej zanieczyszczonymi bakteriologicznie surowcami przed obróbką termiczną był owies, śruta pszenna, pszenica oraz mieszanka dla drobiu KDKA F35%. Natomiast najmniej zanieczyszczeń posiadała kukurydza i mieszanka dla trzody Prowit. Większość zidentyfikowanych gatunków pleśni należała do rodzajów *Aspergillus* i *Penicillium*, czyli grzybów powszechnie występujących w środowisku.

Otrzymane wyniki wskazują na skuteczność zastosowanej obróbki termicznej w ograniczaniu zanieczyszczenia bakteriologicznego pasz i przedłużenia ich trwałości.

Ocena pasz jest niezwykle ważna, gdyż informacje na temat częstotliwości i wielkości ich zanieczyszczenia mikroorganizmami mogą być wykorzystane do oceny ryzyka zakażeń pokarmowych zarówno u zwierząt oraz ludzi.

Bardzo istotne, aby producenci i rolnicy przestrzegali zasad higieny i dobrej praktyki, zwracali szczególną uwagę na etapy suszenia i przechowywania surowców paszowych, co pozwoli ograniczyć zagrożenie ze strony drobnoustrojów.

Introduction

Industrial production of animal feeds in Poland reaches from 5 to 8 million tonnes a year. If this number would be added directly by animal breeders, then the production of feeds reaches up to 16 million tonnes a year (Anklam and Battaglia, 2001). The policy of the European Union impacts the production of the animal feed sector by improving its quality and safety during all stages of animal feeds production and circulation according to the concept – from the field to the table. Each animal production is possible only when needs for adequate quantities of animal feeds with proper hygienic and microbiological parameters are met. Pathogenic microorganisms which can occur in animal feeds constitute a serious threat not only to animals but also indirectly to humans through resources and products yielded for humans (Davis et al., 2003; Meng and Doyle, 2002). Pathogenic microbes which can be found in animal feeds constitute a threat of transferring them through animals to consumer products of animal origin such as meat, milk or eggs, which could endanger human health (Pusta et al., 2008; Sadkowska-Todys et al., 2005). Moreover, constant exposure of animals to saprophytic microbes which dwell in the environment and animal feeds may induce the production of proinflammatory cytokines, supress appetite, accelerate metabolism and stimulate the production of leptin. All these factors impact the decrease of productivity of animals for slaughter (Colditz, 2002).

Among microorganisms which contaminate animal feeds the fungi can be identified. Fungi are ubiquitous microorganisms thanks to abundantly produced spores resistant to many environmental factors. The main source of moulds in animal feeds is feed resources of plant origin, i.e. cereals, in which moulds contamination is found very often even at the amount of 10^4 cfu·g^{-1} (Bauduret, 1990; Żukiewicz- Sobczak et al., 2012, 2013a). The amount of moulds in material depends on animal feed moisture, temperature, availability of nutrients, oxygen or pH of the substrate. The presence of moulds carries the risk of fungal infections, allergies, and above all mycotoxicoses – diseases caused by the influence of mycotoxines (secondary moulds metabolites, mainly: *Penicillium, Aspergillus, Fusarium*). Mycotoxins are unusually dangerous due to their effects: carcinogenic, mutagenic, teratogenic, and estrogenic. In animals mycotoxines cause decreased digestibility of feed, worse state of health and immunosuppression. It has also been noticed that in animals mycotoxines decrease the intake of feed, cause worse state of health and immunosuppression. Animal mycotoxicoses, through contaminated food products of animal origin may cause mycotoxicoses in humans (Grajewski et al., 2012; Hinton, 2000; Jenerowicz et al., 2012; Kolenda and Mroczkowski, 2013; Żukiewicz-Sobczak et al., 2013b).

In the assessment of microbiological quality of animal feeds, apart from moulds, pathogenic microbes such as: *Salmonella* species, *Clostridium* species, *Staphylococcus aureus, and Bacillus anthracis* have a significant role. The assessment of animal feeds is especially important due to many recorded cases of food poisoning and food infections in humans. As a result they cause, apart from health consequences, economic losses as well (Buzby and Roberts, 1997; Rocourt et al., 2003; Sinell, 1995). The reasons for this phenomenon, apart from negligence during the stage of production and food circulation, are sought early at the stages of primary production, which involves, among others, the production of resources for animal feeds. Epizootic and epidemiologic data prove that feeds can constitute an indirect cause of foodborne illnesses connected with the food chain: animal feed – animal – human (Davis et al., 2003; Hinton, 2000). Due to these reasons information on frequency and amount of microorganisms contaminating feeds may be used to assess the risk of food infections both in animals and humans.

The aim of the study was to assess the microbiological cleanness of selected components of animal feeds and mixtures which underwent thermal processing. The general number of aerobic mesophilic bacteria as well as quantitative and qualitative composition of moulds occurring in the samples were assessed.

Material and methods

The research material constituted components of animal feeds: barley, oats, wheat, maize, sunflower middlings, rapeseed middlings, soya middlings, wheat middlings and feed mixtures: Prowit (livestock feed) and KDKA F35% (poultry feed – "Finisher"). The samples of feed were collected at one time and than thermal processed.

Thermal processing

Each of the resources underwent thermal processing, and then the second microbiological cleanness test was conducted to define the results of the process. The control material was the resource and mixtures without thermal processing.

Thermal processing of feed resources was conducted in a heating apparatus, in which 100 g samples were placed. The resources were spread creating a thin layer in a flat container at 80 °C. The sample was kept in the apparatus until it reached 80 °C. The temperature of the resource was measured with a laser thermometer. The resources processed in this way underwent the assessment were considered as microbiologically pure.

Microbiological purity

During the initial stages of the research 10-fold serial dilutions were made saline solution (0.9% NaCl) with an addition of 100μl of TWEEN® 20, and then the total number of aerobic mesophilic bacteria was defined. The process was conducted on the basis of methodology according to the PN-R-64791:1994 standard. The research was carried out in two repetitions. After the inoculations the samples were

incubated for 48h at 37 °C, then the results were collected and the number of microbes was calculated per 1g of animal feed.

Moulds

Later in the research the assessment of quantitative and qualitative composition of moulds was conducted in feed resources. Two culture media were applied for the growth of moulds: PDA (Potato Dextrose Agar, SigmaAldrich) and MEA (Malt Extract Agar, SigmaAldrich) with an addition of chloramphenicol (5 mg into 100 ml of the medium). Inoculations from each dilution were made in two repetitions. Petri dishes with PDA medium were incubated at 24 °C for 72 hours, whereas Petri dishes with MEA medium at 30 °C for 72 hours. During the consecutive 96 hours the dishes were incubated at ambient temperature. On the basis of macro- and microscopic characteristics the analysis of qualitative composition of moulds was conducted based on the taxonomic literature (Larone, 2011; Ramirez, 1982; Samson et al., 2002).

Results and discussion

The degree of contamination with aerobic mesophilic bacteria from the examined animal feed resources and mixtures is presented in Table 1.

The most bacteriologically contaminated resources prior to thermal processing were oats ($15.9 \cdot 10^4$ in 1g of feed), wheat middlings ($8.6 \cdot 10^4$ in 1g of feed), wheat ($4.1 \cdot 10^4$ in 1g of feed) and poultry feed mixture KDKA F35%. The least contaminated feed resources prior to thermal processing were: maize ($0.3 \cdot 10^4$ in 1g of feed), and livestock feed mixture – Prowit ($0.3 \cdot 10^4$ in 1g of feed). The results presented in Table 1 indicate the efficiency of the applied thermal processing in the limitation of bacteriological contamination in animal feeds.

Table 1. The degree of contamination of the examined animal feed resources with aerobic mesophilic bacteria

Tabela 1. Stopień zanieczyszczenia badanych surowców paszowych przez tlenowe bakterie mezofilne

Examined animal feed resource (Badany surowiec paszowy)		L (number of microbes in 1g of feed) [·10^4] L (liczba drobnoustrojów w 1g paszy) [·10^4]
Barley (Jęczmień)	raw (surowy)	3.2
	after thermal processing (po obróbce termicznej)	0.8
Oats (Owies)	raw (surowy)	15.9
	after thermal processing (po obróbce termicznej)	0.5
Wheat (Pszenica)	raw (surowy)	4.1
	after thermal processing (po obróbce termicznej)	1.9
Maize (Kukurydza)	raw (surowy)	0.3
	after thermal processing (po obróbce termicznej)	0.1
Sunflower middlings (Śruta słonecznikowa)	raw (surowy)	0.4
	after thermal processing (po obróbce termicznej)	0.1
Rapeseed middlings (Śruta rzepakowa)	raw (surowy)	0.8
	after thermal processing (po obróbce termicznej)	0.1
Soya middlings (Śruta sojowa)	raw (surowy)	0.6
	after thermal processing (po obróbce termicznej)	0.3
Wheat middlings (Śruta pszeniczna)	raw (surowy)	8.6
	after thermal processing (po obróbce termicznej)	2.2
Prowit	raw (surowy)	0.3
	after thermal processing (po obróbce termicznej)	0.05
KDKA F35%	raw (surowy)	6.1
	after thermal processing (po obróbce termicznej)	0.5

Table 2. Types and species of fungi isolated from resources and feed mixtures

Tabela 2. Rodzaje i gatunki grzybów pleśniowych wyizolowanych z surowców i mieszanek paszowych

Examined feed resource (Badany surowiec)	Filamentous Fungi (Wyizolowane grzyby pleśniowe)
Barley (Jęczmień)	*Aspergillus fumigatus* *Panicillium purpurogenum*
Oats (Owies)	*Acremonium strictum* *Alternaria alternate*
Wheat (Pszenica)	*Aspergillus candidus* *Mucor racemosus* *Penicillium purpurogenum*
Maize (Kukurydza)	*Acremonium strictum* *Penicillium glabrum*
Sunflower middlings (Śruta słonecznikowa)	*Aspergillus candidus* *Aspergillus tamarii* *Penicillium italicum*
Rapeseed middlings (Śruta rzepakowa)	*Aspergillus candidus* *Aspergillus flavus* *Aspergillus sydowii* *Penicillium nordicum* *Penicillium purpurogenum* *Penicillium solitum*
Soya middlings (Śruta sojowa)	*Aspergillus candidus* *Aspergillus fumigatus* *Epicoccus sp.* *Penicillium nordicum* *Penicillium solitum*
Wheat middlings (Śruta pszeniczna)	*Aspergillus candidus* *Penicillium crateriformis* *Penicillium purpurogenum* *Penicillium solitum* *Rhizopus oryzae*
Prowit	-
KDKA F35%	*Acremonium strictum* *Aspergillus candidus* *Aspergillus wentii* *Penicillium purpurogenum* *Penicillium solitum*

The research of mycological contamination of feed resources proved very low contamination with moulds. Regardless of the culture medium single colonies were identified, which did not exceed the number of 10 in the lowest dilution, which according to the standard (PN-R-64791: 1994) is defined by the number of fungi: less than $1.0 \cdot 10^2$ cfu·g^{-1}. The exception was feed mixture for livestock KDKA F35%, in which contamination with moulds was $18.5 \cdot 10^3$ cfu·g^{-1}, while after thermal processing – $4.75 \cdot 10^3$ cfu·g^{-1}. The mixture for livestock Prowit was mycologically clean, in which no moulds were identified. All grown colonies of fungi were identified by species, which is presented in Table 2.

The Polish Standard PN-R-64791:1994 provides the total permissible number of fungi in 1 g of material up to 70000 for postextractive middlings of oilseeds, 200000 for feed mixtures and premixes and 100000 for feed concentrates. Comparing the values proposed by the standard with the permissible values of microbiological contamination binding in other countries, it can be stated that the Polish norms are not among the most restrictive ones (Colditz, 2002). The research proved that the examined feed resources were characteristic of lower contamination with bacteria and fungi, which was in accordance with the requirements of the standard.

Kwiatek et al. (2008) in their research of microbiological quality of animal feeds conducted in years 2003-2006 proved contamination at the level above 10^6 cfu·g^{-1} in relation to the number of aerobic mesophilic bacteria which occur within the range 2.3-19.4% of the examined samples of feed mixtures for poultry and 4.3-9.0% for livestock. A specific analysis of levels of contamination at an angle of the quantity of aerobic bacteria allows stating that mixtures for livestock are characteristic of a lower contamination as compared to poultry feeds. Most often the recorded contamination was at the level of 10^3-10^6 cfu·g^{-1}. The level of feed contamination was not significantly different over the period of four years of the research. Other research proved 10-fold higher average levels of contamination for aerobic mesophilic bacteria (Vlachou et al., 2004). The results of the research proved the lowest contamination of feed with mesophilic bacteria as compared to results achieved by Kwiatek et al. (2008), they also confirmed the results of the research conducted by this author showing higher contamination of feed for poultry as compared to mixture for livestock. Microbiological tests of animal feeds also involve defining the quantity of fungiin the material. Kwiatek et al. (2008) proved contamination of resources at the level above 10^5 cfu·g^{-1} for 0.7-4.0% poultry feed, 1.8%-7.7% cattle feed and 2.2-5.3% in samples of feed for livestock. Other Polish authors obtained similar results of contamination with fungi of animal feeds coming from Poland (Miklaszewska et al., 2004). These research proved much lower contamination of feed resources with moulds as compared to the above-mentioned authors. The only, more contaminated feed mixture was poultry feed KDKA F35%, but still the quantity of fungi did not exceed 10^5 cfu·g^{-1}. The most often isolated moulds by Cegielska-Radziejewska et al. (2013) were fungi of the following types: *Aspergillus, Mucor,* and *Rhizopus*. The average contamination of poultry feed type “Finisher” amounted to $1.6 \cdot 10^3$ cfu·g^{-1}. The poultry feed of the same type examined in the present research was contaminated with moulds to a similar degree ($18.5 \cdot 10^3$ cfu·g^{-1}), whereas *Aspergillus* and *Penicillium* being the dominant species.

Both bacteriological and mycological research proved the efficiency of thermal processing in the limitation of microbiological contamination, and at the same time

this process improves the digestibility of feed by animals (De Vries et al., 2014). For instance, in maize, which is rich in non-starch polysaccharides, the extrusion technology (i.e. barothermal processing) increases their degradation, thanks to which their application as a potential source of nutrients for animals is improved (De Vries et al., 2013). As indicated by the research results even thermal processing itself may be used to limit the enormous threat from mycotoxines. Ryu et al. (2002) proved that processing at high temperature and pressure may decrease the concentration of zearalenone in food products (predominantly in cereals), and also decrease its estrogenic properties. Cazzaniga et al. (2001) proved the efficiency of extrusion in inactivating deoxynivalenol and limited efficiency of inactivating aflatoxin B1 in samples of maize flour. Other authors have confirmed the efficiency of extrusion in inactivating fumonisin in corn flakes. Approx. 60-70% of the initial quantity of this mycotoxine is inactivated during the whole cycle of extrusion (De Girolamo et al., 2001). All the research is essential to improve the method of processing resources. To sum up, methods of thermal processing, apart from improving digestibility of food, may also contribute to its increased microbiological cleanness and decreased threat to animals and humans.

Conclusions

Microbiological safety and cleanness of animal feeds is essential not only due to the sanitary conditions of animals, but also because of indirect impact on health of consumers. Therefore, it is important the producers of animal feeds, having in mind health consequences in animals and humans who consume food products of animal origin, provide the highest possible microbiological cleanness of their products. An essential role is also played by the farmers, who are producers of plant resources – the basic animal feeds for animals. Both farmers and producers within the framework of hygiene and good practice must pay attention particularly to the stage of drying and storing feed resources, in order to prevent microbiological contamination. Moreover, as it can be seen from the research results, thermal processing may be valuable to extend the durability of animal feeds and eliminate the threat posed by microbes.

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