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EFFECT OF STORAGE CONDITIONS ON THE MICRBIOLOGICAL QUALITY OF FEED

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ABSTRACT

The microbiological quality of feed is affected by a range of factors throughout the food chain. The aim of this study was to analyze the effect of feed storage conditions on its microbiological quality. Poultry feeds were sampled in three periods: winter, summer and autumn from four domestic farms. At the same time, air temperature and humidity, as well as indoor microbiological air quality were measured. The transfer of contaminants from the air to feed that was not properly protected was demonstrated. Contamination of feed with fecal bacteria occurred regardless of the animals' access to feed stores. The study showed the influence of indoor conditions on air and feed contamination. In winter and autumn, where high air humidity (>51%) was recorded, higher concentrations of total yeast and mold counts were isolated for feed 2 in winter and feed 1 in autumn in the inverted rooms. Only in winter was there a correlation between the physical parameters of the air samples. The effect of temperature on the microbiological quality of feed was determined only during the autumn period.

Key words: bacteria and mould, fungi, microbiology of poultry feed, animal housing, air quality, feed hygiene

INTRODUCTION

The global feed and livestock industry has been facing serious food safety issues for the past two decades. This also applies to poultry feed, which is considered to be one of the important sources of contamination of the products obtained and of economic losses. Poultry feed is prepared primarily from vegetable raw materials [Krnjaja et al. 2010]. They provide a good environment for the development of microorganisms naturally occurring on them, which, through their enzymatic activity, support the digestive processes of the feed. This is important as the birds simple digestive system and specific gut microbiota contribute little to the digestion of food [Stanley and Bajagai 2022]. Each feed ingredient used in it is preparation also carries a unique risk of contamination by pathogenic microorganisms. Therefore controlling the quality of feed is an important element to ensure safety in the food chain. The occurrence of aberrations has an impact on animal health therefore also on consumers consuming animal products. Although hygiene standards are observed in feed production, secondary contamination could occur. The storage and distribution stages of the feed are promoting this phenomenon. Depending on where it is stored, the product is exposed to contamination from the air, animals and equipment [Karol 2008].

Most microorganisms present in feed do not constitute a risk to animals. However, the presence of contaminants results in a reduction in the hygienic quality of the feed, which may result in facilitated transmission of pathogenic microorganisms [Kwiatek 2010]. Significant attention is focused on the presence of bacteria considered as hygiene indicators, which include: total aerobic bacterial counts and fungal counts, *Enterobacteriaceae* and *Clostridium* sp. [Kukier et al. 2014]. The degree of contamination of raw materials, the effectiveness of sterilisation methods and the introduction of additives is illustrated by the total number of microorganisms and aerobic bacteria [Brzozowska 2018]. According to the degree of biohazard, Kukier et al. [2014] divided microorganisms into three groups. The first, most dangerous group includes *Salmonella* sp. The presence of these bacteria has the potential to cause disease in animals and in humans, thus it is known as a zoonotic

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agent. The presence of these microorganisms is most commonly determined in broiler meat and pig meat, as well as on the eggshell of laying hens, in eggs and egg products [EU One Health Zoonoses Report 2021]. The second group includes the spore-producing anaerobic rods *Clostridium* sp., aerobic *Bacillus* sp. and *E. coli* O157:H7 and *Listeria monocytogenes*. The third includes antibiotic-resistant bacteria [Kukier et al. 2014]. In addition to bacterial pathogens, feed can also be a source of devastating viruses and toxigenic fungi.

One of the many factors influencing the microbiotic quality of feed is the microbiological quality of the air. Particular attention should be paid to the housing in which the feed is stored. The qualitative and quantitative diversity of the airborne microbiota depends on many factors. One is the intensity of the airflow, changes in temperature and humidity levels the source can be animals, the litter or humans. Due to the greater stability of the indoor air microbiota, contamination with microorganisms or mould spores contributes to respiratory diseases in animals [Chmiel et al. 2015].

Therefore, the aim of the research was to analyse the influence of storage conditions of feeds and compound feeds, intended for poultry, on their microbiological quality.

MATERIAL AND METHODS

Feed for the laying hens was taken from four different domestic farms in Poland. Three samples were taken from each farm at different times: winter, summer and autumn. Feed samples were taken according to ISO/TS 17728:2015 technical specifications. Farms 2, 3 and 4 used ready-mixed feed in 25 kg bags. Only farm 1 used grain grown by itself. Each time, attention was paid to how the feed was stored and macroscopic evaluation was conducted for the presence of myotoxins. On the other hand, the following scale was used to grade the premises: 3 – cleaned area, good air circulation; 2 – unclean area, good air circulation; 1 – uncleaned area, no air circulation. The temperature and humidity in the livestock housing were also monitored [Ropek and Frączek 2016]. A thermohygrometer (Terdens s.c., Poland) was used to measure the mentioned parameters.

Analysis of the microbiological quality of feed

All microbiological analyses were performed in accordance with accepted standards [PN-EN ISO 2013b]. Each feed sample was ground with a blender (SilverCrest, Germany). Subsequently, 25 g was dehydrated into sterile BagPage bags (Interscience, France) and 225 ml of 1% buffered peptone water was added. Samples were homogenised for 90 s in a BagMixer stomacher (Interscience, France). All samples were preincubated to determine the presence of *Salmonella* sp. [PN-EN ISO1 2020]. The following ISO standards were used to determine specific groups of microorganisms: total psychrophilic bacterial count (TPB) [PN-EN ISO 2013a], total mesophilic bacterial count (TMB) [PN-EN ISO 2022a], coliform (TCC) and *E.coli* (TECC) [PN-EN ISO 2017] using ChromoColi chromogenic medium (MERC, Germany), *Enterococcus* sp. (TEC) [PN 1993] using Slanetz medium, *Staphylococcus* sp. (STP) [PN-EN ISO 2022b] using Chapman's medium (Oxoid, United Kingdom), total count of yeasts and moulds (TYMC) [PN-ISO 2009], anaerobic spore-forming bacteria [PN-EN ISO 2005] using Liver Broth liquid medium (Oxoid, United Kingdom) and aerobic spore-forming bacteria (TASB) [ISO 2020] using PEMBA medium (Oxoid United Kingdom). Colonies were counted according to [PN-EN ISO 2013b].

Air quality analysis

A sedimentation method was used to determine the microbiotic diversity of the air. Petri dishes were exposed in the tested livestock housing for 10 minutes in 3 replicates for each parameter tested. Results were recalculated according to Kubera et al. 2015. The total number of gram negative bacteria (TCN) was determined on McConkey medium (BTL, Poland). Total number of coagulase-positive *Staphylococcus* sp. was determined on Baird Parker medium (Merck, Germany) [PN-EN ISO 2022b]. Other parameters were analyzed as in the feed analysis, i.e. TMB, STP, TASB, TYMC.

Identification of characteristic colonies

Microscopic preparations were made of all characteristic colonies. The Gram staining technique was used. It was the basis for the selection of API (bioMérieux, France) biochemical tests, which confirmed microbial species affiliation. Identification of moulds was carried out by microscopic and macroscopic observation according to the Samson and van Reenen-Hoekstra [1988].

Statistical analysis

All experiments were repeated three times, taking 3 samples each season. All data were expressed as mean value ±standard deviation (SD). Student's *t*-test was carried out by using the software Statistica 13.03 (StatSoft, Poland). The compared values were considered as significantly different when $P < 0.05$.

Correlations between the variables feed quality, air quality, temperature and humidity were assessed using Pearson's correlation coefficient (r) at a significance level of $P < 0.05$.

RESULTS

Visual evaluation of feed, livestock housing and physical parameters

During the assessment of domestic farms, only two of them had a dedicated room for its storage. Feed 1 and 4 were accessed by animals affecting more pollen and less air circulation. In addition, livestock housing 2 was the only one to be thermally isolated. In summer the lowest temperatures were in livestock housings 1 and 3, while in winter and autumn the highest temperature was determined in 2. The lowest humidity was recorded in summer and the highest in autumn. Only in livestock housing 1 was the humidity above 70%.

Microbiological analysis of feed

In winter and summer, the TMB for the analysed feeds was at a comparable level. In contrast, statistically significant differences between the samples were indicated during the autumn period. The highest TPB count was determined in feed 1 and the lowest in feed 2. The total number of psychrophilic bacteria in the samples differed for each feed. Analysing TCC in winter confirmed statistical differences between feeds 2 ($7.90 \cdot 10^4$ cfu $\cdot$ g^{-1}) and 3 ($3.40 \cdot 10^4$ cfu $\cdot$ g^{-1}) and 4 ($3.05 \cdot 10^4$ cfu $\cdot$ g^{-1}), whereas in summer between feeds 1 ($3.33 \cdot 10^3$ cfu $\cdot$ g^{-1}) and 2 ($2.33 \cdot 10^3$ cfu $\cdot$ g^{-1}). Statistically significant differences in TEC were confirmed in winter between feed 2 ($4.90 \cdot 10^3$ cfu $\cdot$ g^{-1}) and 3 ($1.25 \cdot 10^4$ cfu $\cdot$ g^{-1}), in autumn between sample 3 ($4.00 \cdot 10^2$ cfu $\cdot$ g^{-1}) and 4 ($1.03 \cdot 10^3$ cfu $\cdot$ g^{-1}). In summer, TEC was not determined in any of the feeds (Table 3). *Staphylococcus* sp. was not detected in any feed regardless of the season.

Feed 1 presented the highest concentration of total bacterial count throughout the study period: winter, $2.01 \cdot 10^6$ cfu $\cdot$ g^{-1}, summer, $2.91 \cdot 10^6$ cfu $\cdot$ g^{-1}, and autumn, $6.16 \cdot 10^5$ cfu $\cdot$ g^{-1}. The highest total yeast and mould count (TYMC) was determined in summer in feed 1 ($1.27 \cdot 10^5$ cfu $\cdot$ g^{-1}). In winter and autumn, the TYMC ranged from $2.03 \cdot 10^3$ to $2.96 \cdot 10^4$ cfu $\cdot$ g^{-1}.

Significant correlations between physical factors and microbiological parameters of feed were determined only in autumn. The effect of temperature on the amount of TPB and TYMC determined was shown. In addition, a positive correlation was found between an increase in the amount of TPB and the amount of TYMC (Table 2). In none of the seasons was there a correlation between moisture content and the amount of determined microorganisms in the tested feeds.

The feeds were also assessed for the presence of anaerobic bacteria. For this, the most probable number technique was used. The highest MPN of *Clostridium* sp. (Table 4) was determined in all samples collected in the autumn season. In samples collected in the winter season, the determined MPN for *Clostridium* sp. and *Cl. perfringens* was at a higher level and ranged from $2.3 \cdot 10^1$ to $>1.1 \cdot 10^4$ cfu $\cdot$ g^{-1} . In feed samples tested in the summer season, the presence of *Clostridium* sp. was determined only for feeds 1 and 3, while *Cl. perfringens* was not detected in any of the feeds.

Identification of microorganisms isolated from animal feed

The presence of *Rhizobium radiobacter* was confirmed by microscopic analysis (gram-negative bacilli) and API 20 NE tests (Biomérieux, France). *Actinomyces naesnundii* was also isolated from each feed, which grew as small, white, round and dry colonies on DRBC medium (Oxoid, United Kingdom). The presence of *Bacillus mycoides*, growing as planar, rhizoidal, turquoise colonies on PEMBA (Oxoid, United Kingdom) medium, was confirmed in feed 1 during the summer and in each of the samples tested in the autumn. In contrast, *Areococcus viridans* and *Leuconostoc* sp. were identified in samples taken only during the winter period (feeds 2 and 3). As with feeds 2 and 3, *Citrobacter* sp. was also isolated in all samples taken in autumn. *Salmonella* sp. was not identified in any of the feeds regardless of the season.

Considering the presence of yeasts in winter and summer, the same species predominated in each feed (Table 5).

Microbiological analysis of air

In summer, the lowest count was determined for air 2, which indicated a divergence from air 1 and 3. In contrast, TASB were not isolated from air 1 and air 4 in winter. STP CPS and TCN concentrations were not determined in any of the samples, regardless of the season. The highest TNY in winter and summer was determined in the air of livestock room 3 ($9.44 \cdot 10^2$ cfu $\cdot$ g^{-1}). In winter, the lowest TNY was determined in room 2 ($1.97 \cdot 10^2$ cfu $\cdot$ g^{-1}). TNM in winter dominated the air sampled in livestock housing 2 ($2.01 \cdot 10^3$ cfu $\cdot$ g^{-1}). In the other samples, the determined levels showed no significant differences.

In autumn, the highest TNM contamination occurred in the air of room 1 ($1.40 \cdot 10^4$ cfu $\cdot$ g^{-1}). The statistical analysis performed did not confirm significant differences between the samples.

In the air sampled from livestock housing 1, 2 and 4, the highest number of TYMC was determined in autumn ($2.64 \cdot 10^3$ to $1.40 \cdot 10^4$ cfu $\cdot$ m^{-3}). Only in livestock housing 3 was the highest number determined in summer. In winter, the total number of yeasts and moulds was at a comparable level.

A significant correlation was found between temperature and humidity in the tested livestock housing air samples only during the winter period ($R = 0.7$) at $p < 0.05$ (Table 7). No significant correlation was found between

Table 1. Visual assessment of the livestock housing, feed storage and physical parameters

Area surveyed	Area 1	Area 2	Area 3	Area 4
Type of area	Livestock housing with access for animals	Livestock housing designated for storage of feed	Poultry house	Livestock housing with access for animals
Area assessment	1	3	3	2
Sample	Feed 1	Feed 2	Feed 3	Feed 4
Method of storage	Animal trough	Uncovered bag	Uncovered bag	Plastic, closed container
		Temperature, °C		
Winter	11.2	18.0	10.0	9.8
Summer	22.8	24.5	22.9	23.0
Autumn	14.8	18.0	17.0	16.0
		Humidity, %		
Winter	57.0	66.0	51.0	62.0
Summer	40.0	39.0	45.0	41.0
Autumn	71.0	66.0	60.0	65.0

3 – cleaned area, good air circulation; 2 – unclean area, good air circulation; 1 – uncleaned area, no air circulation.

Table 2. Correlation between physical and microbiological parameters in tested feeds during the autumn period

Variable	Temperature, °C	Humidity, %	TPB	TMB
Humidity, %	–0.60	1.00	–	–
TPB	–0.97*	0.76	1.00	–
TMB	–0.29	–0.20	0.24	1.00
TYMC	–0.95*	0.79	0.98*	0.03

*difference significance at $P < 0.05$; TMB – total number of mesophilic bacteria; TPB – total number of psychrophilic bacteria, TYMC – total number of yeasts and moulds.

the physical parameters during the other periods tested. In addition, there was no effect of temperature and humidity on the determined microorganisms.

In winter, *Zygosaccharomyces* sp. was identified in each air sample, with the exception of room 2 (Table 8). *Saccharomyces cerevisiae* was determined in the air of livestock housing 2 similarly to the autumn. *Rhodotorula* sp. was only present in the air taken from the room in winter. Regardless of the season, moulds of the types *Penicillium* sp. and *Cladosporium* sp. were dominant in each sample analysed. In summer, *Microsporum* sp. was identified in every sample except sample 4, while the types *Bysochlamys* sp. and *Fusarium* sp. were isolated in the air of livestock housing 3 and 4, respectively.

DISCUSSION

Nutrition is a key stage in animal husbandry on which the efficiency of production depends. It is well known that feed is not sterile and can be contaminated with biological substances at any stage of its production. For microbiological contamination, the presence of pathogenic and non-pathogenic microorganisms is considered. Humidity, temperature and airflow have a significant impact on the microbiotic structure of the feed and the diversity of microorganisms present. This is of particular importance in cases where feed is stored on the premises where farm animals are housed. Dust and microorganisms released from litter and animals form a bio-sol in which fungi, viruses and endotoxins are present in addition to bacteria, which account for about 80. For this reason, the number of airborne microorganisms in livestock buildings is considered an important indicator of environmental quality [Stanley and Bajagai 2022].

The largest amounts of bacterial cell fractions are isolated from dust present in barns or livestock housing [Ropek and Frączek 2016]. Matusiak et al. [2017] reported that 10^6 cfu $\cdot$ g^{-1} of fungi and 10^9 cfu $\cdot$ g^{-1} of bacteria could be present in sedimented dust and higher levels were observed in rooms with animal access. In the research presented here, feed storage rooms without animal access (2 and 3) also demonstrated high lev-

Table 3. Results of bacterial analysis of feed (cfu/g)

Feed	TMB	TPB	TASB	TECC	TCC	TEC	TYMC	TBC
				Winter				
Feed 1	$5.70 \cdot 10^4$ ±1.84	$1.88 \cdot 10^6$ ±3.89	$5.00 \cdot 10^2$ ±1.41	$5.00 \cdot 10^2$ ±1.41	$1.70 \cdot 10^{3\ abcd}$ ±0.5	$6.25 \cdot 10^4$ ±1.77	$8.65 \cdot 10^3$ ±3.29	$2.01 \cdot 10^{6\ abc}$ ±0.61
Feed 2	$6.05 \cdot 10^4$ ±1.91	$4.30 \cdot 10^5$ ±0.85	$1.85 \cdot 10^3$ ±1.34	$1.85 \cdot 10^3$ ±1.34	$<10^{2\ ab}$ ±0.00	$7.90 \cdot 10^{4\ abc}$ ±0.41	$2.86 \cdot 10^4$ ±1.22	$5.76 \cdot 10^{5\ ac}$ ±1.26
Feed 3	$1.05 \cdot 10^4$ ±0.77	$3.68 \cdot 10^{4\ ab}$ ±1.14	$5.50 \cdot 10^2$ ±0.70	$5.50 \cdot 10^2$ ±0.70	$5.00 \cdot 10^{1\ ac}$ ±0.02	$3.40 \cdot 10^{4\ ab}$ ±0.21	$8.40 \cdot 10^3$ ±4.31	$1.08 \cdot 10^{5\ b}$ ±0.07
Feed 4	$4.58 \cdot 10^4$ ±2.03	$1.14 \cdot 10^{5\ ab}$ ±0.57	$8.00 \cdot 10^2$ ±2.83	$8.00 \cdot 10^2$ ±2.83	$<10^{2\ ad}$ ±0.00	$3.05 \cdot 10^{4\ ac}$ ±0.22	$6.13 \cdot 10^3$ ±1.92	$1.92 \cdot 10^{5\ ac}$ ±0.47
				Summer				
Feed 1	$4.10 \cdot 10^{5\ a}$ ±0.14	$2.50 \cdot 10^{6\ abcd}$ ±0.15	$<10^2$ ±0.00	$4.25 \cdot 10^2$ ±1.19	$3.33 \cdot 10^{3\ ab}$ ±0.42	$<10^2$ ±0.00	$1.27 \cdot 10^{5\ ab}$ ±0.65	$2.91 \cdot 10^{6\ abcd}$ ±0.71
Feed 2	$5.38 \cdot 10^4$ ±0.82	$4.89 \cdot 10^{4\ ab}$ ±0.49	$1.10 \cdot 10^3$ ±0.58	$<10^2$ ±0.00	$2.33 \cdot 10^{3\ ab}$ ±0.31	$<10^2$ ±0.00	$1.14 \cdot 10^{4\ ab}$ ±0.49	$1.06 \cdot 10^{5\ abc}$ ±0.31
Feed 3	$5.30 \cdot 10^4$ ±0.90	$1.90 \cdot 10^{5\ ac}$ ±0.35	$2.30 \cdot 10^3$ ±1.45	$<10^2$ ±0.00	$2.83 \cdot 10^4$ ±0.89	$<10^2$ ±0.00	$1.19 \cdot 10^{3\ a}$ ±1.34	$2.74 \cdot 10^{3\ abcd}$ ±0.62
Feed 4	$4.06 \cdot 10^{4\ a}$ ±0.81	$8.54 \cdot 10^{4\ ad}$ ±0.5	$6.00 \cdot 10^2$ ±1.00	$<10^2$ ±0.00	$2.65 \cdot 10^3$ ±0.49	$<10^2$ ±0.00	$4.45 \cdot 10^{3\ ab}$ ±1.34	$1.29 \cdot 10^{5\ abcd}$ ±0.38
				Autumn				
Feed 1	$2.87 \cdot 10^{4\ acd}$ ±0.46	$5.45 \cdot 10^{5\ abc}$ ±1.06	$1.50 \cdot 10^2$ ±0.70	$4.50 \cdot 10^2$ ±0.54	$3.60 \cdot 10^4$ ±1.00	$2.87 \cdot 10^{4\ acd}$ ±0.46	$2.96 \cdot 10^4$ ±0.26	$6.16 \cdot 10^{5\ abc}$ ±2.08
Feed 2	$8.16 \cdot 10^{3\ abcd}$ ±0.78	$2.61 \cdot 10^{4\ abcd}$ ±0.91	$1.00 \cdot 10^2$ ±0.00	$1.00 \cdot 10^2$ ±0.00	$2.28 \cdot 10^3$ ±1.01	$8.16 \cdot 10^{3\ abcd}$ ±0.78	$2.03 \cdot 10^{3\ a}$ ±0.78	$3.74 \cdot 10^{4\ abcd}$ ±1.10
Feed 3	$4.70 \cdot 10^{4\ abcd}$ ±0.94	$6.18 \cdot 10^{4\ abcd}$ ±0.22	$5.00 \cdot 10^2$ ±1.24	$<10^2$ ±0.00	$1.25 \cdot 10^3$ ±0.21	$4.70 \cdot 10^{4\ abcd}$ ±0.94	$4.45 \cdot 10^3$ ±1.51	$1.11 \cdot 10^{5\ abc}$ ±0.31
Feed 4	$1.53 \cdot 10^{5\ abcd}$ ±0.08	$3.20 \cdot 10^{5\ abcd}$ ±0.75	$1.53 \cdot 10^3$ ±0.91	$7.33 \cdot 10^2$ ±1.06	$2.77 \cdot 10^3$ ±0.86	$1.53 \cdot 10^{5\ abcd}$ ±0.08	$1.22 \cdot 10^4$ ±0.05	$4.79 \cdot 10^{5\ abcd}$ ±1.23

± standard deviation, ^{a,b,c,d} – column difference significance at $P < 0.05$, TMB – total number of mesophilic bacteria, TPB – total number of psychrophilic bacteria, TASB – total number of aerobic spore–forming bacteria, TECC – total number of *E. coli*, TCC – total number of coliforms, TEC – total number of *Enterococcus* sp., TYMC – total number of yeasts and moulds, TBC – total number of bacterial count.

Table 4. MPN of *Clostridium* sp. and *Clostridium perfringens* in the investigated feeds

Feed	MPN, cfu/g	
	Clostridium sp.	*Clostridium perfringens*
	Winter	
Feed 1	$>1.1 \cdot 10^4$	$2.3 \cdot 10^1$
Feed 2	$2.3 \cdot 10^1$	$2.3 \cdot 10^1$
Feed 3	$>1.1 \cdot 10^4$	$2.4 \cdot 10^3$
Feed 4	$2.3 \cdot 10^1$	$2.3 \cdot 10^1$
	Summer	
Feed 1	$2.3 \cdot 10^2$	<0.30
Feed 2	<0.30	<0.30
Feed 3	$2.3 \cdot 10^1$	<0.30
Feed 4	<0.30	<0.30
	Autumn	
Feed 1	$>1.1 \cdot 10^4$	$2.3 \cdot 10^1$
Feed 2	$>1.1 \cdot 10^4$	<0.30
Feed 3	$>1.1 \cdot 10^4$	$>1.1 \cdot 10^4$
Feed 4	$>1.1 \cdot 10^4$	$>1.1 \cdot 10^4$

Table 5. Identified species occurring in the feed

Feed	Bacteria	Yeast	Moulds
		Winter	
Feed 1	*Rhizobium agrobacterium*, *Actinomyces naesnundii*	*Zygosaccharomyces* sp.	*Penicillium* sp.
Feed 2	*Rhizobium agrobacterium*, *Actinomyces naesnundii* *Areococcus viridans*, *Leuconostoc* sp. *Citrobacter* sp.	*Candida pelliculosa*	
Feed 3			*Microsporum* sp.
Feed 4	*Rhizobium agrobacter*, *Actinomyces naesnundii*	*Zygosaccharomyces* sp., *Candida pelliculosa*	
		Summer	
Feed 1	*Rhizobium agrobacter* *Bacillus mycoides*, *Actinomyces naesnundii*	*Candida pelliculosa*, *Candida valida*	*Penicillium* sp., *Cladosporium* sp., *Bysochlamys* sp.
Feed 2	*Rhizobium agrobacter*, *Actinomyces naesnundii*	*Candida pelliculosa*, *Candida valida*, *S. cerevisiae*	*Penicillium* sp., *Bysochlamys* sp.
Feed 3		*Candida valida*	*Bysochlamys* sp.
Feed 4		*Candida pelliculosa*, *Candida valida*, *Zygosaccharomyces* sp.	*Microsporum* sp., *Bysochlamys* sp.
		Autumn	
Feed 1	*Rhizobium agrobacter* *Bacillus mycoides*, *Actinomyces naesnundii* *Citrobacter* sp.	*Candida pelliculosa*, *S. cerevisiae*	*Penicillium* sp., *Microsporum* sp.,
Feed 2			*Microsporum* sp.
Feed 3			*Microsporum* sp., *Bysochlamys* sp.
Feed 4			*Penicillium* sp. *Microsporum* sp. *Bysochlamys* sp.

els of contamination as did rooms where animals were present (1 and 4). The ventilation system which is the most important element in maintaining proper air quality is also significant [Wójcik et al. 2010]. The tested livestock housing had a gravitational airflow system.

In the livestock housing designated 2 and 3 similar temperature values were recorded in autumn (Table 1). In contrast to Ropek and Frączek [2016] who reported lower temperature values in livestock housing without animals present. In addition, a similar summer temperature was recorded in livestock housing 1 and 3 (22.8°C and 22.9°C respectively), despite the presence of animals in area 1. Temperatures in winter were quite low (Table 1), which apart from the season was due to the lack of space heating and the low density of birds. In livestock housing 2 on the other hand the temperature was 18°C in autumn and winter due to the thermal protection of the building. In tested spaces 1 and 4 (with animal access) higher humidity was recorded in autumn than in the facilities studied by Bródka et al. [2012] where mechanical ventilation was used.

The quantitative analysis of STP carried out, indicates significantly lower counts of these bacteria ($<10^2$ to 10^2 cfu · m^{-3}) compared to published information. Popescu et al. [2011] determined STP at levels of 10^3–10^4 cfu · m^{-3}, with a humidity of more than 80%. The spaces analysed (1 and 4) did not record such high humidity, which may have contributed to keeping the number of STPs low.

Anaerobic spore-forming bacteria (*Bacillus* sp.) are widely distributed in the environment. They are isolated from the bioaerosol of farm and livestock housing and from dust [Bródka et al. 2012, Ropek and Frączek 2016]. In winter, TASB was not determined in livestock housing 1 and 4, after which in summer Bacillus sp. was already isolated from each space. The occurrence of *Bacillus* sp. in livestock housing is associated with the presence of favourable growth conditions in the bioresol [Ropek and Frączek 2016].

No TCNs were detected in any spaces as in the research conducted by Ropek and Frączek [2016]. In contrast, Zucker et al. [2000] detected TCN in 6 livestock housing at $7.20 \cdot 10^1$ to $5.05 \cdot 10^2$ cfu · m^{-3}. In addition, they have shown that the air of the livestock housing mainly contained bacteria from the *Enterobacteriaceae* family with a predominance of *E. coli.*

The amount of TYMC is usually in the range of 10^3 to 10^4 cfu · m^{-3} [Witkowska et al. 2010, Lawniczek-Walczyk et al. 2013, Pavan and Manjunath 2014], which was also determined in the current study. *Penicillium* sp.,

Table 6. Results of the microbiological air quality, cfu/m^3

Livestok housing	TMB	TASB	STP	TNY	TNM	TYMC
			Winter			
Air 1	$3.62 \cdot 10^{3\ acd}$ ±0.56	$0.00^{\ ac}$ ±0.00	$4.33 \cdot 10^{2}$ ±1.90	$7.47 \cdot 10^{2}$ ±1.01	$1.61 \cdot 10^{3}$ ±0.28	$2.36 \cdot 10^{3}$ ±0.60
Air 2	$3.11 \cdot 10^{3\ bd}$ ±0.29	$2.08 \cdot 10^{3}$ ±0.39	$4.33 \cdot 10^{2}$ ±0.59	$1.97 \cdot 10^{2\ bc}$ ±0.59	$2.01 \cdot 10^{3}$ ±0.61	$2.20 \cdot 10^{3}$ ±1.40
Air 3	$2.48 \cdot 10^{4\ ac}$ ±0.65	$2.20 \cdot 10^{3\ ac}$ ±0.34	$7.87 \cdot 10^{2}$ ±0.56	$9.44 \cdot 10^{2\ bc}$ ±1.01	$1.46 \cdot 10^{3}$ ±0.17	$2.40 \cdot 10^{3}$ ±0.32
Air 4	$2.97 \cdot 10^{4\ abd}$ ±0.10	$0.00^{\ c}$ ±0.00	$6.29 \cdot 10^{2}$ ±1.12	$4.82 \cdot 10^{2}$ ±0.8	$1.22 \cdot 10^{3}$ ±0.28	$1.70 \cdot 10^{3}$ ±0.49
			Summer			
Air 1	$1.81 \times 10^{4\ ab}$ ±0.04)	$1.65 \cdot 10^{3}$ ±0.89	$9.83 \times 10^{2\ abcd}$ ±1.07	$5.11 \times 10^{2\ acd}$ ±0.52	$4.44 \cdot 10^{3}$ ±1.31	$4.95 \cdot 10^{3}$ ±3.08
Air 2	$7.86 \times 10^{3\ abc}$ ±0.78	$1.89 \cdot 10^{3}$ ±0.45	$0.00^{\ ab}$ ±0.00	$7.90 \times 10^{1\ abc}$ ±0.00	$1.10 \cdot 10^{3\ bc}$ ±0.22	$1.18 \cdot 10^{3\ a}$ ±0.60
Air 3	$3.92 \times 10^{4\ c}$ ±0.87	$2.04 \cdot 10^{3}$ ±0.67	$0.00^{\ ac}$ ±0.00	$9.44 \times 10^{2\ abcd}$ ±1.11	$9.83 \cdot 10^{3\ bcd}$ ±0.23	$1.08 \cdot 10^{4\ ab}$ ±0.51
Air 4	1.18×10^{4} ±0.39	$1.97 \cdot 10^{3}$ ±0.22	$0.00^{\ ad}$ ±0.00	$7.90 \times 10^{1\ acd}$ ±0.00	$2.36 \cdot 10^{3\ cd}$ ±0.45	$2.44 \cdot 10^{3\ b}$ ±1.34
			Autumn			
Air 1	$1.53 \cdot 10^{3\ ac}$ ±0.78	$3.54 \cdot 10^{2}$ ±0.52	$4.72 \cdot 10^{2}$ ±1.00)^{ad}	$3.54 \cdot 10^{2\ ac}$ ±0.52	$1.40 \cdot 10^{4}$ ±0.81	$1.40 \cdot 10^{4}$ ±0.93
Air 2	$2.79 \cdot 10^{3\ bc}$ ±0.94	$3.93 \cdot 10^{2}$ ±1.22	$3.94 \cdot 10^{2}$ ±1.11	$3.93 \cdot 10^{2}$ ±1.02	$2.44 \cdot 10^{3}$ ±1.01	$2.60 \cdot 10^{3}$ ±1.80
Air 3	$1.58 \cdot 10^{4\ abcd}$ ±0.02	$1.58 \cdot 10^{2}$ ±1.11	$3.04 \cdot 10^{2\ cd}$ ±0.16	$1.58 \cdot 10^{2\ ac}$ ±0.81	$2.81 \cdot 10^{3}$ ±0.05	$4.56 \cdot 10^{3}$ ±1.77
Air 4	$2.24 \cdot 10^{3\ cd}$ ±0.94	$4.33 \cdot 10^{2}$ ±1.66	$4.65 \cdot 10^{1\ acd}$ ±0.59	$4.33 \cdot 10^{2}$ ±0.66	$2.44 \cdot 10^{3}$ ±0.21	$2.64 \cdot 10^{3}$ ±1.78

± standard deviation, ^{a,b,c,d} – column difference significance at $P < 0.05$, TMB – total number of mesophilic bacteria, TASB – total number of aerobic spore–forming bacteria, STP – total number of *Staphylococcus* sp., TNY – total number of yeasts, TNM – total number of moulds, TYMC – total number of yeasts and moulds.

Table 7. Correlation between physical and microbiological parameters in the tested livestock housing during winter

Variable	Temperature, °C	Humidity, %	TMB
Humidity, %	0.70*	1.00	–
TMB	–0.68	–0.33	1.00
TYMC	0.17	–0.54	–0.54

* difference significance at $P < 0.05$; TMB – total number of mesophilic bacteria; TYMC – total number of yeasts and moulds.

Cladosporium sp. and *Aspergillus* sp. are identified as the dominant mould species in poultry houses [Jo and Kang 2005]. In this paper, only the presence of *Aspergillus* sp. fungi was not confirmed in the samples analysed (Table 8). A study by Jo and Kang [2005] confirms the dominance of *Penicillium* sp. in laying hen houses. Lugauskas et al. [2004] also identified 38 species of moulds present in the feed storage area, with a dominance of *Penicillium* sp, *Cladosporium* sp. and *Fusarium* sp., the presence of which was also detected in other investigated farm buildings [Lawniczek-Walczyk et al. 2013, Pavan and Manjunath 2014]. Lugauskas et al. [2004] identified the genus *Microsporum* sp. only within the air of the poultry house, whereas in the study presented here both in the air of the poultry house (air in room 1) and in the livestock housing (air in rooms 2 and 3).

Yeasts are among the forms commonly found, also in air. Of the species determined, the frequent occurrence of *S. cerevisiae* is indicated in the air samples studied, which is also confirmed by Górny and Dutkiewicz

Table 8. Identified species occurring in the air

Livestok housing	Yeast	Moulds
	Winter	
Air 1	*Zygosaccharomyces* sp.	*Penicillium* sp., *Cladosporium* sp.
Air 2	*S. cerevisiae, C. pelliculosa*	
Air 3	*Zygosaccharomyces* sp.	
Air 4	*Rhodotorula, Zygosaccharomyces* sp.	
	Summer	
Air 1	*C. valida, C. pelliculosa*	*Penicillium* sp., *Cladosporium* sp., *Microsporidium* sp.
Air 2	*C. valida*	*Penicillium* sp., *Cladosporium* sp., *Microsporidium* sp.
Air 3	*S. cerevisiae, Zygosaccharomyces* sp.	*Penicillium* sp., *Cladosporium* sp., *Microsporidium* sp., *Bysochlamys* sp.
Air 4	*C. valida*	*Penicillium* sp., *Cladosporium* sp., *Fusarium* sp.
	Autumn	
Air 1	–	*Penicillium* sp., *Cladosporium* sp.
Air 2	*S. cerevisiae, C. pelliculosa*	*Cladosporium* sp., *Penicillium* sp., *Fusarium* sp.
Air 3	*C. pelliculosa*	*Penicillium* sp., *Cladosporium* sp., *Microsporum* sp., *Bysochlamys* sp.
Air 4	*S. cerevisiae, C. pelliculosa*	*Penicillium* sp., *Cladosporium* sp.

[2002]. Lawniczek-Walczyk et al. [2013] primarily isolated yeasts belonging to the genus *Candida* sp., including *C. pelliculosa*, which was one of the dominant yeast species isolated from culture houses [Sowiak et al. 2012].

In winter (Table 1), the higher counts of TPB than TMB, probably related to the temperature range of 9.8–18.0°C favourable for the growth of psychrophilic bacteria. Analysing the results from the summer period, an increase in the number of TMB could be expected, while no dominance of this group is observed. The direct reason could be the non-optimal temperature for their growth [Mycielski 1984]. Comparing the air results obtained (Table 6) with those from the feed (Table 3), a similar range of TASB counts is found.

Hossain et al. [2020], during testing of three types of poultry feed (depending on the age of the animals), found *Bacillus* sp. contamination of 10^5–10^6 cfu · g^{-1}, which is significantly higher than the results obtained in the present study (Table 3). *Actinomyces naesnundii*, of which the soil is also the main reservoir in each study period, was identified. The presence of these microorganisms may be due to the contamination of the raw materials used to form the feed mixtures and, consequently, their presence in the analysed samples. On the other hand, it is a component of the soil microbiota and was isolated from all feed regardless of the season.

The determination of TCC, TECC, TEC provides information on faecal contamination of the feed. In each feed, regardless of the season, TCC was determined at a similar level. The analysis of 20 different poultry feeds by Sule and Ilori [2017] allowed an indication of coliform contamination in the range of 10^3–10^4 cfu · g^{-1}. The same level of contamination was obtained in the results presented (Table 3). The counts of TECCs varied according to the season and the type of feed analysed (Table 3). Feed 1, stored in a room with access to the animals, was contaminated with *E. coli* regardless of the season, with the highest contamination in winter. In addition, feed 1 was own grown grain not excluding natural soil fertilisation. Gazal et al. [2015] demonstrated the survival of *Escherichia coli* in organic manure after the composting process, which may also favour contamination of the grown grain [Mgbeahuruike et al. 2023].

Storage of feed 4 in the poultry house should indicate a higher *E. coli* count. However, feed 4 was stored in a plastic sealed container which significantly reduced the possibility of contamination. During winter and autumn, TEC was determined at similar levels in each feed, while this microbial group was not recorded during summer. Analysis of the commercial feed showed contamination levels of *Enterococcus* sp., 10^2 cfu · g^{-1} [da Costa et al. 2007]. In the results presented here, the same values were obtained for feeds 2 and 3 during the autumn period (Table 4).

In the samples tested regardless of the season, none of the STPs were determined. Counts of *Staphylococcus* sp. determined in the dust, among others, at levels as high as 10^9 cfu · g^{-1}, are not consistent with their presence in the feed [Stanley et al. 2012].

During the winter, a gram-positive granuloma, *Areococcus viridans*, was isolated in feed 2 and 3 (Table 5), *Areococcus* sp. are widely distributed in the environment

including in the air, water or on the skin of humans and animals [Larson et al. 2008]. Bacteria from the family *Leuconostocaceae* populate the gastrointestinal tract in large numbers and constitute the natural intestinal flora of healthy poultry. The presence of the micro-organism in feed may suggest contamination from animals [Stanley et al. 2012].

In the feeds analysed regardless of the season, no *Salmonella* sp. was detected. In contrast, *Citrobacter* sp. was isolated in feeds 2 and 3 in winter and autumn. The presence of the microorganism in the feed may also be a result of contaminated air at the storage site. Zucker et al. [2000] isolated *Citrobacter freundii* species in the air of livestock housing. Despite the absence of *Salmonella* sp. in the analysed feeds, it is a microorganism of concern in terms of contamination of compound feeds [Maciorowski et al. 2007].

The highest counts of *Clostridium* sp. in feed were determined in feed samples taken during the winter and autumn seasons. An important source of *Clostridium* sp. is the digestive system of animals and organic dust floating on farms [Bindari et al. 2021]. Of concern is the presence of *Cl. perfringens*. Particularly high amounts were determined during the autumn season. *Cl. perfringens* is isolated in feed, including meat meal, feather meal, indicating that feed may be the initial source of *Cl. perfringens* to the farm. Once established in the soil, workers footwear, fans and ventilation ducts, *Cl. perfringens* persists on the farm, even if the feed is pathogen-free [Stanley and Bajagai 2022]. The predisposition of poultry to infections is related to the presence of lesions and intestinal dysbiosis. *Cl. perfringens* are also isolated from faeces, from litter and from water [Immerseel et al. 2004].

Poultry feeds are mainly based on plant raw materials, hence the high percentage of contamination recorded [Krnjaja et al. 2010]. In the case of the analysed feed samples, the highest TYMC concentrations were recorded in the summer for feed 1. The access of animals to it is storage room may have contributed to increased contamination with mycocenoses. In feed 4 (stored in the poultry house), high TYMC counts were found ($1.22 \cdot 10^4$ cfu $\cdot$ g^{-1}) during the autumn period, which is also linked to high TYMC concentrations in the room air ($2.64 \cdot 10^3$ cfu $\cdot$ g^{-1}). The differences in TYMC between seasons are due to changes in atmospheric conditions. Kwiatek et al. [2008] when analysing cereal grains, over a three-year period, showed a persistent fungal contamination of 10^6 cfu $\cdot$ g^{-1}. The storage of feed from winter to summer promotes its exposure to adverse effects: oxygen, carbon dioxide and humidity, resulting in an increase in the development of so-called storage fungi (e.g. *Penicillium* sp.) [Adelusi et al. 2022]. The type *Penicillium* sp. very commonly contaminates poultry feed [Sukmawati et al. 2018]. Moreover, it is one of the toxigenic fungi having the ability to produce mycotoxins, i.e. ochratoxin, patulin or citrulline [Szymczak and Bogusławska-Wąs 2020]. During the macroscopic evaluation of the feed, there were no visible changes indicating the possibility of mycotoxin production, i.e. no clumping of the feed, no discolouration of the grain. As in the air samples (Table 8), the type *Penicillium* sp. was isolated regardless of the season.

As with the confirmed presence of yeast in the air of the feed storage livestock housing (Table 5), the presence of yeast was also determined in the feed. The species/genus consistency of yeast occurrence in both environments was established (Table 4 and Table 6). The exception is *Rhodotorula* sp., strains of which were isolated from the air of room 4 during winter, but not isolated from feed 4.

CONCLUSIONS

The way in which feed is stored and the physical conditions, i.e. temperature and humidity, can significantly affect the microbiological quality of the air and feed. In winter and autumn, where high air humidity (>51%) was recorded, higher concentrations of total yeast and mold counts were isolated for feed 2 in winter and feed 1 in autumn in the inverted rooms. Only in winter was there a correlation between the physical parameters of the air samples. Temperature determined the type of microbes that appeared in both feed and the air of livestock housing. However, animal access and cleanliness of the housing also affect the microbiological quality of the stored feed. Inadequate protection of the feed results in the transmission of microorganisms present in the bioareosol.

Air and feed contamination mainly represented the genus *Penicillium* sp. Moreover analysis of faecal bacteria showed their presence both in rooms with and without animal access.

The level of feed contamination varied over time. *Salmonella* sp. was not detected in any of the feeds. However, they show a questionable level of hygiene, in terms of high concentrations of *Enterobacteriaceae* regardless of the season. The use of feeds after the last sampling – in autumn, is not recommended, due to the significant exceedance of the hygiene criterion for *Clostridium* sp., *Enterobacteriaceae* and the high concentration of *Clostridium perfringens*.

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WPŁYW WARUNKÓW PRZECHOWYWANIA NA JAKOŚĆ MIKROBIOLOGICZNĄ PASZ

STRESZCZENIE

Na jakość mikrobiologiczną paszy wpływa szereg czynników w obrębie całego łańcucha żywnościowego. Celem badań była analiza wpływu warunków przechowywania pasz na ich jakość mikrobiologiczną. Próbki pasz dla drobiu pobierano w trzech okresach: zimowym, letnim i jesiennym z czterech krajowych ferm. Wykonano pomiary temperatury i wilgotności powietrza oraz jakości mikrobiologicznej powietrza w pomieszczeniach. Wykazano przenoszenie zanieczyszczeń z powietrza do paszy, która nie była odpowiednio zabezpieczona. Zanieczyszczenie paszy bakteriami kałowymi występowało niezależnie od dostępu zwierząt do magazynów paszowych. Badanie wykazało wpływ warunków panujących w pomieszczeniach na zanieczyszczenie powietrza i paszy. Zimą i jesienią, gdy notowano wysoką wilgotność powietrza (>51%) w pomieszczeniach inwentarskich, izolowano wyższe stężenie całkowitej liczby drożdży i pleśni dla paszy 2 zimą oraz paszy 1 jesienią. W okresie zimowym wykazano korelację pomiędzy parametrami fizycznymi prób powietrza. Wpływ temperatury na jakość mikrobiologiczną paszy oznaczono tylko w okresie jesiennym.

Słowa kluczowe: bakterie i grzyby pleśniowe, mikrobiologia pasz dla drobiu, pomieszczenia dla zwierząt, jakość powietrza, higiena pasz

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