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The Effect of Storage Temperatures on the Microbiological Quality of Fish Feeds

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Abstract

Temperature is a factor which affects microbiological quality of feeds during their storage. Three storage temperatures of the feeds for fish were taken into account in this study: -11°C, 5°C and 20°C. Analyses comprised the survival of proteolytic, ammonifying, psychrophilic and mesophilic bacteria as well as fungi. It was found that after 72-day storage of fish feed at the three temperatures, fungi showed the highest survival (56% - 80%). As regards the four physiological groups of bacteria, the highest survival was observed for mesophilic bacteria (6.25% - 9.58%), followed by psychrophilic ones (2.5 - 3.25%) and ammonifiers (0.07 - 0.11%), while proteolytic bacteria showed the lowest survival (0%). No live cells of the latter bacteria were observed after 64-day storage of the feed at -11 and 5°C, and after 72-day storage at 20°C.

Keywords: micro-organisms, survival, fish feed.

Introduction

Complete feeds produced commercially are both important and indispensable in fish farming. They are usually in form of dry complex feeds composed of plant (cereal seeds, bran, rapeseed or soybean meal or cake, legume seeds) and animal components (meat-bone and fish meal, poultry off-fall, meat, powdered milk, animal fats) supplemented with vitamins and minerals [1].

Protein represents the major component of fish feeds. It is a source of energy for the fish, but also a medium for micro-organisms, especially proteolytic bacteria and ammonifiers. Good quality of the products used and proper hygiene of the technological processes decrease the risk of microbiological contamination of fish feeds.

Storage conditions, especially temperature and humidity, represent another important factor affecting microbiological quality of feeds. Improper storage temperature may prolong survival of the micro-organisms present in the feed [11], or even enhance their multiplication and production of toxic substances. Toxin-producing fungi are especially dangerous, most of all those producing aflatoxins, patulins, and trichotecens which are strongly cancinogenic and mutagenic [6].

Microbiological analysis should be used in classifying a feed as suitable for use; its results should not exceed the respective standards. At present, fish feeds used in Poland must conform to the Polish Standard (PN-76/R-64791) [9] referring to dry feeds. This standard defines necessary microbiological examination of the presence of active micro-organisms, such as: proteolytic and ammonifying bacteria, saprophytic and toxin-producing fungi. No works have been found in the available literature on the survival of micro-organisms present in fish feeds during storage, although there are papers devoted to the survival of bacteria in other environments, e.g. lake water [3, 7, 10], fish, meat, milk, cheese, broth and soil [3, 4, 5]. These studies dealt with the survival of single strains of bacteria.

The objective of the present study was to examine survival of the physiological groups of proteolytic and ammonifying bacteria, fungi (mentioned in the standard PN-76/64791), as well as psychrophilic and mesophilic bacteria present in fish feeds during their storage at -11, 5 and 20°C.

Materials and Methods

Materials

Materials consisted of the feed for whitefish fry composed of: yeast, wheat flour, fish meal, meat-bone meal, fish oil, mineral mixture (Polfamix B, Polfa-Kutno) and a vitamin mixture of Poczyczyfiski [8].

Preparation of the Samples

30 weighed 10 g portions of the examined feed were prepared. Ten portions were placed in each of the three temperatures (-11, 5 and 20°C) and stored for 72 days.

Microbiological Examinations

Quantitative analyses were carried out of selected micro-organisms present in fish feed:

- proteolytic bacteria developing on a medium with gelatine, at 20°C/48 h,
- ammonifying bacteria developing in nutritive agar medium at 37°C/24 h,
- saprophytic fungi developing on wort-agar medium at 28°C/72 h,
- toxin-producing fungi developing on modified Czapek medium at 28°C/3 to 5 days,
- psychrophilic bacteria growing on common agar me dium at 20772 h,
- mesophilic bacteria developing on common agar medium at 37°C/24 h.

The first four determinations were carried out according to the Polish Standard (PN-76/R-64791) (Tab. 1), while the other two - according to the generally used microbiological methods [2]. 0.85% NaCl solution was used as a diluent. CFU were determined after the required time and recalculated into 1 g of the feed.

Table 1. Permissible maximal numbers of bacteria and fungi in 1 g of the analyzed feeds.

Micro-organisms	In bulk feeds of plant origin and in feed mixtures
Proteolytic bacteria	50,000
Ammonifiers	250,000
Saprophytic fungi	25,000
Toxin-producing fungi	50

Determination of Micro-Organism Survival in the Samples

The first microbiological examination of the feed was performed before storage. Other examinations were carried out at 8-day intervals until day 72 of storage. The feed was stored at three temperatures: -11, 5 and 20°C.

Micro-organism survival was given in days and percentages, taking into account the last day of storage (72nd), or the last examination revealing live bacteria cells.

Results and Discussion

Feed used in the experiment fulfils the requirements of the Polish Standard (PN-76/R-64791). No toxin-producing fungi were found. Results pertaining to the survival of the five groups of micro-organisms in fish feed are presented in Figs. 1-5 (in a logarithmic scale) and in Table 2.

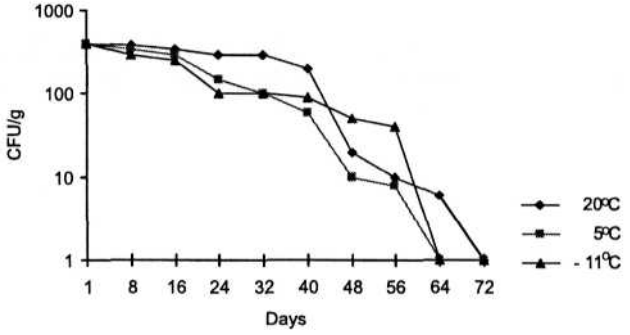

Fig. 1. Survival of proteolytic bacteria in the feed.

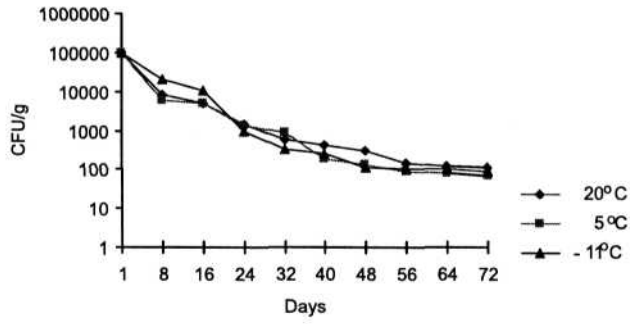

Fig. 2. Survival of ammonifying bacteria in the feed.

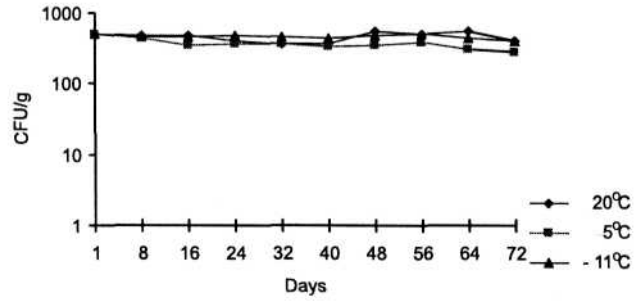

Fig. 3. Survival of fungi in the feed.

Table 2. Survival of the examined micro-organisms in fish feed (in per cents and days).

Groups of micro-organisms	Initial number of micro-organisms in 1 g of the feed	Temperature					
		-11°C		5°C		20°C	
		%	days	%	days	%	days
Proteolytic bacteria	400	0.00	64	0.00	64	0.00	72
Ammonifying bacteria	102,000	0.09	> 72	0.07	> 72	0.11	> 72
Fungi	500	80.00	> 72	56.00	> 72	80.00	> 72
Psychrophilic bacteria	4,000	2.75	> 72	3.25	> 72	2.5	> 72
Mesophilic bacteria	2,400	6.25	> 72	7.5	> 72	9.58	> 72

Numbers of all bacteria decreased during feed storage in all temperatures throughout the whole study period, but the decreased trends were different for particular groups of micro-organisms.

Temperature affected survival of proteolytic bacteria in a different way (Fig. 1). This group of bacteria showed the highest survival up to 40 days of storage at 20°C; later on rapid reduction of bacteria numbers was noted. At -11°C a decrease of bacteria numbers was fairly regular until day 56 of storage. On day 64 there was a rapid decrease of live bacteria numbers, and no live cells were obtained on day 72.

Ammonifying bacteria (Fig. 2) as well as psychrophilic (Fig. 4) and mesophilic (Fig. 5) survived in a similar way at all three temperatures. A slow but regular decrease of bacteria numbers was observed throughout the study.

Survival of saprophytic fungi (Fig. 3) in the feed was similar at the three tested temperatures and remained at the same level throughout the experiment.

Based on the results of the studies, the highest survival after 72-day storage of the fish feed in three different temperatures was noted in the case of mesophilic bacteria (Tab. 2): from 6.25% to 9.58%. It was a little lower for psychrophilic bacteria, from 2.5% to 3.25%, followed by ammonifiers (0.07% to 0.11%), being the lowest for proteolytic bacteria, which disappeared until day 72 of feed storage.

Low survival of proteolytic bacteria in the feed might have been caused by their high susceptibility to external conditions or low initial numbers which amounted to 400 cells in 1 g of the feed. Initial numbers of mesophilic bacteria (2400) were lower than of psychrophilic ones (4000 in 1 g of the feed), but on the last day of studies mesophilic bacteria showed higher survival (6.25% - 9.58%) than psychrophilic (2.5% - 3.25%), suggesting higher resistance of the first bacteria to storage conditions.

Initial samples contained the highest numbers of ammonifying bacteria (102,000 in 1 g), but after 72 days of storage the number of these bacteria decreased considerably and amounted to only 0.07-0.11% of the initial levels.

Survival of fungi in the fish feed (Tab. 2) on the last day of storage at -11 and 20°C was 80%, while at 5°C - it amounted to 56%. Due to high survival of fungi during feed storage attention should be paid especially to initial contamination of the feeds with these micro-organisms, as well as care taken to ensure proper storage conditions and feed sampling during storage.

Conclusions

1. Fungi showed the highest survival during storage of the fish feed. After 72 day-storage at -11 and 20"C survival of these group amounted to 80%, and at 5"C - to 56%.

2. From among the four physiological groups of bac teria, the highest survival after 72 days of feed storage at the three temperatures was observed for mesophilic bac teria (from 6.25 to 9.58%). It was lower for psychrophilic bacteria (2.5 - 3.25%) and ammonifiers (0.07 - 0.11%), and the lowest (0%) for proteolytic bacteria.

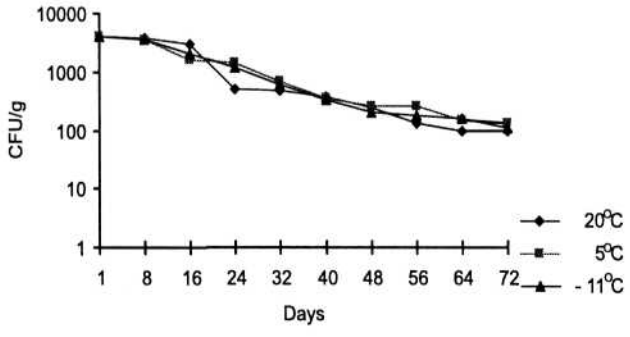

Fig. 4. Survival of psychrophilic bacteria in the feed.

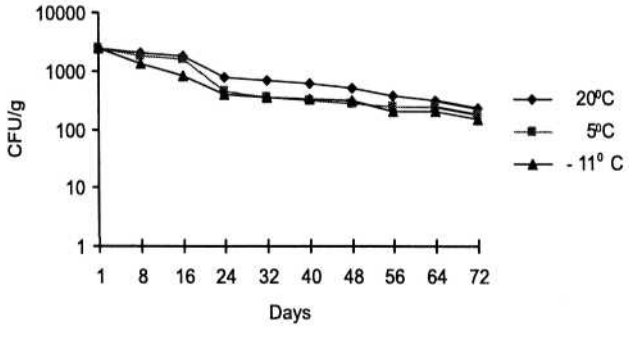

Fig. 5. Survival of mesophilic bacteria in the feed.

3. It is advised to perform microbiological control of the feed immediately after its production as well as during storage.

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