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THE EFFECT OF USING LOW-PHYTATE CEREAL VARIETIES ON PHOSPHORUS DIGESTIBILITY AND SELECTED PRODUCTION INDICES

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Abstract

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The pollution of surface waters by excessive biogenic elements, especially phosphorus, is a highly topical subject. Fishery management on ponds may be one of the potential sources of phosphorus in waters. Cyprinid fish generally have a very poor digestion of the phosphorus contained in the plant-based components of feeds. In them, phosphorus is deposited in the form of phytic acid which is almost indigestible for cyprinids. The conducted testing was focused on affecting the digestibility of phosphorus contained in plant-based feeds. Two of the cereals most frequently grown in the Czech Republic were tested, namely, wheat and barley. Control feeds were varieties commonly grown in our country, i.e. Vánek and Bojos. Furthermore, we used special cereal mutant lines with a decreased content of the phytic acid, JS-12/IDO 563 and M955. The test results showed that when using the low-phytate wheat line, the digestibility of phosphorus was increased by up to 11.21% ($P < 0.01$); and by 5.89% ($P < 0.05$) in the case of barley. Both low-phytate lines of the cereals used significantly affect phosphorus digestibility and decrease the loading of the environment with phosphorus. When using the low-phytate wheat line, a significant effect ($P < 0.05$) was found on the fat content in the fish body compared to the control variety but also compared to the low-phytate barley line.
No significant effect was found comparing both control varieties and comparing the barley varieties mutually.

Keywords: phosphate, phytate, lpa cereals, barley, wheat, digestibility of phosphorus

INTRODUCTION

Phosphorus is among the most common and most important elements on earth. It is represented in many forms both in the animal and the plant kingdoms. It is also a part of countless numbers of minerals. In the animal tissues, it is mostly deposited in bones and also in teeth (Jelínek *et al.*, 2003). In these tissues, phosphorus is in large part contained in the form of hydroxyapatite or various kinds of phosphates (Lall, 1991). In fish, substantial percentage of phosphorus is contained in the scales. Chow and Schell (1980) further report that same as the skeleton, the skin can also be an important depository of digestible phosphorus in some species. Phosphorus is further represented by a large percentage (10–20%) in other tissues, especially in the muscle, nervous, and heart tissues, and also in the liver and kidney tissues. In these soft tissues, phosphorus functions mainly as a construction element and is part of the intermediary metabolism (Jirásek *et al.*, 2005). Pointillart (1987) and Leeson and Summers (2001) point out the importance of phosphorus in the formation and metabolism of proteins, lipids, saccharides or individual amino acids. It is an integral part of blood and its formation, or of the DNA (Pointillart, 1987), coenzymes, vitamins, macroergic compounds (Jirásek *et al.*, 2005; Leeson and Summers, 2001).

Phosphorus is thus an important element for the fish organism. It is found in several forms in fish feeds, of which the most important are inorganic phosphorus supplemented to the feed, and organic phosphorus naturally occurring in the feed components. In the case of inorganic phosphorus, it is mainly monocalcium phosphate and dicalcium phosphate (Leeson and Summers, 2001). Jirásek *et al*., (2005) report up to 94% digestibility for phosphorus from monocalcium phosphate, and only 46% for dicalcium phosphate. It follows from these data that using other form than monocalcium phosphate becomes very uneconomical. In its natural form, phosphorus comes from two sources. The first source is the animal component. The most important source of animal-based phosphorus is undoubtedly the fish meal. The production of complete compound feeds, however, takes a different route, trying to find substitutions for fish meal (Carter and Hauler, 2000). This fact is affected by the ever increasing price of fish meal which in turn increases the price of the feed (Baruah *et al*., 2004; Dutta, 2009) and also by the very high phosphorus content within the fish meal. Jirásek *et al*., (2004) mention only a 25% digestibility of phosphorus from the fish meal. Feed producers, therefore, have tried to substitute fish meal in the feed by balancing the composition of amino acids obtained from various plant sources (BioMar Group, 2017).

Phosphorus is found in plants in the form of the phytic acid, or phytate (Jirásek *et al*., 2005). The phytate molecule, and therefore, also the phosphorus deposited in it, is very difficult to digest for fish. Apart from phosphorus, phytate further binds also divalent and trivalent cations of various other elements, most frequently those of zinc, calcium or magnesium, thereby rendering these elements unusable for the organism (Kumar *et al*., 2011). Digestibility of phosphorus from phytate ranges between 0–18% in salmonids and 8–35% in cyprinids (Jirásek *et al*., 2005) which is very negative in terms of undigested phosphorus. Phosphorus is contained in the phytic acid by up to 80%. Digestibility of phosphorus from phytate can be influenced by several ways. The already proven method is the adding of commercially produced phytases, especially in salmonid fish breeding. Phytases are enzymes that make possible the decomposition of the phytic acid, thereby making the phosphorus from this complex accessible. In the case of cyprinid fish, the use of phytase is somewhat more problematic (Cao *et al*., 2007). Their activity is affected by the temperature and pH value of the digestive tract (Simmons, 1990). Phytases are very susceptible to higher temperatures. Most commercial phytases lose their activity at temperatures above 70 °C (Cao *et al*., 2007). Cain and Carling (1995) further report that the use of phytases becomes problematic even in salmonid fish breeding when feed mixtures are produced by granulation or extrusion. In the production process of these feeds, the temperature highly exceeds the limit temperature, and the phytase mixed into the feed becomes unusable. The ideal method is by spraying a liquid form of the phytase onto the surface of granules (Vielma *et al*., 2004). The ideal pH value for the appropriate enzyme function is 2.5–5. This complies with the digestive system of salmonids whose stomach has an acidic pH. This factor is limiting for the cyprinids as they do not have a stomach and the pH value of their digestive system is around 7. In such case, the enzyme is not activated and therefore does not influence the digestibility of phosphorus from phytate (Cao *et al*., 2007). The enzyme can be activated by acidifying the feed mixture by adding one of the organic acids, e.g. the citric acid (Baruah *et al*., 2005). Another possibility is applying a neutral type of phytase which finds ideal conditions in neutral pH (Yu and Wang, 2000; Zeng *et al*., 2001 in Cao *et al*. 2007).

Another possibility of decreasing the phosphorus load upon the environment is the genetic modification of the plant components used for feed production. Phosphorus is contained in plants in the form of phytic acid by up to 80% of the total phosphorus content. This presents a big problem when fish in ponds are supplemented with cereals. Commonly used feeds contain high concentrations of phosphorus due to the phytate content. A wheat grain contains approximately 3 g of the total phosphorus; triticale contains approximately 3.4 g phosphorus, same as barley (Jirásek *et al*., 2005). Lolas *et al*. (1976) report the phytate content in wheat to be between 0.62 and 1.35%. What is even more important, these figures represent 61.7–79.9% of total phosphorus. In barley, phytate makes approximately 0.98–1.16%. In terms of the phytate content of total phosphorus, it is 66.1–69.6%. Generally, it can be said that phytate makes more than two thirds of total phosphorus. For more effective utilization of phosphorus from plant components, special cereal varieties have been bred with a lower phytate content by the hybridization, mutation, of standard cereal varieties with low-phytate ones. Low-phytate mutant lines, so called low phytic acid (lpa) ones, are special lines with decreased phytate content in the grain. Despite the low phytate, however, the total phosphorus content in the cereal is preserved (Raboy, 2000).

The study of Vaculová *et al*., (2012) reports that approximately 31% of phosphorus is deposited in the form of phosphates, i.e. digestible components. The rest of phosphorus makes a phytate component. The study further mentions that in newly bred lpa lines, this proportion has been exceeded many times, namely from 138.4% up to 770%. The proportion between phytate and phosphate is mainly influenced by the variety, line, and also by the conditions in which the cereal was grown. Raboy (2000) reports that by obtaining lpa barley varieties, the phytic acid content was decreased by 50 to 95%. Especially M955 is one of the best lpa, where the decrease in the phytic acid content reaches up to almost 95%; as one of the few lpa mutants, it is viable even with a major decrease of the phytate content in the grain. In contrast to M955, various other varieties of e.g. corn with a relatively identical phytate content decrease are no longer viable. For this reason, M955 is a very important step in breeding lpa cereal lines, albeit this variety has a substantially lower yield compared to common varieties.

In terms of fish breeding in ponds, the issue of solving phosphorus residua from supplemental feeding is highly topical because of water pollution by phosphorus. Therefore, in our experiment we studied the effect of low-phytate wheat and barley lines mainly on phosphorus digestibility. The evaluation of digestibility was complemented with the assessment of the effect of the variety used on the production variables and tissue composition of the fish bred.

MATERIALS AND METHODS

Environmental characteristics – the system

For the feeding test, special tanks were produced, divided into two parts based on the model published by Stejskal (2012). The principle was in collecting the fish excreta in a sedimentation cone located at one side of the tank and separated from the breeding tank by a partition, so that the excreta could further be used for various analyses. A total of six tanks of the volume of 106 litres were used. These tanks were connected to the recirculation system. Part of the system is the Nexus 310 biofilter of a volume of 840 litres, where water is purified and then drawn by a pump into the storage reservoir. This tank of a volume of approximately 451 litres is located above the biofilter, and it is here where the chemistry of the purified water is stabilized. Water from the storage reservoir is distributed into the tanks. For good functioning of the tanks, appropriate water circulation needs to be secured. This can be achieved by the right combination and placing of the air stone, adjusting the inflow of purified water and setting the partition separating the breeding part of the tank. For our feeding test, we placed the partition approximately 1 cm from the bottom of the tank, so that sediments could flow to the adjacent part and into the sedimentation cone.

Analysis of the main components – the cereals

Samples of the cereals used were analysed at the University of Chemistry and Technology Prague, using the capillary electrophoresis method, conductivity and with UV detection for the phosphate and phytate contents (Blatný *et al.*, 1995). Analyses were done in order to verify information on the higher proportion of digestible phosphorus in low-phytate cereal lines, or on the lower phytate content in the caryopsis.

Test 1 – wheat

For this monitoring, 60 pieces of common carp (*Cyprinus carpio* L.) of the mean weight of 138.19 g were selected. Fish were divided into six groups of ten pieces, and stocked into tanks. After stocking, fish were left to acclimate for seven days, and for further seven days to adapt to the new feed that was to be used subsequently in the testing. Before initiating the test, the fish were measured and weighed individually in order to establish the right feed ration for individual tanks.

In the test, fish were fed two wheat-based feed mixtures. Both of the lines used were provided by the Agrotest Fyto, Ltd. Kroměříž. The feed in the control group was based on the variety Vánek, one of the varieties commonly grown in the Czech Republic, registered in 2004 in Czech Republic. For the tested feed with expected higher percentage of phosphorus digestibility, we used a hybrid of the Vánek variety and a selected low-phytate line labelled JS-12/IDO 563. The feeds fed to the fish were based on both variants. The basis of the feed was made up of the used cereal which was shredded. Furthermore, 1% molasses and 1% sweet whey were added to the feed mixture to improve the intake by fish. Moreover, 1% Pellet Dur was added to the feed for its connective function.

The testing itself was conducted for 14 days. The feed ration was set as 2% of the stock weight, divided into two partial rations. Each group underwent three repetitions in the test. Before each feeding, basic properties of water – the temperature, pH, and oxygen – were measured using the multimeter device HachLange HQ 40d (Germany). Furthermore, contents of ammonia ions, nitrites, and chlorides were determined × 3 weekly. The mean temperature during the testing was 24.2 ± 0.3 °C. Water saturation with oxygen did not fall below 95%. Other measured properties ranged within the values complying with carp breeding. Samples of excreta were taken × 1 daily always before the morning feeding. They were sucked from the sedimentation cone using a pipette and filtrated through filtration paper. After excess water was sucked out, samples were deposited into appropriate sample containers, and then stored in a freezing box.

Test 2 – barley

For the second experiment, 60 pieces of common carp were used that had been used for the previous experiment with wheat. A period of 7 days was left in between the two experiments, for adjusting to the new feed containing barley. The fish were again divided into six groups and measured and weighed individually.

In this experiment, fish were given barley-based feed. Both monitored lines were provided by the Agrotest Fyto, Ltd. Kroměříž. Control feed was represented by the malting variety Bojos which is one of the most widely grown varieties in the Czech Republic.

The line tested was a lpa mutant line labelled M955. This line was obtained by chemical mutageneses method, without genetic localization of sing, by prof. V. Raboy from University Idaho, USA. The M955 ranks among the most favourited of the lpa line. The feed fed to the fish was prepared from both variants. The feeds were produced in the same composition as in the first testing.

This experiment was also conducted for 14 days. The feeding ration was set at 2 % of the weight of the aquarium stock; fish were fed twice daily and samples were always taken before the morning feeding and stored in sample containers in the freezing box. Also in this experiment, basic hydrochemical properties of water such as the temperature, pH value, oxygen concentration and water saturation with oxygen were determined regularly. The mean water temperature during the experiment was 24.1 ± 0.5 °C and water saturation with oxygen did not drop below 93 %.

Analyses of samples and feed mixtures

After completion of the testing, the excreta samples taken were pre-dried, homogenized, and analysed for the contents of phosphorus, fibre, and dry matter, for correction of results. Digestibility of phosphorus from the feeds was determined using the indicator method (MENDELU in Brno, 2017). As the indicator, we chose fibre naturally occurring in plant components of feeds, i.e. the cereals used. The indicator method calculation is presented in Fig. 1.

Test 3 – production indices

Recirculation system with eight tanks of the volume of approximately 160 litres was prepared for the experiment. Parts of the system are the Nexus biofilter and a storage reservoir for stabilizing the chemistry of purified water. The tanks are employed in two rows in the system. When using four feed variants, this set up allows us a double repetition in one testing.

A total of 160 pieces of common carp were selected for the experiment, of a mean weight of 97.95 g and of the same origin as for the previous experiments. Fish were stocked into the tanks by twenty pieces so that the individual tank stocks were even. Before commencing the testing, fish in tanks were acclimated for 14 days to the new conditions and to the newly administered feed. For comparison of the effect of feed both on phosphorus digestibility and on the selected production indices, the fish were fed the same feed as in previous experiments.

The testing itself was planned to last 56 days. Before its commencing, all stocked fish were measured and weighed individually using the measuring board. Before each feeding, basic properties of water chemistry were determined, such as the temperature, pH value, oxygen concentration and water saturation with oxygen, using the multimeter device HachLange HQ 40d (Germany). The feeding ration was set at 2 % of the weight of stock. After correction of the feeding ration, the fish were re-weighed regularly once in 14 days and the feeding ration was recalculated. The fish were fed twice daily, at 8:00 h in the morning and at 18:00 h in the afternoon. Each of the feeds used was fed in two repetitions. After the testing was completed, the fish were measured and weighed again individually. At the completion of testing, tissue samples were taken from each variant for subsequent analyses.

Analysis of samples and indices determined

Using the data from individual measuring and weighing of fish, basic length-weight parameters, as Fulton's condition factor (F_C), Highbackedness index (I_V), Widebackedness index (I_W), were determined at the beginning and at the end of tests (Gela and Linhart, 2000)

Contents of fat, nitrogen substances and dry matter contents were analysed as per the methodology presented in Kacerovský *et al.*, (1990).

Furthermore, values of the Feed conversion ratio (FCR) and values of the specific growth rate (SGR), and their mutual ratio (FCR/SGR), were determined (Mareš *et al.*, 2015a).

The obtained data were mutually compared in the program Microsoft EXCEL 2010 using the method of single factor analysis of variance (ANOVA).

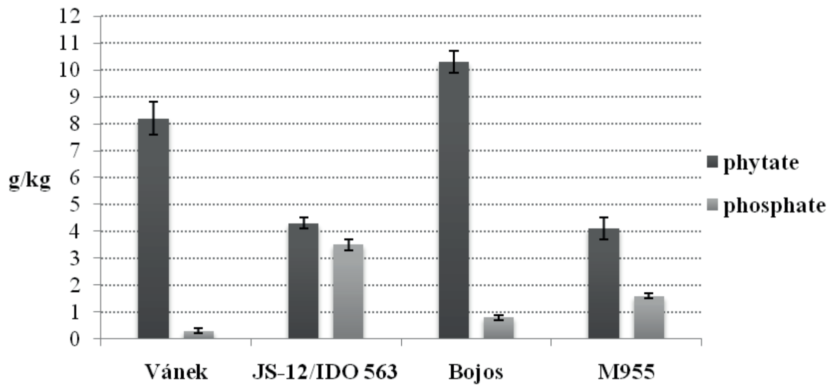
RESULTS AND DISCUSSION

Analysis of cereals

Before the initiation of the testing, samples of the cereals used were analysed for the contents of digestible phosphate and indigestible phytate. The results of analyses confirm the decrease of phytate in lpa mutant lines while maintaining approximately the same content of total phosphorus. Fig. 2 shows that both control varieties Vánek and Bojos have a very high phytate content

$$digestibility\,coefficient = 100 - \frac{I\,diet \times N\,excreta}{I\,excreta \times N\,diet} \times 100$$

1: *Digestibility coefficient by indicator method*
* I diet, I excreta – content of indicator in diet and excreta (%)
* N diet, N excreta – content of nutriment in diet and excreta (%)



2: *Phytate and phosphate contents in the cereals used*

I: *Content of crude fibre in experimental diets and excreta samples (%)*

	Wheat C*	Wheat lpa*	Wheat C** ±SD	Wheat lpa** ±SD
CF (%)	3.00	3.06	21.04 ±1.04	19.22 ±0.23

* fish feed, ** excreta, CF – crude fibre, C – control variety, lpa – low phytic acid lines

II: *Total phosphorus content in experimental diets and excreta samples (%)*

	Wheat C*	Wheat lpa*	Wheat C** ±SD	Wheat lpa** ±SD
P (%)	0.2622	0.3557	0.9951 ±0.079	0.9573 ±0.027

* fish feed, ** excreta, P – phosphorus, C – control variety, lpa – low phytic acid lines

compared to an almost negligible content of phosphate. In both varieties, it makes more than 92%. In terms of loading the environment with phosphorus from the excrements, this fact is very negative as the digestibility of phytate from plant components of the feed is only 8–38% in carp (Jirásek *et al.*, 2005). In contrast, new lpa mutatn lines have a considerably more even proportion of the phytate and phosphate contents. Especially the lpa wheat line has a very favourable proportion of these two forms of phosphorus. The results of the analyses are in accordance with the results of the study by Vaculová *et al.*, (2012) which reports that lpa mutatn lines have a decreased phytic acid content while maintaining the same content of total phosphorus. For the M955 line, Raboy (2000) reports a decrease of the phytate content by up to 95% agains the primary barley variety called Harrington. In this study the phytate content was decreased by approximately 62% compared to the control variety.

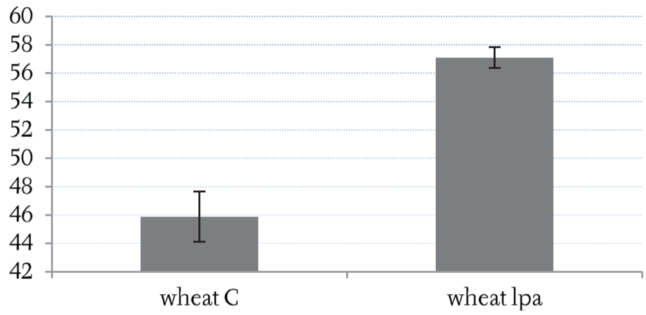
Test 1 – wheat

Samples of excreta and feeds were pre-dried, homogenized, ground and then analysed for the contents of phosphorus and fibre. Based on the data found, phosphorus digestibility was calculated using the indicator method. The results of analyses of fibre and phosphorus content are presented in Tabs. I and II.

The fibre content in the wheat variants used in the testing was almost even. The mean fibre content was found to be 3.03%. From the excreta analyses, differences between individual grain variants are more apparent. A higher mean fibre content was found for the control group, however, also with a higher variability of analysis results in individual repetitions. The results of wheat analysis are higher compared to the results of the study by Lee *et al.*, (2016) who report a mean of 2.49% for wheat. Zeman *et al.*, (1995) report a fibre content in wheat in the range 2.27–3.27% depending on the line.

For wheat feeds, a mean of 0.309% of phosphorus was found. It is apparent from the table that the tested lpa mutatn line had a higher total phosphorus content compared to the control variety which does not correlate with the results of other studies (Vaculová *et al.*, 2012). Regarding the total phosphorus content, the results of our study are in agreement with Lee *et al.*, (2016) who report a mean of 0.30% phosphorus for wheat, and also with Jirásek *et al.*, (2005) who also report a mean of 0.3% for wheat. Zeman *et al.*, (1995) report a range of 0.3 to 0.44% of total phosphorus for wheat.

After the calculation, the following values were found for phosphorus digestibility (Fig. 3).



3: *Digestibility of phosphorus from experimental diets (%)*

III: *The content of crude fibre in experimental diets and excreta samples (%)*

	Barley C*	**Barley M955***	**Barley C** ±SD**	**Barley M955** ±SD**
CF (%)	4.29	4.89	23.83 ±1.00	25.33 ±1.17

* fish feed, ** excreta, CF – crude fibre, C – control variety, M955 – low phytic acid lines

IV: *The content of total phosphorus in experimental diets and excreta samples (%)*

	Barley C*	**Barley M955***	**Barley C** ±SD**	**Barley M955** ±SD**
P (%)	0.2875	0.3455	0.8397 ±0.045	0.8351 ±0.035

* fish feed, ** excrements, P – phosphorus, C – control variety, M955 – low phytic acid variety

For the control feed, phosphorus digestibility of 45.89 ± 1.78% was found. For the tested lpa wheat mutant line, phosphorus digestibility of 57.10 ± 0.93% was found. Thus, a highly statistically significant ($P < 0.01$) increase of phosphorus digestibility (by 11.21%) was found when using low-phytate wheat line compared to the commonly used varieties.

Test 2 – barley

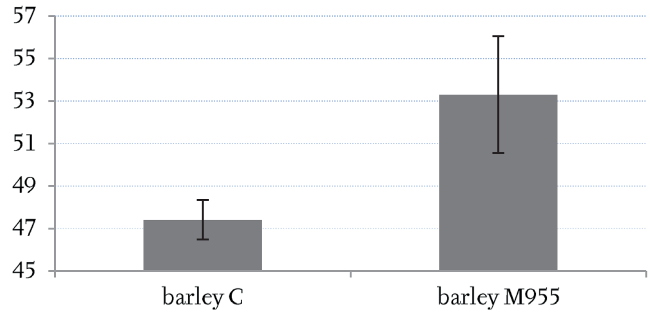
Tabs. III and IV present the results of analyses of feeds and collected excreta samples for the contents of phosphorus and fibre when using different barley varieties.

The analyses results show an apparent difference (0.6%) in the fibre content between individual variants. Zeman *et al.*, (1995) report the fibre content within the range of 5.01–6.94% for barley; in the case of naked barley they report only 2.49% of fibre. Lee *et al.*, (2012) report a mean of 4.96% of fibre for barley. In both cases of the barley we used, lower fibre content was found compared to other studies. However, fibre content differs based on the lines grown, growing conditions, and grain quality and maturity.

In barley-based feeds, a mean of 0.317% of phosphorus was found. Zeman *et al.*, (1995) report the range of 0.35 to 0.45% of phosphorus for barley. In contrast, Lee *et al.*, (2012) report a lower phosphorus content of 0.25–0.28%. The low-phytate line M955 contains a higher amount of total phosphorus compared to the variety Bojos, however, Vaculová *et al.*, (2012) notes that lpa lines have maintained the total phosphorus content.

Fig. 4 presents the results of the determined digestibility of phosphorus from barley-based feeds. For the control group, phosphorus digestibility of 47.41 ± 0.93% was reached. For the tested line M955, an increase of phosphorus digestibility by 5.89% was found compared to the control group. Thus, phosphorus digestibility reached 53.3%. This difference was evaluated as statistically significant ($P < 0.05$).

Malý (2015) reports a 63.89% digestibility of total phosphorus from the complete feed mixture KP1. Higher phosphorus digestibility is mainly due to its source. Phosphorus is added to complete feed mixtures in the form of monocalcium phosphate, digestibility of which is up to 94% (Jirásek *et al.*, 2005). In the feed in this study, a mean of 0.7% of total phosphorus was found. However, the feed producer does not mention what proportion of total phosphorus is made by the supplemental form. In the same study, Malý (2015) further reports verification of the supplementation of phytase enzymes, however, without a significant increase of phosphorus digestibility. Other authors (Phromkunthong *et al.*, 2010, Nwanna and Schwarz, 2007) report a positive effect of adding the enzyme on the digestibility of phosphorus from the feed. Nwanna and Schwarz (2008) further mention that in the common carp, phosphorus digestibility



4: *Digestibility of phosphorus from experimental diets (%)*

V: *Determined condition and exterior indices*

	F_C n = 10 ±SD		C_C n = 10 ±SD		I_H n = 10 ±SD		I_W n = 10 ±SD	
	in	**out**	**in**	**out**	**in**	**out**	**in**	**out**
Wheat C	3.17 ± 0.39	3.64 ± 0.45	2.85 ± 0.25	2.88 ± 0.25	2.94 ± 0.20	2.95 ± 0.16	19.08 ± 1.30	19.53 ± 1.47
Wheat lpa	3.19 ± 0.37	3.90 ± 0.42	2.85 ± 0.25	2.83 ± 0.26	2.88 ± 0.15	2.76 ± 0.24	19.06 ± 1.08	19.97 ± 1.33
Barley C	3.15 ± 0.40	3.97 ± 0.38	2.85 ± 0.25	2.77 ± 0.20	2.88 ± 0.13	2.80 ± 0.20	22.85 ± 7.74	20.14 ± 2.60
Barley M955	3.13 ± 0.24	3.52 ± 0.47	2.85 ± 0.25	2.71 ± 0.24	2.94 ± 0.13	2.93 ± 0.13	18.51 ± 0.79	20.18 ± 2.53

FC – Fulton's condition factor, CC – Clark's condition factor, IH – Highbackedness index, IW – Widebackedness index, C – control variete, lpa, M955 – low phytic acid lines

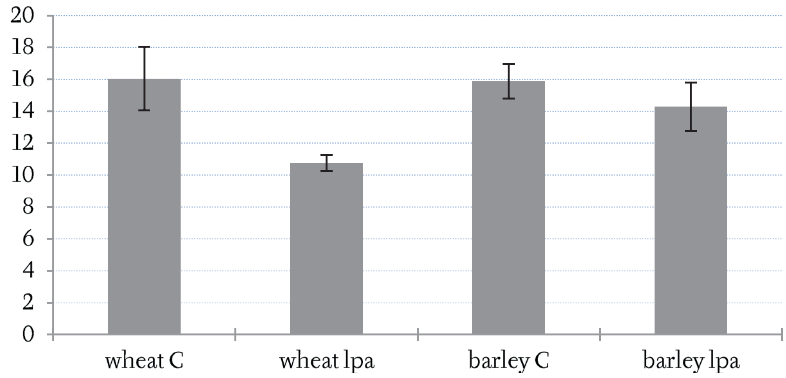
VI: *Determined production indices*

	FCR	**SGR**	**FCR / SGR**	**HSI n=10**	**VSI n=10**
Wheat C	6.3	0.256	24.61	2.29	10.72
Wheat lpa	5.29	0.303	17.46	2.06	11.04
Barley C	5.6	0.337	16.62	2.09	10.40
Barley M955	9.09	0.169	64.01	2.02	11.49

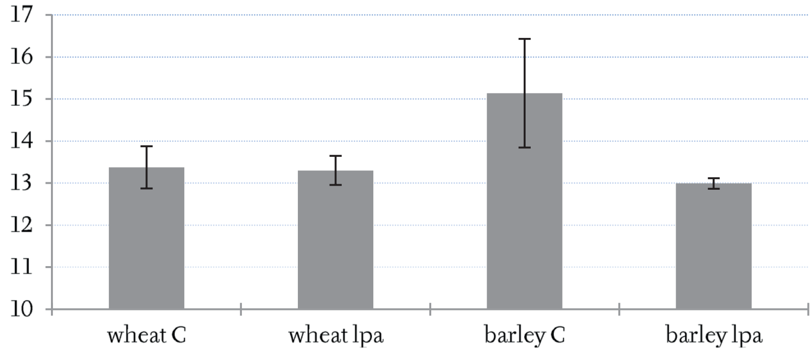
C – control vriety, lpa, M955 – low phytyic lines

was increased after adding phytase to the feed, however, in a diet with the addition of monocalcium phosphate, the digestibility was even much higher. This fact demonstrates the excellent digestibility of the supplemental form of phosphorus but it also points out that phytate phosphorus contained in the feed does not get digested due to the sufficiency of available phosphorus. An important factor for the evaluation of this study will be the economic effect of the addition of the enzyme or monocalcium phosphate. Depending on the fish species, phosphorus content in the feed is not directly proportional to the amount of phosphorus loading of the environment. This is mainly due to the incapability of cyprinid fish to digest phytate phosphorus. In the carp, the loading of the environment by phosphorus during production of one tonne of fish is reported to be approximately 8.9–26.4 kg phosphorus (Watanabe *et al.*, 1999); in contrast, for salmonid fish it is less than 3 kg (Cho and Bureau, 1997). At present, even lesser load may be presumed, as the issue of phytase supplementation in salmonid breeding has been well managed by now (Cheng and Hardy, 2002; Hua *et al.*, 2008).

Other studies mention that high phosphorus content in feed negatively influences its digestibility (Rodehutscord *et al.*, 2000; Sugiura *et al.*, 2000); in contrast, older studies (Nakamura, 1982; Satoh *et al.*, 1996) disprove this fact. Generally it holds true that total phosphorus digestibility is given especially by the source of the phosphorus in feed. Various phosphorus components are digested with different dynamics. Another important factor for phosphorus digestibility which is connected with its source is also the fish species, i.e. differences in the digestive tract construction (Hua and Bureau, 2006).



5: *Fat content in fish body samples (%)*



6: *Content of NL in fish body samples (%)*

Test 3 – production indices

During the experiment, basic water chemistry properties were measured twice daily. The water temperature reached 25 °C on average (within the range of 23.1–27.3). The mean oxygen concentration in water was 7.4 mg/l and the mean water saturation with oxygen was 92.6% (72.3–101.3). The pH value during the experiment was 7.71. The indices monitored during the experiment ranged within the values complying with carp breeding (Svobodová *et al.*, 2007). During the experiment, no fluctuations of the above mentioned indices occurred that might negatively influence the results of the experiment. The fluctuations of oxygen concentration and water saturation with oxygen can be due to the loading of the biofilter.

Part of the experiment was evaluation of the effect of the feed used on selected condition and exterior indices. Among the selected were Fulton's condition factor (F_C), Clark's condition factor (C_C), Highbackedness index (I_H), and Widebackedness index (I_W). The results of these measurements are presented in Tab. V.

No statistically significant difference was found between individual groups or the input and the output data. In the case of F_C, Mareš *et al.*, (2015b) obtained similar results (3.05–3.23) in a study monitoring the effect of adding a coloured wheat line.

In the following phase, results of the monitored production indicators were evaluated. Based on the obtained data, the Feed Conversion Ratio (FCR) and the Specific Growth Rate (SGR) were calculated.

The results of the FCR and SCR values and their mutual ratio are very different for individual groups. However, such marked differences especially in barley testing are probably not due to the effect of the feed used. The most probable cause was the method of feed administration, when the amount of unaccepted feed was probably not evaluated; in other words, the daily feed ration did not reflect the intensity of feed intake by fish, and the feed was not accepted by the fish. Consequently, the feed conversion ratio grew substantially.

As regards HSI (hepatosomatic index) and VSI (viscerosomatic index), these values present the content of visceral fat, or fat and glycogen deposited in the hepatopancreas, for all cereal groups. In both groups of lpa grains, the VSI value increased by 2% and 10%, respectively, compared to the control groups. In contrast, the HIS value was decreased by 10% and 4%, respectively, compared to the control groups.

Fig. 5 presents the results of the statistical evaluation of analyses of fish body samples for fat content. Statistically significant decrease of fat content was found in the mixed sample of fish body ($P < 0.05$) when using the low-phytate wheat line. When comparing both control groups, no significant difference was found, and no statistically significant difference was found between the control and the low-phytate barley line. A significant difference ($P < 0.05$) in fat content decrease in a fish body sample was found also when using the low-phytate wheat line compared to the low-phytate barley line. In their study, Mareš *et al.*, (2015) report a fat content in the fish body in the range of 11.96 to 14.73%. Likewise, Hůda (2009) who tested various cereal species in pond and model conditions, reports a fat content of 13.27% in carp muscle which is substantially higher than in this study. This fact, however, is due to the type of feed used.

Results of analyses for nitrogen substances showed no significant changes as a result of using different feed types. The analysis results are depicted in Fig. 6.

CONCLUSION

The aim of this study was to find whether feeding special low-phytate cereal lines has an effect on decreasing the phosphorus load on the environment, and on selected production indices in carp breeding. In three partial experiments, fish were fed feed mixtures based on wheat and barley. As control food, commonly grown varieties Vánek for wheat and Bojos for barley were chosen. Alongside, the low-phytate line of wheat JS-12/IDO 563 and the low-phytate line of barley M955 were chosen. After conducting the experiments, we determined the digestibility of phosphorus from these different feeds. For the wheat control group, digestibility of 45.89 ± 1.78% was found. For the experimental variant, digestibility of 57.10 ± 0.73% was found, i.e. almost by 11.21% higher. The increase of digestibility in the experimental diet was evaluated as statistically highly significant ($P < 0.01$).

For barley, digestibility of 53.30 ± 2.75% was found in the experimental diet which is a statistically significant ($P < 0.05$) increase of digestibility compared to the control group, where digestibility reached 47.41 ± 0.93%. In both of the feeds used, barley and wheat, a significant increase of phosphorus digestibility occurred when low-phytate lines were used which may positively affect the decrease of the load on the water environment by phosphorus from fish breeding.

When evaluating the selected production indices, no statistically significant influence on these indicators was found from the use of the low-phytate lines. In another phase, we determined the effect of the feed used on the fat content in various parts of the fish body. A statistically significant difference ($P < 0.05$) was found when the low-phytate wheat line was used compared to control wheat, and also compared to the low-phytate barley line. In contrast, no significant difference was found when studying the effect of the low-phytate line of barley compared to control barley. No significant difference was found when comparing both control groups mutually, either. No significant differences we found when comparing the feeds in terms of nitrogen substances content, either.

In conclusion, it can be said that the use of low-phytate cereal lines, and especially wheat line, has a positive effect both on the increase of phosphorus digestibility, and on the decrease of the fat content in the fish body. The use of low-phytate barley line also increases phosphorus digestibility, however, as regards the fat content, no difference was noted compared to the control group.

By selecting the right feed, we may therefore both improve the quality of the environment by decreasing the load on water by phosphorus from carp breeding, and affect the quality of fish meat. Price of these special lines of cereals could be decisive factor, but monitoring of economical side of carp production will be subject of further studies.

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