HUNGARIAN UNIVERSITY OF
AGRICULTURE AND LIFE SCIENCES

ASSESSING THE APPLICABILITY OF DDGS IN THE HUNGARIAN AQUACULTURE

Thesis

DOI: 10.54598/001660

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Gödöllő

2021

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1. BACKGROUND AND AIMS OF THE STUDY

Scientific research and innovative solutions are needed to provide a sufficient amount of animal protein for a growing population and to reduce the costs of fish farming at the same time. Their task is to help and guide the transformation of industry worldwide so that animal protein can be produced in large quantities, in sufficient quality and in a sustainable manner. This would also benefit for the feed manufacturers, breeders, processors, retailers, consumers and future generations.

Currently, 76% of the world's fisheries are depleted or overfished, so aquaculture will play a critical role in the present and in the future. However, the production of compound feed for aquaculture production depends to a large extent on finite marine resources, especially in terms of affordable, sustainable sources of protein and omega-3 - EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) fatty acids in fishmeal and fish oil.

Today, 50% of the world's cereal production and 70% of soy are used as animal feed. These are grown on finite lands, and as the demand for animal proteins increases, it is increasingly urgent to convert terrestrial crop production to animal feed, which is one of the main causes of biodiversity loss. In practice, larger quantities of feed must be produced from smaller land in such a way that the change in the way land is used is reduced. This can be achieved by increasing the digestibility and utilisation of feed materials and by-products.

As animal and fish farming contributes 14.5% of the world's greenhouse gas emissions, it is extremely important to do so as soon as possible, especially considering that by 2026 the world's meat and fish demand will be 40 million tonnes higher and fish demand by 25 million tonnes. It is also very important to address the problem of nitrogen and phosphorus emissions from livestock production, which are crucial for the eutrophication of land and waters and the loss of biodiversity.

The morbidity and mortality of livestock represents a loss of USD 300 billion per year, and the associated environmental damage is also significant. Food waste can be reduced at all stages of the food supply chain by food measures that lead to an increase in the incorporation of animal proteins.

I am convinced that the development of a sustainable food system and the internal transformation of the livestock farming system can be part of solving this problem. I wish to contribute to this transformation by developing feasible and tangible solutions for an environmentally conscious development.

Due to its high energy, medium protein and highly digestible phosphorus content, corn DDGS (Dried Distiller's Grain with Solubles) is an extremely promising alternative feed material suitable for fully or partially replacing the much more expensive, traditional energy sources (maize), protein sources (fish meal, soy) and phosphorus sources (MCP - monocalcium phosphate, DCP - dicalcium phosphate). Mixing DDGS in well-designed feed in the right proportions resulted in excellent health, good growth performance and product quality for previously tested species. The first most important step in the use of DDGS in feedingstuffs is to determine digestibility, which varies from species to species, depending on dietary habits.

1.1 Primary goals

- Determination of the apparent digestibility coefficient of DDGS by means of an indicator method for carp (*Cyprinus carpio L.*) of paramount importance in hungarian aquaculture and European catfish (*Silurus glanis L.*)
- Development and testing of complex feed with optimal DDGS content depending on digestibility in complex feeding experiments. Study of the effects on growth, feed utilization, nutrient uptake, metabolism and health in particular.
- Examination of the performance, meat quality, certain blood parameters and cost-effectiveness of carp stock reared in semi-operational small ponds with semi-intensive technology while feeding feed with a high DDGS content.

2. MATERIALS AND METHODS

My experiments (Table 1.) were conducted in the recirculation aquaculture system (RAS) and experimental fish ponds of the NAIK Research Institute of Fisheries and Aquaculture (HAKI). The tested fish species were Hungary's most important fish specie, the common carp (*Cyprinus carpio*) and one of the most promising, the European catfish (*Silurus glanis*).

In the beginning, the new I have assessed the new raw material's apparent digestibility coefficients (ADC) with indicator method. Yttrium-oxide was added to the diets, what were planned and produced by NOFIMA (Norwegian Institute of Food, Fisheries and Aquaculture Research) and University of Novi Sad, Feed Technology Centre. After the feeding period of experiments the faeces were collected by stripping method after the fish were gently anesthesied. The faeces samples were analysed for trace minerals, proximate composition and selected nutrients. Based on these results I was able to calculate the ADCs.

In the second part of my studies, long term (12 and 8 weeks) nutritional experiments were conducted in RAS with common carp and european catfish as well. During these trials elevated DDGS contents were tested in artrificial diets. In term of common carp the feed recipes were based on terrestrial plants and industrial by-products, what were produced in an industrial feed mill factory (Nagyhegyesi Takarmány Zrt.). The experimental feeds for european catfish were produced in a pilot scale feed mill at University of Novi Sad. The idea was to replace soybean meal and wheat by DDGS. In the end of the experiments growth performance, feed efficiency, whole body proximate composition, blood plasma biochemistry, fatty acid analysis and histology of liver were assessed.

Finally, I have observed the possibilities of feeding a mixed age population of common carp in semi-industrial conditions. Six earthen ponds with an average surface of 1808 (± 53) m^2 and a depth of 1.5 m were used for the experiment which lasted for a production season. The feeding technology during the experiment was set in line with the principles of carp nutrition under semi-intensive technology as described by Ruttkay (2016). In the first part of the season, nutrient supply was ensured by high natural yield (zooplankton and zoobenthos) (Körmendi & Hancz, 2000), what was supplemented with wheat grain as recommended by Horváth et al. (2002). In the second part of the rearing, formulated feeds were tested. As control, commercially available feed was chosen, what was produced by Haltáp Kft (tilápia-ponty nevelő, d= 4.5 mm). The idea of the experimental diet was to replace the control diet's soybean meal and other vegetal components to DDGS.

Picture 1. - DDGS - Dried Distiller's Grain with Solubles

Picture 2. - Location of the experiments: NAIK Research Institute of Fisheries and Aquaculture (HAKI), Szarvas, Hungary

Table 1. Summary table of experiments

Type of experiment	Studied fish specie	Initial weigh of fish	Period of time	Aim of the study	Treatments	Observed parameters
Digestibility trial	common carp	40 ± 7 g	4 weeks	· to evaluate the nutrient digestibility of corn DDGS to develop sustainable low cost feed · investigation of the interaction between water temperature and diet digestibility	4	Apparent digestibility coefficients of the diets and DDGS; growth performance; feed utilization; whole body proximate composition.
Digestibility trial	european catfish	154,3 ± 2,7 g	2 weeks	to determine the apparent digestibility coefficients of the diets and the DDGS	2	Apparent digestibility coefficients of the diets and DDGS
Nutritional trial in RAS	common carp	63,1 ± 11,4 g	12 weeks	test of terrestrial plant and industrial by-product based diets with elevating DDGS levels (0 - 20 - 40%)	3 DDGS 0 DDGS 20 DDGS40	Growth performance; feed utilization; whole body proximate composition; blood plasma biochemistry; fatty acid analysis and histology of liver
Nutritional trial in RAS	european catfish	272,7 ± 37,8 g	8 weeks	to evaluate the effect of partial replacement of soybean and wheat by DDGS (0 - 10 - 20 - 30%) in the diets.	4 DDGS 0 DDGS 10 DDGS 20 DDGS 30	Growth performance; feed utilization; whole body proximate composition; blood plasma biochemistry; fatty acid analysis of liver; histology of liver and intestine
Pilot scale nutritional trial in earthen ponds	common carp	1 years old (1050 pc): 45 ± 1 g 2 years old (70 pc): 362 ± 10 g	from 2nd May, 2018. - to 3rd October (154 days)	comparison of a 40% DDGS based diet to a commercially avaiable feed within a mixed age population, semi-intensive earthen pond system	2 Control DDGS 40	Growth performance; feed utilization; meat quality; blood plasma biochemistry; economical evaluation

3. RESULTS

3.1 Digestibility study with common carp

Among treatments, the DDGS group of 20 ºC has the best growth rates. A similar result can be seen for the Feed Conversion Ratio (FCR) and the specific growth rate (SGR), however, there is a significant difference between SGR and FCR only in temperatures (p-value = 0.014) and no between feeds. However, there is also a significant difference in protein efficieny ratio (PER) between the diets at 20°C (p-value = 0.019). During the experiment, mortality occurred in 1-1 cases, so there was no difference between the survival rates (SR).

Based on the proximate measurements of faeces, I calculated the digestibility coefficients of the diets for dry matter, crude protein and phosphorus at two different temperatures. The dry matter digestibility coefficient varied between 67%and 76% and differed significantly between diets and water temperatures, except for control. At lower temperatures, ADC values are higher for both feedingstuffs than at 30°C. The same trend can be seen for protein and phosphorus.

For the crude protein digestibility of DDGS as a feed ingredient, I set a value of around 86 % at both temperatures. I calculated 45-50 % for the apparent digestibility coefficient of dry matter. For the digestibility of DDGS phosphorus, I set a value of between 81 % and 83 %.

3.2 Digestibility study with European catfish

Using the analytical test results, I calculated the apparent digestibility coefficients (ADC) of feedingstuffs and the DDGS test substance for dry matter, crude protein, crude fat, phosphorus and essential amino acids. The digestibility coefficient of each of the nutrients tested was significantly higher for control feed with a high fishmeal content, as opposed to DDGS, excluding phosphorus. With regard to amino acids, I received a significant difference between control and experimental feed for cystine, lysine, histidine and arginine. DDGS' apparent digestibility coefficient for crude protein and crude fat was relatively high at 73,4 % and 77,4 %, all with a high phosphorus digestibility of 88 %. As regards amino acids, lysine, cystine, arginine and histidine were lower.

3.3 Nutritional study with common carp in RAS

After the 12-week trial, statistical differences in terms of growth and feed conversion parameters were observed. C. carpio demonstrated significant advantages for groups fed with DDGS-containing experimental feeds compared to the control in weight gain, daily growth

index, feed conversion rate and specific growth rate. Significant differences were not detected between DDGS 20 and DDGS 40, but slightly higher values were measured in DDGS 40 for most of the parameters. Protein efficiency ratios were significantly different between all groups, and the highest nitrogen efficiency use was found in the DDGS 40 group. Previous advantages, like better nitrogen utilization of DDGS groups were strengthened by protein production values. Fish mortality was below 4% in each group. The examined fish did not showed differences in biometric indices, irrespective of the dietary composition. The results of whole-body composition were showed that, crude protein content was significantly higher ($p<0.05$) in the DDGS fed groups compared to the zero level. Parallel with this, crude fat content increased, while crude ash level decreased with higher DDGS inclusion levels.

The blood plasma parameters studied did not show a statistically verifiable difference due to the high standard deviation, but the total choleserine (TC) and triglyceride (TG) levels appear to be a trend-like correlation with a decrease in body fat. These parameters are closely related to fatty acid metabolism and the quality of feed, as they are cell membrane creators and precursors of steroid hormones, as well as indicate the vitality and energy supply of the organism. The activity of the alkaline phosphatase (AP), gamma-glutamyltransferase (GGT) alanine aminotransferase (ALT), aspartate aminotransferase (AST) enzymes may be associated with liver damage. The GGT of the plasma was below 2,5 U/L, which is a normal value for a healthy carp (Velisek et al., 2009) or tilapia (Chen et al., 2003). From the low ALT and the same ALP levels, I concluded that DDGS did not cause liver damage to carp. Besides this, histological analysis in the liver of C. carpio showed that the group fed with 40% DDGS had hepatocellular necrosis, tight sinusoids and hypertrophia. The experimental diets did not affect gut health, and only generic differences (thickness of epithelium, size and number of goblet cells) were found in histological sections.

Regarding the fatty acid composition of the liver, the level of linoleic acid (18:2 n-6) in the tissues reflects the dietary trends. The synthesis of a higher homologue such as arachidonic acid was detectable. The EPA and DHA levels were extremely low in the liver. Statistically significant differences were found for oleic acid (18:1n-9), and the highest deposition was observed in the DDGS 00 group (Table 7), where more than 40% of the lipid were composed from this fatty acid. A similar tendency could be observed for the total MUFA level, which decreased with an increasing inclusion level of DDGS in the diet, while the total PUFA level increased.

3.4 Nutritional study with european catfish in RAS

After the 8 weeks of feeding trial, statistical differences in terms of growth performance and nutrient protein utilization were not observed for European catfish juveniles. FCR values varied between 1.29–1.36 g/g, SGR 1.43–1.50 g/day, PER and PPV 1.78–1.94, and 27.7–30.2 % respectively. No mortality was observed during the trial. The examined fish did not showed differences in biometric indices, irrespective of the dietary composition.

The plasma biochemical parameters such as Glucose, Phosphatase, Ca, Total protein, Globulin, Alanine aminotransferase, Alkaline phosphatase, Cholesterol, Triglyceride and Amylase were not differing significantly.

Proximate composition was determined in the whole body and filet on dry weight basis. In the filet, crude protein level was found between 79.0–80.5 %, the crude fat between 11.5–12.1 % and crude ash had approximately 5.4 %. Significant differences were not detected between treatments. Crude fat content of the whole fish body differs significantly at $p = 0.070$, the highest level in the DDGS 30 treatment was observed. The crude protein content was similar in all of the treatments.

Structure, shape and consistency of hepatocytes, shape and localization of cell nucleus of liver were studied. The liver histopathological observations showed that 20 % and 30 % DDGS fed groups had less vacuolized hepatocytes than the other groups. There were no dif- ferences were observed between fishes of different experimental groups on gut morphology in respect of length of epithelial cells and number and size of goblet cells.

Fatty acid composition of the liver samples presented differences in some of the fatty acids. 16:0 palmitic acid differs significantly in DDGS 20 and 30 to control represent in almost 21 % of the fatty acids. Mono-unsaturated fatty acids, as 16:1n-7 and 18:1n-9 decreased significantly with increase of DDGS level in the diet. Consequently, total saturated and total unsaturated fatty acids follow similar trend with individual fatty acids, namely total SFA increased with DDGS inclusion, while total MUFA decreased. Enrichment with total PUFA of the liver is also observed, but this difference is not significantly demonstrated at $p < 0.05$. Total lipid content in liver tissue varied between 8.46 and 17.31 mg FA/g, less amount in DDGS 30 treatment was determined.

ADC for dry matter and crude protein were not significantly ($p < 0.05$) differing with DDGS inclusion, but for crude fat was highly digestible in all of the diets (ranging between 96–98 %) except DDGS 20 treatment where an outliner values were found (88 %). Phosphorus digestibility was determined for these diets and significant differences found between the treatments. The lowest digestible diet was the control DDGS 0 diet with ADC for P around 29

%, compared to DDGS 10 with 54 %, DDGS 20 with 44 % and DDGS 30 with 47 % phosphorus ADC values.

3.5 Pilot scale nutritional trial in earthen ponds

In Hungary, the traditional carp rearing season starts in April and closes in October. During the season, depending on the water temperature, the biomass of plankton change frequently. Meanwhile feeding compound feeds, plankton biomass varied from 0.8 to 3 ml/100 liters. All along the 155 days of the experiment, the one-year-old group increased their body weight nearly tenfold in both groups. The SGR and weight gain of juveniles were significantly higher in the experimental group. Although statistical significances in these indicators were not detected in the adult group, the final body weight differed significantly here, as well. The mortality rates over the season for the control group was 12.86 %, for the DDGS group was 3.33 % in term of the older age class, and for the juvenile's stock for control group was 12.86 % and for the DDGS group was 13.33 %. Feed as well as protein utilization efficiency also differed significantly in favour of experimental feed (FCR: 1.56 vs 1.78; PER: 2.32 vs 2.08). The protein production value (PPV) reached 36.2 % in the experimental group compared to 31.6 % in the control group. Considering both age classes, per hectare gross was significantly higher under experimental diet than in the control group (3520 vs 3020 $kg.ha^{-1}$).

Composition, slaughtering indices and some physical quality parameters of the market size fish flesh were determined at the end of season from adult fish population. The crude fat content of the meat was measured in a wide range, between 3.14 % and 10.96 % (mean: 6.16 %) in the experimental group, and between 2.51 % and 8.92 % (mean: 6.43 %) in the control group. A similar result was obtained for conventional meat quality parameters as dripping loss, cooking loss, thawing loss, pH and colours. No statistically significant difference could be detected between the control and the experimental group for these parameters. We did not find significant differences (except for the hepatosomatic index) in the dressing indices at the end-of-season be- tween the groups, although the higher filleting yield, hepatosomatic index and viscera index indicate a better weight gain of the experimental group.

For the fatty acid profile, minor difference was found in the total polyene fatty acids (TOTAL PUFA), TOTAL omega-6 content, but substantial individual differences suppressed the feed effect and statistically significant difference was not observed in most parameters. Level of the essential long chain polyunsaturated fatty acids, EPA, DHA, and ARA, was relatively low, the amount varied between 0.19 and 0.65 mg/g. Significant differences were

recorded for linoleic acid, 18:2n-6, in favour to the experimental group due to the inclusion of DDGS. Oleic acid (18:1n-9) level is almost higher in the control group (18.9 mg/g) compared to DDGS containing group (17.4 mg/g).

All the plasma biochemical indices analysed were not influenced significantly by the diet, except the phosphate, when higher values were measured for experimental group in both age classes. Significant differences between the age classes were found only in the amylase activity, but interaction within both factors were not observed.

Economic simulations indicate that calculated profit, which is defined as 'income above feed, seed and labour costs', is higher for experimental groups than for control groups (9264 vs. 7938 €. ha^{-1}). This is mainly attributed to increased revenue resulting from higher carp yields under DDGS-based diets. Feed costs between diets did not differ significantly, because savings associated with reduced feed cost (0.56 vs. 0.60 €.kg $feed^{-1}$ for experimental and control feed, respectively) was eliminated by increased use of feed due to better growth and higher standing biomass in experimental groups in the second part of the season. Calculated benefit-cost ratio is also significantly higher for the experimental group than for the control group. Sensitivity analysis shows that even a 200 % increase in DDGS prices, raising the price of the experimental feed from 0.56 to 0.74 €.kg $feed^{-1}$, would still result in a significantly better economic performance of the experimental group.

4. NEW SCIENTIFIC RESULTS

1. In the case of carp, the dry matter and protein digestibility of compound feedingstuffs containing DDGS has decreased with an increase in water temperature (20–>30 ˚C), which confirms the direct correlation between temperature and digestibility.
2. I have defined and quantified the apparent digestibility coefficients of DDGS for carp. This confirmed that DDGS' crude protein has good digestibility and can therefore be used effectively in carp feed as an alternative source of protein. I also found that maize DDGS is a well-digestible source of phosphorus for carp.
3. I have defined and quantified the apparent digestibility coefficients of DDGS for European catfish, which has shown that predatory catfish have a lower digestibility than carp.
4. I found that carp statistically justified effectively utilize compound feed with a content of 40% DDGS. Liver fat content decreased significantly after a three-month feeding period with a high DDGS content (40%) feed. By biochemical testing of blood plasma, I successfully monitored the metabolic status and health status of the fish. These parameters were well in line with histological results.
5. I have demonstrated by experimentation that catfish have made positive use of feed containing 30 % DDGS without slowing down growth. This ratio is higher than the recommended DDGS mixing rate of 10-20% for other species. Furthermore, the amount of available phosphorus for this fish species has also increased depending on the DDGS content of the feed, which is beneficial for phosphorus uptake and the excretion of excess phosphorus.
6. In a semi-operational feeding experiment, I have demonstrated that feed containing DDGS performs better than commercial feed. Feed containing DDGS did not have a negative impact on fillet yield and meat quality. By economic calculations, I have observed better financial performance when the feeding was performed with DDGS based compound feed.

5. CONCLUSIONS AND RECOMMENDATIONS

Based on the apparent digestibility coefficients of dry matter and protein, I found that carp can digest DDGS to a similarly favourable extent to maize. The digestibility of phosphorus is extremely higher than that of other plant ingredients, thanks to its low phytic acid content. Thus, maize DDGS is a well-digestible source of phosphorus for fish, making inorganic phosphorus (such as dicalcium phosphate, monocalcium phosphate) used as a feed supplement less needed. Hence, not only the cost of feed, but also the amount of phosphorus selected by the fish can be reduced. In the case of carp, using a water temperature of 20 °C, I observed better nutrient digestibility and higher growth performance than at a water temperature of 30 °C. Ultimately, it can be concluded that corn DDGS can be promising to feed carp.

In the case of European catfish, I found that the digestibility of DDGS is less than that of crude protein, of crude fat, and higher than that of carp for phosphorus. For some essential amino acids, I determined a higher ADC than the crude protein (e.g. leucine, isoleucine, methionine, proline, treonin, valine), while others lower (e.g. lysine, arginine, histidine). This allows for a more precise addition of DDGS-containing feedingstuffs with synthetic amino acids.

High inclusion levels (up to 40%) represents an appropriate dietary protein and fat source for C. carpio. The study demonstrated that the decrement of liver fat deposition is remarkable after a three-months feeding period using diet with high percentage (40%) of DDGS. Blood plasma biochemical tests could successfully be used to monitor the metabolic balance and health status of fish. In our study, these parameters correlated with the histological results.

The apparent digestibility coefficients (ADC) of different experimental diets for dry matter and crude protein were similar, this fact pointed out that 30 % inclusion of DDGS in the diet containing 20 % fishmeal is preferable and utilizable by European catfish. Moreover, the available phosphorus increases with inclusion of DDGS in the diet, which is a benefit for phosphorus intake and waste discharge. In conclusion, the results revealed that ADCs of crude fat and phosphorus of corn DDGS were high and reflected in the DDGS as a suitable ingredient for the use in European catfish diet up to 30 %.

Based on a carp feeding experiment conducted throughout the season, it can be said that the feed containing 40 % DDGS performed well in terms of production and feed utilization parameters and could be a promising component of carp feed to be placed on the market in the future. Based on the results of the economic assessment, the use of experimental feed formula in carp culture generates much higher net income per hectare compared to the use of

conventional feeds. At the same time, quality of the fish produced with DDGS-based diet is favor- able and they lead to similar fillet quality values as commercial feeds, which has been used in production for a long time.

6. PUBLICATIONS RELATED TO THE THESIS

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