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***Article***

# Effect of different dietary lysine level on the functioning of genes participating in buildup of intramuscular fat in pork

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***ABSTRACT*** – The eating quality of pork, e.g. its taste and tenderness are favourably affected by inter- and intramuscular fat (IMF) content. Many genes are involved in forming the fat deposited in the meat and especially in between the muscle fibres, among others, the genes of the FABP family (FABP3, FABP4), the LEPR gene, the SDC and the FASN. The objective of this trial was to study whether a moderate change in dietary Lys content (approximately 6%) through the growing and fattening period results in a shift in the expression of genes involved in fat metabolism (FABP3, FABP4, LEPR, SDC and FASN). In the study, Danbred pigs were assigned from a fattening study that involved 96 pigs (50-50% barrows and gilts) from about 25 kg to 125kg live weight. The animals received three-phase feeding and the feeds were formulated according to their requirements. The dietary treatments were set by diets containing different Lys levels in all phases: 10.9, 9.1 g/kg, and 8.3 g/kg vs 11.5, 9.6 and 9.0 g/kg Lys, respectively. The pigs were slaughtered in 125 kg live weight and carcass classification through lean meat % was performed. Meat samples from the carcass were taken within 30 minutes post-mortem from the longissimus dorsi muscle. Gene expression levels were quantified using RT-qPCR analysis. The results indicated that variations in dietary lysine (Lys) content did not significantly influence slaughter quality or the expression levels of genes associated with fat metabolism. Consequently, it can be inferred that a 10% difference from the recommended Lys content does not alter lipid synthesis in the longissimus dorsi muscle of pigs.

***Keywords***: pork quality, intramuscular fat, pig, lysine, gene expression

## INTRODUCTION

Pork contributes to the meat consumption of the world by 40 %, and its quality has a high impact on the preference of consumers. It is widely accepted that a high level of intramuscular fat content (IMF) influences positively the culinary quality of pork (*Laack* et al., 2001). In recent decades the selection of swine primarily focused on growth, feed efficiency and the ratio of lean meat. Due to

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the negative correlation between the ratio of lean meat and IMF content, current commercial swine strains contain less fat and have lower backfat thickness and fat content in the muscle than indigenous breeds. Because of this, the swine industry is highly interested in the increase of IMF content to satisfy consumer needs. IMF content shows a significant difference between breeds and between different lines within a breed (*Pena* et al, 2016). It is also influenced by sex, age and feeding strategy.

IMF incorporates all of the lipids which are found in the muscles and can be functional (cholesterol and phospholipids) or reserve lipids (triglycerides). Recently multiple genes have been identified that participate in the development of IMF and which play a key role in fat metabolism and the appearance of fat in muscle tissue (reviewed by *Malgwi* et al, 2022).

Nutrigenomic research has provided evidence that the bioactive components of food have an impact on the animal genome and the expression profile of genes. Nutrigenomic studies explain the effect of dietary components on transcriptomics and proteomics influencing the physiological state of the organism (*Fenech* et al, 2011).

It has been repeatedly reported that low lysine (Lys) content feed promotes the accumulation of IMF in the muscle of swine. There is evidence that Lys-deficient feed changes gene expression involved in fat synthesis (*Katsumata* et al., 2018), however, it is not clear whether moderately reduced Lys feed could result in a shift in gene expression responsible for fat metabolism. A dose-response relationship between dietary lysine (Lys) levels and intramuscular fat (IMF) content in pork has been observed. This relationship provides the basis for determining the optimal Lys level that maximizes both IMF content and growth performance in pigs (reference). Specifically, lower dietary Lys levels can enhance IMF content; however, they are associated with reductions in growth performance parameters such as body weight gain and daily feed intake (*Katsumata*, 2011).

Additionally, multiple studies have shown that most genes with relation to fat metabolism has indirect effects on the IMF content of pork. The effect of these genes, however, has been observed to be diverse in different muscles (*Malgwi* et al. 2022). Fatty acid binding proteins (FABPs) are intracellular proteins which take part in fatty acid transportation from the plasma membrane to the place of β-oxidation or to the synthesis of triacylglycerol and phospholipids. This group of proteins is built up by different types, including liver, heart, myelin, epidermis, brain, adipocytes and the bowel system (*Becker* et al, 1994; *Chmurzynka* et al 2006). The intracellular long-chain fatty acids in muscle cells primarily originate from substances transported by H-FABP (*Glatz* et al. 2003). *Damon* et al. (2006) reported that the level of FABP4 (A-FABP) in the longissimus dorsi muscle has doubled in swine with higher IMF content than in the ones with lower IMF content, and positive correlation have been found between the level of FABP4 (A-FABP) and adipocyte number and lipid content. Stearoyl-CoA desaturase enzyme is encoded by the SCD gene which converts saturated fatty acids into mono-unsaturated (MUFA) ones. The SCD gene is an important regulator of lipid deposition and fatty acid synthesis in swine (*Malgwi* et al, 2022). The LEPR gene is known in context with the leptin receptor. It takes part in the take-up of nutrients and in regulation of energy metabolism. LEPR is a functional genetic marker which affects the growth and fat deposition of swine. An enhanced expression of this gene can be anticipated in animals with a higher level of fat deposition. The fatty acid synthase (FAS) enzyme is encoded by the FASN genes. This gene regulates the *de novo* synthesis of fatty acids from acetyl-CoA and malonyl-CoA in the presence of NADPH. The FASN gene initiates the synthesis of fatty acids and mono-unsaturated fatty acids during the early stages of lipid metabolism (*Malgwi* et al., 2022).

Therefore, the aim of this study was to investigate whether a moderate alteration in dietary lysine (Lys) content (approximately 6%) during the growing and fattening phases influences the expression of genes associated with fat metabolism.

## MATERIALS AND METHODS

### Experimental Design

The muscle samples were taken from experimental pigs fed different dietary Lys during the growing and fattening period. In the experiment, a total of 96 Danbred pigs (50-50% barrow and gilt) with an initial body weight of 25 kg ± 5 kg (SD) were involved. The animals were kept in groups, 8 pigs in each pen. Pigs were allocated to two treatment groups (n=48) that differed in dietary Lys content. Three-phase-feeding was applied, the compound feeds in the growing and fattening phase contained 10.9, 9.1, and 8.3 g/kg or 11.5, 9.6, and 9.0 g/kg Lys in treatment A and B, respectively. Methionine and Cystine, Threonine and Tryptophan to Lysine ratio was formulated according to the ideal protein concept. The feed was pelleted and offered *ad libitum* and water was freely available from drinkers.

### Feeding

The feeds were formulated isonitrogenous and isocaloric on net energy bases. The crude protein and amino acid content of the experimental feeds except for

Lys were formulated according to the breeder's nutritional guidelines (*SEGES*, 2020). The composition and nutrient content of experimental feeds are shown in Table 1, 2, 3.

The pigs were slaughtered in 126 kg ± 4 kg (SD) live weight and carcass qualifications were performed in the slaughter house of the *MSC Vágóhíd* in Mohács.

**Table 1.**
The experimental feeds for 25-40 kg pigs

| **Components g/kg** | **Treatments** A | B |
|---|---|---|
| Corn | 0 | 0 |
| Soybean meal | 183 | 178 |
| Wheat | 401.5 | 399.7 |
| Barley | 350 | 355 |
| Plant oil | 24 | 24 |
| Lyine-HCL | 0.6 | 1.5 |
| DL-Methionine | 0.4 | 0.8 |
| L-Threonine | 0.4 | 0.8 |
| L-Tryptophan | 0.1 | 0.2 |
| Premix 0,3% | 40 | 40 |
| **Sum** | **1000** | **1000** |
| | | |
| **Nutrients g/kg** | | |
| Dry matter | 889.9 | 890.1 |
| Net energy MJ/kg | 10 | 10 |
| Crude protein | 159.9 | 159.8 |
| Crude fat | 42.6 | 42.5 |
| Crude fiber | 34.2 | 34.1 |
| Crude ash | 53.4 | 53.2 |
| Lysine | 10.9 | 11.5 |
| Methionine+Cystine | 6.1 | 6.5 |
| Threonine | 7.1 | 7.5 |
| Tryptophan | 2.2 | 2.3 |
| Ca | 8 | 8 |
| P | 5.3 | 5.3 |
| AID* Lysine | 9.9 | 10.5 |
| AID M+C | 5.5 | 5.9 |
| AID Threonine | 6.2 | 6.6 |
| AID Tryptophan | 2.0 | 2.1 |

* AID = apparent ileal digestible

**Table 2.**
The experimental feeds for 40-80 kg pigs

| | Treatments | |
|---|---|---|
| **Components g/kg** | A | B |
| Corn | 10 | 15 |
| Soybean meal | 163 | 158 |
| Wheat | 399.1 | 397.5 |
| Barley | 380 | 380 |
| Plant oil | 17 | 17 |
| Lysine-HCL | 0,6 | 1,4 |
| DL-Methionine | 0 | 0,3 |
| L-Threonine | 0.2 | 0.6 |
| L-Tryptophan | 0.1 | 0.2 |
| Premix 0,3% | 30 | 30 |
| **Sum** | **1000** | **1000** |
| | | |
| **Nutrients g/kg** | | |
| Dry matter | 888.2 | 888.2 |
| Net energy MJ/kg | 9.9 | 9.9 |
| Crude protein | 150.4 | 149.8 |
| Crude fat | 35.7 | 35.7 |
| Crude fiber | 34.4 | 34.2 |
| Crude ash | 50.9 | 50.7 |
| Lysine | 9.1 | 9.6 |
| Methionine+Cystine | 5.1 | 5.4 |
| Threonine | 6 | 6.3 |
| Tryptophan | 1.9 | 2 |
| Ca | 7.5 | 7.5 |
| P | 5.2 | 5.2 |
| AID* Lysine | 8.1 | 8.6 |
| AID M+C | 4.5 | 4.8 |
| AID Threonine | 5.1 | 5.4 |
| AID Tryptophan | 1.6 | 1.7 |

* AID = apparent ileal digestible

**Table 3.**
The experimental feeds for 80-120 kg pigs

| **Components g/kg** | **Treatments** A | B |
|---|---|---|
| Corn | 50 | 50 |
| Soybean meal | 131 | 128 |
| Wheat | 400.4 | 401.7 |
| Barley | 380 | 380 |
| Plant oil | 8 | 8 |
| Lysine-HCL | 0.5 | 1.4 |
| DL-Methionine | 0 | 0.2 |
| L-Threonine | 0 | 0.5 |
| L-Tryptophan | 0.1 | 0.2 |
| Premix 0,3% | 30 | 30 |
| **Sum** | **1000** | **1000** |
| | | |
| **Nutrients g/kg** | | |
| Dry matter | 886.4 | 886.6 |
| Net energy MJ/kg | 9.9 | 9.9 |
| Crude protein | 139.9 | 140.2 |
| Crude fat | 27.3 | 27.2 |
| Crude fiber | 34 | 33.9 |
| Crude ash | 48 | 47.9 |
| Lysine | 8.3 | 9 |
| Methionine+Cystine | 4.9 | 5 |
| Threonine | 5.5 | 5.9 |
| Tryptophan | 1.7 | 1.8 |
| Ca | 7 | 7 |
| P | 0.5 | 0.5 |
| AID* Lysine | 7.4 | 8 |
| AID M+C | 4.1 | 4.5 |
| AID Threonine | 4.7 | 5 |
| AID Tryptophan | 1.5 | 1.6 |

* AID = apparent ileal digestible

### *Assessment in the abattoir*

At the slaughterhouse the swine were first lowered in a shaft where the concentration of carbon-dioxide is about 90-95%. The animals spent 1-1.5 minutes down in this shaft. The stunned swine were hanged by their feet with their head hanging down. Then they are transported on a conveyor belt where they are stabbed once and they are exsanguinated. Post mortem they are scalded and their hair is removed, followed by their processing and dismemberment.

At this point carcass qualification and trade classification is performed. During the estimation of lean meat content (%) ultrasonic measuring equipment is used (UltraFom 300). About 7 cm from the plane of cut, between the second and third ribs the loin muscle diameter and backfat thickness is measured (in mm). The lean meat % is calculated automatically from loin area (converted from longissimus muscle diameter) and backfat thickness.

## *Gene expression studies*

### *Sample collection and storage conditions*

Meat samples were collected from randomly assigned 10 carcasses from each treatment group. Samples were taken from the left carcass in less than 45 minutes following the slaughter, from the *Musculus longissumus dorsi* between the 13$^{th}$ and 14$^{th}$ vertebra. The samples were placed in 2 mL DNase/RNase free Eppendorf tubes containing RNA stabilizing liquid (RNAlater™, Thermo Fisher Scientific. 168 Third Avenue. Waltham, MA USA 02451) with a 1:10 sample:liquid volume ratio to stabilize and protect the RNA content of the tissues from degradation with immediate RNase inactivation. After keeping the samples on 4 °C overnight, they were moved to a freezer and kept at -80 °C until further processing.

### *Sample Preparation, and RNA extraction*

Prior to initiating RNA extraction from muscle tissue, ribonucleases (RNases), which are responsible for RNA degradation, are inactivated as an additional precaution to protect the integrity of the RNA. To achieve this, β-mercaptoethanol was used as the deactivating agent, ensuring the RNA remains intact and suitable for the subsequent downstream analyses. The process of RNA extraction started with homogenisation of the tissues in the lysis buffer of RNeasy Mini Kit (Qiagen, 19300 Germantown Road, Germantown, MD 20874, USA) in a Tissuelyser II high-throughput sample disruptor (Qiagen, 19300 Germantown Road, Germantown, MD 20874, USA). To avoid RNA degradation, the adapter of the homogenizer was cooled down to -20 °C and all the tools for sample preparation as well. RNA extraction was carried out according to the instructions of the manufacturer. To extract the RNA 30 mg of muscle tissue was used. For the homogenisation, RTL buffer (This is a lysis buffer which is used for the lysis of cells and tissues) was used, provided by the manufacturer. This was followed by a precipitation with alcohol. After which the resulting solution was pipetted on the "RNeasy Mini spin column", which was centrifuged and flow-through was discarded. Centrifugation steps followed with the use of multiple washing buffers (RW1 buffer and RPE buffer). At last the RNA attached to the column was dissolved with RNA-ase free water with RNase free water.

All the tools for sample preparation and manipulation were pre-sterilised by heat-air steriliser (Celsius 2000 SLE 600, MEMMERT, Germany) on 180 °C for the elimination of microbes and the inactivation of RNases. The quantity and quality of the extracted RNA were measured by Thermo Scientific™ NanoDrop™ OneC Microvolume UV-Vis Spectrophotometer (Thermo Scientific™ 840274200, 168 3rd Ave, Waltham, MA 02451, Massachusetts, USA).

*cDNA preparation*

The cDNA preparation procedure was applied according to (*Korenková* et al., 2015) starting with the same initial and highest possible concentration of the extracted RNA (140 ng/µL), for which every sample was adjusted to, followed by cDNA transcription.

As the first step of cDNA transcription genomic DNA has to be eliminated. For this gDNA wipe-out buffer, the template RNA and RNase free water was brought into reaction mixture which was incubated for 2 minutes at 42 °C (as determined by the manufacturer and provided in the QuantiTect Reverse Transcription Kit). In the next step the mater mix needed for reverse-transcription is assembled for which the necessary reverse-transcriptase enzyme, the RT buffer and the RT primer mix was provided by the manufacturer. The RNA resulting from the first step was used as template (which does not contain genomic DNA). The resulting mixture was first incubated at 42 °C for 15 minutes, then at 95 °C for 3 minutes (QuantiTect Reverse Transcription Kit /Qiagen, 19300 Germantown Road, Germantown, MD 20874, USA/) and used for downstream application without dilution, since inhibitory effect using undiluted cDNA was not experienced (*Whelan* et al., 2003).

*Primer information*

The genes analyzed in this study included *LEPR* (Leptin Receptor), *FABP3* (Fatty Acid Binding Protein-3), *FABP4* (Fatty Acid Binding Protein-4), *SCD* (Stearoyl-CoA Desaturase), and *FASN* (Fatty Acid Synthase). The *β-ACTIN* gene was used as a reference housekeeping gene for normalization. The primer sequences for the analyzed genes are detailed in Table 4.

**Table 4.**
Primer sequences of the genes used for the gene expression study

| Gene name | Forward- and reverse primer sequence | Reference |
|---|---|---|
| ***H-FABP (FABP3)*** | F: 5'AGTTTGATGAGACAACAGCAGATGA 3'<br>R: 5'CAAGTTTGCCTCCATCCAGTGT 3' | *Tyra et al, 2011* |
| ***A-FABP (FAPB4)*** | F: 5' CTGGTACAGGTGCAGAAGTGG 3'<br>R: 5' TTCTGGTAGCCGTGACACCT 3' | *Meng et al., 2018* |
| ***LEPR*** | F: 5' ACATTGCAGGGAAGGCATTT 3'<br>R: 5'CAGTTTGCACCTGTTTGTTGAAA 3' | *Tyra et al, 2011* |
| ***SCD*** | F: 5' TTGCTCTGGGCGTTTGC 3'<br>R: 5' CGAGCTTTGTAAGTTCGGTGACT 3' | *Meng et al., 2018* |
| ***FASN*** | F: 5'-CGTCCTGCTGAAGCCTAACTC-3'<br>R: 5'-GCTCCTTGGAACCGTCTGTGT-3' | *Zhu et al, 2019* |
| ***β-ACTIN*** | F: 5'-ACTGCCGCATCCTCTTCCTC-3'<br>R: 5'-CTCCTGCTTGCTGATCCACATC-3' | *Zhao et al, 2009* |

*qRTPCR conditions*

Real-time PCR was performed using SYBR Premix and Mx3000P Real-Time PCR System (Agilent Technologies). Amplifications were performed in 10 µL reaction volume containing 5 µL of SYBR Premix 0.2 µL (stock concentration:10µM) of each primer,1 µL of diluted cDNA, and sterile water. The PCR amplification was carried out as follows: 95 °C for 15 min, then 40 cycles of 95 °C for 1s and 60 °C for 30 sec in normal two steps settings. PCR tests were performed with three parallels in case of every sample.

## Calculations and statistical analysis

The $C_t$ (threshold cycle) values given by qRT-PCR were evaluated according to the following conditions. The applied method for the determination of relative amount was the $2^{-\Delta\Delta Ct}$ method.

Change of relative gene expression= $2^{-\Delta\Delta Ct}$
Where:
$\Delta\Delta C_t = \Delta C_{t\ treated} - \Delta C_{t\ untreated}$
$\Delta C_t = (C_{t\ target\ gene} - C_{t\ reference\ gene})$

To better visualize the results, the log10 of $2^{-\Delta\Delta Ct}$ values were taken into account.

The gene expression and meat quality values were evaluated with a Student's t-test considering significant differences at the $p<0.05$ level. The data showed normal distribution. The statistics were performed using R-program (R 4.1.2 for Windows, Rcmdr package).

## RESULTS AND DISCUSSION

Pigs were slaughtered over 120 kg body weight in a commercial abattoir. There was a 3.5 kg difference in live weight at slaughter and a 2.7 kg difference in carcass weight ($p$=0.052). The higher Lys feed tended to increase the live weight and the carcass weight, however, there was no difference in backfat thickness, loin diameter and lean meat percentage between pigs of each treatment (Table 5). Our data suggest that Lys recommendation may be slightly overestimated since an approximately 6 % reduction in dietary Lys did not compromise the body weight and the lean meat content of the body. In line with our results, others have also found that a slight reduction in dietary Lys does not change the pig growth. Kumar et al. (2016) reported that 10 and 15% reduction in dietary Lys had no impact on growth performance and carcass parameters in crossbred Landrace pigs either. Results of *Jin* et al. (2010) indicated that finishing pigs fed a diet with 15% lysine restriction had no detrimental effects on growth performance and N utilization but could achieve substantial increases in marbling and longissimus fat content of pork.

**Table 5.**
Slaughter weight and carcass quality traits measured in the abattoir

| | A treatment | | B treatment | | |
|---|---|---|---|---|---|
| | mean | SD | mean | SD | P-value |
| Live weight (kg) | 124.4 | 2.4 | 127.9 | 4.5 | 0.052 |
| Carcass weight (kg) | 98.8 | 1.9 | 101.5 | 3.6 | 0.052 |
| Backfat thickness (mm) | 14.6 | 2.1 | 15.1 | 3.9 | 0.73 |
| Loin diameter (mm) | 63.5 | 2.9 | 63.0 | 4.9 | 0.80 |
| Lean meat % | 59.8 | 1.4 | 59.4 | 2.8 | 0.72 |

Treatment A: feed was formulated to contain 10.9 g/kg, 9.1 g/kg, 8.3 g/kg digestible Lys in grower 1, 2, and finisher phases respectively; Treatment B: feed was formulated to contain 11.5 g/kg, 9.6 g/kg, 9.0 g/kg in grower 1, 2, and finisher phases respectively

In nutrigenomics studies, the general objective is to evaluate whether expression of genes is affected by dietary treatments. In this research, the expression of genes determining IMF was measured in meat samples that originated from pigs fed by different Lys content feed. The dietary contrast in Lys was 5.5% in phases of grower 1 and 2, and 8.4 % in the finishing phase.

The results of the gene expression in different dietary treatments are shown in Figure 1. Dietary Lys content had no significant impact on the expression of measured genes.

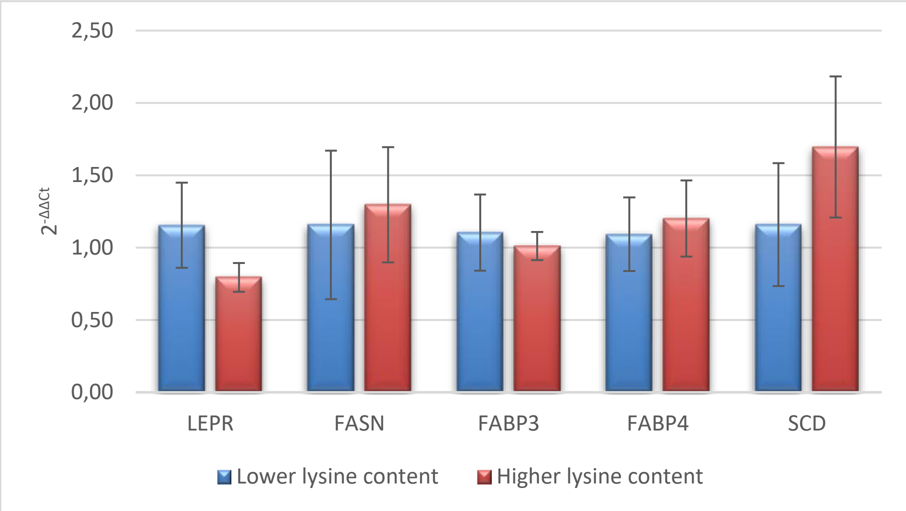


**Figure 1.** Level of gene expression in *m. longissimus dorsi* samples of pork when pigs were feed diets differed in digestible Lys content between 25-125 kg live weight.

**Table 6.**
Result of two-sample Student's t-test

| | *LEPR* | *FASN* | *FABP3* | *FABP4* | *SCD* |
|---|---|---|---|---|---|
| **two-sample t-test** | 0.21 | 0.67 | 0.99 | 0.91 | 0.30 |

The blue columns show the results of gene expression for feeding lower Lys content feeds (10.9 g/kg, 9.1 g/kg, 8.3 g/kg in grower 1, 2, and finisher phases respectively), the orange columns show the gene expression results for feeding high Lys content feeds (11.5 g/kg, 9.6 g/kg, 9.0 g/kg in grower 1, 2, and finisher phases respectively). In the case of *SCD* and *LEPR* genes, the means differ, but the individual variance within the group was high, and thus no reliable statistical difference could be found between the treatment groups with different dietary Lys content. The treatment did not affect the activity of *FABP3, FABP4* and *FASN* genes either. In general, the individual variances within the groups were relatively high with exception of *LEPR* and *FABR3* in high Lys group.

In this study, the experimental contrast was set to achieve a moderate difference in dietary Lys content and this may have been insufficient, or the longissimus dorsi muscle (*M. longissimus dorsi*) may not have been the most suitable tissue for detecting differences in the expression of the target genes with the levels of Lys supplied in the diets. *Katsumata* (2011) reported that IMF content of longissimus dorsi muscle was twice as high in pigs fed lysine-depleted diet, compared to control pigs (0.70% vs. 1.15% lysine; respectively). In a further study of Katsumata et al. (2018) when pigs were fed with significantly reduced Lys content diet (1.37% vs 0.78%) the level of Fatty Acid Synthase mRNA in the liver increased. The mean level of *FAS* mRNA in *Longissimus dorsi* muscle was also higher for those swine that received a diet with low Lys content (*Katsumata* et al, 2018). In line with that *Palma-Granados* et al. (2019) found that the effect of lysine deficiency on lipid metabolism was tissue-specific, with an activation of lipogenesis in longissimus and biceps femoris muscle but no apparent stimulation in backfat adipose tissue. However, it has to be stressed that the experimental contrast was really huge in both trials, the reduction in dietary Lys was more than 40% and 50% in the referred studies (*Katsumata* et al, 2018 and *Palma-Granados* et al., 2019, respectively).

The excessively low intramuscular fat (IMF) content in lean pig genotypes negatively impacts pork eating quality, necessitating precision feeding strategies informed by nutrigenomic insights. *Palma-Granados* et al. (2019) propose that lysine (Lys)-deficient diets could effectively increase IMF content in both lean and fat pig genotypes. However, it is critical that such strategies do not compromise growth performance, as reduced growth rates carry adverse economic and environmental consequences. Precision feeding approaches tailored to individual genotypes and production goals could include carefully timed and targeted Lys restrictions during the fattening phase, optimizing IMF content while maintaining efficient growth and minimizing resource use.

## CONCLUSIONS

In conclusion, a 6-8% variation in dietary lysine (Lys) content relative to the recommended level did not significantly impact slaughter quality or fat synthesis in the longissimus dorsi muscle of lean pigs. Moreover, the expression of genes involved in fat metabolism, particularly those encoding fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD), exhibited considerable individual variability, even within a genotype subjected to intensive genetic selection. This suggests that genetic factors may play a substantial role in regulating fat metabolism, and minor dietary adjustments in Lys may not be sufficient to induce notable changes in fat deposition at the molecular level.

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***Review***

# The role of nutrition in achieving more sustainable and environmentally friendly aquaculture

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***ABSTRACT*** – Sustainability applies to almost all aspects of human activity, and the ever-growing aquaculture industry is no exception. Feeding aquatic animals is of paramount importance in terms of economic and environmental sustainability. This review discusses practices and promising new results for improving feed efficiency at different levels of production intensity. Special emphasis is placed on demonstrating how semi-intensive pond technology can be considered *ab ovo* sustainable also from a social point of view. Recent achievements in the field of alternative protein sources to replace fishmeal are also discussed, as well as the beneficial properties of special feed additives such as probiotics and phytochemicals.

***Keywords***: aquaculture, sustainability, nutrition

## INTRODUCTION

The concept of sustainability has become a prominent buzzword in the field of research and development, and with good reason, as it relates to almost all aspects of human activity and has implications for the future of our planet. However, reviewing the core principles of sustainability from an aquaculture perspective may be useful. The primary objectives of sustainable aquaculture are to produce food to minimize adverse environmental impacts and promote social and economic well-being. The following key principles are worth noting:

- *Environmental Sustainability* - Natural habitat disruption should be kept to an absolute minimum, responsible waste management practices should be used, water quality should be protected, and biodiversity should be conserved.
- *Economic Sustainability* – Key issues are: efficient production, fair trade, community development.
- *Social sustainability* - Labour rights and community involvement are involved.
- *Animal welfare issues*

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*Specific practices for sustainable aquaculture* include recirculating aquaculture systems (RAS)., integrated aquaculture with agriculture or livestock, organic aquaculture, and using sustainable feed sources. Integrated multi-trophic aquaculture (IMTA) represents an approach to sustainable aquaculture that involves farming species from different trophic levels in a single system (Fang et al., 2016). The system offers both environmental and economic benefits, including improved nutrient cycling, reduced environmental impact, and increased profits (Granada et al., 2016). IMTA can be applied in freshwater, brackish, and marine environments, with various approaches tailored to local conditions (Azhar and Memiş, 2023). Research has shown that IMTA can enhance sustainability in aquaculture by reducing nutrient outputs, improving resource utilization, and potentially increasing public acceptance of aquaculture operations (Granada et al., 2016). It should be noted that traditional semi-intensive fishpond polyculture, although it is not usually assigned to this category, is also essentially an IMTA. Furthermore, the polyculture of giant freshwater prawn, silver, and bighead carp with freshwater pearl mussel and silver and bighead carp has also demonstrated considerable promise in inland ponds (Tang et al., 2024). Similarly, the cultivation of mussels and seaweed in a biculture has also yielded favourable results (Michler-Cieluch and Kodeih, 2008). This category also encompasses aquaponics, in which the waste produced by farmed fish or other aquatic creatures is used to nourish plants grown hydroponically. The automatic recirculating system allows the purification of water, which is then used again for the next cycle. Aquaponics requires little monitoring or measuring, making it a relatively simple and low-maintenance system.

Further detailed and pertinent general information on sustainable aquaculture can be found in several publications, including those by Costa-Pierce (2002), "SustainAqua" (2009), Shepon et al. (2021), Austin et al. (2022), Pounds et al. (2022), Barbosa et al. (2024), Garlock et al. (2024), Keer et al. (2024), Tucciarone et al. (2024) and Zhang et al. (2024) which all discuss the object from a different point of view.

While economic sustainability remains a primary objective, all other criteria mentioned above must also be considered to achieve sustainability in aquaculture. Pursuing continuous improvement in production methods is imperative while simultaneously reducing their environmental and energy footprint. The nutritional requirements of the cultured species are undoubtedly a crucial factor in this regard. The objective of this review is to identify the principal elements of nutrition and feeding technology that can facilitate the enhancement of the sustainability and environmental friendliness of the aquaculture industry, while simultaneously promoting economic sustainability. The optimization of feed efficiency represents a pivotal concern in animal production, given that feed constitutes a substantial proportion of overall costs. Fortunately, economic and environmental protection objectives are aligned in this case, thereby promoting sustainability. The objective of this study was to conduct a review of the principal fields of feeding and feed development and manufacturing related to aquaculture's sustainability.

## TECHNOLOGICAL METHODS OF FEEDING USED TO IMPROVE FEED EFFICIENCY

Rational feed development and feeding practice are based on a thorough understanding of the nutritional requirements of cultured animals. The nutritional requirements of aquatic animals, being poikilothermic organisms, require consideration of factors that differ from those applicable to warm-blooded (homeothermic) animals. The temperature of the water in which the animals are kept is a significant factor in their metabolism, which in turn affects their energy requirements and therefore their feed intake. It is also important to consider the age of the animals, as this affects their growth rate in a similar way to homeothermic organisms. It is also important to consider the age of the animals, as this affects their growth rate. Figure 1. depicts the theoretical relationship between feeding level, growth rate, and feed conversion, which collectively determine the efficacy of production.

To optimize feed efficiency, it is indispensable simultaneously to meet both qualitative and quantitative nutrient requirements. The primary objective of the aquaculture industry is to develop the most economically viable feed that meets the nutritional requirements of a given age group of cultured species. Other key considerations are reducing feeding costs and improving water quality through optimal feeding technology. It is essential to minimize feeding loss, which includes both the indigestible portion and all metabolic losses. However, it is important to remember that a non-negligible amount of the offered feed is typically uneaten. The proportion of uneaten feed varies considerably, but an average of 10% has been estimated (Craig, 2009). Commercial feed pellets must remain intact in water until consumed, which is especially important for slow-feeding aquaculture species such as shrimp (Lovell, 1991). Extrusion can be an alternative process for aquaculture feed production, increasing digestibility, and functional properties of the aquaculture feed, such as water stability and floatability. The thermal process during extrusion decreases the antinutritional factors present in legumes or other agro-industrial by-products, such as trypsin inhibitors and lectins. The beneficial effects of extrusion are detailed by Delgado and Reyes-Jaquez (2018). The use of binders as feed additives to ensure water stability has been a standard practice for some time (Tacon, 1987).

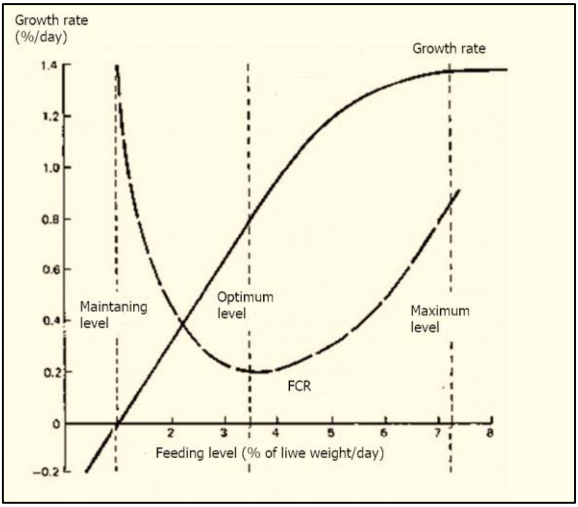


**Figure 1.** Correlation between feeding level, growth rate, and feed conversion ratio (Modified after *Lovell*, 1998)

However, this does not address all issues related to feed loss. It is crucial to identify the optimal feeding frequency to minimize this direct loss and improve the feed conversion ratio (FCR). To this end, published feeding rate tables are available for the majority of commonly cultured species (Craig, 2009). In the current market, there is a wide range of automatic feeders available for programmed feed delivery in cages, raceways, ponds, and RAS operations. These can help to ensure optimal feeding. In the future, monitoring systems could play a significant role in optimizing feeding regimes by providing information about actual feed loss. This is an area that has been explored by Parra et al. (2018). It is also essential to consider the key issues of nutrient sensing and feeding stimulation in this context (Hancz, 2020).

The frequency of feeding was proven to have a significant effect on the growth, feed conversion, and even reproductive performance of different fish species. Optimal growth and feed utilization of juvenile Nile tilapia was achieved by providing food four to five times per day (Daudpota et al., 2016).

In contrast, rainbow trout *(Oncorhynchus mykiss)* showed the greatest food intake and growth with only two feedings per day, with minimal effect on body composition (Grayton and Beamish, 1977). Two feedings per day were found to be optimal for the growth and reproductive performance of the ornamental Siamese fighting fish *(Betta splendens)* (James and Sampath, 2004). Juvenile estuarine groupers *(Epinephelus salmoides)* exhibited optimal growth, food utilization, and survival when fed to satiation once every two days, which corresponds to their 36-hour food deprivation period (Thia-Eng and Seng-Keh, 1978). These studies indicate that the optimal feeding frequency varies among fish species and is influenced by factors such as metabolism, digestive rate, and environmental conditions. To optimize the feed's daily ration, a growth model was developed based on data from Mexican tilapia farms by Domínguez-May et al. (2024). In their model equations, the researchers employed all of the available variables. Therefore, it is crucial to determine species-specific feeding regimens to maximize growth and efficiency in aquaculture. Nowadays the biggest fish feed manufacturing firms generally can supply producers with these regimes, at least in the case of the most important species reared in intensive systems.

## The special features of semi-intensive systems

Semi-intensive aquaculture systems account for about 70% of tropic fish and crustacean production (Tacon, 1996). Large pond areas in Central and Eastern Europe also produce in this system, using a range of feeding methods, from low-cost pond fertilization to supplemental feeding of energy-providing cereals, or even high-cost complete diets. However, all forms emphasize the importance of natural food organisms as protein sources. The other cornerstone is polyculture, based on the synergy of species with different feeding habits, a practice derived from traditional Chinese aquaculture (Edwards, 2009).

To gain a deeper understanding of the functioning of a polyculture fish pond from a production biology perspective, it is first necessary to recall the Elton pyramid (Figure 2.), which is a well-known model in this field. The primary producers in a water body are the phytoplankton and higher plants. The decomposers are the bacterioplankton and bacteria as well as invertebrates that dwell in the sediment. The primary consumers are the zooplankton taxa, with the second level comprising mostly omnivorous fish, which serve as food for the carnivores at the highest levels. The red percentage indicates the ratio of energy or weight at a given trophic level. In the case of a semi-intensive fish pond, the feed is a significant input of energy, incorporated in the nutrients, of course.

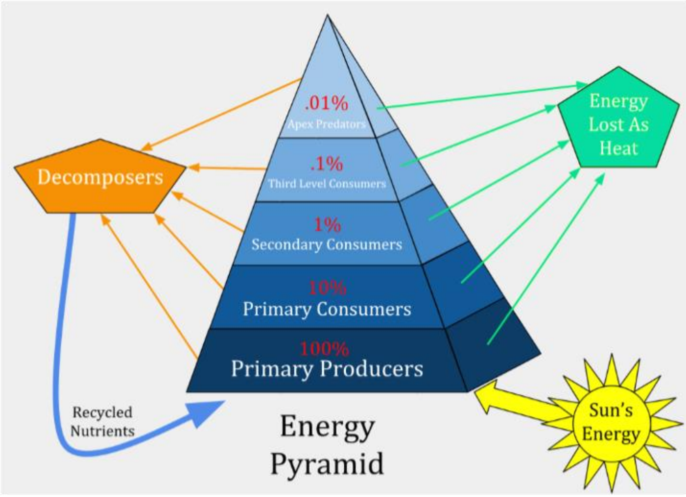


**Figure 2.** Elton's pyramid on energy relations of ecosystems (Source: Wikipedia)

In the context of the practice of feeding, the factors related to fish are species, variety, sex, and especially age. This is because the efficiency of digestion improves with age. The most significant environmental factor is water temperature. (An increase of 10°C results in a doubling of the metabolic rate.) However, the impact of atmospheric pressure must also be considered. It is of the utmost importance to determine the daily ration, as feeding is typically allowed ad libitum, which makes it easy to commit feed waste. As it is not feasible to directly observe consumption, the only viable approach is to assess the quantity of unconsumed grains at designated feeding locations after a few hours and then modify the daily ration accordingly on the subsequent day. Although feeding once a day is not the most optimal practice, it is common due to the significant costs associated with labour. In the case of female breeders in the period preceding artificial propagation, a restricted feeding regimen is employed. The production of natural feeds is promoted by the use of organic or inorganic fertilizers. In a well-designed polyculture, all trophic levels are exploited by fish. Green fodder is consumed by grass carp, *(Ctenopharyngodon idella)* and the unconsumed part, in addition to the fish faeces, serves as fertilizer. It should be noted that grains are not the only substance that can be used for feeding; many kinds of by-products from the milling and food industries can also be employed, thus contributing to an environmentally friendly, circular economy (Hancz and Horváth, 2007).

Last but not least socio-economic concerns of low and medium-intensity aquaculture need also be mentioned, general aspects and methods of evaluation of which are discussed by Bhari and Visvanathan (2018). Aquaculture has been recognized as a potential tool for poverty reduction and food security enhancement, particularly in low-income countries (Viswanathan and Genio, 2001). While intensive aquaculture has raised environmental concerns, semi-intensive systems are being explored as a more sustainable alternative (Pomeroy et al., 2014). Studies in the Philippines have shown that aquaculture provides new opportunities for poor households, women, and youth, despite challenges such as limited access to credit and skills (Philminaq et al., 2007). The socioeconomic impacts of aquaculture are complex, affecting factors such as employment, food security, and overall well-being. To maximize benefits and minimize negative impacts, integrated farming systems, stakeholder involvement, and well-defined rights are recommended (Pomeroy et al., 2014).

## CURRENT ACHIEVEMENTS OF THE AQUATIC ANIMAL NUTRITION AND FEED INDUSTRY

### Alternative nutrient sources

In response to the mounting costs and environmental concerns associated with global fisheries, the aquaculture industry has undergone a significant transition, moving away from its historical reliance on fishmeal (FM) and fish oil (FO) as feed ingredients and towards more sustainable alternatives. McLean (2023) provides an overview of animal, microbial, and plant-based feedstuffs that have been examined as potential substitutes for FM/FO. In this regard, plant-based feedstuffs and rendered meat products have been the subject of the most extensive research, and a substantial body of literature exists on their use in the feeding of a wide range of important species cultivated globally. This research is ongoing and is yielding valuable insights. However, alternative sources of nutrients, including single-celled products such as fungi and yeasts, bacteria, and microalgae, are also being investigated as potential sources of protein, lipids, pigments, and enzymes. The role of feed additives, including exogenous enzymes (such as phytases, lipases, proteases, and carbohydrates), is also being evaluated as a means of potentially enhancing the nutritional profile of aquafeeds.

Additionally, chemoattractants and palatants, as well as pre-, pro-, and synbiotics, are being explored as potential tools for improving the digestibility and palatability of aquafeeds. However, due to the limitations of this review, our focus is limited to the latter issues.

## Soybean

The use of soybean meal (SBM) and soy protein concentrate (SPC) as alternatives to fishmeal in aquafeeds is becoming increasingly prevalent due to the growing demand and limited supply of fishmeal (Dersjant-Li, 2002; Gatlin, 2003). SBM is a highly nutritious plant protein source, often constituting 50-60% of the diet of omnivorous freshwater fish species (Gatlin, 2003). However, SBM contains anti-nutritional factors that can restrict its inclusion levels (Dersjant-Li, 2002). SPC, which contains reduced anti-nutritional components, has demonstrated potential for partial or complete replacement of fishmeal without adverse effects on growth performance in a range of aquatic species (Dersjant-Li, 2002; Gyan et al., 2019). In recent years, there has been a growing interest and adoption of enzymatically hydrolyzed soybean as a fishmeal substitute in aquafeeds. Huang et al. (2024) demonstrated that replacing up to 45% of dietary fishmeal with enzymatically hydrolyzed soybeans did not negatively impact the growth performance of juvenile Chinese mitten crabs (*Eriocheir sinensis*). Similarly, Tibaldi et al. (2006) demonstrated that substituting 50% of dietary fishmeal with enzymatically hydrolyzed soybean meal has no adverse effects on the growth performance and whole-body composition of European sea bass (*Dicentrarchus labrax*). Furthermore, the use of a multi-enzyme strategy for the hydrolysis of soybean has been demonstrated to be more effective than a single-enzyme approach in terms of substituting fishmeal in aquafeeds. It has been demonstrated that protease-treated soybean meal can substitute for 20% of fishmeal in the diets of largemouth bass (*Micropterus salmoides*). Furthermore, combination treatment with protease and non-starch polysaccharide enzymes has been shown to facilitate the replacement of up to 47.27% of dietary fishmeal with soybean meal for largemouth bass (Zhang et al., 2019). Therefore, soybean protein hydrolyzed through multiple enzymes represents an efficacious substitute for fishmeal in aquafeeds. Xu et al. (2024) obtained similar results on juvenile American eel (*Anguilla rostrata*).

The utilization of organic acids in the domain of aquaculture and the aquafeed industry has recently witnessed a surge in interest. Farmers are keen to enhance their yields through the culture-based system and the commercialization of organic acids in aquaculture, to improve growth performance and disease control. As evidenced by the research reviewed, numerous studies have demonstrated that organic acids, their salts, or blends in feed enhance growth, feed utilization, gut health, and disease resistance in aquatic animals. In general, studies on organic acids have indicated that they can enhance growth performance and nutrient utilization in aquaculture. The growth factor appears to be contingent upon the specific type of organic acid employed and the host organism in question (Ng and Koh, 2011). The utilization of organic acids in aquaculture is gaining considerable traction. The results of research studies indicate that organic acids, their salts, or mixtures can enhance growth, feed utilization, gut flora health, and disease resistance in aquatic animals. However, the beneficial effects vary by species and type of acid, and research suggests that further investigation is needed to understand the mechanisms involved. The economic feasibility of incorporating organic acids is limited, so scientific evidence is needed to support the benefits of their use ("Organic acids in aquafeeds – A sustainable alternative to antibiotics" 2019).

## Microorganisms: microalgae, yeasts, fungi, and bacteria

Microalgae, in particular, have high biomass productivity and a low environmental footprint, making them an environmentally sustainable option (Nagappan et al., 2021). However, challenges remain in commercial production, including high costs and the need for novel processing technologies to improve digestibility and reduce antinutritional factors (Sarker, 2023; Siddik et al., 2023). Despite these hurdles, ongoing research and technological innovations are focused on optimizing microbial-derived nutrients for aquaculture, with the potential to significantly contribute to the industry's sustainable growth (Gamboa-Delgado and Márquez-Reyes, 2018; Nagappan et al., 2021).

Sarker et al. (2023) gave an excellent overview of the benefits of using microorganisms in aquafeeds, mentioning first the microalgae protein and oil that has gained momentum for their use in aquaculture feeds. Marine microalgae, particularly have great potential to replace fishmeal and fish oil in salmonids and other finfish feeds because of their high levels of fatty acid and protein content. Marine microalgae, *Nannochloropsis oculata, Isochrysis sp*., and *Schizochytrium sp.* showed promise in aquafeed because they are rich in EPA, DHA, protein, key amino acids (methionine and lysine), lipids, and are good sources of minerals. Defatted *N. oculata* coproducts (leftover biomass oil extraction) contain approximately 20% to 45% crude protein, with good amino acid profiles. Including defatted *N. oculata* as a protein source into diets up to 33% for tilapia and up to 10% for Atlantic salmon did not affect their performance or health status. Another unique benefit of the defatted microalgae is their function in serving not only as an excellent protein source but also as a source of polyunsaturated fatty acids (PUFAs) to enrich n-3 fatty acids. Defatted *N. oculata* is more nutrient-dense compared to the whole cells. The digestibility of lysine (often deficient in terrestrial crop protein) was higher], and the EPA was also highly digestible, making it a good candidate for EPA supplementation in tilapia feed (Sarker et al., 2018) A recent study showed that *Isochrysis* *sp*. is a highly digestible protein, amino acid, lipid, and fatty acids source for rainbow trout. This species could be a good candidate for fishmeal and fish oil replacement in rainbow trout diets and can be used as a health-promoting omega-3, DHA supplement in diets. Research showed that lipids extracted from *Desmodesmus sp*. could be used (20%) in the salmon feeds without any negative effect on growth and fillet composition. A *Spirulina* algal meal could be also incorporated in 10% of the rainbow trout feed without any adverse effect on fish performance (Kiron et al., 2016, Sirakov et al., (2012).

Yeast has also emerged as a viable substitute protein source in the aquaculture industry due to its potential as a nutritional supplement, as illustrated in Table 1. Furthermore, yeast has the potential to act as an effective immune-stimulating agent, thus helping to prevent disease. However, except methionine, lysine, arginine, and phenylalanine, which are typically the limiting essential amino acids in various fish species, the various yeast species have amino acid profiles that are advantageous compared to fishmeal (Sultana et al., 2024).

**Table 1.**
Chemical composition of *Saccharomyces cerevisiae* yeast meal and fishmeal.

| Composition | *Saccharomyces cerevisiae* from beer fermentation | Menhaden fishmeal |
|---|---|---|
| Dry matter% | 93 | 91.2–92 |
| ME (kcal/kg) | 1990 | 3370 |
| Crude protein% | 44.4 | 59–68.5 |
| Crude fat% | 1 | 9.1–10.4 |
| Crude fiber% | 2.7 | 0.9 |
| Ca% | 0.12 | 4.87–5.34 |
| P% | 1.4 | 2.93–3.05 |

Source: *Hicks* et al., 2016; *Bertolo* et al., 2019.

Research indicates that yeast antimicrobial peptides can replace up to 40% of fishmeal in fish diets, with a 1% supplementation significantly improving disease resistance (Gyan et al., 2019). The utilization of yeast in aquafeeds could potentially reduce global fishmeal usage by 13.94% and decrease the carbon footprint of aquaculture, contributing to improved sustainability in the industry. FM replacement using brewer's spent yeast (BSY) is 30–50% for carnivores and 35–80.8% for omnivore fish. Also, the utilization of BSY in the global aquafeed industry could reduce fishmeal usage by up to 13.94% (0.369 MMT) globally and reduce the carbon footprint by about 1.79 megatonnes of $CO_2$ and fish-in-fish-out ratio (FIFO) from 0.82:1 to 0.71:1. Thus, utilization of

BSY in the aquaculture sector improves circular bio-economy and environmental sustainability in fish production. (Gokulakrishnan et al., 2022).

## Edible insects

The production of edible insects for animal feed has seen significant growth over the past decade, due to several key factors, as:

- The increasing global demand for animal protein, driven by population growth and changing dietary preferences, has created a need for alternative protein sources, further stimulating the expansion of the insect farming industry.
- Sustainability concerns include the reduction of greenhouse gas emissions, the conservation of land and water resources, and the optimization of feed conversion ratios.
- The nutritional value of insects is a rich source of protein, essential amino acids, vitamins, and minerals, rendering them an attractive alternative to conventional protein sources such as soy and fishmeal in animal feed.
- Research and development: The increased research into the benefits of insect-based feed has contributed to a greater awareness and acceptance of this practice among farmers and consumers.
- Technological advancements: Technological advancements have led to improvements in farming and processing technologies, thereby enhancing the viability of insect production.

In their introduction to "Insects as Sustainable Food Ingredients," Dunkel and Payne (2016) provide a comprehensive overview of the global significance of edible insects. They highlight the growing demand for animal-based protein, the efficient use of land and water, and the limitations of non-renewable energy sources. In light of the mounting concerns surrounding sustainability, Guiné et al. (2021) present significant findings regarding the efficiency of insects in comparison to other livestock. Furthermore, they indicate that the environmental impact of insect production considers factors such as feed conversion, land use, and water consumption. In comparison to other land animals, insects require the least amount of feed, land, and water. This is followed by chickens, pigs, and cows. In his book chapter, Riddick et al. (2014) provided a comprehensive overview of the utilization of insects as a protein source in aquaculture at the time. This review of the research discusses four key species—the black soldier fly, the common house fly, the silkworm moth, and the yellow mealworm—which were selected as model insects to illustrate the progress been made. It is crucial to consider the variations in protein and fat content across these species, given that they differ not only between species but also between developmental stages within a species. The primary findings of the study indicated that the inclusion of insects in the form of meals or pellets could provide an adequate protein source to partially substitute for conventional fish meals in the diets of omnivorous fish species, such as catfish and carp, as opposed to carnivorous fish, including trout and salmon. It is imperative to develop cost-effective, large-scale farming techniques to meet the increasing demand for farmed fish.

Additionally, it is important to consider that insects serve not only as meal replacements but also as probiotics due to their chitin and AMP content. The inclusion of insect meal in fish diets, even at relatively low levels, has the potential to enhance the immune system of fish and improve their performance, as evidenced by previous studies in other livestock species. It is also important to note that there are more than 200 species of farmed fish, and their dietary requirements remain poorly understood. Furthermore, the process of insect meal elaboration before its incorporation into animal feed warrants consideration (Nogales Mérida et al., 2018). In their 2022 overview, Hameed et al. discussed the potential benefits and opportunities of insect-based feed, emphasizing its capacity to enhance the sustainability and efficiency of aquaculture practices. The authors of the review article in question cite recent research and studies on the use of insects as a feed source for fish and other aquatic species.

## Feed additives

Modern aquafeeds generally contain the following basic ingredients:

- Fishmeal: A primary source of protein derived from fish, providing essential amino acids and omega-3 fatty acids.
- Soybean meal: A common plant-based protein source, often used to supplement fish meal.
- Corn gluten meal: Another plant-based protein source, adding both protein and energy.
- Wheat gluten: Provides protein and helps improve the binding and texture of the feed.
- Fish oil: A source of essential fatty acids, particularly omega-3, important for growth and health.

Using the following kinds feed additives in different concentrations depends on factors like species, age group, heath status and production goals and keeping conditions.

- Vitamins: Essential vitamins such as A, D, E, K, and B vitamins to support overall health and growth.
- Minerals: Essential minerals like calcium, phosphorus, magnesium, and trace minerals such as zinc, copper, and selenium.
- Binders: Ingredients like wheat bran or starch are used to improve pellet stability and texture.
- Probiotics and prebiotics: To enhance gut health and improve nutrient absorption.
- Antioxidants: Such as tocopherols or ascorbic acid to maintain feed quality and stability.
- Flavoring agents: To enhance palatability and stimulate feeding.
- Coloring agents: To improve the appearance of the feed or the coloration of the fish.

The specific composition of the ingredients may vary depending on the particular nutritional requirements of the aquaculture species in question, and may also be adapted to reflect local availability and cost-effectiveness.

A feed premix is defined as a concentrated blend of vitamins, minerals, amino acids, and other nutrients that have been formulated for addition to animal feed. The objective is to guarantee that the ultimate feed mixture provides the essential dietary requirements for any livestock in general, and also for aquaculture species, of course3. The use of a premix allows producers to efficiently balance the nutritional content of the feed, thereby enhancing animal health, growth, and productivity. Feed premixes can be formulated to meet the specific nutritional requirements of a given species or production goal. They are typically mixed with a base feed ingredient before administration to animals. A premix is defined as a blend of micro-ingredients mixed into a carrier substance. The addition of a premix to a feed is often the smallest, yet has the potential to have the most significant impact on the nutritional value of the feed. An effective premix should facilitate the uniform distribution of micro-ingredients and enhance their absorption. The selection of an appropriate carrier for the micro-ingredients can facilitate the attainment of optimal characteristics concerning flowability and homogeneity. Furthermore, the utilization of suitable and stable forms of vitamins and minerals is crucial to guarantee optimal bioavailability for the intended species. Furthermore, innovative oral delivery systems, including bio-encapsulation and enteric-coated beads, are being developed to enhance the efficacy of in-feed medications (Daniel, 2009). The objective of these advancements in aquafeed premixes is to facilitate sustainable growth in aquaculture while addressing challenges related to fish health and environmental concerns.

Modern aquafeed premixes have undergone significant developments to address concerns related to sustainability and to enhance the health of fish. While fishmeal and oil were historically the primary ingredients, their limited supply has prompted the investigation of alternative sources (Hardy, 2009). Microalgae biomass is emerging as a promising sustainable feed ingredient, offering essential nutrients and bioactive compounds that can enhance fish survival, colouration, and fillet quality (Nagappan et al., 2021). Probiotics, prebiotics, and synbiotics are increasingly incorporated into aquafeeds to improve growth performance, immune competence, and overall fish well-being (Rohani et al., 2021). These bio-friendly additives can potentially mitigate stress and enhance the composition of the intestinal microbiota. The extensive research conducted on prebiotics and probiotics has resulted in the rapid production and broad-scale application of these bioactive compounds in various fields, including medicine, nutrition, and agriculture. However, the intensification of aquaculture practices has increased the stress for aquatic animals and the environment. Various chemicals and antibiotics have been applied that cause serious problems and indirectly affect human health and even directly by producing antibiotic-resistant bacteria strains. Nevertheless, novel products and applications may significantly alter the profile of good practices in many fields, from disease prevention to water quality management, and usher in a new era of sustainable development in this field (Hancz, 2022).

The use of phytochemicals (also called phytoactive or phytobiotics) is also flourishing in aquaculture. These are alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids, and essential oils of plant-derived compounds associated with maintaining good health in the human cultural heritage of many countries. The phytochemicals contained in herbs can enhance the innate immune system and possess antimicrobial capabilities that can be used without causing environmental and/or hazardous problems. Most phytochemicals are redox-active molecules having antioxidant properties that can improve the overall physiological condition of fish, thus acting as growth promoters. Their endocrine-modulating ability can even be used for sex reversal (Chakraborty and Hancz, 2011; Chakraborty et al., 2014). Tastan and Salem (2021) and Dev et al. (2024) provided comprehensive overviews of the most recent advances in this field, emphasizing the necessity for further studies to investigate the potential synergistic effects of combined phytochemicals. Additionally, they underscored the importance of conducting more extensive research to assess the industrial applications of phytochemicals on a larger scale.

## CONCLUSIONS

Sustainability has become a key issue in all areas of human activity concerning the environment, climate, social stability and well-being, and ultimately the future of humanity in a thriving globe. Aquaculture has an important role to play as a supplier of healthy food and as a possible guardian of the good quality and biodiversity of natural waters.

Semi-intensive pond aquaculture has evolved *ab ovo* according to the principles of sustainability and so provides a model for a holistic approach to sustainability.

Feed production and feeding technology are certainly the most important areas of the aquaculture industry, both from an economic and environmental point of view, especially in different types of intensive systems. The production of balanced diets that meet the physiological needs of an ever-increasing number of aquatic animals has already yielded tremendous results and is in constant progress.

In addition to the traditional components of feed additives such as vitamins, minerals, binders and antioxidants, relatively new supplements such as prebiotics, probiotics and phytochemicals are particularly important. The latter can help reduce the use of hazardous chemicals and antibiotics.

Fortunately, all of the above areas of aquatic animal nutrition, which are of immense economic importance, are undergoing intense research and development that will help achieve sustainability goals.

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***Preliminary results***

# Comparative analysis of environmental enrichment preferences in poultry

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***ABSTRACT*** – The study, conducted at the Hungarian University of Agriculture and Life Sciences, involved five poultry species across 25 genotypes (N = 174). Environmental enrichment elements such as pumpkins, apples, corncobs, and hay were tested in pens designed to simulate real farm conditions. We monitored the consumption and weight change of these elements over a week, aiming to identify preferences and practical benefits for different poultry genotypes, contributing to improved animal welfare and potential economic efficiencies in production. TETRA SUPER HARCO consumed pumpkin and hay at rates over twice those of other layer hybrids (20 g/hen/day vs. 10 g/hen/day). The preference for red apples was markedly higher in TETRA-L SUPERB and TETRA-SL LL, with up to tenfold greater consumption compared to green apples (5 g/day/hen vs. 0.5 g/day/hen). Meat hybrid genotypes like TETRA-HB COLOR and ROSS 308 showed significant hay consumption (25 g/day/hen), surpassing layer hybrids. Native dual-purpose breeds preferred pumpkin (10 g/day/hen) and had lower consumption of hay, especially the Transylvanian bald-necked hens (3 g/day/hen). All hen genotypes showed reduced interest in enrichment elements over time. Ducks, particularly the Hungarian white, showed high consumption rates for pumpkin (up to 15 g/day/duck) and meadow hay (up to 51 g/day/duck), significantly more than other genotypes. Geese exhibited the highest consumption across all elements, with up to 74.8 g/day/goose of hay, reflecting their grazing nature. Turkeys consumed the most apples, averaging 28.3 g of red apples per individual, while guinea fowls showed lower consumption rates. Generally, softer elements like pumpkin were preferred, with the consumption of harder items such as corn being minimal. These results highlight differences in enrichment use based on genotype behavior and size, suggesting practical implications for enrichment strategies in avian management. Environmental enrichment enhanced the behavioral repertoire of all poultry species, benefiting their welfare. Laying hens preferred red apples over green, likely due to color attraction. Meat-type hens favored hay, reflecting their larger appetite and calmer behavior. Indigenous dual-purpose genotypes used enrichment elements more than intensively reared hybrids. Corn cob was minimally consumed, suggesting it's less effective as an enrichment material. Geese utilized enrichment the most, while Hungarian guinea fowl showed minimal interest, possibly due to their wilder nature. Turkey genotypes varied in their enrichment use, with a tendency towards hay. Further research with larger sample sizes and diverse enrichment forms is recommended.

***Keywords***: environmental enrichment, poultry behaviour, genotype preferences, animal welfare, consumption patterns

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## INTRODUCTION

The development and study of various environmental enrichment processes is currently in its heyday across Europe. One of the main reasons for this is that the European Council banned the keeping of laying hens in traditional cages from January 1, 2012. In addition, the fact that a significant part of the population of the developed world has increased concern for the welfare of animals also plays a big role. This is perfectly demonstrated by a 2019 survey, which clearly shows that the majority of customers (regardless of their financial situation) prefer products that state that they come from appropriate animal welfare practices (*Cornish et al.,* 2020).

Compared to other poultry raised for meat use (broiler chicken, broiler duck, broiler goose), laying hens spend much more time, up to 1-1.5 years, in production compared to 5-9 weeks. It is important that this long period of time is spent under the conditions of the husbandry technology. That is why laying hens receive the greatest professional and scientific attention among poultry species and utilization directions in terms of husbandry technology and environmental enrichment issues.

It is known that physical constraints, especially housing, significantly influence animal behavior (*Black and Hughes* 1974). Comfortable behavior can be associated with a positive emotional state in domestic hens (*Zimmerman et al.,* 2011).

According to *Jacobs et al.* (2023), the activity level of animals can be improved by modifying the environment. Enrichment, which increases the complexity and diversity of the environment, can have great benefits for poultry welfare. Playful behavior leads to a positive feeling of well-being and, although we do not yet know how much play would be optimal, it has been proven that their deprivation reduces the well-being of animals. One of the goals of environmental enrichment is to reduce or even prevent the occurrence of harmful behaviors. Overall, environmental enrichment serves to preserve the mental and physical health of poultry, thereby improving commodity production.

Continuing to highlight the importance of environmental enrichment, it can be said that visual environmental enrichment can enhance neural development. Enrichments that test the left/right hemisphere of the brain and target behavioral characteristics can prepare the birds for the specific type of adult housing environment (e.g. cage, indoor, outdoor). In addition, the use of structural enrichment elements is also necessary for the optimal development of the skeleton. Enrichment elements can improve the function of the immune system by applying mild stress factors that promote adaptability. Housing systems with a rich stimulus environment can have many benefits, including reducing fear, which facilitate later transitions to multilevel technology. Overall, it can be said that environmental enrichment is necessary for the birds' physical and "mental" health, since the final product, be it meat or eggs, largely depends on these factors. Of course, breeders must adapt to different herd preferences in order to provide each group with the most suitable environmental enrichment according to age and genotype (*Campbell et al.,* 2019).

Researchers have already tried a wide variety of environmental enrichment elements, primarily in hen keeping, such as litter materials (*Huber,* 2001; *De Haas* (2014), beak abrasive blocks (*Farkas et al.*, 2021), special beak-wearing feeders (*Runion,* 1993), brightly colored bottles, balls , rattles (*Reed,* 1993), perching bars (*Gunnarsson*, 1999; *Huber*, 1999) and we could list more. In this research, we are looking for an answer to whether a the extent to which the environmental enrichment elements (hay, apples, corncobs, pumpkins) placed in different poultry were used and consumed by the different poultry species and genotypes. Which one they like the most, and which one may be justified to use for poultry species. With the results and conclusions of our study, we hope to be able to help practitioners in creating a more stimulating and well-being environment for animals, as well as in achieving the most economical production possible.

## MATERIALS AND METHODS

The study was conducted at the Kaposvár Campus of the Hungarian University of Agriculture and Life Sciences with five different poultry species, with a total of 25 genotypes (N = 174).

Transdanubia's largest animal breeding exhibition, the "KÁN University Days" is the defining high-ranking professional event of the Hungarian University of Agriculture and Life Sciences in the autumn, at the economic poultry exhibition, the dominant breeders and breeding organizations of our country present the genotypes of various species and utilization types. We conducted our study at this location, where we created environmental conditions and conditions suitable for keeping poultry species.

After the exhibition, we carried out the tests for one test week.

The conditions of our study and the number of elements per genotype were given, and an increase in the number of elements may be justified in a further study.

At the same time, the uniqueness and specialness of our research is that we examined the preferences and practical experiences of different environmental enrichment elements used in both large and small farms in the same conditions in several genotypes of hens, turkeys, guinea fowls, ducks and geese.

**The tested genotypes were**

**Commercially available laying hybrid hen genotypes**
1. TETRA SUPER HARCO parent stock (17 weeks of life) (n = 14) 2 roosters, 12 hens (Bábolna TETRA Kft.)
2. TETRA-L SUPERB parent stock (21st week of life) (n = 13) 1 rooster, 12 hens (Bábolna TETRA Kft.)
3. TETRA-SL LL parent stock (20 weeks of life) (n = 14) 2 roosters, 12 hens (Bábolna TETRA Kft.)
4. TETRA-SL LL commercial hybrid (21st week of life) (n = 15) 15 hens (Bábolna TETRA Kft.)

**Commercially available meat-type hen genotypes:**
1. TETRA-HB COLOR parent stock (17th week of life) (n = 17) 3 roosters, 14 hens (Bábolna TETRA Kft.)
2. ROSS 308 parent stock (19 weeks of life) (n = 15) 1 rooster, 14 hens (Poultry-Tím Kft.)
3. ROSS 308 commercial hybrid (5th week of life) (n = 11) 5 roosters, 6 hens (Agro-Ciko Kft.)

**Native dual purpose hen genotypes:**
1. Hemp-seeded Hungarian hen (19 weeks of life) (n = 4) 1 rooster, 3 hens (NBGK, MGE*)
2. White Hungarian hen (19 weeks of life) (n = 3) 1 rooster, 3 hens (NBGK, MGE)
3. Yellow Hungarian hen (19 weeks of life) (n = 3) 1 rooster, 3 hens (NBGK, MGE)
4. Captive colored Hungarian hen (19 weeks of life) (n = 3) 1 rooster, 3 hens (NBGK, MGE)
5. Hemp-seeded Transylvanian bald-necked hen (19 weeks of life) (n = 3) 1 rooster, 3 hens. (NBGK, MGE)
6. Black and white Transylvanian bald-necked hen (19 weeks of life) (n = 8) 2 rooster., 6 hens. (NBGK, MGE)

(*Breeder: National Center for Biodiversity and Gene Preservation; Breeding Organization: Hungarian Livestock Gene Preservation Association)

**Turkey indigenous genotypes:**
1. Bronze turkey (1 year old) (n = 3) 1 gobbler, 2 hens (NBGK, MGE)
2. Copper turkey (1 year old) (n = 3) 1 gobbler, 2 hens (NBGK, MGE)

**Guinea fowl:**
1. Hungarian guinea fowl (1 year old) (n = 6) (NBGK, MGE)

**Duck genotypes:**
1. STIMUL-MG AS (mulard) parent stock (28 weeks of life) (n = 4; 1 drake, 3 hens) (ORVIA Magyarország Kft.)
2. White Hungarian duck (2 years) (n = 4; 1 drake, 3 hens) (NBGK, MGE)
3. Variegated (wild-colored) Hungarian duck (2 years old) (n = 4 pcs; 1 drake, 3 hens) (NBGK, MGE)
4. ST5 LOURD parent stock (65th week of life) (n = 4; 1 drake, 3 hens) (ORVIA Magyarország Kft.)

**Goose genotypes:**
1. Gray goose SI 14 parent stock (1 year) (n = 4; 1 gander, 3 geese) (ORVIA Magyarország Kft.)
2. Hungarian goose (18 weeks of life) (n = 4; 1 gander, 3 geese) (NBGK, MGE)
3. INTEGRÁL-MB 09 geese (5.5 years) (n = 4; 1 gander, 3 geese) (Integrál-Group Kft.)
4. Dunai Magyar Lúd Egyes (9th week of life) (n = 6; 1 gander, 3 geese) (ANABEST Kft.)
5. White goose SI 4 parent stock (1 year) (n = 4; 1 gander, 3 geese (ORVIA Magyarország Kft.)

All hen genotypes were of almost the same age (17-21 weeks of life).

The observation of the flock and the collection of data started after a 5-day adaptation period.

The pens with a floor area of 4 m$^2$ (2 x 2 m) were littered with dust-free softwood shavings (10 cm thick) (picture 2, picture 3). A suspended hand-filled self-feeder was placed in the scratching area littered with wood shavings, from which the animals could consume the commercially available feed ad libitum, drinking water from a manually filled open surfaced self-drinker (picture 3).

The temperature in the barn during the test was usually 15-18ºC. We used LED (Dilaco Lighting Agro Star LED Spot) lighting for 16 hours a day (sunrise: start: 3:45, duration: 30 minutes, light: 4:15; sunset: start: 19:45, duration: 30 minutes, total darkness: 20:15).

**Picture 1.** Placement of the pens, part of the stock

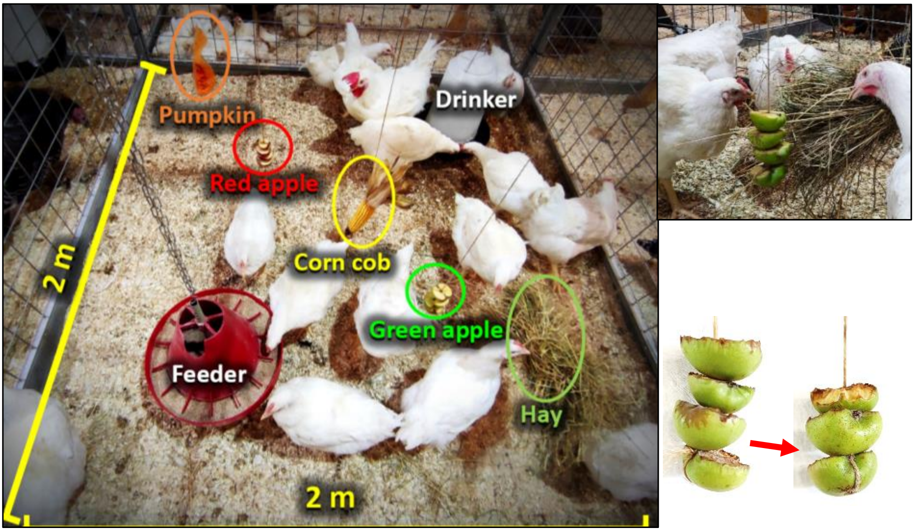
Five different environmental enrichment elements were suspended in the pens, the center of which reached the height of the animals' backs (picture 3). The tested environmental enrichment elements were the following:

1. Pumpkin (Canadian pumpkin, 'Orange' variety) quartered along the longitudinal axis
2. Red 'Jonathan' apple (4 half apples strung on top of each other, small juice apple category, more acidic, sour taste)
3. Corn cob
4. Green 'Mutsu' apple (4 half apples strung on top of each other, small juice apple category, sweet taste)
5. Meadow mixed hay

The environmental enrichment elements were suspended in the same order and distance from each other and other technological elements in all pens for all genotypes (picture 3).

If there were more replicates per genotype, we would have suspended them randomly.

If an environmental enrichment element was completely used up, it was immediately replaced.




**Picture 2.** The layout of the test pen and the environmental enrichment elements, consumption and weight loss

The observation of the flock, the installation of environmental enrichment elements and the collection of data started after a 5-day adaptation period and took place over the course of a test week.

**Picture 3.** The layout of the test pens and the placement of the inserted environmental enrichment elements, with the turkey genotypes in front

During the observations, we remeasured the weight change of the various environmental enriching elements daily, thus the extent of their weight loss. We also monitored the feed consumption of the animals.

In our research, we deliberately used environmental enrichment elements that could also function as food. After all, the wild ancestor of the domestic fowl, the red jungle fowl, spends most of its active time almost constantly searching for and eating food (*Dawkins*, 1989; *Deemling and Bubier*, 1999) and even caged laying hens spend around 40% of their time feeding filled (*Horn,* 1981). Stimulus enrichment related to nutrition is the one that is most suitable for arousing and binding the animal's interest compared to other diverse but less used stimulus enrichment elements (e.g. chain, ball, mirror, shelf, perch, etc.). In case of a larger consumption, it may be necessary for economical production to calculate the amount and content of the expected consumption of stimulus-enriching elements during feeding and production. Examining production indicators was not the aim of the research.

## RESULTS

### Use of environmental enrichment elements in the case of different genotypes of laying hens

Among the layer hybrid genotypes, we had the opportunity to observe three groups of parent stock and the TETRA-SL LL commercial hybrid.

The consumption of pumpkin and hay by TETRA SUPER HARCO, shown in the first half of the diagram, proved to be exceptionally high, more than twice that of the other three test groups, even the red apple was preferred by the light-bodied TETRA-L SUPERB parent stock with a lively temperament and the medium-heavy TETRA-SL LL hybrid (Figure 1). The green apple consumption was almost exactly the same for TETRA SUPER HARCO and the TETRA-SL LL hybrid, while the other two genotypes only consumed half of it during the study period. In the case of all genotypes, it is clear that the attention of the layer hybrids was captured by the corncob the least during the observation, they only consumed a few grams of it in a week. Due to its physical properties, this environmental enrichment element was less able to be consumed by the hen.

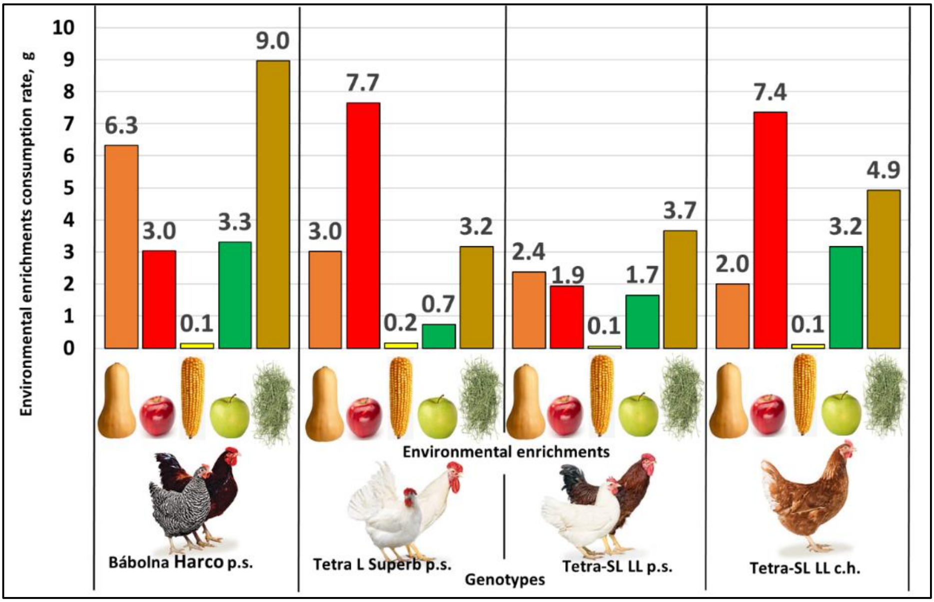



**Figure 1.** Average daily weight loss of different environmental enrichment elements during the test week for the different genotypes of laying hens (g/bird)

By inserting the two different apples, we searched for the answer to which one the hen would prefer if she chose the apple. It is worth using red or green apples in practice, if it comes down to it.

Overall, it can be said that we experienced a higher consumption of red apples, since all genotypes, except for TETRA SUPER HARCO, consumed red apples to a greater extent, which difference in the evening of the TETRA-SL LL hybrid is double, and in the case of the light-bodied TETRA-L SUPERB parent stock, more than it was tenfold.

## Use of environmental enrichment elements in the case of different meat hybrid genotypes

Among the environmental enrichment elements, the hay consumption of the TETRA-HB COLOR and ROSS 308 parent stock was outstanding. It is interesting that the parent stock TETRA-HB COLOR, which gives premium meat quality, and which grows more slowly, and ROSS 308, which is used for the production of typical large-scale chickens, consumed hay in a similar proportion (picture 4), which surpassed the hay consumption of the previously presented layer hybrids (TETRA SUPER HARCO except).

One of the reasons for this high consumption rate may be that these genotypes have a high appetite and feed consumption. It is also characteristic that these calmer, more phlegmatic genotypes ate more of the easier-to-consume environmental enrichment element.

By the way, it is a well-known fact from practical application that straw, which is somewhat similar to hay, attracts the attention of broiler chickens to a large extent and also has a positive effect in reducing foot diseases (*Baxter et al.,* 2018).

The consumption of pumpkin and apples was similar for these two genotypes, showing a value of about one third of the hay, while the meat-type hens paid almost no attention to corn, and used roughly the same amount as the layer hybrids.

The evaluation of the ROSS 308-a broiler final product was left to the end because this genotype was a rather special case. There was hardly any measurable consumption of environmental enrichment elements by these birds. On the other hand, their consumption of mixed feed was exceptionally high compared to the other genotypes, well representing the meat type's high appetite serving its outstanding growth potential.

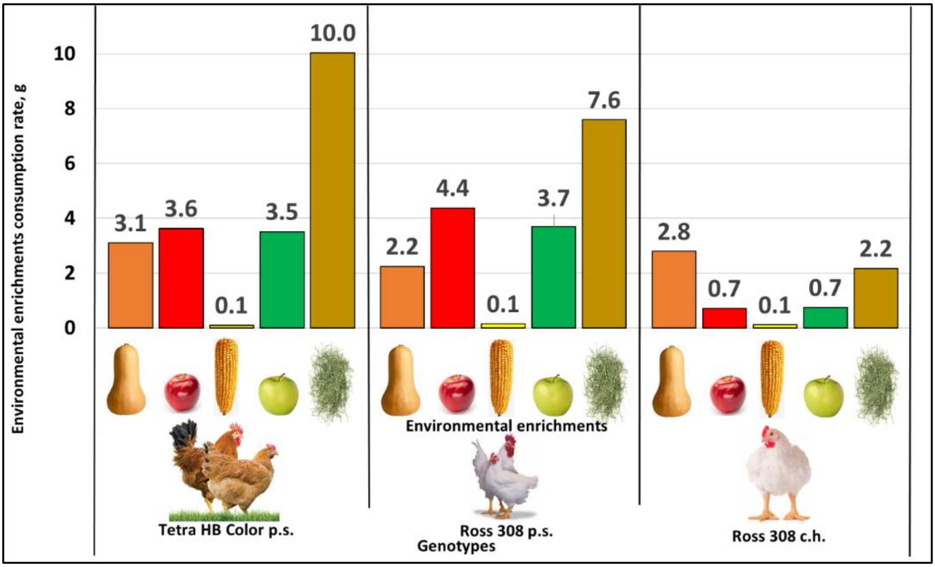



**Figure 2.** Average daily weight loss of different environmental enrichment elements during the test week for different meat type hen genotypes (g/bird)

There was no meaningful, significant difference between the consumption of red and green apples, they were consumed to approximately the same extent.

With their phlegmatic and calm temperament, we can explain that they "concentrated" only on eating the food placed in front of them, and then on resting afterwards.

**Picture 4.** Hay consumption of the meat-type TETRA-HB COLOR and ROSS 308 parent stock

We also found that the interest in environmental enrichment elements decreased somewhat during the study period, even in the study by *Ohara et al.* (2015), where it was established that the use of environmental enrichment elements gradually decreased as the age of the birds increased.

## Use of environmental enrichment elements in the case of native dual-use hen genotypes

The native dual-purpose breeds shown in the diagram represented a completely different category than the genotypes discussed so far.

On the other hand, they paid at least as much, if not more, attention to the environmental enrichment elements placed in the voliere than their egg and meat hybrid counterparts (Figure 3). In the pens of almost all indigenous poultry breeds, pumpkin consumption was the highest with values of around 10 g/hen, followed by red apples and hay, not far behind. Green apple was consumed moderately in the pens of most genotypes, except for Hemp seed color Hungarian hens, where the amount used by the animals was a slightly higher

6.8 g/hen. These birds also ate about as much of the corn cob as the previously mentioned genotypes.

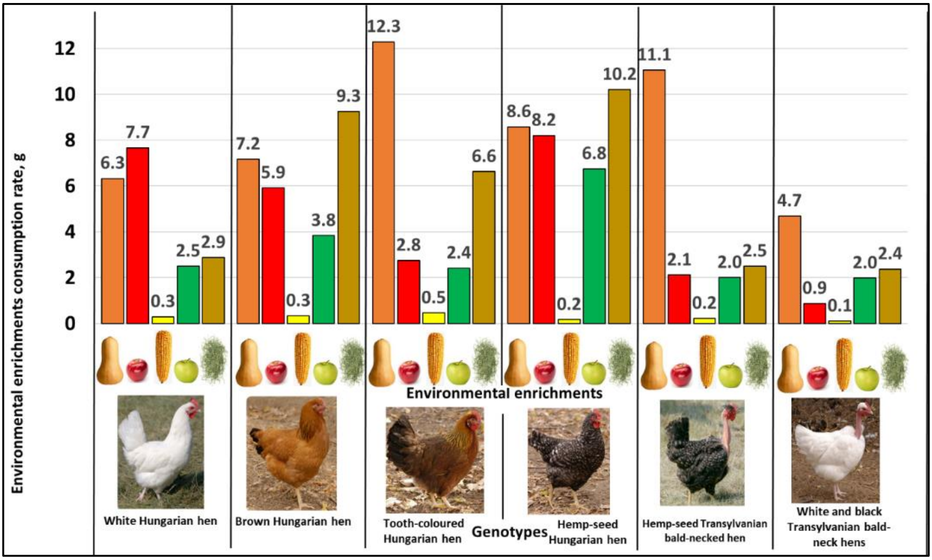


**Figure 3.** Average daily weight loss of different environmental enrichment elements during the test week for the different native dual purpose hen genotypes (g/bird)

Overall, it can be said that among the six indigenous breeds, the black and white Transylvanian bald-necked hens had the lowest weight loss from all environmental enrichment elements. The bald-necked ones consumed less hay specifically, one of the reasons for which may be that the protruding harder, sharper straw fibers could have irritated or poked the bald neck area, which could have disturbed them in eating hay.

These dual-use breeds, suitable for indoor or outdoor conditions, made extensive use of all environmental enrichment elements, one of the reasons for which is the wilder, more natural life instinct that can be traced back to the wilder genotype.

In addition, it may be worth mentioning that the genotypes of TETRA and ROSS were kept in intensive housing conditions before the test, so they did not encounter hay and other environmental elements and factors occurring in semi-intensive conditions.

On the other hand, the indigenous dual-use Hungarian genotypes were raised in semi-intensive conditions. That is why we prefer to compare them with each other, which is also why they are included in a diagram.

## Use of environmental enrichment elements in the case of the studied duck genotypes

The consumption of environmental enrichment elements of the studied duck genotypes is in some cases much higher compared to the hen-shaped ones discussed so far (Figure 4).

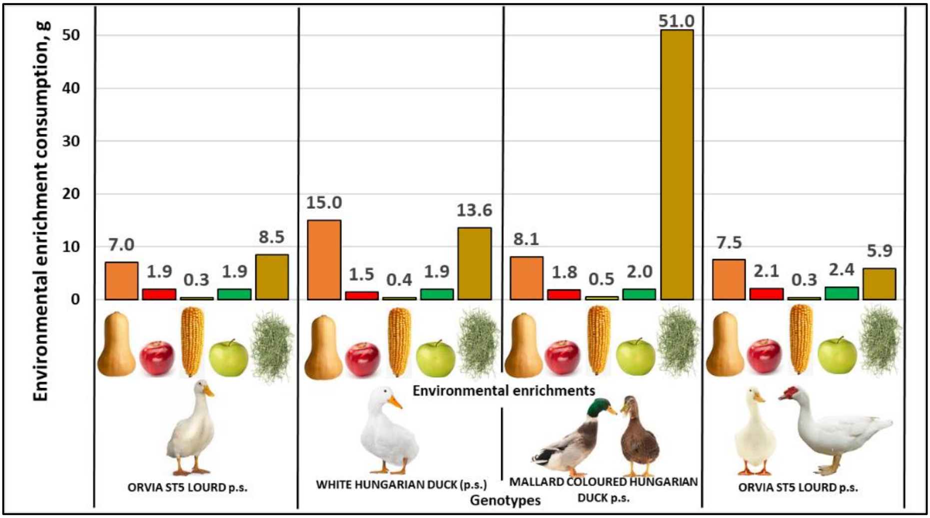



**Figure 4.** Average daily weight loss of different environmental enrichment elements during the test week for different duck genotypes (g/duck)

This can be explained by their visibly larger larger body size and the fact that ducks especially like to peck and search with their beaks. With the exception of the white Hungarian duck, we measured approximately the same pumpkin weight loss in the pens of the other three genotypes, 7-8.1 grams per duck. The Hungarian white duck consumed 15 grams/duck of this environmental enrichment element, which is twice that of the other breeds. The consumption of the two types of apple and the corn was similar for the four genotypes, even the inserted meadow hay was used quite differently, because in the pen of the variegated (wild-colored) Hungarian ducks, we measured a weight loss of 51g/duck during the test period, one of the reasons for which is the genetic background, i.e. it is also to be found in the wilder, more natural temperament. In the case of the other genotypes, this was only between 5.9 and 13.6 g/individual.

Overall, we observed that the duck breeds, like the other genotypes participating in the study, first consumed the soft, easier-to-eat inside of the pumpkin, and then switched to its harder flesh. In relation to their consumption of hay, we noted that they did consume part of the ducks as an element that enriches the environment, but they just "played" with the other part.

## Use of environmental enrichment elements in the case of the investigated goose genotypes

It can be confidently stated that for the five environmental enrichment elements, the highest weight loss was measured in the pens of the goose genotypes (Figure 5).

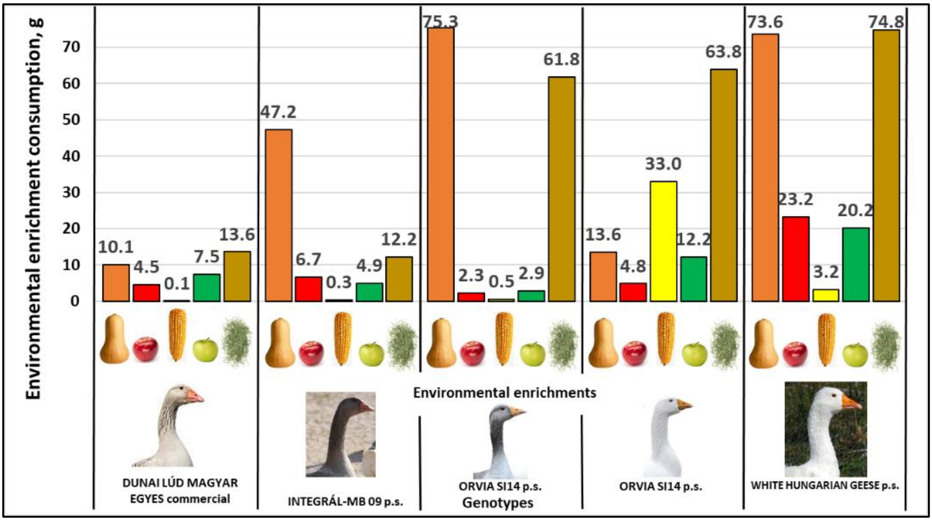



**Figure 5.** Average daily weight loss of different environmental enrichment elements during the test week for different goose genotypes (g/goose)

Geese are known to be excellent grazers, they really like to peck and use their strong beaks to dig into harder materials. Wild specimens and their domesticated but free-range relatives both graze a lot and are very curious, which is why they taste almost everything (*Vickery and Gill,* 1999). For this reason, we fixed the environmental enrichment elements in the pens of the geese participating in the study with a material that they cannot catch. Regardless of this, there was an example of some environmentally enriching element being pinched to such an extent that it ended up in the litter. In such a case, the inserted food was completely consumed within a short time, pointing out that the spatial location of the potential food intended as an environmental enrichment element can also affect the level of consumption. If an experimental item ran out, we naturally replaced it as soon as possible.

We can cite the INTEGRÁL-MB 09 breeding animals, the two parent stock of geese, and the bar chart of the Hungarian goose as a perfect example of this, as it perfectly illustrates how much higher weight loss could be measured in the event that the environmental enrichment element was added to the litter. It can therefore be said that the geese were most interested in the five environmental enrichment elements when they were below the litter level.

However, striking differences in the consumption of individual environmental enrichment elements can also be observed between the investigated goose genotypes. We measured weight loss of 10.1-13.6 g/goose even in the pen of the parent stock of the Dunai lúd goose and the white goose, while the other three genotypes consumed almost five times and seven times this amount during the test period.

The same division can be said about the hay consumption of goose breeds. Individuals of the two genotypes shown in the first half of the table consumed only 12.2-13.6 grams, and in the pens of the other three groups of geese, We measured a weight loss of 61.8-74.8 g/goose during the week.

## Use of environmental enrichment elements for the examined turkey genotypes and guinea fowls

The consumption of the environmental enrichment element developed very differently for these three genotypes. These breeds were not overly interested in corn either, the consumption of the individuals was between 0.2 and 0.7 grams. Pumpkin was consumed by turkeys to about the same extent, guinea fowls were somewhat less interested in it (Figure 6).

The two types of apples were consumed prominently by the turkey compared to the other two genotypes. In this booth, an individual consumed an average of 28.3 grams of red apples in one week, and 18.4 grams of green apples. The apple consumption of the other two genotypes was only a fraction of these values. The two genotypes of turkeys used at least twice as much hay as the Hungarian guinea fowl.

We would like to note that for these breeds (bronze turkey, copper turkey, Hungarian guinea fowl), I measured the greatest weight loss on environmental enrichment elements in the last two days.

It may be that even according to our experience, these wilder genotypes had the hardest time getting used to their new place, perhaps the five-day adaptation period before the test proved to be too little for them and they only start to show their natural behavior after that.

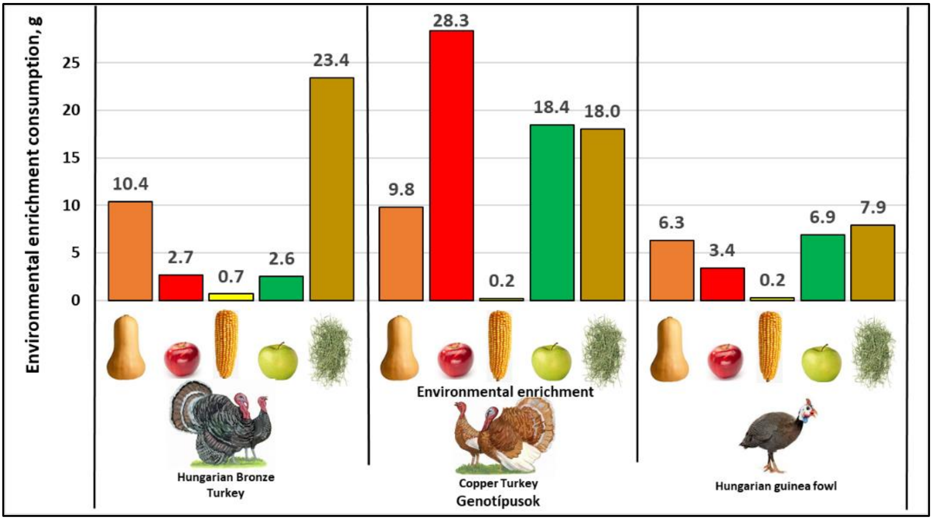



**Figure 6.** Average daily weight loss of different environmental enrichment elements during the test week for turkey and guinea fowl genotypes (g/bird)

## Other observations and comments

In summary, we found that based on our observations, the hen genotypes used in the production of hybrids and their end products proved to be the most suitable for rapid adaptation, their rhythm of life and behavior were least affected by the new, never-before-seen environment. In the case of native genotypes, it is conceivable that during semi-intensive education, they could have encountered more diverse environmental factors and been familiar with them.

Focusing on the corncob as the environmental enrichment factor used in our study, our view is that the majority of the animals were not so interested in this element because it proved to be difficult to obtain.

Because if the corn had been placed in front of the animals not in the form of a tube, but crumbled, so that we could have measured a higher weight loss, it would have functioned more as feed than as an environmental enrichment element.

Most of the examined genotypes usually only consumed the softer, seedier, fibrous, easier-to-squeeze part of the pumpkin placed in the booth, which, in turn, helped the longer-term use of this element.

Hay was one of the first to attract the attention of birds everywhere, but mostly this element was not consumed for nutritional purposes, rather they pulled the fibers out of the bundle out of curiosity and then threw them into the litter.

In practice, for large farms, other, simpler forms of placement of these environmental enrichment elements are recommended, which are less labor-intensive, such as distribution in a feeding basket, net or trough system, depending on the material and species.

During the test week, we did not experience any injuries or deaths, in which the use of environmental enrichment elements could have played a role, because according to *Reed et al.* (1993), environmental enrichment during production can be an important factor that affects the level of fear of adult birds and can reduce the risk of injuries.

In addition, according to *Son et al.* (2022), alfalfa hay effectively alleviates the stress that occurs in animals during keeping, and is also able to improve the production of laying hens in flocks kept in aviaries.

The aim of the thesis was not to examine the production and feeding of animals. However, we considered it important to record the amount of daily feed consumption of the different genotypes, which has an informative nature.

Table 1 only describes the feed consumption of those hen genotypes that are in domestic and international trade, have high production potential and economic weight. In addition, the daily feed consumption data corresponding to the given age were presented from the public product brochures of the breeding companies.

In relation to almost all parent pairs, it can be clearly seen that the laying hens in our study consumed more feed than the daily feed consumption amounts given by the breeding company, even with the consumption of stimulus-enriching elements that can function as feed. The simple reason for this is that, in this life stage, the parent pair is kept with naturally dosed feeding based on the recommendation of the breeding company, while in my study we fed ad libitum. It is also known that laying hens consume more in addition to ad libitum feeding.

From the point of view of production and feed consumption, we obtain truly relevant and usable data regarding the bottom line, in the case of the final product ROSS 308. Commercially available feed was available to them ad libitum both in colony conditions and in the study. In our study, they consumed 25% less feed than the daily feed consumption data provided by the breeding company. From this, I conclude that the consumption of stimulus-enriching elements could also reduce the amount of feed intake. Although, according to our measurements, 6.5 g/day/individual was lost, which does not necessarily explain the 46 g lower feed consumption. There is probably another reason for this. For example, the feed we feed is not exactly the same as described in the ROSS 308 final product brochure, and in our study there was 16 hours of lighting, while the ROSS 308 final product brochure states 18 hours of lighting at this age, which of course affects feed consumption.

**Table 1**

Development of daily feed consumption of different hen genotypes

| Genotype | Feed consumption g/day/bird | Literature data (g) | Difference (g) |
|---|---|---|---|
| TETRA SUPER HARCO p.s. (12 h., 1 r.) ***17th week of life*** | 133 | 87[1] | +46 |
| TETRA-L SUPERB p.s. (12 h., 1 r.) ***21st week of life*** | 83 | 95[2] | -12 |
| TETRA-HB COLOR p.s. (14 h., 3 r.) ***17th week of life*** | 203 | 115[3] (21. week of life) | +88 |
| TETRA-SL LL p.s. (12 h., 2 r.) ***20th week of life*** | 102 | 90[4] | +12 |
| TETRA-SL LL c.h. (15 h.) ***21st week of life*** | 104 | 97[5] | +7 |
| ROSS 308 p.s. (14 h., 1 r.) ***19th week of life*** | 223 | 99[6] | +124 |
| ROSS 308 c.h. (11 ) ***5th week of life*** | **134** | **180[7]** | **-46** |

[1]: BÁBOLNA HARCO LAYER Parent Stock Chart and Graphs (*URL1*); [2]: TETRA L SUPERB Parent Stock Management Guide (*URL2*); [3]: TETRA HB COLOR Parent Stock (*URL3*); [4]: TETRA-SL LL Parent Stock Management Guide (*URL4*); [5]: TETRA-SL LL tojóhibrid Táblázatok és grafikonok (*URL5*); [6]: ROSS 308 Performance Objectives 2016 (*URL6*); [7]: ROSS 308 ROSS 308 FF Performance Objectives 2022 (*URL7*)

## CONCLUSIONS

Based on the results of our study, we concluded that the presence of environmental enrichment elements may have increased the behavioral repertoire of all poultry species, since they dealt with these different diverse environmental enrichment elements, which is particularly beneficial from an animal welfare point of view. The enrichment elements, to varying degrees, attracted the animals' interest as they consumed and utilized them. Since laying hybrid genotypes generally consumed more red apples than green ones, we conclude that laying hens prefer red apples over green ones as an enrichment element.

We hypothesize that this preference may be due to a greater attraction to the color red rather than green, with taste likely playing a secondary role.

The meat-type parent stock hens predominantly consumed hay, which we attribute to their larger appetite and calmer temperament, leading to a feeding behavior that prioritizes easily, quickly, and abundantly available enrichment elements.

Indigenous dual-purpose genotypes, reared semi-intensively, made greater use of the enrichment elements compared to intensively reared modern laying and meat hybrid parent stock and commercial genotypes.

Only a few grams of corn cob, used as a environmental enrichment, were consumed by the various poultry species over a week. From this, we infer that among the tested enrichment elements (pumpkin, red apple, green apple, hay, corn), corn is the least effective as a stimulant. We believe this may be due to its physical properties, making it difficult to consume in this form. It may be beneficial to fix it in place, such as on a wall, to make it easier to consume.

Despite this, they may have dealt with it relatively much, tweaked it, it may have achieved its goal of enriching the environment, but they could not consume it.

Since the ROSS 308 mixed-sex final product consumed the least amount of all the enrichment elements, it can be concluded that among all the studied poultry species and utilization directions, the use of these elements is least justified in broiler chicken farming.

Geese, compared to other commercial poultry species, utilized the enrichment elements the most, especially hay, which is due to their species-specific characteristics. As they are highly inclined to use enrichment elements, their application is recommended, particularly in housing conditions and age groups where reducing stress and aggressive interactions is necessary.

The Hungarian guinea fowl consumed relatively small amounts of all the enrichment elements placed in their enclosure. This could be due to their wilder nature, as they are at a lower level of domestication and are more cautious. Further research is recommended to study the use of enrichment elements in larger housing systems and under calmer conditions.

The two turkey genotypes showed different usage patterns of the enrichment elements. However, like the geese, they consumed relatively more hay, likely due to their lower level of domestication, large body size, and strong beaks.

For more comprehensive and in-depth conclusions, further studies with larger sample sizes and more repetitions are recommended. Standardizing rearing conditions for the same poultry genotypes is also important. Additionally, it is crucial to examine the effects of different enrichment materials on production and aggression. It is also recommended to explore offering the enrichment elements we studied in different, fixed forms, as well as testing other enrichment materials across all poultry species.

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***Irodalmi áttekintés***

# Fumonizin mikotoxinok átalakulása az emésztés során, toxikus hatásuk, valamint biológiai hatástalanításuk

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***ABSTRACT*** - **Fumonisin mycotoxins: the effect of digestion on the different forms. Toxic effects and biological detoxification. - Review**
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Mycotoxin contamination can occur at almost all levels of food production, processing, storage and distribution, and causes significant economic damage in animal husbandry, animal and crop production. Ingestion of foodborne mycotoxins can cause numerous diseases and health impairments. This review presents the different forms of fumonisin that can occur in foods and feeds, as well as the possible effects of digestion on these forms. The description of the toxicity of fumonisins includes the biochemical background, the different degree of toxicity of individual fumonisin metabolites, and the caused detrimental health effects on different species. The biological detoxification of mycotoxins has an advantage over physical and chemical methods with respect to nutrient losses, therefore this review focuses on the biological processes that can lead to the elimination of fumonisins. The presented methods involve bacterial binding and degradation that can promote the detoxification of fumonisins consumed with food or feed.

***Keywords***: fumonisin, gastrointestinal tract, food matrix, digestion, bioaccessibility

## BEVEZETÉS

A penészgomba populációk szaporodásuk azon fázisában, mikor képződésük és pusztulásuk egyensúlyba kerül, szekunder anyagcseretermékeket hoznak létre. Egyes penészgomba fajok szekunder anyagcseretermékként mikotoxinokat is termelhetnek. A mikotoxinok kialakulásához nem elégséges csupán az adott faj genetikai potenciálja, hanem az alábbi környezeti feltételeknek is teljesülniük kell: 1. megfelelő hőmérséklet, 2. megfelelő nedvességtartalom, 3.

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elegendő oxigén, 4. megfelelő és elegendő mennyiségű szubsztrát, 5. szükséges relatív páratartalom. A biogazdálkodás térhódítása és a növényvédőszerek egyre hangsúlyosabb kiszorulása növeli a penészgombák túlélési esélyeit, ezáltal a mikotoxinok termelődése sem kerül kellő mértékben visszaszorításra. Ezen felül egyes rovarok hozzájárulnak a penészgombák terjesztéséhez, melynek következtében a növények nagyobb arányban fertőződhetnek (*Freire* és mtsai, 2017; *Kovács*, 2019). A mikotoxinok kis molekulatömegük miatt kifejezett antigénhatással nem rendelkező toxikus vegyületek. Elviselik a magas hőmérsékletű (100-110 °C) hőkezelést, nem bomlanak le a főzés, melegítés során. Kémiai stabilitásuknak köszönhetően az élelmiszerek tárolása és feldolgozása során sem bomlanak le (*Dawlal* és mtsai, 2019). A gyomornedv sósavtartalmának ellenállnak, a szervezetben akkumulálódhatnak. A placentán átjutva a magzatra is veszélyt jelentenek ezek a metabolitok, valamint tejjel kiválasztódva az újszülött egészségét is károsítják. A *FAO* adatai szerint a világ gabonatermelésének körülbelül 25%-át az FB1 szennyezettségnek leginkább kitett gabonák - az árpa, búza és kukorica - adják, melyek nem csak a humán táplálkozásnak, hanem az abrakfogyasztó haszonállatok (sertés, baromfi, kérődzők) takarmányainak alapját is képezik. Súlyos esetben a szennyezett tételek fogyasztásra, takarmányozásra már nem alkalmasak, ezért azok növelik az élelmiszerveszteség mértékét. Továbbá kiemelendő tény, hogy a mikotoxinok által kiváltott betegségek gyógykezelési költségei növekvő tendenciát mutatnak. A mikotoxinnal szennyezett élelmiszer vagy takarmány elfogyasztása a gyomor-bélrendszeri traktust érinti elsőként. A bélrendszer normál működése, a szervezet teljes egészére hatással van (emésztés, abszorpció, helyi immunválasz, mikrobiom összetétele). Az emésztőtraktus egyik feladata a patogén kórokozókkal és toxinokkal szembeni védekezés. A fumonizinek és származékaik a vékonybélből szívódnak fel, a véráramon keresztül számos szervhez (máj, vese, tüdő, nyelőcső, agy) eljutnak és kifejtik káros hatásaikat. Mindezekből következik, hogy a mikotoxinok jelenlétével és a mikotoxinok és származékaik okozta megbetegedések (mikotoxikózisok) veszélyével fokozottan számolni kell a jövőben, amennyiben nem teszünk hatékony lépéseket annak érdekében, hogy termelődésüket visszaszorítsuk, vagy mennyiségüket a kontaminálódott táplálékban csökkentsük.

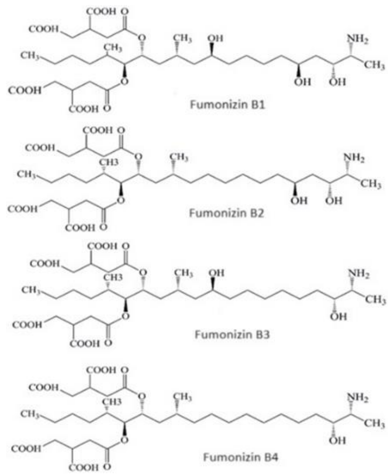
Az 1960-as évek óta a mikotoxinok mintegy 400 féle változatát fedezték fel (*Liu* és mtsai, 2022). Világszinten a táplálékban leggyakrabban előforduló mikotoxinok az *aflatoxinok* (AFB1, AFB2, AFG1, AFG2, AFM1); a *zearalenon* (ZEA); az *ochratoxinok*; a *fumonizinek* (főként FB1, FB2, FB3, FB4); a *trichotecének* (főként DON, T-2, HT-2) és a *patulin* (PAT) (*Liu* és mtsai, 2022).

## A FUMONIZINEK SZERKEZETE ÉS ELŐFORDULÁSI FORMÁI A TÁPLÁLÉKBAN

A fumonizinek a poliketidek csoportjába tartozó természetes bioaktív anyagok, amelyeket különböző mikroorganizmusok termelnek és a gazdaszervezetre nézve káros hatással rendelkeznek (*Ridley*, 2009; *Braun* és mtsai, 2017). A fumonizineket főként a *Fusarium* penészgombafajok termelik, elsősorban a *F. verticillioides* és a *F. proliferatum*, de a fekete *Aspergillus* és néhány *Tolypocladium* penészgomba faj esetében is megfigyelték már a fumonizinek termelődését (*Frisvad* és mtsai, 2007; *Mogensen* és mtsai, 2010). A fumonizineknek többféle szerkezeti formája ismert, a szakirodalom négy fő fumonizinanalóg csoportot különböztet meg (A, B, C, P), melyek közül a legelterjedtebb a fumonizin B1, B2, B3 és B4. A B-csoporton belül a fumonizin B1 fordul elő a leggyakrabban (*Kovács*, 2019). A fumonizin B-csoportot képviselő vegyületek közül az FB1-3 előfordulási aránya kukoricában a következőképpen alakul: FB1:FB2:FB3 – 12:4:1 (*Li* és mtsai, 2024). A FB4 a többihez képest nagyon kis mennyiségben fordul elő. Magyar kutatók írták le először, hogy egyes F. verticillioides törzsek képesek termelni zsírsavval acilezett fumonizineket (*O- és N*-acil-FB1 származékok) is, amelyek jóval toxikusabbak, mint maga az FB1 toxin (*Bartók* és mtsai 2010; 2013; *Csenki* és mtsai, 2023).

A fumonizinek szerkezetileg hidroxi-eikozán származékok észterei (*Kovács*, 2019). A 20 szénatomos aminopentol alapvázhoz két propán-1,2,3-trikarbonsav oldallánc kapcsolódik észterkötéssel, illetve egy szabad aminocsoporttal is rendelkeznek (*Braun* és mtsai, 2017). Az FB analógok szerkezetükben csak kis mértékben térnek el egymástól. A FB1 toxin három hidroxilcsoportot, az FB2 és FB3 toxinok kettőt (az egyik hidroxil-csoport helyzetében különböznek egymástól), míg a FB4 csak egyetlen hidroxilcsoportot tartalmaz (1. ábra)

A fuzárium fajok számos szántóföldi kultúrnövényen elszaporodhatnak, ezek közül a kukorica és a búza a legjelentősebbek. Jelenlétük befolyásolja a növény növekedését és a termésmennyiséget. A fuzárium gomba a növény fejlődésének minden szakaszában képes megtámadni a gyökeret, szárat és a kalászt, mialatt a penészgomba már elkezdi a mikotoxin termelését. A növények betakarítása, szárítása, tárolása során is folytatódik a mikotoxinok kialakulása, ezzel szennyezve az élelmiszer-, és takarmány-alapanyagot (*Iqbal* és mtsai, 2021). A fertőzött növény a penészgombával szembeni védekezés során képes a mikotoxinok szerkezetét módosítani, az így keletkező vegyületeket „maszkolt” mikotoxinoknak nevezzük (*Rychlik* és mtsai, 2014). A „maszkolt” mikotoxin kémiai módosítás révén jön létre xenobiotikumok hatására. A folyamat következtében a mikotoxinok számos folyamat (konjugáció, hidrolízis, redukció, oxidáció) eredményeként a növény számára kevésbé toxikus molekulákká alakulnak át, mint amilyen az eredeti forma volt. A módosítás elősegíti a növénynek, hogy ezeket az átalakított metabolitokat a vakuólumokba zárja, majd ezen molekulák jellemzően egyesülnek a növényi sejtfal komponensekkel (*Freire* és mtsai, 2017). A glikozilálással megvalósuló konjugáció a növény számára védelmet nyújt, azonban az ember és az állatok szervezetében az emésztőrendszerben az endogén enzimes és/vagy a mikrobiális folyamatok dekonjugációt eredményezhetnek, azaz a glikozidkötés hidrolízisével felszabadulhat az eredeti forma, ezáltal „aktiválódik" a mikotoxin (*Rychlik* és mtsai, 2014; *Jin* és mtsai, 2021).




**1. ábra.** A fumonizin B1-4 analógok kémiai szerkezete (*Kostic* és mtsai, 2019)

A mikotoxinok többféle formában fordulnak elő a táplálékban, amelynek azért van jelentősége, mivel az egyes formák biológiai hozzáférhetősége (bioassessibility) és toxicitása eltérhet egymástól. *Rychlik* és munkatársai csoportosítása szerint (2014) a szabad, módosítatlan forma (1) mellett jelen lehetnek mátrixhoz fizikailag vagy kémiailag kötött formában (2), valamint módosított formában (3) egyaránt. A módosítás lehet biológiai eredetű (3a) (pl. „maszkolt" mikotoxinok a növények esetében) vagy tisztán kémiai eredetű (3b). Utóbbi esetben a kémiai reakciókat az alapanyagok feldolgozása során alkalmazott lépések (pl. termikus eljárások) eredményezik. Emésztés során, illetve az emésztőrendszerben zajló mikrobiális folyamatok révén a módosult formák részben visszaalakulhatnak az eredeti szabad (1) formává.

A mátrixhoz kötött mikotoxinok (2) egy része az élelmiszer- vagy takarmánymátrixban fizikailag körbezárva helyezkedik el, míg más részük kovalens kötéssel kapcsolódik a makromolekulákhoz. A fumonizin mikotoxinok a trikarballilsav-részeik funkciós csoportjai révén képesek kovalensen kapcsolódni a szénhidrátokhoz és fehérjékhez (*Shier* és mtsai, 2000, *Seefelder* és mtsai, 2003). A mátrixhoz kötött mikotoxinok jelentős része felszabadulhat az emésztés során, mikor a fizikai barriert képző makromolekulák hidrolizálnak az endogén enzimek hatására. A keményítőhöz kötött fumonizin mikotoxinok szabad fumonizinekké alakulhatnak az emésztés során (*Humpf* és *Voss*, 2004). A táplálékban a kötött formában jelen lévő FB1 mikotoxint főként kukoricából készült ételekben mutatták ki, azonban a mátrixhoz kötött forma jelen volt ezen kívül kukoricapehelyben, sörben, borban, rizsben, szójában, szárított gyümölcsökben, diófélékben és kutyatápban is (*Manyes* és mtsai, 2013; *Tan* és mtsai, 2022; *Yang* és mtsai, 2023). A mátrixhoz kötött forma azt takarja, hogy a mikotoxin és a tápanyagkomponensek együttesen egy komplexet alkotnak. A mikotoxinok elsődlegesen nagyobb makromolekulákhoz kötődnek (fehérje, keményítő, cellulóz, hemicellulóz) (*Tan* és mtsai, 2022). A kötés módjának abból a szempontból van jelentősége, hogy a bevitelt követően a mátrixból milyen ütemben szabadulnak fel a mikotoxinok az emésztés során, illetve a makromolekulához már nem kötődő forma milyen további átalakulásokban tud részt venni pl. hidrolízis, acilezés, mivel ez befolyásolja az eredeti forma biológiai hozzáférhetőségét.

A biológiailag módosított mikotoxinok (3a) úgy jönnek létre, hogy a mikroszkópikus penészgombák által képzett eredeti mikotoxin-szerkezet módosul, mikor a méreganyag bekerül egy másik biológiai rendszerbe (növények, állatok, gombák vagy mikrobák) és a méreganyagot felvevő szervezet megpróbálja azt detoxifikálni. Például egyes növények képesek a zearalenont és a dezoxinivalenolt glikozid képzéssel átalakítani, amely a növény számára detoxifikációt jelent, azonban a növényt táplálékként felvevő szervezetben ez a módosulat hidrolízis során visszaalakulhat az eredeti formává (*Rychlik* és mtsai, 2014; *Jin* és mtsai, 2021).

A kémiailag módosított mikotoxinok (3b) esetében az áll az átalakulás hátterében, hogy az élelmiszer vagy takarmány előállítása során alkalmazott termikus és egyéb kezelések a mikotoxinok kémiai szerkezetének változását eredményezik. A fumonizin B1 szabad aminocsoportja képes reagálni a redukáló cukrokkal a Maillard reakcióban, így termikus műveletek esetében N-(1-dezoxi-D-fruktóz-1-il)-fumonizin B1, valamint N-(karboximetil)-fumonizin B1 származékok keletkeznek (*Humpf* és *Voss*, 2004; *Falavigna* és mtsai, 2012). Az élelmiszerek lúgos kezelése (*Humpf* és *Voss*, 2004) során a fumonizin B trikarballilsav-részei hidrolizálhatnak (*Du* és mtsai, 2017).

A (3b) csoporthoz abban hasonlít a (2) csoport, hogy a mátrixhoz kötött mikotoxinok esetében is kialakulhat kémiai kötés a mikotoxin és a biomolekulák között, azonban a (2) csoport esetében a mikotoxin makromolekulához kapcsolódik, így a táplálék alap-mátrixához „rögzül", míg a (3b) csoportba tartozó reakcióknál módosul(nak) a mikotoxinok funkciós csoportja(i), ez azonban nem jár makromolekuláris kötődéssel. A kémiai módosulás mindkét esetben (2 és 3b) többnyire az alapanyag feldolgozása során, emberi beavatkozás eredményeként jön létre. Ezzel szemben a (3a) csoport esetében a mikotoxin kémiai szerkezete egy biológiai rendszerben, enzimek által katalizált reakciók révén módosul.

## A FUMONIZINEK LEHETSÉGES ÁTALAKULÁSAI AZ EMÉSZTÉS SORÁN

Az emésztőrendszerben végbemenő folyamatok elősegíthetik a mikotoxinok szabad formájának kialakulását. A mátrixhoz kötött, vagy módosított formában jelen lévő fumonizin emésztés során való felszabadulása, illetve eredeti formába való visszaalakulása állhat annak a tapasztalatnak a hátterében, hogy a természetesen szennyezett táplálékmátrixból emésztést követően jelentősen több fumonizint mutatnak ki, mint emésztés előtt. Egy referenciaanyagban – in vitro emésztést követően – a FB1-3 összege 8010 µg/kg volt, míg a referenciaanyag forgalmazója által deklarált FB1 (2406 µg/kg) és FB2 módosulat (630 µg/kg) összege csupán 3036 µg/kg volt (*Dall'Asta* és mtsai., 2009). Ugyanezen szerzők kukoricalisztben RIMV modellben végzett emésztést követően a fumonizin koncentrációt 30-50%-kal nagyobbnak mérték, mint az emésztés előtt. Ennek hátterében feltehetőleg az állt, hogy a fehérjék és a szénhidrátok által megkötött FB molekulák jelentős hányada a makromolekulák hidrolízise során felszabadult a táplálékmátrixból. A hidrolízis a FB esetében nem következett be, mivel a chimusban nem mutattak ki sem részlegesen, sem teljesen hidrolizált FB származékot (*Dall'Asta* és mtsai., 2009). Az alábbiakból az következik, hogy az emésztési folyamatok a mátrixhoz kötött formák szabaddá válását eredményezhetik, de nem zárható ki a módosított pl. maszkolt formák felszabadulása sem. Összességében elmondható, hogy az emésztés olyan átalakulásokat eredményezhet az egyes formák arányában, amely megnövelheti a FB toxikus hatását.

A mikotoxinok biológiai hozzáférhetőségét (bioaccessibility) az határozza meg, hogy mekkora hányaduk van jelen, illetve alakul át felszívódásra alkalmas formába, miközben áthalad a gyomor-bél traktuson (*Versantvoort* és mtsai, 2004; *González-Arias* és mtsai, 2013). Egy részük eredendően szabad formában van jelen, míg egy másik részük az emésztés során a makromolekulák hidrolízisét követően válik szabaddá, illetve egy további hányaduk mikrobiális folyamatok következtében szabadul fel. A mikotoxinok vizes oldatának biológiai hozzáférhetősége gyakorlatilag 100 százaléknak tekinthető. A táplálék mátrixok különbözősége hatást gyakorolhat a mikotoxinok biológiai hozzáférhetőségére, azaz különböző táplálékfajták esetében adott mikotoxin mennyiségből eltérő hányad válhat felszívódásra alkalmassá (*González-Arias* és mtsai, 2013).

A mátrixhatás, azaz a fumonizinek kötődése egyes tápanyagkomponensekhez, tehát hatást gyakorolhat a biológiai hozzáférhetőségre. Egy finomra és durvára darált kukoricaliszt in vitro emésztési vizsgálata során a fumonizinek eltérő biológiai hozzáférhetőségét figyelték meg. A modell a monogasztrikus állatok emésztőrendszerét imitálta, annak érdekében, hogy a száj, gyomor és vékonybél fázisban az emésztés alatti biokémiai folyamatokat és a tápanyagok biológiai hozzáférhetőségét tanulmányozni lehessen (*Versantvoort* és mtsai, 2004). A kutatás rámutatott arra, hogy a fumonizinek körülbelül 90%-a a vékonybélben szívódik fel, 10 % pedig a gyomorban és a vastagbélben. Továbbá kiemelték azt, hogy a biológiai hozzáférhetőséget számos tényező befolyásolja (kezelés, élelmiszer típusa, szerkezete, és más vegyületekkel való kölcsönhatása). Az in vitro emésztés során a finomra őrölt kukoricalisztből kisebb arányban (27%) szabadult fel a fumonizin, mint a durvára őrölt kukoricalisztből (35%), tehát a finomra őrölt kukoricaliszt esetében kisebb volt a fumonizin biológiai hozzáférhetősége. Ez a megfigyelés arra utal, hogy a fumonizin szoros mátrixkapcsolatot létesített a rezisztens keményítővel, redukáló cukrokkal, ásványi anyagokkal és lipidekkel, amely komponensek nagyobb arányban voltak jelen a finom kukoricaliszt mintákban, mint a durvában (*Massarolo* és mtsai, 2020). A fumonizinek a táplálékkomponensekkel alakíthatnak ki kovalens kötést, vagy fizikailag körbezárva helyezkednek el a mátrixban. Az emésztési folyamatok a két kötési módra másképpen hatnak. Az a forma, amelyik nem kovalens kötéssel kapcsolódik a táplálékhoz, az emésztés során szabad formájú fumonizinné alakulhat. A kovalens kötéssel a mátrixhoz kapcsolódó fumonizin származékok (pl. *N*-karboxi-metil-FB1) *in vitro* emésztése során azt találták, hogy ezek nem szabadulnak fel az emésztés során, az emésztőenzimek nem képesek a kialakult kovalens kötéseket felhasítani az *N*-alkil-FB1 és az N-acil-FB1 konjugátumokban (*Falavigna* és mtsai, 2012; *Braun* és mtsai, 2017). Ez számos kérdést és megoldásra váró feladatot felvet élelmiszerbiztonsági és egészségügyi szempontból is.

Emésztés során a fumonizin biotranszformációja az emésztőrendszerben lévő mikrobák tevékenysége révén is megvalósulhat. In vitro emésztésszimulációs vizsgálat igazolta, hogy a sertés vakbélben található mikrobióta képes a fumonizint először részlegesen hidrolizált fumonizinné, majd aminopentollá hidrolizálni (*Fodor* és mtsai, 2007). A trikarballilsav- részletek elvesztése miatt a HFB1 kevésbé poláris, mint az FB1. Emiatt a felszívódásban különbségek adódhatnak a két forma között. *Bouhet* és munkatársai (2007) patkányokon végzett kísérlete során megállapították, hogy az oldalláncok elvesztése elősegíti a HFB1 felszívódását a bélben (*Bouhet* és mtsa, 2007). *Ferrara* 2021-ben munkatársaival emésztés szimuláció során a fumonizinek mikrobiális biodegradációját tanulmányozta. A kutatás során a lebontásban a *Firmicutes* és a *Bacteroides* törzsek voltak a legdominánsabbak (*Ferrara* és mtsai, 2021).

## A FUMONIZINEK TOXICITÁSA ÉS HATÁSMECHANIZMUSA

A mikotoxinok biológiai elérhetőségét (bioavailability) az határozza meg, hogy mekkora hányaduk jut felszívódást követően a szisztémás keringésbe, tehát potenciálisan azt a részt jelenti, amely a szövetekben ki tudja fejteni káros hatását. A biológiai elérhetőség kisebb, vagy legfeljebb akkora, mint a biológiai hozzáférhetőség. Az elméletileg létező legrosszabb eshetőség az, amikor az elérhetőség azonos a hozzáférhetőséggel, ez azt az esetet jelentené, amikor a mikotoxin potenciálisan felszívódásra alkalmas hányada teljes egészében felszívódik és a keringéssel eljut a szövetekhez. A gyakorlatban nyilvánvalóan az oldott hányad nem teljes egészében szívódik fel, illetve a felszívódást követően is végbe mennek olyan folyamatok, melyek a szövetek terhelését csökkentik. A szisztémás keringésbe jutás előtt, az ú.n. „first pass effect” során a mikotoxinok szerkezete átalakulhat, mikor az intoxifikált szervezet megpróbálja őket hatástalanítani (biotransformation), illetve jelenős részük kiürülhet a szervezetből. Míg a hozzáférhetőséget (bioaccessibility) alapvetően a táplálékmátrix és az emésztésfiziológiai jellemzők, valamint a bélmikrobiom befolyásolják, addig a biológiai elérhetőséget (bioavailability) – ezeken kívül – a szervezet mikotoxinokkal kapcsolatos anyagcserefolyamatai is (*González-Arias*, 2013).

A fumonizin mikotoxinok mérgező hatását jelentősen befolyásolja azok biológiai elérhetősége, amely fajonként változó értéket mutat. Általánosságban elmondható, hogy szájon át a szervezetbe juttatott FB1 biológiai elérhetősége viszonylag kis mértékű, sertés esetében 3-6% között van (*Prelusky* és mtsai, 1994), míg tojótyúkoknál még az egy százalékot sem éri el ez az érték (*Vudathala* és mtsai, 1994). Teheneknél szájon át történő FB1 bevitelt követően az FB1 és annak metabolitjai nem mutathatóak ki a vérplazában (*Prelusky* és mtsai, 1995), amely azt jelzi, hogy kérődzőkben, feltehetően az előgyomrokban zajló mikrobiális folyamatok miatt, ezen mikotoxin biológiai elérhetősége nagyon csekély.

Összességében elmondható, hogy a takarmánnyal felvett fumonizinnek csak kis hányada jut el felszívódást követően az állat szervezetének többi részébe, azonban ez a kis mennyiség is jelentős egészségkárosító hatással rendelkezik, és ezt a humán vizsgálatok is alátámasztják. Az FB1 toxin emberre lehetségesen rákkeltő hatású (2B csoport) besorolást kapott (*IARC*, 2002; *Soriano* és mtsai, 2005).

A toxikus hatás kifejtésében a molekula szerkezetén belül kétféle funkciós csoport játszik szerepet, az amino-csoport, illetve az észterkötésben lévő trikarballilsav-rész (*Vanhoutte* és mtsai, 2016). A fumonizin B1 szerkezete hasonló a szfinganinéhoz (*Soriano* és mtsai, 2005). A szabad aminocsoporttal rendelkező fumonizinek kompetitív módon gátolják a *ceramid szintáz* működését (*Riley* és mtsa, 2019), a *ceramid szintáz* gátlás miatt pedig a szfingolipidek anyagcseréjében zavar keletkezik (*Vanhoutte* és mtsai, 2016). A szfingolipidek olyan másodlagos lipidek a sejt membránszerkezében, melyeknek szfingozin az alapváza. A fumonizinek kötődnek a *ceramid szintáz*okhoz, és erős inhibitorai annak. Ezek az enzimek (CerS1–CerS6) katalizálják a ceramid képződését szfinganinból (vagy szfingozinból) és palmitátból vagy más hosszú szénláncú zsírsavakból. A *ceramid-szintáz* gátlása tehát megzavarja az általános szfingolipid metabolizmust, és többek között a sejtekben lévő szfinganin és szfingozin koncentrációjának növekedéséhez, ezen szfingoidbázisok 1-foszfát metabolitjainak növekedéséhez, valamint a sejt ceramid és komplex szfingolipidek mennyiségének csökkenéséhez vezet (*Haschek* és mtsai, 2002). *In vitro* patkány hepatocitákon végzett vizsgálatok alapján a szfinganin (Sa) felhalmozódása volt megfigyelhető a vérben, vizeletben, májszövetben FB1 hatására (*Riley* és mtsa, 2019). A megemelkedett szint oka az, hogy mivel a *ceramid szintáz* enzim szabályozza a *szerin-palmitoiltranszferáz* enzim működését (SPT) – az SPT Sa-t termel – FB1 hatására a megemelkedett Sa szint nem gátolja az SPT-t, hanem az SPT továbbra is folyamatosan termeli a Sa-t (*Riley* és mtsa, 2019). A Sa sokkal gyorsabb ütemben halmozódik fel, mint a szfingozin (So), az arányuk így felborul és megzavarja a szfingolipidek normál bioszintézisét. Ezzel igazolható, hogy a Sa/So aránya indikátorként használható a FB kitettség megfigyelésére. Mivel a Sa és a So is átjut a membránon, ezért megjelennek vizelet és vérmintákban, ahol kimutatható, hogy az FB1 kitettség miatt az arányuk a kedvező mértéktől eltér (*Chen* és mtsa, 2018, *Riley* és mtsa, 2019). A Sa felhalmozódása proapoptotikus, citotoxikus hatást okoz (*Soriano* és mtsai, 2005; *Lallés* és mtsai, 2009). *Soriano* és munkatársai (2005) vizsgálatai szerint már 0,2 mg/kg feletti FB fogyasztás esetében gátlás alá kerül a szfingolipidek bioszintézise, és ez a hatás néhány órán belül jelentkezik.

Az FB1 másik célpontja a mitokondrium, ahol a sejtlégzés csökkentésével az oxidatív szabadgyökök (ROS) (főleg a szuperoxid) túltermelődését eredményezi. Az FB1 az I. mitokondriális komplex gátlásán keresztül depolarizálja a mitokondriális membránt, amely végső soron ROS termeléshez, a $Ca^{2+}$ szintjének hibás jelzéséhez, és a $Ca^{2+}$ arány felborulásához vezet. A $Ca^{2+}$ arány felborulásának eredményeként – a citoplazmában nő a szintje, miközben a mitokondriális $Ca^{2+}$ felvétel csökken – sejthalál következik be (*Domijan* és mtsai, 2011). *In vitro* és *in vivo* kísérletekben az FB1 bevitelét követően a normális értéktől magasabb *szuperoxid dizmutáz*-aktivitást és malondialdehid szintet mértek (*Domijan* és mtsai, 2011; *Braun* és mtsai, 2017). Az oxidatív stressz hatására a DNS is károsodhat a megnövekedett lipid peroxidáció miatt. A mikotoxin jelenléte a szervezetben stresszhatást produkál, ami sejtszinten oxidatív károsodást okoz (*Zeebone*, 2023).

*In vivo* és *in vitro* kísérletek alapján a FB-re elsősorban a lovak és a sertések érzékenyek. Lovaknál *leukoencephalomalaciát* (agyvelő elhalásos elváltozása), sertéseknél tüdővizenyőt (*pulmonary oedeme*) vált ki, szív- és érrendszeri betegségeket okoz, illetve máj-, és vesekárosító hatású. Patkányoknál in vivo kísérletekben máj-, és veseelfajulást, idegrendszeri tüneteket figyeltek meg (*Kovács* és mtsai, 2016). Ezen kívül nyulaknál, bárányoknál is hepatoxikózist, nefrotoxikózist, agyvérzést okoz az FB1 toxin (*Soriano* és mtsai, 2005; *Zeebone* és mtsai, 2020). Baromfinál erős immunszupresszív hatást, míg majmoknál érelmeszesedést okoz (*ateroszklerózis*) az FB1 toxin bevitele (*Soriano* és mtsai, 2005). Humán vonatkozást tekintve velőcsőzáródási rendellenességet, növekedési zavart, kardiovaszkuláris defektust figyeltek meg, illetve a nyelőcsőrák kialakulásában is szerepet játszik (*Andrade*, 2023). Az afrikai „kukoricabetegség” is a mikotoxin szennyezettségnek köszönhető. Ez összetett tüneteket produkál, a nyelőcsőrákon kívül a korai nemi érés, a kariopátia, a férfiak mellmegnagyobbodása, ízületi elváltozások, toxikus leukémia is a toxikózisnak tulajdonítható (*Dutton*, 2009). Az emberek és állatok védelme érdekében számos országban meghatározták a táplálékban és takarmányban jelen levő FB koncentráció maximális beviteli értékét (*Hahn* és mtsai, 2014). Az EFSA 2018-as határozata alapján a fumonizinekre vonatkozó napi tolerálható beviteli érték (TDI) 1 mg/kg/nap (*EFSA*, 2018). A fumonizin egyes szerkezeti módosulatainak toxikus hatása különbözhet egymástól, valamint az általuk kiváltott szervi tünetek is eltérőek lehetnek. A módosult mikotoxinok toxicitásban betöltött szerepe alábecsült, és még több kutatást kíván ezen metabolitok hatásainak a felderítése, és ezzel párhuzamosan a TDI értékek változtatása is fontos szempont.

Kezdetben úgy vélték, hogy a hidrolizált FB1 metabolit (HFB1, aminopentol) toxikusabb, mint az FB1, mivel a trikarballilsav-részletek hiánya csökkenti a molekula polaritását, amely elősegítheti az abszorpciót (*Hopmans* és mtsai, 1997). Ezzel szemben, egy állatkísérletben, malacokkal végzett vizsgálatok szerint, a hidrolízis hatására nem nőtt, hanem csökkent az emésztőrendszerre és a májra gyakorolt toxikus hatás (*Grenier* és mtsai, 2012). Az FB1 toxin hidrolizált formájával (HFB1) végzett kutatás során sertés epitel sejteket vizsgáltak. A sertéseket FB1-el és HFB1-el mesterségesen szennyezett takarmánnyal etették, majd összehasonlították a toxicitásukat. Arra az eredményre jutottak, hogy a HFB1 nem okozott hepatotoxikózist, nem rontotta a bél morfológiáját, viszont kissé módosította az immunválaszt az epitel sejtekben. Ez arra enged következtetni, hogy a HFB1 a Sa/So arányt kevésbé borítja fel, mint az FB1 (*Grenier* és mtsai, 2012). 2019-ben IPEC-J2 sejtvonal összehasonlító vizsgálata szintén azzal zárult, hogy a HFB1 kevésbé rontja a bél egészségi állapotát, mint az FB1 (*Gu* és mtsai, 2019). Ez ellentétben áll azzal a kutatással, ahol patkányban vizsgálták a HFB1 toxicitását. *Bouhet* és munkatársai (2007) a HFB1-et jóval toxikusabbnak találták, mint az eredeti formát. A HFB1 a májban és a vesében az FB1-hez hasonló rákkeltő hatású metabolitnak bizonyult (*Manyes* és mtsai, 2014). Az ételkészítési eljárások közül a nixtamalizálás (hőkezelés lúgos közegben) szerepet játszhat a HFB1 kialakulásában. HT-29 sejttenyészetben a HFB1 toxicitását vizsgálták, ahol a HFB1-ről megállapították, hogy a ceramid szintáz inhibitora és szubsztrátja is egyben, a kapott N-palmitoil-AP1 (PAP1) pedig a ceramid szintáz még erősebb inhibitora. A PAP1 10-szer toxikusabb, mint az FB1 vagy a HFB1 (*Merrill* és mtsai, 2001).

A gasztointesztinális traktus érintettsége nem elhanyagolható, mivel a fumonizinekkel kontaminálódott táplálék elsődleges expozíciós szerve a gyomor, a bélrendszer és a kapcsolódó szervek. A bélrendszer megfelelő állapota nélkülözhetetlen az optimális emésztés fenntartásához, az abszorpcióhoz, a patogénekkel szembeni működőképes immunválaszhoz és védekezési reakcióhoz. IPEC-2 sejtmodellben *Wen* és munkatársai (2024) vizsgálták a fumonizinek bélgyulladásban betöltött szerepét. Azt találták, hogy a nukleáris faktor kappa B (NF-κB) p65, az extracelluláris szignál-szabályozott kináz (ERK), az interleukin 6 (IL-6) és az IL-1β fontos célpontok, mert a fumonizin ezeken keresztül hat a gyulladás kiváltásában. Az Nf-κB a citoplazmában lokalizált nukleáris faktor, amely kötődni képes a limfocitákhoz és többek között az immunválaszban vesz részt, orvosi jelentősége a sejtproliferációban van tumorok kifejlődésénél. Az IL-6 és a IL-1β makrofágok, limfociták által kiválasztott citokinek. Az IL-1β az autoimmun hálózat részeként más citokineket mobilizál, ezen felül a pankreász sejtjeinek pusztulásáért is felelőssé tehető (*Musker* és mtsai, 2018).

A bélrendszerben található epitel sejtek képviselik az elsődleges védvonalat az immunválasz kialakításában, úgy, hogy mucint szekretálnak, ezáltal fenntartják a mikrobák optimális életműködéséhez szükséges környezeti igényeket. Az intesztinális szimbionta mikrobióta támogatja a védőfunkciók működését, azáltal, hogy szabályozza a gyulladásgátló faktorokat és az IgA (immunglobulin A) termelődését, illetve anyagcserefolyamataik révén szénhidrátokat fermentálnak, aminek következtében rövid szénláncú zsírsavak (SCFA) jönnek létre. Ezeknek a zsírsavaknak az a jelentősége, hogy elősegítik az epitel sejtek regenerációját és zavartalan működését (*Ding* és mtsai, 2023).

Az emésztés során a táplálékban lévő mikotoxinok és az emésztőrendszer mikrobiotája kölcsönös kapcsolatban áll egymással. Az emésztőrendszerben oldott formában jelen lévő mikotoxinok mennyiségét megkötés vagy bontás révén befolyásolhatják a mikrobapopulációt alkotó törzsek; valamint a mikotoxinok jelenléte azzal járhat, hogy hatásukra módosul a mikrobiom összetétele és az epitel sejtek normális működése és morfológiája is károsodik (*Lallés* és mtsai, 2009; *Zeebone*, 2023). Az alacsony dózisú FB1 csökkenti a bélhámsejtek citokinválaszát, a sejtek életképességét a sejtproliferáció gátlása és az apoptózis indukciója révén, míg a nagyobb dózisok citotoxikusak (*Lallé* és mtsai, 2009). In vivo kísérlet alapján egerekben az FB1 módosította a glikolipidek eloszlását a vékonybélben található epitel sejtekben, valamint felborította a Sa/So arányt (*Enongene* és mtsai, 2000; *Lallé* és mtsai, 2009). Az FB1 kitettséget a hősokkfehérjék (HSP25 és HSP70) megemelkedett szintje is jelzi a májban, vesében és az immunrendszer egészében. *Zeebone* (2023) sertésekkel végzett kísérlete során szövettani vizsgálattal hepatotoxikus és nefrotoxikus hatás jelenlétét detektálta, amiről a májban és vesében található HSP70 fehérje magas aktivitása is visszaigazolást adott. *Lallé* (2009) és munkatársai szintén sertésekkel végzett kutatásukban rávilágítottak arra, hogy 25-30 mg/kg FB1-el kiegészített takarmány etetésének hatására az alfa-B krisztallin és a COX-1 enzim aktivitása megemelkedett a vastagbélben, amely azt jelezte, hogy a vastagbélben a mikotoxin káros hatása jobban érvényesült, mint a gyomorban vagy jejunumban. A károsodás mértéke azonban ezekben a traktusokban is számottevő volt, annak ellenére, hogy mérsékeltebb HSP70 szintet mértek a gyomorban és ezen vékonybéli szakaszban (*Lallé* és mtsai, 2009).

## A FUMONIZINEK BIOLÓGIAI HATÁSTALANÍTÁSA

Az eddigieknél hatékonyabb prevenciós, elimináló vagy detoxifikáló módszerekre van szükség, annak érdekében, hogy a mikotoxinok okozta egészségi és gazdasági károk ne növekedjenek tovább. A mikotoxinok fizikai vagy kémiai úton történő átalakításához többnyire olyan körülmények szükségesek, melyek az élelmiszerek vagy takarmányok tápértékének csökkenésével járnak, mivel az értékes makro- és mikronutriensek is károsodhatnak a magas hőmérsékletű és/vagy a kémhatás megváltozásával járó kezelések során. Az élelmiszerek előállításakor alkalmazott eljárások hatást gyakorolhatnak a mikotoxinok szerkezetére és mennyiségére. A kémiai szerkezet megváltozása révén kialakuló vegyületek toxicitása azonban nem feltétlenül lesz kisebb, mint az eredeti metabolité, ennek akár az ellenkezője is előfordulhat.

A biológiai hatástalanítási módszerek előnye a kémiai vagy fizikai kezelésekkel szemben az, hogy többnyire enyhébb körülmények alkalmazásával és kevesebb tápanyag veszteséggel járnak (*Liu* és mtsai, 2022). Lényegük, hogy egyes baktériumok képesek a mikotoxinokat a felületükön megkötni, vagy enzimes úton olyan módon átalakítani, hogy a képződött metabolit kevésbé legyen toxikus, mint az eredeti forma. A mikotoxinok baktériumok felületén való megkötődéséhez nem feltétlenül szükséges aktív anyagcserével rendelkező baktérium, elég, ha a sejtfal intakt állapotban van. A sejtfalban található peptidoglikánhoz köthető ez a fajta képesség. A tejsavbaktériumoknak tipikus, Gram-pozitív baktériumokra jellemző sejtfala van, amely vastag, többrétegű peptidoglikán réteget tartalmaz.

2001-ben egy olyan *Sphingopyxis* törzset fedeztek fel, melyről később bebizonyították, hogy karboxilészteráz enzimjei képesek az FB1-et hidrolizálni, miközben aminopentol (HFB1) keletkezik; illetve izoláltak benne egy aminotranszferáz enzimet kódoló gént is, ez az enzim a HFB1-et képes dezaminálni piruvát jelenlétében (*Heinl* és mtsai, 2010). Több kutatás is arra az eredményre jutott, hogy az FB1 mikrobiális megkötése elterjedtebb jelenség, mint az enzimes bontása (*Niderkorn* és mtsai, 2009; *Pizzolitto* és mtsai, 2012; *Zhao* és mtsai, 2016; *Dawlal* és mtsai, 2019). *Dawlal* és munkatársai (2019) fluoreszcens festéssel bizonyították, hogy a fumonizineket számos tejsavbaktérium képes megkötni. A vizsgált *Lactobacillus plantarum, Lactobacillus delbureckii* és *Pediococcus pentosaceus* törzsek közül a *L. plantarum* élő állapotban és elhalt állapotban lévő sejtjei egyaránt nagyobb arányban voltak képesek a fumonizin megkötésére, mint a vizsgálatban részt vevő többi törzs (*Dawlal* és mtsai 2019). *Khalil* és munkatársai (2015) azt tapasztalták, hogy patkányok vérszérum paraméterei a normális, elfogadható szintre álltak vissza, mikor 50, 100 és 200 mg/kg dózisú FB1-t tartalmazó tápjukat tejsavbaktériummal (*L. delbureckii*, és *Pediococcus acidilactici* törzsekkel) egészítették ki. A hematológiai vizsgálatok eredményei arra mutattak rá, hogy a kontroll csoporthoz képest, a csak FB1 mikotoxinnal kezelt patkányok biokémiai paramétereinek értékei emelkedtek, ami szervkárosodásra utal (máj, vese). A tejsavbaktériummal és mikotoxinnal is egyszerre kezelt patkányoknál a vérszérumparaméterek normalizálódtak (ALT, AST, bilirubin szint, összfehérje). A kísérlet során figyelték a DNS károsodását a vérplazmában. A kontroll csoporthoz képest a második és harmadik héten a csak FB1 mikotoxinnal kezelt állatoknál vettek észre károsodást. A tejsavbaktériummal és az FB1 toxinnal is kezelt csoportban viszont csökkent a DNS károsodásának aránya. Mindez mutatja, hogy a tejsavbaktériumok jelenléte a tápban azzal járt, hogy csökkent az FB1 DNS károsító hatása a vérplazmában, valamint javultak a vérszérumparaméterek. *Ezdini* és munkatársai (2020) tunéziai vajból izolált *Lactobacillus paracasei* törzset keverek egerek takarmányához, melyhez FB1 mikotoxint is adtak (100 mg/kg). A vizsgálat kimutatta, hogy a *L. paracasei* törzs csökkentette az FB1 biológiai hozzáférhetőségét egerek gyomrában és bélrendszerében, amely azt eredményezte, hogy az FB1 által generált károsodás (oxidatív stressz, hisztopatológiai elváltozások) mértéke csökkent.

## KÖVETKEZTETÉSEK ÉS JAVASLATOK

A fumonizin szennyezettség elkerülésének legjobb eszköze a prevenció. A szennyezett tételek esetében szóba jöhet azok detoxifikálása. A mikotoxinok hatástalanítására alkalmazott fizikai és kémiai módszerek jelentős tápanyagveszteséggel járnak, ezért nagyobb hangsúlyt kell fektetni olyan biológiai módszerek kidolgozására, melyek kellően hatékonyak. A fumonizin biotranszformációja megvalósulhat mikrobiális átalakítás révén, azonban a fumonizinekből keletkező származékok toxikológiai megítélése nem egységes. További kutatások szükségesek a fumonizinek hidrolizált, dezaminált és egyéb származékai toxicitásának tisztázására. Egy másik lehetséges mód az emésztőrendszerben oldott állapotban lévő, biológiailag hozzáférhető fumonizinek mennyiségének csökkentése, olyan baktériumtörzsek felkutatásával, melyek felületi megkötés révén képesek hatékonyan csökkenteni az oldott formában lévő fumonizinek mennyiségét, ezáltal kevésbé tudnak felszívódni a fumonizinek az emésztőrendszerben.

Több vizsgálat eredménye alátámasztja, hogy az intakt táplálékból meghatározott fumonizin-szint eltér attól, mint amit az emésztésszimulációt követően mérünk; feltehetőleg azért, mivel a mátrixhoz kötött, vagy módosított formák egy része felszabadul az emésztés során. Ebből következik, hogy a fumonizin expozíció szempontjából pontosabb képet kapunk, amennyiben a mintaelőkészítés során – az extrakciós lépést megelőzően – emésztésszimulációt alkalmazunk, és az in vitro emésztést követően oldott állapotban jelen levő fumozininek mennyiségét határozzuk meg, mivel ezzel az értékkel tudjuk megbecsülni a fumonizinek felszívódásra potenciálisan rendelkezésre álló mennyiségét.

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