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Evaluation the nutritive value of abiotic stress resistant oat and barley varieties as poultry feedstuffs

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BACKGROUND OF THE WORK AND OBJECTIVES

Climate change is considered crucial environmental factors in crop production. Increasing tendencies of extreme weather events could be unfavourable for certain crops like corn and other crops. The aim of the breeding programs is to increase abiotic and biotic stress resistance of the major crops because this is an essential point of adaptation and sustainable agriculture. However, much less attention has been paid to the alternative crops such as barley and oats, despite their great potential as feed sources in Europe or even worldwide. The selection of abiotic stress resistant cereals is a main research focus of the Agricultural Institute of the Centre of Agricultural Research of Hungarian Research Network in Martionvásár. Beside corn and wheat, promising new barley and oats varieties are tested. In the frame of this doctoral work the nutritive value of these new barley and oats varieties were tested with analytical and in vivo trials. Cereal grains are mostly energy sources, but their protein content and the digestibility of their amino acids are also important nutritional materials. Oats and barley protein has good nutritional value for monogastric animals and can be used as high-quality protein in diets for poultry due to its amino acid composition. Amino acid (AA) digestibility is an important measure of protein quality. Compared with corn, barley and oats are more resistant to abiotic stresses, such as drought or high temperature and can be alternatives of both corn and wheat in the future. However, both grains are rich in insoluble and soluble non-starch polysaccharides (NSPs). The insoluble fibre of oats and barley are related to the hulls of the grain. Although insoluble fibre is not digestible for poultry, the structural properties of hulls can stimulate the gizzard and enhance the energy utilization and protein digestion of birds **(Svihus 2011)**. Several studies have proven, for example, the positive effects of oats hull supplementation of broiler diets **(Jiménez-Moreno et al. 2013).** The soluble NSP in barley and oats are mainly β-glucans containing 1–3 and 1–4 linkages and represent about 60–70% of the total beta-glucans **(Jeroch et al. 1999)**, which has positive physiological effect on decreasing blood cholesterol and glucose level and decreased risk of cardiovascular disease in humans and prebiotic, immune stimulatory characteristics in farm animals **(Svihus and Gullord 2002)** . This NSP fraction, due to its unique physicochemical properties, increases

digesta viscosity, decreases the passage rate, and this way impairs the digestibility of nutrients and the performance of chickens **(Smits and Annison 1996**; **Bautil et al. 2020)**. The decreased digestion results in more substrates for the gut bacteria, causing increased bacterial content and modified bacteriota composition in the gut. β-glucans, on the other hand, can improve the immunity of pigs and poultry **(Mirjana et al. 2013; Moon et al. 2016).** They have antioxidant potential and prebiotic effect in the hind gut segments **(Marco Castro et al. 2021).** To eliminate the negative effects of the soluble NSP fractions, β-glucanase enzyme supplementation of diets is a common practice in the nutrition of poultry and swine species. Previous studies described that enzyme supplementation increased weight gain, apparent metabolizable energy, and fat digestibility. As well as its positive effect on the production parameters, β-glucanase may reduce the weight of the gut **(Friesen et al. 1992; Brenes et al. 1993).** Nevertheless, the exact mechanism behind the positive effects of NSP-degrading enzymes is not fully clarified yet **(Lazaro et al. 2003; Jozefiak et al. 2004).** Because of their hulls, oats and barley are also rich in cellulose and contain less starch and protein than wheat **(Bach Knudsen 1997)**. Certain amounts of structural insoluble fibre in poultry diets stimulates gizzard and can also improve the growth and feed conversion of broilers or the incidence of cannibalism in laying hens **(Aerni et al. 2000; Jiménez-Moreno et al. 2016). Carré et al. (1990)** found, however, that high ratio of oats hulls decreases the metabolizable energy content of broiler diets and impair the feed conversion **ratio (Carré et al. 1990)**. At limited inclusion rates, however, structural fibre can improve the digestion of starch, enhance the performance of the chickens, and reduce the cannibalism in laying hens **(Aerni et al. 2000; Jiménez-Moreno et al. 2016).** Other scientists (**Denayrolles et al. 2007; Dunkley et al. 2007)** found that mostly the fibre characteristics, its soluble and insoluble fractions, affect the bacterial profile of the gut. Quite a lot of research results are available on the effects of arabinoxylans (AX) on the gut microbiota composition. The AX and xylan-oligosaccharides (XOS), the products of arabinoxylans after xylanase breakdown, are known to have positive effects on the bacteriota composition of the caeca, increasing the number of butyrate producing bacteria, such as *Lachnospiraceae* or *Ruminococcaceae* **(Immerseel et al. 2017)**. **Donaldson et al. (2021)** published recently that the latest rye varieties can also be competitive feedstuffs of corn and wheat. In their trial, feeding rye increased the absorptive surface of the small intestine in broiler

chickens **(Donaldson et al. 2021).**The barley and oat crop varieties are predicted to have increasing importance in the near future. Barley and oat varieties have excellent adaptability to the climatic extremes and also to the extensive cultivation techniques. Both in oat and barley, the aim is to minimise the strong yearly fluctuations. In the frame of this research selected plant materials with excellent environmental plasticity. So, the main objective of the work is to determine the nutritive value of both grains, based on their nutrient contents, ant nutritive factors, digestibility, and growth test to. In order to validate the biological values and the effectiveness of the new final products, like oat and barley-based compound feeds. Since there are only few literature data on the maximal inclusion rates of oats and barley in broiler diets, our PhD aim was to find out the potentials of oats and barley as poultry feedstuffs. The detailed evaluation steps were the follows:

a) To determine the nutritive value of different oat and barley varieties, based on their chemical composition.
b) To determine the variance of the main nutrient categories and the correlation between them.
c) Evaluation the protein quality of oats and barley as poultry feedstuffs.
d) Determination the nutrient digestibility of selected oat and barley varieties at different inclusion rates.
e) Evaluation the effect of exogenous beta glucanase on the nutrient digestibility of barley- and oat-based diets.
f) Conducting a feeding trial with broiler chickens to determine the production traits of chickens when barley and oat containing diets are fed.
g) Investigating the effects of oat and barley containing diets on several gut parameters and gut microbiota composition.

MATERIALS AND METHODS

Determination the nutritive value of oats and barley varieties

Spring oat (36), winter oats (35) and winter barley (36) varieties selected in the Agricultural Research Centre in Martonvásár, Hungary have been evaluated. The chemical analysis procedures for oat and barley samples were conducted at the Institute of Physiology and Nutrition of the Hungarian University of Agriculture and Life Sciences, in Keszthely, Hungary. The grains were analyzed for proximate analyses such as; dry matter (ISO 6496: 2001), crude protein (ISO 5983-2:2009), ether extract (ISO 6830- 19: 1979), crude fibre (ISO 6865:2001) and crude ash (ISO 5984: 1992). The determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was based on ISO 16472:2006 and ISO 13906:2008, respectively. Insoluble dietary fibre (IDF) and water-soluble dietary fibre precipitated (SDFP) in 78% aqueous ethanol were determined according to the AOAC method 2011.25. The total β-glucan content was determined according to the method of Bach Knudsen (1997).

Digestibility trial with broiler chickens

The digestibility trial, the feeding expeteriment was conducted in Georgikon Campus, Hungarian University of Agriculture and Life Sciences, Deák Ferenc Street 16, 8360 Keszthely, Hungary under the license number MÁB-1/2017. All husbandry and euthanasia procedures were performed in accordance with the Hungarian Government Decree 40/2013 and in full consideration of animal welfare ethics.

Five-week-old Ross 308 broiler cockerels were kept in individual cages and fed the experimental diets in 6 replicates. Three determinant varieties form winter barley (Mw118-7,

Mw05-17, Mv- Initium), winter oat (Mv- Kincsem, Mv- Istrang, Mv- Imperialand) and spring oat (Mv- pehely, Mv- Mene, Mv 9-14) samples were incorporated into the test diets at 20 and 40% on the expense of starch. Beside the effect of barley and oats on the nutrient digestibility of the diets, the amino acid digestibility of barley and oat was also calculated the linear regression approach, as described by Rodehutscord et al. (2004). In this arrangement the increase in protein and amino acid intake was related only to the test cereals. Beside the effect of barley and oats on the nutrient digestibility of the diets, the amino acid digestibility of barley and oat was also calculated by the

linear regression approach, as described by Rodehutscord et al. (2004). From the diets and excreta samples their dry matter, nitrogen, crude fat, starch and TiO_2 contents were measured. The TiO2 measurement was done by spectrophotometer (Jenway 6100, Dunmow, UK) at 410 nm, according to the method of Short et al. (1996).

Feeding trial with broiler chickens

A total of 600, Ross 308, day old male broilers were purchased from a commercial hatchery (Gallus Company, Devecser, Hungary). Birds were allocated randomly to one of the 25 pens at a stocking rate of 24 birds per pen (cage; 10 bird/m2). Computer controlled housing and climatic conditions were maintained according to the breeding company's suggestion **(Aviagen, 2018).**

The animal experiment was approved by the Institutional Ethics Committee (Animal Welfare Committee, Georgikon Campus, Hungarian University of Agriculture and Life Sciences) under the license number MÁB—10/2019.

The light intensity was 30 lux in the first week and 10 lux thereafter, with a constant day length of 23 hours from day 0 to day 7 and 20nhours light and 4 hours dark period thereafter. The room temperature was set to 34 °C on day 0 and reduced gradually to 24 °C at day 18.

Beside the barley and oats containing diets a commercial corn – wheat – soybean-based control diet was fed. Four treatments in 5 replicate pens have been used. The winter barley (Mw 05-17) was used at 20 (WB20) and 40% (WB40), while the winter oats (MV Hópehely) at 10 (WO10) and 20% (WO20) inclusion rates. The feed mixtures were prepared for each treatment with a precision feed mixer constructed for small scale experimental diets. Supplements such as amino acids and premix were stepwise homogenized until 10 kg in corn prior to final mixing. All the diets contained exogenous glucanase enzyme and the diets formulated to be identical in almost all nutrients, except fibre. The composition of diets is shown in ***Table 1.***

The starter diets were fed from day 1 till day 10, the grower from day 11 till day 24 and the finisher from day 25 till day 39. All diets were fed in mash form, and were formulated to be isocaloric and isonitrogenous, and to fit to the requirements of this breeds of chickens **(Aviagen, 2018)**. Water and feed were offered ad libitum throughout the whole experiment.

Table 1. Composition of oats and barley containing diets used in the feeding trial (g/kg)

	Starter diets					Grower diets					Finisher diets				
	Control	WB20	WB40	SO10	SO20	Control	WB20	WB40	SO10	SO20	Control	WB20	WB40	SO10	SO20
Corn	430.0	229	28.0	325.0	219.0	400.0	289.0	92.0	381	277.0	459.0	349.0	152.0	440.0	336.0
Wheat						100.0					100.0				
Extracted soybean meal	464.0	449.0	435.0	454.0	444.0	397.0	397.0	382.0	402	392.0	342.0	342.0	327.0	348.0	337.0
Sunflower oil	56.0	72.0	88.0	71.0	86.0	59.0	71.0	84.0	73	88.0	57.0	68.0	81.0	70.0	85.0
Limestone	18.0	18.0	18.0	18.0	17.0	15.0	15.0	15.0	15	14.0	14.0	14.0	15.0	14.0	14.0
MCP	16.0	16.0	15.0	16.0	17.0	15.0	14.0	13.0	15	15.0	14.0	13.0	12.0	14.0	14.0
Barley		200.0	400.0				200.0	400.0				200.0	400.0		
Oats				100.0	200.0				100	200.0				100.0	200.0
Lysine	2.0	2.0	2.0	2.0	2.0	1.0	1.0	1.0	1	1.0	1.0	1.0	1.0	1.0	1.0
DL-methionine	4.0	4.0	4.0	4.0	4.0	3.0	3.0	3.0	3	3.0	3.0	3.0	2.0	3.0	3.0
Threonine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1	1.0	0.5	0.5	0.5	0.5	0.5
Valine					0.5										
Premix [1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5	5.0	5.0	5.0	5.0	5.0	5.0
NaCl	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3	3.0	3.0	3.0	3.0	3.0	3.0
$NaHCO_3$	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1	1.0	1.0	1.0	1.0	1.0	1.0
Phytase [2]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.100	0.1	0.1	0.1	0.1	0.1	0.1
NSP enzyme [3]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.100	0.1	0.1	0.1	0.1	0.1	0.1

C: control diet; WB20: diet that contained winter barley at 20%; WB40: diet that contained winter barley at 40%; WO10: diet that contained spring oats at 10%; WO20: diet that contained spring oats at 20%;

[1] Premix was supplied by UBM Ltd. (Pilisvörösvár, Hungary). The active ingredients in the premix were as follows (per kg of diet): retinyl acetate—5.0 mg, cholecalciferol—130 µg, dl-alpha-tocophero-lacetate-91 mg, menadione-2.2 mg, thiamin — 4.5 mg, riboflavin—10.5 mg, pyridoxin HCl—7.5 mg, cyanocobalamin—80 µg, niacin—41.5 mg, pantothenic acid—15 mg, folic acid—1.3 mg, biotin—150 µg, betaine—670 mg, Ronozyme® NP—150 mg, monensin—Na—110 mg (only grower), narasin—50 mg (only starter), nicarbazin—50 mg (only starter), antioxidant—25 mg, Zn (as $ZnSO_4 \cdot H_2O$)—125 mg, Cu (as $CuSO_4 \cdot 5H_2O$)—20 mg, Fe (as $FeSO_4 \cdot H_2O$)—75 mg, Mn (as MnO)—125 mg, I (as KI)—1.35 mg, Se (as Na_2SeO_3)—270 µg.[2] Phytase: Quantum Blue® (AB Vista, Marlborough, UK). [3] NSP enzyme: β-glucanase, Econase GT 200 P® (AB Vista, Marlborough, UK).

The weight of all chickens and the feed intake on pen level were measured at the end of each phase. From the measured data the growth rate and feed conversion ratio (FCR) were calculated. The experimental unit was the pen. Mortality was registered daily. The weight of death birds was also measured and used in correction of FCR.

On day 40, two animals per pen, 10 chickens per treatment were euthanized, slaughtered by bleeding out of the jugular vein. Immediately, abdominal cavities of animals were opened, and intestinal tracts were removed. The different gut segments were separated (crop, gizzard, duodenum, jejunum, ileum, caeca) and the following measurements have been carried out: the length of the small intestine parts, the empty weight of gizzard, the viscosity of the ileal contents, the pH of the different gut contents (crop, gizzard, jejunum, ileum, caeca), SCFA content of the caeca, gut morphology of jejunum and ileum, and microbiota composition of the jejunum content (JC), jejunum mucosa (JM) and caeca content (CC).

About 200 g excreta samples were collected from each pen on nylon foils. Samples were mixed thoroughly, frozen, and stored at -20 C until further processing. The dry matter content of excreta samples was measured in drying oven at 100 °C for 24 h. The caecal SCFA, the gut morphometry and microbiota measurements have been carried out only form the control and WB40 and WO20 treatments.

The gut morphology samples were taken from the middle of jejunum and from the ileum, 10 cm distal to the Meckel's diverticulum. The 1 cm-long histology samples were put into Eppendorf tubes, containing phosphate buffered formalin and stored at −20 °C.

For next generation sequencing he jejunal content (JC) was collected before the vitelline diverticulum, from a 10 cm long gut segment. Caecal contents (CC) from the right sac were collected for analysis of microbiota composition, and the remainder used for analysis of SCFA. After the gut content collection, the jejunum was washed with sterile ice-cold phosphate buffer solution (PBS) until the mucosa was completely cleaned from the digesta. Mucosa samples (jejunum mucosa, JM) were collected aseptically by scraping off the mucosa from the internal wall of the gut with a glass slide. All samples for microbiota analysis were homogenized and stored at −80 °C until further processing occurred. Before DNA extraction, the samples of two birds of the same pen were pooled. Thus, the microbiota analysis of each gut segments was carried out in 5 replicates.

For pH measurements the gut contents were homogenized. In the case of the crop, gizzard and the left sack of caeca the total contents have been used. The sampling place of jejunum was an about 10 cm long segment, between 10 and 20 cm before the vitelline diverticulum, while from the ileum the proximal segment, between 10 and 20 cm after the vitelline diverticulum.

Approximately 1.0 g digesta samples were collected from the left caecal sack into 2 ml Eppendorf tubes for SCFA analysis.

For viscosity about 2 g ileal chyme was taken from the 10 cm long gut segment, directly proximal to Meckel's diverticulum. The samples for viscosity and SCFA analyses were stored on ice during the sample collection period, and then stored at −20 °C until further analysis.

Histological analyses and small intestine morphology

On day 37 of life, 2 chickens per pen, 10 birds per treatment were slaughtered and the following parameters investigated histomorphology of the duodenum, jejunum and ileum. For the histomorphological examination duodenum and jejunum tissue samples were taken 10 cm after distal half of each part, while ileal tissue samples were taken the proximal part of the junction of Meckel's diverticulum. Tissue sections were washed with 2% phosphate buffered saline (PBS) and fixed in 10% phosphate buffered formalin. Sections from the middle of the duodenum and jejunum (about 5 cm in length) were excised and flushed with cold saline and immediately placed in 10% formalin solution. Samples were cleared and embedded in paraffin and sectioned (5 μM in thickness). A routine staining procedure was carried out using hematoxylin and eosin. Intestine parts sections were measured using a microscope (Leica DMi8 Microscope, Leica Microsystems CMS GmbH, Germany 2015). Villus height, muscle layer thickness and crypt depth were determined with ImageJ software (Version 1.47) developed by National Institutes of Health (Maryland, USA)

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DNA Extraction, 16S rRNA Gene Amplification and Illumina MiSeq

Bacterial DNA was extracted from 15 mg samples using the AquaGenomic Kit (Mo-BiTec GmbH, Göttingen, Germany) and further purified using KAPA Pure Beads (Roche, Basel, Switzerland) according to the manufacturer's protocols. The concentration of genomic DNA was measured using a Qubit 3.0 Fluorometer with the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Bacterial DNA was amplified with tagged primers (forward, 50TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG,andreverse,50GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) covering the V3–V4 region of the bacterial 16S rRNA gene. Polymerase chain reactions (PCRs) and DNA purifications were performed according to Illumina's demonstrated protocol (Illumina Inc., 2013). The PCR product libraries were quantified and qualified by using the High Sensitivity D1000 ScreenTape on the TapeStation 2200 instrument (Agilent Technologies, Santa Clara, CA, USA).

Bioinformatics

The microbiome bioinformatics were performed with the Quantitative Insights Into Microbial Ecology 2 (QIIME2) version 2020.2 software package (Bolyen et al., 2019). Raw sequence data were demultiplexed and quality filtered using the q2-demux plugin, followed by denoising with Deblur (Amir et al., 2017). Sequences were filtered based on quality scores and the presence of ambiguous base calls using the quality-filter q-score options (QIIME2 default setting). Representative sequences were found using a 16S reference as a positive filter, as implemented in the Deblur denoise-16S method. Alpha diversity metrics (Chao1, Shannon, Simpson, and phylogenetic distance (PD)) and beta diversity metrics (Bray–Curtis dissimilarity) were estimated using the QIIME2 diversity plugin and MicrobiomeAnalyst (https://www.microbiomeanalyst.ca/, accessed on 1 September 2020) online software after samples were rarefied to 10,000 sequences per sample (Chong et al., 2020).

Statistical analysis

Evaluation the chemical composition of oats and barley

All statistical analyses were performed by SPSS 23.0 software. Data were assessed for normality prior to statistical analyses. The level of significance was set at ($p<0.05$). The nutrient composition of the grains was analysed by one way ANOVA. Differences between groups were determined by Duncan´s post hoc tests. The nutrient contents of the spring and winter oats genotypes were compared by t-test. The variances of the nutrients were expressed by the coefficient of variation (CV). The interaction between the different nutrient categories was evaluated by linear regression model. Multiple linear regression was used to predicted and determine the relationship between grain

viscosity and the different fibre fractions containing soluble components (NDF, SDFP and β-glucan).

Digestibility trial

The nutrient digstibility of the experimental diets were comapred by one way ANOVA, using the Duncan´s post hoc tests. The homogeneity test was carried out by Levene's test. If the Levene's test was significant Dunnett's test was used for the evaluation. The response between AA intake and pre-caecally absorbed AAs was evaluated by linear regression. Al the statistics have been done with the SPSS 23.0 software.

Feeding trial

All the measured production and gut parameters were evaluated by one way ANOVA, using Kruskal-Wallis test and the post hoc Dunn's multiple comparisons test with Bonferroni correction.

RESULTS AND DISCUSSION

Chemical composition of the investigated oats and barley varieties

The measured chemical composition of the 36 varieties of winter barley (WB), 35 winter oats (WO) and 36 spring oats (SO) varieties is summarized in ***Table 2.*** From the data it can be seen that the dry matter content of oats is about 1-1.5% higher, than that of barley. It has not real practical importance since diet formulation is based on the nutrient contents of feedstuffs as fed basis. The measured DM contents correspond with those of **NASEM (2016).** Regarding crude protein, the lowest mean concentration was found in WO (138 g/kg), and the highest in SO (144 g/kg). Although the differences were significant, the CP contents were close to each other. The results agree with the earlier data **(Beloshapka et al. 2016; Rodehutscord et al. 2016; Evonik 2017).** As it is well known, oats contain higher amounts of either extract (EE) among cereal grains. In our investigations the EE content of SO was two times higher (43.9 g/kg), while that of WO three times higher (59.4 g/kg) than the EE in winter barley (20.5 g/kg). It could be a further important advantage in the chase of new WO varieties besides their higher yield. Our data on EE corresponds to those of **NRC, (1994)** and **NASEM (2016)**, except winter oats. Only very few published data exist on the EE content of WO. The crude ash content of all three grain groups was similar, around 2%. Winter barley had significantly higher nitrogen-free extract (NFE) compared with SO or WO. The difference is about 10%, which means that WB contains about 14-15% higher starch content. These results agree with the values reported by **Rodehutscord et al. (2016), Prates & Yu, (2017), Saccomanno et al. (2017) and Sukhdeep et al. (2019)**. Among the mentioned parameters the CV of DM was the lowest (0.2 – 0.5%), while those of CP (4.9 – 10.4%) and EE (10.3 – 14.9%) was the highest. However, the CV value of crude protein was higher in spring oats (10.35) than other grains. The CV value for EE was particularly high in spring oats (14.87%). From plant breeding aspects, the lower variance of EE in WO could also be a potential advantage comprising to SO. The CF concentration of grains ranged from 50.9 g/kg in winter barley to 119.2 g/kg in spring oats. The two-times higher CF of oats means an important constraint for feeding oats with monogastric animals. The ADF contents of samples were in the range of 61.0 – 145.5 g/kg, while the NDF between 212.0 and 328.5 g/kg. Regarding these fibre fractions, there were only minor differences between SO and WO. The concentrations

of CF, NDF and ADF in WB, WO and SO were in generally good agreement with the values of **Bach Knudsen, (2014); Rodehutscord et al. (2016)** and **Bach Knudsen et al., (2017)**. The insoluble dietary fibre (IDF) category is used mainly in human nutrition, but recently when the gut health of farm animals getting more and more important, the measurement of IDF and the soluble fibre fractions is getting common. Both oats and barley contain significant amounts of soluble β-glucans, which can modify the digestion and the gut microbiota composition of animals **(Svihus and Gullord 2002)**. Therefore, the higher molecular weight, precipitable soluble fibre (SDFP) fractions have also been determined. The IDF content of the samples was in the range of 167.6 – 331.9 g/kg, with the lowest concentration in WB and the highest in SO. The opposite trend was true for SDFP. In this case WB had the highest content (44.5 g/kg) and WO the lowest (29.8 g/kg). The SDFP content of SO (30.8 g/kg) was close to that of WO. The concentrations of both SDFP and IDF agreed with those of **Menkovska et al. (2017).** These authors mentioning that IDF and SDFP concentration of grains depends on the agricultural circumstances.

According to the literature data **(Choct 2015)** the main SDFP compounds of oats and barley is β-glucan. So, the total β-glucan contents were also analysed in order to find correlations between the β-glucans, the SDFP contents and the viscosity of the grains. The SDFP and the β-glucan content of WB was 48 and 40 % higher than that of oats. On the other hand, the grain viscosity was not in line with these two parameters. The concentrations of β-glucan in the present study corresponded with several previous studies **(Bach Knudsen 2014; Beloshapka et al. 2016; Rodehutscord et al. 2016)**. Similarly, to the SDFP and β-glucan results, the viscosity of WB was the highest (9.0 mPas), but it was only 5.9% and 36% higher than those of WO and SO respectively. The reason for the difference in grain viscosity between WO and SO is not known. The interval of our viscosity results is in the range can be found in the relevant publications **(Dusel et al. 1997; Svihus et al. 2000)**.

The variance of all fibre fractions, the grain viscosity and β-glucan were high, below 10% in the case of CF, ADF and NDF, but 18 % for SDFP and viscosity.

Table 2. Chemical composition, fibre fractions, viscosity of oats and barley varieties

		Dry matter	Crude protein	Crude fat	Crude ash	NFE[1]	Starch	Crude fibre	NDF[2]	ADF[3]	IDF[4]	SDFP[5]	Viscosity	β-Glucan
							[g/kg DM]						[mPas]	[mg/g]
Winter barley (n=36)	Mean	888.0[c]	141.0[b]	20.5[c]	2.0[b]	767.0[a]	531.0[a]	50.9[c]	212.0[b]	61.0[c]	167.6[c]	44.5[a]	9.0[a]	46.1[a]
	Min	877.3	120.0	17.1	1.8	739.0	488.0	32.6	165.9	41.8	138.2	33.1	5.5	31.9
	Max	902.9	177.0	24.9	2.4	789.0	554.0	66.7	268.6	77.1	203.5	60.0	15.6	57.7
	SD[6]	4.7	13.7	2.1	0.1	12.6	17.1	8.2	26.9	9.1	17.5	8.2	2.4	5.7
	CV[7](%)	0.5	9.8	10.3	7.1	1.6	3.2	16.1	12.7	14.9	10.5	18.3	27.1	12.4
Winter oats (n=35)	Mean	902.0[b]	138.0[b]	59.4[a]	2.2[a]	664.0[c]	393.0[b]	114.1[b]	327.7[a]	145.5[a]	311.9[b]	29.8[b]	8.5[a]	34.2[b]
	Min	897.0	128.0	45.7	2.0	652.0	353.0	93.8	267.9	118.6	105.9	20.3	6.0	28.3
	Max	909.0	146.0	74.7	2.6	681.0	430.0	134.0	443.7	163.3	368.9	52.0	12.5	42.3
	SD[6]	3.2	4.7	6.3	0.2	7.9	21.2	8.8	34.4	9.9	45.6	7.2	1.6	3.7
	CV[7](%)	0.4	4.7	10.6	7.3	1.2	5.4	7.7	10.5	6.8	14.6	24.3	18.2	10.8
Spring oats (n=36)	Mean	906.0[a]	144.0[a]	43.9[b]	1.9[b]	672.0[b]	384.0[b]	119.2[a]	328.5[a]	137.6[b]	331.9[a]	30.8[b]	6.6[b]	32.7[b]
	Min	902.0	120.0	33.4	1.8	638.0	345.0	97.7	282.5	121.2	220.5	20.4	4.4	26.4
	Max	912.0	163.0	63.6	2.2	703.0	440.0	135.6	383.4	159.6	425.9	41.5	9.8	39.8
	SD[6]	2.1	10.5	6.5	0.1	14.4	22.6	9.0	22.6	10.9	44.4	5.5	1.2	3.3
	CV[7](%)	0.2	10.4	14.9	4.9	2.1	5.9	7.5	6.9	7.94	13.4	18.0	18.3	10.1

Notes: [1]NFE nitrogen-free extract; [2]NDF neutral detergent fibre; [3]ADF acid detergent fibre; [4]IDF insoluble dietary fibre, [5]SDFP, soluble dietary fibre precipitated, [6]SD standard deviation; [7]CV coefficient of variation; [a-c] Means within a column without common superscript letter are significantly different between grain types.

The interactions between the crude protein content with the other nutrients

As expected, the different fibre categories of WO showed negative correlation with the crude protein content of grains (***Table 3).***

On the other hand, the starch and EE change parallel with CP. According to our knowledge no such nutrient interactions have been published so far for WO. In the case of SO, only starch showed significant correlation with CP, but in this case the connection was negative with weak correlation coefficient. No significant interactions between the CP and the other parameters were found in WB samples.

Table 3. Significant correlation between the nutrient categories

winter oats		
equation	r^2	p
crude protein = 17.235 – 0.366 x ADF	0.663	0.0001
crude protein = 5.838 + 0.187 x starch	0.400	0.0001
crude protein = 15.711 – 0.11 x NDF	0.377	0.0001
crude protein = 16.821 – 0.426 x CF	0.669	0.0001
crude protein = 8.952 + 0.66 x EE	0.302	0.001
spring oats		
equation	r^2	p
crude protein = 21.959 – 0.256 x starch	0.291	0.001

Prediction of viscosity and β-glucan contents from the fibre fractions

Data in ***Table* 6.** shows a positive correlation between viscosity and β-glucan in both winter and spring oats. In the case of barley, its beta-glucan content was predictable from the NDF and SDFP contents. This result for barley agrees with the findings of **Sukhdeep et al. (2019)** , who observed also positive interaction between β-glucan and the main soluble dietary fibre compounds. The result is however in opposite with the findings of **(Rodehutscord et al. 2016)**, who reported that there is no significant correlation between the grain's extract viscosity and NDF in any grain type.

Comparison the measured chemical compositions with the table values

The measured CP contents of barley and oats were higher than the values of the EVONIK table **(EVONIK 2017)**. The data of ***Table 4.*** shows, that in the case of WB the measured CP was 22%, in the case of SO 27% higher than the table values. No

specific table values exist for WO, the tables contain the results of both oat types. Starch was the other nutrient where differences were found. In this case the measured values were lower. The measured starch content of WB was 14% and that of SO 16% less. All the other measured parameters, the EE and different fibre contents were similar to the table values.

Comparing the nutrient content of oats and barley with the main cereal ingredients of the Hungarian poultry diets, corn, and wheat, we can conclude, that oats and barley contain more protein and fibre, while less starch. The crude fat content of WB is similar to that of wheat, and the crude fat of oats is close to that of corn

Table 4. Comparison the measured nutrient contents with those of the table values

	Winter barley (n=36)	Winter oats (n=35)	Spring oats (n=36)	Winter barley (Evonik, 2017)	Oats (Evonik, 2017)	Wheat (Evonik, 2017)	Corn (Evonik,2017)
	g/kg DM						
Crude protein	140.0	137.6	143.8	115.3	112.6	131.6	84.7
Crude fat	20.5	59.4	43.9	27.2	51.3	22.4	42.6
Crude ash	19.5	22.2	19.4	24.2	28.2	17.5	13.6
Starch	531.3	393.0	383.8	603.6	443.5	683.2	748.4
Crude fibre	50.9	114.1	119.2	49.0	122.8	26.0	22.0
NDF	212.0	327.7	328.5	199.8	331.7	127.3	110.7
ADF	61.0	145.5	137.6	63.3	154.8	36.4	31.5

The amino acid composition of oats and barley proteins

The amino acid composition of the investigated grains was also compared on the same protein basis ***(Table 5.).*** In this case the AA contents are expressed as percentage of the protein (g/16 g N). In this comparison the differences between the three groups declined. Still the relative EAA contents of oats were higher than those of barley, but no significant difference remained for MET. Comparing of AA composition in WO and SO protein, only TYR and ARG was different. TYR was higher in SO, while ARG was higher in WO. The previously mentioned differences in GLU and PRO between barley and oats was true also for the relative AA values. Since the protein content of SO was higher than that of WO, the comparison on the same protein bases resulted higher AA contents in WO. The total EAA ratio in WB, WO and SO proteins were 43, 49 and 46 % respectively. The differences were significant, which suggests higher protein quality of oats.

The data of ***Table 5.*** show also, that the AA composition of barley protein is more balanced, which is important when the AA composition is calculated from the CP value. The CV values in WB's amino acids were in all cases below 10%, for several AAs only 5 - 6 %. The variance in WO and SO protein AA compositions was higher. The highest CV value belonged to HIS in SO protein (15.96%) and to MET in WO protein (18.16%).

The differences between the AA profile of barley and oats proteins are related to the different storage protein molecules of the grains. The major storage proteins of barley are hordeins (30–50%), of which dominating AAs are proline and glutamine, and the limiting AAs are lysine and tryptophan. Not only lysine but also cysteine content of barley is lower than in other grains **(Siebenhandl-Ehn et al. 2011; Šimić et al. 2019).** Globulins represent 70–80% of the total protein fraction of oats storage proteins. This high concentrate of globulin storage proteins in oats grain may contribute to its high nutritional value when compared with other cereals **(Shewry and Halford 2002)**. Although lysine, methionine and threonine are limiting amino acids in oats but still its lysine content is higher than that of the other cereals **(Sukhdeep et al., 2019)**. Our findings on the difference in the cystine concentration oats than winter barley is in line with the findings of **(Rodehutscord et al. 2016)**.

Correlations between the protein content of grains and the essential amino acid contents of the grains' protein

The linear regression results on WB and WO are summarized in ***Table 6***. No significant regression was found in the case of SO. More significant correlations were found in WB, which means that the AA composition of the barley's protein is not constant.

Table 5. Amino acid composition of barley and oats proteins (g/16 g N)

	Winter Barley (n=36)					Winter Oats (n=35)					Spring Oats (n=36)				
	Mean	Min	Max	SD[1]	CV[2] (%)	Mean	Min	Max	SD	CV (%)	Mean	Min	Max	SD	CV (%)
Cystine	2.14[b]	1.83	2.40	0.16	7.43	3.06[a]	2.46	3.70	0.36	11.87	3.04[a]	2.59	3.46	0.25	8.23
Methionine	1.76	1.51	1.97	0.13	7.54	1.81	1.35	2.75	0.33	18.16	1.85	1.47	2.07	0.17	9.41
Threonine	3.42[b]	2.87	3.97	0.25	7.27	3.72[a]	3.02	4.92	0.46	12.24	3.67[a]	2.15	4.26	0.47	12.84
Valine	4.95[b]	4.54	5.32	0.25	5.00	5.42[a]	4.37	7.55	0.66	12.09	5.26[a]	4.22	5.93	0.46	8.84
Isoleucine	3.60[b]	3.29	3.91	0.20	5.60	4.06[a]	3.25	5.98	0.58	14.30	4.08[a]	3.37	4.63	0.33	8.00
Leucine	6.83[b]	6.08	7.47	0.38	5.57	7.79[a]	6.50	10.94	1.02	13.04	7.70[a]	6.54	8.70	0.68	8.86
Tyrosine	3.02[c]	2.59	3.56	0.26	8.61	3.93[b]	3.02	4.83	0.42	10.71	4.04[a]	3.32	4.44	0.28	6.83
Phenylalanine	5.48	4.98	5.96	0.29	5.32	5.37	4.26	7.32	0.71	13.17	5.40	4.67	6.10	0.42	7.70
Histidine	2.27[b]	1.87	2.94	0.22	9.80	2.49[a]	1.67	3.54	0.37	14.72	2.53[a]	2.03	4.44	0.40	15.96
Lysine	3.62[b]	3.15	4.24	0.25	6.95	4.45[a]	3.42	5.20	0.44	9.91	4.43[a]	3.79	4.80	0.25	5.55
Arginine	5.21[c]	4.79	5.69	0.25	4.82	6.81[a]	5.30	10.15	0.81	11.92	6.49[b]	5.83	7.86	0.52	8.05
Aspartic acid	6.15[b]	4.97	6.67	0.36	5.91	8.62[a]	7.05	10.86	1.06	12.32	8.34[a]	6.96	9.73	0.80	9.54
Serine	4.24[b]	3.73	4.66	0.27	6.39	5.05[a]	4.03	5.90	0.51	10.07	5.06[a]	3.84	5.96	0.55	10.88
Glutamic acid	25.43[a]	22.73	27.84	1.16	4.56	20.45[b]	17.89	28.72	1.82	8.92	19.97[b]	18.24	21.59	0.80	4.00
Proline	11.79[a]	9.40	13.94	1.09	9.25	5.29[b]	4.25	6.22	0.55	10.40	5.43[b]	4.63	6.28	0.46	8.53
Glycine	4.05[b]	3.48	4.49	0.27	6.72	5.07[a]	4.24	6.11	0.51	10.09	5.20[a]	4.34	5.91	0.48	9.14
Alanine	3.98[c]	3.50	4.47	0.25	6.18	4.89[b]	4.12	7.40	0.60	12.30	5.08[a]	4.22	6.37	0.48	9.41

[1] SD standard deviation, [2]CV coefficient of variation, [3]Total EAA total essential amino acids, [4]Total NEAA total non-essential amino acids, [5]Total AA total amino acids, [6]EAA ratio essential amino acid ratio, [7]NEAA ratio non-essential amino acid ratio, [a-c] Means within a raw without common superscript letter are significantly different between grain types.

Table 6. Significant correlations between the protein content and essential amino acids

Winter barley		
Equation	r^2	p
LEU = 8.649 - 0.13 x crude protein	0.217	0.004
TYR = 4.20 - 0.008 x crude protein	0.198	0.007
LYS = 5.231 - 0.11 x crude protein	0.390	0.0001
ASP = 8.343 - 0.16 x crude protein	0.348	0.0001
PRO = 3.47 + 0.059 x crude protein	0.555	0.0001
GLY = 5.479 - 0.010 x crude protein	0.261	0.001
ALA = 5.028 - 0.008 x crude protein	0.175	0.011
Winter oats		
Equation	r^2	p
CYS = 6.678 - 0.026 crude protein	0.118	0.044
LYS = 8.696 - 0.031 crude protein	0.110	0.05

Only the relative PRO content increases if the protein content of the grain increases. The concentration of the other AAs in the table (LEU, TYR, LYS, ASP, GLY and ALA) decrease with the increase of CP. Since the prediction of the AA content of feedstuffs based on the assumption, that the AA composition of the feedstuffs is constant, these results suggest, that in the case of barley it can cause inaccuracies. It could be important mainly for LYS prediction, which is the first or second limiting AA in monogastric animal.

In winter oats, only the CYS and LYS content of the oat's protein decline with the increase of the protein content of the grain. Among the three cereal groups, the AA composition of SO protein seems to be the most stable.

Our results on barley are in some aspects agree with the findings of **(Rodehutscord et al. 2016).** The variability in AA composition of barley protein could be the result of the differences in the prolamin protein deposition and the variance in the different prolamin proteins **(Shewry 2007; Klose and Arendt 2012; Šimić et al. 2019).**

Comparison the measured relative amino acid contents with the table values

In this comparison the measured protein AA composition was compared with the AA composition of oats, barley, wheat, and corn protein's, can be found in the tables. The

results are summarized in ***Table 7***. No big differences exist between the measured AA composition of WB and that can be found in the EVONIK table. On the other hand, the table values of oats are almost for all amino acids lower in the tables. Among the first limiting essential amino acids the differences are the highest for MET, VAL, ILE, LYS and ARG. Looking at the EAA ratios, the measured and table value of WB is identical (0.43). On the other hand, the table value for oats is between the spring (0.46) and winter (0.49) varieties. Both oats and barley show higher EAA ratio than wheat. The EAA ratio of the corn's protein is similar to barley and oats. The ratio of MET, HIS and LEU in corn protein is higher than the other cereals.

Table 7. Comparison the amino acid composition of different cereal grain proteins

Items	Winter barely measured	Winter oats measured	Spring oats measured	Winter barley (Evonik, 2017)	Oats (Evonik, 2017	Wheat (Evonik, 2017)	Corn (Evonik, 2017)
				(g/16gN)			
Cystine	2.14	3.06	3.04	2.17	2.72	2.25	2.28
Methionine	1.76	1.81	1.85	1.67	1.61	1.55	2.15
Threonine	3.42	3.72	3.67	3.35	3.33	2.85	3.62
Valine	4.95	5.42	5.26	4.93	4.94	4.23	4.70
Isoleucine	3.60	4.06	4.08	3.45	3.53	3.37	3.36
Leucine	6.83	7.79	7.70	6.80	7.16	6.56	11.95
Phenylalanine	5.48	5.37	5.40	4.93	4.94	4.49	4.83
Histidine	2.27	2.49	2.53	2.17	2.12	2.25	2.82
Lysine	3.62	4.45	4.43	3.55	4.04	2.76	3.09
Arginine	5.21	6.81	6.49	5.02	6.36	4.75	4.83
Aspartic acid	6.15	8.62	8.34	5.91	7.67	5.01	6.71
Serine	4.24	5.05	5.06	4.24	4.54	4.58	4.83
Glutamic acid	25.43	20.45	19.97	22.96	19.17	28.32	18.12
Proline	11.79	5.29	5.43	10.64	5.15	9.76	8.86
Glycine	4.05	5.07	5.20	4.04	4.84	4.06	4.03
Alanine	3.98	4.89	5.08	4.04	4.54	3.45	7.38
EAA ratio (%)	**43.2**	**49.7**	**49.7**	**43.0**	**47.0**	**38.0**	**46.0**
NEAA ratio (%)	**56.8**	**50.3**	**50.3**	**57.0**	**53.0**	**62.0**	**54.0**

[1] SD standard deviation, [2] CV coefficient of variation, [3] Total EAA total essential amino acids, [4] Total NEAA total non-essential amino acids, [5] Total AA total amino acids, [6]EAA ratio essential amino acid ratio, [7] NEAA ratio non-essential amino acid ratio, [a-c] Means within a raw without common superscript letter are significantly different between grain types

The results of the digestibility trial

Nutrient digestibility of oats and barley containing diets

The inclusion rate of oats and barley did not affect the digestion of the nutrients. Compared with the control diet, the faecal digestibility of crude fat was significantly higher when barley and oats containing diets were fed (***Table 8).*** The opposite was true for starch digestion. In this case the digestibility of the barley and winter oats diets were significantly lower than that on the control. The reason for the significant main effect interaction was, that the 40% inclusion rate reduced the starch digestion of the barley and winter oats containing diets, but no change was found in the of spring oats. The highest cereal effect was found in the ileal digestibility of nitrogen. All the three cereal grain increased the N absorption by 7,7-11 %.

Table 8. Nutrient digestibility of the experimental diets

Cereal	Inclusion rate	Faecal rude fat digestibility	Faecal starch digestibility	ileal N digestibility
Barley	20%	89.58	94.23	80.15
	40%	89.13	93.87	81.06
Winter oats	20%	90.65	94.44	80.42
	40%	91.94	91.62	80.04
Spring oats	20%	89.30	94.64	78.98
	40%	89.20	94.61	75.49
Control	-	84.29	95.70	69.63
SEM		0.0039	0.0019	0.0046
		Main effects		
Inclusion rate				
	20%	89.85	94.44	79.85
	40%	90.10	93.36	78.86
Cereal grain				
	Barley	89.36 [a]	94.06 [b]	80.60 [a]
	Winter oats	91.29 [a]	93.03 [b]	80.23 [a]
	Spring oats	89.25 [a]	94.63 [ab]	77.28 [a]
	Control	84.29 [b]	95.70 [a]	69.63 [b]
		p values		
Inclusion rate		0.749	0.226	0.218
Cereal grain		**0.047**	**0.001**	**0.01**
Inclusion rate x cereal grain		0.605	**0.002**	0.072

[a-b] Means within a column not showing common superscript letter are significantly different (P<0.05)

Amino acid digestibility of the barley- and oats-based diets

Similarly to the fat, starch and nitrogen digestibility, no significant inclusion rate effect was found for the digestion of amino acids (**Table 9.).** The grain type modified the digestion significantly only in four cases. The ARG, ILE and PHE digestibility of the spring oats diets was the highest, but the difference was significant only in comparison with the barley (ARG, ILE) and control (PHE) diets. The CYS digestibility of both oats was significantly lower than that of the barley and control diets.

Table 9. Apparent ileal amino acids digestibility of the experimental diets (%)

Cereal	Inclusion rate	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	VAL	CYS	TYR
Barley	20%	84.7	81.2	80.5	84.7	82.4	88.0	82.1	74.5	81.0	75.0	77.1
	40%	84.1	81.2	81.3	85.8	80.7	88.3	83.3	74.0	80.5	74.9	75.1
W. oats	20%	88.3	85.0	83.1	85.0	83.6	87.1	85.4	75.2	83.2	61.6	75.9
	40%	89.5	84.3	86.0	86.9	84.6	87.4	86.0	74.3	83.3	58.0	73.7
S. oats	20%	90.0	83.8	85.6	85.4	83.7	87.5	85.7	75.7	81.5	63.1	76.3
	40%	90.0	82.9	84.3	86.8	83.9	85.5	86.9	75.6	82.7	58.8	74.9
Control	-	86.6	84.0	81.7	86.0	82.6	88.5	82.0	76.5	81.2	71.5	79.0
SEM		0.004	0.004	0.004	0.003	0.003	0.003	0.004	0.004	0.004	0.008	0.004
Main effects												
Inclusion rate												
20%		87.7	83.3	83.1	85.1	83.2	87.5	84.4	75.1	81.9	66.8	76.5
40%		87.9	82.8	84.0	86.5	83.1	87.1	85.4	74.6	82.2	63.6	74.5
Cereal grain												
Barley		84.4 ^{c}	81.2	80.9 ^{c}	85.3	81.6	88.1	82.7 ^{bc}	74.3	80.8	74.9 ^{a}	76.1
W. oats		89.0 ^{ab}	84.6	84.7 ^{ab}	86.0	84.1	87.3	85.7 ^{ab}	74.7	83.2	59.6 ^{b}	74.7
S. oats		90.0 ^{a}	83.3	85.0 ^{a}	86.1	83.8	86.5	86.3 ^{a}	75.6	82.1	61.0 ^{b}	75.6
Control		86.6 ^{bc}	84.0	81.7 ^{bc}	86.0	82.6	88.5	82.0 ^{c}	76.5	81.2	71.5 ^{a}	79.0
						p values						
Inclusion rate		0.759	0.483	0.205	0.45	0.784	0.474	0.131	0.495	0.705	0.062	0.345
Cereal grain		**0.001**	0.088	**0.001**	0.534	0.136	0.091	**0.001**	0.320	0.232	**0.001**	0.374
Incl. rate x Cereal gr		0.452	0.894	**0.037**	0.874	0.210	0.195	0.927	0.907	0.587	**0.044**	0.908

$^{a-d}$ Means within a raw not showing common superscript letter are significantly different (P<0.05)

The amino acid digestibility coefficients of oats and barley were the slopes of the linear regression equation, that described the relationship between the daily AA intake and daily ileal absorbed AAs.

The regression between the AA intake and pre-caecally absorbed AA was in all cases significant, with high correlation coefficients. The regression equation parameters of barley and oats varieties are shown in ***Table 10. and 11***. The tables also contain the average of the varieties. Among essential AAs of barley, the highest and lowest digestibility coefficients belonged to MET and LYS, respectively. In both oats types the cystine digestibility was the lowest. In winter oats the highest absorption belonged to VAL, while in spring oats to LEU.

Table 10. Ileal amino acid digestibility coefficients (slope) of the barley-based diets

	Experimental diets						
	WB 1		WB 2		WB 3		Average
	WB1/20+WB1/40		WB2/20+WB2/40		WB3/20+WB3/40		
	Slope	r^2	Slope	r^2	Slope	r^2	
Arginine	0.6274	0.8327	0.7902	0.9742	0.863	0.9587	**0.760**
Histidine	0.8585	0.8079	0.7671	0.9501	0.8678	0.9155	**0.831**
Isoleucine	0.744	0.8999	0. 7026	0.9213	0.8448	0.9335	**0.794**
Leucine	0.8698	0.9708	0.8337	0.9569	0.9362	0.9459	**0.880**
Lysine	0.6307	0.8416	0.7196	0.9606	0.7654	0.9304	**0.705**
Methionine	0.9434	0.8878	0.8395	0.9719	0.9677	0.9671	**0.917**
Phenylalanine	0.8211	0.924	0.7754	0.9431	0.8511	0.9613	**0.816**
Threonine	0.6457	0.8664	0.7873	0.9707	0.6961	0.9102	**0.710**
Valine	0.9159	0.9731	0.6986	0.929	0.8512	0.9415	**0.822**
Cysteine	0.7514	0.8811	0.7306	0.9591	0.7613	0.9181	**0.748**
Alanine	0.6871	0.932	0.750	0.9593	0.8529	0.9568	**0.763**
Aspartic acid	0.7322	0.9078	0.7844	0. 9568	0.8271	0.9415	**0.781**
Proline	0.8075	0.9013	0.781	0.9609	0.8837	0.9747	**0.824**
Glutamic acid	0.8011	0.8839	0.7803	0.9818	0.9621	0.9782	**0.848**
Glycine	0.8154	0.8839	0.6854	0.9439	0.8375	0.9278	**0.779**
Serine	0.7501	0.8888	0.7155	0.9796	0.8322	0.8841	**0.766**
Tyrosine	0.6736	0.7637	0.6778	0.9144	0.6966	0.9126	**0.683**

WB 1-3/20: diet that contained winter barley at 20%; WB 1-3/40: diet that contained winter barley at 40%;

Table 11. Ileal amino acid digestibility coefficients (slope) of the oats-based diets

	WO1		WO2		WO3			SO1		SO2		SO3		
	WO1/20+ WO1/40		WO2/20+ WO2/40		WO3/20+ WO3/40		Average	SO1/20 + SO1/40		SO2/20 + SO2/40		SO3/20 + SO3/40		Average
	Slope	r^2	Slope	r^2	Slope	r^2		Slope	r^2	Slope	r^2	Slope	r^2	
Arginine	0.9518	0.9837	0.8676	0.9667	0.7295	0.9372	**0.850**	0.7446	0.9279	0.90	0.946	0.9662	0.9941	**0.870**
Histidine	0.7501	0.8727	0.7886	0.9064	0.8818	0.9149	**0.807**	0.8206	0.8724	0.8593	0.927	0.8607	0.9561	**0.847**
Isoleucine	0.876	0.9469	0.8679	0.9502	0.8329	0.9655	**0.859**	0.863	0.9432	0.8453	0.948	0.9355	0.9887	**0.881**
Leucine	0.7349	0.9699	0.8742	0.9431	0.8878	0.9717	**0.832**	0.8887	0.8375	0.8745	0.959	0.9469	0.9944	**0.903**
Lysine	0.7553	0.9015	0.8945	0.9687	0.7633	0.941	**0.804**	0.7379	0.9209	0.9244	0.941	0.7569	0.98	**0.806**
Methionine	0.8648	0.9492	0.8132	0.9768	0.7387	0.9416	**0.806**	0.7944	0.9025	0.9356	0.914	0.852	0.9758	**0.861**
Phenylalanine	0.8456	0.9605	0.8834	0.9756	0.6621	0.9115	**0.797**	0.7453	0.8574	0.8426	0.937	0.8877	0.9883	**0.825**
Threonine	0.567	0.7008	0.6867	0.944	0.7059	0.811	**0.653**	0.8386	0.93	0.8632	0.96	0.7839	0.9421	**0.829**
Valine	0.9414	0.9627	0.9307	0.9712	0.7989	0.9133	**0.890**	0.7448	0.9421	0.7207	0.944	0.8423	0.9749	**0.769**
Cysteine	0.3321	0.4743	0.5019	0.8639	0.4429	0.894	**0.426**	0.5633	0.7269	0.5678	0.875	0.6565	0.921	**0.596**
Alanine	0.8457	0.9482	0.8502	0.9563	0.8324	0.8848	**0.843**	0.9074	0.9057	0.7166	0.901	0.9153	0.9925	**0.846**
Aspartic acid	0.6732	0.8411	0.8025	0.9647	0.7653	0.8907	**0.747**	0.5955	0.8072	0.7697	0.946	0.7941	0.9728	**0.720**
Proline	0.9219	0.9456	0.7423	0.9684	0.7835	0.9314	**0.816**	0.8101	0.9521	0.8416	0.953	0.8553	0.9781	**0.836**
Glutamic acid	0.9323	0.962	0.8419	0.9733	0.8738	0.9545	**0.883**	0.9188	0.9615	0.9397	0.982	0.8904	0.9739	**0.916**
Glycine	0.8402	0.9028	0.6347	0.8955	0.7068	0.8642	**0.727**	0.4987	0.7063	0.6718	0.859	0.6596	0.9059	**0.610**
Serine	0.5825	0.759	0.7016	0.8849	0.8391	0.8705	**0.708**	0.6905	0.7983	0.8126	0.965	0.7393	0.9213	**0.747**
Tyrosine	0.7766	0.8992	0.7939	0.9313	0.6204	0.837	**0.730**	0.675	0.8432	0.8319	0.949	0.7617	0.9412	**0.756**

WO 1-3/20: diet that contained winter oats at 20%; WO 1-3/40: diets that contained winter oats at 40%; SO 1-3/20: diet that contained spring oats at 20%; SO 1-3/40: diets that contained spring oats at 40%;. (CVB Feed Table "Standardized Ileal Digestibility of Amino Acids in Feedstuffs for Poultry," 2017)

Except of LEU and, MET the measured ileal AA digestibility coefficients of barley were lower than the values showed by **EVONIK, table (2017)** ***(Figure 1.).*** The biggest differences existed between the measured and EVONIK published CYS and LYS digestibility (13 and 17.5% respectively). The **NRC, (1994)** and Centraal Veevoederbureau **CBV (2017)** values showed less differences in comparison with the measured coefficients. The reason for the differencesdiffreences between the EVONIK table values and our results is mainly the differences in the methods of animal experiments. The **NRC, (1994)** data are the oldest and American recommendation is based on the literatureliterutre data. That time in the american continent still the precision feeding method was the most common (Sibbald 1976). The feedstuffs were fed alone, the endogenous AA losses have been determined and total excreta collection was used. The **EVONIK (2017) and CBV (2017)** results are based on ileal sampling. In this case the feedstuffs were incorporated into a basal diet and the apparent digestibility coefficients corrected with the basal endogenous AA losses.

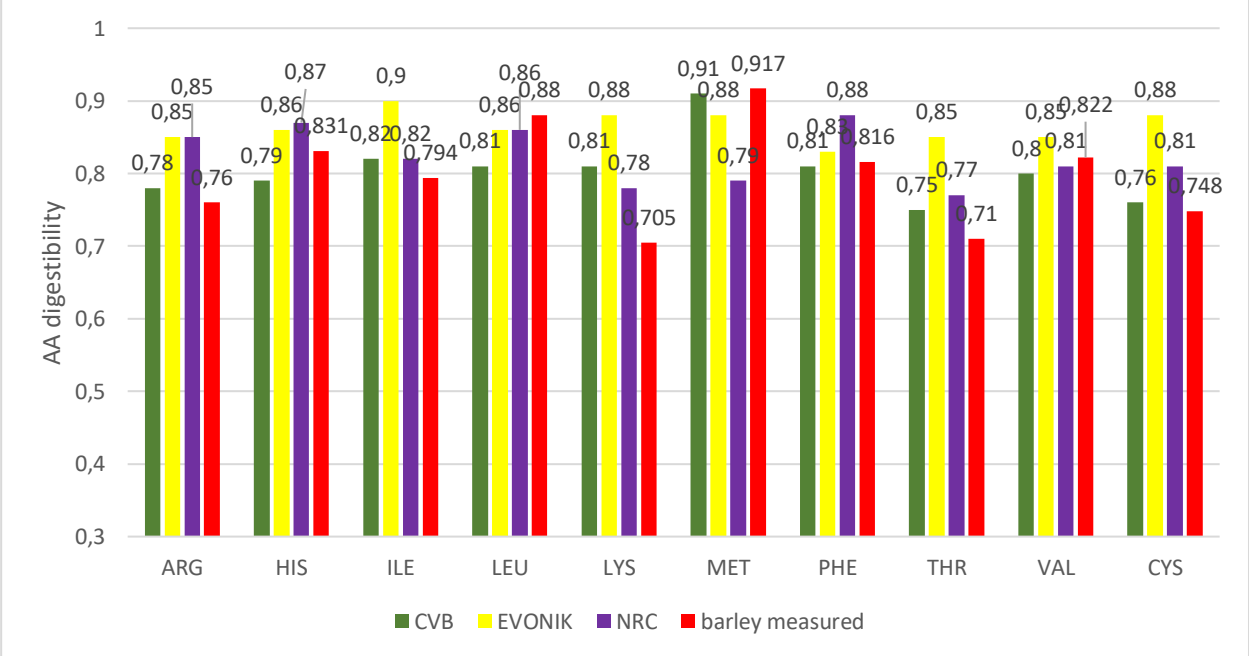

Figure 1. Comparison the measured ileal AA digestibility of barley with the table values (CVB, 2017;) EVONIK, 2017)

Comparing the measured AA digestibility of winter and spring oats, surprisingly even 18% difference was found for example in the digestibility of THR. The CYS digestibility of WO was

the lowest in the measured AAs (0.43). On the contrary the digestibility of VAL was higher in the WOs than SOs ***(Figure 2.***). It is well known that the yield of the cereal grains and legume seeds affects the protein content and also its composition **(Rodehutscord et al. 2016)**. However, not too many results are available on the differences in the digestibility of AAs. According to my knowledge these are the firs results, that prove the differences in the ileal AA digestibility of WO and SO varieties. Comparing the measured values with those of the earlier published values, the NRC and EVONIK coefficients were in most cases higher than the values measured in our experiments. The CVB coefficients showed more similarity. The biggest differences in data measured in our experiments and the values published in the tables mentioned above were found for PHE, THR and CYS. The reason for these alterations could be the different genotypes of oats and the previously mentioned differences in the animal digestibility models.

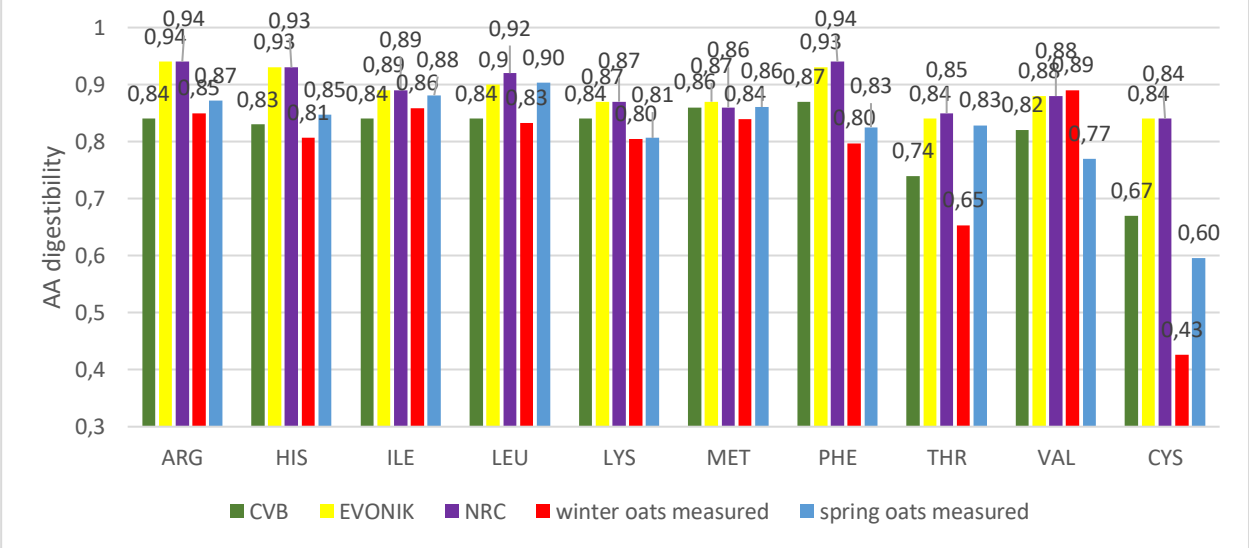

Figure 10. Comparison the measured ileal AA digestibility of oats with the table values (CVB, 2017;) EVONIK, 2017)

Effects of using of exogenous β-beta glucanase on the nutrient digestibility of oats- and barley-based diets

The xogenous β-glucanase enzyme supplementation of the barley and oats containing diets improved significantly the fat and protein digestion of chickens ***(Table 12.).*** On the other hand, starch digestion was not affected by the enzyme. It is known, that all exogenous enzymes increase the digestibility of such nutrients of which basic absorption rate is lower **(Aftab and Bedford 2018)**. Our result on the improvement of fat and protein digestion supports this finding. It is also

documented, that increase in gut viscosity resullts increased bacteriota abundance in the small intestine and these bacteria can conjugate bile acids and this way impair fat dgestion **(Choct & Annison, 1992; Choct et al., 1996; Choct, 2006)**.

Using the 40% inclusion of oats and barley was not depressive on fat digestion, moreover the fat digeestibility of the winter oats based diet was significantly higher than that of the control group. Interestingly winter oats decresed significantly the starch digestion compared with the three other treatments. Protein digestion was not affected significantly by the grain type. No such comparison results are available in the literature, where oats and barley at 40% incluson rate were used.

Table 12. Effect of exogenous β-glucanase on the apparent digestibility of crude fat, starch, and nitrogen

Cereal	Enzyme	Faecal crude fat digestibility	Faecal starch digestibility	ileal N digestibility
Barley	+	88.2	95.0	79.3
	-	82.0	94.3	70.7
W. oats	+	93.4	90.5	81.0
	-	84.5	92.6	68.5
S. oats	+	87.5	95.0	74.0
	-	88.3	93.7	67.3
Control	+	84.3	95.7	69.6
	-	84.9	95.4	71.2
SEM		0.006	0.0025	
		Main effects		
Enzyme				
	+	88.3 [a]	94.0	76.0 [a]
	-	85.0 [b]	94.0	69.4 [b]
Cereal grain				
	Barley	85.1 [ab]	94.6 [a]	75.0
	W. oats	89.0 [a]	91.5 [b]	74.7
	S. oats	87.9 [ab]	94.3 [a]	70.6
	Control	84.6 [b]	95.6 [a]	70.4
		p values		
Enzyme		**0.004**	0.954	**0.001**
Cereal grain		**0.021**	**0.001**	0.06
Enzyme x Cereal grain		**0.004**	**0.016**	**0.039**

The incorporation of barley and oats into basal diet and using β-glucanase resulted in amino acid dependent changes and failed to cause negative effects on the digestibility of amino acids (**Table**

13.). Both enzyme and grain effects were significant. Exogenous glucanase improved the digestibility of ARG, ILE, LEU, THR, CYS and TYR significantly. No differences were measured in the digestion of the remaining essential amino acids. The digestibility coefficient of ARG and LYS was significantly higher in the oats containing diets, than that of the barley-based diet. In the case of LEU and VAL only difference between the barley and spring oats treatments was significant. The PHE digestibility of the oats diets was significantly higher than those of the barley and control groups. Both oats treatment decreased the digestibility of CYS and all three cereals the absorption rate of TYR. No such amino acid dependent digestibility differences have been published yet when the oats and barley at 40% inclusion rate, with and without exogenous β-glucanase were fed.

Table 13. Ileal amino acid digestibility values of the experimental diets (%)

Diet	Enzyme	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	VAL	CYS	TYR
Barley	+	83.5	82.0	81.0	85.5	79.7	87.5	83.3	73.7	78.6	75.2	75,1
W. oats	+	88.8	83.1	84.3	87.7	83.7	85.6	85.2	71.2	81.8	58.2	70,8
S. oats	+	89.3	81.6	83.1	88.2	83.8	86.1	87.6	74.7	83.2	59.0	73,5
Control	+	86.6	84.0	81.7	86.0	82.6	88.5	82.0	76.5	81.2	71.5	79,0
Barley	-	81.5	78.1	79.3	80.5	77.3	85.3	81.5	69.7	76.6	71.3	71,1
W. oats	-	84.3	81.9	81.9	83.3	83.4	85.0	84.3	70.6	81.2	55.3	70,0
S. oats	-	86.4	82.8	81.9	85.3	83.3	85.8	84.1	72.8	81.7	56.8	70,8
Control	-	85.0	83.5	78.5	85.9	81.7	88.8	82.4	71.9	80.0	67.1	77,6
SEM		0.55	0.54	0.50	0.50	0.54	0.47	0.55	0.53	0.55	1.07	0.62
Main effects												
Enzyme												
Enzyme +		87,1 ^{a}	82.7	82.5 ^{a}	86.9 ^{a}	82.5	86.9	84.5	74.0 ^{a}	81.2	66.0 ^{a}	74.6 ^{a}
Enzyme -		84,3 ^{b}	81.6	80.4 ^{b}	83.8 ^{b}	81.4	86.2	83.1	71.3 ^{b}	79.9	62.6 ^{b}	72.4 ^{b}
Cereal grain												
Barley		82,5 ^{b}	80.1	80.2	83.0 ^{b}	78.5 ^{b}	86.4	82.4 ^{b}	71.7	77.6 ^{b}	73.3 ^{a}	73.1 ^{b}
W. oats		86,6 ^{a}	82.5	83.1	85.5 ^{ab}	83.6 ^{a}	85.3	84.8 ^{a}	70.9	81.5 ^{ab}	56.8 ^{b}	70.4 ^{b}
S. oats		87,9 ^{a}	82.2	82.5	86.8 ^{a}	83.6 ^{a}	86.0	85.9 ^{a}	73.8	82.5 ^{a}	57.9 ^{b}	72.2 ^{b}
Control		85,8 ^{ab}	83.8	80.1	86.0 ^{ab}	82.2 ^{ab}	88.7	82.2 ^{b}	74.2	80.6 ^{ab}	69.3 ^{a}	78.3 ^{a}
						p values						
Enzyme		**0.008**	0.324	**0.036**	**0.001**	0.333	0.484	0.195	**0.010**	0.241	**0.002**	**0.038**
Diet		**0.002**	0.192	0.078	**0.019**	**0.002**	0.143	**0.034**	0.087	**0.014**	**0.000**	**0.000**
Enzyme x Diet		0.768	0.385	0.889	0.275	0.896	0.856	0.577	0.542	0.979	0.838	0.720

$^{a-d}$ Means within a column not showing common superscript letter are significantly different (P<0.05)

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The results of the feeding trial

The effect of feeding the barley and oats containing diets on the production traits

No significant difference was found in the feed intake of chickens in the starter and grower phase ***(Table 14.)***. However, in the finisher phase the feed consumption from the barley and oats containing diets declined compared with the control. Due to the higher feed consumption in the finisher phase this trend was true also for the whole production period. Feed intake was the lowest in WB40 group. The dietary treatments did not result significant difference in the growth rate of animals in the starter phase. In the grower phase the highest weight gain was measured in the control group, which was significantly higher, than those of the higher inclusion rates of barley (WB40) and oats. Surprisingly, in the finisher phase the two oats-based diets resulted the highest gain, which exceeded significantly the three other treatments. The opposite trends in the grower and finisher phases equalized each other, so no significant differences existed in the cumulative weight gain. Regarding the feed conversion of birds, the best FCR in the starter phase belonged to the two barley-based diets, which were significantly lower than that of the control. No significant differences were found in the grower phase, but the FCR values in the finisher phase and for the whole production period were more favourable when barley and oats were fed. In both cases, the treatment of WO20 resulted the best FCR. From these results it can be concluded, that even young chickens can tolerate the higher fibre content of barley and oats. The barley and oats inclusion rates should not exceed 20 and 10% respectively in the grower phase, but their higher inclusion rates in the finisher phase can improve the growth rate and the feed conversion of chickens. For the reason of this positive effect the structural fibre of both grains and their gizzard stimulation could be mentioned. Many research results prove the efficiency of oats hulls as feed additive in broiler nutrition **(Svihus 2011); (Mateos et al. 2013).**

The effects of treatments on different gut parameters

The viscosity of the ileal gut content was the highest in the barley-based diets. Bot the 20 and 40% inclusion rates resulted in significant increase which was in line with the highest soluble β-glucan content of barley ***(Table 15)***. The measured ileal viscosity of this trial was in the range of the published values **(Shakouri et al., 2009; Konieczka & Smulikowska, 2018; Wang et al., 2017).**

In our study ileal chymus of birds fed the barley-based diets was more viscous compared with the two other treatments. The reason for this could be, that barley contains higher amounts of soluble NSP than oats, and at 40% inclusion rate the exogenous glucanase was probably not efficient enough to degrade the high concentration of β-glucan in the ileal digesta. The soluble dietary fibre content of the barley-based diet was higher than that of the oats and control treatments. This result reaffirms the need for a more precise fibre evaluation of poultry feedstuffs and considering both the structural and soluble fractions. Exogenous enzyme supplementation of the diets and the dosage/activity of enzymes should be in accordance with the real soluble fibre content. The other explanation of the results can be that not all the soluble, high molecular weight fibre of the grains are β-glucan. There is also arabinoxylan and other viscous compounds in barley and oats **(Choct 2006)**, but their ratio and variance are not investigated in detail yet.

The excreta dry matter was not affected significantly by the treatments. The gizzard weight, as expected increased significantly when the diets contained oats. Both inclusion rates of oats significantly increased the gizzard weight. The diets with different compositions did not modify the length of the small intestine parts. On the other hand the pH of the crop and caecal contents showed significant differences. In the case of the crop the barley and oats containing diets increased the pH, and significantt difference was observed between the control and WB40 treatment. This result could be the reason of the quicker enptying of crop if structural fibres are fed. In this case the lactic acid bacteria in the crop has less chance to reduce the pH. Similarly, in the caecum barley increased the pH in chickens fed WB diet. Interestingly, no significant difference was found in the WB40 group. This result is hard to explain, since soluble fibre and their ezymatic breakdown products work as prebiotics in the caecum. However, this mechanism is proved only for the soluble arabinoxylan and its fermented product, the xilan oligosacharides (XOS) **(Castro et al. 2024)**. No such research results are available on soluble β-glucans yet.

Table 14. Effects of the experimental diets on the production traits of broiler chickens

	Feed intake (g)				Body weight gain (g)				Feed conversion ratio (FCR)			
	10 days	24 days	39 day	1-39 day	10 days	24 days	39 day	1-39 day	10 days	24 days	39 day	1-39 day
	Starter	Grower	Finisher	Total	Starter	Grower	Finisher	Total	Starter	Grower	Finisher	Total
Control	311	1398	2540[a]	4249[a]	245	967.5[a]	1408.2 [b]	2620.5	1.27[a]	1.44	1.80[a]	1.62[a]
WB20	313	1358	2408[ab]	4078[ab]	267	942.6 [ab]	1405.0 [b]	2613.3	1.17[ab]	1.44	1.71[ab]	1.56[ab]
WB40	308	1354	2347[b]	3992[b]	258	914.9 [b]	1406.3 [b]	2586.3	1.16[b]	1.47	1.67[bc]	1.55[ab]
WO10	310	1386	2434[ab]	4130[ab]	253	933.5 [ab]	1487.3 [a]	2673.6	1.23[ab]	1.49	1.64[bc]	1.55[ab]
WO20	312	1352	2444[ab]	4105[ab]	256	892.5 [b]	1536.2 [a]	2684.3	1.22[ab]	1.52	1.59[c]	1.53[b]

C: control diet; WB20: diet that contained winter barley at 20%; WB40: diet that contained winter barley at 40%; WO10: diet that contained spring oats at 10%; WO20: diet that contained spring oats at 20%; [a-b] Means within a column not showing common superscript letter are significantly different (P<0.05)

Table 15. Effects of the experimental diets on gut parameters

	Ileal digesta viscosity (mPa.s)	Excreta DM (%)	Gizzard empty weight (g)	Intestine length (cm)			pH				
				Duodenum	Jejunum	Ileum	Crop	Gizzard	Jejunum	Ileum	Caecum
C	3.29 [c]	16.25	40.21[c]	30.8	77.05	78.8	5.0 [b]	4.14	6.30	6.37	6.90 [cb]
WB20	4.13 [b]	15.79	37.03 [c]	30.9	79.3	80.8	5.27 [ab]	4.05	6.29	6.38	7.53 [a]
WB40	4.97 [a]	17.35	38.19 [c]	30.7	77.1	77.6	5.61[a]	4.49	6.31	6.34	6.61[c]
WO10	3.42 [c]	16.65	50.20 [b]	31.4	78.55	77.9	5.38 [ab]	3.90	6.29	6.36	7.22 [ab]
WO20	3.44 [c]	17.71	57.67 [a]	30.9	82.5	79.7	5.13 [b]	4.17	6.26	6.36	7.10 [b]

C: control diet; WB20: diet that contained winter barley at 20%; WB40: diet that contained winter barley at 40%; WO10: diet that contained spring oats at 10%; WO20: diet that contained spring oats at 20%.; [a-b] Means within a column not showing common superscript letter are significantly different (P<0.05)

In the frame of gut morphology evaluation only the higher incorporation rates of barley (40%) and oats (20%) were used. In the jejunum villus height was decreased by oats, compared with the WB40 and C treatments ***(Table 16.).*** No differences were found in the depth of crypth and the thickness of lamina propria. The reason for the shorter villi of the oats fed birds could be the higher erosion of the epithelial cell due to the higher structural fibre. It is a well known effect of dietary fibre which cause higher endogenous losses **(Mateos et al. 2013; Svihus 2014).** The ileal villi were higher in the barley diet fed birds, without difference between the control and oats treatments. On the other hand, the crypt depth was decreased by oats. Lower crypth depth means less intensive recovery of the villi, which result is in opposite with the findings in jejunum for oats. The reason for this difference is hard to explain. Usually, more fibre results more intensive gut motility and more developed gut muscle. In spite in the ileum both barley and oats decreased the diameter of lamina propria. The reason of this contradictory results is also unknown.

Table 16. Effects of dietary treatments on the gut morphology

Jejunum (μm**)**			
	Villus height	Crypt depth	Lamina propria
C	1688.7 [a]	149.2	174.3
WB40	1734.8 [a]	153.5	168.7
WO20	1571.7 [b]	152.6	170.9
Ileum (μm**)**			
	Villus height	Crypt depth	Lamina propria
C	1395.9 [b]	161.4 [a]	170.8 [a]
WB40	1562.6 [a]	166.8 [a]	134.5 [b]
WO20	1364.0 [b]	145.3 [b]	124.1 [b]

C: control diet; WB20: diet that contained winter barley at 20%; WB40: diet that contained winter barley at 40%; WO10: diet that contained spring oats at 10%; WO20: diet that contained spring oats at 20%.; [a-b] Means within a column not showing common superscript letter are significantly different ($P<0.05$)

In the caecum content acetate was the determinant volatile fatty acid followed by butyrate and propionate ***(Table 17.)***. The dietary treatments did not modify this main trend. However, all measured SCFA concentration decreased in the treatment group WB40. Barley incorporation significantly reduced the acetate, propionate and total SCFA compared to contents of the caeca compared with the control group. This finding is in opposite with the results of the wheat based and xylanase supplemented diets. It is well known that xylanase splits the long chain arabinoxylans to smaller molecular weight xylan oligosaccharides (XOS), which increase the microbial activity

in the caeca and the abundance of the butyrate producing bacterial genera **(Immerseel et al. 2017).** Comparing the effects of corn and wheat-based diets on the caecal SCFA concentration of 35-day old broilers, wheat increased the amounts of acetate and butyrate significantly, but failed to modify the concentration of propionate **(Nguyen et al., 2021).** We also found, that feeding wheat-based diets with xylanase supplementation, increase the SCFA content, decrease the pH in the caeca and this way significantly decreased the *Campylobacter jejuni* counts significantly 14 days post infection **(Molnár et al. 2015).** It seems that the degradation of beta glucans does not provide such oligosaccharides that mean extra substrate for the bacteria in the caeca. The negative effect of barley on the caecal SCFA production remains unclear.

Table 17. Effects of dietary treatments on the caecal short chain fatty acid concentrations (µmol/g)

	Dietary treatments			p—Value
	C	**WB40**	**WO20**	
	Mean ± SD	Mean ± SD	Mean ± SD	
Acetate	49.71 ± 7.63 [a]	36.54 ± 15.95 [b]	35.81 ± 17.17 [ab]	**0.041**
Propionate	7.27 ± 2.36 [a]	3.23 ± 1.95 [b]	6.46 ± 3.85 [ab]	**0.007**
n—Butyrate	0.49 ± 0.18	0.38 ± 0.23	0.50 ± 0.25	0.318
Butyrate	13.54 ± 3.99	9.81 ± 4.92	10.03 ± 5.37	0.191
n—Valerate	0.46 ± 0.25	0.34 ± 0.29	0.55 ± 0.31	0.201
Valerate	0.81 ± 0.16 [ab]	0.49 ± 0.28 [b]	0.79 ± 0.40 [a]	**0.026**
Total SCFA	72.29 ± 11.07 [a]	50.79 ± 22.14 [b]	54.15 ± 26.17 [ab]	**0.037**

C: control diet; WB40: diet containing winter barley at 40%; WO20: diet containing winter oats at 20%, SCFA: short chain fatty acid; [a. b:] values within the mean rows with different lowercase letters were significantly different ($p < 0.05$).

Microbiota composition of the different gut segments

Diversity of gut microbiota

As expected, the highest bacterial alpha diversity was found in the caecal content (CC) and lower species richness was true for the jejunal content (JC) and jejunal mucosa (JM) ***(Table 18.)***. The Chao1 and Shannon indexes were more sensitive than the Simpson index. Significant dietary treatment effects were found only with Chao1 and Shannon.

Feeding barley, increased tendentially ($p = 0.056$) the number of species in the jejunum content (Chao1) compared with the control treatment. In JM, both barley and oats resulted lower species richness according to the Chao1 index, however, according to the Shannon diversity index tendentially ($p = 0.093$) higher diversity was found in WB40 treatment group.

In CC, WB40 dietary treatment significantly reduced (p = 0.009) the number of species compared to C and WO20 groups (Chao1). Barley also reduced the Shannon diversity index. The difference was in this case significant (p = 0.017) between treatments WB40 and WO20.

Alpha diversity is a measure of microbiome diversity or species richness of a local site, in our experiment of the gut different sampling places. It is assumed that higher diversity means more stable and balanced microbial community. The dietary treatments in our trial had different effects in the different digestive tract parts. The oats-based dietary treatment did not cause significant differences in this index. The barley-based diets increased in tendency with the bacterial diversity in the jejunal content and jejunal mucosa. On the other hand, this diet significantly decreased the alpha diversity index in the caeca. The lower caecal diversity of treatment WB40 is in accordance with the SCFA results when barley also had a depressed effect. The reason for the results could be that barley resulted in higher gut viscosity, this way decreased nutrient digestibility **(Choct and Annison 1992)**. The impaired digestibility means more available substrate for the bacteria, resulting in higher bacterial counts and probably also higher diversity. The reason for the decline of diversity and SCFA production in the caeca after feeding of the barley-based diets is not known. We did not find research results to compare the effects of barley on these parameters.

Table 18. Alpha diversity indices of the intestinal microbiota of broiler chickens

		Chao1 Mean ± SD	Shannon Mean ± SD	Simpson Mean ± SD
JC	C	129.94 ± 23.95^{B}	2.30 ± 0.41	0.83 ± 0.05
	WB40	184.85 ± 26.21^{A}	2.68 ± 0.49	0.85 ± 0.10
	WO20	157.93 ± 43.21^{B}	2.27 ± 0.30	0.81 ± 0.07
	P	0.056	0.336	0.404
JM	C	209.99 ± 96.05	2.73 ± 0.47^{B}	0.87 ± 0.05
	WB40	164.21 ± 89.54	3.44 ± 0.44^{A}	0.92 ± 0.03
	WO20	144.92 ± 27.01	2.94 ± 0.36^{B}	0.89 ± 0.04
	P	0.357	0.093	0.177
CC	C	501.04 ± 18.95 ^{a}	4.64 ± 0.08 ^{ab}	0.98 ± 0.004
	WB40	406.75 ± 28.01 ^{b}	4.40 ± 0.14 ^{b}	0.97 ± 0.01
	WO20	496.48 ± 18.59 ^{a}	4.72 ± 0.14 ^{a}	0.98 ± 0.01
	P	**0.009**	**0.017**	0.459

JC: jejunum chymus; JM: jejunum mucosa; CC: caecal content; C: control diet; WB40: diet containing winter barley at 40%; WO20: diet containing winter oats at 20%, $^{a, b}$: values within the mean columns with different lowercase letters were significantly different ($p < 0.05$). $^{A, B}$: p values between 0.05 and 0.1 were considered as a trend.

Jejunal and caecal microbial abundances

The composition of microbiota is affected mostly on the cross-feeding interactions between the groups that degrade complex carbohydrates, simple sugars, or amino acids. Feeding diets with high fibre content increase the abundance of *Firmicutes* and *Actinobacteria* **(Koh et al. 2016);**

(González-Ortiz et al. 2019). In the jejunum *Firmicutes* was the dominant phylum both in chymus and mucosa ***(Table 19).*** No significant dietary treatment effect was found in the jejunal content. However, the abundance of *Firmicutes* was 6—7 % lower in the barley fed birds in the jejunal mucosa. The difference was in the comparison of treatments WB40 and C. The other significant difference in the phyla above 1% abundance was *Proteobacteria*. The difference in this comparison was also only significant between the barley containing and control diets. Phylum *Tenericutes* could be detected only in the mucosa of the barley treated group. Treatment WB40 resulted also increase in *Bacteroidetes* and *Actinobacteria*, but the differences in these cases were not significant.

Table 19. Relative abundance of bacterial phyla in the different sampling places (%)

		Actinobacteria	*Actinobacteria*	*Bacteroidetes*	*Cyanobacteria*	*Deinococcus—Thermus*	*Firmicutes*	*Patescibacteria*	*Proteobacteria*	*Tenericutes*	*Verrucomicrobia*
JC	C	0.00	1.85	0.01	0.15	0.00	97.93	0.01	0.05	0.00	0.00
	WB40	0.00	4.75	0.13	0.12	0.00	94.86	0.01	0.13	0.00	0.00
	WO20	0.00	3.19	0.01	0.08	0.00	96.59	0.02	0.11	0.00	0.00
	Pooled SEM	0.00	1.05	0.07	0.07	0.00	1.05	0.01	0.04	0.00	0.00
	Asymp. Sig.	1.000	0.179	0.426	0.779	1.000	0.208	0.580	0.230	0.368	0.368
JM	C	0.01	0.44	1.03	0.05	0.00	97.69 ^{a}	0.01	0.76 ^{b}	0.00 ^{b}	0.01
	WB40	0.01	2.36	3.87	0.70	0.03	89.42 ^{b}	0.00	3.47 ^{a}	0.11 ^{a}	0.02
	WO20	0.00	1.53	0.48	0.05	0.06	96.24 ^{ab}	0.01	1.62 ^{ab}	0.00 ^{b}	0.00
	Pooled SEM	0.01	0.88	1.45	0.25	0.03	1.18	0.01	0.35	0.06	0.01
	Asymp. Sig.	0.409	0.185	0.281	0.426	0.161	**0.004**	0.581	**0.006**	**0.032**	0.291
CC	C	0.00	0.29 ^{ab}	9.28	0.10	0.00	89.59	0.00	0.20	0.07 ^{ab}	0.46
	WB40	0.00	6.73 ^{a}	8.20	0.07	0.00	84.21	0.00	0.25	0.53 ^{a}	0.01
	WO20	0.00	0.17 ^{b}	11.32	0.24	0.00	87.64	0.00	0.40	0.07 ^{b}	0.15
	Pooled SEM	0.00	0.95	1.43	0.05	0.00	1.29	0.00	0.08	0.06	0.23
	Asymp. Sig.	1.000	**0.008**	0.210	0.061	1.000	0.069	1.000	0.310	**0.007**	0.193

JC: jejunum chymus; JM: jejunum mucosa; CC: caecal content; C: control diet; WB40: diet containing winter barley at 40%; WO20: diet containing winter oats at 20%; $^{a, b}$: values within the mean rows with different lowercase letters were significantly different ($p < 0.05$). Results between 0.05 and 0.1 ($0.05 < p < 0.10$) were considered a trend (^{T}).

Interestingly, the significant increase of *Tenericutes* of treatment WB40 remained also in the caeca. In the caeca still *Firmicutes* was the determinant phyla, but its abundance was lower than in the jejunum. On the expense of *Firmicutes, Bacteroidetes* increased to 8-11%. Oats increased, but barley decreased the abundance of this phyla, but the differences were not significant. The most significant difference in CC was the increase of *Actinobacteria* to 6.73% in treatment WB40. Its abundance in the two other groups was below 0.3%.

CONCLUSIONS AND RECOMMENDATIONS

Our results indicated that oats and barley are not only more abiotic stress resistant cereal grains and could be at least partly replace corn in the Hungarian poultry diets, but their nutrient composition is also competitive. The climatic change, the disappearance of the frosty winter periods could give further chance for example to cultivate more winter oats.

Both barley and oats are specific among cereal gains since they contain hulls and this way higher fibre content. The other specificity of oats and barley are, that they contain soluble β-glucan, that can modify the gut parameters, the digestion, and the production of animals. There is plenty information available on the effects of soluble arabinoxylans and using exogenous xylanases if wheat, rye or triticale are fed at higher inclusion rates. However, only limited scientific data are available on oats, barley, and the effects of their β-glucan.

We can conclude that, although cereal grains are mainly energy sources, but their protein content and protein quality are also important quality measures. From this aspects it is important, that oats have high quality protein, rich in cysteine and arginine and can decrease the amount of crystalline amino acids in the diets. It is also an important finding that the amino acid composition of the grain proteins is not constant. In barley the lysine, tyrosine, and leucine, in winter oats the lysine and cysteine contents of the protein decrease if the crude protein of the grains increases. It has also practical relevance, since in most cases the amino acid contents of feedstuffs are calculated form the protein content.

Surprisingly, oats and barley have no depressive effects on the digestion of nutrients even at 40% inclusion rate. Their fibre fractions can modulate the gut parameters. Oats significantly increase the gizzard weight but decrease the villus length in the jejunum and ileum. Barley results increased viscosity of the ileal content in spite of the β-glucanase enzyme supplementation of the diets and affects the microbiota diversity in the different gut segments (jejunum mucosa, jejunum content, caecal content) and the SCFA composition of the caeca. It means that the routinely used β-glucanase enzyme supplementation of the barley-based diets is not always efficient enough.

From practical point of view of course the most important the effects of barley and oats on the production traits of broiler chickens. Recently we do not have exact recommendations on the maximal inclusion rates of oats and barley in the different phases of production. Our results suggest that even young chicken can tolerate the high fibre content of oats and barley. In the grower phase the 40% barley and 20% of oats was already depressive, so about 20% barley and

10% oats seems to be the limit in the starter and grower phase. The most interesting finding was the significant weight gain supporting effect of oats in the finisher period. It seems that the chicken has at this age period higher fibre requirements. The positive effect of oats could relate of its gizzard stimulating effect.

In conclusion, both grains have promising characteristics, that make them possible to use in poultry nutrition even at higher inclusion rates. Beside the broiler chickens, they could also be used in the laying hen, turkey, and waterfowl nutrition.

NEW SCIENTIFIF RESULTS

1. Among essential amino acids LYS and LEU contents of barley protein declines with the increase of crude protein. In winter oats the same negative correlation exists for CYS and LYS.
2. Feeding barley and oats increases the faecal digestibility of fats and the ileal absorption of dietary nitrogen. Using exogenous β-glucanase in barley and oats containing diets, improves the faecal digestibility of crude fat and ileal crude protein. β-glucanase supplementation the 40% barley and 40% oats containing diets improves the ileal digestibility only of arginine, isoleucine, leucine, threonine, cysteine, and tyrosine.
3. Feeding oats at 20% in the finishing period significantly increases the growth rate and improves the FCR of broiler chickens
4. Feeding oats at 10 and 20% increases significantly the gizzard weight and at 20%, significantly decreases the villus height of the jejunum and the crypt depth of the ileum of broiler chickens.
5. In comparison with the corn- and oats-based diets, feeding barley with broiler chickens at 40% increases significantly the viscosity of the ileal digesta, decreases the short chain fatty acid (acetate, propionate and total SCFA) concentration and the microbiota diversity in the caecum. Feeding barley at 40% decreases the abundance of Firmicutes and increases the ratio of Proteobacteria in the jejunal mucosa of chickens.

Publications related to the dissertation

Scientific journal articles

1. Farkas Valéria, Molnár Andor, Menyhárt László, Márton Aliz, Csitári Gábor, Pál László, Such Nikoletta, Koltay Ilona Anna, **Mohemed Ali Rawash**, Mezőlaki Ákos, Dublecz Károly (2018): Új kutatási eredmények a házityúk emésztőtraktusának bakteriótaösszetételéről. Magyar Állatorvosok Lapja, 141. 485-494.

2. **Rawash, M. A.,** N. Such, I. Koltay, L. Pál, L. Wágner, Á. Mezőlaki, J. Poór, A. Márton, A. Molnár, K. Dublecz (2019): Comparing the ileal amino acid digestibility of barley, winter oats and spring oats and the effect of using beta glucanase with broiler chickens. 22nd European Symposium in Poultry Nutrition, 10-13 June. ISBN: 978-83-942760-6-5

3. Such Nikoletta - **Mohamed Ali Rawash** - Koltay Ilona Anna - Mezőlaki Ákos - Farkas Valéria - Pál László - Dublecz Károly (2020): Baromfitápok zab és árpa kiegészítésének hatása a baromfi alomból felszabaduló ammónia mennyiségére. XXVI. Ifjúsági Tudományos Fórum, május 21. Keszthely. konferenciakiadvány 1-7. o. ISBN 978-963-396-143-8

4. **Rawash, M. A**.; Rizk, R.; Mezőlaki, Á.; Molnár, A.; Pál, L.; Wágner, L.; Such, N., Koltay, I. A.; Strifler, P.; Dublecz, K. (2021): Evaluation the fibre composition and grain viscosity of different spring oat, winter oat and winter barley varieties. 19th BOKU-Symposium Tierernährung. April 15. Wien, Austria. Proceeding book, 203-206. ISBN 978-3-900932-72-5

Conference papers

5. Farkas Valéria, Gábor Csitári, László Menyhárt, Nikoletta Such, László Pál, Ferenc Husvéth, **Mohamed Ali Rawash**, Ákos Mezőlaki and Károly Dublecz (2022): Microbiota Composition of Mucosa and Interactions between the Microbes of the Different Gut Segments Could Be a Factor to Modulate the Growth Rate of Broiler Chickens. Animals 12. 10. 1296. (Q1, IF:3,231)

6. **Rawash, M.A**.; Farkas, V.; Such, N.; Mezőlaki, Á.; Menyhárt, L.; Pál, L.; Csitári, G.; Dublecz, K. (2023): Effects of Barley- and Oat-Based Diets on Some Gut Parameters and Microbiota Composition of the Small Intestine and Ceca of Broiler Chicken. Agriculture 2023, 13, 169. https://doi.org/10.3390/agriculture13010169 (Q2; IF: 3,6)

7. **Rawash M. A**., Evaluation the protein quality of oats for poultry. 6th Conference of Agronomy Students, on 14 -16 August 2019, Faculty of Agronomy in Čačak, Serbia. P 381-387. ISSN 2334-9883 DOI: 10.13140/RG.2.2.20195.71209