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

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Contents

LIST OF ABBREVIATIONS AND ACRONYMS

AA	Amino acid
NSPs	Non-starch polysaccharides
AX	Arabinoxylans
XOS	Xylan-oligosaccharides
OLPs	Osmotin-like proteins
PR-5	Pathogenesis-related
DM	Dry matter
CF	Crude fibre
NDF	Neutral detergent fibre
ADF	Acid detergent fibre
ADL	Acid detergent lignin
DF	Dietary fibre
NSP	Non-starch polysaccharides
SCFA	Short-chain fatty acids
NSPase	NSP degrading enzyme
SCFA	Short chain fatty acids
VFA	Volatile fatty acids
CAID	Coefficient of apparent ileal digestibility
AGP	Antibiotic growth promoters
DF	Dietary fibres
GIT	Gastro-intestinal tract
RS	Resistant starch
FOS	Fructo-oligosaccharides
MOS	Mannan-oligosaccharides
HCl	Hydrochloric acid
WG	Weight gain
FI	Feed intake
F/G	Poorest feed to gain ratio
OH	Oats hull
IDF	Insoluble dietary fibre
SDFP	Soluble dietary fibre precipitated
NDO	Non-digestible oligosaccharides
AEV	Acid extract viscosity
CV	Coefficient of variation
EAAI	Essential amino acid index
AMEn	Apparent metabolizable energy
GE	Gross energy
FCR	Feed conversion ratio
JC	Jejunum content

JM	Jejunum mucosa
CC	Caeca content
PBS	Phosphate buffer solution
JM	Jejunum mucosa
PBS	Phosphate buffered saline
PCRs	Polymerase chain reactions
NCBI	National Center for Biotechnology Information
SRA	Sequence Read Archive
PD	Phylogenetic distance
PCoA	Principal coordinate analysis
CCA	Canonical correspondence analysis
WB	Winter barley
WO	Winter oats
SO	Spring oats
EE	Either extract
NFE	Nitrogen-free extract
IDF	Insoluble dietary fibre
SDFP	Soluble dietary fibre precipitated
EAA	Essential AAs
NEAA	Non-essential AAs
LP	Low protein
CVB	Centraal Veevoederbureau
XOS	Xilan oligosacharides
IC	Ileal content
IM	Ileal mucosa
PCoA	Principal coordinate analysis
FCR	Feed conversion ratio
GIT	Gastrointestinal tract
WHO	World Health Organization
FAO	Food and Agriculture Organization
UNU	United Nations University
Arginine	ARG
Histidine	HIS
Isoleucine	ILE
Leucine	LEU
Lysine	LYS
Methionine	MET
Phenylalanine	PHE
Threonine	THR
Valine	VAL
Cysteine	CYS
Alanine	ALA

Aspartic acid	ASP
Proline	PRO
Glutamic acid	GLU
Glycine	GLY
Serine	SER
Tyrosine	TYR

1. INTRODUCTION

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).

2. OBJECTIVES

The main goal for breeding new lines and varieties of barley and oats was to get cereals that can acclimate to weather changes (drought, hot temperature, frost) to supply stable feedstuffs for animal nutritiom. Poultry production in Hungary increaseing staeadily and has high cereal requirements. Barley and oats are not typical poultry feeds, but since their higher fibre can have special effects in stimulating the gizzard of birds. Beside that, both cereals conatin soluble β-glucans. There are plenty informtion available on the soluble arabinoxylans of wheat or rhy, but not too much information exist on the effects of soluble β-glucans on the gut physiology and gut health. So, our main goal to evaluate the nutritiv value of the new barley and oats varieties for poultry species. The detailed evaluation steps were the follows:

a) To determine the nutritive value of different oats 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 oats and barley varieties at different inclusion rates.
e) Evaluation the effect of exogenous beta glucanase on the nutrient digestibility of barley- and oats-based diets.
f) Conducting a feeding trial with broiler chickens to determine the production traits of chickens fed barley and oats containing diets.
g) Investigating the effects of oats and barley containing diets on several gut parameters and gut microbiota composition.

2. LITERATURE OVERVIEW

2.1. Climate change and its effects on cereal production

The environmental factors have a significant effect on crops production and crops availability. It has rapid consequences on agricultural industry. At the same time, raising the cost is one of the negative effects of the environmental factors, which causing food and feed demand escalate globally. Plants endure various unfavourable climatic conditions during their growth cycles. Such conditions are comprised of biotic stresses, including infection by pathogens, and abiotic stresses, including heat and cold, drought, scarcity of nutrients, higher levels of salt, and hazardous metals and metalloids (arsenic, cadmium, and aluminium) in the soil. Temperature (heat or frost), drought, and salt are the primary and most frequently encountered climatic factors that reduce agricultural crop yields. Such impacts are a dangerous sign for food security and impact the geographical distribution of plants in nature. Climate change, i.e., long-term changes in weather patterns, is a source of significant abiotic stress (YOUNIS et al. 2014, 2020). Exposure to drought can have severe impacts on plant physiology, often leading to reduced growth and photosynthetic capacity, leading to reductions in crop yields. These consequences are primarily mediated through plant physiological and molecular responses to drought stress, and the associated changes can also influence the population dynamics, fitness, phenology, biology, and behaviour of herbivorous insects (ZEPPEL et al. 2014; KANSMAN et al. 2020; LIN et al. 2021). Within the world agricultural area, 91 % of that area is under stress, and 50% of agriculture production loss is due to such stresses. The strength and harmful effects of abiotic stress may be accelerated with changes in climate. Improvement in agronomic management and stress-resistant genotype promotion in breeding programs can reduce this impact (MINHAS et al. 2017). Heat and drought are the two major stresses that adversely affect the yield and productivity of a crop. Such abiotic stresses reduce farm income and agricultural benefits. The reduction of water by up to 40% causes the lowering of corn yields to 40% and wheat to 21% of the former yield (DARYANTO et al. 2016). The remaining 90% is facing one or more environmental stresses. Plants continue to adapt to abiotic stress biochemically, physiologically, molecularly, and phenotypically. Still, a persistent need exists for additional efforts to improve stress tolerance by improving plant defences

genetically, promoting technologies for resource conservation, and adopting other approaches (KANG et al. 2017).

Barley and oats varieties have excellent adaptability to the climatic extremes and also to the extensive cultivation techniques. In dry vegetation conditions the production of winter barley and winter oats is still possible because the harvest of these plants is prior to the dry period of summer. In these conditions the yield of these crops is safer and may be larger than that of corn. The extended cultivation of barley and oats helps to include areas with less favourable conditions into the agricultural production. Barley has a strong root system which gives it supersite in growth and develops in the dry environments. That root system provides higher barley yields compared to other crops (VALENZUELA et al. 2002; CHLOUPEK et al. 2010)**.** On the other hand, many studies illustrated that barley has a positive effect in break insect and weed cycles (BROWN 2003). The level of nitrogen fertilizer used for barley is lower than other grains like corn and wheat due to the higher photosynthetic activity in barley (VERGÉ et al. 2009; KARLEY et al. 2011). Barley has several advantages more than traditional grains like wheat and corn. It can grow up in areas less suitable for corn; it is a short-season, early maturing crop that has been adapted to a wide variety of climates. Meanwhile, barley is tolerating humidity, warm climates of the tropics (BADR et al. 2000). It has very good heat and drought tolerance, making it a valuable plant for semiarid areas. Barley is also the most salt-tolerant among cereal crops. It grows at soil pH between 5.0 and 8.3. It thrives also in cool, dry conditions. In Hawaii, barley grows year-round at elevations above 500 feet. Barley considered one of the highest crops in terms of dehydration stress resistant due to production of dehydrin proteins. Barley contains about 10 of 13 dehydrin genes. Dehydrins are actively synthesized in response to strong dehydration stress like frost, drought, and salt (KOSAVA et al. 2014; YOUNIS et al. 2020). Many studies illustrated that oats (*Avena sativa*) and barley (*Hordeum vulgare*) seeds contain osmotin-like proteins (OLPs). Those OLPs belong to the pathogenesis-related (PR-5) protein family and can protect plants from different biotic and abiotic stresses (MANGHWAR et al. 2018). Under oxidative stress they are expressed in the meristematic region of the shoot apex and the quiescent (inactive) region of the root apex (KUMAR et al. 2015). Oats competes with wheat and barley for a place in rotations where it features interesting attributes including high competitiveness with weeds and tolerance to major cereal diseases (SADRAS et al. 2017).

Globally, the European Union being the largest producer of barley and oats in follow by Russia and Ukraine (ULLRICH 2011). Now, barley is also grown specifically for food and animal feed. Globally, up to 85% of the barley produced is used for feeding animals, including cattle (beef and dairy), swine, and poultry. Barley is the main feed ingredient in many countries in Europe. In Scandinavia, barley and oats are the only cereals that can be successfully grown for use in animal feeds.

2.2. Nutrient content of oats and barley

The oats (*Avena sativa*) has always been a favourite cereal for ruminant and horses but has been less popular in poultry feeding because of its comparatively high fibre content and low energy value. The nutritive value of oats depends to a large extent on the proportion of kernel (groats) to hull (STERNA et al., 2016). The structure of cereal grains is shown in ***Figure 1.***

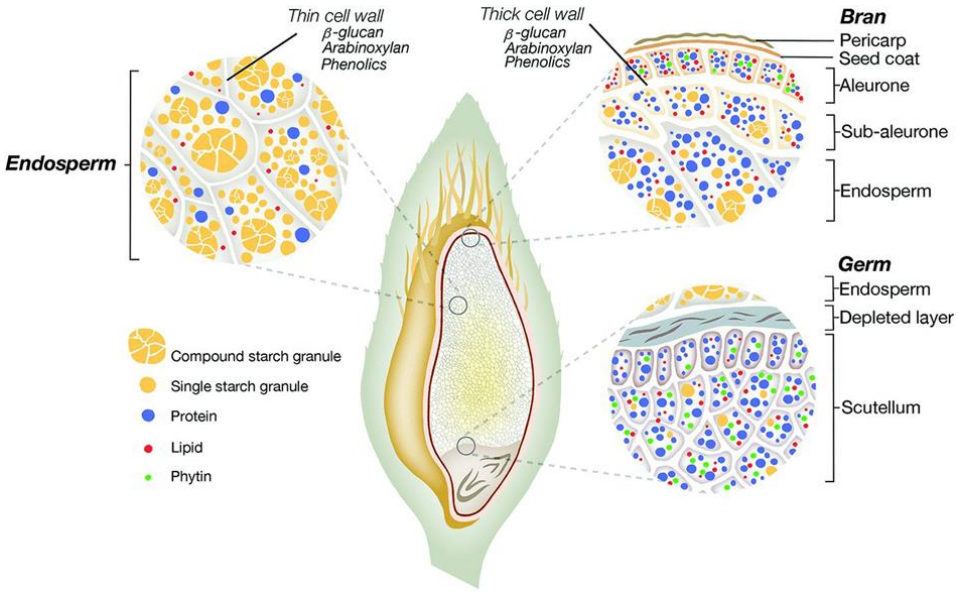

Figure 1. The structure of cereal grains (The source of this figure should be indicated)

The proportion of hull in the whole grain depends upon the variety, environment, and season, and can vary from 23 to 35% (average 27%). Oats of high hull content are richer in crude fibre and have a lower metabolizable energy value than low-hulled oats. The crude protein content, which ranges from 70 to 150 g/kg dry matter (DM), is increased by the application of nitrogenous fertilisers (literature has to be ind. Oats have well-balanced amino acid composition without anti-

nutritional factors. Oats' protein comprises globulin, albumins, prolamins, and glutelins (KLOSE AND ARENDT 2012). Compared to other cereals like corn and wheat, oats protein is considered having higher nutritive value, with adequate essential amino acid content (MOHAMED et al. 2009). Prolamins are the most dominant protein in most cereals, such as wheat, barley, and rye, whereas the prolamin content of oats is only 4-15 % of total protein. Compared to other cereals, the consequence of low-prolamin and high-globulin in oats provides balanced amino acids essential for monogastric animals and humans (KLOSE AND ARENDT 2012). The lysine content is also low but is slightly higher than that of the other cereal proteins. Glutamic acid is the most abundant amino acid of oats protein, which may be contained up to 200 g/kg. On the contrary, (RODEHUTSCORD et al. 2016) found that the mean lysine (LYS) concentration ranged from 2.7 g/16 g N in wheat protein to 4.2 g/16 g N in oats protein and differed significantly between all cereal grains ($p \leq 0.05$). The mean tryptophane (TRP) concentration from 0.8 g/16 g N (correlation variance, CV 8.5%) in corn protein to 1.4 g/16 g N (CV 4.1%) in oats protein. The oil content of oats is higher than that of most of the other cereal grains, and about 60% of it is present in the endosperm. As mentioned earlier, the oil is rich in unsaturated fatty acids and has a softening effect on the body fat. The husk of a variant of oats, naked oats (Avenanuda), is removed easily during threshing, leaving the kernel. Naked oats have about 130—140 g crude protein, 6 g lysine and 100 g oil/kg DM. Although oats are the third most important cereal feed grain in the United States, they accounted for only 1.4% of feed grain use. Oats do not yield as much as the other grains and, considering the hull of the whole grain, the feeding value is relatively lower than that of the other grains. The protein content of oats is relatively high (11% to 14%), and the amino acid distribution is the most favourable in any of the cereal grains; the ranking from the point of amino acid composition generally is the following: oats, followed, barley, wheat, corn, rice, rye, sorghum, and millet (MC'DONALD et al. 2002). The amino acid content in 100 g oats protein is calculated to predict biological value or the anticipated ability of the absorbed test protein to fulfil amino acid requirements (W.H.O./F.A.O./U.N.U. 2007). The result of investigation shows that amino acid composition of hulled and naked oats varieties and breeding lines is close to optimal. Just lysine and methionine are below FAO recommended reference standard. Some studies reported that the mean lysine concentration of protein was the highest in oats (4.2 g/ 16 g N) and the lowest in wheat (2.7 g/16 g N). Oats contain significant amounts of soluble dietetic fibre, β-glucans, fat-soluble

vitamin E and polyunsaturated fatty acids. Some obtained results showed a wide range of fat (4.9 to 10.5 g/100 g DM), α-tocopherol (4.5 – 12.3 mg/kg DM), essential amino acid (35-45g/ kg DM) and unsaturated fatty acid (78–81.5% of total fatty acids) contents. Results of evaluation lead to conclusion that oats grains are rich with biologically significant substances and their consumption diets is beneficial. Influence of Inclusion Level of Barley in Wheat-Based Diets and Supplementation of Carbohydrase on Growth Performance, Nutrient Utilisation and Gut Morphometry in Broiler Starters (PERERA et al. 2019a). Barley (*Hordeum sativum*) has always been a popular grain in the feeding of farm animals. In most varieties of barley, the kernel is surrounded by a hull, which forms about 10—14 % of the total weight of the grain. The metabolizable energy value (MJ/kg DM) is about 13.2 for poultry. The crude protein content of barley grain ranges from about 60 to 160 g/kg DM with an average value of about 120 g/kg DM (MC'DONALD et al. 2002). As with all cereal grains the protein is of low quality, being particularly deficient in lysine. High-lysine mutants of barley have been produced by plant breeders and the superior nutritional value of two such mutants is described (KELLEMS et al. 2010). Unfortunately, with many of these mutants the yields of grain are much lower (about 30 %) than from parent varieties, and the starch contents may be reduced. The crude fat content of barley grain is low; usually less than 25 g/kg DM. Barley forms are the main concentrate in the diets of pigs in many parts of the world. Hull-less varieties of barley are roughly equivalent to wheat or corn and are thus more sui for swine and poultry feeding; however, not much hull-less barley is produced world while (PERERA et al. 2019a). Pearled barley, from which the hull and most of the bran have been removed, has a high feeding value, but it is used primarily as a human food. A considerable amount of making barley is grown (LUKINAC AND JUKIĆ 2022). Such barleys are generally higher in protein content with heavier bushel weights than feed grain varieties.

2.3. Oats and barley in human and animal nutrition

Livestock plays a key role in alleviation of poverty as well as food scarcity. Among livestock, poultry is one of the significant commodities that provide high quality protein and micronutrients through meat and eggs. Nutrients from those are more easily taken up by the human organism than plant-based foods. In poultry farming, feed constitutes 70 to 75% of total production cost. Poultry feed is based primarily on cereal grains mainly corn, corn, wheat, sorghum, and vegetable protein

meals which are supplied to meet energy and protein requirements of poultry diets. Recently, these grains are also being used for bio-fuel production. Due to this paradigm shift of farming from the food industry to bio-fuel industry there is an increase in the prices of these feedstuffs. The more affordable ingredients including barley, oats, triticale, rye, olive cake and sunflower meal (AL-HARTHI 2017; WAITITU et al. 2018) play important role in substitution of corn, wheat, and soybean, but have some anti-nutritional factors which may affect growth performance and intestinal health of the birds. Oats is an important cereal crop throughout the world, which is the sixth highest amongst the global cereal production in tons (STATISTA 2020). For ruminants and horses, oats are a favoured feed for breeding stock or in creep feeds for young animals. The worldwide production of oats and barley is around 144 million tons for barley and 23 million tons for oats annually (F.A.O. 2014). The daily use of oats in human diet can reduce the risk of heart diseases; it possesses the unique class of antioxidants. Oats grains contain sufficient amounts of proteins, which makes them a staple diet for humans and cattle (IBRAHIM et al. 2020). Barley and oats are unique among the cereals, due to their relatively high protein content, distinct protein compositions, high levels of soluble dietary fibre and antioxidants. Regular consumption of whole-grain foods is correlated with a lower body mass index. Eating whole grain products helps to reduce hunger and increase feelings of fullness. The high dietary fibre content effectively lowers cholesterol and blood glucose level, preventing the formation of various diseases. Producing new varieties with higher levels of beta-glucan will be one of the most important topics of the targeted breeding strategies. Oats and barley are also common in the aspect, that most of the cultivated species are hulled, decreasing the inclusion rates of nutrients when fed to animals, and requiring extra steps in the processing when used for human consumption. The less exploited hull-less cultivars thus also represent an important source of the aim-oriented breeding. Barley and oats can be utilized more as special healthy food. To fulfil these requirements, breeders must consider some novel aspects and methods. The targeted crop species are predicted to have increasing importance soon, and they have much wider untapped developmental potential than major crops. Improving the variety assortments and the agronomy practices of these species may efficiently help to reduce the yield fluctuation year by year and in addition, the extended cultivation of barley and oats helps to include areas with less favourable conditions into the agricultural production. In fact, these crops could also be important as protein sources in the future.

To solve the dual demands of protein deficit and increased nutritional value, the harvested yield must be increased. Both in oats and barley, the aim is to minimize the strong yearly fluctuations in yield and the differences in average yield levels existing between West-Europe and South-, East-Europe. While barley is already a medium crop in Europe, in the case of oats it is also a challenge to increase the sowing area via ensuring its more profitable production. According to some studies, increasing barley inclusion increases nutrient and energy utilization, presumably by lowering digesta viscosity and improving gizzard function. Supplementing carbohydrate in barley-based diets can improve feed efficiency and nutritional and energy utilization, regardless of how much barley is included. Barley is a very palatable feed for horses and ruminants, particularly when steam rolled before feeding, and few digestive problems result from its use, although it is more prone to cause bloats in feedlot cattle when used as a major portion of the ration than are the other cereal grains. Barley, as the only grain source, will not allow maximum gains or optimum feed efficiency for swine, and the fibre level is too high for use of more than small amounts in poultry rations (JACOB AND PESCATORE 2012). There are some debates of recommendations for barley or oats inclusion in broiler diets is partly because most studies replaced other cereals with barley either on a weight-to-weight basis or by using nutrient composition data for barley and the substituted grain from established sources such as (NRC 1994) or chemical analysis (BRAKE et al. 1997). Moreover, most of the available recommendations on inclusion levels of barley have overlooked the influence of the hull, NSP and starch type on the feeding value of barley for poultry. Only minimal attempts have been made to elucidate the possible interaction between barley and oats inclusion level and enzyme addition on the utilization of nutrients and performance of broilers, and this aspect merits further evaluation.

2.4. The fibre composition of oats and barley

Based on the analytical methods the dietary fibres are mainly classified as crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). These dietary fibres have also been classified as insoluble dietary fibres (RAZA et al. 2019). Dietary fibre (DF) can be defined in many ways; most commonly being based on the chemical composition and the physiological functions. Based on chemical composition, DF is the sum of non-starch polysaccharides (NSP) and lignin (TEJEDA AND KIM 2021). From a nutritional point of view, it can be simply defined as indigestible cell wall compounds. The utilization of DF in poultry diets depends on the fibre content, its solubility, and the degree of microbial fermentation in the caeca (MATEOS et al. 2013; JHA et al. 2019). Soluble fibre sources are rapidly fermented by resident microbes in the distal small intestine and large intestine, increase digesta viscosity, reduce digesta passage rate through the intestine, and can decrease feed intake due to increased satiety (JHA et al. 2019). It is well-known that DF can contribute nutritional value to animals, directly by providing energy and indirectly by improving gut health and immune function (Pieper et al., 2008; Tejeda and Kim 2021). The different categories of DF are summarized in ***Figure 2.***

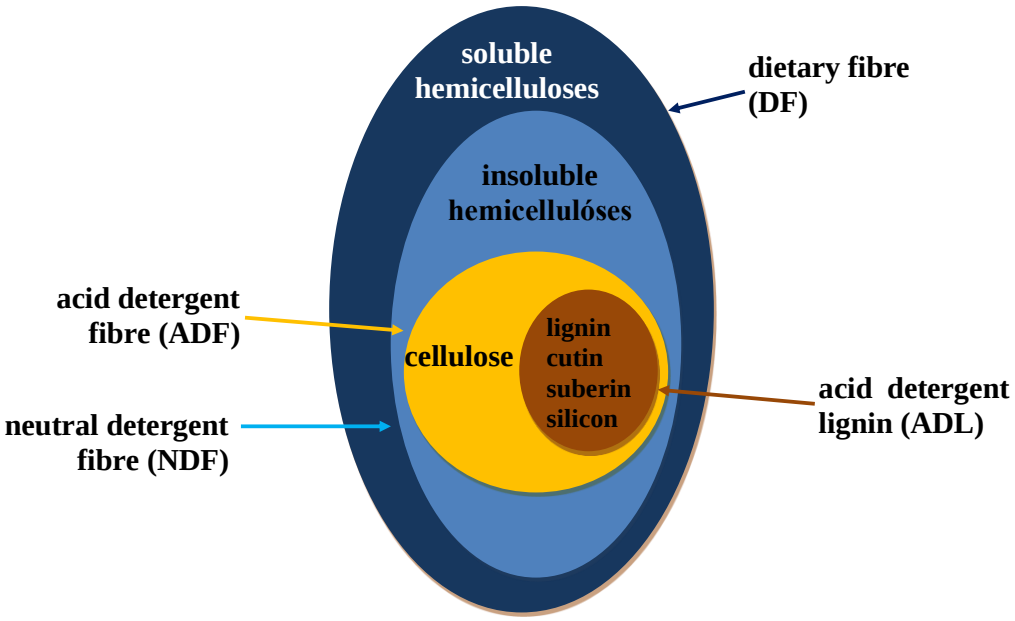

Figure 2. The different fibre fractions of feeds and foods (Bach Knudsen 2014),

Traditionally DF has been considered as an antinutritional factor due to its negative impacts on nutrient utilization (JHA et al. 2019). However, DF has recently gained special attention due to its functional value in improving gut health of monogastric animals (JHA et al. 2019). Maintaining or improving gut health is essential to enhance feed efficiency, promote growth performance, and maintain the overall health of monogastric animals. Dietary fibre compounds are naturally occurring compounds with a diverse composition and are present in all plant-based feedstuffs including cereals, tubers, and agro-industrial byproducts (TIWARI AND JHA 2017; JHA et al. 2019). Despite some adverse effects on nutrient and energy digestibility, there is growing interest for including DF in monogastric animal diets due to its potential beneficial effects on the gut health, welfare, and the environment (JHA AND BERROCOSO 2016). Dietary fibres escape digestion by host endogenous enzymes in the proximal small intestine and is utilized by the residing microbial population as a fermentative substrate in the distal small intestine and large intestine (TEJEDA AND KIM 2021). Microbial fermentation of DF produces metabolites including short-chain fatty acids (SCFA), which in turn, inhibits the metabolism and multiplications of bacteria wich can be harmful for the host animal., supports intestinal integrity, and proper immune function. Some studies with poultry showed that fermentation characteristics and their beneficial effects on gut health vary widely based on type, form, and the physio-chemical properties of the DF (JHA et al. 2019; TEJEDA AND KIM 2021) as well as the matrix in which it lies (JHA AND BERROCOSO 2015). Dietary Fibres can affect intestinal health directly by functioning as a direct source of energy and extra nutrients or indirectly by causing the modulation of intestinal microbiota and, subsequently gut function (JHA et al. 2019). Intestinal health is directly affected by arabinoxylans, β-glucans, and pectin (HETLAND et al. 2004). The soluble carbohydrates, including oligosaccharides and polysaccharides, are the most influential in terms of growth performance, nutrient absorption modulation, and intestinal welfare. In general, water-soluble NSPs have β-1,4 glycosidic linkage backbones with β-1,3 linkages. The degree of solubility is associated with the degree of branching of the NSP molecule.

It has been reported in many studies that DF can modulate physiological structure and functionality of the gastrointestinal tract differently (SADEGHI et al. 2015). All these changes present an overall modulation of the nutrient metabolism that might result in impacts on performance, gut morphology, organ growth, general nutrient digestibility, and microbiota (TEJEDA AND KIM

2021). Soluble NSP act through various mechanisms to provoke anti-nutritive effects. When these soluble dietary fibres fed in bulk amount increase the viscosity of intestinal contents by making viscous gels which decrease the rate of diffusion of endogenous digestive enzymes and substrates with hampered interaction at the mucosal surface. This increased viscosity also induces thickening of the mucous layer in the intestine (KALDHUSDAL 2000; TEJEDA AND KIM 2021). Consequently, soluble fibre slow down rate of passage and decrease the absorption of nutrients, increase the number of bacteria in the small intestine, decrease feed intake and body weight gain. Therefore, there is a need to find efficient and effective solutions for these problems.

2.5. Using exogenous enzymes in oats and barley containing diets

Supplementation of NSP degrading enzyme (NSPase) has an important role in poultry diets with high NSP contents (KALDHUSDAL 2000). SHAKOURI et al. (2009), comparing intestinal viscosity of broilers fed barley, corn, sorghum, and wheat, reported greater digesta viscosity in the birds fed wheat-based diets compared to barley-based diets. This surprising observation on decreasing digesta viscosity with increasing inclusion of barley confirmed that digesta viscosity is dependent not only on the concentration of NSP, but also on its molecular weight. Therefore, a grain with a low content of soluble NSP might result in high viscosity if the NSP is of a high molecular weight (COWIESON et al. 2005). In agreement with SHAKOURI et al. (2009), PERERA et al. (2019a) suggested that reason for the higher viscosity of wheat diets could be that wheat cultivars contain higher molecular weight NSPs.

Exogenous feed enzymes are being used from years to enhance growth performance and digestibility but have limited activity for selective ingredients. Arabinoxylans are composed of a linear backbone of β (1-4) linked xylose units with side chains of arabinose and other sugars linked to carbon 2, 3, or 5 on xylose. β-glucans are composed of β-D-glucose molecules joined by (1-3) and (;1-4) β glycosidic bonds. Parts of arabinoxylans and β-glucans are soluble in water. The solubility is related to both molecular size and the types of bonds in the polymer. The longer the sequence of β (1-4) bonds, the more it behaves like cellulose. The degree of branching varies between cereals as wheat and barley have higher degree of polymerization and molecular weight than that in corn. Exogenous microbial enzymes are essential for fibre degradation. Wheat contains larger amounts of high molecular weight arabinoxylans with 7.3% of total dry matter and showed

considerable anti-nutritive properties (BACH KNUDSEN 2014), while barley contains large amounts of beta-glucans with a high ratio of (1-3) and (1-4) β-glycosidic bonds. The gels formed when these two grains are fed together and reduce nutrient digestibility and availability. Non-viscous grains, such as corn, have cell walls made up primarily of low molecular weight arabinoxylans and small amounts of β-glucans, which do not cause the viscosity problems. Soybean and canola meals contain arabinogalactans, galactans, xylans and β-glucans as structural components of cell walls, but their levels are relatively low (KNUDSEN 1997; SLOMINSKI 2011). They have higher levels of the oligosaccharides (stachyose and raffinose) along with pectin. The pectin found in soybean meal are composed of a backbone of galacturonic acids with side chains containing rhamnose, galactose, arabinose, xylose, and fructose (SLOMINSKI 2011). Pectin is associated with cellulose in the cell wall and becomes soluble in the gastrointestinal tract (GIT). The NSPases have numerous mechanisms of actions in GIT. Enzyme supplementation could increase the duodenal crypt depth if barley-based diets are fed, resulting in a significant interaction between barley inclusion and enzyme supplementation. However, this observation was difficult to explain as enzyme supplementation in previous studies reduced crypt depth (REBOLÉ et al. 2010). In addition to direct effects on the gut morphology and physiology, NSPases also have indirect effects. Soluble NSP lower the oxygen tension in the small intestine thereby favours the development of anaerobic microflora that can lead to production of SCFA and toxins by some anaerobic organisms (RAZA et al. 2019). This induces lymphocyte infiltration in the gut wall and apoptosis of epithelial cells (TEIRLYNCK et al. 2009).

Several enzymes are being used to balance the adverse effects of NSP on gut health/performance of poultry (AFTAB AND BEDFORD 2018). Previous studies demonstrated that fungal and bacterial enzymes effectively degrade β-glucans and arabinoxylans present in wheat, barley, rye, and oats-based diets (SILVA AND SMITHARD 2002). Selection of exogenous feed enzymes is an important task which mainly depends on the feed ingredients. (CARDOSO et al. 2018) reported that exogenous enzyme supplementation improved the nutritive value of a wheat-based diet with high extract viscosity and low endogenous and xylanase activity in poultry. Three major modes of action of NSP-degrading enzymes have been proposed in the literature (i) reduction of digesta viscosity, (ii) release of encapsulated nutrients via cell wall degradation and (iii) modification of gut microbiota through supply of prebiotic oligosaccharides (GONZÁLEZ-ORTIZ et al. 2017;

AFTAB AND BEDFORD 2018). The production of fermentable substrates for favourable microbial groups is been proven to have beneficial effect on gut health (JÓZEFIAK et al. 2010) and villus growth (GONZÁLEZ-ORTIZ et al. 2017), and to improve nutrient utilization. MATHLOUTHI et al. (2002) attributed the improved protein and fat digestibility with supplementation of NSP-degrading enzymes in wheat- and barley-based diets to the reduction of total anaerobic bacterial load in the caeca. In addition, the increase of *Lactobacillius* and *Bifidobacter spp.* in barley-based and enzyme supplemental diets (JÓZEFIAK et al., 2010; RODRÍGUEZ et al., 2012) might indirectly enhance the nutrient digestibility in broilers. For degradation of NSP, such as arabinoxylans and β-glucans in poultry feed, xylanases (hemicellulases) and β-glucanases (cellulases) are commonly used enzymes. Xylanases and ββ-glucanases have been shown to reduce the viscosity of wheat and barley digesta at 30-50% and 300%, respectively (JUANPERE et al. 2005). The reduction in viscosity improves protein digestibility, energy utilization, feed intake, body weight gain, and feed conversion. Generally, the new generation carbohydrases with a board range of activity and stability help to degrade the complex substrates and improve growth performance of poultry (RAZA et al. 2019).

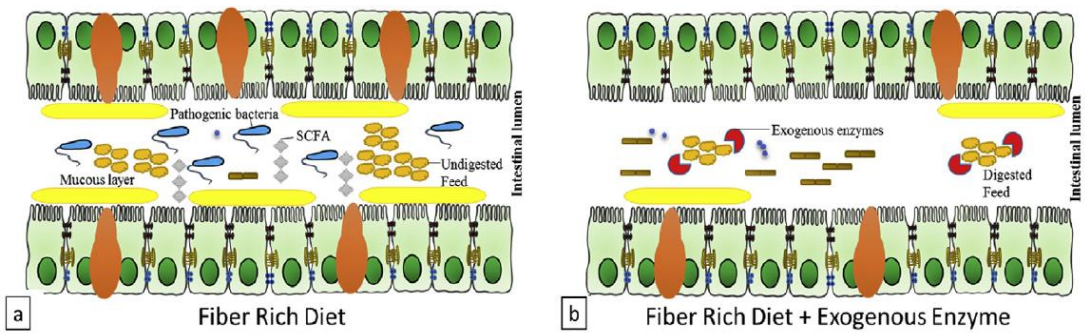

Figure 3. Effect of exogenous fibres NSPase enzyme on intestinal health: (a) without enzyme supplementation intestinal lumen presenting highly viscous environment with increased mucous, undigested feed, competition of host and microbiota for SCFA in small intestine, (b) with enzyme supplementation intestinal lumen presenting carbohydrases, normal mucous, beneficial bacteria, and digested feed (Raza et al., 2019)

Also, using exogenous NSPase supplementation in poultry with high NSP containing diets showed improvement in nutrients availability as well as digestibility. On the other hand, it has impact in improving the intestinal health (histomorphology and microbiota) of birds (SALEH et al. 2018). RAZA et al. (2019) showed the effect of exogenous NSPase enzyme supplementation to barley-

based diets *(Figure 3)* causing an improvement in growth and feed efficiency. When barley β-glucans were added to a corn-based diet there was an increase in the viscosity of the intestinal contents. Moreover, added exogenous enzymes to the β-glucan supplemented diet the intestinal viscosity was returned to near control levels. The researchers concluded that the β-glucans of barley are the cause of poor chick performance, most likely due to the increase in the viscosity of the intestinal contents (JACOB AND PESCATORE 2012). Non-starch polysaccharide degrading enzymes can reduce the intestinal digesta viscosity through partial depolymerization of NSP in cereal grains (JÓZEFIAK et al., 2010; PERERA et al., 2019a), wherein cell wall integrity is disrupted by the enzyme action and encapsulated nutrients are exposed to digestive enzymes to better interaction of endogenous digestive enzymes with their respective substrates.

2.6. Effects of feeding dietary fibre on gizzard function and gut morphology

Intestinal health concept is much comprehensive and dependent on knowledge about the diet, intestinal morphology, and microflora. All these components interact with one another in order to maintain proper functioning and dynamic equilibrium of GIT. From economical point of view, nowadays much attention is given to formulate a least cost balanced poultry diet. Intestinal morphology has an important role in well-functioning GIT for the transport of nutrients from the lumen into systemic circulation (RAZA et al. 2019). Intestinal morphology (villus height, crypt depth and epithelial turnover rate) changes in response to exogenous agents, for example, presence or absence of food and pathological conditions (GOMIDE JUNIOR et al. 2004). Deeper crypts indicate faster tissue turnover as they contain stem cells and considered villus factories (AWAD et al. 2009). Intestinal mucins/ mucous are high molecular weight glycoproteins secreted by goblet cells. In chickens, mucus is observed to be extensively expressed in goblet cells of colon and small intestine (SMIRNOV et al. 2005). NSP has been shown to increase mucin secretion (TANABE et al. 2006). Therefore, NSP decreases the digestion and absorption of nutrients through its physicochemical effect in the intestinal tract. The GIT microflora predominantly consists of bacteria with lesser populations of fungi and protozoa. Some studies showed that barley and oats inclusions have a positive effect on weight of proventriculus and gizzard and gizzard pH. In the barley-based diets, added enzyme increased the duodenal crypt depth with barley inclusion. Supplemental enzyme increased the epithelial thickness in the duodenum. In the jejunum, the

inclusion level of barley had a significant effect on jejunal villus height. Moreover, barley inclusion level tended to have a significant effect on jejunal epithelial thickness. Jejunal epithelial thickness increased with increasing inclusion of barley (RAZA et al. 2019). The proventriculus secreting hydrochloric acids, but due to its small volume, most of the mechanical digestion takes place in the gizzard. One important role the gizzard is to regulate digesta particle size in the gastrointestinal tract (SVIHUS 2011) with the ability to sense and modulate the passage of feed from the upper digestive tract to the small intestine based on particle size. Factors such as fibre type and particle size are determinant factors that stimulate the muscular activity of the gizzard resulting in increased size (HUSVÉTH et al. 2015). The normal retention of feed in the gizzard has been shown to be between half an hour to one hour, which can increase up to two hours when structural components are added to the diets (RAZA et al. 2019; SHANG et al. 2020). Similarly, studies using oats hulls and soyhulls at 3% in the diet have been shown to result in increased proventriculus and gizzard size as well as in improved feed conversion. The increase in particle size and fibre in the diet increases the muscular activity of the gizzard because of the need for particle size reduction, resulting in heavier weights as observed by different researchers in different poultry species (REZAEI et al. 2018). The increase in the size of the proventriculus and gizzard is a logical result of an increased volume due to the slower passage rate of the almost-intact feed particles, which can only be solved by muscular grinding in the gizzard. The presence of insoluble dietary fibre such as cellulose, lignin, and arabinoxylans can also modulate the size of the small intestine, pancreas, and caeca, which can result in improvements of the total tract apparent retention of nutrients and feed efficiency as described by different researchers (KHERAVII et al. 2017; REZAEI et al. 2018). At the same time, increasing inclusions of barley in wheat-based diets improved the coefficient of apparent ileal digestibility (CAID) of all nutrients (PERERA et al. 2019b). Barley contained more insoluble NSP, in consequence development of the gizzard is facilitated and increased weights of the gizzard (SVIHUS, 2011). Using oats hulls has a good impact on gizzard enlargement and mechanical abrasion resulting in disruption of starch particles and modification in gut microflora. Changes in gastrointestinal morphology associated with variation in dietary fibre concentrations were previously observed with special reference to the gizzard (HETLAND et al. 2003; AMERAH et al. 2009). A more developed musculature in the gizzard, as an adaptive response to increased dietary fibre, can lead to increased gizzard weight.

In the experiment of (PERERA et al. 2019b), the complete replacement of wheat with barley resulted in a 37.9% increase in the gizzard weight, from 7.45 to 10.27 g/kg body weight. More extensive grinding by larger gizzards might have facilitated the improvements in body gain/feed intake, AMEn and nutrient utilization at greater levels of barley inclusion. An increase in gizzard size can improve digestive function through increased retention time, lower pH, and better grinding and mixing with digestive enzymes (SVIHUS 2011, 2014). The grinding cycle begins with contraction of the thin muscles followed by opening of the pylorus and a powerful peristaltic contraction in the duodenum. The pair of thick muscles contracts immediately after the commencement of the duodenal contraction, which results in some gastric material being pushed into the duodenum and some material entering the proventriculus. As the thick muscles begin to relax, the proventriculus contracts and returns content to the gizzard. This contraction cycle takes place up to four times per minute and grind material due to rubbing against the koilin layer on the inside of the gizzard and against other particles in the gizzard during contraction of the large muscles, while the small muscles move material towards the grinding zones between contractions of the large muscles. Most of the digestive action of these secretions therefore takes place in the gizzard. Since material is refluxed into the proventriculus during contractions of the large gizzard muscles, this will allow for additional HCl secretions. It has been shown that the volume of the gizzard may increase substantially when structural components are added to the diet, sometimes increasing to more than the double (AMERAH et al. 2009). At high fibre inclusion levels, a decreased feed intake would even be expected due to the need for the gizzard to grind fibre material before it passes through the gizzard. On the other hand, birds have a desire for structural components, and those birds will search for structural components in the environment in a situation. Using oats and barley as structural components causes an improvement in nutrient digestibility. It has been shown repeatedly that when structural components such as whole or coarsely ground cereals are fed, pH of the gizzard content decreases by a magnitude of between 0.2 and 1.2 units (SENKOYLU et al. 2009; SVIHUS 2014; PERERA et al. 2019b). The logical explanation for this is an increased gizzard volume and thus a longer retention time which allows for more hydrochloric acid secretion, combined with a stimulative effect of gizzard activity on acid secretion. In addition to the indirect potential beneficial effects of a reduced pH due to less

pathogenic microflora in the digestive tract, a reduced pH may also contribute to an improved gastric digestion and gut health.

Animal performance, feed efficiency, and overall health are heavily dependent on gut health. This is because the gut contains more than 600 species of bacteria and more than 20 hormones associated with endocrine, paracrine and autocrine modulation; it also digests and absorbs nutrients and consumes about 20% of the incoming energy (CANT et al. 1996). Besides those important roles, the gut accounts for a substantial number of immune cells in the body, being critical in terms of overall animal health (RATCLIFFE 2002). One mechanism by which DF improves gut health is through maintenance of an anaerobic intestinal environment that subsequently prevents facultative anaerobic pathogens from flourishing. Among alternatives to antibiotic growth promoters (AGP), the inclusion of DF in monogastric diets has been attempted with some success. Dietary fibres stimulate the growth of health-promoting gut bacteria, and degraded to SCFA producing s, and have beneficial effects on the immune system (JHA et al. 2019; MAHMOOD AND GUO 2020) (*Figure 4.*). Poultry species require a certain amount of DF for normal intestinal physiology functions. The mechanisms by which DF functions in the gastrointestinal tract depend on the chemical structure, particle size, and amount being fed. Across poultry species, a rapid and relatively consistent intestinal response to changes in DF has been reported resulting in modification of intestinal length, villus height, crypt depth as well as the passage rate and size through different segments of the intestines (REZAEI et al. 2018); (TEJEDA AND KIM 2021).

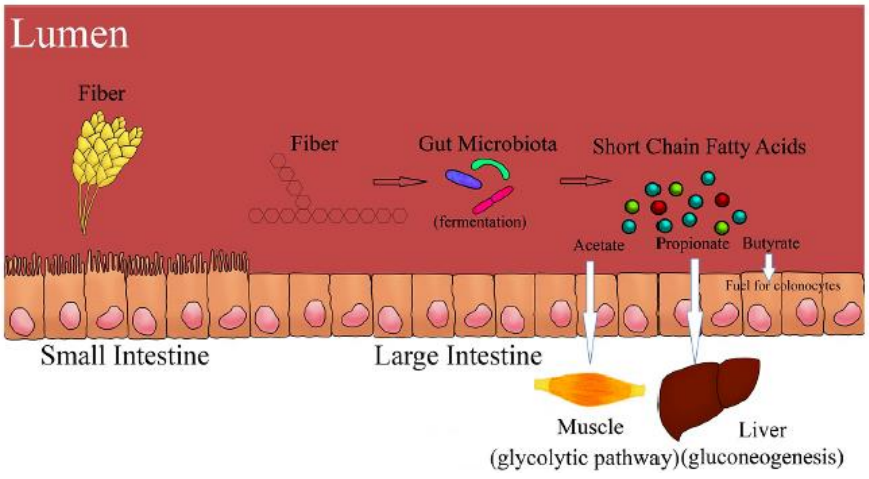

Figure 4. Fibre fermentation and its primary utilization pathways (Jha et al., 2019)

The improvements in villus height and overall epithelial cell arrangement have been regarded as desirable due to the potential increase in nutrient absorption. Similar results have been reported in quails fed 1.5% micronized wheat fibre, which usually results in an increase in relative length of intestinal segments, villi height, villus thickness, and villi to crypt proportions. In geese, increase in villus height were reported with inclusions of alfalfa, rice hulls, or pectin; no changes with inclusion of barley hulls or cellulose; and reductions in villus height with inclusions of lignin. However, the inherent increment in nutrients for the maintenance of such tissues is generally ignored (TEJEDA AND KIM 2021). In a study of KLUTH AND RODEHUTSCORD (2009) it was reported that inclusion of 8% cellulose in broiler diets resulted in higher crude protein and amino acid (i.e., glutamic acid (GLU), aspartic acid (ASP) and threonine (THR)) losses compared to diets fed 3% cellulose.

Gut health is essential to maintain growth performance and overall health of monogastric animals. The primary role of intestinal mucosa layer is digestion and absorption of nutrients. Feed ingredients are hydrolysed and broken down in the intestine into smaller compounds. The DF fermentation resulting in SCFA, which promote proliferation of the mucosal epithelium and villus height. The epithelial layer of mucosa regulates the exchange of nutrients to the body (MONTAGNE et al. 2003). Besides the intestinal secretions and glycoproteins produced by the brush border membrane, mucosal epithelium also greatly influences the adherence capacity and the metabolic activity of intestinal microbes. Hence, the intestinal mucosa acts as a barrier to the pathogenic bacteria and toxic compounds. Both innate and adaptive immune systems participate in the building of intestinal mucosal barrier. The inclusion of DF often increases the endogenous losses, resulting in a perceived decrease in the digestion of energy and nutrients in monogastric animals. Therefore, DF has been recognized as “anti-nutritive compounds” for monogastric animals. However, moderate levels of dietary fibre may increase gut size, length, volume, and morphological structure of poultry and other non-ruminant animals. The villus height to crypt depth ratio is a useful criterion for estimating the likely digestive capacity of the small intestine. The inclusion of high fibre in diets also increased the rate of cell proliferation and crypt depth in the large intestine, when compared to the same diet containing no additional fibre supplementation (JIN et al. 1994). However, the height of villus and the depth of crypt in the gut is not similar in the different sections of the intestine; it changes with the location of the small intestine. Therefore,

it is critical to understand the mechanisms of nutrient absorption, and the location of specific nutrient utilization in the gut to develop the optimal feeding system to obtain the best production performance.

2.7. Effect of dietary fibres on gut health

Since the constraints of using antibiotics in poultry diets, intestinal health has been one of the hot topic issues of poultry production (CHOCt et al. 1996). AWAD et al. (2016) reported that the caeca of birds younger than two weeks had more *Proteobacteria* (increasing the production of pro-inflammatory cytokines), whereas *Firmicutes* and *Tenericutes* (increasing the production of anti-inflammatory cytokines) dominated in birds older than two weeks. Nutrition and microbiota share a very tight interrelationship with each other. With the removal of antibiotics, we have realized that not only the animals should be fed, but the gut bacteria as well. In this way indigested fibres and other nutrients can be used by harmful bacteria that causes dysbiosis or by commensal bacteria that can yield SCFAs that are utilized by the host (MAKI et al. 2019) and some of them (i.e., butyrate) are associated to intestinal health improvement (AHSAN et al. 2016). Therefore, at the end, the type of fibre (solubility and fermentability) determines the type of bacteria that dominates the gut, and the immune response the host will activate in response to such changes. Therefore, it is important to have information on the different types of DF and their specific roles in optimizing gut health of monogastric animals. On the other hand, insoluble fibre passes through the intestine undigested, increases passage rate and faecal bulking; however, monogastric species have a limited capacity to ferment insoluble fibre as they lack specific microbial species (HETLAND et al. 2004; JHA et al. 2010; TEJEDA AND KIM 2021). Therefore, it is essential to understand the components of DF and its nutritional and physiological effects in animals before incorporating it into monogastric diets. Dietary fibre regulates also intestinal morphology (REZAEI et al. 2018), modulates the digestive enzyme secretion (SITTIYA et al. 2020), consequently, changes in nutrient utilization and growth performance. Intestinal health can influence both nutrient uptake and disease status in animals and is impacted by both the gut microbiota and host immune function. As a result, understanding how both factors influence production parameters, it is important to develop alternative tools that provide outcomes on poultry health and growth. On the other hand,

strategies that enhance immune efficiency can be used to selectively inhibit potentially pathogenic populations, limiting incidences of animal diseases and foodborne illnesses. The age of birds is also a significant factor influencing gut microbiota composition, and the metabolites produced by different bacterial populations can impact the development and maintenance of immune cells in the intestinal tract. Understanding these processes can provide unique production strategies that maximize production and promote animal health in the absence of antibiotics (MAKI et al. 2019).The chicken GIT includes compartments with varied physiological roles and environments that drive a spatial distribution of microbial populations. The GIT serves as the home for anywhere between 500–1000 bacterial species, comprising up to 100 trillion cells in total (GILBERT et al. 2018; SHANG et al. 2018). In mature birds, *Lactobacillus* is the dominant genus in the crop and gizzard. The duodenum and jejunum are colonized at low densities, in part, due to high bile acid concentrations and low pH, but *Lactobacilli, Enterococci,* and *Clostridiaceae* are commonly detected (GONG et al. 2007). The ileum is the terminal segment of the small intestine and has the greatest microbial density and diversity of the small intestine, where *Lactobacillus, Enterococcus, Clostridium,* and *Turicibacter* are found in high abundance among other genera (HAN et al. 2016; BORDA-MOLINA et al. 2018). In the caeca, the bacterial community peaks in complexity and density, with strict anaerobes from the phylum *Firmicutes,* composed of the genera *Clostridium, Enterococcus, Bacillus, and Ruminococcus,* being found in high abundance (XIAO et al. 2016; XI et al. 2019). Successional patterns and mature community compositions are important for bird health, with increased microbiota diversity associated with reduced rates of enteric diseases in poultry (OCEJO et al. 2019). For example, exposing chicks to the mature microbiota of adult birds increases the speed of microbial succession in the gut, resulting in the establishment of a mature microbiota at a younger age. While there are health benefits from increased diversity, the presence of individual microbes is also associated with specific health outcomes (OCEJO et al. 2019). *Lactococcus* colonization of the caeca promotes weight gain in chicks, while the presence of *Akkermansia* and *Prevotella* are negatively correlated with weight gain (ZHAO et al. 2016). Other studies associate specific genera such as *Lactobacillus, Ruminococcus,* and *Clostridium clusters IV* and *XIVa* with enhanced bird performance (EECKHAUT et al. 2011). As a result, understanding the microbial succession in a healthy avian gut and how production practices impact this process is important if alternative intervention strategies for disease are to be examined. After the bacterial

inoculum introduced at hatch, the diet plays the most crucial role in determining the composition and density of the intestinal microflora (YADAV and JHA 2019). As specific bacterial species have substrate preferences, it would follow that bacterial populations in the intestines are influenced by changing the diet. The cecum is considered the main site of bacterial activity in the gastrointestinal tract in poultry and is, generally, the organ used for determination of bacterial populations in broilers. The carbohydrate fraction is the most important dietary component regulating the intestinal microbial activity in broilers, particularly with regards to DF, which escapes digestion. The magnitude of the effects of the dietary carbohydrates depends on the type and amount of carbohydrate. Most data have indicated that water-soluble NSP are the most influential compounds, as these can be degraded to be utilized as substrate by intestinal bacteria (MIRZAIE et al. 2012). These soluble components provide the energy for bacteria, allowing them to use other nutrients (i.e., nitrogen) as substrates to produce metabolites. The presence of viscous-forming carbohydrates in the digestive tract has adverse effects on performance, but the presence of bacteria appears to aggravate the problem. The complex carbohydrates and plant polysaccharides indigestible by monogastric animals provide an essential fermentative substrate to the microbiome (including bacteria, fungi, protozoa, and archaea) and are known to impact bacterial composition, diversity, and metabolic capabilities (SONNENBURG AND SONNENBURG 2014). It must be taken into consideration that the nutritional and health benefits residing bacteria provide to their host is a result of the entire community and their metabolic capabilities, not the presence or absence of a single species. It is through glycoside hydrolases, polysaccharide lyases, and carbohydrate esterase that gut- associated bacterial communities are able to breakdown and ferment complex carbohydrates into SCFAs (FLINT et al. 2012). The microbial process of fibre fermentation is considerably more variable than host macronutrient digestion due to the range in fibre sources and the physicochemical properties of that fibre (i.e., solubility, viscosity, and water-holding capacity) (ZIJLSTRA et al. 2012). It has been recognized recently in humans the substantial effect colonic transit time on the microbial composition (VANDEPUTTE et al. 2016). Therefore, soluble fibre can increase the viscosity of intestinal digesta and the transit time, hence increased intestinal mass. Retained digesta in intestinal lumen for longer time provides opportunity for proliferation of selective microbiota. This might be the probable mechanism which causes fibre and its type alter microbial profiles. Resistant starches are

also involved in increasing the viscosity of digesta. However, resistant starch (RS) is easily degraded to small molecular weight residue whereas DF are more resistant to depolymerization. This might be the reason for RS to have better response than DF. Fermentable fibre from barley and oats high in β-glucans also shifts the site of nutrient digestion from the small towards the caeca and increases the relative abundance of *Firmicutes* genera; *Dialister*, *Sharpea*, and *Ruminococcus* (FOUHSE et al. 2017). However, increasing digesta viscosity in poultry with soluble fibre (barley β-glucans or wheat arabinoxylans) has shown to favour expansion of potential pathogens, *E. coli* and *Clostridium perfringens* (SHAKOURI et al. 2009). Viscosity caused by certain fibre results in villus cell loss as it prevents the nutrients to reach the enterocytes. Long term impact of such fibre inclusion results in atrophy of villi. Supplemental enzyme has shown positive response in minimizing this impact (CHOCT 2006). The villus height to crypt depth ratio is a useful criterion for estimating the likely digestive capacity of the small intestine. The literature exploring the complex interactions between gut microbiota and fibre in poultry is scarce. However, recently over 200 different non-starch polysaccharide degrading enzymes (mainly oligosaccharide degrading enzymes vs. cellulases and endo-hemicellulases) were found encoded within the metagenome of broiler microbiota, suggesting poultry microbiota can utilize soluble forms of dietary fibre (SERGEANT et al. 2014). The importance of supplying dietary fibre to the microbiota is truly demonstrated in fibre deficient diets, where resident polysaccharide degrading bacteria begin to utilize the mucus layer of the intestine, which can reduce intestinal barrier function leaving the host increasingly vulnerable to pathogen invasion (DESAI et al. 2016).

Inclusion of dietary fibre can support colonization of beneficial commensal microbiota that competitively exclude pathogens, enhance maturation, and barrier function of the GIT through metabolite production, and directly block adhesion of pathogenic microbes to the intestinal epithelium by providing alternative adhesion sites (DENAYROLLES et al. 2007). Oats hulls are highly insoluble and lignified in nature, they are also able to reduce faecal biogenic amines, cadaverine, and β-phenylethylamine, from protein fermentation, signifying oats hulls can beneficially influence dietary fermentation patterns (KIM et al. 2008). Enrichment of commensal microbiota such as *Lactobacillus* with NSP may induce growth inhibition or competitive exclusion to *E. coli* (KONSTANTINOV et al. 2006). Dietary fibre may reduce pathogen colonization is by improving intestinal barrier function. Necrotic enteritis is a severe intestinal disorder in poultry is

caused by the pathogen *C. perfringens*. Feeding whole wheat has been shown reduce the number of *C. perfringens* in intestinal content, which bacteria are the causal pathogens of necrotic enteritis (BJERRUM et al. 2005). It is suggested by authors that whole wheat improves gut health of chickens by reducing gizzard pH, increasing retention time and viscosity creating an inhospitable environment for pathogen survival into the lower intestinal tract (BJERRUM et al. 2005). Acetylated resistant starch has also been shown to improve gut health and reduce severity of a *C. perfringens* challenge through reducing luminal pH through specific SCFA delivery (M'SADEQ et al. 2015). Controlling *Salmonella* colonization in poultry flocks is another global priority to reduce potential zoonotic contamination of meat products. Other fibre types including fructo-oligosaccharides (FOS) and mannan-oligosaccharides (MOS) have shown to inhibit the growth and colonization of *Salmonella in vitro* and *in vivo* (FERNANDEZ et al. 2002). Although there is much evidence to suggest supplementing dietary fibre to pigs and poultry is beneficial to gut health and disease resistance, research needs to focus on defining the mechanisms of action to help develop optimal nutritional strategies to further improve animal health. It must be recognized that there are likely numerous nutritional strategies that utilize dietary fibre to improve gut health of poultry depending on environment, health status, life stage, and feeding objective.

During microbial colonization the GIT goes from being aerobic to anaerobic. In a homeostatic state the intestine remains anaerobic with anaerobic bacteria outcompeting aerobe and facultative anaerobes. During dysbiosis facultative anaerobic *Proteobacteria*, such as *E. coli* and *Salmonella*, characteristically expand at the expense of oxygen sensitive butyrate producers, disrupting the anaerobic intestinal environment (LITVAK et al. 2017). Inclusion of dietary fibre may help to prevent or ameliorate the micro-aerophilic environment that occurs during dysbiosis by providing a fermentative substrate to anaerobic butyrate-producing bacteria (JHA et al. 2019). In a homeostatic environment host intestinal tissues use butyrate as an energy substrate via β-oxidation, a process that consumes considerable amounts of oxygen helping to maintain an anaerobic environment (BYNDLOSS et al. 2017; LITVAK et al. 2017). In the absence of butyrate, enterocytes use anaerobic glycolysis to obtain energy, a process that increases epithelial oxygen concentrations creating a favourable niche for facultative pathogens such as *Salmonella* to flourish (RIVERA-CHÁVEZ et al. 2016) (LITVAK et al. 2017). To maintain and improve piglet and

poultry gut health, nutritional strategies should aim at restoring the hypoxic intestinal environment through the expansion of butyrate producers to prevent facultative anaerobic expansion.

2.8. Nutrient digestibility of oats and barley in poultry

Ways to improve the feeding value of barley and oats in poultry diets has been studied over the years. However, the published data have been contradictory, resulting in a variable range of inclusion levels being recommended in broiler diets. According to PERERA et al. (2019b) there are many suggestions for barley inclusion rate which ranged between 140, 153, 200 and 300 g barley/kg diet. Some of these studies have been used it in grower and finisher phase of broiler chickens and other use it in the finisher phase only; with taking in consideration β-glucanase enzyme supplementation. Some studies illustrated that using barley had a significant effect on feed intake and body weight gain. On the other hand, the digestibility of some nutrients like starch, protein and fat were progressively increased with increasing inclusion of barley and oats in the diet. Some studies reveals that the barley with complete replacement of wheat shows the highest digestibility of some nutrients (FRIESEN et al. 1992). Also, the digestibility of all nutrients was improved ($P<0.05$) by enzyme supplementation, regardless of the barley inclusion level. Regarding the energy utilization a gradual improvement in AMEn was observed with increasing level of barley in a wheat-based diet. On the other hand, inclusion barley and oats affect positively the starch and protein digestibility for certain limit. The proventriculus and gizzard (ventriculus) are the true stomach compartments in birds. Hydrochloric acid (HCl) and pepsinogen are secreted by the proventriculus and mixed with digesta in the gizzard. The proventriculus is the initial site of protein digestion in chickens where proteins are exposed to HCl, which denatures the protein and then exposes peptide bonds for enzyme hydrolysis. Adequate acid secretion is necessary for conversion of pepsinogen to pepsin, the enzyme initiating protein digestion. The amount of time that feed is retained in the proventriculus is insufficient for adequate exposure to secretions. Extended retention and mixing in the gizzard are necessary to allow for increased contact between feed, gastric juices, and pepsin, thus, facilitating the denaturation and digestion of proteins (RYNSBURGER 2009). Accordingly, the larger gizzards in birds fed greater inclusion levels of barley might have aided in initial protein hydrolysis, and subsequently, resulted in greater CAID

of N. Although the pH of gastric secretions is around 2.0, the retention time and chemical characteristics of the digesta in the proventriculus/gizzard can result in a more variable, and usually higher pH (SVIHUS 2011). Higher feed intake of birds can lead to a higher gizzard pH, unless HCl secretion is able to increase in conjunction with intake (SVIHUS 2014). Moreover, increased grinding in the gizzard and a longer retention time allows for more HCl secretion, resulting in reduced pH. Some studies reveal that using barley or oats could cause feed intake reduction which associated with a reduction in gizzard pH. Besides lower feed intake (FI), the increased size of the gizzard in birds fed greater inclusion levels of barley or oats in the diet might have facilitated higher HCl secretion, resulted a lower pH. It has been observed that using barley diet could increase jejunal villus height, and subsequent greater absorptive area, corresponded with the positive effect of barley inclusion on feed efficiency and CAID of nutrients in some studies. Generally, diets are formulated to contain a maximum of 2–3% CF (CHOCT 2006). Dietary fibre can also increase pancreas enzymatic activity and reverse peristalsis that can lead to an increase in nutrient digestibility (AMERAH et al. 2009).The reverse peristalsis causes bile salts to reach the gizzard, where the bolus is being mixed with gastric secretions. This results in an improved fat emulsification, reducing the potential of fat droplets to coats nutrients, and therefore, nutrients are more readily hydrolysed and absorbed in the gut (HETLAND et al. 2004). However, the results obtained when using dietary fibre can be heavily impacted by the source of fibre and the formulation of iso-nitrogenous and iso-caloric diets. Such changes were due to the prompt ability of the gastrointestinal tract to compensate for changes in dietary fibre, thus increasing the ability to use nutrients. One of the targets when using insoluble dietary fibre is to increase pancreatic secretions (amylases, lipases, proteases) that can improve substrate breakdown and subsequent release of nutrients. It has been reported that additions of insoluble fibres at 1% in diets of pullets can increase the relative weights of proventriculus, gizzard and liver and improve pancreatic proteolytic activity (YOKHANA et al. 2016). Inclusion of insoluble fibres at 3–5% in the diet is commonly known to improve nutrient metabolism due to their ability to modulate gastric secretions from the proventriculus and muscular activity from the gizzard (SACRANIE et al. 2012). Regardless of the initial size, the feed components leaving the gizzard have a consistent particle size range (HETLAND et al. 2004). It would follow that larger particle of DF will help in the retention of bolus in the upper portion of the gastrointestinal tract, slowing down the passage

rate and increasing the exposure of feed components to HCl and enzymes from the proventriculus. This results in the accumulation of insoluble fibre in the gizzard and increases the gastroduodenal reflux and subsequent digestibility of nutrients (HETLAND et al. 2004; SACRANIE et al. 2012). Insoluble dietary fibre has been shown to modulate (oftentimes positively) digestion of starches (AMERAH et al. 2009), fats, and crude proteins when added at 3–5% in the diet. Two of the most prominent factors affecting digestion efficiency of nutrients in the presence of soluble fibre are solubility and fermentability because of their impact on passage rate in the small intestines and the fermentability in the hindgut, respectively (KHERAVII et al. 2018). Both factors are determined by the type of linkages and the amount of branching among sugar units, which allows or prevents interactions with water molecules and/or potential bacterial break down. In poultry nutrition, the term "water-soluble NSP" has been erroneously interchanged with the term "antinutritional fibre". Even though most of the soluble fibres can increase viscosity, there is a small group of soluble fibres that does not. In fact, low-molecular weight carbohydrates such as oligosaccharides are regarded as prebiotics that facilitate the growth of beneficial bacteria from which *Lactobacillus spp.* and *Bifidobacterium spp*. have been targeted as beneficial for intestinal development (RICKE et al. 2020). Therefore, the hygroscopic properties of some oligo- and polysaccharides should not necessarily be directly associated with anti-nutritional factors. The difference in how soluble and insoluble fibre affect intestinal passage rate relies on the site of action of each fibre type. When insoluble fibre is fed as particles bigger than 1.5 mm, it can accumulate in the upper part of the gastrointestinal tract (gizzard and duodenum loop), where most of the bolus mixes with enzymes and where mechanical grinding takes place (in the gizzard). While small (3–5%) additions of insoluble fibres can improve nutrient digestibility, extreme supplementation can interrupt normal digestion metabolism by the formation of coating structures that reduces the accessibility of digestive enzymes to nutrients (JHA et al. 2019); therefore, it is unclear how the threshold for excess DF should be defined. Type and source of fibre, as well as other parameters intrinsic to diet formulation, may influence this threshold. Finally, it is paramount to bear in mind that fibre should be used as a functional nutrient and not as a nutrient *per se*, and the adequate nutritional amendments should be made when using fibrous feedstuffs in terms of energy, protein, and their ratios. In general, improvements in intestinal morphology and organ development can lead to increase nutrient absorption, which will be reflected in enhanced performance (YOKHANA et al.

2016). As it is clear, different carbohydrates from dietary fibre can have different modes of action once ingested by the bird. Therefore, to make conclusions about the effect of fibre, there are different factors that need to be closely considered. Factors such as fibre source (i.e., soluble vs. insoluble), particle size, level of inclusion, species, age, physiological status (i.e., laying hen vs. broiler), dietary energy and protein (i.e., amino acids) levels, and duration of inclusion are among the most influential factors determining the effects of fibres on broiler diets (TEJEDA AND KIM 2021). Because of this intrinsic chemical and structural organization, it is hard to separate soluble from insoluble NSP in feedstuffs, and it is important to understand both fractions individually and in conjunction when formulating diets for poultry species. The ratios of insoluble and soluble components can vary based on grain type, cultivar, environmental conditions, and other associated factors. For insoluble polysaccharides (cellulose, hemicellulose, lignin) the intramolecular interactions are higher (NGUYEN et al. 2019). Soluble fibre is found in association with insoluble fibre mainly as xyloglucan-cellulose and xyloglucan-pectic polysaccharides. The tri-dimensional structure of soluble fibre is referred as matrix polysaccharides, which includes mainly arabinoxylans, beta-glucans, and pectin (HETLAND et al. 2004). The soluble carbohydrates, including oligosaccharides and polysaccharides, are the most influential in terms of growth performance, nutrient absorption modulation, and intestinal welfare.

2.9. Oats and barley in the broiler chicken nutrition

The growth performance in broilers fed barley-based diets has been reported to be poorer compared to corn-based and sorghum (TANG et al., 2017), and this commonly attributed to the greater digesta viscosity in barley-fed birds. SHAKOURI et al. (2009) and TANG et al. (2017) evaluated barley as the sole cereal in the broiler diets in comparison to corn, sorghum and wheat and reported that birds fed barley-based diets had the lowest weight gain (WG), feed intake (FI) and poorest feed to gain ratio (F/G). In contrast, BRENES et al. (1993), who compared barley (cultivar, Scout) with wheat in broilers, reported 58 g superior weight for barley-fed birds at 42 d. However, the F/G of birds fed barley-based diets was impaired by 8 points. The WG and F/G differences caused by the grain type were minimised by the supplemental carbohydrases. Oats has high content of insoluble fibre (JIMÉNEZ-MORENO et al. 2009) and its moderate inclusions in broiler chicken diets have resulted in improvement in nutrient digestibility, and gizza (JIMÉNEZ-

MORENO et al. 2016) Supplementing broiler chicken diets with oats, therefore, has the potential to enhance gut microbiota and SCFA production, thus increasing gut barrier integrity. The structure and mode of feeding of oats fibre have been reported to play an essential role in enhancing intestinal function and modifying the composition and quantity of the microbial population in the chicken gastrointestinal tract (KHERAVII et al. 2018). ADEWOLE et al. (2020) showed that inclusion of 3% fine particle-sized oats hull (OH) has the potential to enhance growth performance and carcass weight of broiler chickens, while OH fed as free-choice did not. GRACIA et al. (2016) reported reduced caecal *Campylobacter jejuni* colonization when 5% OH was fed to broiler chickens in mash form compared to those fed in pelleted form. HETLAND AND SVIHUS (2001) reported a faster feed passage with the inclusion of coarsely ground OH, but no effect of finely ground OH was found. SACRANIE et al. (2017) reported that intermittent feeding of OH showed improvement in nutrient digestibility compared to regular feeding. Coarse OH has high lignin content, which may limit microbial fermentation in the hindgut. Extrusion, a thermal and mechanical processing technique, which combines high pressure with high temperatures, has been known to improve the nutritive value of feed ingredients (ROJAS AND STEIN 2017) and is widely used to improve the functional properties of food. A previous study has reported that physical processing altered the lignin content in oats hulls (PERRUZZA 2010). Oats hulls are considered a lignocellulose biomass, majorly consisting of lignin (16%), hemicellulose (16%) and cellulose (48%) on dry weight basis. It has been noted that physical processing disrupts the bonds between the lignin, cellulose and hemicellulose in OH. Previous research has shown that birds fed coarse diets performed better than those given fine particle-sized diets and that coarse diet increased the efficiency of nutrient retention in broiler chickens (PARSONS et al. 2006).

In summary, barley and oats contain dietary fibre, which can have both positive and negative effects on broiler performance. The fibre content can contribute to improved gut health by promoting the growth of beneficial bacteria and supporting digestive function. However, excessive fibre levels can reduce nutrient digestibility and negatively impact feed intake and growth performance. Barley and oats also provide essential nutrients such as vitamins and minerals, which contribute to the overall nutritional profile of broiler diets. Proper formulation of diets incorporating barley and oats is essential to ensure that broilers receive all necessary nutrients for optimal growth and performance. Barley and oats can affect feed efficiency in broilers. The energy

content of these grains can contribute to efficient growth when properly utilized in balanced diets. However, factors such as fibre content and nutrient digestibility can also influence feed conversion ratios.

3. MATERIALS AND METHODS

Some promising new varieties of barley and oats resistant to drought or cold weather were selected, which were developed for climate changes (heat stress and drought). Nutritive values of these varieties were determined by chemical analyses and broiler chicken experiments.

3.1. The chemical composition of different oats and barley varieties

3.1.1. Cultivation of oats and barleys

A field experiment in the 2017/2018 growing season was conducted in the HUN-REN Centre for Agricultural Research at Martonvásár, Hungary. 35 winter oats (Thirty-five winter oats WO) (5 registered varieties and 30 advanced breeding lines), 36 spring oats (SO) genotypes (6 registered varieties and 30 advanced breeding lines) and 36 winter barley (WB) (6 registered varieties and 30 advanced breeding lines) were involved in the study. Winter genotypes were sown in 17th of October 2017 and the spring genotypes in 3rd of March 2018. The original plot size was 8 m^2, 8 meters in length and 1 meter in width. The row distance was 12.5 cm and 450 plants/m^2 sowing rate was applied by each plot. Excluding the side effect, after the full maturity, 6 m^2 from each plot had been harvested.

After 60-60-60 kilograms nitrogen, phosphorous and potassium active ingredients per hectare basic fertilization, in early spring 40 kg/ha nitrogen top-dress was spilt. The weeds were controlled by spraying U46 (MCPA, 750 g/l, Kwizda Agro Ltd.) and Starane (840 g/l fluroxypyr, Corteva Agroscience Ltd.) herbicides. Against cereal leaf beetle and other insects Fury 10 EW (100 g/l zeta-cypermethrin, Kwizda Agro Ltd.) was used.

The most promising varieties of oats and barley have been selected in Martonvásár in 2020 and sent to Keszthely for the evaluation of their nutritive value.

Thirty-six varieties of winter barley, 35 varieties of winter oats and 36 varieties of spring oats grain samples were analyzed for the following parameters: dry matter, crude ash, crude protein, crude fat, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), starch, water soluble and insoluble dietary fibre, total β-glucan, amino acid content and grain viscosity. From the data the variance of the parameters and the interaction between the nutrient categories were analyzed.

The poetential predictability of the β-glucan content and viscosity of the grains from the different fibre fractions was evaluated with multifactorial linear regressioin equations. Based on the amino acid composition of the grains, the ratio of the essential and non-essential amino acids and the protein quality have been determined.

3.1.2. Chemical analyses

The chemical analysis procedures for oats 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 dry matter (ISO 6496: 2001), crude protein (ISO 5983-2:2009), crude fat (ISO 6830- 19: 1979), crude fibre (ISO 6865:2001) and crude ash (ISO 5984: 1992). The determination of neutral detergent fibre (NDF) and acid detergent fibre (ADF) was based on ISO 16472:2006 and ISO 13906:2008, respectively. The starch content was analyzed by the polarimetric method in line with the European Directive 152/2009. 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 AOAC 2011.25 method is applicable to all samples containing dietary fibre, including resistant starch (RS) and non-digestible oligosaccharides (NDO). The gross energy of varieties was determined with a bomb calorimeter (IKA C6000,). The total β-glucan content was determined according to the method of KNUDSEN, (1997). Acid extract viscosity (AEV) of oats and barley was analyzed as described by SVIHUS et al. (2000) with a Brookfield digital viscometer (Model DV-II., Brookfield Engineering Laboratories, Stoughton, MA 02172, USA) fitted with a C-40 cone and plate. The amino acid analysis was carried out with an automatic amino acid analyzer (INGOS AAA500). The amino acids were separated by ion-exchange chromatography and after reaction with ninhydrin, the photometric detection happened at 570 nm. Tryptophan contents were not measured. Cysteine (CYS) and methionine (MET) were transformed to cysteic acid and methionine sulphone by using performic acid oxidation. and barley the grains

3.1.3 Calculations

The measured oats and barley grain parameters were compared with those can be found in the recently published feedstuff table of EVONIK (2017) and compared also with wheat and corn as

traditional feedstuffs. This data base is one of the most robust information sources containing detailed specific data for the different countries and world regions. In the present comparison the average table values measured in Hungary27 were taken. The protein qualities evaluation of the oats and barley was based on the essential amino acid index (EAAL) calculatuion. The amino acid contents of oats and barley proteins were compared with those of the requirement of the broiler chickens (amino acid composition of the grower diet protein). Not all essential amino acids were involved in the calculations, only those which have requirements for the broiler hcicken (LYS, MET, THR, ARG, VAL, ILE, LEU, VAL). The EAAL was caclulated as the geometcic nean of the amino acid ratios, as in the case of chemical index calculation.

3.2. Digestibility trial with broiler chickens

3.2.1. Animals and diets

The digestibility trial, the feeding experiment, all husbandry and euthanasia procedures were performed in accordance with the Hungarian Government Decree 40/2013 and in full consideration of animal welfare ethics. The animal experiments were approved by the Institutional Ethics Committee (Animal Welfare Committee, Georgikon Campus, Hungarian University of Agriculture and Life Sciences, Deák Ferenc Street 16, 8360 Keszthely, Hungary) under the license number MÁB-1/2017.

Five-week-old Ross 308 broiler cockerels were kept in individual cages and fed ad libitum the experimental diets in 6 replicates. The length of the light and dark periods was 18 and 6 hours respectively. After 5 days adaptation period, the feed intake of birds was measured and about 100g representative excreta samples collected. The excreta samples were stored in a fridge at 4 °C, the samples of the two days mixed, and representative samples taken for the laboratory analysis. The laboratory samples were stored at minus 20 °C until analysis. At day 7 all birds were euthanized with CO_2, slaughtered and immediately the total content of the ileum collected. The ileum was considered as the small intestine part between the Meckel's diverticulum and the ileocecal junction. Three determinant varieties form winter barley (Mw 118-7, Mw 05-17, Mv Initium), winter oats (Mv Kincsem, Mv Istrang, Mv Imperial) and spring oats (Mv Pehely, Mv Mene, Mv 9-14) samples have been incorporated into a basal diet, which composition is described in ***Table***

1. Oats and barley were incorporated into the test diets at 20 and 40% on the expense of starch. In this arrangement the increase in protein and amino acid intake of chickens was related only to the test cereals. The diets were supplemented with exogenous glucanase at 20.000 β-glucanase units (Econase GT 200 P; endo-1,3(4)- beta-glucanase, ABVista, Marlborough, UK). As indicator compound TiO_2 was used to calculate the ileal digestibility of nutrients. The composition of diets is shown in ***Table 1***.

Table 1. Composition of experimental diets

Ingredients	Control	Oats 20%	Oats 40%	Barley 20%	Barley 40%
corn	14.4	14.4	14.4	14.4	14.4
extracted soybean meal	36.2	36.2	36.2	36.2	36.2
corn starch	40	20	0	20	0
barley	0	0	0	20	40
oats	0	20	40	0	0
sunflower oil	4.9	4.9	4.9	4.9	4.9
L-lysine	0.1	0.1	0.1	0.1	0.1
DL-methionine	0.4	0.4	0.4	0.4	0.4
limestone	1.4	1.4	1.4	1.4	1.4
MCP	1.4	1.4	1.4	1.4	1.4
NaCl	0.3	0.3	0.3	0.3	0.3
Sodium bicarbonate	0.1	0.1	0.1	0.1	0.1
premix [1]	0.35	0.35	0.35	0.35	0.35
phytase [2]	0.01	0.01	0.01	0.01	0.01
beta-glucanase [3]	0.01	0.01	0.01	0.01	0.01
TiO2	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100

[1] Premix was supplied by UBM Ltd. (Pilisvörösvár, Hungary). The active ingredients contained in the premix were as follows (per kg of diet): Starter and grower premixes—retinyl acetate—5.0 mg, cholecalciferol—130 g, dl-alpha-tocopherol-acetate—91 mg, menadione—2.2 mg, thia-mine—4.5 mg, riboflavin—10.5 mg, pyridoxin HCl—7.5 mg, cyanocobalamin—80 g, niacin—41.5 mg, pantothenic acid—15 mg, folic acid—1.3 mg, biotin—150 g, betaine—670 mg, monensin-Na—110 mg (only grower), narasin—50 mg (only starter), nicarbazin—50 mg (only starter), antioxidant—25 mg, Zn (as ZnSO4H2O)—125 mg, Cu (as CuSO45H2O)—20 mg, Fe (as FeSO4H2O)—75 mg, Mn (as MnO)—125 mg, I (as KI)—1.35 mg, Se (as Na2SeO3)—270 g; Finisher premix—retinyl acetate—3.4 mg, cholecalciferol—97 g, dl-alpha-tocopherol-acetate—45.5 mg, menadi-one—2.7 mg, thiamin—1.9 mg, riboflavin—5.0 mg, pyridoxin HCl—3.2 mg, cyanocobalamin—19 g, niacin—28.5 mg, pantothenic acid—10 mg, folic acid—1.3 mg, biotin—140 g, L-ascorbic acid—40 mg, betaine—193 mg, antioxidant—25 mg, Zn (as ZnSO4H2O)—96 mg, Cu—9.6 mg, Fe (as FeSO4H2O)—29 mg, Mn (as MnO)—29 mg, I (as KI)—1.2 mg, Se (as Na2SeO3)—350 g; [2] Axtra® Phy 5000 TPT phytase 500 FTU (Danisco Animal Nutrition & Health, USA); [3] Econase GT 200 P; endo-1,3(4)- beta-glucanase, ABVista, Marlborough, UK)

In the frame of the digestibility one barley (MV 05-17), one winter oats (Mv Imperial) and two spring oats (Mv Pehely, Mv 0914) at 40% incorporation rates were fed also without exogenous beta glucanase, to evaluate the enzyme effect on the ileal N and AA digestion and apparent

digestibility of crude fat and starch. The composition of these diets was the same, only the enzyme was a separate treatment ***(Table 2.)***.

Table 2. Compositions of diets, used in the enzyme effect trial

Ingredients	C+	C-	O40+	O40-	B40+	B40-
corn	14.4	14.4	14.4	14.4	14.4	14.4
extracted soybean meal	36.2	36.2	36.2	36.2	36.2	36.2
corn starch	40	40	0	0	0	0
barley	0	0	0	0	40	40
oats	0	0	40	40	0	0
sunflower oil	4.9	4.9	4.9	4.9	4.9	4.9
L-lysine	0.1	0.1	0.1	0.1	0.1	0.1
DL-methionine	0.4	0.4	0.4	0.4	0.4	0.4
limestone	1.4	1.4	1.4	1.4	1.4	1.4
MCP	1.4	1.4	1.4	1.4	1.4	1.4
NaCl	0.3	0.3	0.3	0.3	0.3	0.3
Sodium bicarbonate	0.1	0.1	0.1	0.1	0.1	0.1
premix [1]	0.35	0.35	0.35	0.35	0.35	0.35
phytase [2]	0.01	0.01	0.01	0.01	0.01	0.01
beta glucanase [3]	0.01	-	0.01	-	0.01	-
TiO2	0.50	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100	100

C: control diet; O40: diets containing 40% oats; B40: diets containing 40% barley; +: diets with Econase enzyme supplementation; -: diets without Econase enzyme supplementation [1] Premix was supplied by UBM Ltd. (Pilisvörösvár, Hungary). The active ingredients contained in the premix were as follows (per kg of diet): Starter and grower premixes—retinyl acetate—5.0 mg, cholecalciferol—130 g, dl-alpha-tocopherol-acetate—91 mg, menadione—2.2 mg, thia-mine—4.5 mg, riboflavin—10.5 mg, pyridoxin HCl—7.5 mg, cyanocobalamin—80 g, niacin—41.5 mg, pantothenic acid—15 mg, folic acid—1.3 mg, biotin—150 g, betaine—670 mg, monensin-Na—110 mg (only grower), narasin—50 mg (only starter), nicarbazin—50 mg (only starter), antioxidant—25 mg, Zn (as ZnSO4H2O)—125 mg, Cu (as CuSO45H2O)—20 mg, Fe (as FeSO4H2O)—75 mg, Mn (as MnO)—125 mg, I (as KI)—1.35 mg, Se (as Na2SeO3)—270 g; Finisher premix—retinyl acetate—3.4 mg, cholecalciferol—97 g, dl-alpha-tocopherol-acetate—45.5 mg, menadi-one—2.7 mg, thiamin—1.9 mg, riboflavin—5.0 mg, pyridoxin HCl—3.2 mg, cyanocobalamin—19 g, niacin—28.5 mg, pantothenic acid—10 mg, folic acid—1.3 mg, biotin—140 g, L-ascorbic acid—40 mg, betaine—193 mg, antioxidant—25 mg, Zn (as ZnSO4H2O)—96 mg, Cu—9.6 mg, Fe (as FeSO4H2O)—29 mg, Mn (as MnO)—29 mg, I (as KI)—1.2 mg, Se (as Na2SeO3)—350 g; [2] Axtra® Phy 5000 TPT phytase 500 FTU (Danisco Animal Nutrition & Health, USA); [3] Econase GT 200 P; endo-1,3(4)- beta-glucanase, ABVista, Marlborough, UK)

Beside the effect of barley and oats on the nutrient digestibility of the diets, the amino acid digestibility of barley and oats 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. From the diets and excreta samples their dry matter, nitrogen, crude fat, starch and TiO_2, form the ileal digesta the TiO_2, N and AA contents were measured. The TiO_2 measurement was done by a spectrophotometer (Jenway 6100, Dunmow, UK) at 410 nm,

according to the method of SHORT et al. (1996).

3.2.2. Calculations

The faecal nutrient digestibility and apparent metabolizable energy corrected to zero-nitrogen retention (AMEn) were calculated with the following formulas:

$$\text{faecal digestibility coefficient (\%)} = 1 - \frac{\text{Nutrient diet} - (\text{Nutrient excreta} \times (TiO_2 \text{ diet}/TiO_2 \text{ excreta}))}{\text{Nutrient diet}}$$

where:

Nutrient diet = nutrient content of the diet (g/kg)
Nutrient excreta = nutrient content of the excreta (g/kg)
TiO_2 diet = TiO_2 content of the diet (g/kg)
TiO_2 excreta = TiO_2 content of the excreta (g/kg)

The ileal digestibility coefficients of amino acids (AA) of the experimental diets were calculated as follows:

$$\text{ileal digestibility coefficient of AAs (\%)} = 1 - \frac{\text{AA diet} - (\text{AA ileal content} \times (TiO_2 \text{ diet}/TiO_2 \text{ ileum}))}{\text{AA diet}}$$

Where:
AA diet = amino acid content of the diet (g/kg)
AA ileum = amino acid content of the ileal content (g/kg)
TiO_2 diet = TiO_2 content of the diet (g/kg)
TiO_2 ileum = TiO_2 content of the ileal content (g/kg)

The amino acid digestibility of barley and oats amino acids were calculated by linear regression between the daily amino acid intake and the daily ileal absorbed amino acids. The digestibility coefficient in this case was the slope of the linear regression equation. The AA intake and ileal absorbed AA amounts were calculated as follows:

$$\text{daily AA intake (mg/day)} = FI \times \text{AA feed}$$

where:
FI = daily feed intake (g/day)
AA feed = AA content of the diet (mg/g)

ileal absorbed AA (mg/day) = AA ileum x (TiO_2 diet / TiO_2 ileum)
where:
AA ileum = AA content of the ileal content (mg/g)
TiO_2 diet = TiO_2 content of the diet (g/kg)
TiO_2 ileum = TiO_2 content of the ileal content (g/kg)

3.3. Feeding trial with broiler chickens

3.3.1. Animals, treatments, and samplings

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 3.***

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 3. 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.

3.3.2. Histological analyses and small intestine morphology

On day 40, two chickens per pen, 10 birds per treatment were slaughtered and the following parameters investigated histomorphology of the jejunum, and ileum. For the histomorphological examination jejunum and ileum tissue samples were taken. The jejunum sample originated about 10 cm before the Meckel's diverticulum and the ileal samples were taken about 5 cm after the Meckel's diverticulum. Tissue sections were washed with 2% phosphate buffered saline (PBS) and fixed in 10% phosphate buffered formalin. Each fixed sample was processed on a tissue processor. The samples were dehydrated through graded alcohol concentrations (70%, 95% and absolute alcohol) at ambient temperature, cleared in graded concentrations of isopropyl alcohol to remove any residual alcohol and then impregnated with Histosec pastilles under pressure at 60°C.

Samples were embedded in paraffin blocks 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). The fixed tissue samples in formalin were dehydrated and embedded in paraffin wax. Five-μm-thick sections, in duplicate, were cut by using a Microtome and using Feather S35 disposable blades fixed on slides. A total of 10 intact, well-oriented crypt–villus units were selected for each intestinal cross-sections at 4× magnifications. The principle for villus selection required villi

covered by intact lamina propria. The measurements of villus height (from the apical end of the villus to the lamina muscularis mucosae), crypt depth (from the onset of crypt to the lamina muscularis mucosae) and muscular layer thickness (tunica muscularis) were conducted.

3.3.3. Viscosity measurement

In the feeding trial 4 birds for each pen have been used for ileal viscosity measurement, which means 20 replicates for each treatment. After thawing, viscosity samples were centrifuged (12,000 g for 10 min) and the supernatant (0.5 ml) was measured using a Brookfield DV II+ digital viscometer Model DV2TLV (Brookfield Engineering Laboratories, Stoughton, MA) at 25°C with a CP-40 cone spindle and shear rate of 60–600/s to measure the viscosity. Viscosity measurements were expressed in centipoise (cPs) unit (1 cPs = 1/100 dyne sec/cm2 = 1 mPa.s) prior to statistical analysis.

3.3.4. pH measurement

The pH samples (crop, gizzard, jejunum, ileum, and caecum) were processed immediately after sampling and were diluted with distilled water (1:5) and vortexed thoroughly. The pH was measured using a pH meter (pH 200A portable pH meter; CLEAN Instruments, Shanghai) equipped with an electrode (CS1068 SNEX; CLEAN Instruments). Three values were taken, and the average value was considered as the final pH value.

3.3.5. Short chain fatty acid analysis

The samples were thawed on ice and samples were prepared for gas chromatographic SCFA measurements according to the method of ATTEH et al, (2008) with minor modifications. Briefly, samples were thoroughly mixed and 250 µl digesta were taken and mixed with 600 µl of 1.11 M HCl. The SCFA concentrations were determined by gas chromatograph (TRACE 2000; Thermo Scientific, USA) using a 30 m (0.25 mm i.d.) fused silica column (Nukol column, Supelco Inc., Bellefonte, PA). The detector type was FID with a split injector (1:50), the injection volume was set as 1 µl at 220°C, and the detection was performed at 250°C. Helium was used as a carrier gas with the pressure of 83 kPa. Standard mixtures of SCFAs (1, 4, 8 and 20 mM), comprised of acetate, propionate, n-butyrate, n-valerate, i-butyrate and i-valerate as external standards (Water Soluble Fatty Acid (WSFA) Mixes; WSFA-2, SUPELCO, Sigma-

Aldrich Ltd. Budapest) were applied for calibration. The total SCFA concentration was derived from the sum of all the individual SCFAs in the sample, expressed as μmol/g digesta.

3.3.6 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). Equimolar concentrations of libraries were pooled and sequenced on an Illumina MiSeq platform using a MiSeq Reagent Kit v3 (600 cycle; Illumina Inc., San Diego, CA, USA) with a 300-bp read length paired-end protocol. Raw sequences data of 16S rRNA gene analysis were deposited at the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number PRJNA609272.

3.3.7 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. Sequences were clustered into operational taxonomic units (OTUs) using VSEARCH algorithm open-reference clustering, based on a 97% similarity to the SILVA (release

132) reference database (QUAST et al. 2013) 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). Only features appearing in at least a minimum of 10 reads across all samples were retained in the resulting feature table. To examine differences in microbial community structures between samples, a principal coordinate analysis (PCoA) with the Bray–Curtis dissimilarity was generated using the Microbiome Analyst online software. Permutational multivariate analysis of variance (PERMANOVA, $p < 0.05$) was used to analyse spatial variation in beta diversity and the effects of sampling places (jejunum chymus—JC, jejunum mucosa—JM and caecum chymus—CC). Canonical correspondence analysis (CCA) was used to evaluate the effect of BW on the intestinal tract microbiota structure. Correlations of the canonical axes with the explanatory matrix were reported and the significance of each correlation was determined by 999 permutations with Calypso online software (ZAKRZEWSKI et al. 2017).

The comparison of production traits, gut parameters and caecal SCFA contents were compared with one way ANOVA (SPSS 23 software) using the same tests as described in the previous trials.

3.4. Statistical analysis

3.4.1. 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).

3.4.2. 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.

3.4.3. 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.

4. RESULTS AND DISCUSSION

4.1. 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 4***. 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 AND 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 4. 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.

4.1.1. The interactions between the crude protein content with the other nutrients

As expected, the different fibre categories of WO show negative correlation with the crude protein content of grains (***Table 5).*** 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 5. 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

4.1.2. 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. It is surprising the lack of significant interaction between the SDFP content and viscosity of oats and barley, since the trend of these parameters was similar *(Table 6).* The reason for this contradiction could be that probably not all precipitable oligosaccharides increase viscosity on one side and smaller soluble fractions, that are not measured in SDFP could also modify viscosity. The ratio of these fibre components is different in the cereal grains. The soluble β-glucan content of barley and oats is similar, 24 and 27 g/kg respectively (RODEHUTSCORD et al. 2016). From the results

we can conclude, that unfortunately grain viscosity and β-glucan content cannot be predicted from the routinely measured fibre fractions.

Table 6. Prediction the viscosity and β-glucan contents of oats and barley from their fibre fractions

	Equation	r^2	p
Winter oats	viscosity = 3.276 + 0.153 x β-glucan	0.135	0.03
Spring oats	viscosity = 2.432 + 0.129 x β-glucan	0.123	0.036
Winter barley	β-glucan=50.4 + 2.80 x SDFP - 0.072NDF	0. 258	0.009

SDFP: soluble dietary fibre precipitable; NDF: neutral dietary fibre

4.1.3. 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 7.*** 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 7. 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

4.1.4. The amino acid content of oats and barley

In spite the crude protein content of the grains was close to each other (138 – 144 g/kg DM) significant differences were found in several AAs ***(Table 8.).*** The amino acid content of SO was in all cases higher than in WO, but the differences among the essential AAs (EAA) were only in the case of MET, ILE, TYR, PHE and LYS significant. The content of each measured non-essential AAs (NEAA) was higher in SO than in WO. The AA composition of WB was similar to WO but contained in all cases less EAAs. The highest difference was found in the case of cystine. Its amount in WB was 29 and 32% less, than in WO and SO respectively. WB on the other hand contained more GLU and PRO than oats. GLU was more than 20%, while PRO two times higher in WB. The measured AA contents agree with the EVONIK values (EVONIK 2017).

The variance of EAAs was in the range of 7.39 – 17.01, with the lowest CV of lysine and the highest of histidine. Among NEAAs, the variance of aspartic acid was in the oats varieties higher than that in WB. The opposite was true for proline, of which CV in WB was 18.3%. The table also shows that oats contain more essential amino acids. The essential and non-essential amino acid ratio in oats is about 50:50%, but in barley the essential amino acid ratio is only 43%.

4.1.5. 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 9.).*** 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 9.*** 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%).

Table 8. The amino acid content of oats and barley samples (g/kg DM)

	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 (%)
EAAs [3]															
Cystine	2.99^{b}	2.36	3.82	0.34	11.52	4.20^{a}	3.33	5.10	0.47	11.24	4.39^{a}	3.42	5.29	0.54	12.25
Methionine	2.47^{b}	1.92	3.14	0.31	12.75	2.48^{b}	1.88	3.87	0.45	18.10	2.68 ^{a}	1.88	3.21	0.34	12.69
Threonine	4.78^{b}	3.95	5.88	0.49	10.15	5.11^{a}	4.20	6.51	0.60	11.65	5.31^{a}	2.98	6.73	0.89	16.79
Valine	6.92^{b}	5.75	8.93	0.76	10.96	7.45^{a}	6.12	10.61	0.89	11.94	7.57^{a}	6.06	8.94	0.75	9.91
Isoleucine	5.03^{c}	4.33	6.29	0.51	10.11	5.57^{b}	4.54	8.40	0.77	13.85	5.89^{a}	4.51	6.87	0.64	10.83
Leucine	9.54^{c}	8.38	12.21	0.82	8.62	10.71^{a}	8.86	15.36	1.41	13.20	11.11^{a}	8.95	13.74	1.33	12.01
Tyrosine	4.21^{c}	3.70	5.20	0.40	9.58	5.40^{b}	4.32	6.54	0.57	10.64	5.83^{a}	4.53	6.73	0.59	10.20
Phenylalanine	7.67^{ab}	6.67	9.78	0.91	11.90	7.38^{b}	5.78	10.28	0.96	12.96	7.78^{a}	6.51	9.25	0.66	8.42
Histidine	3.18^{b}	2.47	4.10	0.44	13.70	3.43^{a}	2.34	4.97	0.51	14.90	3.64^{a}	2.76	6.38	0.62	17.01
Lysine	5.05^{c}	4.28	6.22	0.37	7.39	6.11^{b}	4.66	7.29	0.57	9.40	6.39^{a}	5.07	7.60	0.62	9.71
Arginine	7.29^{b}	6.31	9.95	0.80	11.04	9.36^{a}	7.52	14.25	1.16	12.36	9.36^{a}	7.84	12.69	1.00	10.69
NEAAs [4]															
Aspartic acid	8.58^{b}	7.44	10.85	0.67	7.81	11.85^{a}	9.44	15.25	1.45	12.26	12.05^{a}	9.06	14.89	1.66	13.73
Serine	5.93^{c}	4.62	7.42	0.64	10.80	6.94b	5.65	8.29	0.68	9.80	7.30^{a}	5.53	8.73	0.93	12.79
Glutamic acid	35.62^{a}	30.33	45.43	4.16	11.68	28.11^{b}	24.21	40.33	2.47	8.80	28.79^{b}	25.47	34.39	2.24	7.78
Proline	16.60^{a}	12.29	24.64	3.04	18.31	7.27^{b}	5.63	8.75	0.79	10.91	7.83^{b}	5.64	9.70	0.94	11.96
Glycine	5.66^{c}	4.74	6.67	0.47	8.25	6.97^{b}	5.63	7.98	0.72	10.32	7.49^{a}	5.74	8.62	0.75	10.02
Alanine	5.55^{c}	4.51	6.63	0.49	8.83	6.73^{b}	5.64	10.39	0.87	12.91	7.33^{a}	5.85	8.93	0.87	11.93
Total EAAs	52.5	4.63	6.55	0.47	8.87	60.7	5.52	8.26	0.48	7.92	63.4	5.32	7.18	0.49	7.68
Total NEAAs	69.2	5.80	8.91	0.77	11.1	61.3	5.44	8.20	0.44	7.12	64. 2	5.36	7.45	0.49	7.70
EEA ratio (%)	**43.2**	40.1	46.0	0.01	2.88	**49.7**	47.0	53.0	0.015	3.11	**49.7**	47.7	51.5	0.01	2.00
NEEA ratio (%)	**56.8**	54.0	59.9	0.01	2.19	**50.3**	47.0	53.0	0.015	3.08	**50.3**	48.5	52.3	0.01	1.98

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

Table 9. 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.

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).

4.1.6. 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 10***. 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 10. 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)**.**

4.1.7. 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 11***. 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 11. 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 type

4.1.8. Comparison the protein quality of barley, oats, wheat, corn and extracted soybean meal

The protein quality comparison of the different feed sources is shown in ***Figure 5.*** The amino acid composition of the feed proteins was compared with that of the chicken's requirement (AVIAGEN 2014). The amino acid composition of the grower diet's protein was used as the basis of comparison. On the figure the red line shows the relative requirement of the chicken, and the essential amino acids of the feeds are below or above this value. The LYS ratio of WO and SO are the highest among cereal grains, but WB protein contains also higher LYS content, than that of wheat or corn. Furthermore, the protein content of both oats is rich in CYS and ARG, of which ratio is 36-44% above the chicken's requirement. The high CYS content pf oats could be important if low protein (LP) diets are fed, since in this case the MET to CYS transformation could be limited and affected by other amino acids (SIEGERT AND RODEHUTSCORD, 2018).

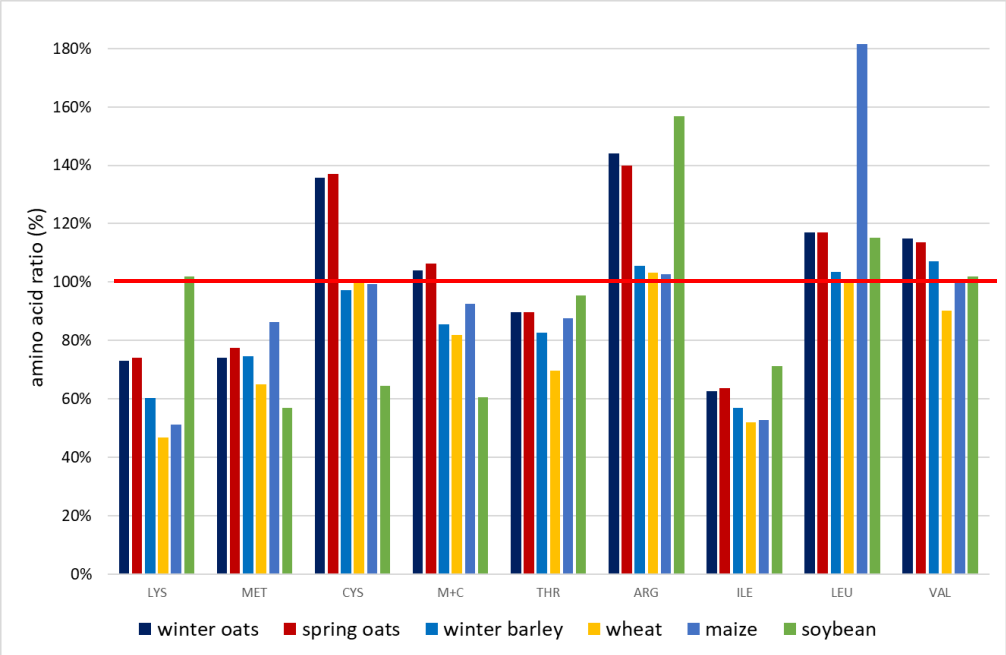

Figure 5. The Essential amino acid ratios of oats, barley, wheat, corn, and soybean proteins as compared with the broiler chicken's requirements

Data in ***Figure 6.*** show that the proteins of both spring and winter oats have higher EAAI than other cereal grains and soybean. From nutrition point of view, it means that the proteins with higher EAAI contain more essential amino acids and their ratio is closer to the requirement of the chicken. It could also be important if low protein (LP) diets are fed, considering that the price of the crystalline AAs is high. The high biological value of oats protein is not well-indicated.

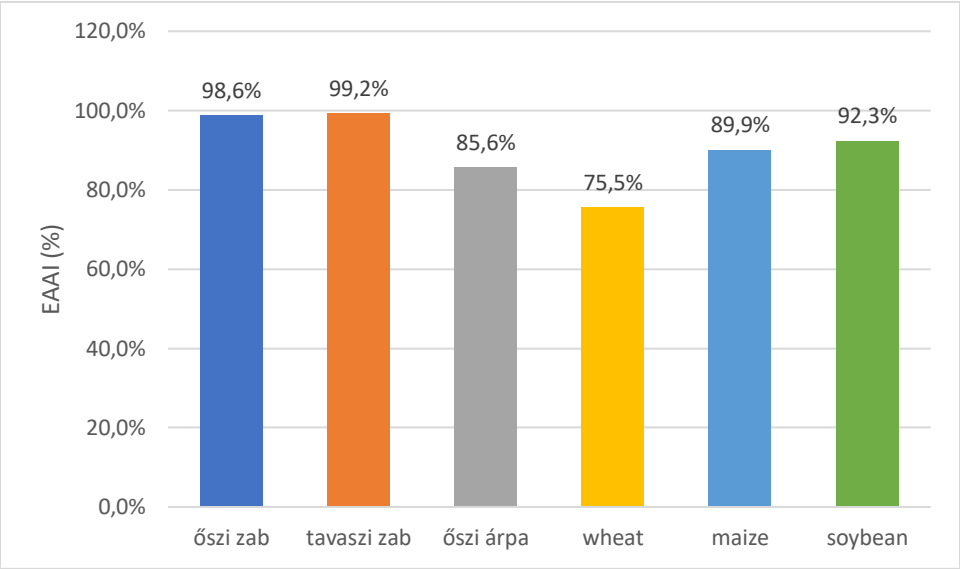

Figure 6. The Essential amino acid index of oats, barley, wheat, corn, and soybean proteins

4.2. Nutrient digestibility of oats and barley containing diets

The nutrient composition of the oats and barley varieties, used in the digestibility trial can be found in ***Table 12.*** One variety of barley (Mw 05-17) and one variety of oats (Mv-Pehely) variety that contained high crude protein level, 13.5 and 14.6%, respectively. Winter barley varieties had lower crude fat (EE), higher starch and higher soluble fibre than oats. The insoluble fibre content was however, two times higher in the oats. The β-glucan content of grains showed high variance without reflecting their SDFP content and viscosity. These results on the main nutrient categories agree with those of Witten et al. (2019). The concentrations of SDFP, IDF and β-glucan were in the range of the relevant literature (BELOSHAPKA et al. 2016; RODEHUTSCORD et al. 2016; MENKOVSKA et al. 2017).

Table 12. Chemical composition of the oats and barley varieties, used in the digestibility trial

		Dry matter	Crude protein	Crude fat	Crude Ash	NFE[1]	Starch	Crude fibre	NDF[2]	ADF[3]	IDF[4]	SDFP[5]	β-Glucan	Viscosity
		g/kg												(mPas)
Winter barley (n=3)	Mw 118-17	892	122.3	17.6	22.5	676.9	478.5	52.9	157.5	64.1	144.3	32.5	31.85	13.25
	Mw 05-17	887	135.0	15.4	19.4	688.2	480.7	28.9	238.2	37.1	122.6	36.6	44.76	15.60
	Mv Initium	887	116.0	18.0	19.5	688.3	474.1	44.8	168.7	53.2	165.5	35.6	52.00	7.60
Winter oats (n=3)	Mv Kincsem	901	120.2	41.2	20.3	614.2	339.7	105.5	312.3	126.6	278.1	23.8	36.51	7.74
	Mv Istrang	897	117.0	47.1	20.9	591.4	326.9	120.1	335.4	141.8	283.6	21.0	28.80	6.59
	Mv Imperial	904	128.4	52.1	21.3	593.3	321.2	108.6	320.1	126.5	292.8	25.0	39.78	11.80
Spring oats (n=3)	Mv Pehely	908	146.4	49.2	20.1	590.5	328.2	101.5	294.1	110.1	292.3	30.2	32.99	9.78
	Mv Mene	905	121.1	31.3	18.7	611.6	344.0	122.4	309.7	132.8	324.2	20.0	28.42	5.73
	Mw 09-14	907	128.8	33	17.9	625.1	346.8	102.5	282.8	128	271.3	28.6	31.92	7.60

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 precipitable.

Data of ***Table 13***. show the amino acid content of oats and barley varieties. The AA compositions were similar to those described in detail in the first part of results.

Table 13. Amino acids content of barley and oats samples (g/kg DM)

	Winter barley (n=3)			Winter oats (n=3)			Spring oats (n=3)		
	Mw 118-17	Mw 05-17	Mv Initium	Mv Kincsem	Mv Istrang	Mv Imperial	Mv PehelyPehely	Mv Mene	Mw 09-14
Cystine	2.80	3.50	2.71	3.33	4.24	4.76	4.63	3.98	4.74
Methionine	2.58	2.71	2.14	2.80	2.00	2.80	2.50	2.10	2.40
Threonine	4.60	5.19	4.85	4.22	4.91	4.54	6.39	4.53	5.62
Valine	6.39	7.67	6.77	7.32	7.81	8.19	8.15	7.84	6.83
Isoleucine	5.27	5.64	4.40	4.55	5.91	6.09	5.73	4.64	6.17
Leucine	9.86	10.03	9.25	9.65	10.37	9.30	13.22	10.50	12.34
Tyrosine	4.60	3.95	3.95	4.44	5.69	5.86	6.72	5.63	6.06
Phenylalanine	6.84	8.34	7.44	7.54	7.14	8.30	8.15	7.62	8.05
Histidine	2.91	2.93	2.71	2.66	3.57	3.87	3.31	2.98	3.64
Lysine	4.82	5.07	4.62	4.66	6.13	6.53	7.05	6.08	6.39
Arginine	6.95	8.12	7.22	10.10	8.59	7.52	9.47	8.29	8.93
Aspartic acid	9.08	8.68	8.35	14.20	9.70	14.16	14.87	10.50	10.47
Serine	6.39	6.09	5.19	6.55	7.25	7.75	8.59	7.51	7.27
Glutamic acid	34.30	41.61	35.53	28.84	26.10	28.22	32.61	25.96	27.33
Proline	16.70	18.49	12.29	7.65	6.13	7.86	9.69	8.40	7.60
Glycine	5.38	5.30	5.53	5.99	5.69	6.20	8.04	6.74	7.94
Alanine	5.27	5.98	5.08	5.99	5.80	6.64	8.26	7.40	6.72

The measured nutrient compositions of the 19 experimental diets are in ***Table 14***. Since the control diet contained 40% corn starch, the substitution of starch with barley and oats increased the crude protein content of the diets. The diets with 40% oats, contained the highest amount of EE and CF. The measured amino acid composition of the diets is found in **Table 15**. In parallel with the crude protein contents, the amino acid composition of diets increased with the barley and oats inclusion rates. This arrangement made it possible to evaluate the response of AA absorption to the increased AA intake.

Table 14. Measured nutrient content of the oats and barley containing diets

		Dry matter	Crude protein	NFE	Crude fat	Starch	Crude fibre	Crude ash
					g/kg			
Control	Control	894.0	184.1	548.4	64.6	447.3	35.5	61.4
Winter barley	WB1/20	894.3	199.3	528.3	67.0	395.7	34.5	65.2
	WB1/40	894.2	218.4	492.2	70.3	334.8	47.0	66.3
	WB2/20	891.9	201.1	528.5	65.9	389.5	32.7	63.7
	WB2/40	890.8	224.4	488.8	69.3	351.4	42.5	65.8
	WB3/20	894.1	202.2	523.4	67.4	395.5	36.2	64.9
	WB3/40	894.0	227.5	470.3	74.0	326.3	53.8	68.4
Winter oats	WO1/20	895.8	203.8	512.5	72.2	368.3	42.7	64.6
	WO1/40	902.6	226.0	463.8	79.7	274.6	67.4	65.7
	WO2/20	897.6	204.8	512.3	73.8	371.8	44.2	62.5
	WO2/40	901.4	222.9	461.2	83.1	286.6	69.3	64.9
	WO3/20	895.7	205.4	500.8	77.7	361.5	46.5	65.3
	WO3/40	903.0	221.0	469.5	85.2	273.5	61.0	66.3
Spring oats	SO1/20	897.0	211.5	506.6	74.1	358.5	41.5	63.3
	SO1/40	901.3	233.5	461.3	82.2	265.7	56.0	68.3
	SO2/20	897.7	201.2	527.0	68.2	373.2	38.1	63.2
	SO2/40	901.1	219.6	474.7	74.8	267.0	64.0	68.0
	SO3/20	896.7	203.8	521.6	68.8	372.6	38.8	63.7
	SO3/40	903.1	225.4	481.7	76.0	283.3	53.5	66.5

WB 1-3 20: diet that contained winter barley at 20%; WB 1-3 40: diet that contained winter barley at 40%; WO 1-3 20: diet that contained spring oats at 20%; WO 1-3 40: diet that contained spring oats at 40%.

Table 15. Amino acids composition of the experimental diets (g/kg)

	Control	Winter Barley (n=6)						Winter Oats (n=6)						Spring Oats (n=6)					
		WB1 20	WB1 40	WB2 20	WB2 40	WB3 20	WB3 40	WO1 20	WO1 40	WO2 20	WO2 40	WO3 20	WO3 40	SO1 20	SO1 40	SO2 20	SO2 40	SO3 20	SO3 40
CYS	1.62	1.71	1.76	1.74	1.84	1.70	1.76	1.75	1.81	1.84	2.01	1.84	2.06	1.83	1.98	1.81	1.96	1.87	2.06
MET	3.94	3.73	3.51	3.69	3.50	3.72	3.54	3.74	3.43	3.67	3.46	3.68	3.51	3.62	3.38	3.66	3.45	3.68	3.45
THR	4.26	4.28	4.23	4.20	4.16	4.19	4.14	4.16	4.06	4.23	4.21	4.24	4.06	4.26	4.26	4.18	4.12	4.26	4.37
VAL	5.18	5.29	5.29	5.24	5.29	5.18	5.17	5.28	5.49	5.33	5.44	5.34	5.40	5.22	5.24	5.31	5.42	5.19	5.17
ILE	4.90	4.81	4.66	4.81	4.73	4.82	4.76	4.76	4.62	4.89	4.89	4.90	4.82	4.75	4.63	4.76	4.64	4.88	4.83
LEU	8.63	8.61	8.47	8.47	8.35	8.55	8.48	8.54	7.94	8.62	8.60	8.64	8.25	8.66	8.67	8.60	8.34	8.73	8.76
TYR	3.86	3.84	3.76	3.74	3.65	3.84	3.83	3.83	3.87	3.96	4.03	3.96	3.98	3.95	4.01	3.94	4.09	3.96	4.01
PHE	5.53	5.66	5.68	5.61	6.05	5.53	5.52	5.60	5.83	5.57	5.59	5.58	5.68	5.52	5.50	5.60	5.65	5.61	6.14
HIS	2.89	2.85	2.77	2.80	2.73	2.83	2.78	2.81	2.84	2.90	2.90	2.90	2.89	2.80	2.72	2.83	2.78	2.88	2.85
LYS	7.10	6.80	6.45	6.67	6.34	6.72	6.42	6.71	6.37	6.86	6.66	6.87	6.60	6.77	6.50	6.83	6.57	6.82	6.57
ARG	7.82	7.69	7.47	7.59	7.25	7.56	7.35	7.87	7.86	7.73	7.65	7.75	7.82	7.61	7.44	7.68	7.56	7.69	7.56
ASP	11.60	11.74	11.18	11.26	10.91	11.64	11.41	12.15	11.91	11.73	11.33	12.28	11.85	11.91	11.64	11.76	11.40	11.69	11.22
SER	5.16	5.40	5.26	5.35	5.25	5.44	5.40	5.46	5.39	5.53	5.57	5.54	5.55	5.52	5.54	5.54	5.59	5.49	5.55
GLU	19.80	21.15	21.88	20.35	22.26	21.30	21.93	20.25	20.00	20.00	20.13	20.04	20.10	20.10	20.10	19.38	20.01	19.94	19.11
PRO	5.74	6.32	6.68	6.74	7.52	6.65	7.87	5.80	5.98	5.66	5.59	5.67	5.77	5.64	5.92	5.86	5.81	5.75	5.99
GLY	4.60	4.65	4.62	4.52	4.45	4.57	4.55	4.64	4.56	4.61	4.62	4.62	4.61	4.72	4.69	4.70	4.65	4.79	4.91
ALA	4.96	4.93	4.82	4.89	4.83	4.87	4.80	4.95	4.76	4.94	4.91	4.95	4.96	4.90	5.09	5.07	4.95	4.98	5.45

WB 1-3 20: diet that contained winter barley at 20%; WB 1-3 40: diet that contained winter barley at 40%; WO 1-3 20: diet that contained spring oats at 20%; WO 1-3 40: diet that contained spring oats at 40%.

4.2.1. Nutrient digestibility of the experimental 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 16).*** The opposite was true for starch digestion. In this case the digestibility of the barley and winter oats diets were significantly lower than that od 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 16. 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)

4.2.2. 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 17.).** 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 17. 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 were the slopes of the linear regression equation, that described the relationship between the daily AA intake and daily ileal absorbed AAs. As examples, ***Figure 7.*** and ***Figure 8.*** show the responses of MET in barley (Mv05-17) and LEU of spring oats (Mv 9-14).

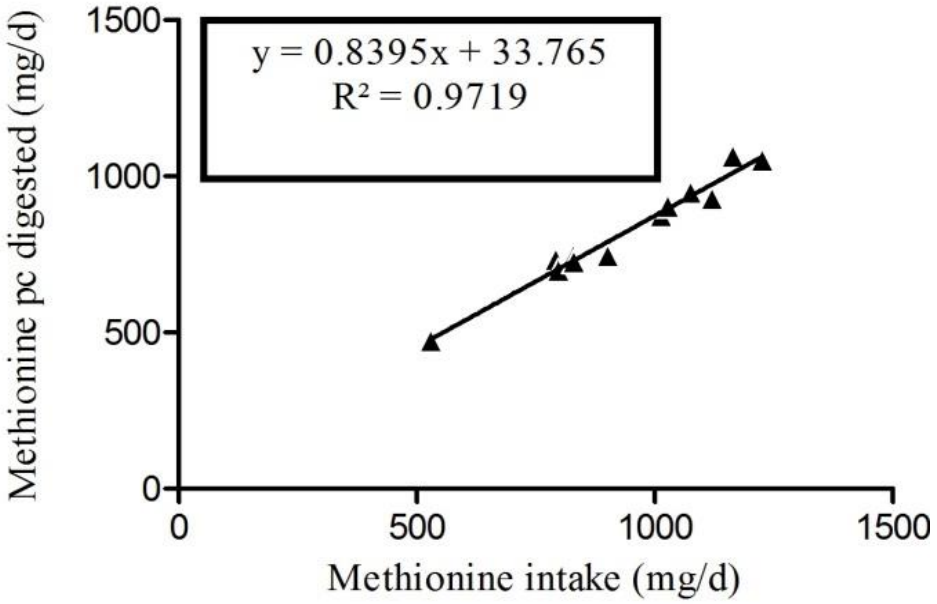

Figure 7. Linear regression between the daily methionine intake and the daily methionine ileal absorption of the Mv05-17 barley

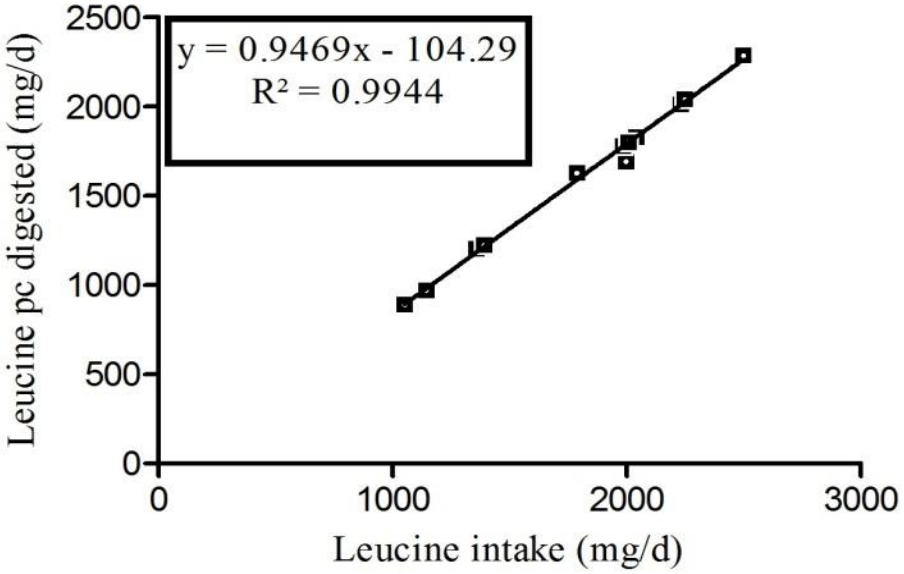

Figure *8*. Linear regression between the daily leucine intake and the daily *leucine* ileal absorption of Mv 9-14 spring oats

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 18. and 19***. 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 18. 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 19. 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 9.)*. 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.

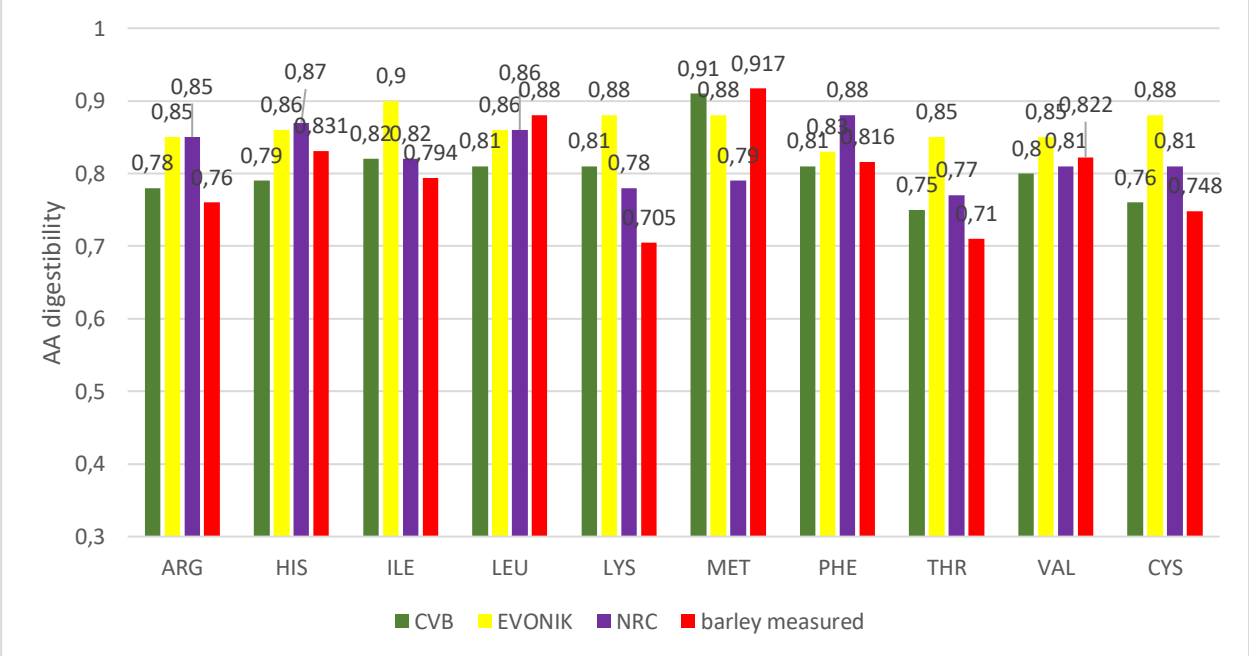

Figure 9. Comparison the measured ileal AA digestibility of barley with the table values (CVB, 2017;) EVONIK, 2017)

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 10.***). 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.

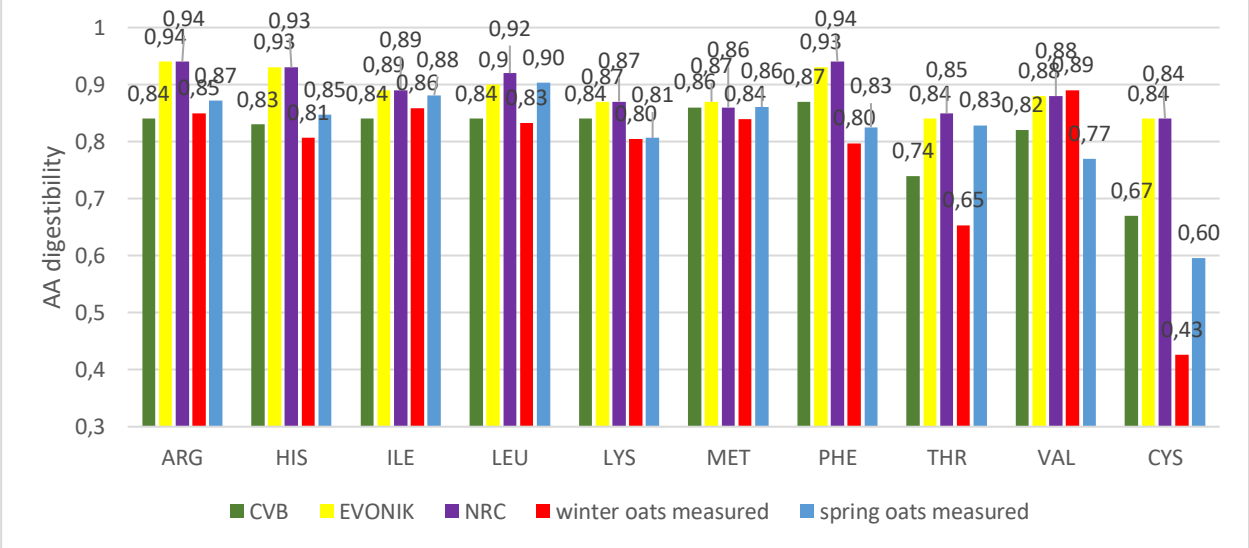

Figure 10. Comparison the measured ileal AA digestibility of oats with the table values (CVB, 2017;) EVONIK, 2017)

4.2.4. 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 20.).*** 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 AND 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 20. 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**

21). 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 21. 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)

4.3. Effect of feeding oats and barley containing diets on the production traits and gut characteristics of broiler chickens

Table 22. shows the chemical composition of the oats and barley varieties, used in the feeding trial. The winter barley contained more starch, energy and less protein, fat and fibre compared with the oats.

Table 22. Chemical composition of barley and oats (%)

	Dry matter	Crude protein	Crude fat	Crude Fibre	Ash	N-free extract	Ca	P	Starch	NDF	ADF	AMEn * (MJ/kg)
Winter barley (Mv 05-17)	91.12	9.57	1.47	3.59	2.15	74.34	0.07	0.35	51.14	18.17	4.41	10.77
Winter oats (MV Pehely)	92.62	10.96	5.3	11.35	2.39	62.62	0.09	0.3	36.99	29.76	14.06	9.92

* AMEn values were calculated according to the equations of the European Table of Energy Values for Poultry Feedstuffs (WPSA, 1989).

The measured nutrient contents of the experimental diets are shown in ***Tables 23.*** The crude protein content of the diets was in the range of the requirements of the chickens. The only inaccuracy was the lower CP content of the WO20 treatment in the starter phase. The diets differed only in their fibre and crude fat contents. The lower energy content of barley and oats were compensated with increased sunflower oil supplementation. In all cases the fibre content of the 20% oats containing diets were the highest. ***Table 24***. contains the measured AA contents of the diets. No big differences were found in the amino acids.

Table 23. The measured nutrient content of experimental diets (g/kg)

		Dry matter	Crude protein	NFE	Crude fat	Starch	Crude fibre	Ash	NDF	ADF	Ca	P	AMEn*
						%							MJ/kg
Starter	C	893	22.87	48.04	8.19	32.94	3.71	6.49	17.08	5.97	0.98	0.71	12.39
	WB20	893	23.99	45.77	9.38	29.23	3.46	6.68	17.91	6.02	1.02	0.74	12.39
	WB40	895	24.24	45.31	9.18	25.85	4.14	6.67	18.84	6.82	1.07	0.75	11.83
	WO10	896	23.05	44.93	11.13	29.29	3.92	6.61	19.45	5.22	1.09	0.71	12.84
	WO20	899	21.88	44.08	12.44	26.73	4.97	6.57	19.93	7.20	1.10	0.72	12.68
Grower	C	896	21.43	51.32	8.46	35.57	2.71	5.71	13.07	4.71	0.86	0.69	12.76
	WB20	899	22.60	48.79	9.29	31.71	3.11	6.06	12.79	4.73	0.95	0.70	12.59
	WB40	898	22.38	47.56	10.06	29.23	3.68	6.15	12.61	5.16	0.95	0.67	12.43
	WO10	899	22.06	47.64	10.12	31.08	3.94	6.17	15.59	5.03	0.98	0.71	12.65
	WO20	902	21.36	46.41	11.77	29.02	4.52	6.12	16.65	5.78	0.97	0.72	12.80
Finisher	C	897	19.91	53.45	8.14	39.41	2.51	5.70	13.91	5.41	0.85	0.70	13.12
	WB20	900	19.53	53.02	8.75	36.80	3.18	5.56	14.43	5.68	0.88	0.61	12.82
	WB40	904	19.96	50.53	10.02	33.86	4.09	5.82	16.16	7.10	0.89	0.60	12.87
	WO10	901	20.23	50.71	9.64	35.60	3.73	5.74	23.66	5.00	0.90	0.66	13.01
	WO20	912	19.63	48.79	11.99	33.18	5.06	5.74	28.99	6.16	0.90	0.66	13.33

* ME values were calculated according to EU regulation 152/20; 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%.

Table 24. The measured amino acids content of the experimental diets (%)

	Starter					Grower					Finisher				
	Control	WB20	WB40	WO10	WO20	Control	WB20	WB40	WO10	WO20	Control	WB20	WB40	WO10	WO20
Cystine	0.35	0.37	0.40	0.36	0.36	0.33	0.36	0.38	0.36	0.37	0.32	0.33	0.35	0.34	0.35
Aspartic acid	2.30	2.34	2.27	2.28	2.17	2.14	2.15	2.06	2.19	2.09	2.02	1.83	1.77	2.03	1.91
Methionine	0.71	0.73	0.71	0.69	0.65	0.61	0.62	0.59	0.62	0.58	0.50	0.46	0.43	0.50	0.46
Threonine	0.90	0.92	0.93	0.89	0.84	0.81	0.85	0.84	0.83	0.83	0.74	0.72	0.72	0.75	0.72
Serine	1.04	1.09	1.07	1.03	1.00	0.99	1.05	1.01	1.03	1.00	0.95	0.87	0.92	0.96	0.91
Glutamic acid	4.19	4.51	4.62	4.32	4.02	3.97	4.33	4.37	4.12	4.01	3.84	3.89	4.12	3.89	3.70
Proline	1.31	1.48	1.54	1.25	1.21	1.24	1.45	1.51	1.22	1.25	1.18	1.34	1.49	1.27	1.29
Glycine	0.89	0.91	0.86	0.85	0.82	0.81	0.87	0.85	0.80	0.82	0.75	0.69	0.78	0.77	0.78
Alanine	1.02	1.08	1.12	1.06	0.97	1.01	1.06	1.02	0.99	0.98	0.93	0.90	0.92	0.95	0.94
Valine	1.08	1.13	1.15	1.08	1.03	1.02	1.08	1.08	1.06	1.03	0.89	0.89	0.92	0.91	0.89
Isoleucine	0.92	0.94	0.95	0.93	0.89	0.82	0.86	0.85	0.85	0.82	0.70	0.68	0.68	0.71	0.69
Leucine	1.84	1.94	1.96	1.89	1.79	1.81	1.89	1.82	1.88	1.75	1.69	1.75	1.62	1.70	1.66
Tyrosine	0.78	0.86	0.87	0.83	0.79	0.77	0.81	0.78	0.81	0.72	0.72	0.70	0.70	0.71	0.72
Phenylalanine	1.12	1.11	1.22	1.13	1.09	1.04	1.12	1.08	1.10	0.99	0.98	0.96	0.99	0.97	0.97
Histidine	0.60	0.56	0.64	0.61	0.57	0.52	0.60	0.58	0.60	0.50	0.55	0.50	0.54	0.52	0.52
Lysine	1.51	1.52	1.48	1.48	1.41	1.22	1.24	1.27	1.28	1.24	1.07	1.01	0.97	1.08	1.03
Arginine	1.58	1.62	1.61	1.61	1.52	1.48	1.50	1.44	1.51	1.55	1.38	1.29	1.28	1.39	1.34

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%.

4.3.1. 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 25.)***. 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)

4.3.2. 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 26)***. The measured ileal viscosity of this trial was in the range of the published values (SHAKOURI et al., 2009; KONIECZKA AND 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 25. 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 26. 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 27.)*.** 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 27. 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 28.)***. 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 28. 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 ($p < 0.05$).

4.3.3. Microbiota composition of the different gut segments

4.3.3.1. 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 29.)***. 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 29. Alpha diversity indices of the intestinal microbiota of broiler chickens

		Chao1	**Shannon**	**Simpson**
		Mean ± SD	Mean ± SD	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.

Beta diversity based on principal coordinate analysis (PCoA) ordination using Bray–Curtis dissimilarity matrix showed significant differences (PERMANOVA global R = 0.69. p = 0.001) among sampling places. High overlap exists for the bacterial structure of JC and JM, but the

species similarity of CC is different ***(Figure 11.)***. The dietary treatment effects on the bacterial community structure were also significant. In the JC the control and oats treatments showed almost 100% similarity. However, feeding the barley—based diets resulted different species too (1B; R = 0.53, p = 0.02). Interestingly, in the jejunal mucosa both cereal treatments modified the species composition of the bacterial community (1C, R = 0.46, p = 0.047). The biggest dissimilarity of the bacterial species was found in the caeca. In this case, beside the overlaps, all the three treatments resulted different beta diversity (1D, R = 0.59, p = 0.001).

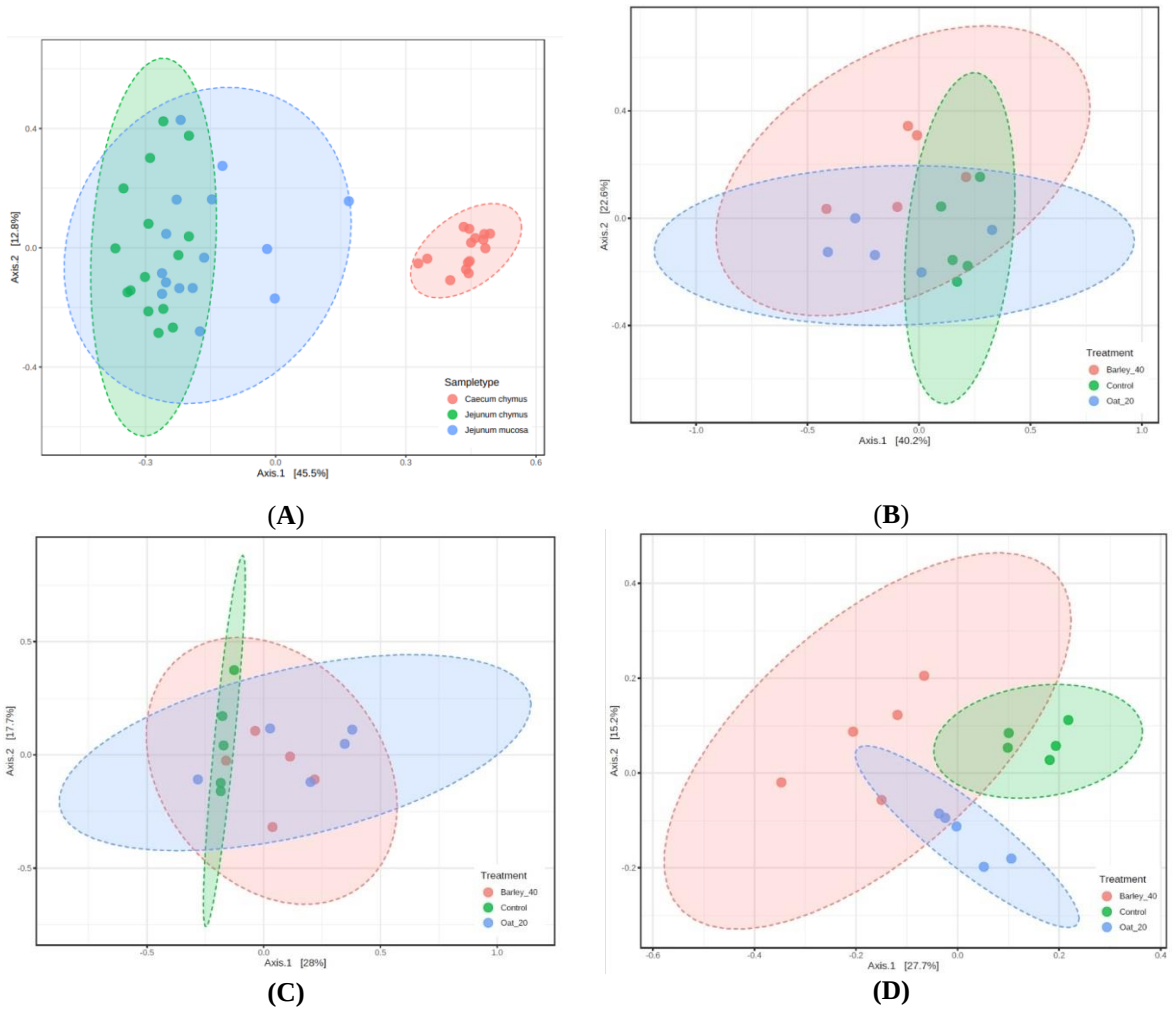

Figure 11. Principal coordinate analysis (PCoA) based on Bray—Curtis dissimilarity matrix of the three sampling places (1A) and dietary effects in the jejunal **chymus (1B),** jejunal **mucosa (1C) and caecal content (1D)**

Beta diversity measures the similarity or dissimilarity of two communities, in our case between the sampling places and between the dietary treatments of a sampling place. As expected, the most significant differences in this index were found between the sampling places. It is not new

since the environment for the bacteria are different in the different gut segments. The jejunum is not a fully anaerobic environment, which is not true for the mucosa and caeca. The nutrient availability and pH are also different in these gut segments. No big differences between the beta diversity indices in the jejunal content was found. In the jejunum the change in the substrates probably cannot cause big differences because of the quick transit time and low bacterial content. However, in the mucosa clear difference can be found between the control group and the groups of barley or oats. It means, the soluble fibre fractions can modify the composition or the thickness of the mucus and this way the translocation of some bacteria from the gut lumen into the mucosal surface. Intestinal mucus, synthetized by the goblet cells, is an important barrier in the gut which acts as a physical fence, participate in bacterial clearance and display antimicrobial activity (ALEMKA et al. 2012). Only few studies have been done on the impact of dietary β-glucan, with and without endo-β-glucanase on the gut morphology and microbiota number and diversity. According to these studies butyrate is the most efficient SCFA believed to have the largest effect on the intestine fibre (PIEPER et al., 2008; JHA et al., 2010; JÓZEFIAK et al., 2010;GORHAM et al., 2017).

4.3.3.2. 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 30).*** 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 30. 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%.

At the genus level in JC *Lactobacillus* was the dominant group with 80.31%, 65.81% and 88.38% abundance in treatments C, WB40 and WO20 respectively. In spite the big decrease in treatment WB40, the differences were not significant. On the other hand, the change of some genera with low abundance was significant. For example, *Curtobacterium* increased in treatment WO20, while *Bifidobacterium* (p = 0.019), *Ruminococcus torques group* (p = 0.032), *Erysipelatoclostridium* (p = 0.019), *Dietzia* (p = 0.032) and *Christensenellaceae R-7* (p = 0.031) in the barley fed birds ***(Figure 12).***

In JM, similarly to the jejunal content, *Lactobacillus* was also the dominant group with 57.7%, 48.36% and 66.86% abundance in treatments C, WB40 and WO20 respectively. The next dominant genus was *Bacteroides*, only with 0.8%, 2.35% and 0.33%. Of the genera with a relative abundance above 1%, only *Pseudomonas* (p = 0.046) showed significant differences between C and WB40 treatments group (0.55% vs. 2.08%; ***Figure 13***.). In JM oats resulted significant increase in the case of some minor genera (*Curtobacterium*, p = 0.031; *Cutibacterium* p = 0.039 and *Methylobacterium*, p = 0.044).

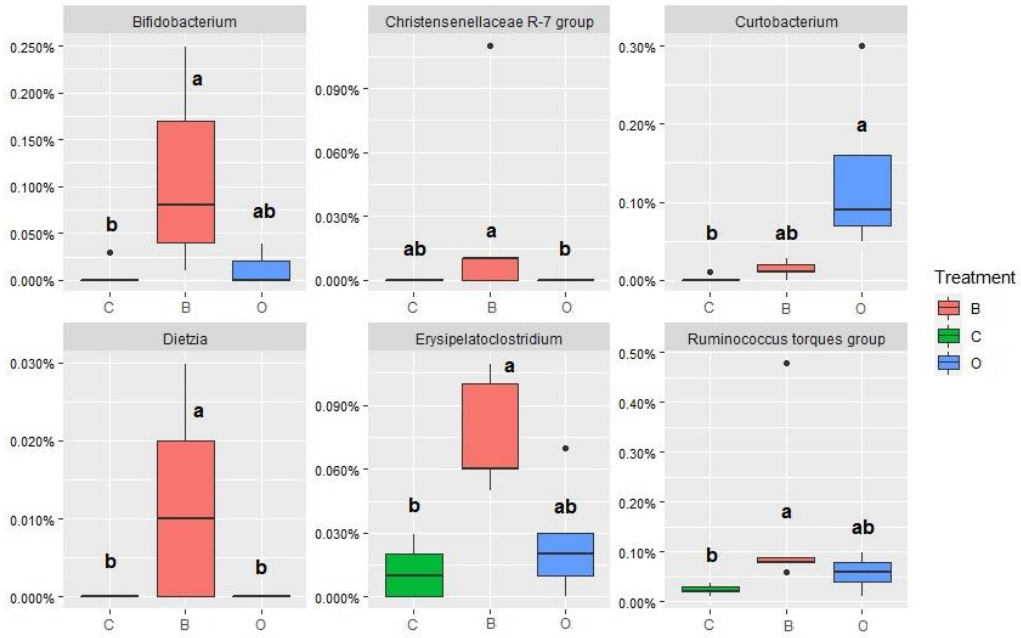

Figure 12. Boxplots showing significant changes in the genera of the jejunum contents

a. b: values marked with different lowercase letters were significantly different (p < 0.05). Dots represent the outlier values.

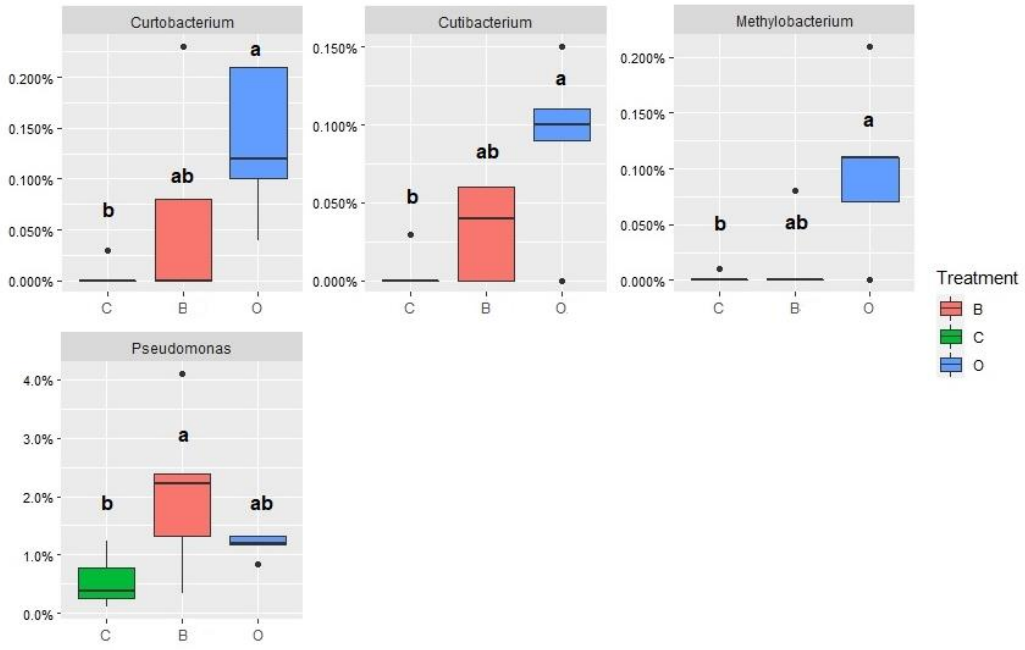

Figure 13. Boxplots showing significant changes in the genera of the jejunum mucosa

a. b: values marked with different lowercase letters were significantly different (p < 0.05). Dots represent the outlier values.

At the genus level in CC the high abundance of *Lactobacillus* decreased to 10.48%, 11.05% and 8.89%, while that of *Bacteroides* increased to 6.61, 4.90 and 6.22 in the treatments C, WB40 and WO20 respectively (***Table 31.***). These dominant genera showed no significant changes due to the diet composition. The most determinant significant treatment effects are shown in Table 33. It can be seen that WO20 decreased the abundance of *Streptococcus* (p = 0.009) and GCA—900066225 (family *Ruminococcaceae*) and increased those of *Christensenellaceae R—7 group* (p = 0.026); some genera from family *Lachnospiraceae* (*Sellimonas* (p = 0.032), *Marvinbryantia* (p = 0.047) and some genera from the family of *Ruminococcaceae* (*Ruminococcaceae UCG—004. —005. —008. —014. —NK4A214 gr., Ruminococcus 2, Anaerotruncus, Anaerofilum*) significantly compared to treatment C. Treatment WB40 resulted significantly higher *Bifidobacterium* (p = 0.005) and *Anaerostipes* (p = 0.01) ratios.

Table 31. Relative abundance of bacterial genera in caecum chymus of broiler chickens as affected by dietary treatments (%)

Phylum Class	Family	Genus	C	WB40	WO20	Pooled SEM	p value
Actinobacteria **Actinobacteria**	Bifidobacteriaceae	Bifidobacterium	0.21 ^{ab}	6.63 ^{a}	0.09 ^{b}	0.950	**0.005**
Bacteroidetes	Bacteroidaceae	Bacteroides	6.61	4.90	6.22	1.093	0.677
Bacteroidia	Rikenellaceae	Alistipes	2.67	3.30	5.10	0.992	0.326
Firmicutes	Lactobacillaceae	Lactobacillus	10.48	11.05	8.89	1.377	0.533
Bacilli	Streptococcaceae	Streptococcus	4.74 ^{a}	2.54 ^{ab}	0.67 ^{b}	0.695	**0.009**
Firmicutes	Christensenellaceae	Christensenellaceae R-7 gr.	1.68 ^{ab}	1.16 ^{b}	2.31 ^{a}	0.216	**0.026**
Clostridia	Peptostreptococcaceae	Romboutsia	4.96	4.10	3.45	0.921	0.275
	Lachnospiraceae	CHKCI001	4.31	1.08	2.97	1.108	0.164
		Ruminococcus torques gr.	2.19	3.82	2.26	0.721	0.228
		Sellimonas	1.10 ^{b}	1.41 ^{ab}	1.90 ^{a}	0.164	**0.032**
		Eubacterium hallii gr.	0.56	1.05	0.98	0.145	0.080
		Anaerostipes	0.22 ^{b}	1.30 ^{a}	0.48 ^{b}	0.215	**0.010**
		Eubacterium ventriosum gr.	0.02 ^{a}	0.00 ^{b}	0.00 ^{b}	0.004	**0.031**
		Marvinbryantia	0.09 ^{ab}	0.07 ^{b}	0.19 ^{a}	0.026	**0.047**
		Blautia	2.91	1.42	2.52	0.486	0.059
		Lachnoclostridium	0.37	0.70	0.50	0.082	0.071
		Lachnoclostridium 5	0.03	0.06	0.01	0.020	0.097
	Ruminococcaceae	Faecalibacterium	7.81	9.34	12.36	1.393	0.125
		Ruminococcaceae UCG-004	0.16 ^{b}	0.19 ^{ab}	0.28 ^{a}	0.024	**0.035**
		Ruminococcaceae UCG-005	4.55 ^{a}	1.15 ^{b}	3.77 ^{a}	0.663	**0.009**
		Ruminococcaceae UCG-008	1.00 ^{ab}	0.00 ^{b}	1.48 ^{a}	0.353	**0.008**
		Ruminococcaceae UCG-014	3.70 ^{ab}	1.87 ^{b}	4.39 ^{a}	0.525	**0.034**
		Ruminococcaceae NK4A214 gr.	0.32 ^{b}	0.23 ^{b}	0.75 ^{a}	0.064	**0.006**
		Subdoligranulum	2.76	3.93	2.62	0.520	0.196
		Butyricicoccus	2.00	1.82	1.48	0.454	0.887
		Ruminococcus 2	0.58 ^{a}	0.02 ^{b}	0.50 ^{a}	0.109	**0.008**
		Ruminiclostridium 5	1.09	1.73	1.05	0.231	0.176
		Eubacterium coprostanoligenes gr.	0.78	0.69	1.01	0.147	0.405
		Anaerotruncus	0.03 ^{ab}	0.004 ^{b}	0.06 ^{a}	0.009	**0.016**
		Anaerofilum	0.08 ^{ab}	0.02 ^{b}	0.09 ^{a}	0.023	**0.025**
		GCA-900066225	0.14 ^{a}	0.065 ^{ab}	0.058 ^{b}	0.023	**0.038**
	Family XIII	Family XIII UCG-001	0.002	0.02	0.00	0.006	0.088
	Clostridiales vadin-BB60 group	uncultured Clostridia bacterium	0.04	0.20	0.14	0.062	0.080
Firmicutes	Erysipelotrichaceae	Turicibacter	3.00	1.69	1.10	0.527	0.112
Erysipelotricha		Erysipelatoclostridium	2.57 ^{b}	7.35 ^{a}	3.94 ^{ab}	0.798	**0.016**
Not Assigned			18.67	17.22	17.84	0.900	0.357
Other genera			7.56	7.86	8.56	1.758	
			100.00	100.00	100.00		

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

Evaluating the jejunal microbiota results, viscous nature and an increase in the retention time of digesta reduces the oxygen pressure in the small intestine, which in turn stimulates the growth conditions for anaerobic microbiota (FEIGHNER AND DASHKEVICZ 1988; Choct 2006). Normally, the small intestine is colonized by a relatively higher proportion of facultative anaerobic microbiota than strict anaerobic microbiota (SALANITRO et al. 1978; LU et al. 2003). The growth of these anaerobic microbiota is affected by the viscosity, which decreases

the oxygen pressure and increases the abundance of anaerobic bacteria. It means not only change in the composition but also higher bacterial count and more competition for nutrients between host and microbiota. Furthermore, favouring the growth of anaerobic microbiota over facultative anaerobic microbiota in the small intestine increases the bile salt hydrolase activity in the digesta, which in turn markedly reduces the digestion of fats (FEIGHNER AND DASHKEVICZ 1988; Choct 2006). Most of the gut microbiota evaluation is published on caecum and in some cases on the ileum. There are no barley or cereal β-glucan published results from the jejunum. This gut segment was used in our case because the viscosity and all the impairments of digestion happening in the jejunum. The disadvantage of using jejunal contents is the high standard deviation of the parameters.

The mucosa is mostly anaerobic, and it is the main environmental factor of the changes. FARKAS et al. (2022) found similar bacterial composition in the mucosa and in the caecum in broiler chickens. In their trial the phylum *Bacteroidetes* increased till 30%. In this trial the increase of *Bacteroidetes* was smaller. The abundance of these determinant phyla was also dietary treatment dependent. Barley significantly decreased the abundance of *Firmicutes* but increased *Proteobacteria* significantly, and *Bacteroidetes* in tendency.

Similarly to JC, in the JM barley also decreased the relative abundance of *Lactobacillus* and *Turicibacter* and increased the ratio of several other genera (e. g. *Corynebacterium-1, Bacteroides, Alistipes, Nosocomiicoccus, Weissella, Romboutsia* and *Pseudomonas*). Like to the jejunal content, oats increased *Lactobacillus*, *Corynebacterium-1* and decreased *Romboutsia* abundances. Of the genera with a relative abundance above 1%, only *Pseudomonas* showed significantly difference between treatment C and WB40. From the results it can be concluded that there are similarities between the bacteriota composition of the jejunal gut content and the jejunal mucosa, but diet composition can slightly modulate it. The mechanism, how and which bacteria can translocate form the gut to the surface of the epithelial cells is not fully understood yet. Changes in gut viscosity can be a factor that modulate the mucosal wall of the small intestine (RAKOWSKA 1993). It has been shown that the intestinal villi of the duodenum can be strongly damaged when high amounts of viscous polysaccharides are fed to broilers (RAKOWSKA 1993; SMULIKOWSKA 1998). Moreover, the interactions of the soluble wheat arabinoxylans with the glycocalyx layer of the intestinal brush border can thicken the unstirred water layer of the mucosa. In this way, the absorption of nutrients along the small intestine is decreased (JOHNSON AND GEE, 1981; Choct et al., 1996; CLASSEN, 1996; MENG et al., 2004).In the caeca of birds usually decreased *Firmicutes* and increased *Bacteroidetes* abundance is measured. The ratio of the two phyla, (*Bacteroidetes*: *Firmicutes*)

is also correlates with the growth rate and fat deposition of humans and animals (DUNCAN et al., 2008; SALAHEEN et al., 2017; WANG et al., 2017). In this trial *Bacteroidetes* increased to 8.2-11.3 % and *Firmicutes* decreased to 84.2-89.6%. Only slight dietary effects have been found in this trial in the caecum. Both *Tenericutes* and *Actinobacteria* increased significantly in the barley treatment. The increase of *Tenericutes* could be positive in the caeca, since it was positively correlated with the final body weight of chicken (FARKAS et al. 2022). On genus level the increase of *Bacteroides* and the decrease of *Lactobacillus* was the most important change of treatment WB40. Besides that, significant differences were found only in the abundances of genera with low incidence, below 1%. Feeding the oats containing diets decreased *Streptococcus* and *GCA-900066225* (family *Ruminococcaceae*) abundances significantly compared to the control treatment.

CHOCT AND ANNISON, (1992) described that xylanase supplementation of broiler diets modify the composition of caecal microbiota in chickens. Members of the family *Lachnospiraceae* and *Ruminococcaceae* become more abundant and are efficient butyrate producers. β-glucan can reduce the colonization of *Salmonella* in the intestinal tract and promote the number of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*. It has also significant immune stimulatory effects against parasitic and viral diseases (SHAO et al. 2016). However, from this results it can be concluded, that β-glucans and their shorter chain oligosaccharides have only limited effect on butyrate production.

5. 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.

6. 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.

7. SUMMARY

Corn is very sensitive for the climatic changes, for heat stress and drought. Therefore, the use of alternative, abiotic stress resistant secondary cereals will increase in the near future. Barley and oats could be potential alternatives of corn and wheat. However, both of these cereals contain hulls and also soluble fibre. Structural fibre in poultry diets can be beneficial because its gizzard stimulatory effect. On the other hand, high dietary structural fibre decrease digestion mostly in young birds. The dominant soluble fibre fraction in barley and oats are β-glucans. It is generally known that the soluble arabinoxylan and β-glucan increase digesta viscosity, decreasing the access of enzymes to the substrates, so impair nutrient digestion and absorption and also, the growth and feed conversion of animals. Exogenous glucanase enzymes are used to split beta glucans and produce shorter chain carbohydrates with lower water holding capacity. Glucanase can therefore decrease digesta viscosity and overcome all the previously mentioned negative effects. NSP degrading enzymes can also provide fermentable substrates for beneficial bacteria, resulting increased SCFA production and less pathogenic bacteria. There are many research data available how arabinoxylans (AX) and xylan-oligosaccharides (XOS) of wheat can modify the gut characteristics and the microbiota composition in the intestine of chickens. However, the information on beta-glucans and their degraded products is very limited. Therefore, in this study barley- and oats-based diets, supplemented with exogenous glucanase, were fed to get more understanding on their effects on some relevant gut parameters and the intestinal microbiota of broiler chickens.

In comparison the composition of oats and barley we can conclude that the crude protein, crude fat, the insoluble dietary fibre (IDF) and the precipitable soluble dietary fibre (SDFP) have the highest variance. In the case of winter oats (WO) the crude protein content of grains was positively correlated with the starch content. The opposite was found for spring oats (SO). The CP of WO change in opposite trend with the fibre content. Interestingly no significant correlations were found between the nutrients of winter barley (WB). The measured CP contents were higher, while the starch content lower than those of the table values. The amino acid (AA) composition of the barley and oats protein is not constant. In WB the LYS and LEU and in WO the CYS and LYS contents of the protein decreased with the increase of CP. Both type of oats contains more CYS and ARG than barley or the other poultry feedstuffs. Compared with the requirements with the broiler chicken’s oats protein has the highest essential amino acid index.

In the frame of a digestibility trial, feeding barley and oats at 20 or 40% did not affect the digestibility of starch, but improved the absorption rate of fat and protein. The reason behind this positive effect could be the more proper gizzard function of the barley and oats containing diets. The AA digestibility of barley and oats varieties have also been determined with linear regression between the daily ingested AA and daily pre-caecally absorbed AA. The digestibility coefficients measured were lower than the table values (NRC, EVONIK, CVB). In the frame of the digestibility trial the effect of exogenous β-glucanase was also determined. Crude fat and crude protein digestibility was improved by the enzyme, but no effect was detected for starch digestion. Among essential amino acids the digestibility of ARG, ILE, LEUTHR, CYS and TYR was improved if the diets contained β-glucanase. No changes in the other AAs were found.
In a feeding trial, oats at 10 and 20% and barley at 20 and 40% was fed with broiler chickens between day 1 and day 39. The feed intake of chickens was affected only in the finisher phase, when barley and oats decreased the feed consumption. Surprisingly, the weight gain of the young animals was not affected by the treatments in the starter phase. The higher inclusion rates of oats (20%) and barley (40%) decreased the weight gain in the grower phase. Barley failed to modify the weight gain in the finisher phase, but oats at both inclusion rate significantly increased the weight gain of chickens. This positive effect was also true for the cumulative weight gain and feed conversion ratio (FCR). According to our knowledge no such positive effects of oats feeding has been published yet.
The gut investigation after the end of the feeding trial proved, that feeding oats at both inclusion rates significantly increases the gizzard weight. Feeding barley at 20 and 40% significantly increased the viscosity of the ileal gut content. Feeding barley at 40% significantly decreased the acetate and propionate contents and also the bacterial diversity of the caeca. Compared with the control animals, barley resulted significant changes in the microbiota composition of the jejunum mucosa. The abundance of *Firmicutes* decreased while those of *Proteobacteria* and *Tenericutes* increased in the barley treatment.
From our result it can be concluded, that both barley and oats can be used in broiler chicken nutrition efficiently, even at higher inclusion rates. Oats had unexpected positive effects on the production traits, while barley modified several gut parameters. The measured chemical composition data were in several aspects different form the table values (NRC, EVONIK, CVB). So, it is important to use the real nutrient composition and their digestibility if barley and oats are used in diets formulations.

8. APPENDIX

A1: Bibliography

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