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Comparison of starch digestibility methods for extruded wheat grains (*Triticum aestivum* L.)

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Abstract: This study compared different methods of determining starch digestibility (*in vivo* vs *in vitro*) in wheat grains and evaluated the influence of extrusion on digestibility. *In vivo* starch digestibility was determined in broiler chickens by calculating the residual starch content in their ilea and the digestibility using a chromium oxide indicator. *In vitro* digestibility was examined using pepsin and pancreatin. During *in vivo* testing, the highest digestibility coefficient (DC) was achieved by the Bonanza variety in its extruded form (91.19 ± 0.40%). In contrast, the lowest DC was achieved by the Tobak variety in its non-extruded form (81.45 ± 1.92%). Generally, a higher DC was observed *in vivo* for extruded forms of wheat. During *in vitro* testing, the highest DC was achieved by the Stefii variety in its non-extruded form (96.10 ± 0.55%), whereas the lowest DC was observed in the Yetti variety in its extruded form (49.72 ± 0.41%). Overall, the *in vitro* experiments did not exhibit significant differences between extruded and non-extruded forms of wheat. Linear regression analysis showed a strong relationship (r^2 = 0.860; 85.98%) between *in vivo*- and *in vitro*-derived DC values in all wheat varieties, both in extruded and non-extruded forms. The study showed that *in vivo* testing is a suitable method for the determination and control of starch levels in extruded materials. However, despite of the accuracy of this technique, it is also very demanding in terms of time, space, equipment, and methodological knowledge. Therefore, based on the strong correlation between the *in vivo* and *in vitro* assays, we recommend *in vitro* digestibility testing as a preferable alternative.

Keywords: chicken broilers; chymus; nutrient digestibility; feed nutrients

Wheat is grown on 250.6 million hectares (FAO 2019) which is more land than any other food crop. World production of wheat in 2019 was 766 million tonnes. As for animal feed use of cereals, similar to 2020/21, a notable feature is anticipated continued growth in the use of wheat for feed, which is seen to rise to 156 million tonnes (FAO 2020). In many countries, wheat varieties are officially reg-

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istered and characterised by agronomic, chemical, physical, and technological parameters. The genetic stability of these properties is often relatively high (Turnbull and Rahman 2002). However, in most cases, the nutritional potential of each variety remains unknown, as most of the technological parameters have been defined only for human use and are not related to poultry feeding (Svihus et al. 2000). For example, in the Czech Republic, the majority of total wheat production (4 896 800 tons in 2019) is used for animal feed, although it is primarily cultivated for use in the bakery industry (SZIF 2019).

Until the 1980s, wheat was considered a common feed ingredient for broiler chickens (Kim et al. 2003). Wheat typically represents a substantial part (55–65%) of the feed mixture; therefore, it is very important to know the exact characteristics and efficiency of its use (Carre et al. 2002). In animal feeds, wheat is the main source of energy; thus, regardless of the wheat variety, utilisation, or growing system, carbohydrates are the predominant nutrient group of wheat grain. Carbohydrate contents vary from 65–85%, and starch contents fluctuate from 60–75% depending on the variety and external factors, such as climate or soil type (Carre et al. 2002).

Extrusion is a common high-temperature short-time mechanical processing method for wheat grains that involves high pressures and temperatures. The aim of such processing is to reduce the content of antinutritional substances and increase the digestibility of nutrients (Scudamore et al. 2008). Starch is the carbohydrate most affected by the extrusion process, as it is broken down into simple sugars to become more accessible to enzymes and transformed into a smear when exposed to high temperatures (Santala et al. 2014). In wheat starch, the optimum temperature for gelation is 120 °C, with a humidity of approximately 20%. Moreover, starch acts as a natural feed binder (Imam et al. 2013). However, the extrusion method also has some risks and disadvantages, such as the Maillard reaction, which leads to poor utilisation of nitrogenous substances (amino acids) and the loss of important substances in the feedstock (e.g., vitamins) at higher temperatures (Santala et al. 2014). The wet extrusion method may also require the material to be dried first because of the risk of undesirable pathogens appearing in the feed, which may affect the health of animals (Xiaoguang et al. 2015).

However, many studies have reported the problematic digestion of fodder wheat types, especially in chicken broilers. The first cases were noted as early as the 1980s and lasted for the next 25 years, during which time the amount of observed digestive disorders increased (Peron et al. 2005). However, subsequent changes related to the selection of more suitable wheat varieties, heat treatment of the feed, and genetic modification have led to an increase in the efficiency of wheat utilisation and reduced the occurrence of digestive problems.

Consequently, for the evaluation of chicken broiler feed, it is important to focus not only on wheat cultivation but also on the selection of suitable wheat varieties, the correct processing technology of the chickens, and their breeding strategy (Carre et al. 2005). Therefore, this study compares the starch digestibility of selected wheat varieties before and after extrusion treatment, as well as the performance of *in vitro* and *in vivo* methods for determining starch digestibility (Wiseman et al. 2000; Akerlind et al. 2011).

MATERIAL AND METHODS

Wheat sample preparation

The experiment involved the following wheat varieties; Bonanza, Gordian, Tobak, Vanessa, Steffi, and Yetti, in which the digestibility of starch was evaluated in both the extruded (E) and non-extruded (N) forms. Additionally, Grizzly and Reform varieties were selected, including samples with and without extrusion, which were used in three different types of processes under defined conditions. The extrusion temperatures in the final chamber ranged from 110 °C to 130 °C with dosing of procedural water from 5% to 8%, depending on the extrusion variant. Before the analyses, each of the tested wheat lines was shredded on a VM3 vertical hammer mill (Taurus s.r.o., Chrudim, Czech Republic) with a 3-mm mesh. After shredding, 1% chromium oxide was added as an external indicator of digestibility. After mixing, the blend was granulated on a JGE 200 granulator (GreenEnergy, Uherský Brod, Czech Republic) with a 2-mm matrix.

Chicken broilers

Six cockerels from ROSS 308 chicken broilers were included in the balanced experiment. At the

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beginning of the experiment, the broilers were 35 days old, and their average live weight was approximately 2.4 ± 0.2 kg. Prior to the experiment, the broilers were housed on loose bedding and fed *ad libitum* with a full-fledged complete feed mixture of type BR1 (first phase of fattening within 10 days of age) and BR2 (second phase of fattening from 10 days to 35 days of chickens age). In the experiment, the cockerels were divided into 19 groups, according to the fed wheat variety and its form, and in pairs housed in balance cages.

Each of the tested feed mixtures and water was administered to two experimental groups for seven days (*ad libitum*), with three days as the preparation period. At the end of the feeding tests, the broilers were slaughtered and the ileal content (chyme) was removed. A mixed sample of ileal content from the two broilers was created from each balance cage. Samples were frozen at –80 °C for 24 h and then lyophilised using a ScanVac CoolSafe lyophiliser (Labogene, Lynge, Denmark). After lyophilisation, the samples were ground in a Cyclotec Sample Mill laboratory grinder (Foss, Hilleroed, Denmark).

Chemical composition

All samples were determined using the methods of the European Commission Regulation (EC) No. 152/2009. The dry matter (DM) content was determined by drying for 5 h at 103 °C. The nitrogen content was evaluated using the Kjeldahl method [International Organization for Standardization (ISO) 5983-1:2005 Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content – Part 1: Kjeldahl method] with the Kjeltec 2400 sanalyser unit (Foss, Hilleroed, Denmark), and the crude protein (CP) level was subsequently calculated using a nitrogen-to-protein conversion factor of 6.25.

Determination of starch *in vitro*

Starch was determined enzymatically using the K-TSTA-100A analytical kit (Megazyme, Bray, Ireland) according to the manufacturer's instructions. To determine the digestibility of starch, two 0.1-g portions of each sample were used, and a solution of 0.075 M hydrochloric acid and 1 140 IU of pepsin was added. All samples were then mixed and incubated for 2 h at 40 °C. After 2 h of incubation, a 0.1 M sodium hydroxide solution was added with an additional 2.67 mg/ml pancreatin to adjust the pH to 6.9. M tris(hydroxymethyl)aminomethane buffer (2 ml) was pipetted into the samples, followed by further incubation for 2 h at 40 °C. After the second incubation, the samples were centrifuged at 3 000 × *g* for 20 minutes. In each sample, 0.1 ml was added to the resulting supernatant, which was further incubated in a water bath for 30 min at 50 °C. After incubation, the volume of the tubes was adjusted to 11 ml by mixing with distilled water, and the samples were then centrifuged at 3 000 × *g* for 10 minutes. From the resulting supernatant, 1 ml was taken and diluted with distilled water to a total volume of 10 ml. 0.1 ml was taken from this aliquot into a new test tube with 2 ml of glucose oxidase/peroxidase and incubated at 50 °C for 20 minutes. The absorbance was measured at 510 nm using an Infinite M200 reader (Tecan, Grödig, Austria).

Statistical analysis

The data obtained from *in vivo* and *in vitro* experiments were evaluated using STATISTICA v12 software (StatSoft CR s.r.o., Prague, Czech Republic). Detailed analysis of variance was determined via ANOVA using the Tukey HSD test, followed by linear regression analysis to show the relationship between the *in vivo* and *in vitro* digestibility coefficients for all wheat varieties in both extruded and non-extruded forms.

RESULTS AND DISCUSSION

A total of eight wheat varieties were tested in 19 treatment variants (11 extruded and eight non-extruded). The DM of the tested materials averaged 91.45 ± 0.22%. The lowest DM was in Bonanza E (89.45 ± 0.07%), the highest value of DM was in the Reform 3 variety (93.08 ± 0.09%), i.e. after extrusion. The CP content averaged 12.74 ± 1.24%. The lowest CP value was in the Yetti N variety (10.66 ± 0.05%). On the other hand, the highest value of CP was in Grizzly G2 (14.58 ± 0.04%), an extruded form of the variety. When comparing extruded and non-extruded variants, the same tendencies in favour of extruded or non-extruded variants were not confirmed. The average value of starch content (SC)

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in the study group was 60.87 ± 4.31%. The minimum starch content was in the Yetti 2 variety (53.15 ± 0.15%), i.e. after extrusion treatment. The highest starch content was found in the sample Steffi N (72.44 ± 6.16%) (Table 1). Wheat determined via the *in vivo* method exhibited an average digestibility of 88.07 ± 1.61%. The average digestibility of extruded samples in the *in vivo* experiments was 89.61 ± 0.85%, whereas the digestibility in the second group, which was not modified by extrusion, was reduced by 3.67% (85.94 ± 1.78%). In the *in vitro* testing, the average digestibility was 75.80 ± 7.19%. Extruded samples displayed average digestibility of 70.50 ± 7.19%, whereas non-extruded wheat exhibited 13% higher average digestibility (83.08 ± 5.41%). Analysis of the correlation between *in vivo* and *in vitro* methods revealed that the coefficient of determination (r^2) was 0.860 for digestibility regardless of the treatment, i.e., 0.875 for untreated wheat and 0.717 for extruded wheat. This proves a strong relationship between *in vivo* and *in vitro* testing methods, both for extruded and non-extruded forms of wheat.

The prediction for lyophilised chyme was based on an r^2 value of 0.863. In the wheat feed, the prediction of the digestibility coefficient was based on an r^2 value of 0.693. These data suggest that the digestibility of starch in common wheat can be predicted using *in vitro* methods. The values of the total starch digestibility determined by the *in vivo* method, regardless of the wheat treatment, ranged from 81.45 ± 1.92% to 91.19 ± 0.40% (Table 1). The Tobak wheat variety exhibited the lowest digestibility, specifically the Tobak whole non-extruded variety, which scored 81.45%. The highest digestibility was determined in the Bonanza variety, particularly Bonanza E, reaching 91.19%. In all wheat varieties treated under *in vitro* conditions, the digestibility coefficient ranged from 49.72 ± 0.41% to 96.10 ± 0.55% (Table 1). The lowest digestibility value was determined in the Yetti variety; specifically, the Yetti 2 ex-

Table 1. Chemical composition

Wheat variety	Treatment	Sample*	DM (%)	CP (%)	SC (%)	DC starch *in vivo* (%)	DC starch *in vitro* (%)
Bonanza	N	Bonanza whole	91.62 ± 0.06	13.48 ± 0.07	58.12 ± 2.20	83.60 ± 0.73	79.65 ± 0.74
Bonanza	E	Bonanza E	89.45 ± 0.07	13.41 ± 0.01	60.14 ± 1.48	91.19 ± 0.40	94.22 ± 0.51
Gordian	N	Gordian whole	90.44 ± 0.06	13.83 ± 0.02	60.62 ± 0.42	82.22 ± 0.97	90.94 ± 2.53
Gordian	E	Gordian E	90.64 ± 0.05	13.69 ± 0.03	62.06 ± 2.65	88.36 ± 1.79	90.48 ± 1.26
Grizzly	E	Grizzly 1	92.95 ± 0.01	14.43 ± 0.12	61.84 ± 4.70	90.31 ± 0.27	87.09 ± 0.73
Grizzly	E	Grizzly 2	92.57 ± 0.06	14.58 ± 0.04	55.09 ± 1.29	90.55 ± 0.32	71.72 ± 1.64
Grizzly	E	Grizzly 3	91.98 ± 0.00	14.43 ± 0.07	56.83 ± 1.40	85.96 ± 0.80	61.14 ± 2.32
Grizzly	N	Grizzly whole	91.14 ± 0.11	14.28 ± 0.02	59.90 ± 2.48	90.21 ± 0.65	86.01 ± 0.78
Reform	E	Reform 1	92.18 ± 0.00	11.49 ± 0.06	65.47 ± 0.86	90.82 ± 0.51	65.62 ± 0.06
Reform	E	Reform 2	92.77 ± 0.18	11.56 ± 0.01	61.90 ± 2.93	89.66 ± 0.23	65.14 ± 1.28
Reform	E	Reform 3	93.08 ± 0.09	11.56 ± 0.05	66.25 ± 4.25	88.56 ± 0.55	64.63 ± 1.46
Reform	N	Reform whole	91.31 ± 0.03	11.24 ± 0.05	64.23 ± 0.45	90.02 ± 0.52	61.75 ± 1.60
Steffi	N	Steffi whole	90.77 ± 0.01	13.15 ± 0.11	72.44 ± 6.16	84.39 ± 0.31	96.10 ± 0.55
Tobak	N	Tobak whole	90.02 ± 0.01	13.49 ± 0.11	62.35 ± 2.75	81.45 ± 1.92	92.79 ± 0.05
Vanessa	N	Vanessa whole	91.01 ± 0.05	13.17 ± 0.03	60.20 ± 0.98	87.99 ± 2.20	79.76 ± 0.78
Yetti	E	Yetti 1	91.60 ± 0.07	10.93 ± 0.00	55.55 ± 3.55	90.84 ± 0.05	58.03 ± 0.25
Yetti	E	Yetti 2	92.18 ± 0.03	10.94 ± 0.02	53.15 ± 0.15	90.28 ± 0.95	68.28 ± 1.48
Yetti	E	Yetti 3	92.93 ± 0.03	11.16 ± 0.00	61.49 ± 1.75	89.20 ± 1.94	49.72 ± 0.41
Yetti	N	Yetti whole	90.11 ± 0.02	10.66 ± 0.05	58.90 ± 1.59	87.65 ± 2.90	70.60 ± 2.78

Data are presented as mean ± SD

CP = crude protein; DC = digestibility coefficient; DM = dry matter; E = extruded form; N = non-extruded form; SC = starch content

*Individual numbers indicate a different extrusion variant and the temperature used, 1 = 110 °C; 2 = 120 °C; 3 = 130 °C

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truded sample, whereas the highest digestibility was determined in the Steffi variety, particularly the non-extruded Steffi whole sample. Linear regression analysis revealed a strong dependence (r^2 = 0.860; 85.98%) between the *in vivo* and *in vitro* digestibility coefficients for all tested wheat varieties, in both their extruded and non-extruded forms (Figure 1).

In vivo digestibility may be slightly biased by the individuality of each animal. Therefore, digestibility *in vivo* may be relatively higher than *in vitro* digestibility, where everything takes place under pre-specified conditions. Another reason for the jumps in results may be the small volume of data, where, especially for non-extruded samples, even one outlier may lead to a jump.

Selle et al. (2021) investigated the basic chemical composition of four wheat varieties. They found that CP averaged 14.7%. This is, on average, about 2% higher than the average of our tested samples. However, the Grizzly variety (all variants) is close to these measured values, with Grizzly 2 (14.58 ± 0.04%) being the closest. However, in the study by Wieser et al. (2020) is the average CP is only 11%. Only the Yetti variants do not reach this value. Specifically, non-extruded Yetti (10.66 ± 0.05%), Yetti 1 (10.93 ± 0.00%) and Yetti 2 (10.94 ± 0.02%).

Hidalgo et al. (2016), among other things, also investigated the dry matter values of wheat grains. According to their results, the dry matter of baking wheat should be around 95% (95.00 ± 0.20%). Our results average 91% (91.45 ± 0.22%). According to Wieser et al. (2020), the average dry matter content of mature wheat grains was 87%. However,

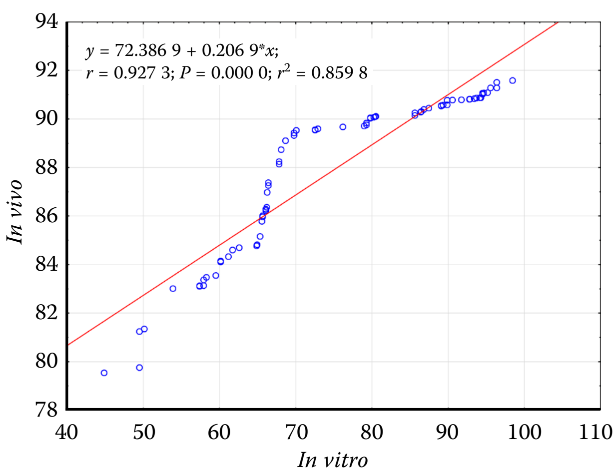

Figure 1. Linear regression analysis of the *in vivo* vs *in vitro* relationship of all wheat varieties

as Biel (2016) points out, the quality of a particular batch of grain grown is also significantly influenced by the environment. Naeem et al. (2012) point out that the nutritional status of the plant can also significantly affect the quality of the wheat grain.

In a previous study, Konieczka et al. (2020) reported that extrusion does not improve digestibility or improve the nutritional value of the material and may adversely affect the activity of microbiota in the appendix. Opoku et al. (2015) also reported that there were no beneficial effects of extrusion on poultry performance. Our study also confirmed that extrusion might not lead to an improvement in digestibility. In the *in vitro* testing, digestibility was not significantly higher in extruded forms than non-extruded forms in all tested wheat varieties. This may be a result of the excessive temperature and pressure during the extrusion process, which could lead to the occurrence of Maillard reactions. This disagrees with the results of Rodriguez et al. (2020). Their results showed that extruded grains had a higher ($P < 0.001$) apparent ileal digestibility of starch than non-extruded grains.

Amerah (2015) investigated the effect of both physical and chemical grain composition, selecting seed hardness as the most important physical property of the grain and the content of non-starch polysaccharides (NSP) as the most important chemical characteristic. Wiseman (2000) also described the effect of high variability in the chemical composition of different wheat species, especially the level of NSP, which affects the performance gains in broilers. Ball et al. (2013a) examined the effect of wheat varieties and their growth on broiler performance and nutrient digestibility, testing 164 wheat samples from different localities, varieties, and years. The research focused on the contents of energy, nitrogenous substances, neutral detergent fibre, lysine, threonine, and amylose, as well as the digestibility of starch. Their results showed that the variety and cultivation conditions of the wheat within wheat grain mixtures significantly affect broiler performance. Additionally, the use and digestibility of nutrients were also affected by the application of nitrogen fertilisers and fungicides (Ball et al. 2013a). In this study, the average digestibility of starch in wheat was 91.7%, with a minimum of 83.2%, a maximum of 97.2%, and a standard deviation of approximately 3.3%. In comparison, the results of our study showed lower wheat starch digestibility. The average val-

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ue of digestibility was 82.17%, with a minimum of 44.86%, a maximum of 98.43%, and a standard deviation for all 19 wheat samples of 8.00%.

The Bedford and Classen study (1993) focused on an *in vitro* test to predict the intestinal viscosity of broilers, and a two-phase *in vitro* test procedure was developed to predict the effectiveness of rye as a food source for broiler chickens. This method used a complete feeding mixture as a basis to predict the *in vivo* intestinal viscosity and final weight of broilers. The results showed that *in vitro* testing is a reliable method for *in vivo* determination. The *in vitro* test accurately predicted the *in vivo* intestinal viscosity as well as the final weight of the birds, suggesting that the *in vitro* test might be a good data predictor for every diet. The correlation between actual intestinal viscosity and *in vitro* viscosity, determined on the same diet, also showed very good results. The viscosity was reduced both *in vivo* (quadratically) and *in vitro* (linearly), indicating a statistically strong dependence between the variables. Karunaratne et al. (2018) also confirmed significant and moderately strong positive correlations between starch digestibility *in vitro* and *in vivo*. Thus, the studies suggested that *in vivo* results should correlate with *in vitro* results. In our research, *in vitro* testing was compared with *in vivo* testing for eight wheat varieties (extruded and non-extruded), which formed the basis of the broiler diet. The results of the comparison were interpreted using a regression analysis three times: 1) *in vivo* vs *in vitro* comparison for all eight wheat varieties, regardless of their treatment; 2) *in vivo* vs *in vitro* testing in wheat without any treatment, and 3) *in vivo* vs *in vitro* testing of 11 samples treated by extrusion. In all three groups, the linear regression graph showed a strong dependence. As the results of all *in vivo* × *in vitro* digestibility dependences were very similar (85.98% for all wheat regardless of treatment, 87.53% for untreated wheat, and 79.32% for extruded wheat), it would be sufficient to perform regression analysis of total wheat varieties only. However, the division into untreated and treated forms provides more accurate results and highlights the importance of considering the treatment of wheat as well as its parameters.

The results obtained from the starch showed that its average content in wheat was 67.22%. According to Amerah (2015), starch is the most abundant carbohydrate in wheat and serves as the primary source of energy, and its content in the grain should range between 60% and 75%. Wiseman et al. (2000) stressed that the starch content in grains depends on the wheat variety and external conditions, ranging from 50% to 70%. The results of our experiment corresponded to this statement because the starch content of samples ranged from 56.03% to 74.51%. Among the commercial types of wheat, the Bonanza variety had the highest starch content (72.29%). In chyme, the average starch content was 13.69%.

Amerah (2015) stated that it is common practice to add xylanase to broiler feeds, which alleviates the side effects of NSP and helps minimise changes in the apparent smetabolisable energy (AME). Ball et al. (2013b) determined the effect of the relationship between the physical and chemical parameters of wheat on the digestibility of feed mixture components in chicken broilers fed wheat. According to their results, the average starch digestibility was 91.7%. One of their key findings was that the degree of starch digestibility plays an important role in the weight gain performance of birds. In addition, the increased NSP content increased the viscosity of the chymus and thus reduced feed conversion. Moreover, NSPs were negatively correlated with AME_n values. More specifically, an increased NSP content may be responsible for the low AME value in some wheat varieties.

Peron et al. (2005) investigated the effect of wheat particle size on the digestibility of nutrients in feed. Whole wheat grains may have a low coefficient of digestibility owing to the hardness and resistance of the seeds. Therefore, they examined the digestibility of dry matter, starch, proteins and lipids, water excretion, and AME_n values. The starch content was determined using the amyloglucosidase-dimethylsulfoxide method. Other nutrients (proteins, lipids, and crude energy) were measured using near-infrared spectroscopy. The results showed that digestibility varied based on the type of grinding. In coarsely ground grains, the starch digestibility reached 85.4%, whereas finely ground grains exhibited higher digestibility (92.5%). Protein digestibility reached 80% in coarse-ground wheat and 81.4% in fine-ground wheat. However, the correlation with AME_n values was not confirmed. Although the AME_n value was higher than the common average values in wheat, there was almost no effect on weight gain or feed intake in finely ground and subsequently granulated particles. The starch digest-

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ibility reported in our study (88.02%) agrees with the findings of Peron et al. (2005). Furthermore, the results showed that the Steffi variety had a lower starch content of 62.77% but very high digestibility (93.78%). Other varieties, such as Bonanza, Gordian, and Tobak, also displayed high digestibility of starch (> 90%). Only the Vanessa variety had very low starch digestibility (80.42%), whereas its starch content was comparable to that of the Steffi variety.

CONCLUSION

The starch content of eight wheat varieties was evaluated, and the digestibility of starch was determined for each variety using both *in vivo* and *in vitro* methods. The average starch digestibility of all wheat varieties was 82.17%. The lowest digestibility was determined in the Yetti variety [in Yetti sample 2 (44.86%)], whereas the highest digestibility was determined in the Bonanza variety [in the Bonanza E sample (98.43%)]. The group of extruded wheat tested *in vivo* had the highest average starch digestibility of 89.61%, whereas the lowest average digestibility was determined for extruded wheat tested *in vitro*, reaching 70.50%. This study confirms that the *in vitro* determination of digestibility is closely correlated with natural digestion and is therefore suitable for determining crude protein and starch digestibility. These results also emphasise the need for selecting an appropriate wheat variety and type of treatment when preparing high-quality feed mixtures for poultry.

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Conflict of interest

The authors declare no conflict of interest.

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