The effect of selected feed mixtures on the duodenal morphology: comparison study

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Short tittle:

Gluten-free and convectional diet effects on duodenal morphology

Summary

The objective of this work was to compare the effect of selected feed mixtures on the duodenal morphology. One-hundred-four rats of the Wistar strain were divided to thirteen groups per eight rats. The experiment started at 35-days rats after birth and lasted for 32-days. The groups (A-M) were fed; commercial diet, 85% wheat and 15% oat diet, 85% wheat and 15% triticale, 85% wheat and 15% barley, 85% wheat and 15% amaranth, 85% wheat and 15% lantern, 85% wheat and 15% buckwheat, 100% wheat, 100% white lupine, 100% flock peas – variety Garden, 100% native peas – variety Garden, 100% native peas – variety Zekon or 100% extruded peas – variety Zekon diet respectively. Samples from the duodenum were taken. The height of the villi and the depth of the crypts were measured. The tallest villi were measured in group F (474.33±114.36 µm) and the shortest villi were observed in group B (294.08±88.52 µm). The deepest crypts were measured in group K (166.41±35.69 µm) and the shallowest crypts were observed in group E (77.85±17.61 µm). The work document, that gluten-free and classical cereals combination can be a better choice for people who want to limit the gluten content of the diet.

Key words

Celiac disease • Gluten-free diet • Cereals • Nutrition • Villi intestinalis

Introduction

Besides cereals like wheat, barley, the food-processing industry also uses some gluten-free products like amaranth, lantern, legumes, lupine and peas. They have prospective uses in human as well as animal nutrition in ecological agriculture. The main group of consumers of such products are people with celiac disease (CD), which is a life-long disorder with intolerance to gluten. Currently, people with this intolerance can only manage their symptoms by following a strict life-long gluten-free diet (Al-Bawardy *et al* 2017, Shannahan and Lefler 2017). The increasing number of newly diagnosed patients has also increased demand for the above-mentioned products in the common food trade network. Otherwise gluten-free products are also often added to classical bakery products to improve the quality of the resulting products. Supplementation of gluten-free cereals to classical bread are currently a common practice in a number of countries. Several studies have shown that there is a possibility to use many gluten-free cereals and legumes in the broader food industry (Belghith-Fendri *et al* 2016, Wu *et al* 2017), but currently not much is known about their effects on small bowel morphology. Part of the population want to live healthy and passing to gluten-free diet, but they are healthy. Some experts warn against this trends, but we are the opinion that combination of classical gluten food with gluten-free diet is possibility for people who want to limit the gluten in their diet. The small bowel is the most important organ of digestion and absorption, and a target for gluten adverse effects. It is obvious that various types of diets may have different effects on digestion, and long-term feeding will affects the mucosal morphology of the small bowel. Changes mainly in the villous-crypts relief, including height of villi and depth of crypts are in relation to nutrients resorption that is considered with the villi growth and crypts deepening. So therefore, the morphometric data of the small bowel can be practically and usefully used to document the nutrition effects on digestible capacity and on health. In addition, the food-processing industry has already been working with the hypotheses of food production on the basis of gluten-free materials, but since today it is not well known which combination is characterized by easier degradation and, possibly, by high nutritional effect. This information would be very useful for food industry. In this study, we tested the effect of selected feed mixtures of wheat, oat, triticale, barley, amaranth, lantern, buckwheat, lupine and peas on duodenal morphology. Duodenal biopsies are currently performed to achieve diagnosis and evaluation of damage from CD in patients. Availability of simple morphometric data addressing to different condition of mucosal health, following a specific diet, can be helpful in managing patients CD, but also for general population. The objective of this work is to compare the height of villi and depth of crypts after ingestion of different feed mixture cereals and gluten-free cereals and legumes in the experimental rat model.

Material and Methods

Ethics

All care and experimental procedures involving animals followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals accepted by Slovak Governmental Veterinary and Food Institute. The study was approved by the Ethics Committee of the Research Institute for Animal Production Nitra in Slovak republic. (Approval number of testing equipment SK P 34004)

Characteristics of the experiment

One hundred and four rats of the Wistar strain were divided into thirteen groups (8 rats per group) housed in conventional cages. The design of the experiment is described also in the Table 1. After birth rats were feed by mother milk to 25 days of their life and after they were weaned and feed with standard commercial diet. Rat mothers were feed before and during pregnancy with standard commercial diet. Diet supplier (Institute of Experimental Pharmacology & Toxicology, SAS Bratislava, Department of Toxicology and Laboratory Animals Breeding Dobra Voda). For composition of the compound feed see also Table 2. Rats were tested on the postnatal day 35 and fed the chosen diet for 32 days. Control group (A) was fed with standard commercial feed for rat and mice used for the duration of the experiment. Group B was fed a mixture of 85% soft wheat

(*Triticum aestivum*) and 15% naked oat (*Avena nuda* – variety Detvan). Group C was fed a mixture of 85% soft wheat (*Triticum aestivum*) and 15% triticale (*Triticosecale* variety – Triamant). Group D was fed a mixture of 85% soft wheat (*Triticum aestivum*) and 15% common barley (*Hordeum vulgare* – variety KM2283). Group E was fed a mixture of 85% soft wheat (*Triticum aestivum*) and 15% amaranth (*Amarantus spp.*, variety Plainsman). Group F was fed a mixture of 85% soft wheat (*Triticum aestivum*) and 15% lantern (*Medicago sativa* – variety Lucia). Group G was fed a mixture of 85% soft wheat (*Triticum aestivum*) and 15% buckwheat (*Fagopyrum esculentum* – variety Spacinska 1). Group H was fed 100% wheat (*Triticum aestivum*). Group I was fed 100% white lupine (*Lupinus albus native* – variety Amiga native). Group J was feed 100% flock peas (*Pisum sativum* – variety Garden). Group K was fed 100% native peas (*Pisum sativum* – variety Garden). Group L was fed 100% native peas (*Pisum sativum* – variety Zekon) and group M was fed 100% extruded peas (*Pisum sativum* – variety Zekon). Food and water were available to all animals *ad libitum*. Standard zootechnic and veterinary conditions were always maintained.

Sampling

Rats were euthanized in CO_2 chamber. Samples from the small intestine were obtained by necropsy less than 30 minutes after death. One sample of the proximal duodenum (the superior duodenal flexure) was taken from each animal and fixed in 4% formalin solution for one week.

Histological methods and samples evaluation

The samples were processed by standard histological methods and embedded in paraffin blocks. 3–5 µm serial sections were cut using a microtome. The slices were placed on standard slides and stained with haematoxylin-eosin. The samples were viewed and evaluated using an Olympus Provis BX50 microscope (Olympus, Japan) equipped with Nikon camera (Nikon Digital Sight DS-Fi1, Japan). Objective assessment achieved by the measurement of the height of villi and depth of crypts was carried out using the NIS-Elements software, version 3.0 (Laboratory Imaging s.r.o., Prague, Czech Republic). In each sample, ten areas were selected, in which ten villi and ten crypts were measured. There were eight measurements for each animal making 80 measurements of height of villi and 80 measurements of depth of crypts in total.

Statistical evaluation of the experiment

All data was statistically analysed using the following tests: basic statistical characteristics, correlations, ANOVA and MANOVA. Statistical computation was carried out using Statistix9 and IfoStat packages. Differences were declared significant at $P<0.05$; $P<0.001$.

Results

As shown in Table 3, the tallest villi were measured in F group (474.33±114.36 µm). Lower heights of the villi values were measured in G (459.36±107.29 µm), E (458.61±65.26 µm), C (457.45±95.25 µm) groups, followed by D (429.50±101.66 µm), M (421.39±71.98 µm), J (419.72±83.76 µm) and A (399.29±75.77 µm) groups. Even shorter villi heights were measured in K (396.35±88.818 µm), H (391.04±82.56 µm), L (388.72±78.40 µm) and I (387.34±55.44 µm) groups. The shortest villi were measured in group B (294.08±88.52 µm).

The deepest crypts were measured in group K (166.41±35.69 µm). Slightly shallower crypts were measured in group J (165.25±38.19 µm), followed by A (138.90±22.03 µm), M (136.31±51.95 µm), I (123.71±24.21 µm), L (103.70±35.53 µm), H (103.32±23.51 µm) and F (101.21±30.65 µm) groups. Very shallow crypts were measured in B (93.37±36.57 µm), D (83.03±24.85 µm), C (82.65±21.93 µm) and G (78.47± 16.20µm) groups. The shallowest crypts were measured in group E (77.85±17.61 µm).

Table 3 shows the basic statistical characteristics of villus height and crypt depth by analyzed groups and correlations between these traits in groups. Minimal average value of villus height was found in the group B 294 µm and maximal was in the group F 474.33 µm. For crypt depth minimal average value was in group E 77.85 µm and maximal one in group K 166.41 µm.

The significant or highly significant correlations between analysed traits were obtained only for groups A, B, D and K, only group B showed a high (r=0.5013).

Table 4 shows the ANOVA for analysed traits. The difference between groups in both traits were highly significant.

Tukey t-test for homogenous groups shows, that there is difference between groups in both analysed traits for α=0.05 and α=0.01. There is not clear significant pattern in significant difference between treatments in height of villous. Generally animals treated by soft wheat with mixture have significantly greater height of villous (except of group A and B). Group B had the smallest height of villous from all tested treatments and highest variance (30%), but still this variance is not so big to show on error in measurement.

Furthermore there is clear influence of treatment on crypt depth. With the exception of treatment A, the depth is significantly smaller for all soft wheat treatments. For detailed difference see table 5.

Discussion

The increasing incidence of diseases, including an increasing percentage of people suffering from allergies, requires special dietary regimes. There are also other diseases that have benefit from special and easily digestible diets (e.g. type 2 diabetes, Morbus Crohn, Pancreatitis diet). At present the food-production industry is focused on exploring the possibilities of producing food with a better content of energetically utilizable components (Schönfeldt and Gibson 2010). A number of additives are being tested, including some gluten-free cereals and legumes used in the form of additives in food products. They are also commonly used in gluten-free diets, but their effects on small bowel morphology is not well known. This is very important to know, because decreased small bowel resorption is a concomitant phenomenon of several diseases and common postoperative consequence. On the other hand, it is also well known that European populations are today affected by several civilisation diseases, including overweight and obesity (Doak *et al* 2012, Honzikova and

Zavodna 2016). Thus, additional knowledge about the effects of individual food additives on the duodenal morphology is necessary and may result in practical output for dietetic indication and preparation of better nutritional products. Some animal models for celiac disease were established and the effects of various diet have been investigated (Costes *et al* 2015, Ju *et al* 2015). Moreover, not much is known with regards to the best supplement options as food additive for healthy people. Consequently, in our experiment we compared the influence of several feed mixture additives on the duodenal morphology. Using an animal model, we have shown some rather impressive changes in villus structure on different diets given over only a period of just a month. This has some intriguing implications, even for adaptation changes in humans on different diets, particularly in the area of food faddism, never mind celiac disease. The histological findings shows normal views and in the representative Figure 1 there are some examples of normal small bowels. On the other hand the results of measurements shows some differences between experimental groups. The results of morphometry show that lantern, buckwheat, amaranth and triticale have positive effects on the height of villi compared with commercial based feed. On the other hand oats have a negative effect on the height of villi. Oats have been studied for several decades and there are different conclusions as to their addition to gluten-free diet. European legislation specifies the use of oats for gluten-free diet. Oats contained in a food presented as "gluten-free" or "very low gluten" must have been specially produced, prepared and/or processed in a way to avoid contamination by wheat, rye, barley, or their crossbred varieties and the gluten content of such oats cannot exceed 20 mg/kg. On the other hand, it was suggested that it may only be certain strains of oats which could produce a toxic response to people with celiac disease (Gimenez *et al* 2017). A long-term dietary intake of oats or oat bran could benefit inflammatory bowel disorders, but the protective effect from colorectal adenoma and cancer incidence has not yet been convincingly shown (Thies *et al* 2014). Our data shows that some cereals have significantly better effects on villous relief compared to commercial based feed. Similar observations are made with legumes and commercial based feed comparisons. Therefore, starting from the working hypothesis of the positive effect of basic feed mixture with additives we can confirm that there are differences in the height of villi and depth of crypts. Our results show that addition of peas to the diet gives better results, compared to commercial based feed, or only wheat feed. From our point of view, peas are a suitable alternative of nutrition or a component of nutrition in diets and our results show that there are some differences between different peas groups. In previous work, no differences were documented in chemical composition and meat quality among experimental pig groups fed peas and peas can be included in diets at 15% for growing-finishing pigs, 30% for the growing phase and 30% during the finishing phase (Chrastinova *et al* 2005). Diets containing peas need to be fortified with synthetic amino acids to balance the composition of the diet. In addition, pea components implementation in the food-processing industry can bring diversity to the diet of people, including celiac disease diet. Buckwheat and amaranth are already used in practice for such purposes and their enhanced final nutritional quality was documented (Gambus *et al* 2009, Sebestyen *et al* 2013). It is mentioned that their nutritional value is interesting mainly because of significantly higher levels of protein, fat, fibre and minerals than the control bread (Alvarez-Jubete *et al* 2009). Today they are globally used in development of functional gluten-free foods as bread, cookies, pasta, noodles and beer (Gimenez-Bastida *et al* 2016). Our results on experimental rats show the positive effects on the villi heights and depth of crypts of different feed mixtures with gluten-free cereals and legumes. Legumes and some cereals are suitable for gluten-free diet, because they have a content of prolamin less than 4% (Mlynekova *et al* 2014). Positive effects of legumes suggest that cellulose fiber may have played a significant role in small bowel villous architecture, which was confirmed also one well written earlier study (Dirk and Freeman 1987). Height of the villi and depth of the crypts are the important parameters of the small bowel morphology. They are dynamically changing and adapting to current nutrition and their parameters are important signs of small bowel absorption possibilities. They were subject of several works dealing with diseases (Rallabhandi 2012), food restriction (Tumova *et al* 2016), different short-term dietary regimes (Pauwels 2011), but also for maintaining certain principles of healthy nutrition (Martinkova *et al* 2000), or genetically modified products (Ibrahim and Okasha 2016). Larger villi relief enables greater contact with chyme and therefore greater absorption of nutrients as there is a greater number of blood and lymphatic capillaries in the villi, which transport the absorbed compounds through the blood. It is well known, that many people decide to adhere to gluten-free diet, even if they don’t have any health problems with gluten. For example in the United States about one-third of people report trying to avoid gluten (Jargon 2014). These facts are supported by many myths about gluten-free diet. It is primarily about slimming, healthy lifestyle, or detoxification and conviction of general gluten harm (Koning 2015, Nash and Slutzky 2014). The work of Reilly (2016) documents that following a gluten-free diet can be at the end harmful for healthy people. On the other hand some studies have shown that non-celiac autoimmune diseases can partially respond to gluten-free diet (Lerner *et al* 2017). In fact the mechanisms by which gluten-free diet can effects, or alleviate non-celiac autoimmune diseases initiation, or progression are today not clear. Every article bringing facts is valuable, and should not nourish false hope in people. Strict gluten-free diet is designed for special dietary purposes, it's not about fashion and cannot be recommended as a miraculous diet with the power to cure everyone. It should also be clear that it is not a guarantee of a better mood, or happiness. Here dietitians play a pivotal role in the education, follow-up and navigation of the special diets for individual disease (Lerner and Torsten 2017). The work of Coattrenec *et al* (2015) state there is no strong evidence for a strict indication to a gluten-free diet in endocrinologic, psychiatric, and rheumatologic diseases, or to improve performance in elite sports. Our work documents, that gluten-free cereals and classical cereals combination can be better choice for people who want to limit the gluten content from body.

Conclusion

This work documents that the combination of gluten-free cereals and classical cereals can be a better choice for people who want to limit the gluten intake in the body, but also to maintain adequate mucosal morphology of the intestine for a valid digestive function.

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Duality of interest

The authors declare that there is no duality of interest associated with this manuscript disclosures to report.

References

AL-BAWARDY B, CCODIPILLY DC, RUBIO-TAPIA A, BRUINING DH, HANSEL SL, MURRAY JA: Celiac disease: a clinical review. *Abdom Radiol* **42**: 351-360, 2017.

ALVAREZ-JUBETE L, ARENDT EK, GALLAGHER E: Nutritive value and chemical composition of pseudocereals as gluten-free ingredients. *Int J Food Sci Nutr* **60**: 240-257, 2009.

BELGHITH-FENDRI L, CHAARI F, KALLEL F, ZOUARI-ELLOUZI S, GHORBEL R, BESBES S, ELLOUZ-CHAABOUNI S, GHRIBI-AYDI D: Pea and broad bean pods as a natural source of dietary fiber: The impact on texture and sensory properties of cake. *J Food Sci* **81**: 2360-2366, 2016.

COATTRENEC Y, HARR T, PICHARD C, NENDAZ M: Benefits of gluten-free diet: myth or reality? *Rev Med Suisse* **11**: 490, 2015.

COSTES LM, MERESSE B, CERF-BENSUSSAN N, SAMSOM JN: The role of animal models in unravelling therapeutic targets in coeliac disease. *Best Pract Res Clin Gastroenterol* **29**: 437-450, 2015.

DIRKS P, FREEMAN HJ: Effects of differing purified cellulose, pectin and hemicellulose fiber diets on mucosal morphology in the rat small and large intestine. *Clin Invest Med* **10**: 32-38, 1987.

DOAK CM, WIJNHOVEN TM, SCHOKKER DF, VISSCHER TL, SEIDELL JC: Age standardization in mapping adult overweight and obesity trends in the WHO European region. *Obes Rev* **13**: 174-191, 2012.

GAMBUS H, GAMBUS F, PASTUSZKA D, WRONA P, ZIOBRO R, SABAT R, MICKOWSKA B, NOWOTNA A, SIKORA, M: Quality of gluten-free supplemented cakes and biscuits. *Int J Food Sci Nutr* **60**: 31-50, 2009.

GIMENEZ MJ, REAL A, GARCIA-MOLINA MD, SOUSA C. BARRO, F: Characterisation of celiac disease related oat proteins: based for the development of high quality oat varieties suitable for celiac patients. *Sci Rep* **17**: 42588, 2017.

GIMENEZ-BASTIDA JA, PISKULA M, ZIELINSKI, H: Recent advances in development of gluten-free buckwheat products. *Trends Food Sci Technol* **44**: 58-65, 2016.

HONZIKOVA N, ZAVODNA F: Baroreflex sensitivity in children and adolescent: physiology, hypertension, obesity, diabetes mellitus. *Physiol Res* **65**: 879-889, 2016.

CHRASTINOVA L, CHRENKOVA M, FLAK P, LAHUCKY R, FORMELOVA Z, CERESNAKOVA Z, MAKOVICKY, P: The effect of including pea in diets for growing-finishing pigs on performance and meat quality. *Arch Zootech* **8**: 91-96, 2005.

IBRAHIM MA, OKASHA EF: Effect of genetically modified corn on the jejunal mucosa of adult male albino rat. *Exp Toxicol Pathol* **68**: 579-588, 2016.

JARGON J: The gluten-free craze: Is it healthy? *Wall Street J* **6**: 22, 2014.

JU JM, MARIETTA EV, MURRAY JA: Generating transgenic mouse models for studying celiac disease. *Methods Mol Biol* **1326**: 23-33, 2015.

KONING F: Adverse effects of wheat gluten. *Ann Nutr Metab* **67**: 8-14, 2015.

LERNER A, MATTHIAS T: Gluten-free diet - Tough alley in torrid time. *Int J Celiac Dis* 5: 50-55, 2017.

LERNER A, SHOENFELD Y, MATTHIAS T: Adverse effects of gluten ingestion and advantages of gluten withdrawal in nonceliac autoimmune disease. *Nutr Rev* 75: 1046-1058.

MARTINKOVA A, LENDHARDT L, MOZES S: Effect of neonatal MSG treatment on day-night alkaline phosphatase activity in the rat duodenum. *Physiol Res* **49**: 339-345, 2000.

MLYNEKOVA Z, CHRENKOVA M, FORMELOVA Z: Cereals and legumes in nutrition of people with Celiac disease. *Int J Celiac Dis* **2**: 105-109, 2014.

NASH DT, SLUTZKY AR Gluten sensitivity: new epdemic or new myth? *Proc (Bayl Univ Med Cent)* **27**: 377-378, 2014.

PAUWELS EK: The protective effects of the mediterranean diet: focus on cancer and cardiovascular risk. *Med Princ Pract* **20**: 103-111, 2011.

RALLABHANDI P: Gluten and celiac disease-an immunological perspective. *J AOAC Int* **95**: 349-362, 2012.

REILLY NR: The gluten-free diet: Recognizing fact, fiction, and fad. *J Pediatr* **175**: 206-210, 2016.

SEBESTYEN A, KISS Z, VECSERI-HEGYES B, KUN-FARKAS G, HOSCHKE A: Experiences with laboratory and pilot plant preparation of Millet and Buckwheat beer. *Acta Aliment* **42**: 81-89, 2013.

SCHÖNFELDT HC, GIBSON N: Food composition data in health communication. *Eur J Clin Nutr* **64**: 28-33, 2010.

SHANNAHAN S, LEFFLER DA: Diagnosis and updates in celiac disease. *Gastrointest Endos Clin North Am* **27**: 79-92, 2017.

THIES F, MASSON LF, BOFFETTA P, KRIS-ETHERTON P: Oats and bowel disease: a systematic literature review. *Brit J Nutr* **112**: S31-S43, 2014.

TUMOVA E, VOLEK Z, CHODOVA D, HARTLOVA H, MAKOVICKY P, SVOBODOVA J, EBEID TA, UHLIROVA L: The effect of 1-week feed restriction on performance, digestibility of nutrients and digestive system development in the growing rabbit. *Animal* **10**: 1-9, 2016.

WU T, TAYLOR C, NEBL T, NG K, BENNETT LE: Effects of chemical composition and baking on in vitro digestibility of proteins in breads made from selected gluten-containing and gluten-free flours. *Food Chem* **233**: 514-524, 2017.

Table 1. The design of the experiment

Group	Composition
A	Commercial base fed.
B	Soft wheat (85%) and naked oat – variety Detvan (15%).
C	Soft wheat (85%) and triticale – variety Triamant (15%).
D	Soft wheat (85%) and common barley (15%).
E	Soft wheat (85%) and Amaranth – variety Plainsman (15%).
F	Soft wheat (85%) and lantern – variety Lucia (15%).
G	Soft wheat (85%) and buckwheat – variety Spacinska 1 (15%).
H	Soft wheat (100%).
I	Lupine – variety Amiga native (100%).
J	Flock peas – variety Garde (100%).
K	Native peas – variety Garde (100%).
L	Native peas – variety Zekon (100%).
M	Extruded peas – variety Zekon (100%).

Table 2. Composition of compound feed (in % dry matter)

Component	An amount equivalent to 10% CP
Saccharose	10 %
Mineral mixture	6 %
Vitamin mixture	2 %
Oil	5 %
Cellulose	to 4 % fiber
Starch	to 100 %

Table 3. Basic statistical characteristics of Villus height and Crypt depth with their correlations in groups

Group	Villus height				Crypt depth				r
	$\overline{x}$	s	$s_{\overline{x}}$	V %	$\overline{x}$	s	$s_{\overline{x}}$	V %	
A	399.29	75.77	8.47	18.98	138.90	22.03	2.46	15.86	0.3812**
B	294.08	88.52	9.90	30.10	93.37	36.57	4.09	39.17	0.5013**
C	457.45	95.25	10.65	20.82	82.65	21.93	2.45	26.54	0.0678
D	429.50	101.66	11.37	23.67	83.03	24.85	2.78	29.93	0.3855**
E	458.61	65.26	7.30	14.23	77.85	17.61	1.97	22.62	0.0386
F	474.33	114.36	12.79	24.11	101.21	30.65	3.43	30.28	0.1228
G	459.36	107.29	12.00	23.36	78.47	16.20	1.81	20.65	0.2075
H	391.04	82.56	9.23	21.11	103.32	23.51	2.63	22.75	0.0704
I	387.34	55.44	6.20	14.31	123.71	24.21	2.71	19.57	0.0185
J	419.72	83.76	9.36	19.96	165.25	38.19	4.27	23.11	0.2081
K	396.35	88.818	9.93	22.41	166.41	35.69	3.99	21.45	0.2545*
L	388.72	78.40	8.77	20.17	103.70	35.53	3.97	34.26	-0.0299
M	421.39	71.98	8.05	17.08	136.31	51.95	5.814	38.11	-0.1727

Legend: $\overline{x}$: average, s: standard deviation, $s_{\overline{x}}$: standard error of mean, V %: percentual variation, r: correlation

Table 4. One-way analyses of variance of analysed traits

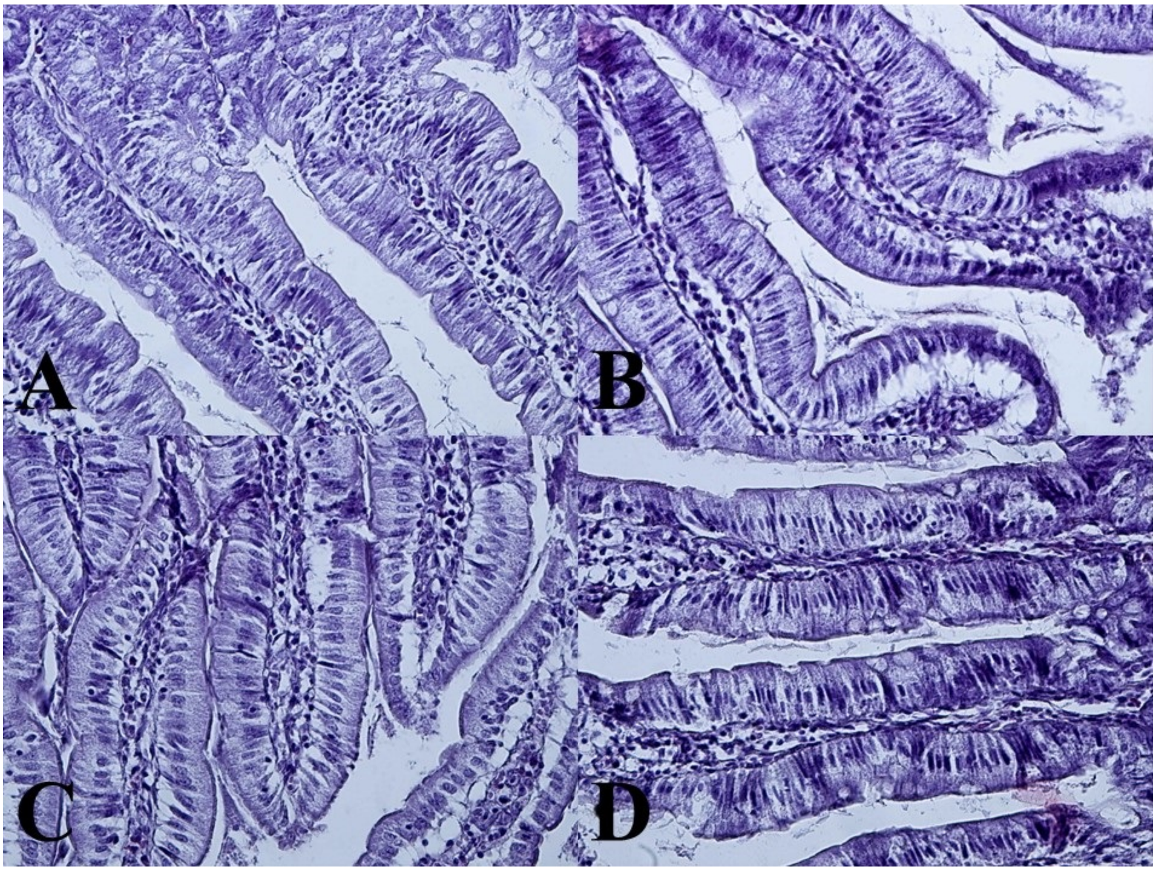
Traits		Groups f_G=12	Error, f_e =1027	F_α (12, 1027)
Villus height	MS	177903.81	7534.83	$F_{0.05}$; 1.762
	F	23.61***		$F_{0.01}$; 2.202
				$F_{0.001}$; 2.774
Crypt depth	MS	78971,89	943.51	
	F	83.70***		

Table 5. Tukey T test – homogenous groups for α = 0.05 and α = 0.01

Villus height				Crypt depth			
Group	$\overline{x}$	Hom. groups, *α*		Group	$\overline{x}$	Hom. groups, *α*	
		0.05	*0.01*			*0.05*	*0.01*
F	474.33	*a*	*a*	K	166.41	*a*	*a*
G	459.36	*ab*	*ab*	J	165.25	*a*	*a*
E	458.61	*ab*	*ab*	A	138.90	*b*	*b*
C	457.45	*ab*	*ab*	M	136.31	*b*	*b*
D	429.50	*abc*	*abc*	I	123.71	*b*	*b*
M	421.39	*bc*	*bc*	L	103.70	*c*	*c*
J	419.72	*bc*	*bc*	H	103.32	*c*	*c*
A	399.29	*c*	*c*	F	101.21	*c*	*cd*
K	396.35	*c*	*c*	B	93.37	*cd*	*cde*
H	391.04	*c*	*c*	D	83.03	*d*	*de*
L	388.72	*c*	*c*	C	82.65	*d*	*e*
I	387.34	*c*	*c*	G	78.47	*d*	*e*
B	294.08	*d*	*d*	E	77.85	*d*	*e*

The treatments with the different letters are significantly different.

Figure 1



A: Commercial base feed. Hematoxylin-eosin: 400x.

B: Soft wheat (85%) and Amaranth – variety Plainsman (15%). Hematoxylin-eosin: 400x.

C: Flock peas – variety Garde (100%). Hematoxylin-eosin: 400x.

D: Native peas – variety Zekon (100%). Hematoxylin-eosin: 400x.