EFFECT OF DIGESTED WHEAT GERM EXTRACT ON THE INTESTINAL MORPHOLOGY IN FARM ANIMALS

Emma Kósa PhD
SZENT ISTVÁN UNIVERSITY, FACULTY OF VETERINARY SCIENCE,
H-1078 BUDAPEST, ISTVÁN ÚT 2. HUNGARY

SUMMARY

The aim of this study was to answer the question, if there is any morphological background connected with the significant improvement of weight gain and feed conversion efficiency in both pigs and chickens due to diet supplemented with digested wheat germ extract (DWGE). In both pigs and chickens, which were fed diet containing zero and 1-2 g/kg DWGE, a complete histomorphological examination of the mucous membrane in the small intestines (duodenum, jejunum, ileum) including histometrical measurement of height and width of the villi and that of the crypt depth was conducted at different age (for pigs on day 1, 28, 90 and 160, for chickens on day 1, 10, 21 and 42) in order to compare the control and experimental groups under both laboratorial and field conditions. In both pigs and chickens fed with DWGE the length of intestinal villi was significantly longer regarding both duodenum and jejunum that results in a larger surface and better conditions for absorption. The difference in the length of villi was greater in the chickens reared in large-scale farms than those kept in laboratory. In the control groups of both chickens and pigs the extent of the epithelial exfoliation of significantly shorter and wider villi, that of the lymphocytic, histiocytic and plasma cell infiltration of stratum villous and that of the fusion of the villi was greater, indicating a less favorable microbial environment in the intestinal content. These healthful and beneficial morphological changes in the gut of animals fed diet containing DWGE could explain the significant improvement of weight gain and feed conversion in both laboratory testing and large-scale pig and poultry farms.

INTRODUCTION

The application of nutraceuticals aims at the more efficient immune system and the improved production of animals through balance of the intestinal flora, more intensive enzyme activity and more effective absorption (Davis et al, 2004; Fuller and Turvey, 1971; Miles et al., 1981; Mohan et al., 1996; Decuypere et al., 1998; Patterson and Burkholder, 2003; Huang et al., 2004; Li et al., 2005; Hahn et al., 2006; Li et al., 2006.; Lien et al., 2007; Kırösiné et al., 2011). Cereal germ as a nutraceutical is the reproductive part of grain kernels that concentrates essential vitamins and fatty acids, important macro- and micorelements, and its fermentation with Saccharomyces cerevisiae glycosides of the germ cells split into benzoquinones (Johanning and Wang-Johanning, 2007).

DWGE is standardized to contain methoxy-substituted benzoquinones (2-methoxy-benzoquinone and 2.6-DMBQ) at a concentration of 0,04% beside other as yet poorly characterized molecules beneficial for immune status, and patented and produced for livestock, poultry and pets (Rafai et al., 2011). In many preliminary studies the immune modulating, antioxidant and growth promoting characteristics of DWGE (as a feed supplement of 1-2 g/kg) were reported, it significantly improved the live weight gain, the feed conversion efficiency, and greatly decreased the premature death in both pigs and chickens under laboratorial and field conditions (Jakab et al., 2008; Kósa and Bajcsy, 2008; Kósa et al., 2008; Kovács et al., 2004, 2008; Nagy et al., 2008; Rafai et al., 2011; Stipkovits et al. 2004). The aim of this study was to reveal the morphological background connecting with the significant improvement of weight gain and feed conversion efficiency in both pigs and chickens due to diet supplemented with DWGE.

MATERIALS AND METHODS

Animals and feeding

Forty-eight Hungarian Large White piglets, weaned at 28 days of age, were randomly allocated into a control and an experimental group of 24 piglets in each group in a large-scale commercial pig farm. Pigs were ear clipped and castrated at 2 days of age according to the Hungarian Animal Welfare Act. In the first three days the pre-starter feed were gradually changed for a starter feed, then grower (28 to 60 d) and eventually fattener (60 to 160 d). Feed of the experimental group was supplemented with DWGE (1-2 g/kg). Antibiotics were excluded from diets. Every pig had an ad libitum access to feed and water. Pigs were put into flat decks equipped with stainless steel feed troughs, nipple drinkers and aluminum-cast slatted floor at a population density of 4 piglets/pen; 0.6 m^2 resting area/pig. The flat decks were housed in climatically controlled chambers at optimum ambient temperature, relative humidity and air velocity. Twelve-hour/day light regime was applied.
Hundred and twenty Ross 308 broiler chickens were involved in the experiment, out of which 40 chickens were randomly allocated into a control and an experimental group of 20 chickens in a large-scale commercial broiler chicken farm, and 80 chickens into a control and an experimental group of 40 chickens under laboratorial conditions. Diets were fed from 1 to 42 day including starter feed (1 to 14 d) and grower feed (15 to 42 d). Feed of the experimental group was supplemented with DWGE (1g/kg). Antibiotics were excluded from diets. Every chicken had an ad libitum access to feed and water. Temperature was maintained at 32^{o}C for the first 5 days and then gradually reduced to 22^{o}C according to usual management practice. Eighteen-hour/day light regime was applied.

Hystomorphological analysis

In pigs fed zero and DWGE supplemented diet a complete histomorphological investigation of the mucous membrane in the alimentary tract (stomach, duodenum, jejunum, ileum, colon) including histometrical measure of the villus height and width and the thickness of the hyperkeratotic layer in the nonglandular region of stomach was conducted at different ages (Day 1, 28, 90 and 160). In both groups 3-3 piglets per group on Day 1, and 7-7 piglets per group on Day 28, 90 and 160 were slaughtered and examined, respectively.
In chickens fed zero and DWGE supplemented diet a complete histomorphological investigation of the mucous membrane in the small intestines (duodenum, jejunum, ileum) including histometrical measurement of height and width of the villus and that of the crypt depth was conducted at different ages (Day 1, 10, 21 and 42). In the laboratory 10-10 chickens per group were slaughtered and examined on Day 1, 10, 21 and 42, in the large-scale chicken farm 10-10 chickens per group on Day 20 and 42, respectively. The samples were taken from the same places of duodenum, jejunum and ileum of the animals in any case. Additionally, samples were taken from the non-glandular region of the stomach from pigs, as well. The histological and histometrical findings in pigs and chickens fed zero and DWGE supplemented diet are compared with each other.
Tissue samples were flushed with physiological saline and fixed in 10% neutral buffered formaldehyde solution then embedded into paraffin. Cross-sections of 3-4 µm width were made of this paraffin blocks that, after extracting the paraffin, were stained with haematoxylin and eosin (Uni et al., 1998).
Histometrical analysis was performed in every group at every sampling time on the mucous membrane. Segments were removed from the duodenum, jejunum, and ileum, in order to measure the length and width of villi, and the depth of crypts in µm by ocular micrometer. A total of 10 intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section (30 measurements for each sample). Villus height was measured from the tip of the villi to the villus crypt junction, and crypt depth was defined as the depth of the invagination between adjacent villi. The villus width was measured at the middle part of the villus.
Furthermore, in the *gross pathological examination* the different layers of the intestinal mucosal membrane, the epithelium and stratum villosum of villi, the possible syncytial fusion

of villi, the lymphocytic, histiocytic and plasma cell infiltration of stratum villosum causing larger membrane thickness, and morphology sings of activity of Goblet cells were evaluated. In pigs the average thickness of hyperkeratotic and degenerated layer in the non-glandular region was examined, as well.
Additionally, the aforementioned parameters were analyzed in terms of that whether they are dependent on the age and independent on the substance tested or the morphological changes can be traced back to the DWGE.

Statistical analysis

Regarding chickens one-way and two-analysis analysis of variance was performed using the general linear model procedure of SAS software (1989). Differences among means were tested using Duncan's multiple-range tests. A significance level of 0.05 was used. Concerning pigs two-way analysis of variance was applied to compare the groups. The magnitude and significance of each difference was evaluated via appropriate contrast within this model. The thicknesses of hyperkeratotic layers were logarithmically transformed before the analysis in order to make the model better fit.

RESULTS

The average parameters (µm) of the small intestines in the control and DWGE group at different age of pigs are shown in *Table 1*, those of chickens in *Table 2*.
In pigs the length of villi of newborn piglets was 567 µm in the duodenum, 733 µm in the jejunum and 633 µm in the ileum. At weaning (Day 28) the significant shortening and widening of villi, the partial exfoliation of epithelial cells, and the infiltration of stratum villosum with lymphocytes, histiocytes and plasmocytes and on several occasions the fusion of villi can be observed. In the pigs fed DWGE supplemented diet this shortening of intestinal villi at weaning and the extent of epithelial exfoliation, the lymphocytic, histiocytic and plasmacell infiltration was milder (regarding duodenum and jejunum the shortening of villi was significantly ($p<0.001$) smaller) and this difference could be observed on Day 90 and 190, too (*Chart 1-3.*).

Chart 1. The impact of DWGE on the height of villi in the small intestine of pigs at different age

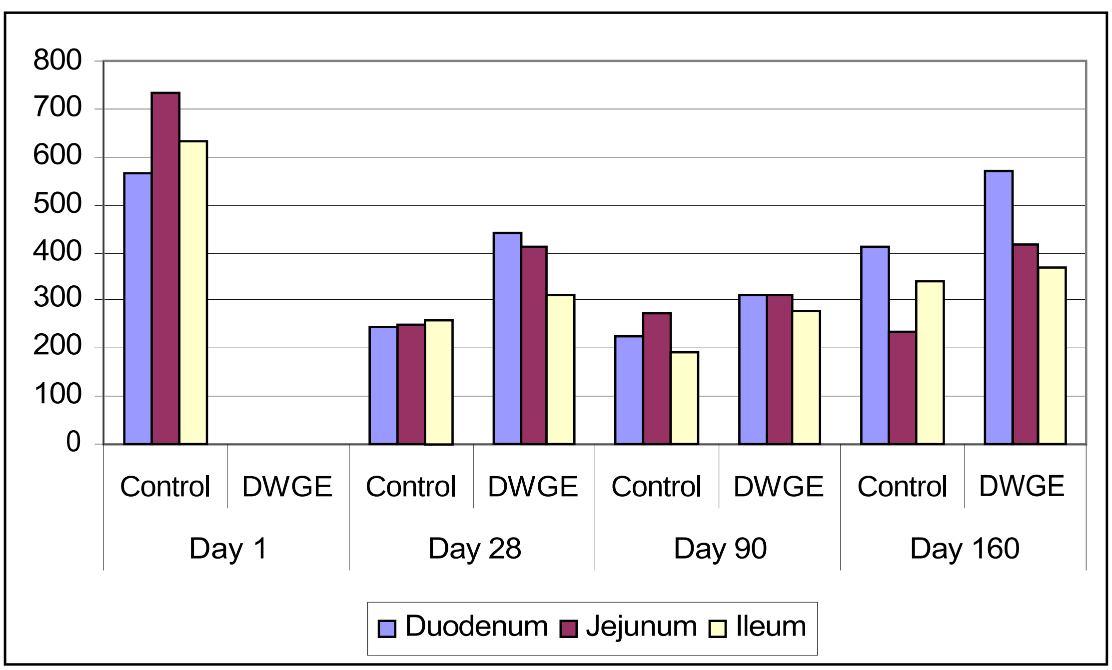


Chart 2. The histopathological photo of villi in the small intestine of pigs in the control group

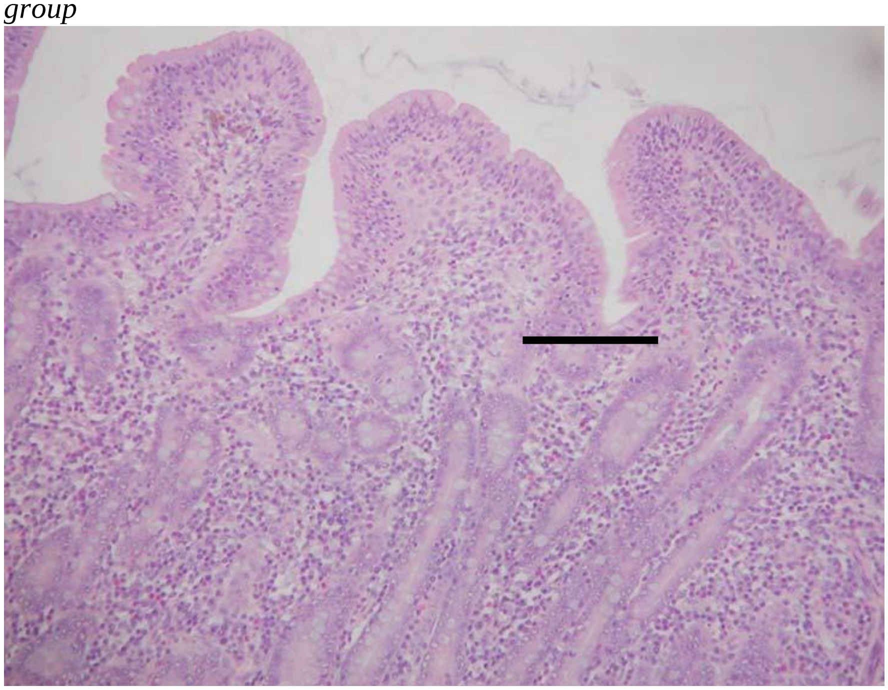
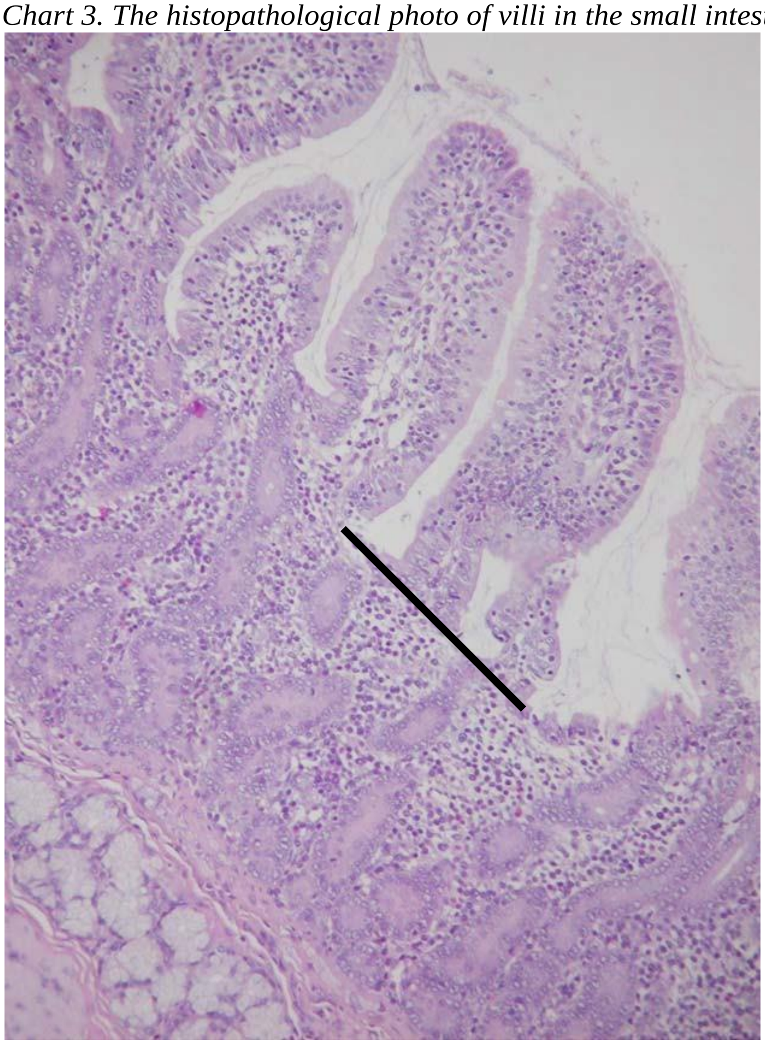


Chart 3. The histopathological photo of villi in the small intestine of pigs in the DWGE group



The average thickness of hyperkeratotic layer in the nonglnadular region of stomach and the extent of its vacoular degeneration was smaller (significantly ($p<0.001$) on Day 90) in the DWGE group (*Charts 4-6.*)

Chart 4. The average width of keratinized and degenerated laye on the nonglandular region of stomach

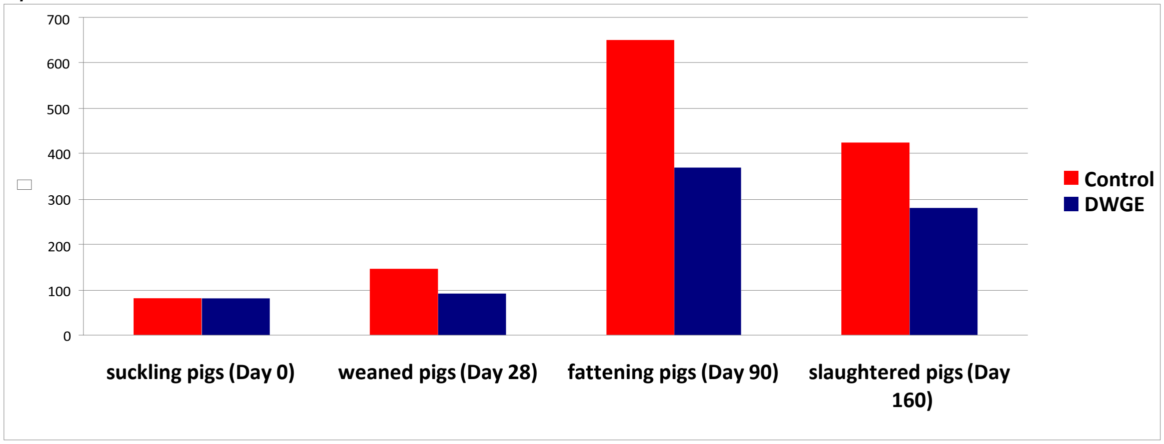


Chart 5. The histopathological photo of the nonglandular region of swine stomach in the control group

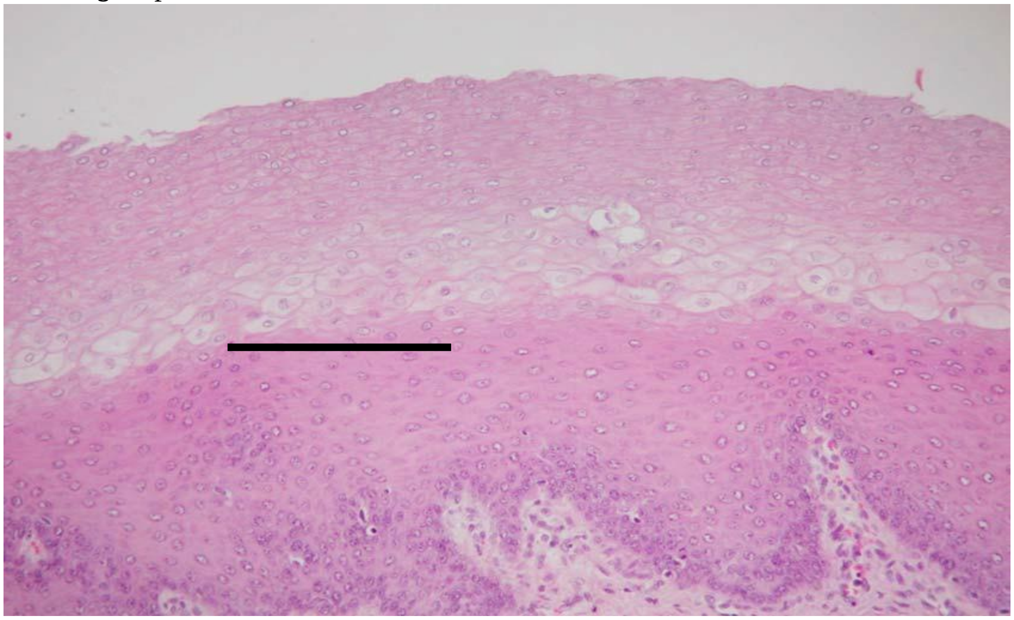
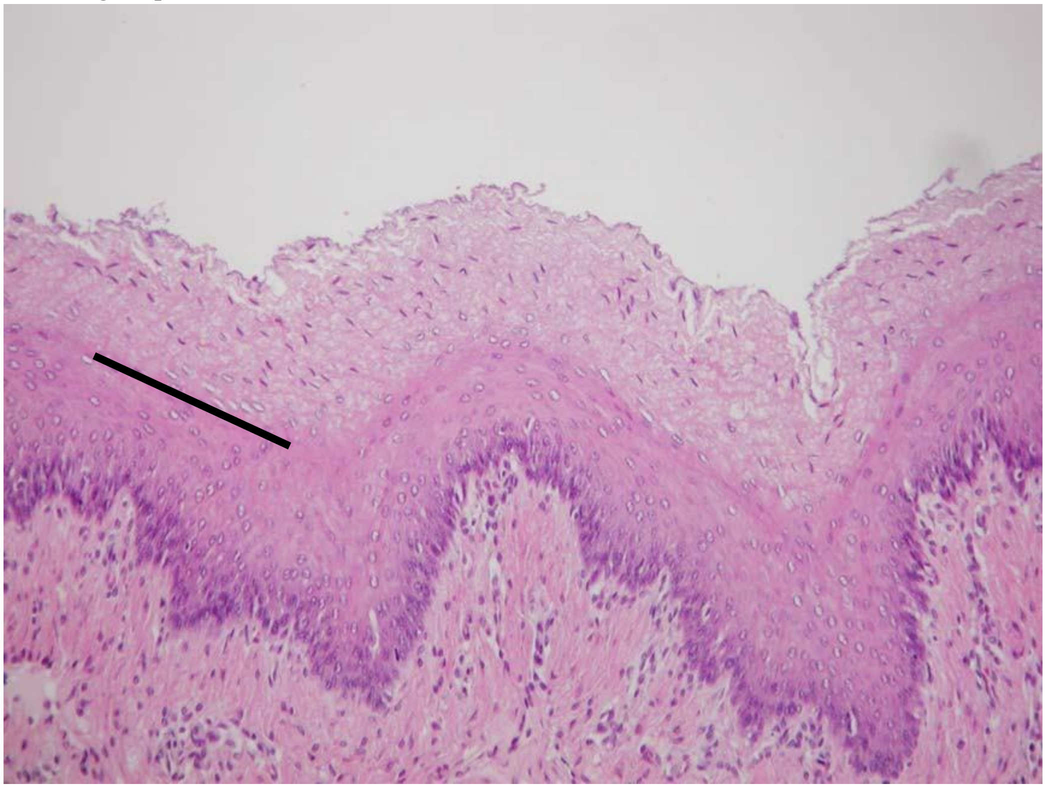


Chart 6. The histopathological photo of the nonglandular region of swine stomach in the DWGE group



In the control group of broiler chickens the height of villi in the small intestine had gradually increased in progress of time until 21 days of age. The average height of villi of the control chickens in the duodenum was 580 µm on Day 1, 1100 µm on Day 10, 1250 on Day 21, and 1100 µm on Day 42, respectively. At every age examined the longest villi could be found in the duodenum, lower ones in the jejunum and the lowest ones in the ileum. The average width of villi has increased on Day 10, 21 and 42, and the infiltration of stratum villosum with lymphocytes, histiocytes and plasmacytes and the fusions of villi could often be observed.
Concerning the chickens fed with DWGE supplemented diet in the laboratory the villi were longer (in the duodenum by 15.7% on Day 10, 21.6% on Day 21 and 33% on Day 42, respectively) and thinner compared to those in the control group (*Chart 7-9.*).

Chart 7. The impact of DWGE on the height of villi in the small intestine of pigs at different age in the laboratory

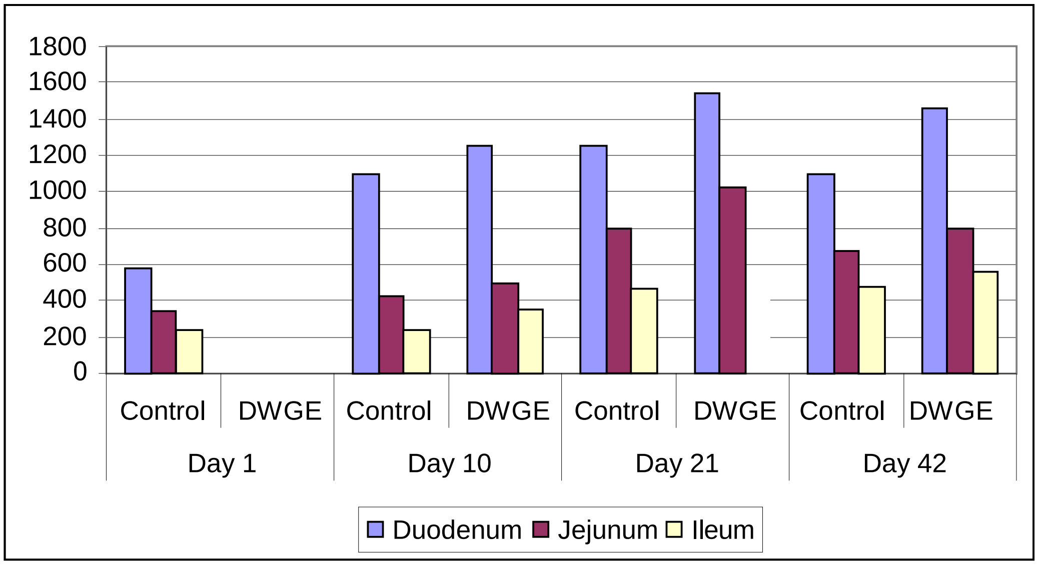


Chart 8. The histopathological photo of villi in the duodenum of broilers in the control group

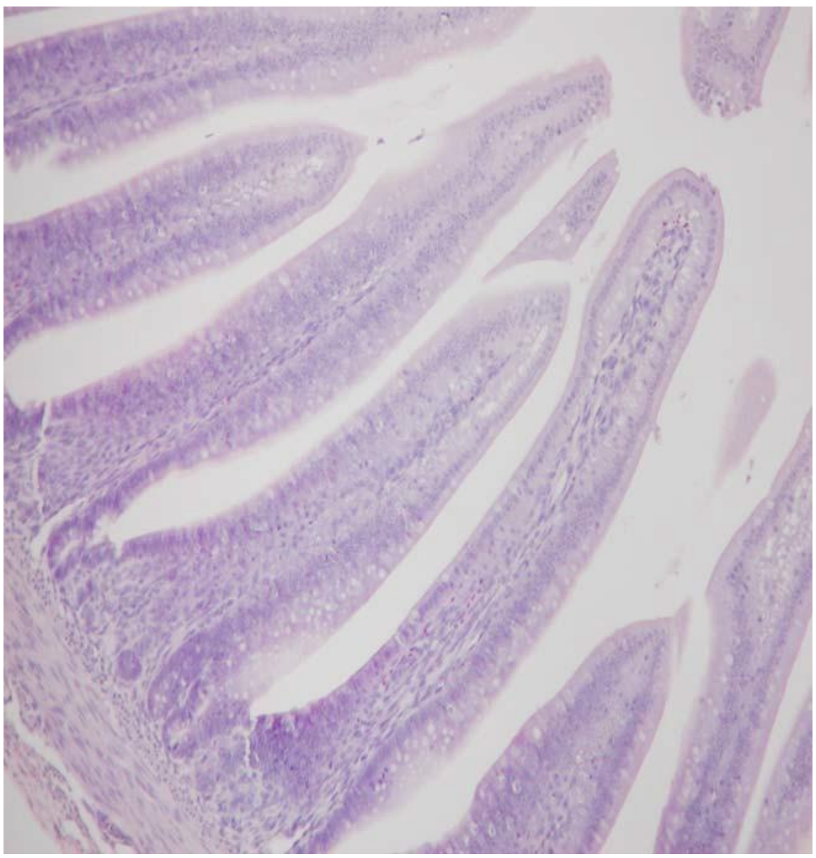
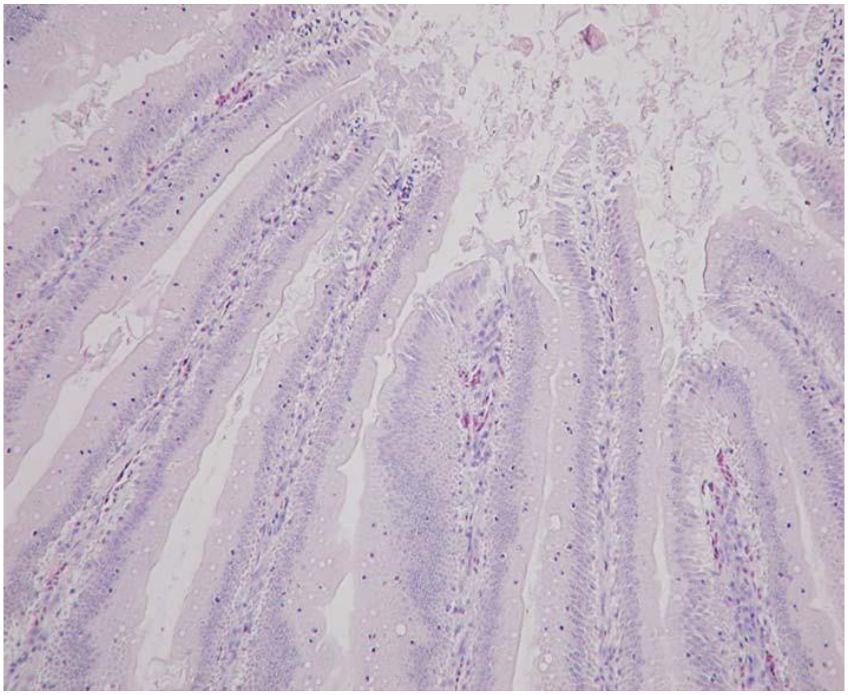
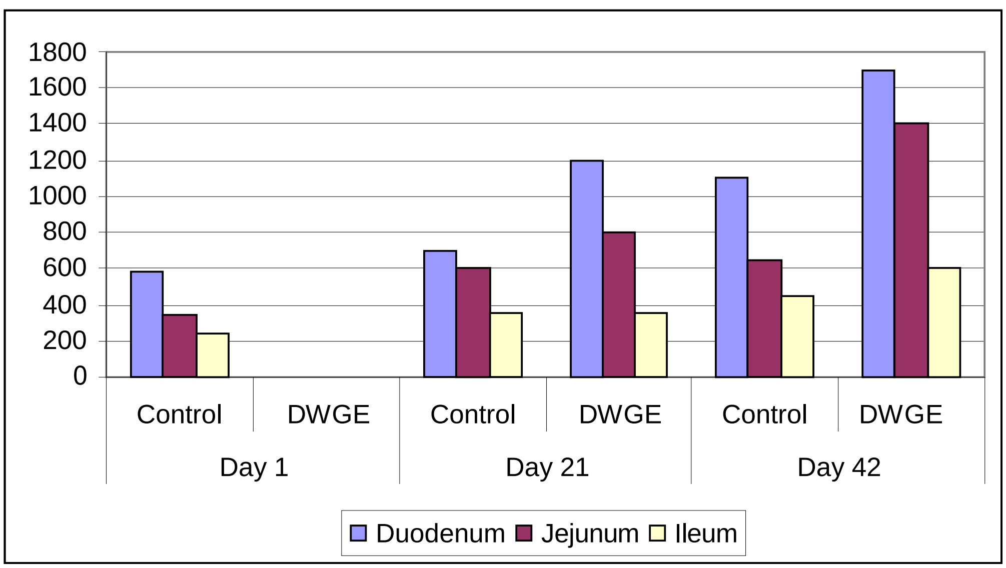


Chart 9. The histopathological photo of villi in the duodenum of broilers in the DWGE group



This difference was even greater under field conditions (in the large-scale commercial broiler flock); in the duodenum the height of villi was greater by 71% on Day 21 and 54% on Day 42 in the DWGE group (*Chart 10.*). Higher villi were detected in the jejunum and ileum of chickens in the DWGE group, as well. The difference was 17.2%, 25.9% and 24.6% in the jejunum, and 14.6%, 10% and 18.9% in the ileum on Day 10, 21 and 42, respectively, in the laboratory. The incidence of villus atrophy, accompanied by widening of the lamina propria, fusion of the villi and leucocytic infiltration of lamina propria in the small intestine was higher in the control group. Regarding the depth of crypts no significant difference was detected between the control and DWGE group at any age.

Chart 10. The impact of DWGE on the height of villi in the small intestine of broilers at different age in the field



DISCUSSION

In animal farming antibiotics are often used for nutritive, preventive and sometimes for therapeutically purposes. Data indicate that antibiotics, administered for nutritive purposes, play significant role in the evolving resistance of some pathogen microorganisms against antimicrobial agents. This is not only a health problem of the animals, but since antibiotics may penetrate to their tissues, thus be converted into human food, they might even threaten human consumption (Danaher et al., 2008). Resistant strains may also cause serious treatment problems in human medicine.

Due to the ban of antibiotic supplementation of farm animals' feed for growth promoting and alleviating the negative effects of the ambient microbiological load extended research has started to substitute the antibiotics in animal nutrition. Large number of dietary substances, e.g. including acidifiers, probiotics, prebiotics, etc. has been investigated with various successes. Among the nutraceuticals DWGE may present promising alternative according to the results of previous experiments and field trials (Jakab et al., 2008; Kósa and Bajcsy, 2008; Kósa et al., 2008; Rafai et al., 2011). The morphological background of gut could have a major part in the revealed significant improvement of weight gain and feed conversion efficiency in both pigs and chickens.

The structure of the intestinal mucosa in the small intestines of chickens and pigs, the parameters of villi (length, width, depth of crypts) have already been surveyed by several researchers (Nabuurs et al., 1993; Pluske et al., 1997; Hetty et al., 1998; Vente-Spreeuwenberg et al., 2003; Yamaguchi et al., 1995; Uni et al., 1998; Xu et al., 2003). In chickens just after hatching the highest villi can be found in the duodenum, while the jejunal villi are lower, but later in the jejunum the villi show a significant growth (Yamaguchi et al., 1995). This explains that the major absorptive part is the duodenum in the early stage of life and then it extends to the jejunum in progress of time. In pigs the significant histological and biochemical changes in the small intestines after weaning play a major role in the decreasing efficacy of digesting and absorption (Nabuurs et al., 1993; Pluske et al., 1997; Hetty et al., 1998; Anonymus, 1999; Vente-Spreeuwenberg et al., 2003). In our experiments and field trials the characteristics of the small intestines and the parameters of villi in the control groups of both pigs and chickens are similar to the findings of other researchers.

The structure of the intestinal mucosa can render some information on gut health. Stressors, which can be found in the digestive tract, can cause relatively quick changes in the intestinal mucosa due to the close proximity of the mucosal surface and the intestinal content. In the small intestines of chickens the length of villi, the depth of crypts and the histological structure of mucosa can be influenced by different factors (Sonmez and Eren, 1999; Xu et al., 2003). Changes in intestinal morphology, such as shorter villi and deeper crypts, have been associated with the presence of toxins (Yason et al., 1987; Anonymous, 1999). Shortening of villi decreases the surface area for nutrient absorption. The crypts can be regarded as the villus factory, and large crypts indicate fast tissue turnover and high demand for new tissue (Yason et al., 1987; Anonymous, 1999). Demand for energy and protein for gut maintenance is higher compared to other organs. A fast-growing broiler devotes about 12% of the newly synthesized protein to the digestive tract (Anonymous, 1999). Any additional tissue turnover will increase nutrient requirements for maintenance and will, therefore, diminish the performance of the animal. These morphological changes (e.g. shortening of villi and larger crypts) can lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, diarrhea, reduced disease resistance, and eventually lower overall performance (Xu et al., 2003).

In our study regarding both pigs and chickens in the DWGE groups the average length of intestinal (duodenum, jejunum, ileum) villi was greater and their width was smaller, representing a larger surface for absorption, in accordance with the better body weight gain and feed conversation ratio. The degree of exfoliation of epithelial cells and the infiltration with lymphocytes, histiocytes and plasmocytes was smaller in the intestinal villi, helping a more effective adsorption.

The average thickness of hyperkeratotic layer of nonglandular region of stomach in pigs and the degree of vacuolar degeneration in this layer was also significantly smaller in the DWGE group, hence, diminishing the risk of oesophageal ulceration, as a common enteral disease in growing pigs.

The special cytological traits of gut associated lymphoid tissue (GALT) reflect its role in the maintenance of immunity of intestinal mucosa. The hyperplasia of GALT and the active germinal centers in the mucous membrane of ileum and in the mesenteric lymph nodes in the DWGE groups could be in connection with the activity of the local immunity of digestive tract, which is in accordance with the experimental findings on the immune- stimulatory effects of DWGE containing (Stipkovits et al., 2004; Kovács et al., 2008; Kósa and Bajcsy, 2008; Rafai et al., 2011). Similar changes in the GALT were observed after administration of different probiotics (Yurong et al., 2005; Huang et al., 2008).

CONCLUSIONS

The healthful and beneficial morphological changes observed in the gut mucosa of farm animals fed DWGE supplemented diet, and consequently the more effective digestion and absorption, could explain the significant improvement of weight gain and feed conversion, and the animals' better immune status in both the laboratory experiments and the field trials conducted in large-scale commercial pig and poultry farms. Nevertheless, further investigations are necessary to evaluate whether the increase of villus height observed in the small intestines is caused by a more favorable intestinal microbial environment due to the DWGE or it is a direct effect of DWGE on the intestinal tissue.

Table 1. The average parameters (μm) of the small intestines in pigs at different age

Intestine / Group / Day of sampling	*DUODENUM*						*JEJUNUM*						*ILEUM*					
	Control			DWGE			Control			DWGE			Control			DWGE		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	C
1	567	65	80				733	68	80				633	63	80			
28	243	140	150	440	95	115	247	140	160	410	98	125	257	145	150	310	95	125
90	225	180	190	310	150	200	275	200	200	310	156	200	190	190	200	280	153	190
160	414	190	200	571	160	230	235	190	210	415	160	300	341	180	200	370	150	220

Note:
a = height of villi
b = width of villi
c = depth of crypts

Table 2. The average parameters (µm) of the small intestines in chickens at different age

	Intestine / Group / Day of sampling	*DUODENUM*						*JEJUNUM*						*ILEUM*					
		Control			DWGE			Control			DWGE			Control			DWGE		
		a	b	C	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
IN THE LABORATORY	1	580	80	70				340	80	35				240	80	38			
	10	1100	170	120	1250	130	120	420	162	100	500	122	101	240	175	62	350	125	64
	21	1250	210	130	1540	155	125	800	200	106	1020	150	110	470	200	95	475	160	95
	42	1100	210	140	1460	165	130	670	200	131	800	173	119	475	186	116	560	171	126
IN THE LARGE-SCALE COMMERCIAL FLOCK	21	700	150	130	1200	150	130	600	200	120	800	148	120	350	200	100	350	164	110
	42	1100	250	170	1700	200	140	650	300	200	1400	200	110	450	300	130	600	195	115

Note:
a = height of villi
b = width of villi
c = depth of crypts