Effects of Energy and Purines in the Diet on Proliferation, Differentiation, and Apoptosis in the Small Intestine of the Pig

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The effects of the energy and purine content in the diet on mucosal cell mitosis, function, and apoptosis in the small intestine of pigs were investigated in two experiments. In experiment I, three groups of five pigs were first fed a commercial diet that contained 9.1 MJ metabolizable energy (ME) per kilogram dry matter (DM) and 16.4% crude protein. It was followed by the experimental diets for 5 days each starting with an energy deficit (5.8 MJ ME/kg DM; 7% crude protein) followed by a high-energy diet with low purine content (14.1 MJ ME/kg DM; 13.6% crude protein; 460 mg purines/kg), or alternatively an isocaloric high-purine diet (2,160 mg purines/kg). During experimental periods, blood samples were drawn daily through catheters for insulin-like growth factor-I (IGF-1) determination. The animals were killed at the end of the corresponding feeding period and gut tissue samples were collected. In tissue samples, IGF-I and parameters for the characterization of mitosis (thymidine kinase [TK], proliferating-cell nuclear antigen [PCNA]) and differentiation (RNA content, alkaline phosphatase, sucrase) were measured. The degree of apoptosis was determined histologically. In experiment II, five pigs were fitted with simple T-cannula at the distal jejunum. They were fed the three experimental diets consecutively for 7 days each and sucrase and alkaline phosphatase were measured in digesta (four samples daily). IGF-I in blood but not in tissue clearly responded to the energy content of the diet with a decrease during the deficit and an increase in the two high-energy groups. However, purines had no additional effect on IGF-I. TK, PCNA, and gut weight showed an energy effect on mitosis, which was paralleled by increased peripheral IGF-I. Purines led to a further increase of mitosis, but IGF-I and gut weight were not increased. The degree of mitosis was correlated with higher activities of sucrase and alkaline phosphatase and also with the number of apoptotic cells. The enzyme activity increased from the deficit to the high-energy group and was further elevated due to purines. The results from experiment II also confirm these effects of energy and purines, because the activities of the enzymes in digesta decreased during energy deficit, but increased due to energy and in addition to purines.

THE GUT IS CHARACTERIZED by a high turnover rate. For the pig, it was estimated that an average 44% of gut protein has to be synthesized daily to maintain the size and function.[1] The daily loss of cells by apoptosis has to be compensated by mitotic divisions of stem cells in the crypts.[2,3] The resulting immature progenitor cells must be differentiated so that their full digestive and absorptive capacity is reached during their migration from the crypt-villus junction to the tip.[4] Maturation was quantified by measuring specific digestive enzymes and transporter proteins.[5]

The cellular processes require an appropriate regulation by endocrine and paracrine factors, which must balance the degree of mitosis with apoptosis.[6] Mitosis is stimulated by an orchestrated action of several growth factors, such as epidermal growth factor (EGF), transforming growth factor-α (TGF-α), and insulin-like growth factor-I (IGF-I).[7,8] The latter is known to play a key role for mitosis in several tissues and has a dual function as a paracrine and endocrine factor.

For differentiation, glucocorticoids seem to play a key role. Such an effect, which is linked to the prenatal rise of glucocorticoids in many species, is well documented.[9,10] Meanwhile a variety of genes that are important for normal function of the epithelium in the gut were shown to be dependent on glucocorticoids.[11] The high concentrations of glucocorticoid receptors in the pig intestine may thus be linked to the assumed function for differentiation of the immature progenitor cells.[12]

The initiation of apoptosis is induced by specific genes, but the mechanisms that balance the degree of apoptosis with mitosis and differentiation are largely unknown.[13] It is likely that glucocorticoids not only initiate differentiation, but also trigger the intracellular mechanisms that ultimately lead to cell death, as supported both by the existence of high concentrations of glucocorticoid receptors in gut tissue[12] and the demonstration of their function as an apoptosis-inducing reagent.[11,14]

This regulatory system also has to ensure that the gut is rapidly adapted to changing demands according to changes in the amount and composition of the diet. IGF-I, for example, mediates the stimulating effect of growth hormone (GH) in an energy-dependent way.[15] Thus, the increased levels of IGF-I-mRNA during refeeding in previously starved rats contribute to the rapid regeneration of the gut epithelium.[16]

Purines in the diet support gut regeneration after severe starvation or surgical resection due to their role as precursors for DNA formation.[17,18] No studies have been conducted yet to determine whether they additionally influence the expression of growth factors. Most of the information was obtained either in cell culture or with experimental animals such as rats after severe surgical pretreatment. The objective of our investigation was to clarify the effects of energy and purine concentrations in the diet on gut adaptation and function in pigs under physiological conditions.

MATERIALS AND METHODS

Experimental Design

Two experiments were performed with pigs fitted with jugular vein catheters.[19] In experiment I, 15 pigs were divided into three groups according to three feeding regimens differing in energy and purine content. They were killed at the end of the feeding period and tissue

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samples were collected for later characterization of growth factor expression and degree of mitosis, differentiation, and apoptosis. In experiment II, five pigs fitted with a simple T-cannula at the distal jejunum[20] were fed consecutively with the three diets and determinations of enzyme activities in digesta were used to characterize the functional state of the gut.

Animals and Feeding

German Landrace pigs (castrated males) were used for the experiments. The 15 pigs in experiment I had an average age of 7 months and weight of 113 kg at the beginning of the experiment. The average age of the five pigs in experiment II was 6 months and the weight 95 kg. The pigs were kept in individual crates and fed the experimental diets (2 kg at 8 AM and 3 PM, respectively). They had free access to water.

The animals were fed with a commercial diet that contained 9.1 MJ metabolizable energy (ME) per kilogram dry matter (DM) and 16.4% crude protein during a 7-day adaptation period. For the experiments, three different diets were designed. One was low in energy (5.8 MJ ME/kg DM; 7% crude protein) and is hereafter referred to the "deficit diet." The second diet had a high energy content (14.1 MJ ME/kg DM; 13.6% crude protein), but a low purine concentration (460 mg purines/kg; "energy diet"); the third had the same high energy and protein content (14.1 MJ ME/kg DM; 13.6% crude protein), but the purine concentration was increased by replacing milk protein with brewers dried yeast (2160 mg purines/kg; "purine diet"). The composition of the diets is given in Table 1.

In experiment I, each diet was fed for 5 days. The first group was killed after feeding the deficit diet by intravenous infusion of 30 to 40 mL of Narcoren (Rhone-Merieux, Laupheim, Germany) and tissue samples were collected. The other two groups then received either the energy or the purine diet for another 5 days and were killed.

In experiment II, all pigs were fed in the same order as described previously, starting with the adaptation ration followed by the deficit diet, the energy diet, and then the purine diet. Each diet was fed for 7 days.

Sampling

Blood samples (10 mL) were taken in both experiments daily at 8 AM and the heparinized plasma was frozen at −22°C until analysis. After each sampling and again in the afternoon, the catheters were rinsed with heparinized saline.

Tissue collection (duodenum and jejunum) in experiment I was performed within 15 minutes after killing. The samples were divided for analytical and histological investigations. The samples were washed with ice-cold physiological saline and were either immediately frozen in liquid nitrogen (analytical samples) before storage at −75°C, or fixed in 4% buffered paraformaldehyde (pH 7.2) overnight at 4°C. Thereafter, these samples were stored in 0.1 mol/L phosphate buffer (pH 7.2) and sent to the histological laboratory, where they were dehydrated in graded ethanol and embedded in paraffin.

Table 1. Composition (%) of the Experimental Diets

Component	Diet		
	Deficit	Energy	Purines
Oat bran (starch-free)	53	—	—
Wheat bran	10	—	—
Wheat	—	42	42
Corn	—	30	30
Oats	—	10	10
Wood powder	25	—	—
Skim milk powder	—	8.7	—
Casein	4	2.3	—
Brewers dried yeast	—	—	11
Mollasses	5	—	—
Soy bean oil	—	4	4
Minerals and vitamins	3	2.8	2.8
Lysine-HCl	—	0.2	0.2

Digesta sampling in experiment II was performed through the cannula in the jejunum four times daily at 9 AM, noon, 3 PM, and 7 PM. The volume varied between 2 and 10 mL depending on the diet. The samples were immediately frozen in liquid nitrogen and then stored at −75°C until enzyme analysis. The skin area around the cannula was carefully washed with warm water after each sampling and then coated with zinc salve to avoid irritation. The cannula were maintained throughout the sampling period without complications.

Histological Evaluation

Histology was used for the characterization of mitosis and apoptosis in experiment I. From all tissue samples embedded in paraffin, sections of 5 to 6 μm were cut and mounted on slides and dried overnight.

To characterize mitosis, the slides were stained for the proliferating-cell nuclear antigen (PCNA), which is a specific marker for cell proliferation.[21,22] The slides were deparaffinized and washed in phosphate buffered saline (PBS; 0.01 mol/L, pH 7.2). To block endogenous peroxidase, the specimen were incubated in 3% H_2O_2 in ethanol for 5 minutes and washed three times for 5 minutes with 0.01 mol/L PBS by aid of a horizontal shaker (all of the following washing steps were performed out similarly). To reduce unspecific binding, the slides were incubated with normal rabbit serum (NRS) in a 1:20 dilution. Thereafter, the PCNA antibody (PCNA AB-1; Dianova Oncogene Science, Cambridge, MA) was applied (1:50) and incubated overnight at 4°C. The slides were washed and the second antibody (Kit K677, Dako, Hamburg, Germany) was added and incubated for 15 minutes. Thereafter, streptavidin-peroxidase was applied and the slides incubated for 15 minutes. Then, the slides were washed again before adding the substrate-chromogen solution and incubated for 5 minutes. After washing with distilled water, they were counterstained with Mayer hematoxilin for 15 seconds, washed with water for 1 hour, and then coverslipped. As negative controls, slides were incubated with PBS instead of the first antibody.

To obtain a proliferation index of PCNA, four different areas from cross sections both of the duodenum and jejunum were randomly evaluated. Each of these areas was composed of six counting fields (161.1 μm × 109.6 μm) piled up in columns from the fundi of the propria mucosal glands via the crypt base of the villi. The PCNA-positive nuclei of epithelial cells were counted using an image analysis system (Softimage software program Analysis 2.0; SCS, Münster, Germany) at a magnification of 40. Thus each index from each cross section was composed of 24 counting fields.

Cells undergoing apoptosis were identified by use of the TUNEL assay (in situ cell death detection kit; POD Boehringer, Mannheim, Germany), which leads to an immunohistochemical staining of apoptosis-specific DNA fragments. After rinsing the deparaffinized specimens with distilled water, they were treated with 20 μg/mL proteinase K in 10 mmol/L TRIS/HCl pH 8.0 for 15 minutes at 37°C followed by washing with 0.01 mol/L PBS. After deactivating endogenous peroxidase as described previously, the slides were washed with PBS followed by incubation with the TUNEL reaction mixture (enzyme and label solution) in a humidified chamber at 37°C for 1 hour. Negative control slides were treated identically, but the enzyme solution was omitted. After rinsing, the sections were incubated with Converter-POD (antifluorescein antibody, Fab fragment from sheep, conjugated with horse radish peroxidase) in the humidified chamber at 37°C for 30 minutes and rinsed again. Incubation with 3-amino-9-ethylcarbazole (AEC) chromogen substrate solution was performed for 15 to 30 minutes at room temperature. The specimens were rinsed with distilled water, mounted with Kaiser glycerol-gelatine (Merck, Darmstadt, Germany),

and coverslipped. The sections were examined by light microscopy at a magnification of 20.

Positive apoptotic reactions were visible as a distinct black total-cell-body precipitation in some of the enterocytes. At least 30 villi per cross section from the duodenum and jejunum of each animal were randomly evaluated.

The evaluation was assessed as a semiquantitative score with grades of 1 to 6, as follows: 1, single apoptotic cells at the villus tip or near the crypt base (Fig 1A); 2, few apoptotic cells at the villus tip; 3, apoptotic cells at the villus tip with decreasing frequency down to the crypt; 4, apoptotic cells at villus tip and along the villus; 5, many apoptotic cells confined to the villus tip (Fig 1B); and 6, many apoptotic cells at the villus tip and occasionally in the crypt base.

Analytical Evaluation of Blood, Tissue, and Digesta

IGF-I determination in blood plasma was performed by radioimmunoassay after acid-ethanol extraction.[23] Similarly, IGF-I in tissue (experiment I) was measured after acid-ethanol extraction of tissue homogenates. The intraassay and interassay variation in pools of tissue homogenates (n = 6) were determined. The intraassay and interassay coefficients of variation were 15.9% and 14.6%, respectively. The lower limit of sensitivity was 13 ng/g tissue. Thymidine kinase (TK) was determined by measuring the conversion of tritiated substrate (thymidine) after removal of unreacted substrate by QAE-Sephadex A-50 columns (Pharmacia, Uppsala, Sweden).[24-26] Alkaline phosphatase was measured by the conversion rate of *p*-nitrophenylphosphate and sucrase by determining the conversion rate of sucrose to glucose.[27,28] The RNA content was measured photometrically after acid guanidinium-thiocyanate-phenol-chloroform extraction.[29]

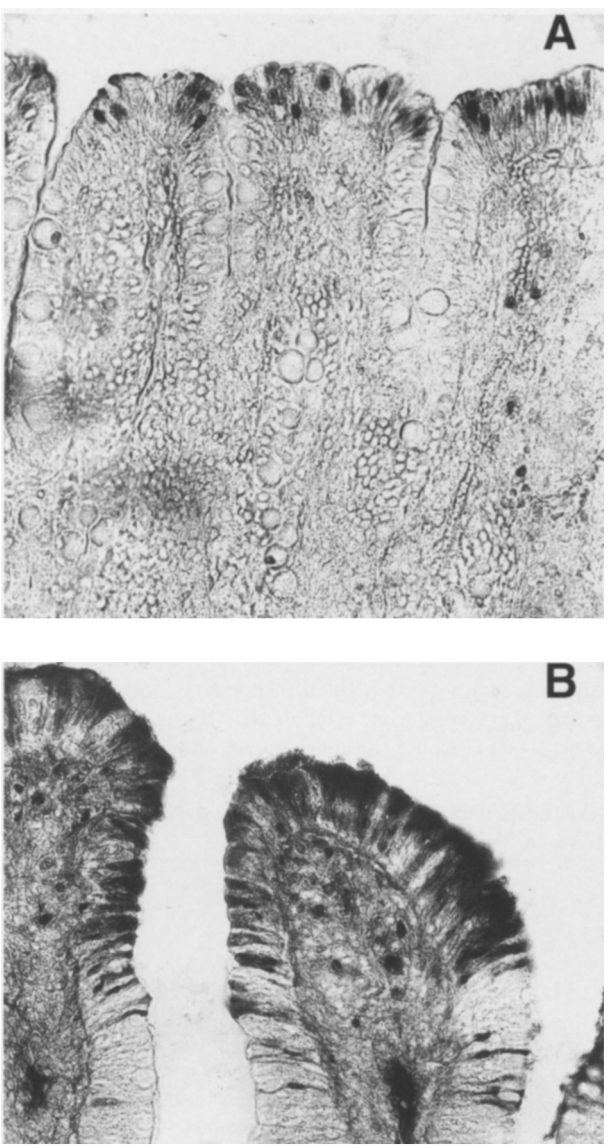

Fig 1. Comparison of apoptosis in gut mucosa from the energy deficit (A; original magnification ×230) and purine group (B; original magnification ×115). These examples correspond to the degree of apoptosis of 1 and 5, respectively.

Table 2. IGF-I Concentrations in Blood Plasma and in Duodenal and Jejunal Tissue, Depending on the Preceding Experimental Diet

Variable	Blood Plasma	Duodenum	Jejunum
LSmeans ± SEM			
Deficit	100.6 ± 3.5 ng/mL	92.3 ± 3.6 ng/g	53.4 ± 3.6 ng/g
Energy	164.2 ± 6.5 ng/mL	112.9 ± 4.9 ng/g	41.8 ± 4.1 ng/g
Purine	160.5 ± 8.6 ng/mL	73.2 ± 3.3 ng/g	43.5 ± 3.3 ng/g
Significances ($P \leq$)			
Deficit *v* energy	.001	.001	.05
Energy *v* purine	NS	.001	NS
Deficit *v* purine	.001	.001	.05

Abbreviation: NS, not significant.

In experiment II, the same enzymes were determined in digesta based on the same methods. Before determination, digesta were centrifuged (1 hour at 3°C; 24.000 × *g*). Aliquot portions of the supernatant were used either directly (alkaline phosphatase) or after removal of endogenous glucose by DEAE anion exchange columns (sucrase). The intraassay and interassay variations in digesta were 2.1% and 3.1% for alkaline phosphatase, respectively. The intraassay and interassay variations for sucrase were 4.1% and 13.5%, respectively.

Statistical Evaluation

The results are given as least squares means (LSmeans) ± SEM and differences between means were tested for significance using the general linear models (GLM) procedure (statistical analysis system [SAS]; SAS Institute, Cary, NC).

In experiment I, differences between the experimental diets were checked for each group separately (main effects: individual and experimental diet). Furthermore, each tissue parameter was analyzed between groups (main effect: group). In experiment II, differences between the experimental diets were analyzed (main effects: individual and experimental diet).

RESULTS

Experiment I

The IGF-I concentrations in peripheral plasma and in duodenal and jejunal tissue are shown in Table 2. In blood plasma, IGF-I was influenced by the level of energy in the diet, because in the high-energy diet, the concentrations were more than one third higher compared with the low-energy diet ($P \leq .001$); purines had no effect on IGF-I concentrations in plasma. The IGF-I concentrations in tissue were usually higher in duodenal compared with jejunal tissue, but a relationship to the diet could not be detected. In the duodenum, a highly significant increase due to energy was obvious when compared with deficit. However, purines caused significantly lower concentrations of IGF-I. In the jejunum, no dependency on the diet was obvious at all.

Table 3. Parameters for the Mitotic Activity in Duodenum and Jejunum and the Resulting Weight, Depending on the Preceding Experimental Diet

Variable	TK		PCNA-Stained Cells		Weight	
	D	J	D	J	D	J
LSmeans ± SEM						
Deficit	4.7 ± 1.4 U/mg protein	4.4 ± 2.3 U/mg protein	14.13 ± 1.24	14.77 ± 1.11	69 ± 13 g	1,558 ± 101 g
Energy	8.2 ± 1.4 U/mg protein	8.4 ± 2.3 U/mg protein	18.28 ± 0.83	22.35 ± 0.89	94 ± 16 g	2,447 ± 124 g
Purine	10.4 ± 1.4 U/mg protein	11.1 ± 2.3 U/mg protein	21.36 ± 1.57	20.92 ± 1.57	101 ± 21 g	2,472 ± 146 g
Significances ($P \leq$)						
Deficit *v* energy	NS	NS	.01	.001	NS	.001
Energy *v* purine	NS	NS	.05	NS	NS	NS
Deficit *v* purine	.01	.05	.001	.001	NS	.001

Abbreviations: D, duodenum; J, jejunum.

The mitotic activity is presented in Table 3. The activity of TK did not differ between the duodenum and jejunum, but depended on the diet; the lowest activities were found for the deficit. The energy diet led to a nearly twofold increase in TK activity. The purine diet led to a further increase in duodenum and jejunum as well. Due to the limited number of animals, the individual evaluation of the two compartments reached the level of significance only for the comparison of the deficit and purine group (duodenum, $P \leq .001$; jejunum, $P \leq .05$). However, when taking the two compartments together, significant differences were demonstrated for the deficit versus energy group ($P \leq .001$), the deficit versus purine group ($P \leq .001$), and the energy versus purine group ($P \leq .05$), respectively.

The results on the number of PCNA-stained cells further support the differences (Table 3). A significant effect of the high-energy diet on the number of mitotic cells was obvious in the duodenum and jejunum, as well when compared with the deficit. However, a further increase due to purines was only demonstrated for the duodenum.

Differences in the weight of the two gut compartments are obvious for the jejunum. Thus, energy led to a significant, 1.6-fold increase in weight compared with the deficit, but purines had no additional effect. The weight of the duodenum tended to show the same food-dependent increase, but the differences were not significant.

The functional state of the small intestine is characterized by the RNA content and the brush-border enzymes (Table 4). The RNA concentrations increased in both compartments depending on the previous dietary treatment. It was lowest in the deficit group, intermediate under high energy, and further increased due to purines. In the jejunum, all values differed significantly. In the duodenum, the increase from the energy to the purine group was not significant.

The activities of alkaline phosphatase and sucrase were also usually higher in the jejunum. The activity of alkaline phosphatase in the duodenum increased from the deficit to the high-energy ration. A further increase was found due to purines. However, all of these values in the duodenum showed high variation, so that the level of significance was not reached in these groups of five animals. However, in the jejunum, both the increase from the deficit to purines and from energy to purines was significant.

Sucrase activity was also higher in the jejunum compared with the duodenum. In the jejunum, no effect of the feeding regimens could be detected, whereas sucrase activity in the duodenum increased due to energy and additionally due to purines, but the differences were not significant.

The degree of apoptosis is presented in Table 4. In Fig 1, histological examples from the deficit group (Fig 1A) and the purine group (Fig 1B) are presented. Within the different feeding groups, the reaction of the individual animals was extremely uniform, so that all individuals within a group showed the same degree of apoptosis. The lowest degree of apoptosis was found in the deficit group. Additionally, the short

Table 4. Parameters for Gut Differentiation, Function, and Apoptosis (scale 1-6) in Duodenum and Jejunum, Depending on the Preceding Experimental Diet

Variable	RNA		Alkaline Phosphatase		Sucrase		Degree of Apoptosis	
	D	J	D	J	D	J	D	J
LSmeans ± SEM								
Deficit	2.26 ± 0.18 mg/g tissue	2.34 ± 0.18 mg/g tissue	149.4 ± 43.3 U/mg protein	333.6 ± 36.0 U/mg protein	1.5 ± 3.2 mU/mg protein	21.4 ± 3.2 mU/mg protein	1	2
Energy	3.36 ± 0.20 mg/g tissue	3.10 ± 2.20 mg/g tissue	202.2 ± 43.3 U/mg protein	329.2 ± 36.0 U/mg protein	2.8 ± 3.2 mU/mg protein	22.5 ± 3.2 mU/mg protein	3	4
Purine	3.73 ± 0.16 mg/g tissue	3.93 ± 0.16 mg/g tissue	224.7 ± 39.5 U/mg protein	432.8 ± 32.9 U/mg protein	4.8 ± 3.0 mU/mg protein	17.8 ± 3.0 mU/mg protein	5	6
Significances ($P \leq$)								
Deficit *v* energy	.001	.001	NS	NS	NS	NS		
Energy *v* purine	NS	.001	NS	.01	NS	NS		
Deficit *v* purine	.001	.001	NS	.01	NS	NS		

villi point to atrophy of gut tissue. In the purine group, the highest degree of apoptosis is visible. Compared with the deficit group, the villi were elongated. In the duodenum, apoptosis increased from degree 1 (Fig 1A) to degree 3 (energy) and even 5 (purines) (Fig 1B). In the jejunum, the degree of apoptosis was also dependent on the feeding regimen (deficit, 2; energy, 4; purines, 6), but was usually higher than in the duodenum.

Experiment II

Experiment II focused on a continuous monitoring of enzymes in digesta during the feeding periods. The results of the determination of alkaline phosphatase are presented in Fig 2. Figure 2 also includes the LSmeans values and the SEMs during the four feeding periods. During the control period, the mean activity was 5.9 U/mL digesta. After a transient increase, the activity decreased continuously during the deficit period to a minimum of 1.5 U/mL at the onset of the energy phase. The high energy led to a continuous increase with a delay of 1 day. The activities remained relatively stable at a high level (11.26 U/mL digesta) during the purine period. The activities between the control period and the deficit did not differ significantly due to the transient increase at the beginning of the deficit. However, the energy and deficit period differed significantly ($P \leq .05$), as well as the energy and purine period ($P \leq .001$) and purine versus deficit period ($P \leq .001$).

The determination of sucrase activity (Fig 3) led to similar results. From an activity of 117.4 mU/ml in the control period, the deficit led to a decrease (61.3 mU/mL; $P \leq .01$). The activity increased again about 3.5-fold due to the energy ration (217.7 mU/mL; $P \leq .001$). An additional increase could be attributed specifically to purine supplementation (341.4 mU/mL). Compared with the control period, the increase due to energy was twofold ($P \leq .001$) and threefold ($P \leq .001$) when comparing energy and purines.

DISCUSSION

A positive effect of the diet on gut proliferation and regeneration was shown in a variety of species mainly by refeeding after starvation or surgical resection of the small bowel.[8,30] For the pig, an influence of nutrition on gut morphology and function was only demonstrated during the early suckling period.[31,32] In

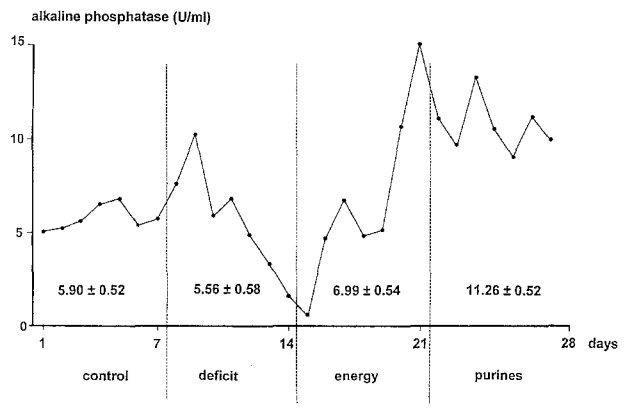

Fig 2. Course of the activity of alkaline phosphatase in digesta (5 pigs, 4 samples/d) during the consecutive feeding periods (LSmeans ± SEM).

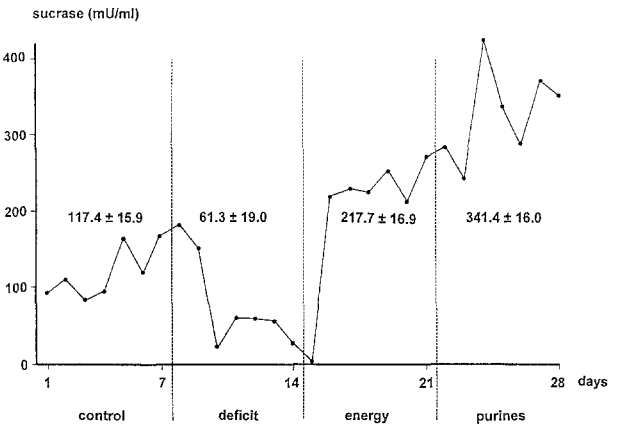

Fig 3. Course of the activity of sucrase in digesta (5 pigs, 4 samples/d) during the consecutive feeding periods (LSmeans ± SEM).

only a few studies, positive effects of the diet on proliferation and function were attributed to specific components of the diet. In rats, for example, it was demonstrated that a deprivation of nucleotides in the diet or total parenteral nutrition depresses regenerative processes in the gut.[33,34] A positive effect of glutamine and polyamines on gut proliferation was also described; the latter group appears to contribute to differentiation.[35,36]

In our study, systematic effects of the energy level and purines in the diet on the regulation of gut cell mitosis, function, and apoptosis were investigated. The ultimate criterion of gut function was the course of enzyme activities in digesta (experiment II) over the consecutive feeding regimens. Whereas the sucrase activity may also have been influenced by substrate induction during the high energy period, the further increase during the purine period rather suggests an indirect influence via an increase in the mitotic rate, as was also confirmed in experiment I. Moreover, the energy-deficit diet contained 5% molasses, but gave the lowest activity of sucrase. The activity of alkaline phosphatase is similar to the course of sucrase and additionally confirms that the changes are predominantly due to changes in gut-cell proliferation. The feeding regimens led to significant differences in the activity of sucrase and alkaline phosphatase even if the duration of the varying feeding periods was limited to 7 days. A still more pronounced effect might have resulted when the enzymes are allowed to reach a steady-state level by extending the duration of the feeding periods.

The results of experiment II are also supported by the results of experiment I. In experiment I, the counteracting phenomena of mitosis and apoptosis could be quantified and attributed to the effects of energy and purines.

A clear effect of the energy content on the proliferation rate could be shown. This effect is probably mediated by IGF-I as was reported for the rat.[13,37] In our study, IGF-I in peripheral plasma responded to the energy supply, whereas tissue concentrations were largely independent on the energy level. The absence of a reaction of tissue IGF-I does not agree with data from the rat. However, in these studies, the increase of tissue IGF-I mRNA occurred only when preceded by complete starvation.[7]

A general effect of energy on circulating concentrations of

IGF-I is well known for the pig.[15] However, the effect of purines on the mitotic activity is not mediated via IGF-I. Rather, the IGF-I–dependent mitotic capacity can only be fully realized when purines are available. A rate-limiting function of purine availability was reported for mitosis in gut tissue and in other tissues with rapid cell turnover.[17,34]

The onset of function in this study was characterized by the determination of sucrase and alkaline phosphatase in tissue and digesta as well. The same criteria were also used in other studies[38] to characterize the functional state. The activities of enzymes in tissue and digesta usually reflect the changes in the mitotic rate. The onset of functional maturity probably is dependent on glucocorticoids. This role was proven for the first onset of function around birth.[10] A specific role of glucocorticoids for gene expression is also known for sucrase and alkaline phosphatase, as well as for other gut-specific enzymes.[11] This role in the gut is also supported by the high and variable amounts of glucocorticoid receptors in gut tissue, whereas changes of cortisol concentrations in peripheral blood did not vary depending on the diet.[12,39]

The results from experiment I demonstrate that a balance exists between mitosis and apoptosis; because apoptosis increased whenever mitosis was accelerated. This balance may be explained by a dialog between glucocorticoids and the IGF-I/GH axis.[40] An interaction between these two systems is also supported by the fact that the GH gene is also regulated by a glucocorticoid-responsive element.[41] On the other hand, glucocorticoids are functioning as a switch for apoptotic signals.[11] Even if a dialog between mitosis and apoptosis does exist, the regulatory system leaves enough space to allow changes in gut morphology and function. In the present study, eg, high energy led to a remarkable increase in gut weight, as well as in enzyme activities. This adaptation is explained by a delay of apoptosis due to IGF-I leading to a prolonged lifespan of mature cells.[42,43]

However, in the case of purine supplementation, the mitotic rate and apoptosis increased without a further increase of IGF-I and tissue weight. However, the RNA content and the enzyme activities increased. It appears, therefore, that the effects of purines do not require additional endocrine or paracrine stimuli, but rather are explained by a higher availability of this rate-limiting precursor for nucleotide synthesis. An effect of a nucleoside-nucleotide mixture in total parenteral solutions on intestinal integrity and DNA content has been shown previously in rat experiments.[44]

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