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The influence of apple- and red-wine pomace rich diet on mRNA expression of inflammatory and apoptotic markers in different piglet organs

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Abstract

Flavan-3-ols are a class of flavonoids that are widely distributed in fruits and beverages including red wine and apples. Consumption of flavanoid-rich food has been shown to exhibit anti-microbial, anti-oxidative, anti-inflammatory, and immune-modulating effects. To test the nutritional effects of flavanols on mRNA gene-expression of inflammatory and apoptotic marker genes, piglets were given two flavanoids-rich feeding regimens: a low flavanoid standard diet (SD) was compared with diets enriched with 3·5% apple pomace (APD) or 3·5% red-wine pomace (RWPD). The influence on mRNA expression levels was investigated in different immunological active tissues and in the gastro-intestinal tract (GIT). The investigation took place from 1 week prior weaning to 19 days post weaning in 78 piglets. The expression of expressed marker genes was determinate by one-step quantitative real-time (qRT-PCR): TNFα, NFκB as pro-inflammatory; IL10, as anti-inflammatory; caspase 3 as apoptosis; cyclin D1 as cell cycle marker; and nucleosome component histon H3 as reference gene.The feeding regimens result in tissue individual regulation of mRNA gene expression in all investigated organs. It was discovered that there were significant differences between the applied diets and significant changes during feeding time curse. Both pomace treatments caused a significant up-regulation of all investigated genes in liver. The effect on mesenterial lymph nodes and spleen was not prominent. In the GIT, the treatment groups showed a inhibitory effects on gene expression mainly in stomach and jejunum (NFκB, cyclin D1 and caspase 3). In colon the trend of caspase 3 was positive with the greatest change in the RWPD group.In jejunum and stomach the cell cycle turn over was reduced, whereas in liver the cell turn over was highly accelerate. The influence on inflammatory marker gene expression is mainly relevant in stomach. It is presume that both flavanoid rich feeding regimens have the potential to modulate the mRNA expressions of inflammatory, proliferation and apoptotic marker genes in the GIT and piglet organs.

Keywords: *apple pomace, genetic markers, grape pomace, piglets, messenger RNA, polymerase chain reaction.*

Introduction

Flavan-3-ols are a class of flavonoids that are widely distributed in fruits and beverages including green tea, red wine, chocolate and apples (Waterhouse *et al.*, 1996; Hammerstone *et al.*, 1999; Damianaki *et al.*, 2000; Guyot *et al.*, 2003). Although their intake levels are not precisely known, they are likely the most abundant flavanoids in the vertebrate diet with estimates of consumption ranging from 0·1 to 0·5 g/day (Santos-Buelga and Scalbert, 2000; Scalbert and Williamson, 2000). In addition to widely reported *in vitro* biological activities of flavan-3-ols (Waterhouse *et al.*, 1996), consumption of purified monomers and food containing predominantly flavan-3-ols has been shown to reduce platelet activity, fatty streak development and certain types of cancers (Eberhardt *et al.*, 2000; Santos-Buelga and Scalbert, 2000). Animal models observed that polyphenols have preventive activity against cancer of the oral cavity, oesophagus, stomach, intestine, colon, liver, lung, prostate, and skin (Lambert and Yang, 2003). Further flavonoids exhibit anti-microbial, anti-oxidative, anti-thromboses, immune-modulated, anti-inflammatory, and showed blood pressure and blood-glucose reducing effects (Leitzmann, 2003). In western countries apples were one of the main sources of dietary flavanoids that showed the strongest associations with decreased human mortality (Boyer and Liu, 2004). The particular mechanisms of action are impossible to establish without a complete understanding of their uptake as well as their metabolism and distribution among tissues and cells (Boyer and Liu, 2004). The average of the total polyphenol content in apple is 3 g per kg fresh weight

(0·5 to 11 g/kg) (Stoll, 1997) and about 2 mg/l red wine (Scalbert and Williamson, 2000). The pomace of apples and grapes also has a high concentration of these polyphenols, whereas the main components are flavan-3-ole and proanthocyanidine (Stoll, 1997; Shrikhande, 2000).

Since 1946, sub-therapeutical levels of antibiotics have been used as growth promoters in pig meat production, and became an important commercial factor in pig production. However, the use of antibiotics as food additives is risky due the development of antibiotic resistance in pathogens (Aarestrup, 1999; Bach Knudsen, 2001). Since 1 January 2006 the antibiotics in animal food is official forbidden by the EU (Regulation 1831/2003/EC). Many studies assume, that antibiotics and special diets change the intestinal morphology and therefore affect health (Hedemann *et al.*, 2002; Boudry *et al.*, 2004; Kotunia *et al.*, 2004). In the recent literature alternative food ingredients are discussed, e.g. probiotics, prebiotics, synbiotics, as well as secondary plant components (Scalbert and Williamson, 2000; Bach Knudsen, 2001).

In this approach two different flavanol-rich feeding regimens were applied in 78 young growing piglets (freeze-dried apple and red-wine pomace). The goal of this study was to show the influence of the different flavanol-rich diets on mRNA expression of pro- and anti-inflammatory, apoptotic and proliferation markers in different organs and the gastro intestinal tract (GIT).

Material and methods

Animals

In this study 78 cross breed piglets (Pietráin × (Deutsche Landrasse × Deutsches Edelschwein)) were given three different food compositions. The animals were housed at the experimental station Osterseeon (Bayerische Landesanstalt für Landwirtschaft, Institut für Tierernährung und Futterwirtschaft). A total of 30 animals were given a control standard weaning diet (SD) (per kg: 500 g wheat, 230 g barley, 220 g soya, 10 g soya oil, 40 g vitamins and minerals), and 24 each received additionally 3·5% dry mass apple pomace (APD) or red-wine pomace (RWPD). Dry matter (DM), crude protein, crude lipids, crude fibre, mineral and marker amino-acid contents were balanced in all three diets (Table 1). Polyphenol analysis was made from the applied diets via high-resolution high-pressure liquid chromatography (HPLC) analysis as described earlier (Treutter, 1989). The individual flavanol peaks were subjected to the procedure of chemical reaction detection using DMAZA (1% DMAZA in MeOH p.A./ H_2SO_4, 1·5 mol/l (11/1) (v/v)) as staining reagent. The phenolic compounds were detected with an inert UV-VIS detector (Gynkotek, FRG) at 640 nm (Treutter, 1989) and calculated as chlorogenic acid. APD contains 5·8 mg/g DM total polyphenols, RWPD 12·8 mg/g DM total polyphenols, whereas SD contains only 3·8 mg/g DM (unpublished data).

Live weight of the piglets was monitored 1 week before weaning (live day 24, treatment day 0), at weaning (live day 31) and at day 36, day 43 and day 50 (treatment day 26) at slaughtering. At weaning, piglets were grouped with identical age (31·0 ± 1·8 days), weight (7·5 ± 1·1 kg) and half male and female in each feeding group. At weaning and every following week 6 SD pigs, 6 APD- and 6 RWPD-diet piglets were slaughtered. Piglets were killed by electric gripper and exsanguinated. A horizontal incision along the mid line was made to open the abdominal cavity, and all the organs and the GIT were excised. Empty body weight, kidneys, spleen and liver weights were measured at sampling. Tissue sample from mesenterial lymph nodes, spleen, liver, kidney, muscle (*longissimus dorsi*), stomach, jejunum, ileum and colon were dissected, cut into small pieces (about 0·5 cm^3), aliquoted and immediately frozen in liquid nitrogen. Tissues were stored at −80°C until total RNA extraction.

mRNA extraction

Total RNA of the different tissues was isolated using TriFast (Peqlap, Erlangen, Germany) according to the manufactures instructions. To quantify the extracted RNA concentration, the optical density was determined in triplicates at three different dilutions of the final total RNA preparations at 260 nm. RNA integrity was verified by optical density $OD_{260\,nm}/OD_{280\,nm}$ absorption ratio $>1{\cdot}90$.

Quantitative one-step real-time polymerase chain reaction

Relative-quantification of mRNA concentration was carried out using a quantitative one-step real-time polymerase chain reaction (qRT-PCR) in the ep realplex Cycler (Eppendorf, Hamburg, Germany). To minimize pipetting errors an eight-channel pipetting robot ep Motion 5075 (Eppendorf) was used. 25 ng total mRNA in 1 μl volume was inserted as RT-PCR template. Further the master-mix components for the qPCR reactions (0·3 μl iScript (Bio-Rad, Munich, Germany), 0·225 μl (20 pmol) of forward and reverse primer (Table 2) synthesized by MWG Biotech (Ebersberg, Germany), 7·5 μl 2× SYBR Green (Bio-Rad) and up to 14 μl water) was assembled by the robot. One-step qRT-PCR was performed with 40 cycles and product-specific annealing temperature, according to the manufactures cycle settings (Bio-Rad). The crossing points (CP) were acquired with the cycle threshold method

Table 1 *Dry matter, protein, fat, fibre, nitrogen-free extract (Nfe), metabolizable energy (ME), minerals and marker amino acids of the different diets in (g) or (mg) per kg dry matter (the feeding was according to GFE and 'Gruber Futterwerttabelle')*

	Dry matter (g)	Crude protein (g)	Crude lipids (g)	Crude fibre (g)	NfE (g)	ME (MJ)	Ca (g)	P (g)	Na (g)	K (g)	Mg (mg)	Cu (mg)	Zn (g)	Lys (g)	Met (g)	Cys (g)	Thr (g)	Trp (g)
Standard diet	878·0	188·8	25·2	38·3	564·6	12·93	8·4	5·1	2·0	7·6	2·5	179·4	182·4	10·5	3·5	4·6	6·9	2·2
Apple pomace diet	880·0	182·7	25·2	42·6	571·6	12·95	8·5	5·0	2·0	7·4	2·5	157·2	184·8	10·9	3·2	4·3	6·8	2·1
Red-wine pomace diet	880·0	188·8	30·5	45·2	556·8	12·95	7·4	4·8	1·7	7·9	2·3	134·0	167·4	10·4	3·2	4·1	6·4	2·2

Table 2 *Sequence of forward and reverse primers for quantitative one-step qRT-PCR*

Gene	Forward primer	Reverse primer
Histon H3	act ggc tac aaa agc cgc tc	act tgc ctc ctg caa agc ac
TNFα	ccc cca gaa gga aga gtt tc	ttg gcc cct gaa gag gac
IL10	act tta agg gtt acc tgg gtt g	gta gac acc cct ctc ttg ga
NFκB	ggt gga gaa ctt tga gcc tc	cca gag acc tca tag ttg tcc
Caspase3	tgt gtg ctt cta agc cat gg	agt tct gtg cct cgg cag
CyclinD1	tcc tgt gct gcg aag tgg a	ggt cca ggt agt tca tgg c

present in the ep realplex analysis software (Eppendorf). Amplified PCR products underwent a melting curve analysis after the last cycle to validate specificity and integrity of amplification. Finally a cooling step was performed. A relative-quantification was applied, using Histon H3 as reference gene and a panel of physiological marker genes shown in Table 2, according to the model established by Livak and Schmittgen (2001). The reference gene Histon H3 mRNA expression remained constant during the entire study and was affected neither by the treatment time nor by the applied diet. Single target genes mRNA expression were normalized by Histon H3 mRNA expression, comparable with the ΔΔCP method, assuming optimal amplification efficiency of two (Schmittgen, 2001; Livak and Schmittgen, 2001), (equations 1 and 2).

$$\Delta CP_{(\text{Histon–marker gene})\ \text{day}\ 0/7/12/19\ \text{or}\ 26} = CP_{(\text{Histon})} - CP_{(\text{marker gene})} \quad (1)$$

In a second step, each ΔCP value at the defined time point at day 7, 12, 19 or 26 was compared with the mean expression CP of the control group at the first slaughter date (treatment day 0), according to equation 2:

$$\Delta\Delta CP = CP_{(\text{Histon–marker gene})\ \text{day}\ 0} - \Delta CP_{(\text{Histon–marker gene})\ \text{day}\ 7/12/19\ \text{or}\ 26} \quad (2)$$

Positive ΔΔCP values represent an up-regulation and negative ΔΔCP values represent a down-regulation of the described mRNA gene expression, compared with the control group at day zero.

Statistical evaluations

Means of reference gene Histon H3 were compared with two-way ANOVA (time point and applied diet). To test the trend of treatment over 26 feeding days a linear regression was made from every tissue, target gene and feeding group using Sigma Stat (1995). P values less than 0·05 were considered significant ($P < 0{\cdot}05$).

Results

Daily gain and organ weights

All animals remained healthy during the feeding experiment and no animal losses were registered. Some of the piglets excreted pasty faeces, but none of the pigs became ill or needed treatment for any disease. Energy, food uptake, and average daily gain did not differ between dietary treatments. Over all feeding groups the daily food intake was 130 g/day in the 1st, 310 g/day in the 2nd and 550 g/day in the 3rd week after weaning. In the first treatment week before weaning, we had weight losses of 152 g/day (no. = 18) and afterwards weight increase of 239 g/day (no. = 18), 299 g/day (no. = 18), and 262 g/day (no. = 18), in the single treatment weeks. At the five different slaughter dates the piglets weighed 8·6 ± 0·7 kg, 7·5 ± 1·1 kg, 8·7 ± 1·0 kg, 10·8 ± 1·4 kg, and 13·15 ± 1·6 kg. Live weight increased significantly with age ($P < 0{\cdot}001$), however, the three feeding groups showed no differences in live weight and daily gain at within different slaughter dates. Therefore the empty body weight at slaughtering was not affected by the dietary treatments.

Relative weights of spleen, kidney and liver, compared with total weight at slaughtering, are shown in Table 3. Relative spleen and two-kidney weights showed no significant differences between the different feeding groups, whereas the absolute weight increased numerically during the observation time. During the study the absolute liver weight increased significantly ($P < 0{\cdot}001$). At the end of the study relative liver weight was significantly ($P = 0{\cdot}003$) higher in the RWPD-group.

mRNA expression and total RNA

Extracted total RNA contents showed no significant variations in RNA integrity and quantity between analysed feeding groups. All tested genes were abundant in all tissues, showing high specificity, single peaks in melting curve analysis (ep realplex software, Eppendorf), and a single band in high-resolution 4% agarose gel electrophoresis (gels not shown). Five different marker genes were determinate in qRT-PCR: (1) pro-inflammatory and apoptosis inducing marker: TNFα; (2) transcription factor: NFκB; (3) anti-inflammatory marker: IL10; (4) apoptotic marker: caspase 3; (5) proliferation and cell-cycle markers: cyclin D1.

Table 3 *Relative organ weights of piglets in (g organ per kg body weight) at slaughter (six piglets were in each feeding group: SD = standard diet, APD = apple pomace diet, RWPD = red-wine pomace diet)*

		Spleen weight (per ml body weight) (g/kg)		2-kidneys weight (per ml body weight) (g/kg)		Liver weight (per ml body weight) (g/kg)	
Feeding	Day	Mean	s.d.	Mean	s.d.	Mean	s.d.
SD	0	2·84	0·34	5·26	0·35	22·21	1·94
SD	7	2·88	0·85	4·95	0·72	19·75	1·25
APD	7	2·61	0·41	5·18	0·43	20·32	2·43
RWPD	7	2·13	0·35	5·50	0·91	21·10	2·40
SD	12	2·37	0·68	5·13	0·88	19·55	1·89
APD	12	2·33	0·58	5·22	1·22	22·08	4·05
RWPD	12	2·24	0·38	4·34	0·74	19·30	1·12
SD	19	2·08	0·30	4·72	0·70	21·07	1·03
APD	19	2·14	0·60	4·35	0·53	20·68	1·49
RWPD	19	1·96	0·27	4·66	0·44	21·93	1·65
SD	26	2·62	0·60	4·77	0·29	21·21[a]	1·43
APD	26	2·93	1·01	5·09	0·28	22·95[b]	1·92
RWPD	26	2·75	0·61	5·18	0·55	25·52[ab]	3·33

[a,b,c]Superscript indicates significant differences between feeding groups ($P < 0{\cdot}05$).

Only significant gene expression results of the analysed marker genes in the GIT are summarized in Table 4, all group differences are listed in Table 6a to d. In the stomach, the mRNA expression of NFκB decreased highly significant over time in the treatment groups (APD and RWPD; $P < 0{\cdot}001$). A slight decrease of NFκB expression in jejunum was measured in the SD group, in colon the APD group showed a slight increase. The trend of TNFα expression was negative in stomach (APD; $P < 0{\cdot}05$), jejunum (SD; $P < 0{\cdot}01$) and ileum (RWPD; $P < 0{\cdot}01$), and positive in colon (SD; $P < 0{\cdot}001$, APD; $P < 0{\cdot}01$). The GIT of the RWPD group showed diverse trends in the IL10 mRNA expression. There was an increase in the jejunum ($P < 0{\cdot}01$) and the colon ($P < 0{\cdot}001$) and a decrease in the ileum ($P < 0{\cdot}001$). The mRNA expression of cyclin D1 showed a lot of expression changes. In the stomach (APD and RWPD; $P < 0{\cdot}001$) and the jejunum (APD $P < 0{\cdot}01$, RWPD $P < 0{\cdot}05$) cyclinD1 expression decreased significantly, whereas the ileum showed an increase in RWPD group. The trend of cyclin D1 expression in the colon showed a decrease in SD group ($P < 0{\cdot}01$) and APD group ($P < 0{\cdot}05$). In contrast caspase 3 expression in the colon increased, and decreased in the jejunum. The trends in the treatment groups where greater than in the control group. The RWPD group showed a positive trend in the jejunum, too.

Gene expression results in immunological tissues, as well as in piglets' organs and are summarized in Table 5. All group differences are listed in Table 6e to i. There were no significant changes in the mRNA expression of mesenterial lymph nodes, kidneys and spleen, except for IL10 and caspase 3 expression. Spleen (SD $P < 0{\cdot}05$) and kidneys (SD $P < 0{\cdot}01$, APD $P < 0{\cdot}01$) showed an increase of IL10 expression, and spleen (APD $P < 0{\cdot}05$) and kidneys (SD $P < 0{\cdot}05$) also showed an an increase of caspase 3 expression over the experimental period.

The expression of NFκB (APD; $P < 0{\cdot}001$, RWPD; $P < 0{\cdot}01$), TNFα (APD; $P < 0{\cdot}01$), IL10 (APD; $P < 0{\cdot}05$, RWPD; $P < 0{\cdot}05$), IGF1 (APD; $P < 0{\cdot}001$, RWPD $P < 0{\cdot}05$), cyclinD1 (APD; $P < 0{\cdot}001$, RWPD; $P < 0{\cdot}001$) and caspase3 (APD; $P < 0{\cdot}001$, RWPD; $P < 0{\cdot}001$) changed significantly in the liver. All genes showed an up-regulation in the APD and RWPD group over the 26 days.

The trend of NFκB expression showed an increase in muscle ($P < 0{\cdot}01$) in the RWPD group. The mRNA expression of TNFα increased in muscle ($P < 0{\cdot}05$). IL10 expression was up-regulated in the SD group ($P < 0{\cdot}001$). The muscle showed a slight increase in the SD group ($P < 0{\cdot}05$) and a decrease in the RWP group ($P < 0{\cdot}05$).

Discussion

Both pomace rich diets had no influence on feeding performance and feeding behaviour. Despite of the two-times more total polyphenol content and the resulting adstringent properties of the red wine pomace, compared with the non-stringent and well tasting apple-pomace or control diet, no differences in food uptake were registered. Further, no effects on piglets' growth performance could be shown.

To test the physiological effects of pomace-derived polyphenols, various GIT tissues and inner organs were investigated in weaned piglets. The impact on the relative mRNA expression level of different anti- and pro-inflammatory marker genes, as well as apoptosis and proliferation marker genes were measured by high sensitive and fully quantitative real-time RT-PCR. Gene quantification at the mRNA level gives an early view of the gene regulation and a fully quantitative answer on various marker genes.

Several cytokines such as TGFα, IL1β and IL6 are constitutively expressed by the intestinal epithelium and may play a role in the basal influx of the immune cells into the mucosa, in epithelial cell growth and in homeostasis (Stadnyk, 1994). Other cytokines such as IL8, IL1β and TNFα are also expressed by normal epithelial cells and markedly up-regulated in response to microbial infection (Jung *et al.*, 1995; Pie *et al.*, 2004). Local expression of IL1β, IL6 and TNFα mRNA has been largely documented after bacterial or viral infection in pigs (Murtaugh *et al.*, 1996; Fossum, 1998). In our study, TNFα as pro-inflammatory marker was generally reduced in the GIT, except in the colon. The process of inflammation is self-limiting because the production of pro-inflammatory cytokines is followed almost immediately by production of anti-inflammatory cytokines like IL10 and IL13 (Philpott and Ferguson, 2004). Herein both pomace groups showed a divergent reaction in the IL10 mRNA expression in GIT, increasing in the stomach and the ileum, and decreasing in the jejunum and the colon.

TNFα and NFκB mRNA expressions react in comparable pattern in most of the observed tissues and organs. TNFα is a potent cytokine produced by many cell types, including macrophages, monocytes, lymphocyte, keratinocytes and fibroblasts, in response to inflammation, infection, injury and other environmental challenges (Baud and Karin, 2001). Exposure of cells to TNFα can result in activation of a caspase dependent cascade leading to apoptosis (Chang and Yang, 2000). TNFα causes activation of two major transcription factors, AP-1 and NFκB, that in turn induce genes involved in chronic and acute inflammatory responses (Barnes and Karin, 1997; Shaulian and Karin, 2001).

Central to the inflammatory response is activation of the transcription factor NFκB. NFκB is the key transcriptional regulator of many pro-inflammatory cytokines (Baldwin, 1996; Ghosh and Karin, 2002). However, NFκB activation also promotes cellular proliferation (Guttridge *et al.*, 1999; Hinz *et al.*, 1999) and protects against apoptosis (Beg and Baltimore, 1996; Van Antwerp *et al.*, 1996). In our piglet study, no consistent correlation of NFκB and Caspase 3 could be found. The NFκB pathway has been rather implicated in the resolution of inflammation. It has been shown that NFκB activation in leukocytes recruited during the onset of inflammation is associated with pro-inflammatory gene expression, whereas such activation during resolution of inflammation is associated with the expression of anti-inflammatory genes and the induction of apoptosis (Lawrence *et al.*, 2002).

Caspase 3 plays a key rôle in the regulation of apoptosis. Inhibition of caspase 3 prevents cell death by apoptosis

Table 4 *Trend of mRNA gene regulation over 26 days in the gastro-intestinal tract of piglets given SD (standard diet), APD (apple pomace diet), RWPD (red-wine pomace diet) (data are shown as of $\Delta\Delta CP$ values from day 0 to 26; positive trend represent an up-regulation; negative trend a down-regulation of mRNA marker gene expression)*

		SD			APD			RWPD		
	Tissue	Trend of regulation	Significance	Pearson correlation coefficient	Trend of regulation	Significance	Pearson correlation coefficient	Trend of regulation	Significance	Pearson correlation Coefficient
NFkB	Stomach				−6·53	***	0·63	−7·90	***	0·68
	Jejunum	−1·86	*	0·46						
	Ileum									
	Colon				+1·61	*	0·44			
TNF alpha	Stomach				−1·74	*	0·41			
	Jejunum	−2·35	**	0·57						
	Ileum							−2·63	**	0·52
	Colon	+0·94	***	0·29	+1·48	**	0·47			
IL10	Stomach				−1·44	*	0·39			
	Jejunum							+3·80	**	0·58
	Ileum							−1·70	*	0·39
	Colon							+2·35	***	0·63
IGF 1	Stomach									
	Jejunum	−1·67	*	0·40						
	Ileum									
	Colon							+2·02	*	0·37
Cyclin D1	Stomach				−3·28	***	0·67	−3·64	***	0·71
	Jejunum				−3·69	**	0·55	−2·89	*	0·49
	Ileum							+2·36	*	0·55
	Colon	−1·25	**	0·50	−1·07	*	0·36			
Caspase 3	Stomach									
	Jejunum	−2·11	**	0·50	−2·86	*	0·43	−3·72	**	0·50
	Ileum							+2·25	***	0·63
	Colon	+0·78	*	0·24	+1·09	*	0·37	+1·32	**	0·51

Table 5 *Trend of mRNA gene regulation over 26 days in mesenterial lymph node (mes LN), spleen, liver, kidney and muscle of piglets given SD (standard diet), APD (apple pomace diet), RWPD (red-wine pomace diet) (data are shown as of $\Delta\Delta CP$ values from day 0 to 26; positive trend represents an up-regulation; negative trend a down-regulation of mRNA marker gene expression)*

SD Trend of regulation	p	SD Trend of regulation	SD Significance	SD Pearson correlation coefficient	APD Trend of regulation	APD Significance	APD Pearson correlation coefficient	RWPD Trend of regulation	RWPD Significance	RWPD Pearson correlation coefficient
NFkB	mes LN									
	Spleen									
	Liver				+3·98	***	0·71	+3·46	**	0·61
	Kidney									
	Muscle							−9·10	**	0·50
TNF alpha	mes LN									
	Spleen									
	Liver				+2·13	**	0·49			
	Kidney									
	Muscle				+1·87	*	0·44			
IL10	mes LN	−1·28	*	0·37						
	Spleen				+1·89	**	0·47			
	Liver				+6·86	*	0·53	+6·94	*	0·49
	Kidney	+7·90	**	0·58	+2·34	*	0·45			
	Muscle	+11·91	***	0·65						
IGF 1	mes LN									
	Spleen									
	Liver				+3·15	***	0·66	+2·19	*	0·42
	Kidney									
	Muscle				−2·81	*	0·41			
Cyclin D1	mes LN									
	Spleen									
	Liver				+2·83	***	0·65	+3·77	***	0·60
	Kidney									
	Muscle									
Caspase 3	mes LN									
	Spleen				+1·56	*	0·42			
	Liver				+8·66	***	0·78	+8·89	***	0·83
	Kidney	+1·34	*	0·40						
	Muscle	+1·95	*	0·44				−2·73	*	0·41

Table 6 *The mRNA expression of gastro-intestinal tract, mesenterial lymph node (mes LN), spleen, liver, kidney, and muscle of piglets given SD (standard diet), APD (apple pomace diet), RWPD (red-wine pomace diet). Data are shown as of $\Delta\Delta CP$ values from day 0 to day 26 (no. = 6; mean ± s.d)*

		day 0	day 7	day 12	day 19	day 26
(a) Stomach						
IL 10	SD	0 ± 0·82	0·27 ± 1·03	−0·34 ± 1·81	−0·08 ± 1·33	−1·05 ± 1·39[a]
	APD	0 ± 0·82[1]	0·31 ± 1·95[2]	0·35 ± 1·82[3]	−2·76 ± 4·17[1,2,3,4]	1·19 ± 2·52[4]
	RWPD	0 ± 0·82	−2·24 ± 1·71	−0·15 ± 1·19	−1·77 ± 2·08	1·67 ± 2·81[a]
TNF a	SD	0 ± 1·33	−0·04 ± 0·57	−0·97 ± 0·46	−1·63 ± 1·53	0·21 ± 1·91
	APD	0 ± 1·33[1]	0·13 ± 1·80	−1·21 ± 0·90[2,3]	−4·19 ± 2·77[1,2,4]	1·85 ± 2·66[3,4]
	RWPD	0 ± 1·33	0·01 ± 1·51	−1·6 ± 3·25[1]	−2·26 ± 1·79[2]	1·54 ± 2·52[1,2]
NFkB	SD	0 ± 1·92	0·75 ± 2·34	0·22 ± 2·03	0·63 ± 2·34	0·78 ± 1·57
	APD	0 ± 1·92	0·58 ± 2·76	1·72 ± 1·84	−1·18 ± 2·73	−0·10 ± 3·00
	RWPD	0 ± 1·92	0·13 ± 1·76	0·05 ± 2·36	−0·44 ± 1·69	−2·18 ± 4·27
Casp3	SD	0 ± 0·63	0 ± 1·08[a]	−0·23 ± 1·27	−1·18 ± 1·16	−1·69 ± 0·71[a]
	APD	0 ± 0·63[1,2]	0·56 ± 2·21[b]	−0·98 ± 2·12	−3 ± 3·57[1,2]	−1·65 ± 1·93[b]
	RWPD	0 ± 0·63[1]	−2·78 ± 2·14[1,2,a,b]	−0·25 ± 0·72	−1·16 ± 1·00	0·89 ± 3·08[2,a,b]
Cyc D1	SD	0 ± 1·41	0·48 ± 1·21	−0·5 ± 1·27	−0·96 ± 1·54	−0·66 ± 1·27[a]
	APD	0 ± 1·41	1·23 ± 1·55	0·28 ± 1·33	−0·65 ± 1·80	−1·12 ± 1·55[b]
	RWPD	0 ± 1·41	−0·74 ± 2·19[1]	−0·2 ± 1·26	−0·80 ± 1·57[2]	1·76 ± 1·62[1,2,a,b]
IGF 1	SD	0 ± 1·65	0·93 ± 1·24	−0·07 ± 1·39	−1·00 ± 1·26	−0·69 ± 0·65
	APD	0 ± 1·65	1·21 ± 1·94	0·66 ± 1·13	0·50 ± 0·69	0·40 ± 1·58
	RWPD	0 ± 1·65	1·13 ± 1·16	−0·11 ± 1·78	0·16 ± 0·54	2·38 ± 1·90
(b) Jejunum						
IL 10	SD	0 ± 1·10	0·3 ± 0·90	−1·24 ± 2·69	−2·73 ± 1·99[a]	−2·08 ± 0·67
	APD	0 ± 1·10	−0·26 ± 1·39	−0·12 ± 1·32	0·73 ± 3·27[a,b]	−1·07 ± 0·69
	RWPD	0 ± 1·10	0·62 ± 1·29	−1·14 ± 1·66	−1·54 ± 1·08[b]	−0·35 ± 1·04
TNF a	SD	0 ± 1·81	0·14 ± 1·28	−0·79 ± 3·36	−2·68 ± 1·61[a]	−2·15 ± 1·17
	APD	0 ± 1·81	−0·67 ± 1·05	−0·97 ± 2·11	0·7 ± 3·20[a]	−1·65 ± 1·26
	RWPD	0 ± 1·81	0·01 ± 1·02	−0·54 ± 0·84	−0·89 ± 1·33	−2·48 ± 1·17
NFkB	SD	0 ± 1·37	0·6 ± 1·10	0·8 ± 2·09	−0·12 ± 1·27	0·12 ± 1·28
	APD	0 ± 1·37	0·14 ± 1·96	−0·67 ± 1·22	1·61 ± 2·23[a]	0·37 ± 1·06
	RWPD	0 ± 1·37	1·04 ± 1·69	0·37 ± 0·64	−0·1 ± 0·63[a]	−1·67 ± 1·72
Casp3	SD	0 ± 0·71	−1·62 ± 1·96	−0·98 ± 3·41	−0·38 ± 0·64	−1·03 ± 0·69
	APD	0 ± 0·71	−0·46 ± 1·11	0·41 ± 1·43	1·07 ± 4·06	−0·59 ± 1·18
	RWPD	0 ± 0·71	−0·18 ± 1·48	−1·68 ± 0·98	−1·94 ± 1·84	−1·82 ± 1·52
Cyc D1	SD	0 ± 0·51	0·81 ± 2·01	0·76 ± 4·041	−0·52 ± 1·37	−0·79 ± 0·60
	APD	0 ± 0·51	−0·01 ± 0·49	1·73 ± 1·68	1·57 ± 3·15	−0·93 ± 0·86
	RWPD	0 ± 0·51	1·44 ± 2·24	−0·03 ± 0·91	−0·96 ± 1·6	−0·01 ± 0·76
IGF 1	SD	0 ± 1·03	0·37 ± 4·48	−0·15 ± 2·79	0·18 ± 1·61	−0·91 ± 1·10
	APD	0 ± 1·03	−0·6 ± 1·17	−0·33 ± 0·86	0·37 ± 3·17	−0·71 ± 0·98
	RWPD	0 ± 1·03	0·47 ± 1·79	−0·64 ± 1·51	−1·12 ± 1·21	−1·38 ± 2·01
(c) Ileum						
IL 10	SD	0 ± 0·49	−0·89 ± 1·06	−0·76 ± 0·91	−1·72 ± 1·88	−0·53 ± 0·55
	APD	0 ± 0·49	−1·13 ± 0·92	−1·02 ± 0·58	−0·85 ± 4·39	−0·92 ± 0·76
	RWPD	0 ± 0·49	−0·76 ± 0·48	−0·28 ± 0·66	−1·11 ± 1·07	−1·99 ± 3·15
TNF a	SD	0 ± 0·38	−0·69 ± 0·89	−0·5 ± 0·88	−1·14 ± 1·42	−0·63 ± 0·51[a]
	APD	0 ± 0·38	−1·07 ± 0·97	−0·87 ± 0·17	0·08 ± 4·09	−0·99 ± 0·64[b]
	RWPD	0 ± 0·38[1]	−1·09 ± 0·37	−0·63 ± 0·73[2]	−1·14 ± 1·27	−3·3 ± 3·20[1,2,a,b]
NFkB	SD	0 ± 0·97	1·63 ± 1·92	−0·44 ± 0·96	−2·05 ± 7·24	−1·43 ± 4·08
	APD	0 ± 0·97	0·73 ± 1·93	0·79 ± 2·60	0·58 ± 6·47	−1·33 ± 2·65
	RWPD	0 ± 0·97	−1·06 ± 0·86	−0·32 ± 0·79	0·65 ± 1·09	−0·27 ± 5·36
Casp3	SD	0 ± 1·18	−0·19 ± 1·01	−0·78 ± 1·01	0·32 ± 1·47[a]	1·28 ± 1·83
	APD	0 ± 1·18[1]	0·77 ± 0·92[2]	0·61 ± 1·32[3]	4·86 ± 4·68[1,2,3,4,a,b]	0·39 ± 0·56[4]
	RWPD	0 ± 1·18	−0·22 ± 0·93	0·26 ± 0·87	1·26 ± 0·65[b]	2·1 ± 1·44
Cyc D1	SD	0 ± 0·56	−0·28 ± 0·91	−0·35 ± 1·22	0·99 ± 0·21	−0·18 ± 1·21[a]
	APD	0 ± 0·56[1]	−0·31 ± 1·27[2]	−0·73 ± 0·50[3]	2·04 ± 3·51[1,2,3,4,a]	0·31 ± 1·37[4,b]
	RWPD	0 ± 0·56[1]	−1·26 ± 0·63[2]	−0·99 ± 0·50[3]	−0·19 ± 0·95[4,a]	2·3 ± 1·76[1,2,3,4,a,b]
IGF 1	SD	0 ± 0·63	−0·53 ± 1·20	−1·22 ± 0·87[a,b]	−0·48 ± 1·82[a]	0·63 ± 1·24
	APD	0 ± 0·63[1]	0·40 ± 0·38	0·48 ± 0·61[a]	2·34 ± 3·48[1,a,b]	0·38 ± 1·47
	RWPD	0 ± 0·63	−0·23 ± 0·76	0·64 ± 0·79[b]	0·15 ± 0·80[b]	0·59 ± 0·47
(d) Colon						
IL 10	SD	0 ± 1·51[1]	1·10 ± 1·10[2]	−0·08 ± 0·90[3]	2·48 ± 0·85[1,2,3,4]	0·79 ± 0·96[4]
	APD	0 ± 1·51[1]	−0·18 ± 1·29[2]	0·58 ± 0·73	1·95 ± 1·18[1,2]	0·56 ± 0·87
	RWPD	0 ± 1·51[1,4]	0·12 ± 1·04[2,5]	0·44 ± 0·92[3,6]	2·07 ± 1·09[1,2,3]	1·98 ± 0·59[4,5,6]
TNF a	SD	0 ± 1·37	0 ± 1·78	0·89 ± 0·54	0·07 ± 1·04	1·15 ± 0·59
	APD	0 ± 1·37	0·02 ± 0·79	1·27 ± 0·90	1·67 ± 1·27	1·06 ± 0·29
	RWPD	0 ± 1·37	0·27 ± 1·15	1·16 ± 0·58	1·31 ± 2·26	−0·09 ± 0·54
NFkB	SD	0 ± 2·29[1]	−0·69 ± 3·17	0·77 ± 1·11	−0·33 ± 0·88	1·97 ± 0·75[1]
	APD	0 ± 2·29	0·45 ± 0·17	1·07 ± 0·62	1·34 ± 1·6	1·62 ± 0·34
	RWPD	0 ± 2·29	0·51 ± 1·62	0·75 ± 0·80	1·52 ± 1·49	0·94 ± 0·81
Casp3	SD	0 ± 1·04[1,3]	1·47 ± 1·11[1,2]	0·11 ± 0·7[2,3]	2·32 ± 0·62[3,4,5]	0·65 ± 0·65[5]
	APD	0 ± 1·04[1]	0·39 ± 1·22[2]	0·63 ± 0·72	1·79 ± 1·08[1,2]	0·71 ± 0·74

Table 6. Continued

		day 0	day 7	day 12	day 19	day 26
	RWPD	0 ± 1·04[1]	0·84 ± 0·98	0·7 ± 0·43	1·73 ± 0·74	1·27 ± 0·70
Cyc D1	SD	0 ± 0·70	−0·43 ± 0·74	−0·45 ± 0·72	−0·56 ± 0·39	−1·53 ± 1·27
	APD	0 ± 0·70	−0·25 ± 0·94	−0·48 ± 0·79	−0·12 ± 1·63	−1·42 ± 0·75
	RWPD	0 ± 0·70	−0·25 ± 0·89	−0·76 ± 0·91	0·47 ± 2·18	−0·53 ± 0·51
IGF 1	SD	0 ± 1·59[1]	0·39 ± 2·70	0·59 ± 1·07	1·43 ± 0·47[1]	0·88 ± 0·87
	APD	0 ± 1·59	1·33 ± 0·58	0·90 ± 0·62	1·35 ± 1·16	0·41 ± 1·23
	RWPD	0 ± 1·59	1·36 ± 1·36	1·24 ± 0·88	2·31 ± 1·03	0·72 ± 0·75
(e) mes LN						
IL 10	SD	0 ± 0·92	0·05 ± 1·17	−1·12 ± 1·00	−0·54 ± 1·37	−1·32 ± 1·58
	APD	0 ± 0·92	−1·49 ± 1·00	−1·16 ± 0·49	−1·17 ± 2·03[a]	0·47 ± 3·02
	RWPD	0 ± 0·92	−1·22 ± 0·66	−0·72 ± 0·79	0·84 ± 1·07[a]	−0·08 ± 1·04
TNF a	SD	0 ± 1·08	−0·18 ± 0·39	−0·73 ± 0·64	0·24 ± 2·57	−0·63 ± 0·51
	APD	0 ± 1·08	−0·31 ± 0·59	−0·81 ± 0·38	−0·05 ± 2·48	0·54 ± 2·32
	RWPD	0 ± 1·08	−0·39 ± 0·47	−0·55 ± 0·50	−0·01 ± 0·41	0·12 ± 0·36
NFkB	SD	0 ± 1·26	0·46 ± 0·85	0·94 ± 0·60	−1·58 ± 2·16[a,b]	0·22 ± 2·38
	APD	0 ± 1·26	0·87 ± 0·64	0·68 ± 0·36	2·47 ± 3·71[1,a]	−0·7 ± 2·48[1,a]
	RWPD	0 ± 1·26	1·09 ± 0·48	0·58 ± 0·82	1·34 ± 2·41[b]	1·82 ± 2·69[a]
Casp3	SD	0 ± 1·84	−0·64 ± 1·20	−1·55 ± 0·28[1]	0·78 ± 1·56[1]	−0·14 ± 0·82
	APD	0 ± 1·84	−1·12 ± 0·38[1]	−0·93 ± 0·32[2]	−0·22 ± 1·94	1·11 ± 2·24[1,2]
	RWPD	0 ± 1·84	−1·11 ± 0·40	−1·12 ± 0·40	0·95 ± 1·11	−0·51 ± 2·31
Cyc D1	SD	0 ± 1·60	−0·73 ± 1·11	−1·14 ± 0·63	0·3 ± 1·71	−0·62 ± 0·80
	APD	0 ± 1·60	−1·25 ± 0·71	−1·33 ± 0·78	−0·60 ± 1·88	0·22 ± 2·59
	RWPD	0 ± 1·60	−1·27 ± 0·43	−1·20 ± 0·54	0·52 ± 1·49	−0·86 ± 2·33
IGF 1	SD	0 ± 3·045	0·70 ± 0·57	0·38 ± 0·64	1·14 ± 1·86	−1·89 ± 1·12
	APD	0 ± 3·045	0·81 ± 0·55	0·44 ± 1·60	2·28 ± 3·15	−1·00 ± 1·93
	RWPD	0 ± 3·045	0·92 ± 0·38	−0·1 ± 1·73	1·89 ± 0·58	0·75 ± 0·37
(f) Spleen						
IL 10	SD	0 ± 0·87	1·45 ± 0·87[a]	0·20 ± 0·80	0·77 ± 1·36[a]	0·13 ± 1·14
	APD	0 ± 0·87[1]	−0·56 ± 0·87[2,a]	0·30 ± 0·50[3]	2·78 ± 1·41[1,2,3,4,a]	0·68 ± 0·97[4]
	RWPD	0 ± 0·87	0·44 ± 1·29	−0·33 ± 0·73	1·57 ± 2·72	−0·34 ± 0·78
TNF a	SD	0 ± 1·21	1·83 ± 0·85[a]	0·45 ± 0·49	1·11 ± 1·07	0·35 ± 0·55[a]
	APD	0 ± 1·21[1]	0·08 ± 0·51[2,a]	0·42 ± 0·64[3]	1·90 ± 1·55[1,2,3,4]	0·05 ± 1·89[4,b]
	RWPD	0 ± 1·21	1·13 ± 1·31[1]	−0·02 ± 0·31	1·31 ± 2·14[2]	−1·39 ± 0·37[1,2,a,b]
NFkB	SD	0 ± 0·82	−0·49 ± 0·93	−0·53 ± 0·79	−0·78 ± 1·69	0·29 ± 0·58
	APD	0 ± 0·82	−0·11 ± 1·50	−0·59 ± 0·83	1·97 ± 2·39	−0·28 ± 1·62
	RWPD	0 ± 0·82	−1·21 ± 1·68	−0·83 ± 1·33	2·12 ± 2·99	−0·31 ± 1·13
Casp3	SD	0 ± 2·62	1·30 ± 0·51	0·12 ± 0·18	1·54 ± 0·55	1·00 ± 0·46
	APD	0 ± 2·62	0·82 ± 0·46	0·73 ± 0·34	1·84 ± 0·69	1·51 ± 0·66[a]
	RWPD	0 ± 2·62	1·21 ± 0·55	0·48 ± 0·71	2·14 ± 1·95[1]	−0·57 ± 1·94[1,a]
Cyc D1	SD	0 ± 1·21	1·83 ± 0·85[a]	0·45 ± 0·49	1·11 ± 1·07	0·35 ± 0·55
	APD	0 ± 1·21[1]	0·08 ± 0·51[2,a]	0·42 ± 0·64[3]	1·9 ± 1·55[1,2,3,4]	0·05 ± 1·89[4]
	RWPD	0 ± 1·21	1·13 ± 1·31[1]	−0·02 ± 0·31	1·31 ± 2·14[2]	−0·96 ± 0·82[1,2]
IGF 1	SD	0 ± 0·88	−0·50 ± 0·35	−0·88 ± 0·64	−1·08 ± 0·21	−0·85 ± 0·80
	APD	0 ± 0·88[1]	−0·75 ± 0·9[2]	−0·48 ± 0·53[3]	2·31 ± 1·71[1,2,3,4]	−1·35 ± 1·33[4]
	RWPD	0 ± 0·88	−0·19 ± 0·7[1]	0·21 ± 1·18	0·34 ± 1·79[2]	−1·07 ± 1·01[1,2]
(g) Liver						
IL 10	SD	0 ± 2·98	−1·00 ± 1·32	−3·02 ± 0·61	−2·38 ± 1·21	−1·85 ± 1·74
	APD	0 ± 2·98	−1·93 ± 0·79	−3·25 ± 1·13	−1·59 ± 3·25	1·09 ± 4·43
	RWPD	0 ± 2·98	−2·33 ± 0·81	−1·77 ± 0·81	−0·75 ± 1·63	−0·37 ± 0·75
TNF a	SD	0 ± 3·19	−0·99 ± 2·31	−0·62 ± 0·52	−1·09 ± 1·15	−0·11 ± 1·59
	APD	0 ± 3·19	−0·38 ± 0·46	−1·02 ± 1·31	−0·20 ± 3·27	−0·10 ± 3·29
	RWPD	0 ± 3·19	−0·58 ± 0·61	−0·05 ± 0·75	0·26 ± 2·72	−0·75 ± 1·54
NFkB	SD	0 ± 2·96	−0·93 ± 1·18	−0·47 ± 0·92	−1·81 ± 1·39	−0·14 ± 0·74
	APD	0 ± 2·96	−0·77 ± 0·40	−0·22 ± 0·48	0·67 ± 3·47	1·12 ± 3·96
	RWPD	0 ± 2·96	0·32 ± 0·85	−0·46 ± 0·66	0 ± 2·20	−0·03 ± 0·09
Casp3	SD	0 ± 2·21	−1·41 ± 1·02	−1·29 ± 1·08	0·39 ± 0·86	−0·47 ± 0·71[a]
	APD	0 ± 2·21	−0·40 ± 0·55	−0·4 ± 0·45	1·76 ± 1·82	1·94 ± 2·16[a]
	RWPD	0 ± 2·21	0·15 ± 1·70	−0·7 ± 0·50	1·33 ± 1·84	0·49 ± 2·00
Cyc D1	SD	0 ± 2·31	−0·78 ± 1·13	−0·61 ± 0·40	−1·62 ± 1·62	−0·13 ± 0·71
	APD	0 ± 2·31	−0·63 ± 0·97	−0·98 ± 0·51	0·32 ± 2·64	0·11 ± 3·49
	RWPD	0 ± 2·31	−0·59 ± 0·34	−0·14 ± 0·45	0·30 ± 2·15	−0·90 ± 0·96
IGF 1	SD	0 ± 1·46	−3·05 ± 2·09	−2·55 ± 0·59	−3·09 ± 0·35	−1·68 ± 1·09[a]
	APD	0 ± 1·46	−0·64 ± 0·75[1]	−1·97 ± 1·19	−0·22 ± 2·45	0·49 ± 0·67[1,a,b]
	RWPD	0 ± 1·46	−0·36 ± 0·82	−1·85 ± 1·04	−0·56 ± 1·72	−1·71 ± 0·70[b]
(h) Kidney						
IL 10	SD	0 ± 2·25	2·41 ± 1·16	1·21 ± 1·34	0·89 ± 0·89	2·34 ± 2·24
	APD	0 ± 2·25	1·05 ± 1·14	1·35 ± 1·15	2·56 ± 0·88	2·26 ± 2·73
	RWPD	0 ± 2·25	0·57 ± 0·94	0·51 ± 1·44	0·08 ± 3·04	1·87 ± 2·01
TNF a	SD	0 ± 1·82	0·86 ± 0·65	−0·33 ± 1·16	−1·00 ± 1·29	0·53 ± 2·94
	APD	0 ± 1·82	0·73 ± 1·29	0·98 ± 2·47	1·74 ± 1·86	−0·11 ± 1·45
	RWPD	0 ± 1·82	−0·14 ± 2·22	−0·44 ± 1·05	−0·41 ± 2·83	0·18 ± 2·59
NFkB	SD	0 ± 1·34	0·18 ± 1·11	0·13 ± 0·83	−0·96 ± 1·82	1·01 ± 1·32

Table 6. Continued

		day 0	day 7	day 12	day 19	day 26
	APD	0 ± 1·34	1·93 ± 0·67	0·52 ± 0·61	1·11 ± 0·37	1·29 ± 2·55
	RWPD	0 ± 1·34	−0·16 ± 1·16	−0·13 ± 0·47	0·75 ± 1·82	0·96 ± 0·40
Casp3	SD	0 ± 0·87	−0·07 ± 0·6	0·60 ± 0·22	−0·09 ± 0·80	1·67 ± 2·07
	APD	0 ± 0·87	−1·63 ± 0·57[1]	1·54 ± 1·36[a]	0·51 ± 1·75[1]	0·33 ± 1·83
	RWPD	0 ± 0·87	−0·09 ± 0·84	−0·19 ± 0·65[a]	0·94 ± 1·05	0·91 ± 1·94
Cyc D1	SD	0 ± 1·29	0·13 ± 0·49	0 ± 0·58	−0·32 ± 0·91	1·18 ± 1·87
	APD	0 ± 1·29	0·36 ± 0·79	1·43 ± 1·80	1·25 ± 1·52	−0·63 ± 3·47
	RWPD	0 ± 1·29	−0·18 ± 0·56	−0·4 ± 0·82	0·45 ± 1·57	0·64 ± 1·59
IGF 1	SD	0 ± 1·16	0·12 ± 1·03	−0·42 ± 0·55	1·02 ± 1·42	−0·75 ± 1·92
	APD	0 ± 1·16	2·77 ± 0·87	1·78 ± 0·95	1·25 ± 0·33	0·97 ± 1·79
	RWPD	0 ± 1·16	1·59 ± 2·95	−0·93 ± 0·63	1·37 ± 1·16	0·36 ± 1·08
(i) Muscle						
IL 10	SD	0 ± 0·44	0·97 ± 1·09	−0·48 ± 1·69	0·50 ± 2·37	3·17 ± 1·18
	APD	0 ± 0·44	0·58 ± 1·73	−0·51 ± 1·49	−0·81 ± 1·03	0·81 ± 1·75
	RWPD	0 ± 0·44	1·15 ± 2·10	−0·39 ± 1·73	0·81 ± 2·13	1·99 ± 2·55
TNF a	SD	0 ± 0·64	−0·45 ± 0·96	−1·11 ± 0·44	−0·39 ± 2·14	0·32 ± 1·26
	APD	0 ± 0·64	0·28 ± 1·99	−1·59 ± 0·76[1]	−0·52 ± 1·26	1·08 ± 1·39[1]
	RWPD	0 ± 0·64	0·35 ± 3·10	−1·62 ± 0·76[1 2]	0·86 ± 1·8[1]	1·26 ± 1·13[2]
NFkB	SD	0 ± 0·76	0·75 ± 1·82	−0·18 ± 1·24	6·56 ± 2·64	6·19 ± 1·87
	APD	0 ± 0·76	1·02 ± 0·42	−0·28 ± 2·02	3·75 ± 4·79	6·61 ± 1·63
	RWPD	0 ± 0·76	−0·04 ± 2·11	−0·10 ± 3·20	7·55 ± 1·78	7·1 ± 1·87
Casp3	SD	0 ± 0·85	−0·13 ± 0·26[a]	−0·33 ± 0·60	0·74 ± 2·00[a]	1·41 ± 1·19
	APD	0 ± 0·85[1 2]	2·69 ± 2·13[a b]	−0·71 ± 1·18[1 3]	−0·28 ± 1·8[b 4]	1·75 ± 1·43[2 3 4]
	RWPD	0 ± 0·85[1]	0·65 ± 1·45[2 b]	−0·56 ± 0·46[3 4]	2·68 ± 0·69[1 2 3 a b]	1·44 ± 0·19[4]
Cyc D1	SD	0 ± 0·59	0·42 ± 1·13	−0·78 ± 0·43[1]	0·89 ± 2·10	1·39 ± 1·49[1]
	APD	0 ± 0·59	1·90 ± 2·05	−0·71 ± 0·53[2]	0·54 ± 1·36	2·01 ± 1·61[2]
	RWPD	0 ± 0·59[1]	0·43 ± 2·39	−0·82 ± 0·3[2]	2·21 ± 1·63[1 2]	0·74 ± 0·67
IGF 1	SD	0 ± 0·61	1·04 ± 0·66[a]	−0·21 ± 0·76	2·63 ± 3·51	0·74 ± 2·60
	APD	0 ± 0·61	−3·29 ± 0·84[1 2 a b]	−0·62 ± 0·72	1·80 ± 2·12[1]	1·92 ± 2·39[2]
	RWPD	0 ± 0·61	1·91 ± 2·35[b]	−0·38 ± 1·16	2·38 ± 2·26	−0·14 ± 2·75

[1,2,3] Superscript indicates significant differences over treatment time (in each row) ($P < 0{\cdot}05$).
[a,b,c] Superscript indicates significant differences between feedings within each marker mRNA (in the same column and marker mRNA) ($P < 0{\cdot}05$).

(Heczko *et al.*, 2001). Caspase 3 mRNA expression showed an increase in the ileum and the colon but in the jejunum, a decrease over experimental period. High and low-molecular weight DNA fragmentation is caused by the action of caspase 3 (Liu *et al.*, 1997; Enari *et al.*, 1998).

The abundance of cyclin D1 is induced by growth factors, including IGF-1 (Albanese *et al.*, 1999). Cyclin D1 forms physical associations with more than 30 transcription factors or transcriptional co-regulators (Inoue and Sherr, 1998; Zhang *et al.*, 1999; Horstmann *et al.*, 2000; Wang *et al.*, 2004). The regulation of the cell cycle is controlled in part by a family of protein kinase complexes, and each complex is composed minimally of a catalytic subunit, and its essential activating partner, the cyclin (Sherr, 1994; Macleod *et al.*, 1995; Jacks and Weinberg, 1996; Collins *et al.*, 1997; Sherr and Roberts, 1999). These complexes are activated at specific intervals during the cell cycle but can also be induced and regulated (Macleod *et al.*, 1995; Sherr and Roberts, 1995). The pomace feeding reduce the cyclin D1 as a marker of proliferation in the GIT. Apples (Eberhardt *et al.*, 2000; Wolfe *et al.*, 2003), especially apple peel, and wine by-products (Carbonneau *et al.*, 1997; Shrikhande, 2000) have been found to have a potent antioxidant activity and can greatly inhibit the growth of liver cancer and colon cancer cells. Therefore apples have been shown to have anti-proliferative activity in several studies in cell culture (Eberhardt *et al.*, 2000; Wolfe *et al.*, 2003). In our study the tested cyclin D1 mRNA expressions showed an up-regulation in liver in the pomace groups, therefore we can argue that pomaces induce cell proliveration. It was reported that the extracts of apple and grape are more potent than onion, tomato and celery in inducing activation of caspase-3/-7 and cleavage of poly-(ADP-ribose) polymerase in intact Jurkat T cells (Chen *et al.*, 2004). In previous investigation, the villi lengths in the jejunum were measured in length and showed an increase over 3 weeks after weaning (data not published). We can confirm the villi growth by less expression of the apoptotic marker caspase 3.

Green tea polyphenols, including EGCG inhibit endotoxin-mediated TNFα production (Fujiki *et al.*, 2001) and blocked the NFκB activation (Yang *et al.*, 2001). Another cell experiment demonstrated that the four apple extracts inhibit TNFα production of LPS-stimulated macrophages at certain concentrations (Adaim *et al.*, 2005). In cell culture silymarin, genistein, and EGCG inhibit cyclin D1 (Agarwal, 2000).

In the liver generally the mRNA expression of the tested genes increased in the two polyphenol-rich feeding regimes. Both pomace treatments have a common effect on mRNA expression. This is in accordance with prior cell studies, where physiological polyphenol concentration stimulates total mRNA expression (Sehm *et al.*, 2005).

Conclusion

We presume that both flavanoid-rich feedings have the potential to modulate the mRNA expressions as such and of proliferation- and apoptotic-markers. In the jejunum and the stomach, the cell cycle turn-over was decreased, whereas

in the liver the cell turn-over was highly increased. The effect of pomace-rich feeding on inflammatory-marker gene expression is mainly relevant in the stomach, by reducing expression of transcription factor NFκB and TNFα. To test which key polyphenol component is relevant and has the most impact on GIT epithelial or organ growth performance and which substance may modulate the GIT intestinal immune-response, further single substance-based cell culture trials must be performed.

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