Effects of plant polyphenols and mannan-oligosaccharides on growth performance, antioxidant defense system and inflammatory responses of ileal mucosa in *Escherichia Coli* Challenged piglets

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Introduction

Polyphenols are a powerful kind of naturally bioactive compounds found in food such as fruits, wine and tea as well as wood waste like larch bark (Heim *et al.*, 2002). Plant polyphenols (PP) had exhibited antimicrobial activity against bacteria causing food-borne disease (Taguri *et al.*, 2004; Percival *et al.*, 2006; Kim *et al.*, 2009). PP had also shown the ability to improve gut microflora balance (Ishihara *et al.*, 2001). MOS is derived from the outer cell wall of yeast. Meta-analyses of literature had shown that MOS dietary supplementation improved body weight gain and feed efficiency in piglets (Eugeniusz *et al.*, 2006). Enterotoxigenic *Escherichia coli* (ETEC), is reported to be one main etiological agent that cause piglet intestine disorder after weanling. It is worthwhile to explore the possible protective role of natural origin feed ingredient against the ETEC infection.

Aim of the study

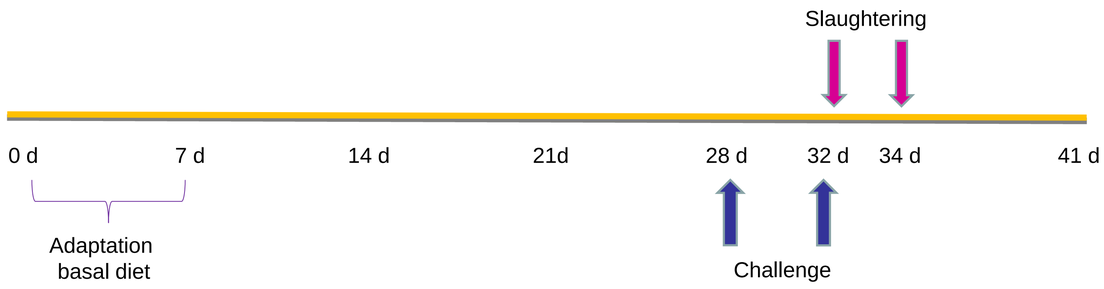
To evaluate the effect of plant polyphenols (PP) (mixture containing anthocyanin, catechins, chlorgenic and oleuropein) and/or mannan-oligosaccharides (MOS) on growth performance, plasma antioxidant capacity and ileal mucosa inflammatory responses in piglets *E. coli* challenged

Material and Methods

- Duration of the study: 41 d
- 96 piglets, (LxLW)xPenerland, 22 days old, 7.43 ± 0.89 kg L.W.
- 4 treatments starting after a 7 d-adaptation to basal diet:

BD (Basal Diet), PP (BD + 0.1 % PP), MOS (BD + 0.1 % MOS), MOS+PP (BD + 0.1 % MOS & PP)

- 48 piglets were orally inoculated with *E. Coli* (4 ml 1x10^9 cfu/ml of virulent *E. Coli* 0149: F4(K88)-positive strain) at 28 and 32 d; the other piglets were orally inoculated with the same amount of saline solution
- Piglets were individually weighed at 7, 14, 21, 28, 32, 34 and 41 d
- Blood samples from 1 piglet/replicate at 7, 28, 32, 34 and 41 d
- Plasma antioxidant capacity: Total antioxidant capacity (T-AOC), Malondialdehyde (MDA), Total Superoxide Dismutase (T-SOD), Catalase (CAT) and Glutathione Peroxidase (GSH-Px)
- 1 piglet/replicate slaughtered at 32 and 34 d
- Ileal mucosa inflammatory response: Inducible nitric oxide synthase (iNOS), Myeloperoxidase (MPO) and Nitric oxide (NO)
- Data analyzed by GLM procedure of SPSS (15.0)



Results

- BD and PP piglets had lower FCR before *E. Coli* challenge (*P*< 0.05) (Tab. 1). MOS supplemented piglets had higher ADG than BD from 32 to 34 d (*P*= 0.017). PP and MOS supplemented alone resulted in lower FCR from 28 to 34 d (*P*= 0.005) (Tab. 3).
- Dietary PP partially enhanced the systemic antioxidant properties with higher T-AOC (*P*= 0.060) and lower MDA (*P*= 0.076) compared to BD or PP+MOS at 28 d (Tab. 2). At 32 d, dietary PP or MOS increased plasma GSH-Px activity compared to BD (*P*= 0.003) (Tab. 4). Challenge increased plasma MDA content (*P*= 0.078) and decreased plasma T-AOC (*P*= 0.047) and CAT activity (*P*= 0.020) at 34 d. At 41 d dietary PP and PP+MOS supplemented piglets had an increased CAT activity compared to basal or MOS diet (*P*= 0.003). Howevere a decrease of T-AOC was also observed in all challenged piglets at the same time (*P*= 0.031) (Fig. 1-2).
- Challenge increased ileal mucosa inflammatory enzyme activities of iNOS and MPO as well as NO production at 32 and 34 d. At 34 d, dietary PP or PP+MOS tended to inhibit the increase of iNOS activity (*P*= 0.077) and NO production (*P*= 0.085) and markedly suppressed the increase of MPO activity post infection (*P*= 0.045) due to challenge compared to BD (Tab. 5).

Conclusions

Plant polyphenols administration had the potential to improve feed efficiency, while a combination PP and MOS had no effect. PP and MOS separately enhanced the antioxidant defense system before challenge. Dietary PP and MOS favorably affected the systemic antioxidant capacity during *E. Coli* post-challenge. Dietary PP shortened the ileal mucosa inflammatory response due to challenge via inhibiting the elevation of MPO and iNOS activity and NO production. The unfavorable response of the combination PP and MOS might be indicative of some unclear interactive effects between the two additives.

References

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Tab. 1 - Growth performance before *E. Coli* challenge

	BD	PP	MOS	PP+MOS	*P* value
ADG(g/d)					
d 7-14	365.3	351.7	335.7	302.8	0.163
d 14-21	482.3	482.8	481.6	460	0.771
d 21-28	553.8	555.2	536.5	499	0.369
d 7-28	467.1	462	451.3	405.2	0.172
FCR					
d 7-14	1.205^{c}	1.245^{bc}	1.402^{ab}	1.432^{a}	0.027
d 14-21	1.364^{ab}	1.317^{b}	1.290^{b}	1.438^{a}	0.029
d 21-28	1.277	1.3	1.315	1.334	0.493
d 7-28	1.288^{b}	1.289^{b}	1.323^{ab}	1.381^{a}	0.045

Means within a row with different superscripts differ significantly (P<0.05)

Tab.2 - Plasma antioxidant capacity in piglets before *E. Coli* challenge

		BD	PP	MOS	PP+MOS	*P* value
d 7	**MDA (nmol/mL)**	5.08	3.64	3.71	3.81	0.305
	T-AOC (nmol/mL)	18.84	17.67	18.06	19.32	0.537
	CAT(U)	16.34	18.89	18.09	12.17	0.565
	GSH-Px (U/mL)	403.75	380.97	414.06	458.27	0.377
	T-SOD (NU//mL)	102.75	94.42	75.75	103.29	0.421
d 28	**MDA (nmol/mL)**	3.64	1.93	3.64	3.79	0.076
	T-AOC (nmol/mL)	17.73	22.08	19.91	18.18	0.060
	CAT(U)	16.37	27.41	17.22	32.25	0.252
	GSH-Px (U/mL)	396.87	410.04	384.41	452.89	0.458
	T-SOD (NU//mL)	96.96	102.73	102.89	90.86	0.383

Fig. 1 - Effect of treatment on T-AOC

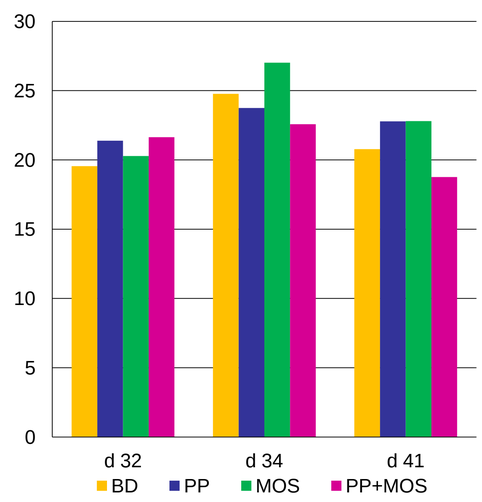
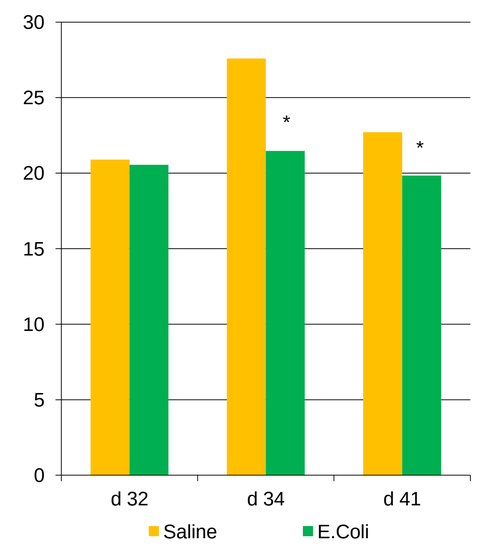


Fig. 2 - Effect of *E. Coli* Challenge on T-AOC (* *P*< 0.05)



Tab. 3 - Growth performance post *E. Coli* challenge

		Diet				Challenge	
		BD	PP	MOS	PP+MOS	Saline	*E. Coli*
ADG (g/d)							
d 28-32		603.6	560.2	581.6	575.4	599.3	561.1
d 32-34		462.1^{b}	596.8^{ab}	696.0^{a}	541.9^{b}	585.7	562.7
d 34-41		718.8	681.0	725.5	739.9	749.3	683.3
d 28-34		556.4	572.4	619.7	564.3	594.8	561.6
d 32-41		661.8	661.0	718.9	694.4	712.7	655.4
d 28-41		643.9	630.0	676.7	657.8	677.8	626.4
FCR							
d 28-32		1.566	1.334	1.331	1.607	1.443	1.476
d 32-34		2.192	1.743	1.635	1.911	1.847	1.894
d 34-41		1.695	1.668	1.643	1.595	1.650	1.651
d 28-34		1.711^{a}	1.465^{b}	1.418^{b}	1.699^{a}	1.539	1.576
d 32-41		1.765	1.678	1.624	1.648	1.683	1.674
d 28-41		1.699	1.582	1.544	1.635	1.613	1.617

Means within a row with different superscripts differ significantly (P<0.05)

Tab.4 - Plasma antioxidant capacity in piglets post *E. Coli* challenge

		Diet				Challenge	
		BD	PP	MOS	PP+MOS	Saline	*E. Coli*
d 32	**MDA (nmol/mL)**	3.68	2.33	4.09	2.73	2.69	3.73
	T-AOC (nmol/mL)	19.55	21.39	20.28	21.64	20.90	20.55
	CAT (U)	9.11	17.98	7.53	18.22	15.26	11.08
	GSH-Px (U/mL)	348.2^{b}	449.1^{a}	485.6^{a}	364.5^{b}	405.2	418.5
	T-SOD (NU//mL)	97.34	101.7	97.79	94.73	100.9	94.89
d 34	**MDA (nmol/mL)**	3.64	2.93	4.24	3.27	2.93	4.11
	T-AOC (nmol/mL)	24.77	23.75	27.02	22.58	27.59^{a}	21.47^{b}
	CAT (U)	11.43	12.83	14.74	17.33	17.94^{a}	10.23^{b}
	GSH-Px (U/mL)	494.8	467.8	394.2	486.8	447.0	474.8
	T-SOD (NU//mL)	97.21	100.2	99.38	95.46	97.92	98.22
d 41	**MDA (nmol/mL)**	8.95	6.33	8.09	7.91	7.76	7.88
	T-AOC (nmol/mL)	20.78	22.78	22.80	18.76	22.71^{a}	19.84^{b}
	CAT (U)	10.61^{b}	18.01^{a}	7.71^{b}	18.96^{a}	15.09	12.73
	GSH-Px (U/mL)	630.6	621.6	623.1	642.3	640.9	617.9
	T-SOD (NU//mL)	89.88	92.34	89.78	94.55	91.06	92.31

Means within a row with different superscripts differ significantly (P<0.05)

Tab.5 - Ileal inflammatory enzyme activity at slaughtering

		Diet				Challenge	
		BD	PP	MOS	PP+MOS	Saline	*E. Coil*
d 32	**MPO (U/g)**	0.38	0.33	0.33	0.37	0.25^{b}	0.46^{a}
	iNOS (U/mg protein)	1.02	0.95	1.00	0.99	0.84	1.14
	NO (µmol/mg protein)	4.60	3.21	5.85	2.25	2.45	5.51
d 34	**MPO (U/g)**	0.36^{a}	0.22^{b}	0.24^{b}	0.30^{ab}	0.23^{b}	0.33^{a}
	iNOS (U/mg protein)	0.98	0.81	0.93	0.94	0.73^{b}	1.09^{a}
	NO (µmol/mg protein)	3.92	2.79	3.12	2.99	2.43^{b}	3.99^{a}

Means within a row with different superscripts differ significantly (P<0.05)