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Conjugated linoleic acid and tryptophan supplementation improves immune response of weaned piglets

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ABSTRACT

Conjugated linoleic acid (CLA) enhances immune function while decreasing the negative effects of inflammatory responses. Aminoacidic profile of the immune system proteins is different than of muscle proteins, as higher Trp: Lys ratio. The aim of this study was to assess the effect of CLA in combination with two different Trp:Lys ratios in piglets in a low-health status nursery unit. There were 4 treatments arranged factorially with 2 CLA contents (0 vs 1%) and 2 Trp:Lys dietary ratios (0.15 vs 0.22). For the experiment, 336 piglets were used (7.6 ± 1.78 kg BW) and allotted in 48 pens. Productive performance was especially poor because of a severe outbreak of diarrhea. Under these special circumstances, CLA supplementation improved average daily gain (ADG) in 80% (P=0.0001) and FGR in 44% (P<0.001). Higher dietary Trp: Lys ratio allowed expressing CLA improvements in performance (P interaction <0.10 in ADG). Trp also improved ADG (170 vs 155 g/d; P=0.02). Mortality rate was not significantly affected, but in the total nursery period the highest risky group (0% CLA & low Trp content) showed the highest % of mortality (10.7%; P=0.02). We conclude that CLA supplementation allows minimizing signs of diarrhoea and improves ADG, used as biomarker of immunity, in the nursery period in presence of a severe outbreak of diarrhoea. Furthermore, higher dietary Trp content facilitates the expression of the CLA effect, demonstrating certain synergy between both (CLA and Trp).

OBJECTIVE

The aims of this study were:

- To study the effect of the CLA supplementation on health status in weaned piglets based on productive performance and susceptibility to different pathologies.
- To study the combination of CLA with the dose of dietary tryptophan on the same parameters.

MATERIALS AND METHODS

Experimental facilities, housing and lodging

The trial was carried out at the PigCHAMP Experimental Farm, a 550 farrow-to-finish farm, placed at Aguilafuente, Segovia (Spain). The animals were housed in a clean disinfected barn.

The nursery barn included identical rooms, each containing 24 pens. Each cage of 7 piglets per cage measured 1.25 m x 1.75 m (0.31 m^2/piglet) and had individual feeder and drinker.

Environmental conditions during the trial (temperature, and ventilation) were automatically controlled, according to the age of the animals. For the experiment, 2 rooms were used (48 pens).

Experimental effects

1. **Conjugated linoleic acid (CLA):** The experiment was performed to assess the effect of CLA on the immune system. In particular, CLA might accelerate immune system maturation. At weaning, these properties of CLA might allow the piglet to face the enteric pathogens that usually cause problems of diarrhoea. These effects could be especially beneficial for piglets or farms with low health status, more sensitive to diarrhoeas in the nursery period.

2. **Tryptophan: Lysine ratio (Trp):** The aminoacidic profile is different among body proteins. While muscular proteins are rich in lysine, other proteins implicate in the immune response, like immunoglobulins or acute-phase proteins, have higher proportion of Trp or threonine in detriment of lysine. Consequently, aminoacids requirements will be different depending on health status. In low-health status farms or in phases (ages) when disease susceptibility is especially high, dietary Trp supplementation should be increased. In these situations, like in post-weaning, Trp and CLA might have a complementary (or synergetic) effect.

Experimental animals

For the experimental trial, 336 piglets (7.6 ± 1.78 kg BW) were used, half males and half females, and allotted at random to the experimental treatments. Piglets were followed up from weaning (28 d of age) until the end of the nursery period at 63 d of age.

Experimental design

In the nursery period, the experimental design was factorial completely randomised block design with two experimental effects: CLA supplementation (CLA effect) and Trp: Lys ratio (TRP effect), resulting in 4 experimental treatments (2 CLA x 2 TRP). Experimental treatments are presented in table 1.

Table 1. Experimental treatments

Treatment	No. of animals	Experimental effects	
		CLA[1]	TRP[2]
T1	84	0	0.15
T2	84	0	0.22
T3	84	1.0	0.15
T4	84	1.0	0.22

[1]Expressed as %
[2]Expressed as Trp:Lys ratio.

Animals were allotted to pens based on the parity of the sow and initial weight. Each treatment was replicated 12 times and 7 pigs caged together were the experimental unit (84 piglets per treatment)

Experimental diets

The experimental diets were formulated according to the raw material composition of FEDNA tables (1999) to meet or exceed the nutrient requirements proposed by the NRC (1998). Diets were presented as meal and no growth promoters or antibiotics were added to any of the experimental diets. All diets were offered *ad libitum* to animals throughout the experiment. The ingredients composition and calculated nutrients content of experimental basal diets are presented in tables 2 and 3.

Table 2. Composition and calculated analyses of the prestarter diets (28-42 d of age).

Ingredients (%)	**PRESTARTER DIETS**[2]			
	T1	**T2**	**T3**	**T4**
Maize	34.76	34.87	34.81	34.95
Wheat	24.86	24.86	24.86	24.86
Soybean meal, 44% CP	9.07	8.83	9.06	8.81
Fish meal LT	6.00	6.00	6.00	6.00
Nutrilac (dehydrated yoghurt)	8.00	8.00	8.00	8.00
Dried whey	5.00	5.00	5.00	5.00
Soy oil	3.07	3.07	2.03	2.01
L-lysine HCl, 78%	0.41	0.42	0.41	0.42
L-threonine	0.20	0.21	0.20	0.21
Methionine-OH, 88%	0.16	0.17	0.16	0.17
L-Tripthophan	0.01	0.11	0.01	0.11
Calcium carbonate	0.41	0.41	0.41	0.41
Dicalcium phosphate	0.30	0.30	0.30	0.30
Salt	0.25	0.25	0.25	0.25
Nucleus – 7% Premix[1]	7.50	7.50	7.50	7.50
CLA	-	-	1.00	1.00
Calculated analyses[2] (%)				
Net energy (kcal/kg)	2,566	2,566	2,566	2,566
Crude protein	19.52	19.52	19.52	19.52
Total Lysine	1.470	1.470	1.470	1.470
Digestible Lysine	1.365	1.330	1.365	1.330
Total Tryptophan	0.226	0.330	0.226	0.330
Digestible Tryptophan	0.200	0.300	0.200	0.300

[1]Includes grains of cereals, protein of potato, extracted soybean, protein of soybean concentrated, vitamins and minerals. Analytical content: ether extract, 5.50%; crude protein, 39.0%; crude fibre, 2.00%; ashes, 10.50%; lysine, 3.65%. Vitamins and addives: Copper, 2.262 mg/kg; Vit A, 168000 IU/kg; Vit D3, 28000 IU/kg; Vit E, 1400 IU/kg; Xylanase (EC3.2.1.8), 150 IU/kg; 3-phytase (EC 3.1.3.8), 7142 FTU/ kg.

[2]Based on FEDNA (1999) values for feed ingredients

Table 3. Composition and calculated analyses of the starter diets (42-63 d of age).

Ingredients (%)	STARTER DIETS[2]			
	T1	T2	T3	T4
Barley	10.00	10.00	10.00	10.00
Maize	36.07	36.00	36.00	36.00
Wheat	20.58	20.78	20.68	20.84
Soybean meal, 44% CP	20.23	20.01	20.21	19.99
Soy oil	3.45	3.45	2.43	2.41
L-lysine HCl, 78%	0.41	0.42	0.42	0.42
L-threonine	0.17	0.17	0.17	0.17
Methionine-OH, 88%	0.17	0.17	0.17	0.17
L-Tripthophan	-	0.08	-	0.08
Calcium carbonate	0.36	0.36	0.36	0.36
Dicalcium phosphate	1.21	1.21	1.21	1.21
Salt	0.35	0.35	0.35	0.35
Nucleus – 7% Premix[1]	7.00	7.00	7.00	7.00
CLA	-	-	1.00	1.00
Calculated analyses[2] (%)				
Net energy (kcal/kg)	2,485	2,485	2,485	2,485
Crude protein	19.10	19.10	19.10	19.10
Total Lysine	1.33	1.33	1.33	1.33
Digestible Lysine	1.25	1.25	1.25	1.25
Total Tryptophan	0.216	0.296	0.216	0.296
Digestible Tryptophan	0.195	0.275	0.195	0.275

[1]Includes grains of cereals, protein of potato, extracted soybean, protein of soybean concentrated, vitamins and minerals. Analytical content: ether extract, 5.50%; crude protein, 39.0%; crude fibre, 2.00%; ashes, 10.50%; lysine, 3.65%. Vitamins and addives: Copper, 2.262 mg/kg; Vit A, 168000 IU/kg; Vit D3, 28000 IU/kg; Vit E, 1400 IU/kg; Xylanase (EC3.2.1.8), 150 IU/kg; 3-phytase (EC 3.1.3.8), 7142 FTU/ kg.
[2]Based on FEDNA (1999) values for feed ingredients

4. SAMPLING AND RECORDS:

Average daily gain (ADG) was individually controlled individually, while feed intake (FI) and feed efficiency (FGR) were evaluated per pen at weaning (28), 42, and 63 d of age, to calculate performance parameters (daily gain, feed intake, feed efficiency). Mortality was daily registered.

At 35 d of age a severe outbreak of diarrhoea occurred. Piglets were severely affected and piglets were treated with colistin through the water. Diarrhoea was persistent and, because of ethical reasons, trial was stopped at 56 days of age. Piglets were weighed at this age instead of 63 d. The last experimental day, faeces consistence was evaluated following a subjective scale: 0-normal consistence, 1-soft faeces, 2-diarrhoea and 3-severe signs of diarrhoea.

STATISTICAL ANALYSIS OF THE DATA

The data were analysed as a completely randomised design by GLM of SAS v. 6.12 (SAS, 1990). Data are presented as least square means corrected by periods and for the complete trial. Effects taken into account were CLA supplementation (CLA effect), dietary Trp:Lys

ratio (TRP effect) and their interaction (CLA x TRP). Initial body weight was included as covariate. Losses were analysed by the FREQ procedure of SAS.

RESULTS

Productive performance

Productive performance is presented in tables 4 and 5.

Table 4. Body weight evolution and average daily gain (ADG) of experimental piglets in the different sub-periods of the nursery phase analysed individually.

		BODY WEIGHT			ADG		
		28	**42**	**56**	**PREST.**	**STARTER**	**TOTAL**
CLA	**0**	7.53	8.31	10.88	48	184	116
	1%	7.58	8.37	13.47	53	364	209
TRP	**High**	7.55	8.42	12.38	56	283	170
	Low	7.56	8.26	11.97	45	265	155
CLA x Trp:							
0% CLA	**High Trp**	7.50	8.39^{ab}	10.94^{c}	54^{ab}	182^{c}	118^{c}
	Low Trp	7.56	8.22^{b}	10.82^{c}	42^{b}	185^{c}	114^{c}
1% CLA	**High Trp**	7.60	8.45^{a}	13.82^{a}	59^{a}	383^{a}	221^{a}
	Low Trp	7.56	8.29^{ab}	13.13^{b}	47^{ab}	346^{b}	196^{b}
Std. Desv.		*1.776*	*1.914*	*3.147*	*44.31*	*135.57*	*75.70*
***P*[1]**	**CLA**	*NS*	*NS*	***	*NS*	***	***
	Trp	*NS*	*	*	*	*t*	*
	CLA x Trp	*NS*	*NS*	*t*	*NS*	*	*t*

[1]Probability: NS, P>0.10; t, P<0.10; *, P<0.05; ***, P<0.001

Table 5. Average daily gain (ADG), feed intake (FI) and feed:gain ratio (FGR) in the nursery period analysed by pen depending on type of sow or parity (multiparous; MULT vs primiparous; PRIM piglets), CLA and TRP content.

		28-42			42-56			28-56		
		ADG	**FI**	**FGR**	**ADG**	**FI**	**FGR**	**ADG**	**FI**	**FGR**
CLA	**0**	39	226	10.21	179	439	2.54	107	330	3.17
	1%	44	208	6.04	355	471	1.38	197	337	1.79
TRP	**High**	45	217	10.42	272	460	2.02	155	336	2.49
	Low	39	217	5.82	262	449	1.90	149	331	2.47
CLA x Trp:										
0% CLA	**High**	41	227	15.15	172	426	2.63	104	323	3.20
	Low	37	225	5.26	186	452	2.45	110	336	3.14
1 % CLA	**High**	48	207	5.69	371	495	1.41	206	348	1.78
	Low	40	209	6.38	339	446	1.35	187	326	1.80
Std. Desv.		*23.9*	*35.4*	*12.96*	*113.2*	*71.5*	*0.71*	*61.5*	*47.1*	*0.86*
***Prob.*[1]**	**CLA**	*NS*	*t*	*NS*	***	*	***	***	*NS*	***
	Trp	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
	CLA x Trp	*NS*	*NS*	*NS*	*t*	**	*NS*	*t*	*	*NS*

[1]Probability: NS, P>0.10; t, P<0.10; *, P<0.05; **, P<0.01; ***, P<0.001

Productive performance was especially poor (12.2 kg BW at 56 d of age) because of the outbreak of diarrhoea. Signs of diarrhoea were evident and seriously affected both, mortality rate and productive performance.

CLA effect

In these circumstances of severe pathology, CLA showed important positive effects on productive performance. In prestarter, no differences were observed in growth rate, but CLA supplementation negatively affected FI (208 vs 226 g/d; P=0.06). These extremely high values of FE evidenced presence of pathology, in particular digestive disease, although clinical signs were not evident in this immediate post-weaning phase. At 42 d of age, strains of beta-haemolytic *E. coli* were isolated from faeces samples.

In the starter phase, CLA significantly affected growth rate (364 vs 184 g/d; P=0.0001) promoting that CLA supplemented piglets reached higher final BW than the control groups (13.5 vs 10.9 kg BW at 56 d of age; P=0.0001). Higher ADG was due to both, higher FI (P=0.02) and lower FGR (1.4 vs 2.5; P=0.0001). Consequently, in the global nursery period CLA supplemented piglets showed (P=0.0001) higher ADG and lower FGR than control groups (0% CLA).

TRP effect

Dietary TRP content (Trp: Lys ratio) also affected growth rate in both, prestarter and starter phases. In the prestarter, high TRP content group showed higher ADG (56 vs 45 g/d; P=0.02) and reached higher BW at 42 d of life (8.4 vs 8.3 kg; P=0.02). In the starter period, ADG also tended to be higher in the high TRP content group (P=0.08). Consequently, ADG in the whole nursery period and final BW were higher in high TRP than in low TRP groups (12.4 vs 12.0 kg; P=0.02). FI and FGR were not affected by dietary TRP content.

CLA x TRP effect

In CLA supplemented piglets, increasing TRP content promoted higher ADG in the starter phase (383 vs 346 g/d; P<0.05), associated with higher FI (495 vs 446 g/d; P<0.05). This effect of TRP on productive performance was not observe in non-CLA supplemented piglets (P>0.10). As a result, the combination of both effects was beneficial in total nursery ADG, and CLA supplemented piglets with high TRP content tended to show the highest final BW (13.8 kg; P CLA x TRP = 0.086).

Clinical signs

Percentage of mortality and faeces consistence at 56 d of age are presented in table 6.

Table 6 – Percentage of mortality in prestarter, starter and in the whole of the nursery period and faeces consistence at 56 d of age depending CLA and TRP contents.

		% DEATHS			FAECES
		PREST	START	TOTAL	CONSISTENCY
CLA	**0**	5.4	1.9	7.1	1.8
	1%	4.2	1.9	6.0	0.9
TRP	**High**	6.0	1.9	7.7	1.2
	Low	3.6	1.9	5.4	1.5
0% CLA	**High Trp**	2.4	1.2	3.6	1.7
	Low Trp	8.3	2.6	10.9	1.9
1% CLA	**High Trp**	4.8	2.5	7.3	0.7
	Low Trp	3.6	1.3	4.9	1.0
Std. Desv.		*0.213*	*0.136*	*0.248*	*1.117*
***Probability*[1]**	**CLA**	*NS*	*NS*	*NS*	**
	Trp	*NS*	*NS*	*NS*	*NS*
	CLA x Trp	*	*NS*	*	*NS*

[1]Probability: NS, P>0.10; t, P<0.10; *, P<0.05; **, P<0.01

Consistency of faeces, as indicator of severity of the diarrhoea, was only affected by CLA supplementation, which significantly reduced the signs of diarrhoea (0.9 vs 1.8; P=0.001). This result is highly correlated with the improvement of growth rate promoted by CLA. It was interesting the faeces consistence assessed by individual treatments, where the normal consistence (the lowest value) was observed in the group supplemented with CLA and high Trp:Lys ratio; 0.7, and the highest value, indicating more severe signs of diarrhoea or faeces more liquid, in the group non-supplemented with CLA and low Trp:Lys ratio; 1.9.

CONCLUSION

In conclusion, CLA supplementation allows minimizing signs of diarrhoea and improves ADG in the nursery period in presence of a severe outbreak of diarrhoea. Although CLA was not able to reduce mortality, in terms of ADG results obtained by its dietary inclusion were comparable with results of antibiotics: final BW was increase in 23.8% and ADG in 80.2% in the nursery period. On the other hand, higher dietary TRP content facilitates the expression of the CLA effect, demonstrating compatibility and certain synergy between both (CLA and TRP).