THE IMPORTANCE OF DIETARY TRYPTOPHAN FOR PRESERVING GROWTH AND CONTROLING INFLAMMATORY RESPONSE OF WEANED PIGS SUBMITTED TO IMMUNE STRESS

Nathalie Le Floc'h[1], Delphine Melchior[1,2], Bernard Sève[1]
[1]INRA, UMRVP, Saint Gilles 35590, [2]Ajinomoto-Eurolysine, Paris, France

Introduction

Immune system stimulation prevents the pigs to express their growth performance. This is explained by a decrease in feed consumption but also by the existence of a competition between immune system and growth process for nutrient utilization. Preliminary results showed that pigs suffering from a chronic lung inflammation had lower plasma tryptophan (Trp) concentrations than healthy pair-fed pigs suggesting an increased Trp utilization for other metabolic purposes than for body protein deposition [3]. Moreover, inflammation caused an induction of indoleamine 2,3 dioxygenase (IDO) activity an enzyme involved in Trp catabolism through the kynurenine pathway [4]. In this article we present experimental data suggesting that Trp availability for growth may be decreased by immune system activation and that Trp metabolism is involved in the control of inflammatory response.

Material and Methods

Experiment 1: this experiment was conducted to assess the limiting character of Trp for post weaning growth performance in pigs submitted to a moderate immune system stimulation obtained by depressing the quality of environment where pigs were kept [2]. To do that, 20 blocks of four littermate piglets from INRA-UMRVP herd and weaned at 28 days of age were constituted according to their body weight (7.8 kg). Within each block, one piglet was affected to one among the four treatments resulting from a 2 x 2 factorial design: two levels of dietary Trp (adequate and deficient diets) and two levels of sanitary status (clean, with dietary antibiotics, *vs* unclean environment, without supplement). The experimental period, from weaning to 50 d post weaning, was divided into 3 periods : the first 20 days with a phase I type diet, the following 20 days with a phase II type diet then a 10 d period following transfer to the growing unit also with phase II diet. Phase I and II Trp adequate diets (Trp-adeq) were formulated to meet nutritional requirements of post weaning piglets [8] whereas Trp supply was decreased by 20% in the deficient diets (Trp-def). Pigs were fed according to body weight at restricted feeding level in order to suppress or limit feed refusals. Pigs were weighed weekly and blood was taken 12, 33 and 47 days post weaning after an overnight fast for analysis of plasma concentrations of amino acids and haptoglobin, a major acute phase protein (APP) in pigs.

Experiment 2: a second experiment was designed to study Trp metabolism and its interaction with pig inflammatory response. Ten blocks of three littermate pigs (40 d of age, 11.8 kg) were constituted on their body weight basis. Pig's genotype and experimental diets (only phase II diet was used) were the same as those used in the previous experiment. Within a block, pigs were affected to one of the three following experimental groups: 1) healthy control pigs fed the Trp-def diet, 2) pigs suffering from lung inflammation induced by an intravenous injection of a single dose of complete Freund's adjuvant (CFA) and fed the Trp-def diet or 3) the Trp-adeq diet. Within a block pigs were fed the same amount of feed. Blood samples were taken every two days and pigs were slaughtered 9 days after the induction of lung inflammation for tissue sampling.

Results

Experiment 1: there were no significant interactions between dietary Trp and sanitary conditions on growth performance, plasma Trp and haptoglobin concentrations. Despite restricted feeding and contrary to what was observed in a previous experiment [2], pigs kept in an unclean environment had lower feed intake (AFI) than control pigs. From weaning to 19 d post weaning then from 40 d to 50 d post weaning (table 1) daily gain (ADG) and feed per gain (F/G) were significantly altered by depressing the quality of the environment.

Table 1. Effect of sanitary status and dietary Trp level on growth performance.

	Trp-def		Trp-adeq		Main effects
	unclean	clean	unclean	clean	
AFI, g/d					
0-19d	256	278	261	285	S
20-40d	744	754	758	765	T
41-50d	859	943	926	977	S, T
ADG, g/d					
0-19d	174	207	194	227	S, T
20-40d	483	476	492	488	
41-50d	322	459	399	508	S, T
F/G					
0-19d	1.71	1.38	1.36	1.26	T
20-40d	1.57	1.57	1.54	1.57	
41-50d	3.62	2.06	2.45	1.94	S

Main effects correspond to a significant effect ($P < 0.05$) of sanitary status (S) and dietary level of Trp (T).

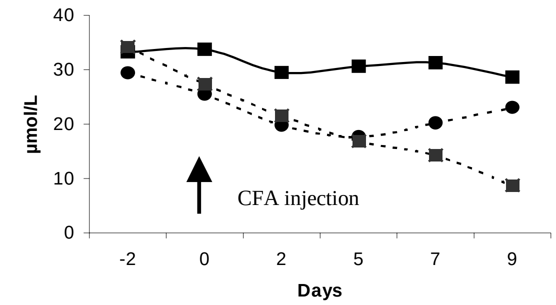
At 12 and 47 d after weaning pigs kept in the unclean environment had lower plasma Trp and higher haptoglobin concentrations (table 2). The effect of sanitary status on Trp concentrations remained significant when values were adjusted to constant feed intake by covariance. Trp deficiency induced lower growth performance and plasma Trp concentrations but did not affect haptoglobin concentrations.

Table 2. Effect of sanitary status and dietary Trp level on haptoglobin and Trp plasma concentrations.

	Trp-def		Trp-adeq		Main effects
	Unclean	clean	unclean	clean	
Haptoglobin, mg/ml					
W+12d	2.11	1.46	2.55	1.16	S
W+33d	0.74	0.87	0.63	0.34	
W+47d	2.12	1.45	2.58	0.93	S
Trp, nmol/ml					
W+12d	18.6	24.7	21.1	29.6	S, T
W+33d	22.9	23.9	27.6	30.3	T
W+47d	14.0	22.8	21.9	28.9	S, T

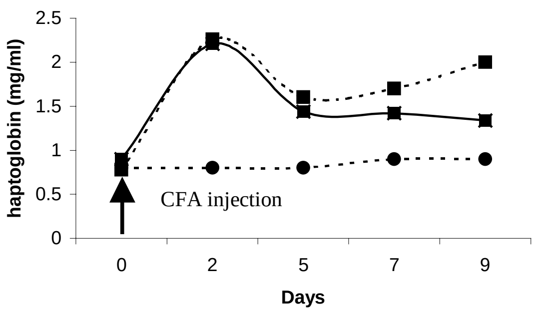
Experiment 2: pigs suffering from chronic lung inflammation exhibited a strong decrease in their plasma Trp concentrations when they were fed a Trp-def diet (figure 1) whereas they were able to maintain plasma Trp concentration when they were fed a Trp-adeq diet.

Figure 1. Plasma Trp concentrations in healthy control pigs fed a Trp-def diet (--●--), CFA fed a Trp-def diet (--■--) or a Trp-adeq (—■—).



In pigs fed the Trp- def diet, IDO activity measured in the lungs was induced by inflammation (1 *vs* 31 nmol of kynurenine produced from Trp/mg of protein/h) but IDO induction caused by inflammation tended to be less pronounced in pigs fed the Trp-adeq diet (12 vs 31 nmol of kynurenine produced from Trp/mg of protein/h). In addition, pigs suffering from chronic lung inflammation had lower signs of inflammation (haptoglobin response presented on figure 2, rectal temperatures and lung lesions observed post mortem) when they were fed the Trp-adeq diet.

Figure 2. Plasma haptoglobin concentrations in healthy control pigs fed a Trp deficient diet (--●--), CFA fed a Trp deficient diet (--■--) or a Trp adequate diet (—■—).



Discussion

Our studies showed increased levels of plasma haptoglobin in pigs suffering from lung inflammation and in pigs kept in unclean conditions. Haptoglobin is an acute phase protein synthesised and released by the liver in response to activation by pro-inflammatory cytokines such as IL-6 and IL-1. This protein is considered as a sensitive and relevant indicator of acute or chronic infectious or non-infectious diseases [6]. In experiment 1, the increase in haptoglobin concentration noticed at 12 then at 47 days post weaning was related to decreased growth performance probably due to a moderate immune response.

Pigs submitted to an immune challenge were not able to maintain their plasma Trp concentration when they were fed a low Trp diet (exp. 1 and 2) but also a Trp-adeq diet (exp.1). Because the decrease in plasma Trp could be related neither to a decrease in food consumption nor to an increase in Trp utilisation for body protein accretion, we hypothesize that immune system stimulation modifies Trp metabolism and may lead to a reduction of Trp availability for growth and other metabolic purposes. In exp. 1, we showed that growth depression caused by immune system activation is limited by increasing the dietary Trp. However, since decreased Trp concentration was also noticed for pigs fed the Trp-adeq diet and submitted to a moderate immune system stimulation, we can speculate that, in these pigs, Trp may be still limiting for growth.

During immune system activation, the decrease in plasma Trp can be explained by the synthesis of APP which are Trp rich proteins [7] and by the degradation of Trp into kynurenine through the induction of IDO activity. This enzyme, located in several tissues but the liver, and immune cells such as macrophages and dendritic cells, is induced by interferon gamma. IDO induction and consecutive Trp depletion might be a T cell proliferation modulator mechanism involved in immune tolerance process [5] but it has been also proposed that the production of some Trp metabolites along the kynurenine pathway could act as free-radical protector [1]. An adequate Trp dietary supply helps pigs submitted to an immune challenge to maintain their plasma Trp concentration and to reduce inflammatory response (experiment 2). The benefit of Trp as a moderator of the inflammatory response may be ascribed to a restoration of inflammation control by T-cells or by the antioxidant properties of Trp and its metabolites but remains to be demonstrated.

Conclusion

In conclusion, these two experiments showed that stimulation of the immune system by an inflammatory challenge or through depressing the quality of the environment where pigs are kept modifies Trp metabolism and probably limits Trp availability for growth performance. Moreover our results showed the importance of maintaining an adequate Trp supply in order to preserve health and growth performance of pigs submitted to immune system stimulation.

Acknowledgements

The authors would like to acknowledge Ajinomoto-Eurolysine for their financial support.

References

[1] Christen S, Peterhans E, Stocker R (1990) Proc. Natl. Acad. Sci. USA 87: 2506-2510.
[2] Le Floc'h N, Jondreville C, Melchior D, Sève B, Matte J (2004) Journées Rech. Porcine 36 : 1159-164.
[3] Melchior D, Sève B, Le Floc'h N (2004) J. Anim. Sci. 82 : 1091-1099.
[4] Melchior D, Mézière N, Sève B, Le Floc'h N. (2004) Journées Rech. Porcine 36 : 165-172.
[5] Mellor AL, Munn DH (1999) Immunology Today 20: 469-473.
[6] Niewold TA, Tousaint MJM , Gruys E (2003) Proceedings 4th European Colloquium on APP (Segovia, Spain) : 57-67.
[7] Reeds PJ, Fjeld CR, Jahoor F (1994) J. Nutr. 124: 906-910.
[8] Sève B (1994) INRA Prod. Anim. 7: 275-291.