RELATIONSHIPS BETWEEN LYSINE LEVEL IN FEED OF WEANERS, ACUTE PHASE PROTEIN AND PERFORMANCES

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ABSTRACT

Close relationships between nutrition, sanitary conditions and immune response have been described. Haptoglobin is an acute phase protein in pig. This biomarker has been shown to be linked to the sanitary conditions of pig farms. The objective of this work is to point out a possible relationship between the characteristics of feed during the post-weaning period and the level of haptoglobin in pig.

We evaluated in three trials the haptoglobin in blood of pigs fed with different lysine, protein or energy levels in feed. The 3 trials were performed on weaners between 7 and 10 weeks of age in two different farms.

In trial 1, pigs with higher level of crude protein (CP), net energy and lysine in feed showed the worse average daily gain (ADG). The level of haptoglobin increased significantly in this group of pigs. In trial 2, feeds only differed by their crude protein and lysine levels. Haptoglobin did not show difference between groups. The feed with higher lysine and CP levels had better ADG. In Trial 3, CP levels of 15, 17, 19% in the feed showed no impact on haptoglobin level. ADG showed a negative correlation with haptoglobin at 10 weeks.

Further work is needed to validate if lysine or protein excess could enhanced an acute phase response in pigs at the end of the post-weaning period. Haptoglobin evaluation, as a routine tool, could help to investigate bad growth performances during the post weaning period in farms.

Keywords: haptoglobin, piglets, lysine, protein, requirements, growth, average daily gain

INTRODUCTION

Haptoglobin is one of the major acute phase proteins in pigs (Eckersall 1996). It has been used to identify clinical, subclinical diseases (Sorensen 2006 – Para 2006) and stress situations (Pineiro 2007). For these reasons it has been proposed as a screening parameter in health management systems in piglet rearing. Haptoglobin could help to detect disturbances of homeostasis leading to poor health and bad growth performances (Gymnich 2004). Bad sanitary conditions during the post weaning period lead to an increase of haptoglobin (Le Floc'h 2006).

During inflammatory states or immune challenges nutrient requirements change (Humphrey 2003 – Obled 2003). The optimal level of amino-acid could be different especially for lysine, essential amino acid for pig growth (Williams 1997). Nutrition is also able to modulate the immune response (Ilsley 2005). The protein metabolism is deeply modified during inflammatory state (Sandberg 2007 – Le Floc'h 2004). Tryptophan and threonine decrease while lysine increases in piglets submitted to bad sanitary conditions during the post-weaning period (Le Floch 2006). In poultry, it has been demonstrated that deficiency of certain amino acids diminished

many components of the immune response (Takahashi 1997– Konashi 2000). Lysine requirement under immunological stress are decreased, and added lysine does not lead to better growth (Klasing and Barnes 1988, Robert 2005).

The objective of this work is to evaluate in 3 nutritional trials the relationship between feed characteristics, haptoglobin level and growth performances of piglets during the post-weaning period. We focused on routine feed characteristics: the level of lysine and protein, because most of the papers deal with specific nutrients or amino acids. Another purpose was to evaluate the interest of routine haptoglobin measurements in nutritional trials.

MATERIALS AND METHODS

Animals and farms

The three trials were performed during the post weaning period (from weaning to 70 and 82 days of age). Trial 1 took place in a commercial farrow to finish farm. The piglets (Landrace X Large White X Pietrain) were weaned at 28 days. Weights were registered by pen. Trials 2 and 3 were conducted at the St. Symphorien research station, in the same post-weaning unit. This post-weaning unit is separated in 48 identical pens of 1.84 m² surface. Sows are crossed Landrace and Large White. The boars are from the commercial bread Master, provided by France Hybrid. All piglets were weaned at 21 days. They were weighted individually.

Haptoglobin analysis

Haptoglobin was quantified in sera. Blood samples were taken from the vena cava. We used a single radial immunodiffusion Plate Test (code P0305 –1 Cardiotech Services Inc, Louisville USA). The agar gel plate contained rabbit antibodies specific for pig haptoglobin. Pig sera were diluted and distributed in the wells of the agar plates. After 24 hours of incubation, a precipitin ring appears. Its diameter is proportional to the concentration of haptoglobin. The test was performed according to the manufacturer except for the reference curve. We used a five points curve – instead of a two points – by diluting the provided standards. The curve requires a $R^2>0.99$ to validate the plate.

Statistical analysis

GLM Univariate procedure (SPSS 14.0) was applied for all growth data. Experimental groups and sex were fixed effect factors. The mixed model procedure (SPSS 14.0) was used for Haptoglobin data when obtained at different times on the same animals. Correlation between parameters was analysed using Pearson's correlation test.

Experimental designs

Trial 1

96 piglets were distributed according to weaning weight in 8 pens of 12 piglets. Feed was provided *ad libitum*. A fixed amount of prestarter diet was fed before changing to the starter diet (table 1). Half of the piglets received feed with lower level of energy, crude protein and lysine (group 1B). The feeding schedule is shown in table 1. The piglets were followed up, in the post-

weaning unit, from 28 to 70 days of age. Pig weights were recorded by pen at 28, 49 and 82 days. Blood was collected from the same 20 pigs in each feeding group at 48 and 70 days.

Table 1. –Trial 1 – Feeding schedule from 28 to 82 days of age (CP crude protein, NE net energy)

	Feed	1A (control)	1B (low level)
	Piglets	48	48
	Pens	4	4
Prestarter diets	Feed 1	2 kg	–
	Feed 2	5 kg	4 kg
Starter diet	NE MJ/kg	9.7	9.5
	CP	18%	17%
	Lysine	1.24	1.05

The pre-starter diets are distributed until the consumption of the specified amount of feed is obtained.
Feed 1: NE 11.5 MJ/kg, CP 20.3, Lys 1.57
Feed 2: NE 10.3 MJ/kg, CP 18.8, Lys 1.33

Trial 2

240 piglets were distributed according to weaning weight in 48 pens of 5 piglets. They were divided into two groups receiving 2 different feeding schedules (table 2). Feed is provided *ad libitum*. The 2B diet has a lower level of crude protein and lysine. The energy level of the 2 starter diets was the same. The piglets were followed up in the post-weaning unit from 21 to 70 days of age. The bodyweight was recorded per piglet at 21, 28, 42 56 and 69 days. Blood samples were taken at 67 days of age from the anterior vena cava of the same 15 pigs in each feeding group.

Table 2. – Trial 2 – Feeding schedule from 21 to 69 days of age (NE net energy, CP crude protein)

		2A (control)	2B (low level)
	Piglets	120	120
	Prestarter diets Weaning – day 42	Feed 1 (2 kg)	–
		Feed 2	Feed 2
Starter diets Day 42 – 69	NE MJ/kg	9.7	9.7
	CP	18%	16.5%
	Lysine	1.24	1.05

The feed 1 is distributed until the consumption of the specified amount of feed is obtained.
Feed 1: EN 11.5 MJ/kg, CP 20.3, Lys 1.57
Feed 2: EN 10.3 MJ/kg, CP 18.8, Lys 1.33

Trial 3

At 42 days, 240 piglets were distributed according to weaning weight in 4 groups (table 3). Each pen included 5 pigs, except the 4th group where one more pig was added to evaluate the impact of density. In each experimental group 24 piglets were randomly selected for blood sampling at 42 and 68 days. The piglets were followed up in the post-weaning unit from 42 to 70 days of age. Bodyweight was recorded per piglet at 42, 56 and 69 days. Blood samples were taken from the same 24 pigs in each group at 42 and 68 days.

Table 3. – Trial 3 – Experimental groups and feeds. (NE net energy, CP crude protein)

	3A	3B	3C	3D
Piglets	24	24	24	24
Pigs / pen	5	5	5	6
NE (MJ/kg)	9.8	9.8	9.8	9.8
CP	15%	17%	19%	17%
Lysine	1.18	1.18	1.18	1.18

RESULTS

Trial 1

Growth performance

Piglets from the control group showed better average daily gain (ADG) during the pre-starter diets period. The diet with lower levels showed better growth during the distribution of the starter diet. There is no difference in the ADG for the whole post-weaning period. (Table 4)

Table 4. – Trial 1 – Average Daily Gain between 28 and 82 days of age – Mean (+/– SD)

		ADG g/d		
	Weight at 28 day	Day 28 to 49	Day 49 to 82	Day 28 to 82
1A – Control group	8.50 (+/–1.25)	424.2 (+/–11.3)	649.2 (+/–27.3)	561.7 (+/–19.9)
1B – "Low level" group	8.39 (+/–1.24)	363.2 (+/–36.5)	720 (+/–44.8)	581.4 (+/–31.8)
F test	*P=0.9*	*P=0.019*	*P=0.035*	*P=0.33*

Haptoglobin

The serum haptoglobin level showed a significant increase ($p<0.001$) from 49 to 70 days of age in the control group (1A). This increase is not observed in the "low level" group. The results between feeding groups differ at both sampling times (figure 1).

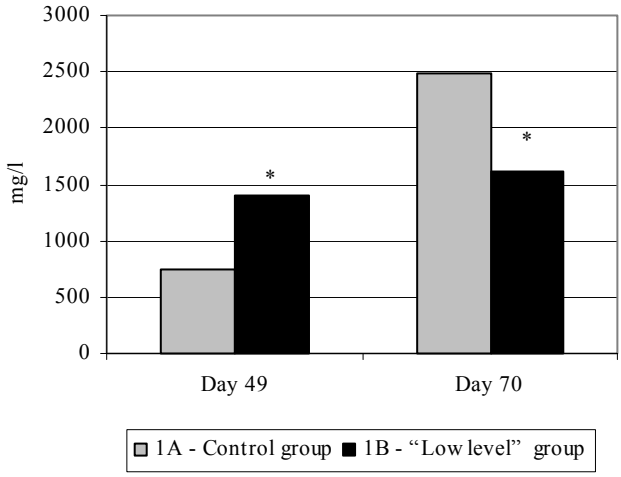

Figure 1. Trial 1 – Serum Haptoglobin – * Mean haptoglobin levels differ between groups ($p<0.05$)

Trial 2

Growth performance

At 21 days the bodyweight was 6.3 (+/–0.9) kg in both groups. The ADG during the prestarter period showed no difference between the groups. The control group had a better growth during the distribution of the starter feed, especially during the first 14 days (table 5).

Table 5. – Trial 2 – Average Daily Gain between 21 and 69 days of age – Mean (+/– SD).

	ADG g/d			
Period (days of age)	21– 42	42–56	56–69	42–69
2A – Control group	399.0 (+/–41.1)	674.5 (+/–64.9)	824.7 (+/–82.2)	752.5 (+/–43.9)
2B – "Low level" group	410.7 (+/–42.5)	581.7 (+/–83.3)	791.1 (+/–98.7)	690.3 (+/–68.8)
F test	*P=0.45*	*P=0.002*	*P=0.32*	*P=0.006*

Haptoglobin

At the end of the post weaning period the average level of haptoglobin was respectively 2226 and 1650 mg/l in the control and the "low level" group (figure 2). There is no significant difference.

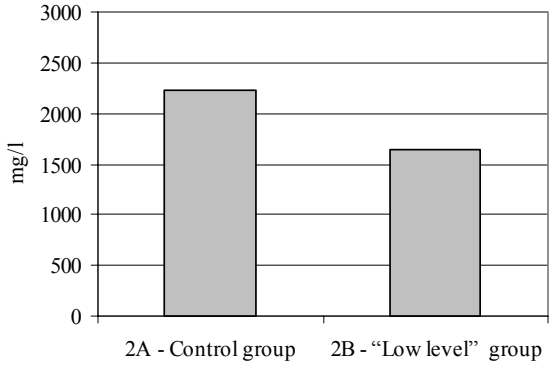

Figure 2. Trial 2 – Serum Haptoglobin at day 67

Trial 3

Growth performance

The group 3A (low level of crude protein) showed the worse. ADG during all the periods (table 6).

Table 6. – Trial 3 – Average Daily Gain between 42 and 69 days of age – Mean (+/– SD)

	N	Day 42 to 56	Day 56 to 69	Day 42 to 69
3A	24	512.8 [a] (+/–110.7)	709.6 [a] (+/–111. 3)	614.8 [a] (+/–104.0)
3B	24	609.2 [b] (+/–95.0)	780.2 [bc] (+/–90.9)	697.9 [b] (+/–66.9)
3C	24	590.4 [b] (+/–97.0)	825.2 [c] (+/–86.8)	712.13 [b] (+/–72.0)
3D	23	590.5 [b] (+/–117.9)	765.1 [b] (+/–85.2)	681.0 [b] (+/–95.1)
F test		*P=0.006*	*P=0.001*	*P=0.001*

[abc] Figures in the same column with different letters differ significantly.

Haptoglobin

There is no correlation between the individual haptoglobin levels of the pigs of 42 and 69 days of age. The haptoglobin level decreases significantly between 42 and 67 days ($p<0.001$) (Figure 3). There is no significant difference in haptoglobin levels between groups at 42 and 67 days.

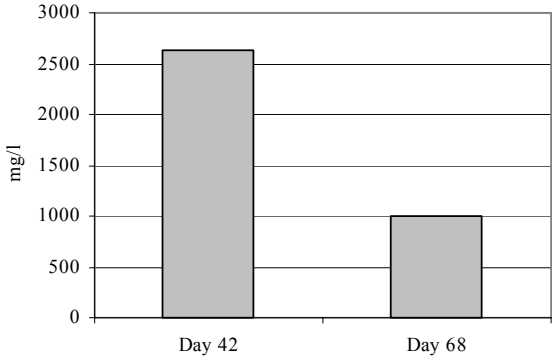

Figure 3. Trial 3 – Serum Haptoglobin at day 67

There was a sex effect at 67 days, males showing a level of 896.8 mg/l and females 1146.7 mg/l (p=0.049). This effect was not observed at day 42.

The pig density (kg/m²) in the pen, calculated with the effective pig weights in each pen at 69 days showed no correlation with the haptoglobin level at 42 and at 67 days of age. The pig density at 69 days showed an average of 86.5 kg/m² and ranged from 65 to 117 kg/m². The ADG from days 56 to 69 and for the whole period showed a significantly negative correlation with the haptoglobin levels at 68 days (fig 1 – table 4).

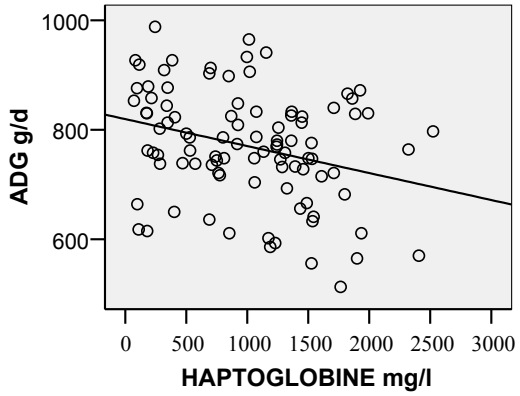

Figure 4. Trial 3 – Relationship between ADG from 56 to 69 days and haptoglobin at 67 days

Table 7. Trial 3 – Correlation between haptoglobin and ADG

	ADG 42–56 days	ADG 56–69 days	ADG 42–69 days
Haptoglobin at 42 days	NS	NS	NS
Haptoglobin at 67 days	NS	R=–0.295 **P=0.004**	R=–0.237 **P=0.021**

DISCUSSION

Trial 1 leads to unexpected results. There is no difference of ADG between the standard and the "low level" feed (1B). The growth pattern between groups is different. During the first period (weaning to 49 days), the ADG obtained with the standard feed (group 1A) is better than the low level feed. But from day 49 to 82, the opposite situation is observed. During the same period of time, the haptoglobin level increases significantly in group 1A. The average pig weight was 39.3 kg at the end of the post-weaning unit. The lower levels in the starter diet of the 1B group were more suitable for pigs with more than 35 kg bodyweight (Brossard 2007). It does not explain the worse ADG of the 1A group, except if an excess of lysine, protein or net energy has negative impact on growth. Sauerwein 2005 observed the same situation. An increase of the haptoglobin level between 7 and 10 weeks was associated with decreased ADG. In Sauerwein's trial the increase of haptoglobin was due to an outbreak of respiratory symptoms. We did not observe any specific symptoms in the pigs of the 1A group. The increase of haptoglobin could be attributed to a subclinical inflammation state (Sorensen 2006). It was not possible to measure individual pigs' weights, because trial 1 was done in a commercial farm. Bodyweight was measured by pen. Only few pens (4 for each experimental group) were used. All the pens were located in the same room. It would be surprising that a subclinical infection affects only the pens of the same experimental group. Nevertheless, it is not possible to strictly conclude that the composition of the diet – higher energy, crude protein and lysine level – is responsible for an enhancement of the acute response. We already observed the same situation in poultry (Robert 2005). A diet with higher level of protein and lysine at the time of vaccination seemed to enhance the inflammatory response of animals.

Trial 2 used the same kind of feeding schedule than trial 1, except the net energy levels which were the same for both starter diets. Unfortunately blood was only sampled at the end of the trial and only from 15 pigs instead of 20 in trial 1. The growth performance of the starter diet (42 to 69 days) was in accordance with our expectation. The 2A group (higher lysine and crude protein level in the starter diet) did not show better ADG after 56 days anymore. That is in accordance with the decrease of lysine needs at the end of the post-weaning period. Haptoglobin levels in the groups are very similar between both trial 1 and 2 at 10 weeks of age (1A, 2480 mg/l; 2A, 2226 mg/L, 1B 1610 mg/l, 2B 1650 mg/l). Nevertheless the haptoglobin difference between groups in trial 2 was not significant.

An inflammatory state between 6 and 10 weeks of age could reduce the needs for lysine and/or protein for growth. This hypothesis is in accordance with Williams 1997. More work have to be done to know if an excess of lysine or protein during this period is able to enhance the acute phase response and could have an additional negative effect on performance of pigs.

The lower level of protein in trial 3 showed the worse ADG. Compared with the other trials the level of haptoglobin at the end of the post-weaning period was low in trial 3. It decreased from 42 to 68 days. Compared with other trials, pigs did not express any acute phase response. The tested situations (different crude protein levels and overcrowding) showed no impact on haptoglobin level. Females showed higher haptoglobin levels. That is in accordance with Clapperton 2005. He found higher haptoglobin level on females at 18 weeks. There was a negative correlation between haptoglobin level at the end of the period and ADG. This negative correlation was highly significant during the two weeks before blood sampling but remains significant for the whole starter diet period (42 to 69 days of age). Haptoglobin at the beginning of this period showed no correlation with ADG. Sauerwein 2005 observed the same. Clapperton

2005 worked with fatteners between 18 and 24 weeks of age. He also showed a negative correlation between ADG and haptoglobin at 24 but not at 18 weeks of age.

CONCLUSIONS

It was not possible to definitely conclude, from this work, that lysine or protein excess could enhance an acute phase response in pigs. Further work is needed to validate this hypothesis. Nevertheless the obtained results demonstrate that pigs can go through an acute phase response without showing any clinical sign. Utilization of feed by the animal is depending on the inflammatory state. So, it seems necessary to measure acute phase protein during feed trials to better analyze the results. Haptoglobin evaluation, as a routine tool, could help to investigate bad growth performances in farms.

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