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INFORMATION N°31

Threonine in pigs and broilers:
A crucial amino acid for growth and gut function

To achieve maximum **growth performance**, feed must satisfy animals' nutritional requirements. However, as **environmental** and **food safety** issues arise, such as reduction of nitrogen (N) excretion and the ban of antimicrobial growth promoters (AGP), new constraints must be taken into account. In this context, the **reduction of dietary protein level** is an important factor in achieving these objectives.

European Union implemented regulations aim to limit and **reduce nitrogen excesses in the environment** (directive 96/61/EC, etc…). Thus, the development of poultry and pig farms is limited by the level of their polluting emissions. By reducing the nitrogen input on farm, reduced protein diets lower the nitrogen output into the environment. For instance, in broilers, a reduction of 2 points of dietary protein content results in a 13 to 14% reduction of nitrogen excretion (Bregendahl *et al.,* 2002; Jialin *et al.,* 2004) and in pigs, a 1 point reduction of dietary protein decreases total nitrogen excretion by 10% (Relandeau *et al.,* 2000).

In addition, **sanitary status can be improved by reducing the dietary crude protein** supply both in pig and broiler productions. Weaning is a critical period for piglets which have to face numerous stress factors such as separation from the sow and transition from milk to solid feed. As a result, **diarrhoea** occurs frequently and could be reduced by limiting the protein flow through the gut. In broilers, crude protein (CP) level is reported as a predisposing factor of **Necrotic Enteritis** (Dahiya *et al.,* 2005; Mc Devitt *et al.,* 2006; Van Immerseel *et al.,* 2004 and Drew *et al.,* 2004). Moreover, when protein is in excess, a greater quantity of water is required to achieve efficient N excretion (Maria, 2005). A practical consequence of this effect in broilers is the degradation of **litter quality** with high CP diets leading to the deterioration of the sanitary environment.

The use of reduced CP diets without a detrimental effect on growth performance is possible through the addition of free amino acids, such as **L-Threonine**.

Regarding European diets, **threonine is the 2nd limiting amino acid after lysine in pigs, and the 3rd after sulphur amino acids and lysine in broilers.** Besides its utilisation for protein synthesis, threonine is involved in biological functions such as gut integrity and immunity. Therefore, the whole threonine requirement is likely to vary according to the importance of each function.

This bulletin aims to review the threonine requirement of pigs and broilers based on recent data and discusses factors which can lead to its variation. The biological functions of threonine are also described with reference to recent research trials and illustrated by practical examples.

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Thr:Lys recommendation for pigs and broilers

Piglet 4-25 kg BW*	:	**65% SID**
Grower pig 15-70 kg BW	:	**67% SID**
Finisher pig 50-110 kg BW	:	**68% SID**
Gestating sow	:	**>70% SID**
Lactating sow	:	**>70% SID**
Broiler 0-42 days	:	**65% SD**
Broiler 42-56 days	:	**>65% SD**

Given values correspond to mean dietary threonine levels that optimise body weight gain and feed efficiency of given animal categories. They are based on current scientific knowledge and experimental evidences available at the time of writing this bulletin.

* BW: Body weight

1- Optimum Thr:Lys ratio increases with animals' age and body weight

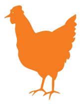
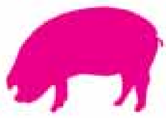
Being the 2nd limiting amino acid in European pig diets and the 3rd in broiler diets, threonine is an essential nutrient for both pigs and broilers. Threonine requirement has been extensively studied ever since L-Threonine has been commercially available because its use allows for a reduction of CP in a diet. Nevertheless, the published results often vary depending for instance, on the statistical model used, unit of expression and age, and are therefore difficult to compare (Leclercq, 1998; Barkley and Wallis, 2001). A good estimation of the threonine requirement should take into account these variation factors.

1.1. Tools for a practical estimation of threonine requirement

The ideal protein concept is defined as the amino acid profile which meets the animals' requirement for protein accretion and maintenance (Fuller *et al.*, 1989). Lysine is the reference amino acid because it is the 1st or 2nd limiting amino acid respectively in pigs and broilers and is mainly used for muscle protein accretion. The ideal protein is thus represented by a profile in which the supply of each essential amino acid is expressed as a percentage of the dietary lysine content. Each of these ratios can be directly introduced as a constraint in feed formulation.

In feedstuffs, **digestibility coefficients** for threonine are generally lower than for lysine and variable among raw materials and species (table 1). This is due to a lower speed of hydrolysis and therefore a slow time for absorption (de Blas *et al.*, 2000). The use of digestible values instead of total values is important for feed formulation, because it takes into account a better knowledge of the raw material quality for all the amino acids.

Additionally, **endogenous losses** are very rich in threonine (Le Bellego *et al.*, 2002). For instance, Thr:Lys ratio in endogenous losses have been measured to be 174% in ileal juice and 132% in poultry excreta (Kadim *et al.*, 2002), and 120% in pig endogenous losses (Mahan and Shields, 1998). This specific threonine trait, linked to its metabolic functions, underlines the importance of using **standardized digestibility** (SD) rather than apparent digestibility, as the latter system does not take into account endogenous losses.

		Broiler		Growing Pigs	
Feedstuffs	**Total threonine (g/kg)**	**SD coeff. (%)**	**SD Threonine (g/kg)**	**SID coeff. (%)**	**SID Threonine (g/kg)**
Wheat	3.1	83	2.6	83	2.6
Corn	3.0	88	2.6	83	2.5
Barley	3.5	76	2.7	75	2.6
Wheat middling	4.9	79	3.9	72	3.5
Soybean meal	17.7	89	15.8	87	15.4
Rapeseed meal	15.5	84	13.0	75	11.6
Sunflower meal	10.0	87	8.7	82	8.2
Peas	7.8	81	6.3	76	5.9

Table 1: Effect of amino acid evaluation systems (in pigs, standardised ileal digestibility (SID) *vs.* total; in broiler, standardised digestibility (SD) *vs.* total) on amino acid level in feedstuff using INRA standardised digestible coefficients (Sauvant *et al.*, 2004).

✔ From a practical point of view, threonine requirement is preferably:
- expressed as a ratio to lysine
- using standardised digestibility.

1.2. SID Thr:Lys requirement changes with pig weight

Several literature references report Thr:Lys requirement values as a function of pigs' body weight (Table 2). NRC (1998), Baker (2000), Jorgensen and Tybirk (2005) and Rostagno *et al.*, (2005) describe an increase of the optimal SID Thr:Lys ratio as the pig is getting heavier. This increase could be explained by an increase of the maintenance requirement of heavier animals (NRC, 1998; Baker, 2000; derived from maintenance requirement of Fuller *et al.*, 1989).

References	Body weight range (kg)	SID Thr:Lys (%)
NRC (1998)	3-5	65
	5-50	64
	50-120	68
Baker (2000)	5-20	65
	20-50	67
	50-110	70
Jorgensen and Tybirk (2005)	9-30	61
	30-55	65
	55-100	67
Rostagno *et al.* (2005)	Starter	63
	Grower	65
	Finisher	67

Table 2: SID Thr:Lys recommendations according to pigs body weight.

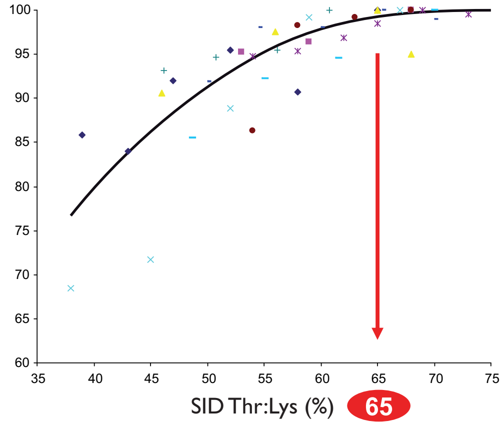
To illustrate the optimum SID Thr:Lys ratio in **piglets** (4-25 kg, 9 trials), **growers** (15-70 kg, 17 trials) and **finishers** (50-110 kg, 13 trials), a literature review on the effect of the SID Thr:Lys ratio on pig performance was conducted. Only articles reporting the composition of the experimental diets, together with amino acid levels were considered for this review. A quadratic regression was fitted to the data. Requirement for the animals was determined as 95% of the ratio that maximises average daily gain (ADG, figure 1) and feed conversion ratio (FCR, figure 2). These average dose response curves show that increasing the SID Thr:Lys ratio through L-Threonine supplementation allows for the optimization of pig performance (weight gain and feed conversion). Moreover, optimal SID Thr:Lys increases with pig weight: 65%, 67% and 68% respectively for piglets, grower and finisher pigs.

- ✔ **As it is described in the literature, the optimum SID Thr:Lys ratio increases with increasing animal weight.**
- ✔ **Optimum SID Thr:Lys ratio is 65% in piglets (4-25 kg BW), 67% in grower (15-70 kg BW) and 68% in finisher pigs (50-110 kg BW).**

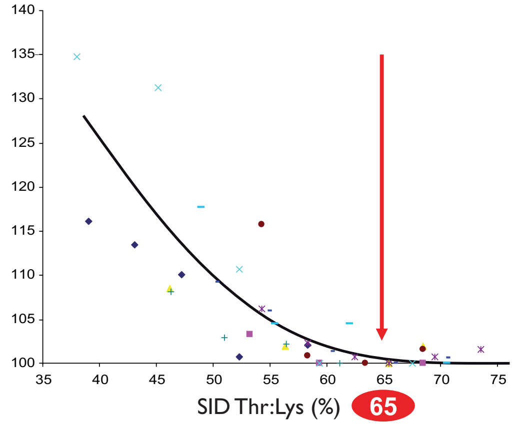
Figure 1: Average daily gain of pigs at various ages, % best performances within each trial

Figure 2: Feed conversion ratio of pigs at various ages, % best performances within each trial

Piglet (4-25 kg BW)

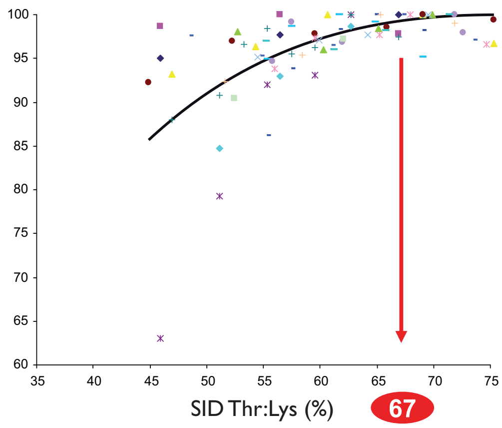


- Lewis & Peo, 1986 (4-14 kg)
- Gatel & Fékété, 1989a (8-25 kg)
- Gatel & Fékété, 1989b (8-25 kg)
- Adeola *et al.*, 1994 (10-20 kg)
- Schutte *et al.*, 1995 (10-20 kg)

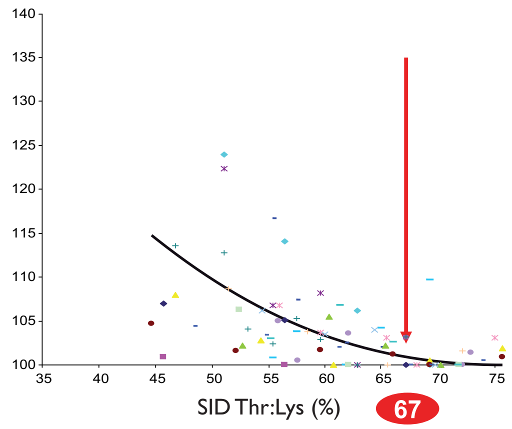


- Usry, 1999 (11-23 kg)
- Lenehan *et al.*, 2004a (10-20 kg)
- Lenehan *et al.*, 2004b (10-20 kg)
- Wang *et al.*, 2006 (10-25 kg)

Grower (15-70 kg BW)

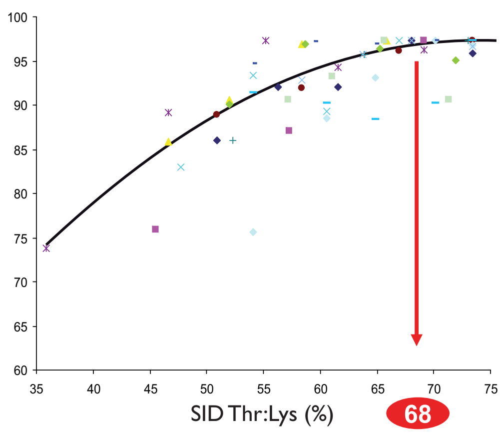


- Chang *et al.*, 2000a (15-55 kg)
- Chang *et al.*, 2000b (15-55 kg)
- Séve et al., 1993 (25-50 kg)
- Lenis et al., 1990a (35-65 kg)
- Conway *et al.*, 1990 (17-50 kg)
- Schutte et al, 1995 (20-40 kg)
- Taylor *et al.*, 1982 (25-55 kg)
- O'Connell *et al.*, 2006a (35-60 kg)
- O'Connell *et al.*, 2006a (35-60 kg)

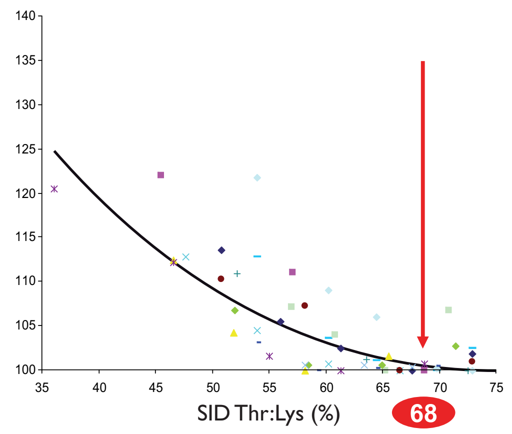


- Buraczewska *et al.*, 2006a (25-50 kg)
- van Milgen & Noblet, 2004 (50-70 kg)
- van Milgen & Noblet, 2002a (20-35 kg)
- van Milgen & Noblet, 2002b (50-70 kg)
- Bikker *et al.*, 2006 (25-45 kg)
- Frantz *et al.*, 2004a (35-60 kg)
- Kluge *et al.*, 2002 30-50 kg
- Bikker *et al.*, 2006 25-45 kg

Finisher (50-110 kg BW)



- Usry, 2000 (90-120 kg)
- Cadogan *et al.*, 1998 (60-100 kg)
- Schutte *et al.*, 1997a (50-95 kg)
- Schutte *et al.*, 1997b (50-95 kg)
- Saldana *et al.*, 1994 (58-96 kg)
- Lenis *et al.*, 1990 (65-95 kg)
- Lynch, 2000a (50-95 kg)



- O'Connell *et al.*, 2006b (80-100 kg)
- O'Connell *et al.*, 2006b (80-100 kg)
- van Milgen & Noblet, 2002c (90-110 kg)
- Frantz *et al.*, 2004b (80-105 kg)
- Quiniou *et al.*, 2006 (60-110 kg)
- Eder, 2004 (50-85 kg)

1.3. SID Thr:Lys requirement is at least 70% for sows during gestation and lactation

1.3.1. Gestating sow: Balanced between protein accretion and maintenance

During pregnancy, sows need nutrients for building up their reserves (fat and muscle) and to support the growth of their conceptus (foetus and associated membranes).

Dourmad and Etienne (2002) used the nitrogen balance technique on 16 animals between day 20 and 41 of gestation to determine the threonine requirement of gestating sows. Sows were allotted to four SID Thr:Lys ratios (61%, 71%, 77% and 84%). They observed that nitrogen retention was significantly increased with increasing threonine supply (P<0.05). Moreover, the best N retention was achieved with a 77% SID Thr:Lys ratio, but was not significantly different from 71% and 84% ratios.

More recently, Kim and Wu (2005) reviewed the amino acid requirement for tissue gain and maintenance of pregnant high-lean type sows, before and after day 70 of gestation.

During the first 2/3rds of the pregnancy, the development of mammary glands and fœtus does not consume high levels of nutrients. However, during the last third of pregnancy, nutrient requirement for accretion in those tissues is highly increased (Perez *et al.*, 1986).

Based on these observations and on literature, Kim and Wu (2005) calculated an ideal amino acid pattern for protein accretion and maintenance (table 3). SID Thr:Lys ratio is equal to 79% in the first 2/3rds of the pregnancy and to 71% in the last third, due to the increase of threonine requirement for accretion. SID Thr:Lys requirement in late gestation decreases due to a dilution of the maintenance requirement within the overall requirement.

	Requirement for	Lysine	Threonine
Day 0 to 70 of gestation	accretion	100	49
	maintenance	100	151
	accretion + maintenance	100	79
Day 70 to 112 of gestation	accretion	100	51
	maintenance	100	151
	accretion + maintenance	100	71

Table 3: Ideal amino acid patterns (SID) for protein accretion and maintenance in pregnant gilts (Kim and Wu, 2005).

- ✔ According to Kim and Wu (2005), SID Thr:Lys ratio is higher during the first 2/3rds of pregnancy due to the lower requirement for protein accretion.
- ✔ The requirement of gestating sows is at least 70% SID Thr:Lys ratio.

1.3.2. Lactating sow: Threonine requirement is linked to tissue mobilisation

During lactation, amino acid metabolism is mainly dedicated to milk protein synthesis: sows use dietary amino acids to produce milk and, when the supply is not sufficient, they use their own tissue.

In lactating sows, reports of the threonine requirement in the literature are scarce and variable. Indeed, Westermeier *et al.* (1998) and Paulicks *et al.* (1998) showed that the total Thr:Lys ratio required to minimize sow body weight losses and maximize milk production and litter weight gain was above 74% (this corresponds to 72% on a SID basis). However, Cooper *et al.* (2001) reported an optimal Thr:Lys ratio of 69% on a total basis (corresponding to about 65% on a SID basis). Nevertheless, in this last trial, sows gained weight during the lactation period, which does not occur under usual farming conditions.

High-lean prolific sows generally have low voluntary feed intake during lactation and have to mobilize their own tissues to meet their requirement. In addition, their reserves are poorly endowed in threonine compared with the requirement for milk production (table 4) (Kim *et al.*, 2001).

	Tissue mobilization	Milk production
Lysine	100	100
Threonine	42	59

Table 4: SID Thr:Lys ratio coming from the tissue mobilization and necessary for milk production in the sow (Kim *et al.*, 2001)

Thus, depending on the level of tissue mobilization, imbalance in amino acids is either more or less important. In order to optimize the utilisation of amino acids released in excess by tissue mobilization (in comparison to threonine), the dietary threonine supply should be higher in sows with low feed intake and with high tissue mobilization.

Kim *et al.* (2001) determined an ideal dietary amino acid pattern for the lactating sow as a function of the level of tissue mobilization (table 5).

Tissue mobilization	No	Low	Medium	High
Lysine	100	100	100	100
Threonine	59	63	69	75

Table 5: Ideal amino acid (SID) pattern depending of tissue mobilization (Kim *et al.*, 2001).

This dynamic ideal protein concept may explain the variability of the recommendation described in the literature. On the one hand, for low prolific sows with high feed intake, the level of tissue mobilization would be low, consequently the SID Thr:Lys requirement would be 59%, which is the requirement for milk production. On the other hand, for high prolific sows showing a low voluntary feed intake and so a substantial tissue mobilization during lactation (i.e. primiparous and second parity sows), threonine is a critical amino acid, and the dietary supply should be around 75% SID.

✔ **During lactation, the Thr:Lys ratio is linked to the tissue mobilization of the sows.**

✔ **Modern high-lean prolific sows have a SID Thr:Lys requirement higher than 70%.**

1.4. Threonine requirement in broilers for growth and carcass performance

Threonine requirement in broilers has been extensively studied over the last decade. In figure 3, optimal weight gain (g/bird/day) of 55 trials and associated threonine intake (g/bird/day) were gathered as a function of the age of the bird. Threonine intake was recalculated and expressed as standardised digestible (SD) using INRA coefficients (Sauvant *et al.*, 2004). The adjusted exponential curve describes a lower marginal efficiency for growth with increasing age and especially in broilers above 42 days of age. This might be related to specific functions of threonine, for instance a higher maintenance requirement for older and heavier birds (Leclercq, 1998).

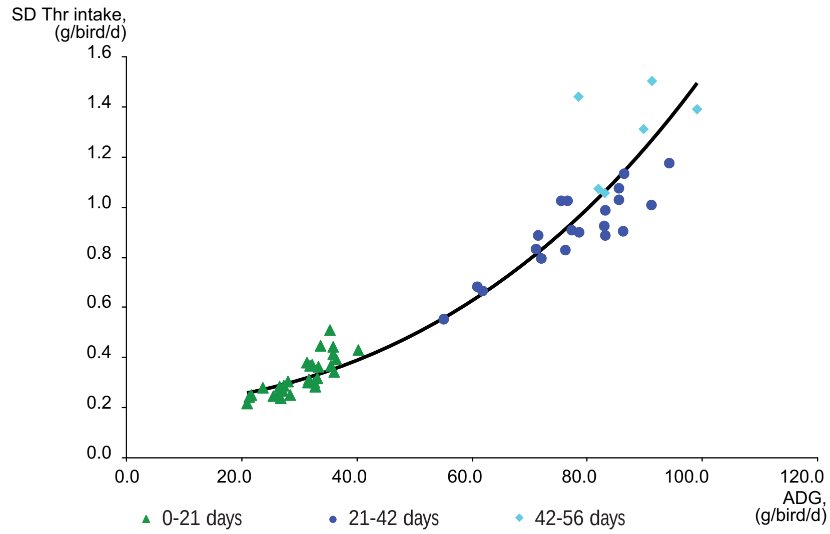


Figure 3: SD Threonine intake (g/bird/d) for optimal weight gain (g/bird/d). 30 data sets reviewed in Relandeau and Le Bellego (2004) (AJINOMOTO EUROLYSINE information N°27) and 25 additionnal trials described in figures 4 and 5

1.4.1. A 65% SD Thr:Lys ratio optimises performance of 0-42 day-old broilers

In order to refine the optimal SD Thr:Lys ratio of broilers between 0-42 days, 27 recent dose response studies (reported after 2000) were used. All the data were calculated in SD and are expressed as a ratio to lysine. A quadratic regression was fitted to the data and the requirement was determined as 95% of the ratio that maximises ADG (figure 4) and FCR (figure 5).

A ratio SD Thr:Lys at 65% optimises weight gain and feed efficiency of broilers for the overall period 0-42 days of age (figures 4 and 5), which is in line with recent recommendations (Rostagno *et al.*, 2005). Moreover, threonine requirement is the same for growth and feed efficiency.

Figure 4: 0-42 day-old broiler average daily gain, % best performance within each trial

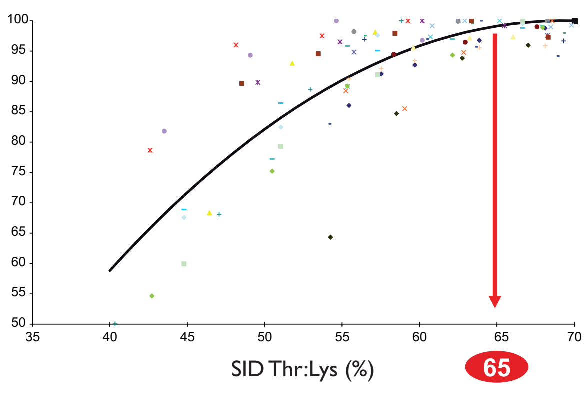
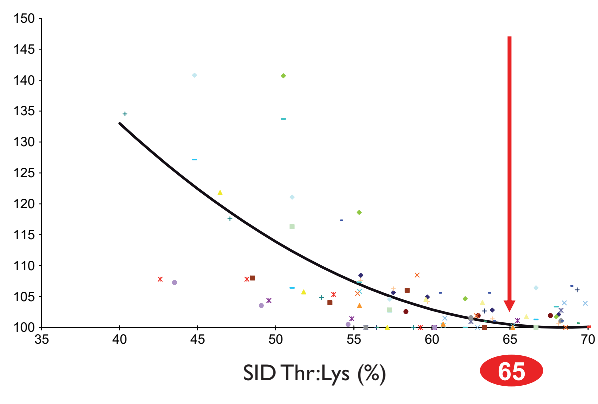


Figure 5: 0-42 day-old broiler feed conversion ratio, % best performance within each trial



- Pos *et al.*, 2005
- Pos *et al.*, 2005
- Atencio *et al.*, 2004
- Ciftci & Ceylan, 2004
- Ciftci & Ceylan, 2004
- Dozier *et al.*, 2003
- Shan *et al.*, 2003
- Shan *et al.*, 2003
- Lensing & Van der Klis, 2006
- Pos *et al.*, 2005
- Pos *et al.*, 2005
- Simon *et al.*, 2002
- Simon *et al.*, 2002
- Ciftci & Ceylan, 2004
- Ciftci & Ceylan, 2004
- Kidd *et al.*, 2004
- Kidd *et al.*, 2004
- Kidd *et al.*, 2004
- Corzo *et al.*, 2007
- Corzo *et al.*, 2007
- Atencio *et al.*, 2004
- Pos *et al.*, 2005
- Pos *et al.*, 2005
- Corzo *et al.*, 2003
- Rosa *et al.*, 2001
- Rosa *et al.*, 2001
- Roth *et al.*, 2004

✔ **A ratio SD Thr:Lys at 65% optimises weight gain and feed efficiency in broilers between 0 and 42 days of age.**

1.4.2. Optimal SD Thr:Lys ratio in broilers above 42 days of age

In order to evaluate the SD Thr:Lys ratio requirement above 42 days, a compilation of studies was carried out using recently published results. Optimal SD Thr:Lys ratios for growth performance (established by the authors), strain and sex in the different studies are presented in table 6 and figure 6.

- Among 6 trials, 4 indicate a SD Thr:Lys ratio requirement above 65% (average value of 67.5%)

	Authors	Strain	Sex
1	Kidd *et al.*, 1999	Ross 308 x Hubbard	M
2	Dozier *et al.*, 2000	Ross 308	M
3	Kidd *et al.*, 2003 a	Cobb 500	M
4	Kidd *et al.*, 2003 b	Cobb 500	F
5	Kidd *et al.*, 2003 c	Cobb 500	F
6	Atencio *et al.*, 2004	Avian Farm	M

Table 6: Trials characteristics used in figure 6

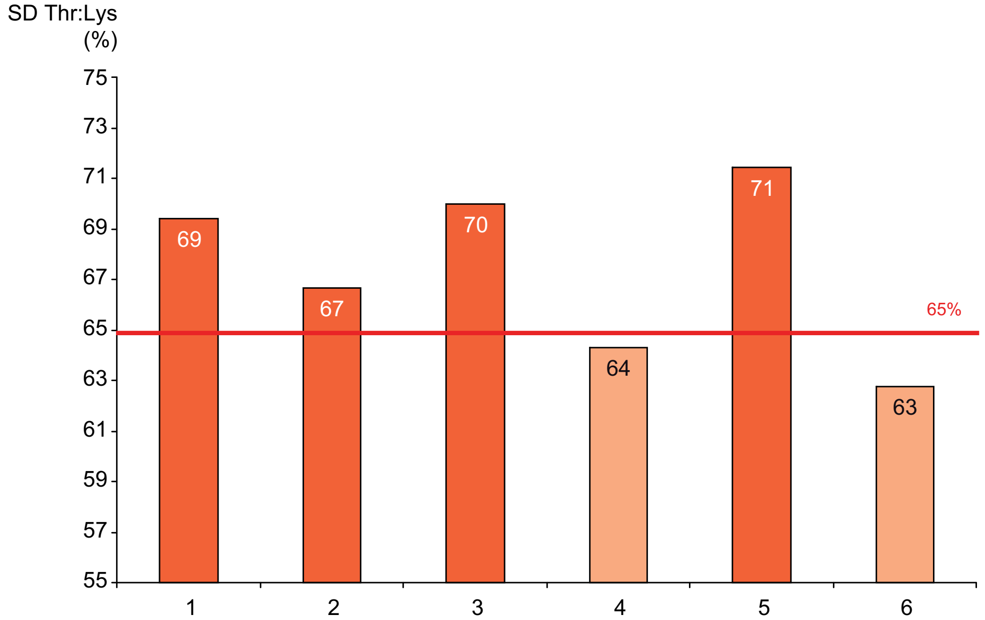


Figure 6: Optimum SD Thr:Lys ratio for average daily gain of broilers older than 42 days

As reported in the literature, Thr:Lys requirement increases with animal age. National Research Council recommendations (1994) indicated an increase of total Thr:Lys requirement for older broilers (from 69 to 74% on a total basis). More recently, Samadi and Liebert (2008) worked on the threonine requirement modelling of Ross 308 broilers and determined a positive correlation between age and total Thr:Lys requirement (from 73% to 80% with age varying from 0 to 8 weeks in a total basis).

✔ **For broilers above 42 days of age, SD Thr:Lys requirement for growth and feed efficiency is higher than 65%.**

1.4.3. Broiler carcass composition and Thr:Lys ratio

The effect of Thr:Lys ratio on carcass traits (breast meat yield and carcass yield) is very low and requirements are described as the same as for body weight gain (Leclerq, 1998; Kidd *et al.*, 2003 b,c; Kidd *et al.*, 2004, Corzo *et al.*, 2007). Thus, below the requirement, increasing threonine level improves carcass traits (Ciftci & Ceylan, 2004), but unlike lysine, increasing the level of threonine in the diet does not seem to increase breast meat yield *per se* (Relandeau and Le Bellego, 2004). Nevertheless, some authors reported linear improvement in the yield of leg quarters (Atencio *et al.*, 2004) and in carcass yield (Corzo *et al.*, 2003) (for SD Thr:Lys ratio from 60 to 80%), which suggests that the optimal Thr:Lys ratios for carcass traits were not matched at 65% SD Thr:Lys.

1.4.4. Practical implication of L-Threonine use in reduced CP diets

Lensing and Van der Klis, in 2006, studied the effect of dietary protein reduction on broiler growth performance with or without addition of L-Threonine.

Three thousand Ross 308 broiler chickens, raised from 0 till 39 days, were allotted to three different diets:

- 21% CP with 65% SD Thr:Lys;
- 19% CP with 55% SD Thr:Lys;
- 19% CP with 65% SD Thr:Lys, *via* L-Threonine addition.

Diets were based on wheat and soybean meal and contained 1.06, 1.02 and 1.00% SD lysine and 12.1, 12.3 and 13.6 MJ/kg AME respectively for 0-14; 15-29; 30-39 days of age. Litter quality was visually scored at 21 days on a scale from 1 (very wet litter) to 10 (very dry litter), thus, higher values describe a better quality of the broiler environment. Results are presented in figures 7 and 8.

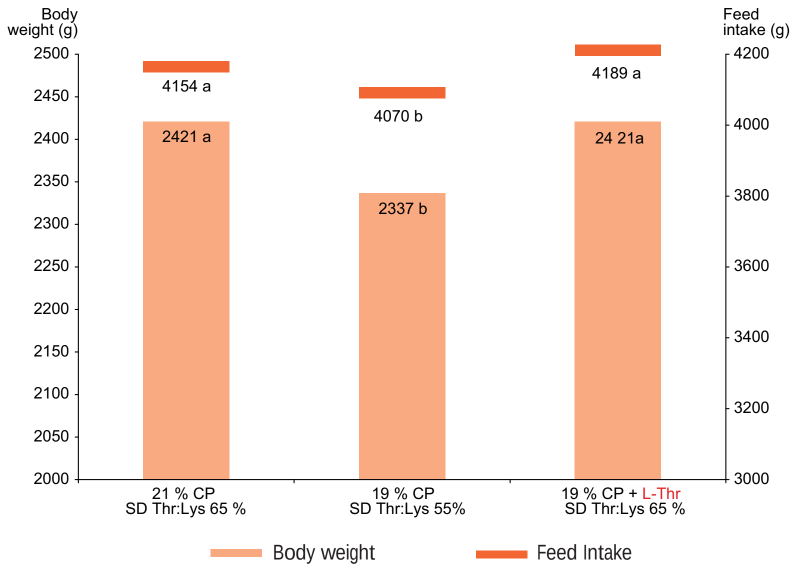


Figure 7: Effect of the crude protein level (CP) and the SD Thr:Lys ratio on body weight and feed intake during the 39 day-study (Lensing and Van der Klis, 2006)

- The reduction of 2 points of protein (21% to 19%) without L-Threonine supplementation resulted in a significant decrease in broiler weight at days 29 ($p<0.05$) and 39 ($p<0.05$) and a significant decrease in feed intake for the overall period (figure 8).
- L-Threonine supplementation in the reduced CP diet allowed the birds to reach the same growth performance when compared with the 21% CP diet.
- The reduced CP diet led to a significantly drier litter than the high CP diet (figure 10). The reduced CP diet balanced with L-Threonine was scored as intermediate.

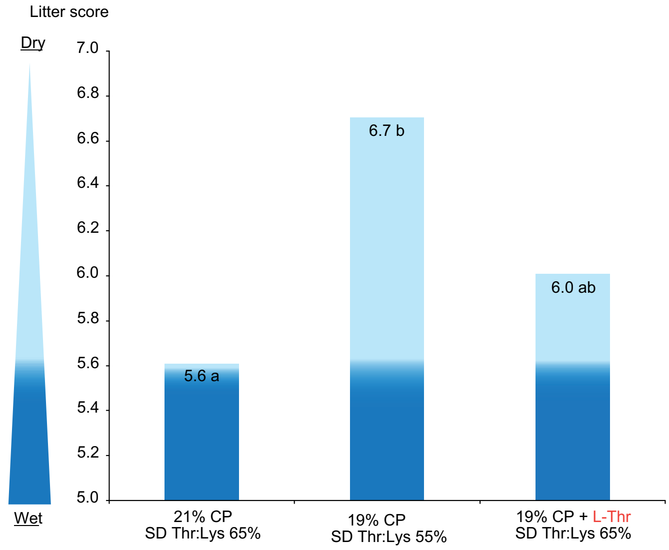


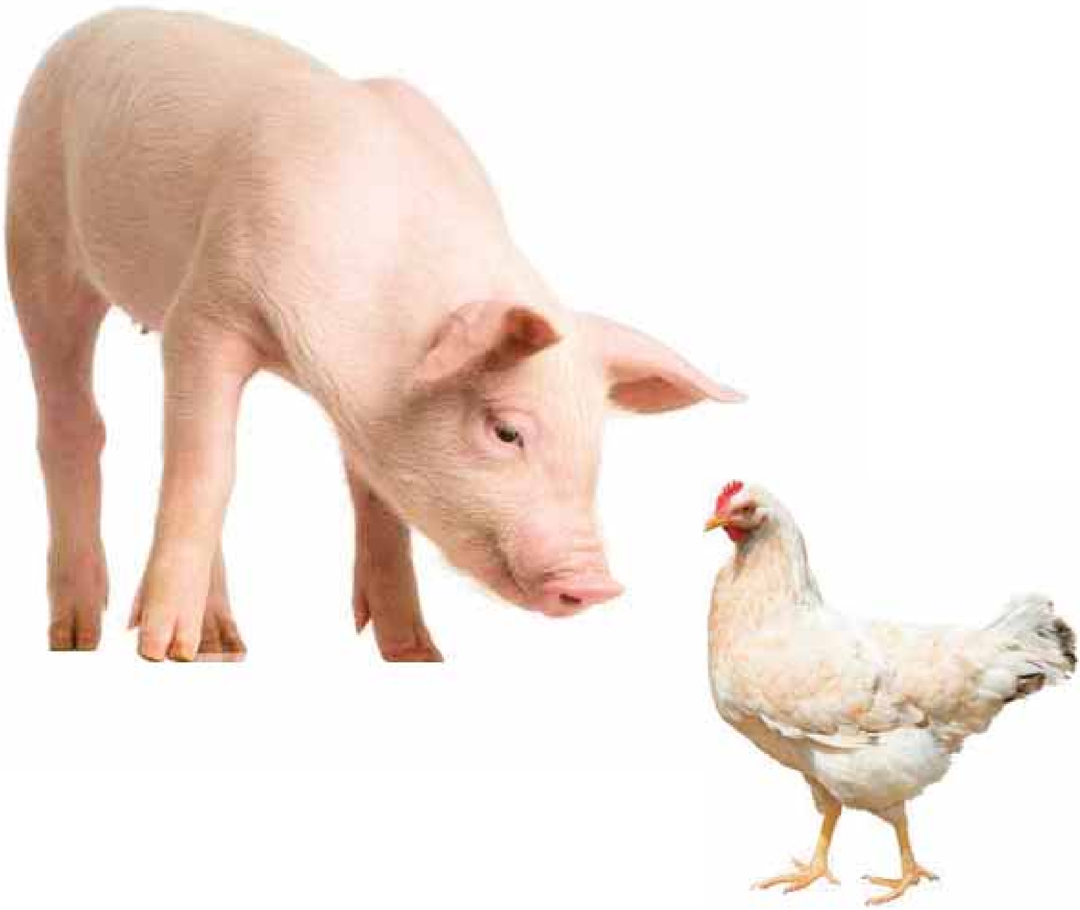
Figure 8: Effect of the crude protein level on litter score (Lensing and Van der Klis, 2006)

✔ **Reduced CP diets are as efficient as high CP diets if L-Threonine is added to reach a 65% Thr:Lys SD ratio.**

✔ **The use of reduce CP diet is a leverage of action on litter quality.**

Conclusions:
Threonine requirement in pigs and broilers

- ✔ Animal age, and therefore body weight, is a factor of variation of the threonine requirement in pigs and broilers.
- ✔ SID Thr:Lys ratios of 65%, 67% and 68% optimise weight gain and feed efficiency in piglets (4-25 kg BW), grower (15-70 kg BW) and finisher pigs (50-110 kg BW) respectively.
- ✔ SID Thr:Lys ratio is higher than 70% in gestating and lactating modern high-lean prolific sows.
- ✔ A SD Thr:Lys ratio of 65% optimises weight gain and feed efficiency in 0-42 day-old broilers.
- ✔ In older broilers, SD Thr:Lys ratio is higher than 65% (average published results is 67.5% SD).



2. **Dietary threonine** supply is essential for adequate gut functions

Similar to other amino acids, digestible dietary threonine is absorbed in the upper part of the intestine (ileum). Besides its utilisation for protein synthesis (growth and milk synthesis), threonine is involved in other physiological functions such as digestion and immunity.

- Immunoglobulin production:

In sows different authors have shown that increasing the dietary threonine content increases gamma immunoglobulin (IgG) in plasma (Cuaron *et al.*, 1984) and in milk at farrowing and for 10 days thereafter (Hsu *et al.*, 2001). In growing pigs, Li *et al.* (1999) and Wang *et al.* (2006) described that animals receiving a threonine enriched diet had higher plasma IgG and specific antigen levels respectively after bovine serum albumin or ovalbumin injection.

- Digestion and gut protection:

Threonine is also found in high concentration in numerous gastrointestinal secretions (Plitzner, 2006; Le Bellego *et al.*, 2002). The mucous gel layer, secreted by Goblet cell scattered along the gut villi, is an important component of the non immune gut barrier that acts to protect the mucosa from digestive enzymes and physical damage by digesta (Faure *et al.*, 2005; Faure *et al.*, 2007). Mucous is mostly made of water (95%) and mucins (5%) which are glycoproteins particularly rich in threonine (Corfield *et al.*, 2001).

2.1. A deficiency in threonine impacts mucin production and villus height

Several literature references (Stoll *et al.*, 1998; Burrin *et al.*, 2001; Bertolo *et al.*, 1998) describe that about 40-50% of the threonine intake by the animals is used by the gut. This implies that a part of threonine requirement is not associated with muscle protein deposition but with gut functions. In fact, the intestine seems to contribute extensively to threonine metabolism. Furthermore, Stoll *et al.* (1998) showed that nearly 90% of threonine used by the intestine was either secreted as mucosal protein or catabolised.
A recent study carried out by Law *et al.* (2007) evaluated the effect of an oral deficiency of threonine on gut function (mucosal mass, mucin production, small intestine histomorphological parameters). Twenty one neonate male piglets (1.8 ± 0.3 kg) were randomly assigned to one of three dietary treatments for 8 days: a threonine adequate diet (0.6 g threonine/kg/day intragastrically), a threonine deficient one (0.1 g threonine/kg/day intragastrically), and the threonine deficient one plus adequate threonine delivered parenterally (0.1 g + 0.5 g threonine/kg/day).

- Piglets fed the deficient diet had higher nitrogen excretion, higher plasma urea and lower plasma threonine concentrations *versus* both other groups ($P<0.05$).
- Mucosal mass and total crude mucin content were lower in colon of the piglets receiving the deficient diet ($P<0.05$).
- Pigs fed the deficient diet had significantly less mucin quantity per length (µg/cm) in the duodenum and proximal colon compared with the two other groups (figure 9).
- Mucin per length (µg/cm) of the gut of piglets receiving a diet adequate in threonine and deficient diet + parenteral threonine was the same (figure 9).
- In mid-jejunum and ileum, villus heights and villus height/crypt depth ratios were lower in piglets fed the deficient diet ($P<0.05$).

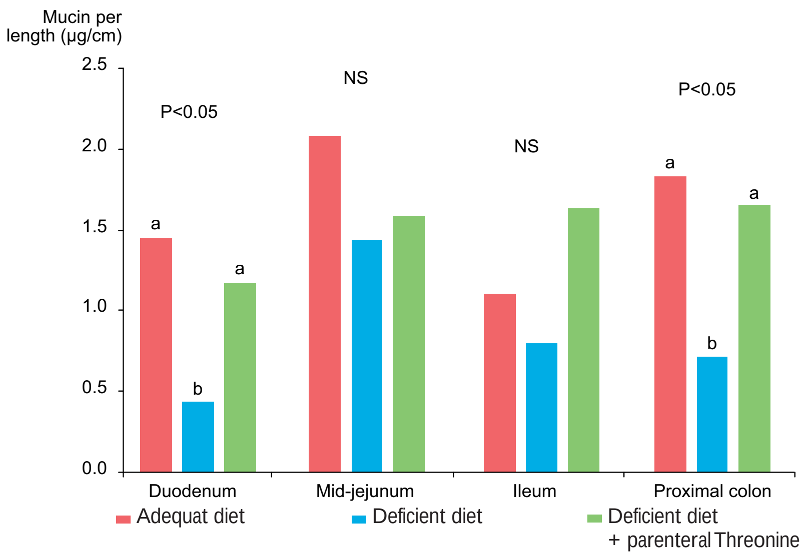


Figure 9: Effect of the type (intragastrically *vs.* parenterally) and the quantity of threonine supplied on weight of mucin per length (µg/cm) in duodenum, mid-jejunum, ileum and proximal colon of piglets (Law *et al.*, 2007).

✔ Piglets receiving diets deficient in threonine increased plasma urea level and consequently decreased protein deposited.

✔ Lower mucin production and mucosal weight could lead to impairments of disease resistance and inability to adapt to dietary changes.

✔ Piglets receiving a deficient threonine supply had lower villus heights, which could result in a decreased intestinal absorptive area.

2.2. Impact of a dietary threonine deficiency on piglet growth and protein synthesis

A recent study carried out by Wang *et al.* (2007) aimed to describe the effect of a deficiency or an excess of dietary threonine on piglet growth performance and on the synthesis of protein in the gut (mucosa, mucins) and in muscle (*Longissimus* muscle). Eighteen piglets weaned at 21 days were used. Three isonitrogenous and isocaloric diets were formulated (14.3 MJ/kg DE, 16.2% CP, 1.33% total lysine). The basal diet (32% total Thr:Lys ratio) was supplemented with different amounts of L-Threonine to reach a total Thr:Lys ratio of 62% and 93%. Feed intake of piglets receiving 62% and 93% total Thr:Lys diets was restricted at the level of 32% total Thr:Lys piglets. Animals were sacrificed on day 14 to measure the protein fractional synthesis rates (FSR) in tissues (gut and muscle).

- Pigs fed the 32% total Thr:Lys diet had lower daily weight gain and feed conversion ratios (P<0.05) than pigs fed the 62% and 93% total Thr:Lys diet.
- FSR of protein was reduced in liver, *Longissimus* muscle, jejunal mucosa and jejunal mucins, (P<0.05) in pigs fed the 32% total Thr:Lys diet compared with pigs fed the 62% total Thr:Lys diet (figure 10).

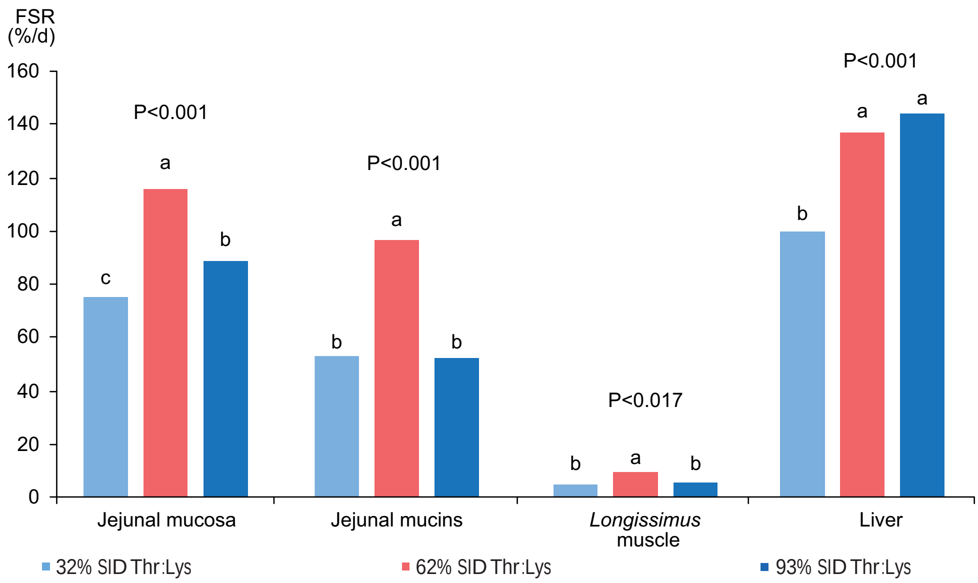


Figure 10: Effect of total Thr:Lys ratio in the diet Fractional Synthesis Rate (FSR) in jejunal mucosa, jejunal mucins, *Longissimus* muscle and liver of piglets weaned at 21 days (Wang *et al.*, 2007)

In addition, a recent study carried out by Hamard *et al.* (2007) showed that a deficiency (control: 9.3 g threonine/kg diet *vs.* low threonine: 6.5 g/kg diet) in threonine leads to a decrease in plasma threonine concentration after 15 days in early weaned piglets (BW=2.5 kg).

A deficiency in dietary threonine did not alter either growth performance or growth of the intestine. However, they noticed a decrease of ileum villus height of piglets receiving the deficient threonine diet.

Moreover, after a period of two weeks, a modification of body composition was observed, such as an alteration of the amino acid composition of the deposited protein. Indeed, a fall in threonine content of the liver and the carcass was observed. These results were obtained after only 15 days and it could be interesting to observe the evolution of piglet growth and carcass composition over a longer period.

✔ **According to Wang *et al.* (2007), a dietary deficiency or excess in threonine, reduces the synthesis of mucosal protein and mucins as well as muscle protein in weaned pigs.**

✔ **The study from Hamard *et al.* (2007) shows that a threonine deficiency during a 15 day-period in early weaned piglets leads to a decrease in plasma threonine concentration and to a modification of the amino acid composition of the carcass and liver.**

2.3. Practical implications of threonine biological functions

As described previously, the threonine fraction absorbed by the ileum is not entirely delivered to the portal blood which collects nutrients from the digestion process. Indeed, a significant part of digestible threonine is used by the digestive tract itself. The threonine use and requirement of the intestinal tract will be dependent on various factors such as the development of gut microflora, activity of lymphatic tissues and digestive disorders. Additionally, endogenous secretions (mucus, enzymes…), amino acid utilisation by bacteria and protein turnover would be increased (Melchior *et al*, 2006). Two recent trials illustrate the practical impact of the sanitary conditions on the threonine requirement: the first one in growing-finishing pigs and the second one in broilers.

2.3.1. Impact of the withdrawal of Antimicrobial Growth Promoters (AGP) on threonine requirement in growing-finishing pigs

In a study carried out by Bikker *et al.* (2006), four dietary threonine levels were tested in growing and finishing pigs (25-110 kg) fed a diet with (30 ppm; AGP+) or without (AGP-) salinomycine. The grower diet was fed to pigs from 45 to 60 kg BW and the finisher diet was fed from 60 to 110 kg BW. Basal SID Thr:Lys ratio were 54 and 55% respectively in the grower and finisher phases (grower: 9.5MJ/kg NE, 15.3% CP and 0.79% SID lysine; finisher: 9.5MJ/kg NE, 13.3% CP and 0.67% SID lysine). The three other steps of threonine were achieved by addition of L-Threonine to the basal diet to reach 60%, 65% and 70% SID Thr:Lys.

- Inclusion of AGP significantly improved the growth of the pigs (P<0.01).
- During the whole experimental period, threonine had no significant effect on daily gain and FCR when the diet contained AGP,
- Whereas dietary threonine linearly increased daily gain (+5.6% from 56 to 70% SID Thr:Lys ; P=0.03) and quadratically decreased FCR (P=0.03) when the diet did not contain an AGP (figure 11).

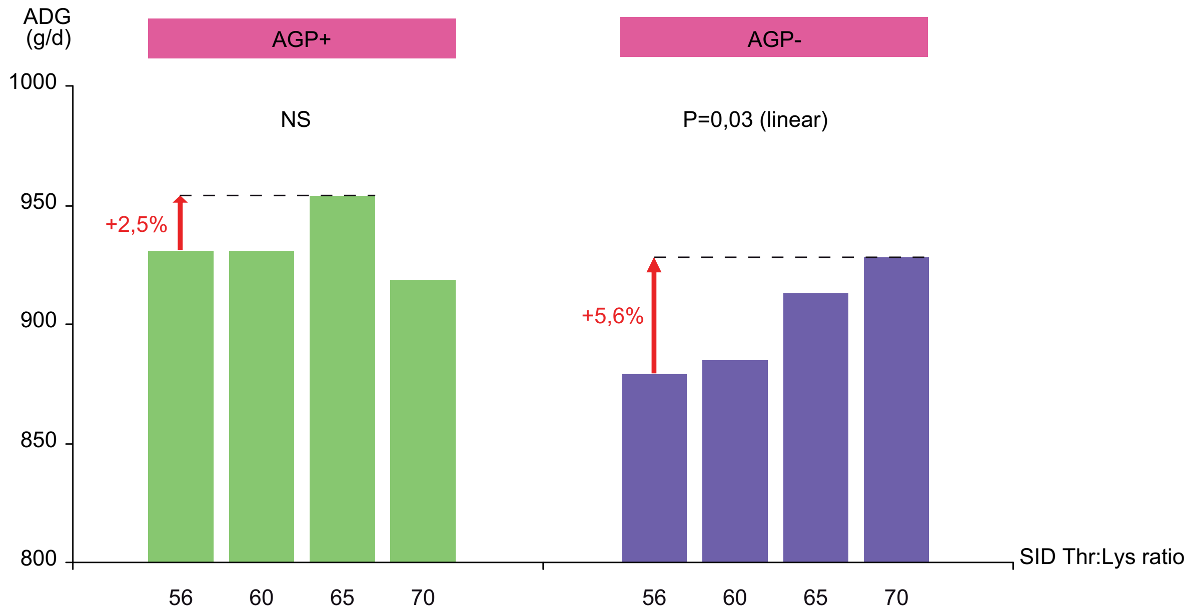


Figure 11: Effect of SID Thr:Lys ratio on Average Daily Gain (ADG) during the whole experiment in 25 to 110 kg body weight pigs receiving diet with or without AGP (Bikker *et al.*, 2006).

✔ **In the group without AGP, increasing Thr:Lys ratio up to 70% allowed pigs to reach the same level of performance as animals fed with dietary AGP.**

✔ **Sanitary status is a factor of variation of the Thr:Lys requirement.**

2.3.2. Impact of sanitary condition on threonine requirement in broilers

Kidd *et al.* (2003) carried out a trial on broilers (Cobb, 42-56 days) raised in different environmental conditions. Performance (ADG and FCR) of birds raised in a clean environment followed a quadratic positive response to the increased Thr:Lys ratio, which means that the threonine requirement of birds was matched (70% SD Thr:Lys). In the dirty environment, birds had a linear positive response suggesting that they did not match their threonine requirement, which was at least 85% SD Thr:Lys.

Corzo *et al.* (2007) worked with younger broilers (21-42 days). They studied the effect of litter status on Thr:Lys requirement of growing broilers (Ross x Ross 708). Birds were raised from 1 to 42 days in floor pens that had either unused new litter (soft wood shaving) or used built up litter from 4 flocks. Experimental corn-peanut meal based diets were fed from 21 to 42 days of age (with 18.6% CP, 1.03% SD lysine and 13.0 MJ /kg ME). By addition of L-Threonine, six SD Thr:Lys levels were tested: 44, 52, 57, 64, 70 and 76% (6 treatments x 8 replicates x 12 birds).

- In both conditions, quadratic responses were observed.
- In the clean environment, body weight gain was optimised for a SD Thr:Lys ratio at 66%.
- Optimal SD Thr:Lys ratio was always higher in dirty conditions than in clean conditions, either for growth performance or carcass traits (figure 12).

For these two experiments, it was hypothesized that the higher threonine requirement of birds raised in a dirty environment reflects the different microbial exposure levels to which these birds were faced and the resulting changes in the maintenance requirement for gastrointestinal functions and immunity (Rostagno *et al.*, 2007, Corzo *et al.*, 2007). Moreover, these two trials illustrate how the optimal SD Thr:Lys ratio increases with age.

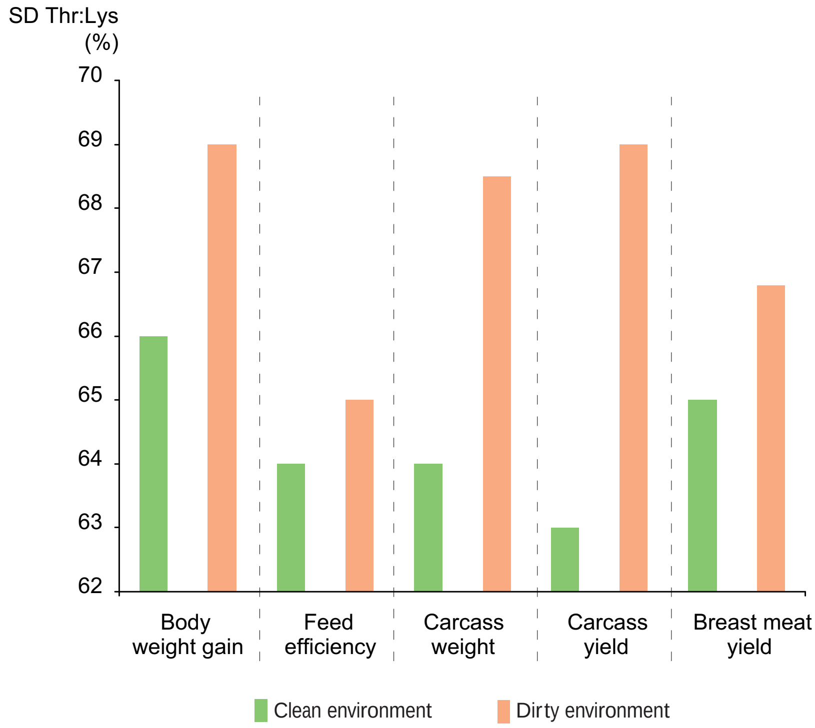


Figure 12: Effect of the sanitary conditions (clean *vs.* dirty) on SD Thr:Lys requirement for maximal body weight, feed efficiency, carcass weight, carcass yield and breast meat yield of 21-42 day-old broilers (Corzo *et al.*, 2007)

✔ Poor sanitary environmental conditions lead to a higher SD Thr:Lys requirement to cover higher maintenance needs

Conclusions:
Dietary threonine supply is essential for adequate gut functions

✔ Threonine is involved in digestion and immunity functions.

✔ A significant part of the threonine intake is used by the gut itself and for the synthesis of endogenous secretions (particularly mucins).

✔ A deficiency in dietary threonine leads to a decrease in protein synthesis in the gut and in muscles.

✔ Sanitary status and animals' environmental conditions are factors of variation of the Thr:Lys requirement.

General conclusions

Threonine in pigs and broilers: A crucial amino acid for growth and gut function

- ✔ SID Thr:Lys ratios 65%, 67% and 68% optimise weight gain and feed efficiency in piglets (4-25 kg BW), grower (15-70 kg BW) and finisher pigs (50-110 kg BW) respectively.

- ✔ SID Thr:Lys ratio is higher than 70% in gestating and lactating modern high-lean prolific sows.

- ✔ A 65% SD Thr:Lys ratio optimises weight gain and feed efficiency in 0-42 day-old broilers. In older broilers, SD Thr:Lys ratio is higher than 65% average published results is 67.5% SD.

- ✔ Age, therefore body weight, and sanitary conditions are variation factor of the threonine requirement in pigs and broilers.

- ✔ Threonine is an amino acid of primary importance for gut functions.

- ✔ L-Threonine supplementation allows the use of reduced CP diets without detrimental effects on growth performance.

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