

Effect of reducing dietary crude protein in hog finisher barrows and gilts on technical performance

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Summary

The objective of this study was to determine the effect of reducing dietary crude protein (CP) levels in hog finisher barrows and gilts on animal performance. Diets were formulated to meet the requirements of the first six essential amino acids. It was hypothesised that lowest dietary CP levels without negative effects on technical performance would be close to treatments D (barrows) and J (gilts). From those CP level onwards, isoleucine (and histidine) likely became limiting factors. The most important conclusions are mentioned below.

Performance

30-55 kg BW:

- The response to CP level was different in barrows compared to gilts (P interaction < 0.05). In barrows, best performance was obtained at a CP level of 14.5% whereas gilts performed best at 15.5% CP. These CP levels also resulted in lowest cost per kg gain for barrows and gilts respectively.
- Broken-line analysis indicated a minimum CP level of 14.7% and 15.1% for barrows and gilts respectively. This is in line with literature. Based on the diet composition in the current trial, soybean meal inclusion at this CP level would be approximately 12.5% and its inclusion could thus be reduced by 36%.

55-80 kg BW:

- No interactions were found between CP level and sex (P interaction>0.05).
- A quadratic response to CP level was observed for ADG and efficiency (both P<0.05), but not for ADFI. ADG and efficiency were highest in animals receiving 13.9% CP or more.
- Barrows had a higher ADFI and ADG (both P<0.001), but were not less efficient compared to gilts (P=0.87).
- Broken-line analysis indicated a minimum CP level of 14.4% for both barrows and gilts. This is partially in line with literature as levels of 11% and 11.9% have been reported to nog negatively affect ADG. However, bodyweight ranges were higher (up to 100 kg). Based on the diet composition in the current trial, soybean meal inclusion at this CP level would be approximately 9.5% and its inclusion could thus be reduced by 32%.

80-115 kg BW:

- No interactions occurred were found between CP level and sex (P interaction>0.05).
- Performance increased with increasing CP level and cost per kg gain decreased with increasing CP level (all P linear<0.001).
- Barrows had a higher ADFI and ADG, but were less efficient compared to gilts (all P<0.001).
- Broken-line analysis indicated a minimum CP level of 11.7% for barrows. Based on the diet composition in the current trial, soybean meal inclusion at this CP level would be approximately 4.5% and its inclusion could thus be reduced by 55%.
- Broken-line for gilts resulted in high standard error with large confidence intervals and were thus not used for interpretation.

Carcass

- Differences in carcass characteristics between treatments occurred, independent of sex (P interaction>0.05). Quadratic responses were observed for final bodyweight, slaughter weight and carcass weight (all P<0.001). Animals receiving the four treatments with highest CP levels had similar weights while the animals with lowest CP level were significantly lighter.
- Quadratic responses for loin depth and lean meat percentage were observed (both P quadratic=0.04). However, expressed as a percentage of slaughter weight these responses were not observed anymore (resp. P quadratic= 0.54 and 0.16).
- Barrows were significantly heavier at the end of the trial (P<0.001). Expressed, as a percentage of slaughter weight, barrows had higher backfat but lower loin depth and lean meat compared to gilts (all P<0.01).

<u>Carbon Footprint</u>

- Dietary Carbon Footprint (CFP) increased with decreasing dietary CP level. This was due to the increase in synthetic amino acid inclusion in the formulations and its high impact on Carbon Footprint.
- Animals receiving the low CP diets were more efficient converting this to gain compared to animals receiving high CP diets.
- The assessment on overall CFP could not be made as the tool is not able to adjust to actual performance and a three phase feeding program.

Introduction 1

Providing diets with a reduced crude protein (CP) content, that are supplemented with increased contents of free amino acids (AA) to cover the AA requirement, might be helpful in reducing the soybean meal content of Latin American origin, and thus in reducing the amount of non-EU protein in the diet. An adequate supply of AA supply is essential for maintaining performance levels of pigs that are fed low CP diets. Pig diets usually at least are optimised for the AAs lysine, methionine + cysteine, threonine, and tryptophan. In low CP diets, the requirements for valine, isoleucine, histidine and leucine should be taken into account as well (Gloaguen et al., 2014).

In Table 1, various recommended amino acid ratios, including valine, isoleucine, histidine and leucine, relative to lysine for growing pigs are compared, as provided by Guillou and Molist (In progress).

Table 1 Different amino acid patterns relative to lysine for growing pigs, as provided by Guillou and Molist (In progress).

	BSAS 2003 (UK+IRL)	Van Milgen et al., 2005- INRAporc	FEDNA, 2006 (ESP)		CVB 2012 (NL+B)		VSP 2013 (DK)	NRC 2012 (USA) SID
Amino	Growing	(FR) Growing	Adaptation	20-60	Starter	Grower	Growers	25-50 kg
Acid	pigs (10 to 120 kg)	pigs	fattening	kg			and Finishers	
LYS	100	100	100	100	100	100	100	100
MET	30	30	31	29-32			31	29
MET+CYS	59	60	60	58-60	59	60	56-61	57
THR	65	65	64	63-64	57	59	63-70	63
TRP	19	18	19	17-19	19	19	20	17
ILE	58	60	59	58-60			58	51
LEU	100	100					102	101
HIS	34	32					34-36	34
PHE	57	50					59-61	60
PHE+TYR	100	95					116-118	95
VAL	70	70					70	66
ARG		42						46

As shown in Table 1, European recommendations for isoleucine are higher than in the USA (58-60 vs. 52% of lysine). Leucine recommendations are more homogeneous (100-102% of lysine). Histidine recommendations ranged from 32 to 36% of lysine (INRA and VSP, respectively), where those for valine varied from 66% (NRC) to 70% (BSAS and Van Milgen et al., 2005).

In addition to Table 1, recent literature was reviewed to determine whether there is any evidence for changing the recommended AA patterns relative to lysine, especially in pigs fed low protein diets.

Lysine level

In 88-116 kg gilts fed low CP diets, the optimum SID Lys content to maximize ADG and optimize FCR as well as to minimize serum urea nitrogen levels were 5.7, 5.8 and 6.1 g/kg using a linear-break point model and 6.5, 6.5 and 6.6 g/kg using a quadratic model, respectively (Ma et al., 2015a).

Total Sulpher Amino Acids (TSAA)

In 96 to 120 kg gilts fed low CP diets, the SID TSAA to lysine ratio for lowest FCR was estimated as 58%, which is in accordance with the recommend ratio by the NRC (2012) (Ma et al., 2016).

Threonine

In a study of Zhang et al. (2013), in which 22-50 kg pigs were fed low CP diets supplemented with free AA, by using a linear-breakpoint analysis, the optimum SID Thr to Lys ratio was estimated as 68%, 67% and 61% for ADG, FCR and serum urea nitrogen, respectively. The ratios for optimized ADG and FCR were higher than recommended by NRC (2012). By use of linear break point analysis the optimum SID Thr to Lys ratio for 90-118 kg gilts fed low CP diets was estimated as 61%, 63% and 64% whereas the quadratic analysis estimated the optimum SID Thr to Lys ratio as 70%, 74% and 72% to maximize ADG, minimize FCR and serum nitrogen levels, respectively (Ma et al., 2015a). The ratios estimated with the linear break point model were slightly higher than the recommend ratio by the NRC (2012). Xie et al. (2013), who conducted an experiment in finishing pigs (72 kg BW at the start of the experiment), that were fed low CP diets, concluded that the SID Thr to Lys ratios for maximal weight gain, and minimal FCR and serum urea nitrogen were 67%, 71% and 64%, respectively, using a linear broken-line model. These ratios are also higher than recommended by NRC (2012).

Tryptophan

Zhang et al. (2012) investigated the SID Trp to Lys ratio in 25-50 kg pigs, fed low CP diets supplemented with free AA. The SID Trp to Lys ratios that optimized weight gain, feed conversion ratio, and serum urea nitrogen were 19.7, 20.0, and 20.8% using a linear-breakpoint model, and 22.6, 23.2, and 23.6% using a curvilinear-plateau model, respectively. Taking an average of these six values, the authors concluded that the optimum SID Trp to Lys ratio is at least 22%. This value is higher than currently recommended by NRC (2012). By use of linear break point analysis the appropriate SID Trp to Lys ratio for 89-121 kg gilts fed low CP diets was estimated as 17% using G:F as the response criteria (Ma et al., 2015b). This estimation was similar to the recommend ratio from the NRC (2012). In a study of Xie et al. (2014), conducted in 67-96 kg barrows fed low protein diets, maximized weight gain using estimates provided by the linear broken line and quadratic model were 20.3% and 25.1%, respectively, while 19.7% and 22.4% for optimized FCR, and 21.4% and 24.9% for minimized serum urea nitrogen. These data suggested that the SID Trp to Lys ratio is at least 20.3% for barrows fed a low protein corn, wheat bran, and soybean meal based diet. This estimate is higher than the current NRC recommendation (NRC, 2012).

Valine

By use of a linear broken-line model, the dietary SID Val:Lys ratios required for 26 to 46, 49 to 70, 71 to 92, and 94 to 119 kg pigs fed low CP diets were estimated to be 62%, 66%, 67%, and 68%, respectively (Liu et al., 2015). These estimations are in line with the recommend NRC ratios (2012).

Isoleucine

Barrows from 15 to 30 kg, fed diets with a low crude protein content (14%), showed to have maximal daily gain and protein deposition at a SID isoleucine to lysine ratio of 58% (Lazzeri et al., 2017). This ratio is higher than the recommended NRC (2012) value, but in line with several European recommendations (Table 1).

From the available literature, it can be concluded that the in Table 1 recommended AA patterns from EU origin seem to meet the AA requirements of pigs fed low CP diets, where NRC recommendations appear to underestimate those requirements. Only for Trp, requirement in low CP diets seems to be higher than most of the values recommend in Table 1.

The current experiment was conducted to determine the effect of low CP diets, with a (partial) replacement of soybean meal by free AA, on technical performance, slaughter quality, animal welfare, economic performance, and the ecological footprint in hog finisher barrows and gilts.

Materials and Methods 2

2.1 Experimental design

A total of 192 grower finisher pigs (Topigs 20 x Topigs Pietrain), blocked by body weight, were allocated in two units at the hog finisher research facilities of Cargill Animal Nutrition in Velddriel (barrows and gilts were split). The trial lasted for 14 weeks and there were three phases; 25-45 kg BW (day 0-27), 45-75 kg BW (day 27-57) and 75-115 kg BW (day 57-98). Feed was provided ad libitum during the entire trial.

Parameters of interest were ADG, ADFI, efficiency and carcass characteristics. Tail and ear scores were performed to test whether there was a negative effect of protein level on these parameters as this was recently observed by van der Meer et al. (2017). Methodology for tail and ear scores was adopted from this same author. Tails from each individual animal were scored as follows: 1 No tail damage, 2 bite marks, 3 small wound, 4 medium wound/part of the tail missing or 5 severe wound/no tail left. The score of both ears was as follows: 1 no ear damage, 2 top or bottom lesions, 3 top and bottom lesions 4 severe damage/part of ear missing or 5 ear necrosis. In addition, cost and carbon footprint per diet were calculated. Feed cost per kilogram gain could be calculated for economical assessment.

Table 1 contains the treatments per phase. Under Dutch practical conditions, CP levels of treatments E and F (and K and L) are mostly observed. CP levels in treatments A (and G) are slightly lower compared to what is applied until now in literature. It is expected that this treatment set will lead to a clear dose response from which optimum CP levels can be analyzed.

Table 1: Treatment table

Treat.	Р	rotein leve	el	Sex
	Phase 1	Phase 2	Phase 3	
Α	12.5	10.9	9.3	Gilt
В	13.5	11.9	10.3	Gilt
С	14.5	12.9	11.3	Gilt
D	15.5	13.9	12.3	Gilt
E	16.5	14.9	13.3	Gilt
F	17.5	15.9	14.3	Gilt
G	12.5	10.9	9.3	Barrow
н	13.5	11.9	10.3	Barrow
ı	14.5	12.9	11.3	Barrow
J	15.5	13.9	12.3	Barrow
K	16.5	14.9	13.3	Barrow
L	17.5	15.9	14.3	Barrow

The crude protein levels in treatment E and K are close to Dutch practical conditions, while the lowest protein levels (treatments A and G) are slightly lower compared to what is used until now in literature.

2.2 **Diets**

Batches of raw materials were reserved at the ABZ-diervoeding production factory, Leusden, The Netherlands. Reserved batches were analysed on crude protein, crude fat, crude ash, moisture, crude fibre (NIRS) at Provimi Rotterdam Laboratory, The Netherlands.

Formulation of diets was based on the analysed nutrient content of the ingredients. In agreement with the F4F project team, amino acid ratios (Thr, Trp, Met and Met+Cys) were based on INRA recommendations (formulated on CVB SID values). For SID valine, a ratio of 67% was set as a minimum to better reflect current Dutch standard (instead of 70%, as recommended by INRA). Energy levels were set at 1.10 EW. SID Lysine levels were based on actual requirement of the animals. Treatments with lowest and highest crude protein levels were produced and mixed before pelleting to obtain the other treatments. Diet formulations are included in appendix I.

2.3 Animals, Environment, and Sample Management

Animals (Topigs 20 x Topigs Pietrain) were housed at the hog finisher facilities at GIC Velddriel. Each pen contained 4 animals, until experimental day 77. At this day one pig per pen was removed. At that age, animals were estimated to weigh approximately 100 kg and due to EU regulation regarding housing conditions (space allowance) of the pigs it is not allowed to have 4 animals per pen anymore. The animal that came closest to the average body weight of that specific pen was removed. Barrows and gilts were equally distributed among treatments. Temperature, humidity and ventilation were automatically controlled.

At slaughter, pigs were weighed and transported to a commercial processing plant (Vion, Boxtel, The Netherlands). Each pig received an ear tag according to pen number to allow for data retrieval by pen and carcass data collection at the packing plant. Hot carcass weights (HCW) were measured immediately after evisceration and each carcass was evaluated for backfat, loin depth, and lean percentage. Backfat depth and loin depth were measured with an optical probe inserted between 3rd and 4th last rib. Lean percentage was provided from the packing plant by using a proprietary equation. Percentage yield was calculated by dividing HCW by live weight obtained before transport to the packing plant.

2.4 **Analysis**

Diets were routed to the Rotterdam lab to be analysed by wet chemistry. In addition, full amino acid profiles were analysed by University of Missouri (appendix II).

Statistics

Data was analysed using Mixed Model analysis (Version 9.3, SAS Institute Inc., NC, 2007) according to the statistical model as described below. Differences between treatment means were assumed to be significant based on the probability of P<0.05 (Student t-test).

The following contrast questions were applied:

- Is there a linear response to decreasing CP level?
- Is there a quadratic response to decreasing CP level?
- Is there a linear interaction between CP level and sex?
- Is there a quadratic interaction between CP level and sex?

```
Y_{ijk} = \mu + BW + a_i + \beta_j + ai*\beta_j + C_k + \epsilon_{ijk}
```

Where:

 Y_{ijk} = a specific trait per experimental unit.

= overall mean μ

RW = co-variable of body weight at start of the trial (day 0)

= fixed effect of CP level (i = 1-6) a_{i} β_j = fixed effect of sex (j = barrow - gilt) $a_i*\beta_j$ = interaction between CP level and sex = random effect of weight block (k = 1 - 4) C_{k}

Eiik

In case of quadratic responses, broken line analysis could be performed to determine minimum crude protein level. In case of an interaction between CP level and sex, broken-line analysis was performed for barrows and gilts separately.

2.5 Results and Discussion

2.6 Diet analyses (Appendix II)

Diets were analysed on total amino acid content at the University of Missouri. For lysine, the difference between analyses versus formulation ranged between minus 2% to plus 8%, which is within an acceptable range taking the analysis error into account. For methionine and cysteine however, analysed values were respectively $\approx 15\%$ and $\approx 18\%$ lower compared to the formulation. In general, deviations seemed similar across all treatments with minor exceptions for the two treatments with lowest CP levels (slightly lower SID M+C/SID Lys in phase 2 and 3 and SID Val/SID Lys in phase 3 compared to the other treatments). However, differences are very small and the analysis error needs to be taken into account. Therefore, no inference can be made to what extend this potentially affected the observed responses.

2.7 Performance and broken-line analyses (Appendix III and IV)

Overall (30-120 kg BW)

General health status was high in the current trial as medication usage was low and animal performance high (>900 gram/day). During the entire period, a total of 7 animals were removed from trial because of minor health issues ranging from animals lacking growth for no obvious reason, rectal prolapse to leg injury. At day two of the trial, four animals had tail bite marks (score 2), three animals had small wounds (score 3) and one animal had a medium wound (score 4) on his tail. In addition, 17 animals received a score 2 on ear biting (top or bottom lesion). All of these animals were equally divided over the treatments with 12.5%, 13.5%, 14.5% and 15.5% CP. However, since animals were only on trial for one day it is more likely that this was left over from the nursery or the result of reallocation (hierarchy determination) rather than dietary treatment. Since tail and ear biting was virtually not observed throughout the rest of the trial, no statistical analyses was performed on these parameters.

ADG of the entire trial was 906 gram/day (Dutch average 811 gram/day) with an ADFI of 2.3 kg/day on average. FCR was on average 2.55 when all treatments were included. However, animals that had received treatments with lowest CP levels clearly had worse efficiency. Excluding this treatment results in an average FCR of 2.51 (Dutch average 2.57). With 956 gram/day, barrows gained on average 100

gram/day more compared to gilts (P<0.001). Together with a 17% higher feed intake (P<0.001), barrows were 4% less efficient compared to gilts (P<0.001) in the overall trial.

Phase 1 (30-55 kg BW) 2.8

The response to CP level was different in barrows compared to gilts. Figure 1 shows that gilts reached highest gain of 910 gram/day at 15.5% CP level whereas barrows reached highest gain of 948 gram/day at a CP level of 14.5% (P interaction=0.05). In both sexes, CP levels of 12.5% and 13.5% resulted in the lowest ADG (790 and 841 gram/day for gilts and barrows respectively). For gilts this could have been caused by the much lower feed intake. ADFI at lowest CP levels was on average 1.56 kg/day whereas the highest feed intakes were reached at 14.5% and 15.5% CP level (on average 1.70 kg/day).

In addition, a significant interaction occurred on FCR (P=0.04). Gilts receiving 15.5% CP clearly showed highest efficiency compared to all other treatments whereas barrows obtained highest efficiency at 14.5% CP and higher. Consequently, lowest feed cost per kg gain was obtained at 15.5% CP level in gilts and 14.5% CP level in barrows (Figure 2, P interaction=0.05).

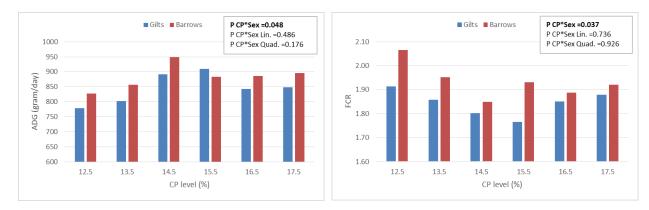


Figure 1: The effect of CP level on ADG (left) and FCR (right) in gilts and barrows from 30-55 kg BW

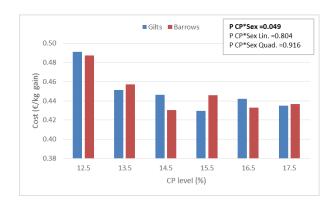
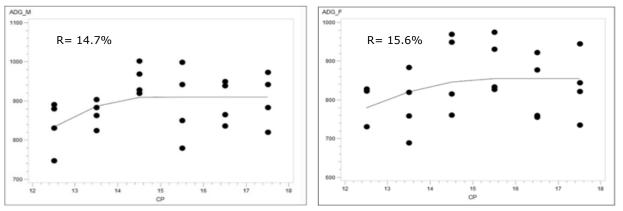


Figure 2: The effect of CP level on cost per kg gain (€) in gilts and barrows from 30-50 kg BW Minimum CP level for barrows is 14.7% and gilts is 15.1%

Since significant interactions between CP level and sex occurred (showing that barrows responded differently to the CP levels compared to gilts), broken-line analyses to determine minimum CP levels were performed for gilts and barrows separately (Appendix V). To achieve optimum ADG and efficiency, CP level should be at least 14.7% (Figure 3, left) and 14.6% in barrows respectively. Due to the lack of a quadratic response, broken-line analyses could not be performed to determine optimum feed intake. For gilts, minimum CP levels to obtain optimum ADG, ADFI and efficiency are

respectively 15.6% (Figure 1, right), 15.5% and 14.1%. For mixed sex, an average minimum of 15.0% should be taken in to account.

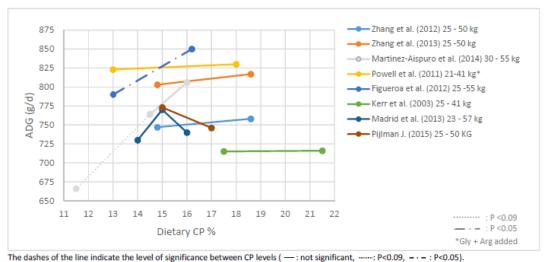


Minimum CP level for gain in barrows (left) and gilts (right) of 30-55 kg BW determined Figure 3: by broken line analysis.

It must be noted that these minimum CP levels are valid when diets are formulated on the first six essential amino acids. In the phase 1 formulations of the current trial, isoleucine levels became limiting from CP level 15.5% and lower (assuming INRA recommendation of SID Ile/SID Lys: 60%). For leucine and histidine this was the case at CP level 13.5% and lower (INRA: 100% and 32% respectively). Most likely, the combination of limiting isoleucine, leucine and histidine caused animals to perform worse in the treatments with 13.5% and 12.5% CP. Lower CP levels might be feasible when animals' requirements on these amino acids are also taken into account.

Minimum CP level determined by current trial is in line with literature

From 2000 onwards, a series of papers have been published in which the response to different CP levels was investigated (Figure 4). It must be noted that in these studies, diets have been formulated to meet animal requirements on the first six essential amino acids and if needed these were added synthetically. In three studies, CP levels were decreased to a similar extend as in the current trial and also similar body weight ranges were applied (25-55 kg BW). Figueroa et al. (2012) reported a significant (P<0.05) decrease in ADG when CP level was reduced from 16.2% to 13.0% whereas Martinez-Aispuro et al. (2014) observed a negative trend when going from 14.5% to 11.5% CP (P<0.09). For FCR however, the latter study showed no negative response to the low level (P>0.10). These studies are very much in line with the current trial in which CP levels below 14.5% also showed to have a negative effect on performance. In the study of Powell et al. (2011), 13% CP did not negatively affect AGD (P>0.1). However, it must be noted that synthetic glycine and arginine were added to the diets which most likely mitigated the negative response to low CP.



Effect of reducing dietary CP level on ADG in 20-60 kg BW animals as reported in Figure 4: literature

2.9 Phase 2 (55-80 kg BW)

In 55-80 kg BW animals, no interactions occurred between CP level and sex, indicating that the response to CP level was similar in barrows and gilts. A quadratic response to CP level was observed for ADG and efficiency, but not for ADFI. Figure 5 shows that ADG was highest in animals receiving 13.9% CP or more (P quadratic=0.003). FCR was highest (2.82) in animals receiving the low CP level and lowest (2.49) at 13.9% (P quadratic=0.011).

CP levels of 13.9% and higher resulted in the lowest feed cost per kg gain, being on average € 0.54 (P quadratic= 0.007).

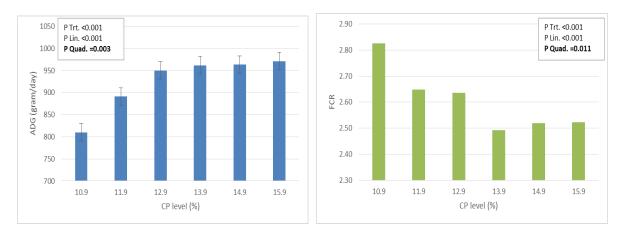
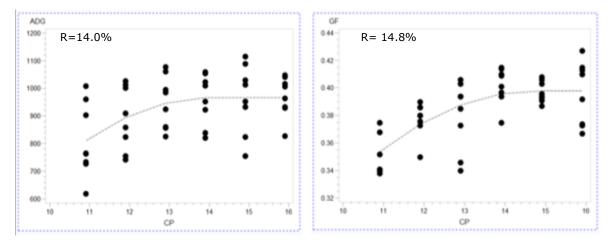


Figure 5: The effect of CP level on ADG (left) and FCR (right) in 55-80 kg BW animals

Note: error bars contain all sources of variation in the experiment, including that from random factors. However, only residual error variance is used for significance testing.

Barrows had an ADG of almost 1 kg per day, whereas gilts gained almost 17% less at 851 gram/day (P<0.001). A similar difference between barrows and gilts (18%) was observed for ADFI (P<0.001). In terms of efficiency however, both sexes were similar at an FCR of 2.6 (P=0.87). So, barrows had a higher ADFI and ADG, but were not less efficient compared to gilts.

Since no interaction between CP level and sex occurred, broken-line analyses to determine minimum CP level was performed for both sexes combined. These analyses show that minimum CP level to obtain optimum ADG and efficiency should be respectively 14.0%, and 14.8% (Figure 6). For ADFI, the minimum level is 13.4%. However, the standard error of this fit is relatively high (5.1) with large confidence intervals which makes this level less reliable. On average, minimum CP level for barrows and gilts is 14.4%.



Minimum CP level for gain (left) and efficiency (right) in barrows and gilts (55-80 kg BW) Figure 6: determined by broken line analysis.

Similar to the phase 1 formulations, phase 2 formulations are based on INRA recommendations for the first 6 essential amino acids (except valine which is set on 67% compared to lysine). Based these recommendations, isoleucine became limiting in diets from 13.9% CP and lower whereas leucine and histidine became (close to) limiting in the treatments with 11.9% and 10.9% CP. Similar to phase 1, ADG of animals receiving these treatments was significantly worse. For efficiency it was less evident.

The minimum CP level determined in the current trial is partially in line with literature. Figure 7 shows that a CP level of respectively 11.9% and 12.1% did not negatively affect ADG (both P>0.1) in studies of Martinez-Aispuro et al. (2014) and Carpenter et al. (2004). This is in contrast with findings from the current trial which clearly showed decreased ADG below a CP level of 12.9%. However, it must be noted that the body weight range in which these trials were performed were higher (up to 100 kg BW) compared to the current trial and thus different responses can be expected. In addition, Martinez-Aispuro et al. (2014) performed a trial in 45-95 kg animals and did observe a negative response when decreasing CP level from 14.0% to 11.0% (P<0.09).

In terms of efficiency, only in studies from Martinez-Aispuro et al. (2014), Kerr et al. (2013) and Madrid et al. (2013), CP levels below 14.0% did not have a negative effect (all P>0.1).

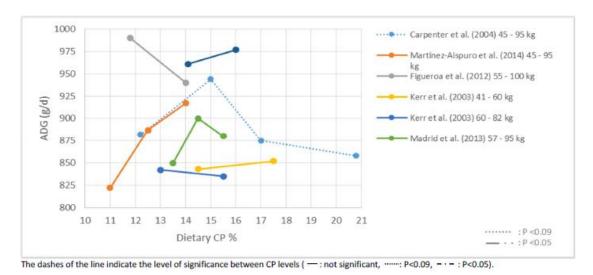
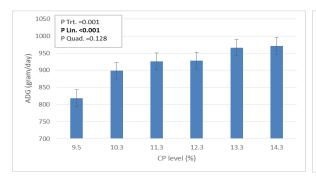


Figure 7: Effect of reducing dietary CP level on ADG in 40-100 kg BW animals as reported in literature

2.10 Phase 3 (80-120 kg BW)

A linear response to CP level was observed for all performance parameters. ADG linearly (P<0.001) increased with increasing dietary CP level but remained at approximately 968 gram/day from 13.3% CP onwards (Figure 8). A similar linear response was observed in ADFI with a difference of 14% between animals receiving the treatment with lowest and highest CP level (P linear<0.001). Consequently, FCR tended to decrease (P linear=0.095) with lowest level observed at 12.3% CP (2.90). For both ADG and FCR, the CP level of 9.5% stands out compared to all other treatments indicating that at this level the negative response was really evident.



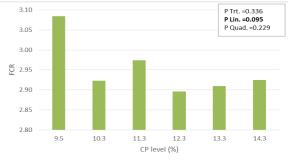


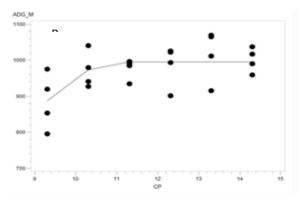
Figure 8: The effect of CP level on ADG (left) and FCR (right) in 80-120 kg BW animals

Note: error bars contain all sources of variation in the experiment, including that from random factors. However, only residual error variance is used for significance testing.

The formulation cost of dietary treatments in the current phase was relatively high due to the difficulty of reaching 9.5% CP. As a result, differences between treatments were also higher (€7/ton) compared to the other two phases (€2/ton). The linear response observed for efficiency, on top of the formulation cost, resulted in a linear (P<0.001) decrease in cost/kg gain when CP level increased. As a result of the poor performance in animals receiving the diets with lowest CP level throughout the entire trial, final bodyweight was approximately 10% lower in this treatment (110 kg) compared to all other treatments (average 121 kg). In addition, at the end of the trial (day 77 and day 98) the standard errors of the mean for bodyweight in animals receiving lowest CP levels were approximately 7% higher compared to all other treatments indicating that more variation within this treatment group started to occur.

Compared to gilts, barrows had a 12% higher ADG and 22% higher ADFI (both P<0.001). As a result, gilts were 8% more efficient (P<0.001) with a FCR of 2.85.

Since no interaction between CP level and sex occurred, broken-line analyses to determine minimum CP level should have been performed for both sexes combined. However, broken-lines could not be fitted well since responses were linear and not quadratic. Therefore, sexes were separated and conclusions were drawn on broken line with good fit with low standard error and small confidence intervals. This was only the case for ADG and ADFI in barrows. Models showed that the minimum CP level should be 11.1% and 12.3% respectively (Figure 9). So, on average, minimum recommended CP level for barrows is 11.7%



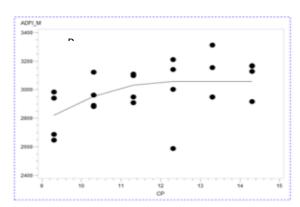


Figure 9: Minimum CP level for gain (left) and feed intake (right) in barrows (80-120 kg BW) determined by broken line analysis.

The minimum CP level determined by current trial not completely in line with literature. From authors included in Figure 10, Ma et al. (2015), Kerr et al. (2003) and Pijlman et al. (2015) used animals that were in terms of bodyweight most close to phase 3 of the current trial. In these trials, dietary CP levels were reduced to 10.0%, 11.0% and 13.0% respectively without negatively affecting ADG and efficiency (all P>0.1). However, data is limited and only two trials represent animals with relatively high performance (Ma et al. 2015, and Pijlman et al. 2015) similar to the current trial.

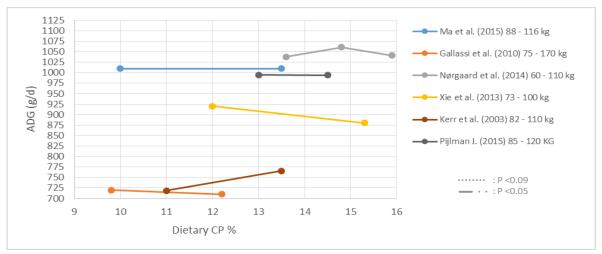


Figure 10: Effect of reducing dietary CP level on ADG in 60-170 kg BW animals as reported in literature

The current study has been performed to determine the optimum crude protein levels in finishers and, as a consequence, to what extend dietary soybean meal can be decreased. From the current trial it can be concluded that a relative reduction up to 36%, 32% and 55% can be achieved in 30-55 kg BW, 55-80 kg BW and 80-120 kg BW animals.

Table 2: Soybean meal (SBM) reduction per phase

Phase	SBM level in control diet (F) (%)	SBM level at optimum performance (%)	SBM saving - Absolute (Column 2 - column 3) (%)	SBM saving – Relative (Column 4/column 2 *100) (%)
1	19.5	12.5	7.0	36
2	14.1	9.5	4.6	32
3	9.9	4.5	5.4	55

2.11 Carcass (Appendix IV)

Independent of sex, differences in carcass characteristics between treatments were observed. Final bodyweight ranged from 110 kg until 123 kg from the treatment with lowest CP to the treatment with highest CP level. Animals receiving the four treatments with highest CP levels had similar final body weights leading to a quadratic response (P<0.001). Similar responses were observed for slaughter weight and carcass weight (both P quadratic <0.001).

Backfat was on average 15.7 mm with lowest level at 14.67 mm in treatment D+J (P quadratic=0.042. Loin depth averaged 68.75 mm and increased with increasing CP level but remained similar in the four treatments with highest CP level (P quadratic=0.043). Consequently, lean meat percentage was highest in animals that received treatment D+J at 58.71% (P quadratic=0.035).

Expressing backfat, loin depth and lean meat as a percentage of slaughter weight is a method to correct for the differences in slaughter weight. Hereby it is possible to determine whether differences between treatments are caused by differences in slaughter weight or due to the actual treatment. In the current trial, the conclusion for backfat remained the same using this method but the response was less pronounced (Figure 11). For loin depth however, no differences between treatments existed anymore when expressed as a percentage of slaughter weight (P=0.845). This indicates that the responses observed in absolute loin depth were caused by the differences in slaughter weight.

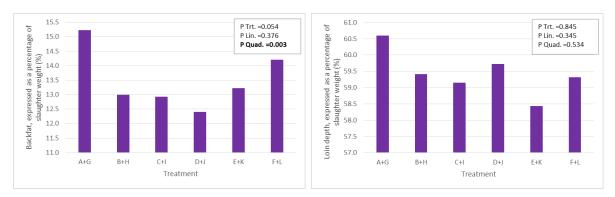


Figure 11: Backfat (left) and loin depth (right), expressed as a percentage of slaughter weight

In addition, lean meat percentage linearly decreased (P<0.001) when dietary CP level increased (Figure 12).

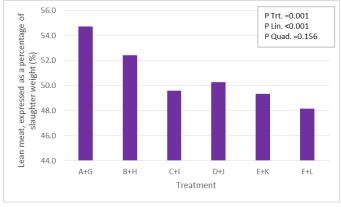


Figure 12: Lean meat, expressed as a percentage of slaughter weight

As a result of the big difference in final bodyweight between barrows and gilts, all carcass characteristics were different between the sexes as well. Expressed as a percentage of slaughter weight, backfat was still higher whereas loin depth and lean meat were lower in barrows compared to gilts (all P<0.01). However, differences became less evident compared to the absolute values.

2.12 Carbon Footprint

Carbon Footprint (CFP) is the total set of greenhouse gas emissions (CO₂, CH₄ and N₂O), expressed as CO₂ equivalents. In animal feed production, CFP of a diet can be calculated as each ingredient has its own CFP value. Figure 13 represents the CFP of diets A and F of each phase in the current trial. Unlike hypothesized, CFP levels of low CP diets were on average 90 CO2 equivalents higher compared to the high CP diets. It was expected that decreasing soybean meal would positively affect the CFP in low CP diets. However, synthetic amino acids have extremely high CFP values (on average 13 times higher compared to soybean meal) and their relatively high inclusion in the low CP diets result in high CFP in these diets.

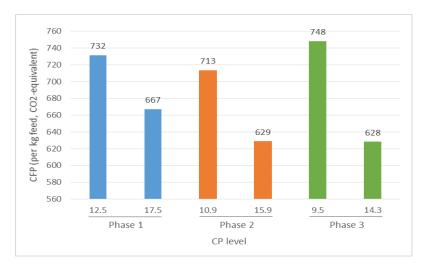


Figure 13: Carbon Footprint of treatments A and F per phase

Conclusions

The most important conclusions are mentioned below.

Performance

30-55 kg BW:

- The response to CP level was different in barrows compared to gilts (P interaction < 0.05). In barrows, best performance was obtained at a CP level of 14.5% whereas gilts performed best at 15.5% CP. These CP levels also resulted in lowest cost per kg gain for barrows and gilts respectively.
- Broken-line analysis indicated a minimum CP level of 14.7% and 15.1% for barrows and gilts respectively. This is in line with literature. Based on the diet composition in the current trial, soybean meal inclusion at this CP level would be approximately 12.5% and its inclusion could thus be reduced by 36%.

55-80 kg BW:

- No interactions were found between CP level and sex (P interaction>0.05).
- A quadratic response to CP level was observed for ADG and efficiency (both P<0.05), but not for ADFI. ADG and efficiency were highest in animals receiving 13.9% CP or more.
- Barrows had a higher ADFI and ADG (both P<0.001), but were not less efficient compared to gilts (P=0.87).
- Broken-line analysis indicated a minimum CP level of 14.4% for both barrows and gilts. This is partially in line with literature as levels of 11% and 11.9% have been reported to nog negatively affect ADG. However, bodyweight ranges were higher (up to 100 kg). Based on the diet composition in the current trial, soybean meal inclusion at this CP level would be approximately 9.5% and its inclusion could thus be reduced by 32%.

80-115 kg BW:

- No interactions occurred were found between CP level and sex (P interaction>0.05).
- Performance increased with increasing CP level and cost per kg gain decreased with increasing CP level (all P linear<0.001).
- Barrows had a higher ADFI and ADG, but were less efficient compared to gilts (all P<0.001).
- Broken-line analysis indicated a minimum CP level of 11.7% for barrows. Based on the diet composition in the current trial, soybean meal inclusion at this CP level would be approximately 4.5% and its inclusion could thus be reduced by 55%.
- Broken-line for gilts resulted in high standard error with large confidence intervals and were thus not used for interpretation.

Carcass

- Differences in carcass characteristics between treatments occurred, independent of sex (P interaction>0.05). Quadratic responses were observed for final bodyweight, slaughter weight and carcass weight (all P<0.001). Animals receiving the four treatments with highest CP levels had similar weights while the animals with lowest CP level were significantly lighter.
- Quadratic responses for loin depth and lean meat percentage were observed (both P quadratic=0.04). However, expressed as a percentage of slaughter weight these responses were not observed anymore (resp. P quadratic= 0.54 and 0.16).
- Barrows were significantly heavier at the end of the trial (P<0.001). Expressed, as a percentage of slaughter weight, barrows had higher backfat but lower loin depth and lean meat compared to gilts (all P<0.01).

<u>Carbon Footprint</u>

- Dietary Carbon Footprint (CFP) increased with decreasing dietary CP level. This was due to the increase in synthetic amino acid inclusion in the formulations and its high impact on Carbon Footprint.
- Animals receiving the low CP diets were more efficient converting this to gain compared to animals receiving high CP diets.
- The assessment on overall CFP could not be made as the tool is not able to adjust to actual performance and a three phase feeding program.

References

- Carpenter, D.A., O'Mara, F.P. and O'Doherty, J.V. 2004. The effect of dietary crude protein concentration on growth performance, carcass composition and nitrogen excretion in entire grower-finisher pigs. Irish Journal of Agricultural and Food Research, 43: 227 - 236.
- Figueroa, J.L., Estrada, J., Zamora, Z., Cordero, J.L., Sanchez-Torres, M.T., Nieto, R. and Copado, J.M.F. 2012. Digestible lysine levels in low-protein diets supplemented with synthetic amino acids for nursery, growing and finishing barrows. Irish Journal of Agricultural and Food Research 51: 33 - 44.
- Gallasi, G., Colombini, S., Malagutti, L., Crovetto, G.M. and Rapetti, L. 2010. Effects of high fibre and low protein diets on performance, digestibility, nitrogen excretion and ammonia emission in the heavy pig. Animal Feed Science and Technology, 161: 140 - 148.
- Gijsberts K. and Doppenberg J. Lineaire programmeringen rundvee-, varkens- en pluimveevoeders. Schothorst Feed Research. 27 October 2016. Nr. 10: p21-24.
- Gloaguen, M., N. Le Floc'h, E. Corrent, Y. Primot, and J. van Milgen. 2014. The use of free amino acids allows formulating very low crude protein diets for piglets. J. Anim. Sci. 92(2):637-644.
- Guillou, D., and F. Molist. In progress. Literature review: Reduction of crude protein in growing pigs and lactating sows diets. Report Schothorst Feed Research (Project VA15-04).
- Kerr, B.J., Southern, L.L., Bidner, T.D., Friesen, K.G. and Easters, R.A. 2003. Influence of dietary protein level, amino acid supplementation, and dietary energy levels on growing-finishing pig performance and carcass composition. Journal of Animal Science, 81: 3075 - 3087.
- Lazzeri, D. B., L. D. Castilha, P. B. Costa, R. V. Nunes, M. S. D. Pozza, and P. C. Pozza. 2017. Standardized ileal digestible (sid) isoleucine requirement of barrows (15-to 30-kg) fed low crude protein diets. Semin.-Cienc. Agrar. 38(5):3283-3293.
- Liu, X. T., W. F. Ma, X. F. Zeng, C. Y. Xie, P. A. Thacker, J. K. Htoo, and S. Y. Qiao. 2015. Estimation of the standardized ileal digestible valine to lysine ratio required for 25-to 120-kilogram pigs fed low crude protein diets supplemented with crystalline amino acids. J. Anim. Sci. 93(10):4761-4773.
- Ma, W., J. Zhu, X. Zeng, X. Liu, P. Thacker, and S. Qiao. 2016. Estimation of the optimum standardized ileal digestible total sulfur amino acid to lysine ratio in late finishing gilts fed low protein diets supplemented with crystalline amino acids. Anim. Sci. J. 87(1):76-83.
- Ma, W. F., X. F. Zeng, X. T. Liu, C. Y. Xie, G. J. Zhang, S. H. Zhang, and S. Y. Qiao. 2015a. Estimation of the standardized ileal digestible lysine requirement and the ideal ratio of threonine to lysine for late finishing gilts fed low crude protein diets supplemented with crystalline amino acids. Anim. Feed Sci. Technol. 201:46-56.
- Ma, W. F., S. H. Zhang, X. F. Zeng, X. T. Liu, C. Y. Xie, G. J. Zhang, and S. Y. Qiao. 2015b. The appropriate standardized ileal digestible tryptophan to lysine ratio improves pig performance and regulates hormones and muscular amino acid transporters in late finishing gilts fed lowprotein diets. J. Anim. Sci. 93(3):1052-1060.
- Madrid, J., Martínez, S., López, C., Orengo, J., López, M.J. and Hernández, F. Effects of low protein diets on growth performance, carcass traits and ammonia emission of barrows and gilts. 2013. Animal Production Science, 53: 146 – 153.

- Martínez-Aispuro, M., Figueroa-Velasco, J.L., Zamora-Zamora, V., Cordero-Mora, J.L., Narciso-Gaytán, C., Sánchez-Torrez, M.T., Carillo-Domínguez, S. and Castillo-Domínguez, R.M. 2014. Effect of CLA supplementation on low-protein diets on the growth performance, carcass characteristics, plasma urea nitrogen concentration, and fatty acid profile in the meat of pigs. Brazilian Archives of Biology and Technology, 57 (5): 742 - 754.
- Meer van der Y., Gerrits W.J.J., Jansman A.J.M., Kemp B. and Bolhuis J.E. 2017. A link between damaging behaviour in pigs, sanitary conditions, and dietary protein and amino acid supply. PLoS ONE 12(5): e0174688.
- Nørgaard, J.V., Hansen, M.J., Soumeh, E.A., Adamsen, A.P.S. and Poulsen, H.D. 2014. Effect of protein level on performance, nitrogen utilization and carcass composition in finisher pigs. Acta Agriculturae Scand Section A, 64 (2): 123 - 129.
- Pijlman, J. Effect of low crude protein diets on performance and carcass characteristics of growerfinisher pigs. 2015. Schothorst Feed Research. Presentation at the 21e Orffa Benelux Symposium. 28-5-15, Fletcher hotel Trivium, Etten-Leur, The Netherlands.
- Powell S., Bidner, T.D., Payne, R.L. and Southern, L.L. 2011. Growth performance of 20- to 50kilogram pigs fed low-crude-protein diets supplemented with histidine, cystine, glycine, glutamic acid, or arginine. Journal of Animal Science, 89: 3643 – 3650.
- Xie, C., S. Zhang, G. Zhang, F. Zhang, L. Chu, and S. Qiao. 2013. Estimation of the optimal ratio of standardized ileal digestible threonine to lysine for finishing barrows fed low crude protein diets. Asian-Australas. J. Anim. Sci. 26(8):1172-1180.
- Xie, C. Y., G. J. Zhang, F. R. Zhang, S. H. Zhang, X. F. Zeng, P. A. Thacker, and S. Y. Qiao. 2014. Estimation of the optimal ratio of standardized ileal digestible tryptophan to lysine for finishing barrows fed low protein diets supplemented with crystalline amino acids. Czech J. Anim. Sci. 59(1):26-34.
- Zhang, G. J., Q. L. Song, C. Y. Xie, L. C. Chu, P. A. Thacker, J. K. Htoo, and S. Y. Qiao. 2012. Estimation of the ideal standardized ileal digestible tryptophan to lysine ratio for growing pigs fed low crude protein diets supplemented with crystalline amino acids. Livest. Sci. 149(3):260-
- Zhang, G. J., C. Y. Xie, P. A. Thacker, J. K. Htoo, and S. Y. Qiao. 2013. Estimation of the ideal ratio of standardized ileal digestible threonine to lysine for growing pigs (22-50 kg) fed low crude protein diets supplemented with crystalline amino acids. Anim. Feed Sci. Technol. 180(1-4):83-91.

Appendix 1: Diet formulations

Note: price formulation (€/ton) based on raw material prices as stated in Lineaire programmeringen rundvee-, varkens- en pluimveevoeders (Gijsberts K. and Doppenberg J., 2016). No margins added.

Phase 1

Treatment	A +0	G	В+Н	C+I	D+J	E+K	F+	L
Bestmix code	101/	570.1	01				101,	/570.105
Priceformulation	23	5.91	224 200	222 506	220 804	220 102		227.40
(€/ton)			234.208	232.506	230.804	229.102		
Ingredients								227.40
Vleesvark. 0.75/0.5%			0.750	0.750	0.750	0.750	0.750	0.750
Barley			37.000	36.766	36.532	36.297	36.063	35.829
•								
Corn			43.936	42.149	40.362	38.574	36.787	35.000
Soybean meal HP			4.049	7.132	10.215	13.298	16.381	19.464
Wheat middlings			9.000	8.170	7.341	6.511	5.682	4.852
Salt			0.215	0.262	0.309	0.356	0.403	0.450
Limestone			1.197	1.184	1.171	1.158	1.145	1.132
L-Valine			0.176	0.141	0.106	0.070	0.035	0.000
L-Threonine			0.292	0.249	0.206	0.164	0.121	0.078
L-Tryptophan			0.072	0.058	0.043	0.029	0.014	0.000
Monocalciumphosphate			0.737	0.714	0.690	0.667	0.643	0.620
Sodium Bicarbonate 24.1%			0.357	0.286	0.214	0.143	0.071	0.000
Potassium Bicarbonate			0.456	0.365	0.274	0.182	0.091	0.000
Fats/oils, Palm oil			0.815	0.951	1.087	1.224	1.360	1.496
L-Lysine HCL			0.745	0.649	0.553	0.457	0.361	0.265
DL-Methionine			0.203	0.175	0.147	0.120	0.092	0.064
Nutrients								
Dry matter	%		89.011	88.897	88.783	88.668	88.554	88.440
Moisture	%		10.989	11.103	11.217	11.332	11.446	11.560
Crude protein	%		12.500	13.500	14.500	15.500	16.500	17.500
Crude fat	%		3.454	3.508	3.562	3.615	3.669	3.723
Ash	%		4.685	4.698	4.712	4.725	4.739	4.752
Starch (Ewers)	%		48.377	47.087	45.798	44.508	43.219	41.929
Calcium	%		0.613	0.613	0.613	0.613	0.613	0.613
Phosphorous P	%		0.519	0.520	0.522	0.523	0.525	0.526
Magnesium	%		0.137	0.143	0.150	0.156	0.163	0.169
Sodium	%		0.180	0.180	0.180	0.180	0.180	0.180
Potassium	%		0.670	0.684	0.698	0.712	0.726	0.740
Chloride	%		0.334	0.341	0.348	0.356	0.363	0.370
EW (energy value pigs)	_	CVB	1.100	1.100	1.100	1.100	1.100	1.100
SID Lysine (pigs)	%	CVB	0.930	0.930	0.930	0.930	0.930	0.930
SID Leucine (pigs)	%	CVB	0.841	0.917	0.993	1.070	1.146	1.222
SID Arginine (pigs)	%	CVB	0.553	0.643	0.733	0.823	0.913	1.003
SID Histidine (pigs)	%	CVB	0.252	0.281	0.310	0.339	0.368	0.397
SID Phenylalanine (pigs)	%	CVB	0.469	0.526	0.510	0.641	0.699	0.756
31D Thenylalanine (pigs)	70	CVD	0.403	0.520	0.304	0.041	0.055	0.750

SID Tyrosine (pigs)	%	CVB	0.325	0.365	0.406	0.446	0.487	0.527
SID Threo./SID Lys. (pigs)	-	CVB	0.650	0.650	0.650	0.650	0.650	0.650
SID Trypt./SID Lys. (pigs)	-	CVB	0.180	0.180	0.181	0.181	0.182	0.182
SID Val./SID Lys. (pigs)	-	CVB	0.670	0.688	0.706	0.723	0.741	0.759
SID Isoleu./SID Lys. (pigs)	-	CVB	0.369	0.425	0.482	0.538	0.595	0.651
SID Meth./SID Lys. (pigs)	-	CVB	0.399	0.384	0.370	0.355	0.341	0.326
SID M+Cyst./SID Lys. (pigs)	-	CVB	0.600	0.600	0.600	0.600	0.600	0.600
SID Leu/SID Lys. (pigs)		CVB	0.904	0.986	1.068	1.150	1.232	1.314
SID His/SID Lys. (pigs)		CVB	0.271	0.302	0.333	0.365	0.396	0.427
SID Arg/SID Lys. (pigs)		CVB	0.595	0.691	0.788	0.885	0.982	1.078
SID Phe/SID Lys. (pigs)		CVB	0.504	0.566	0.628	0.689	0.751	0.813
SID Tyr/SID Lys. (pigs)		CVB	0.349	0.393	0.436	0.480	0.523	0.567
Fe total	mg		262.907	265.459	268.011	270.562	273.114	275.666
Cu total	mg		18.684	18.906	19.128	19.350	19.572	19.794
Zn total	mg		106.067	106.517	106.968	107.418	107.869	108.319
Mn total	mg		80.331	80.419	80.508	80.596	80.685	80.773
Se total	mg		0.490	0.483	0.475	0.468	0.460	0.453
Co total	mg		0.149	0.154	0.159	0.164	0.169	0.174
I total	mg		4.109	4.110	4.111	4.111	4.112	4.113
Vitamin A	IU		7500.000	7500.000	7500.000	7500.000	7500.000	7500.000
Vitamin D3	IU		1500.000	1500.000	1500.000	1500.000	1500.000	1500.000
Vitamin E	mg		60.000	60.000	60.000	60.000	60.000	60.000

Phase 2

Treatment			A+G	В+Н	C+I	D+J	E+K	F+L
Bestmix code		1	01/570.10 1					01/570.10
Price formulation (€/ton)			220.46	218.802	217.14 4	215.48 6	213.82 8	212.17
Ingredients								
Vleesvark. 0.75/0.5%			0.500	0.500	0.500	0.500	0.500	0.500
Barley			36.500	35.926	35.352	34.777	34.203	33.629
Corn			47.605	45.084	42.563	40.042	37.521	35.000
Soybean meal HP			0.000	2.823	5.645	8.468	11.290	14.113
Wheat middlings			10.374	10.799	11.224	11.650	12.075	12.500
Salt			0.249	0.286	0.324	0.361	0.399	0.436
Limestone			1.223	1.225	1.228	1.230	1.233	1.235
L-Valine			0.152	0.122	0.091	0.061	0.030	0.000
Potassium carbonate 56%			0.429	0.343	0.257	0.172	0.086	0.000
L-Threonine			0.260	0.219	0.178	0.138	0.097	0.056
L-Tryptophan			0.067	0.054	0.040	0.027	0.013	0.000
Monocalciumphosphate			0.748	0.675	0.602	0.528	0.455	0.382
Sodium Bicarbonate 24.1%			0.331	0.265	0.199	0.132	0.066	0.000
Fats/oils, Palm oil			0.700	0.938	1.175	1.413	1.650	1.888
L-Lysine HCL			0.703	0.610	0.518	0.425	0.333	0.240
DL-Methionine			0.159	0.131	0.104	0.076	0.049	0.021
Nutrients								
Dry matter	%		89.129	89.031	88.934	88.836	88.739	88.641
Moisture	%		10.871	10.969	11.066	11.164	11.261	11.359
Crude protein	%		10.900	11.900	12.900	13.900	14.900	15.900
Crude fat	%		3.488	3.644	3.800	3.957	4.113	4.269
Ash	%		4.517	4.523	4.529	4.535	4.541	4.547
Starch (Ewers)	%		50.407	48.760	47.112	45.465	43.817	42.170
Calcium	%		0.612	0.610	0.608	0.606	0.604	0.602
Phosphorous P	%		0.516	0.514	0.511	0.509	0.506	0.504
Magnesium	%		0.129	0.138	0.146	0.155	0.163	0.172
Sodium	%		0.190	0.188	0.186	0.184	0.182	0.180
Potassium	%		0.670	0.675	0.680	0.686	0.691	0.696
Chloride	%		0.347	0.350	0.353	0.355	0.358	0.361
EW (energy value pigs)	-	CVB	1.100	1.100	1.100	1.100	1.100	1.100
SID Lysine (pigs)	%	CVB	0.800	0.800	0.800	0.800	0.800	0.800
SID Methionine (pigs)	%	CVB	0.310	0.296	0.283	0.269	0.256	0.242
SID Cysteine (pigs)	%	CVB	0.170	0.184	0.197	0.211	0.224	0.238
SID Meth.+Cyst. (pigs)	%	CVB	0.480	0.480	0.480	0.480	0.480	0.480
SID Threonine (pigs)	%	CVB	0.520	0.520	0.520	0.520	0.520	0.520
SID Tryptophan (pigs)	%	CVB	0.144	0.146	0.148	0.149	0.151	0.153
SID Isoleucine (pigs)	%	CVB	0.276	0.326	0.376	0.425	0.475	0.525
SID Leucine (pigs)	%	CVB	0.749	0.818	0.888	0.957	1.027	1.096
SID Valine (pigs)	%	CVB	0.536	0.556	0.576	0.596	0.616	0.636
SID Arginine (pigs)	%	CVB	0.438	0.528	0.618	0.709	0.799	0.889
SID Histidine (pigs)	%	CVB	0.216	0.245	0.273	0.302	0.330	0.359
SID Phenylalanine (pigs)	%	CVB	0.395	0.450	0.505	0.559	0.614	0.669
SID Tyrosine (pigs)	%	CVB	0.274	0.313	0.351	0.390	0.428	0.467
SID Threo./SID Lys. (pigs)	-	CVB	0.650	0.650	0.650	0.650	0.650	0.650
SID Trypt./SID Lys. (pigs)	-	CVB	0.180	0.182	0.184	0.187	0.189	0.191
SID Val./SID Lys. (pigs)	-	CVB	0.670	0.695	0.720	0.745	0.770	0.795
SID Isoleu./SID Lys. (pigs)	-	CVB	0.345	0.407	0.470	0.532	0.595	0.657
SID Meth./SID Lys. (pigs)	-	CVB	0.387	0.370	0.353	0.336	0.319	0.302

SID M+Cyst./SID Lys. (pigs)	-	CVB	0.600	0.600	0.600	0.600	0.600	0.600
SID Leu/SID Lys. (pigs)		CVB	0.936	1.023	1.110	1.197	1.283	1.370
SID His/SID Lys. (pigs)		CVB	0.270	0.306	0.342	0.377	0.413	0.449
SID Arg/SID Lys. (pigs)		CVB	0.548	0.660	0.773	0.886	0.999	1.111
SID Phe/SID Lys. (pigs)		CVB	0.494	0.562	0.631	0.699	0.768	0.836
SID Tyr/SID Lys. (pigs)		CVB	0.343	0.391	0.439	0.487	0.536	0.584
Fe total	m g		216.347	213.771	211.194	208.618	206.041	203.465
Cu total	m g		13.336	13.617	13.899	14.180	14.462	14.743
Zn total	m g		80.766	81.873	82.981	84.088	85.196	86.303
Mn total	m g		60.369	61.711	63.053	64.395	65.737	67.079
Se total	m g		0.393	0.392	0.391	0.390	0.389	0.388
Co total	m g		0.141	0.145	0.150	0.154	0.159	0.163
I total	m g		2.773	2.772	2.771	2.771	2.770	2.769
Vitamin A	IU		5000.000	5000.000	5000.000	5000.000	5000.000	5000.000
Vitamin D3	IU		1000.000	1000.000	1000.000	1000.000	1000.000	1000.000
Vitamin E	m a		40.000	40.000	40.000	40.000	40.000	40.000

Phase 3

Treatment		A+G	В+Н	C+I	D+J	E+K	F+L
Bestmix code Price formulation (€/ton)		101/570.101 234.47	229.337	222.600	215.864	1 209.127	.01/570.105 202.39
Ingredients							
Vleesvark. 0.75/0.5%		0.500	0.500	0.500	0.500	0.500	0.500
Soybean hulls		7.000	5.880	4.410	2.940	1.470	0.000
Barley		25.712	25.976	26.322	26.668	27.014	27.360
Corn		59.768	57.405	54.304	51.203	48.101	45.000
Soybean meal HP		0.000	1.591	3.679	5.766	7.854	9.942
Wheat middlings		0.000	2.240	5.180	8.120	11.060	14.000
Salt		0.350	0.346	0.342	0.337	0.333	0.328
Limestone		0.710	0.738	0.774	0.811	0.847	0.884
Fibrecell		2.000	1.680	1.260	0.840	0.420	0.000
L-Valine		0.131	0.110	0.083	0.055	0.028	0.000
Potassium carbonate 56%		0.396	0.333	0.249	0.166	0.083	0.000
L-Threonine		0.219	0.191	0.154	0.117	0.080	0.043
L-Tryptophan		0.069	0.058	0.043	0.029	0.014	0.000
Monocalciumphosphate		0.878	0.806	0.712	0.618	0.524	0.430
Sodium Bicarbonate 24.1%		0.518	0.462	0.389	0.315	0.242	0.168
Fats/oils, Palm oil		1.000	1.016	1.038	1.059	1.081	1.102
L-Lysine HCL		0.616	0.556	0.477	0.397	0.318	0.239
DL-Methionine		0.133	0.112	0.085	0.058	0.031	0.004
Nutrients							
Dry matter	%	89.325	89.237	89.122	89.006	88.891	88.775
Moisture	%	10.675	10.763	10.879	10.994	11.110	11.225
Crude protein	%	9.550	10.310	11.308	12.305	13.303	14.300
Crude fat	%	3.942	3.921	3.894	3.867	3.840	3.813

Ash	%		4.115	4.105	4.092	4.080	4.067	4.054
Starch (Ewers)	%		50.613	49.769	48.660	47.552	46.443	45.335
Calcium	%		0.461	0.461	0.461	0.462	0.462	0.462
Phosphorous P	%		0.445	0.455	0.468	0.481	0.494	0.507
Magnesium	%		0.108	0.117	0.129	0.140	0.152	0.164
Sodium	%		0.265	0.251	0.234	0.216	0.198	0.180
Potassium	%		0.620	0.621	0.623	0.625	0.626	0.628
Chloride	%		0.374	0.362	0.346	0.331	0.315	0.299
EW (energy value pigs)	-	CVB	1.103	1.103	1.102	1.101	1.101	1.100
SID Lysine/EW		CVB	0.632	0.632	0.632	0.632	0.632	0.632
SID Lysine (pigs)	%	CVB	0.697	0.697	0.696	0.696	0.695	0.695
SID Methionine (pigs)	%	CVB	0.270	0.260	0.247	0.235	0.222	0.209
SID Cysteine (pigs)	%	CVB	0.148	0.160	0.175	0.190	0.205	0.220
SID Meth.+Cyst. (pigs)	%	CVB	0.418	0.420	0.422	0.424	0.427	0.429
SID Threonine (pigs)	%	CVB	0.453	0.453	0.453	0.452	0.452	0.452
SID Tryptophan (pigs)	%	CVB	0.125	0.126	0.127	0.128	0.129	0.130
SID Isoleucine (pigs)	%	CVB	0.247	0.280	0.324	0.368	0.411	0.455
SID Leucine (pigs)	%	CVB	0.739	0.785	0.845	0.905	0.966	1.026
SID Valine (pigs)	%	CVB	0.467	0.483	0.505	0.526	0.548	0.569
SID Arginine (pigs)	%	CVB	0.358	0.424	0.510	0.596	0.682	0.768
SID Histidine (pigs)	%	CVB	0.193	0.214	0.242	0.270	0.297	0.325
SID Phenylalanine (pigs)	%	CVB	0.353	0.391	0.441	0.491	0.541	0.591
SID Tyrosine (pigs)	%	CVB	0.255	0.281	0.315	0.348	0.382	0.416
SID Threo./SID Lys. (pigs)	-	CVB	0.650	0.650	0.650	0.650	0.650	0.650
SID Trypt./SID Lys. (pigs)	-	CVB	0.180	0.181	0.183	0.185	0.186	0.188
SID Val./SID Lys. (pigs)	-	CVB	0.670	0.694	0.725	0.756	0.788	0.819
SID Isoleu./SID Lys. (pigs)	-	CVB	0.355	0.403	0.466	0.528	0.591	0.654
SID Meth./SID Lys. (pigs)	-	CVB	0.388	0.374	0.355	0.337	0.318	0.300
SID M+Cyst./SID Lys. (pigs)	-	CVB	0.600	0.603	0.606	0.610	0.613	0.617
SID Leu/SID Lys. (pigs)		CVB	1.060	1.127	1.214	1.301	1.389	1.476
SID His/SID Lys. (pigs)		CVB	0.277	0.307	0.347	0.387	0.427	0.468
SID Arg/SID Lys. (pigs)		CVB	0.514	0.608	0.732	0.856	0.981	1.105
SID Phe/SID Lys. (pigs)		CVB	0.506	0.561	0.633	0.706	0.778	0.850
SID Tyr/SID Lys. (pigs)		CVB	0.366	0.403	0.452	0.501	0.550	0.599

Fe total	mg	252.194	244.272	233.875	223,478	213.080	202.683
Cu total	mg	12.626	12.888	13.233	13.577	13.922	14.266
Zn total	•	75.591	77.286	79.510	81.734	83.958	86.182
	mg						
Mn total	mg	48.790	51.658	55.422	59.187	62.951	66.715
Se total	mg	0.337	0.346	0.358	0.371	0.383	0.395
Co total	mg	0.113	0.118	0.125	0.132	0.139	0.146
I total	mg	2.769	2.769	2.768	2.767	2.767	2.766
Vitamin A	IU	5000.000	5000.000	5000.000	5000.000	5000.000	5000.000
Vitamin D3	IU	1000.000	1000.000	1000.000	1000.000	1000.000	1000.000
Vitamin E	mg	40.000	40.000	40.000	40.000	40.000	40.000
Vitamin K3 (3a711)	mg	0.700	0.700	0.700	0.700	0.700	0.700
Vitamin B1	mg	0.700	0.700	0.700	0.700	0.700	0.700
Vitamin B2	mg	2.700	2.700	2.700	2.700	2.700	2.700
Vitamin B6	mg	0.700	0.700	0.700	0.700	0.700	0.700

APPENDIX II: Diet analyses

Complete diet: analyses versus formulation

Note: Analyses performed at Provimi B.V. laboratory in Rotterdam

Values in red indicate deviation higher than measurement uncertainty for that specific analysis

Crude protein determined by wet chemistry

Phase	ase 1					2						3						
Treatment	A+G	B+H	C+I	D+J	E+K	F+L	A+G	B+H	C+I	D+J	E+K	F+L	A+G	B+H	C+I	D+J	E+K	F+L
Sample ID	GF1603-1	GF1603-2	GF1603-3	GF1603-4	GF1603-5	GF1603-6	GF1603-7	GF1603-8	GF1603-9	GF1603-10	GF1603-11	GF1603-12	GF1603-13	GF1603-14	GF1603-15	GF1603-16	GF1603-17	GF1603-18
Formulated																		
Crude protein (%)	12.50	13.50	14.50	15.50	16.50	17.50	10.90	11.90	12.90	13.90	14.90	15.90	9.55	10.31	11.31	12.31	13.30	14.30
Crude fat (%)	3.45	3.51	3.56	3.62	3.67	3.72	3.49	3.64	3.80	3.96	4.11	4.27	3.94	3.92	3.89	3.87	3.84	3.81
Crude fibre (%)	3.74	3.72	3.70	3.68	3.66	3.64	3.79	3.87	3.95	4.03	4.11	4.19	4.59	4.62	4.66	4.69	4.73	4.76
Moisture (%)	11.0	11.1	11.2	11.3	11.4	11.6	10.87	10.97	11.07	11.16	11.26	11.36	10.68	10.76	10.88	10.99	11.11	11.23
Calcium (%)	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.60	0.60	0.46	0.46	0.46	0.46	0.46	0.46
Phosphorus (%)	0.52	0.52	0.52	0.52	0.52	0.53	0.52	0.51	0.51	0.51	0.51	0.50	0.45	0.45	0.47	0.48	0.49	0.51
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.19	0.19	0.19	0.18	0.18	0.18	0.27	0.25	0.23	0.22	0.20	0.18
Potassium (%)	0.67	0.68	0.70	0.71	0.73	0.74	0.67	0.68	0.68	0.69	0.69	0.70	0.62	0.62	0.62	0.62	0.63	0.63
Analyzed																		
Crude protein (%)	12.9	13.9	14.8	15.9	16.4	18.1	11.1	11.8	12.6	14.2	14.7	15.6	10.0	10.3	11.4	12.90	14.1	14.6
Crude fat (%)	4.0	3.9	4.0	4.0	3.9	3.8	4.3	4.8	4.7	4.8	5.0	5.3	4.8	4.9	5.0	5.00	4.9	4.9
Crude fibre (%)	3.2	3.2	3.2	3.2	3.2	3.3	3.7	3.8	3.8	3.8	3.9	4.0	3.7	3.7	3.8	3.70	3.6	3.6
Moisture (%)	11.4	11.0	11.5	11.7	11.5	11.0	12.1	11.5	12.2	11.9	11.7	11.5	11.1	10.7	10.8	10.80	10.7	10.7
Calcium (%)	0.64	0.64	0.63	0.62	0.63	0.66	0.64	0.66	0.62	0.62	0.63	0.63	0.50	0.50	0.49	0.49	0.49	0.49
Phosphorus (%)	0.56	0.52	0.53	0.53	0.54	0.57	0.55	0.57	0.56	0.56	0.56	0.55	0.49	0.51	0.52	0.54	0.56	0.58
Sodium (%)	0.19	0.18	0.20	0.19	0.19	0.19	0.19	0.20	0.19	0.18	0.18	0.18	0.29	0.28	0.24	0.23	0.21	0.19
Potassium (%)	0.69	0.69	0.71	0.72	0.75	0.79	0.68	0.71	0.71	0.71	0.72	0.73	0.65	0.67	0.67	0.69	0.69	0.71
Difference analysis versus formulation (%)																		
Crude protein (%)	103	103	102	103	99	103	102	99	98	102	99	98	105	100	101	105	106	102
Crude fat (%)	116	111	112	111	106	102	124	130	123	122	122	123	122	125	128	129	128	129
Crude fibre (%)	86	86	86	87	87	91	99	98	96	93	95	94	81	80	82	79	76	76
Moisture (%)	104	99	103	103	100	95	111	105	111	107	104	101	104	99	99	98	96	95
Calcium (%)	104	104	103	101	103	108	104	108	102	102	105	104	108	108	106	106	106	106
Phosphorus (%)	108	100	102	101	103	108	107	110	110	110	111	110	110	112	111	112	113	114
Sodium (%)	106	100	111	106	106	106	101	106	102	97	101	99	109	111	103	107	106	106
Potassium (%)	103	101	102	101	103	107	101	105	104	103	104	104	105	108	108	110	110	113

Total amino acids: analyses versus formulation

Note: Amino acids analyzed at University of Missouri, USA

For phase 2, only treatments A and F (sample ID GF1603-7 and GF1603-12) were analyzed. Values from treatments

B – E were extrapolated from those.

	Phase 1					2					3								
	Treatment	A+G	B+H	C+I	D+J	E+K	F+L	A+G	B+H	C+I	D+J	E+K	F+L	A+G	B+H	C+I	D+J	E+K	F+L
	Sample ID	GF1603-1	GF1603-2	GF1603-3	GF1603-4	GF1603-5	GF1603-6	GF1603-7	GF1603-8	GF1603-9	GF1603-10	GF1603-11	GF1603-12	GF1603-13	GF1603-14	GF1603-15	GF1603-16	GF1603-17	GF1603-18
Formulated																			
Lysine		1.04	1.04	1.05	1.06	1.06	1.07	0.90	0.90	0.91	0.92	0.92	0.93	0.79	0.79	0.80	0.81	0.81	0.82
Methionine		0.41	0.39	0.38	0.37	0.36	0.34	0.34	0.33	0.32	0.31	0.29	0.28	0.30	0.29	0.28	0.27	0.26	0.25
Cysteine		0.25	0.27	0.29	0.30	0.32	0.34	0.23	0.25	0.27	0.29	0.30	0.32	0.20	0.22	0.24	0.26	0.28	0.30
Threonine		0.67	0.67	0.68	0.68	0.69	0.69	0.58	0.59	0.59	0.60	0.60	0.60	0.51	0.51	0.52	0.52	0.53	0.53
Tryptophan		0.19	0.19	0.20	0.20	0.20	0.21	0.16	0.16	0.17	0.17	0.18	0.18	0.14	0.14	0.15	0.15	0.15	0.16
Valine		0.71	0.74	0.76	0.78	0.81	0.83	0.62	0.65	0.67	0.70	0.73	0.75	0.54	0.56	0.59	0.62	0.65	0.68
Analyzed																			
Lysine		1.03	1.05	1.04	1.04	1.04	1.10	0.91	0.90	0.89	0.89	0.88	0.87	0.84	0.86	0.82	0.85	0.86	0.87
Methionine		0.35	0.35	0.32	0.32	0.32	0.32	0.29	0.28	0.27	0.26	0.25	0.24	0.24	0.24	0.23	0.22	0.22	0.21
Cysteine		0.22	0.23	0.23	0.26	0.28	0.29	0.17	0.18	0.20	0.21	0.23	0.24	0.16	0.18	0.19	0.21	0.23	0.25
Threonine		0.63	0.64	0.65	0.65	0.65	0.69	0.56	0.56	0.57	0.57	0.58	0.58	0.51	0.52	0.50	0.51	0.52	0.53
Tryptophan		0.22	0.23	0.23	0.23	0.23	0.26	0.18	0.19	0.20	0.20	0.21	0.22	0.17	0.18	0.19	0.18	0.19	0.19
Valine		0.72	0.73	0.77	0.78	0.84	0.89	0.60	0.63	0.65	0.68	0.70	0.73	0.54	0.56	0.57	0.61	0.65	0.69
Difference a	nalysis versu	s formulation	າ (%)																
Lysine		99	101	99	99	98	103	101	100	98	97	95	93	106	108	103	106	106	106
Methionine		86	89	84	87	90	93	85	85	85	85	85	85	79	82	82	81	85	85
Cysteine		87	85	80	86	87	86	73	73	74	74	75	75	80	83	80	82	83	84
Threonine		94	95	96	95	95	100	96	96	96	96	96	96	101	102	97	98	99	100
Tryptophan		118	120	118	116	113	126	113	114	116	117	118	120	120	125	128	119	123	120
Valine		101	101	99	100	96	93	103	103	103	103	103	103	100	100	104	101	99	98

SID amino acids ratios: analyses versus formulation

Note: ratios are extrapolated from above table.

Ratios marked yellow are below (INRA) requirement

Phase				1				2 3										
Treatment	A+G	B+H	C+I	D+J	E+K	F+L	A+G	B+H	C+I	D+J	E+K	F+L	A+G	B+H	C+I	D+J	E+K	F+L
Sample ID	GF1603-1	GF1603-2	GF1603-3	GF1603-4	GF1603-5	GF1603-6	GF1603-7	GF1603-8	GF1603-9	GF1603-10	GF1603-11	GF1603-12	GF1603-13	GF1603-14	GF1603-15	GF1603-16	GF1603-17	GF1603-18
	101/570.101					101/570.105	101/570.201				101/570.205	101/570.205	101/570.301					101/570.305
Formulated																		
SID Met/SID Lys	0.40	0.38	0.37	0.36	0.34	0.33	0.39	0.37	0.35	0.34	0.32	0.30	0.39	0.37	0.36	0.34	0.32	0.30
SID M+C/SID Lys	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
SID Thre/SID Lys	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
SID Tryp/SID Lys	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.19	0.19
SID Val/SID Lys	0.67	0.69	0.71	0.72	0.74	0.76	0.67	0.70	0.72	0.75	0.77	0.80	0.67	0.69	0.73	0.76	0.79	0.82
Analyzed																		
SID Met/SID Lys	0.35	0.34	0.31	0.31	0.31	0.29	0.32	0.31	0.31	0.30	0.29	0.28	0.29	0.28	0.28	0.26	0.26	0.24
SID M+C/SID Lys	0.52	0.52	0.50	0.53	0.54	0.52	0.48	0.48	0.49	0.50	0.51	0.52	0.45	0.46	0.47	0.46	0.48	0.48
SID Thre/SID Lys	0.61	0.61	0.63	0.63	0.63	0.63	0.62	0.63	0.64	0.65	0.66	0.67	0.61	0.61	0.62	0.60	0.61	0.61
SID Tryp/SID Lys	0.21	0.22	0.22	0.21	0.21	0.22	0.20	0.21	0.22	0.23	0.24	0.24	0.20	0.21	0.23	0.21	0.22	0.21
SID Val/SID Lys	0.68	0.69	0.70	0.74	0.73	0.69	0.68	0.72	0.76	0.80	0.84	0.88	0.63	0.64	0.73	0.73	0.74	0.75
Difference analysis versi	us formulation	ı (%)																
SID Met/SID Lys	87	88	85	88	92	90	83	85	86	88	90	91	75	76	80	77	80	80
SID M+C/SID Lys	87	87	83	88	91	87	79	80	82	83	84	86	75	76	79	77	79	79
SID Thre/SID Lys	94	94	97	97	97	97	95	96	98	100	101	103	95	94	95	93	93	94
SID Tryp/SID Lys	118	120	119	117	116	122	111	114	118	121	125	128	113	115	125	113	116	113
SID Val/SID Lys	102	100	99	102	98	90	102	103	105	107	109	110	94	93	101	96	94	92

APPENDIX III: Results table performance

Main effects

Treatment	A+G	B+H	C+I	D+J	E+K	F+L				_				
CP Level Phase 1	12.5	13.5	14.5	15.5	16.5	17.5	SEM	Р	P Linear	P	Cilka	D	CENA	D
CP Level Phase 2	10.9	11.9	12.9	13.9	14.9	15.9	SEIVI	CP level	CP Level	Quadratic	Gilts	Barrows	SEM	P sex
CP Level Phase 3	9.5	10.3	11.3	12.3	13.3	14.3				CP Level				
Body weight (kg)														
Day 0	30.12	30.18	29.93	30.00	30.18	30.12	1.88	0.941	0.949	0.558	29.39	30.78	1.874	0.000
Day 14	40.53	40.45	41.86	41.77	40.98	41.14	0.20	0.000	0.015	0.000	40.84	41.41	0.095	0.003
Day 27	51.36	52.42	54.87	54.23	53.35	53.58	0.38	0.000	0.000	0.000	52.77	53.83	0.297	0.000
Day 42	63.60	66.00	69.55	68.83	68.18	68.51	0.56	0.000	0.000	0.000	65.72	69.17	0.395	0.000
Day 57	75.67	79.15	83.38	83.09	82.26	82.72	0.83	0.000	0.000	0.000	78.30	83.79	0.575	0.000
Day 77	93.20	96.60	102.75	102.33	101.85	102.05	1.18	0.000	0.000	0.000	95.77	103.82	0.745	0.000
Day 98	109.98	116.23	121.57	121.34	122.09	122.74	1.48	0.000	0.000	0.000	114.09	123.90	0.953	0.000
						Phase 1 (d	ay 0-27)							
ADG (gram)	802.85	829.39	919.77	896.44	864.05	872.54	14.00	0.000	0.000	0.000	845.65	882.70	10.828	0.000
ADFI (gram)	1637.49	1604.03	1731.10	1700.34	1646.12	1670.74	27.70	0.003	0.153	0.034	1628.73	1701.21	21.920	0.001
GF	0.48	0.52	0.53	0.53	0.52	0.52	0.00	0.000	0.000	0.000	0.52	0.52	0.004	0.729
FCR	2.08	1.94	1.88	1.90	1.91	1.92		0.000	0.000	0.000	1.94	1.93		0.729
Cost (€/kg gain)	0.49	0.45	0.44	0.44	0.44	0.44	0.00	0.000	0.000	0.000	0.45	0.45	0.003	0.809
Phase 2 (day 27-57)														
ADG (gram)	810.34	891.19	950.49	961.70	963.56	971.12	19.74	0.000	0.000	0.003	850.67	998.80	11.087	0.000
ADFI (gram)	2345.93	2358.36	2512.02	2398.80	2427.52	2458.72	42.30	0.053	0.058	0.276	2214.61	2619.18	27.268	0.000
GF	0.35	0.38	0.38	0.40	0.40	0.40	0.01	0.000	0.000	0.011	0.38	0.38	0.003	0.869
FCR	2.82	2.65	2.63	2.49	2.52	2.52		0.000	0.000	0.011	2.60	2.60		0.869
Cost (€/kg gain)	0.62	0.58	0.57	0.54	0.54	0.54	0.01	0.000	0.000	0.007	0.56	0.57	0.004	0.957
						Phase 3 (da	ay 57-98)							
ADG (gram)	818.20	899.02	926.26	927.87	966.18	970.81	24.53	0.001	0.000		864.19	971.92	12.897	0.000
ADFI (gram)	2497.85	2639.49	2762.41	2667.40	2827.12	2854.11	50.56	0.000	0.000		2438.04	2978.09	31.276	0.000
GF	0.32	0.34	0.34	0.35	0.34	0.34	0.01	0.336	0.095		0.35	0.33	0.003	0.000
FCR	3.08	2.92	2.97	2.90	2.91	2.92		0.336	0.095		2.85	3.06		0.000
Cost (€/kg gain)	0.73	0.67	0.66	0.62	0.61	0.60	0.01	0.000	0.000	0.161	0.62	0.67	0.006	0.000
						Overall (d	ay 0-98)							
ADG (gram)	814.02	877.80	932.23	929.92	937.61	944.19	15.09	0.000	0.000		855.94	955.98	9.722	0.000
ADFI (gram)	2198.22	2243.81	2362.96	2297.28	2349.80	2381.69	35.14	0.001	0.000		2129.60	2481.66	23.652	0.000
GF	0.37	0.39	0.39	0.41	0.40	0.40	0.00	0.000	0.000		0.40		0.002	0.000
FCR	2.73	2.55	2.53	2.46	2.50	2.51		0.000	0.000	0.000	2.50	2.60		0.000
Cost (€/kg gain)	0.63	0.58	0.57	0.54	0.54	0.53	0.01	0.000	0.000	0.000	0.55	0.57	0.003	0.000

Note: GF data (mean, SEM and P-value) is obtained by statistical analyses, FCR data (means and P-value) is derived from GF.

<u>Interactions</u>

Treatment	Α	В	С	D	E	F	G	Н	ı	J	К	L		D	P Linear	Р
Sex			Gilt	ts					Barro	ws			SEM	CP * Sex		Quadratic CP * Sex
Body weight (kg)																
Day 0	29.41	29.46	29.26	29.21	29.46	29.55	30.83	30.90	30.59	30.79	30.90	30.69	1.89	0.987	0.748	0.663
Day 14	40.13	40.12	41.52	42.06	40.78	40.41	40.93	40.77	42.21	41.48	41.17	41.86	0.29	0.055	0.739	0.017
Day 27	50.41	51.70	54.12	54.64	52.80	52.96	52.32	53.13	55.61	53.83	53.90	54.21	0.49	0.048	0.196	0.065
Day 42	61.23	63.74	67.97	68.37	66.09	66.92	65.96	68.27	71.13	69.29	70.27	70.10	0.76	0.106	0.196	0.121
Day 57	72.19	75.78	80.40	81.87	79.00	80.56	79.16	82.52	86.36	84.30	85.51	84.88	1.12	0.235	0.167	0.484
Day 77	89.03	91.08	99.12	99.82	97.08	98.48	97.37	102.11	106.37	104.84	106.62	105.63	1.72	0.396	0.475	0.684
Day 98	104.26	109.87	116.48	117.74	116.85	119.35	115.71	122.59	126.66	124.94	127.34	126.13	2.14	0.511	0.137	0.949
							Phase 1 (day 0-27)								
ADG (gram)	778.88	802.07	891.22	910.30	842.69	848.75	826.82	856.71	948.33	882.59	885.42	896.33	18.79	0.048	0.486	0.176
ADFI (gram)	1571.95	1541.91	1711.16	1697.26	1626.17	1623.95	1703.03	1666.14	1751.04	1703.41	1666.07	1717.54	35.40	0.239	0.194	0.056
GF	0.48	0.52	0.52	0.54	0.52	0.52	0.48	0.51	0.54	0.52	0.53	0.52	0.01	0.037	0.736	0.926
FCR	2.09	1.93	1.92	1.86	1.93	1.91	2.07	1.95	1.85	1.93	1.89	1.92		0.037	0.736	0.926
Cost (€/kg gain)	0.49	0.45	0.45	0.43	0.44	0.43	0.49	0.46	0.43	0.45	0.43	0.44	0.01	0.049	0.804	0.916
							Phase 2 (d	ay 27-57)								
ADG (gram)	725.97	802.15	875.69	907.49	873.13	919.57	894.72	980.24	1025.28	1015.91	1053.99	1022.66	28.24	0.597	0.291	0.932
ADFI (gram)	2135.59	2124.82	2299.26	2257.60	2195.67	2274.71	2556.27	2591.90	2724.77	2540.00	2659.38	2642.73	58.50	0.584	0.538	0.806
GF	0.35	0.38	0.38	0.40	0.40	0.41	0.36	0.38	0.38	0.40	0.40	0.39	0.01	0.548	0.092	0.875
FCR	2.89	2.65	2.62	2.49	2.52	2.47	2.76	2.64	2.65	2.49	2.52	2.58		0.548	0.092	0.875
Cost (€/kg gain)	0.64	0.58	0.57	0.54	0.54	0.53	0.61	0.58	0.58	0.54	0.54	0.55	0.01	0.588	0.109	0.742
							Phase 3 (d									
ADG (gram)	751.18	827.19	875.83	870.84	918.54	941.58	885.21	970.84	976.69	984.91	1013.83	1000.04	37.50	0.821	0.206	0.754
ADFI (gram)	2198.49	2334.50	2519.64	2365.22	2582.04	2628.31	2797.21	2944.47	3005.18	2969.57	3072.21	3079.90	70.71	0.748	0.243	0.818
GF	0.33	0.35	0.35	0.36	0.36	0.36	0.32	0.33	0.33	0.33	0.33	0.32	0.01	0.973	0.419	0.955
FCR	3.01	2.82	2.88	2.79	2.80	2.79	3.16	3.03	3.08	3.01	3.03	3.08		0.973	0.419	0.955
Cost (€/kg gain)	0.71	0.65	0.64	0.59	0.59	0.57	0.74	0.70	0.69	0.65	0.64	0.62	0.02	0.986	0.626	0.858
							Overall (
ADG (gram)	755.59	812.90	880.31	893.21	884.04	909.57	872.45	942.70	984.15	966.63	991.17	978.80	21.88		0.137	0.949
ADFI (gram)	1996.56	2036.70	2209.24	2133.35	2179.55	2222.20	2399.89	2450.92	2516.68	2461.21	2520.05	2541.19	47.96		0.257	0.597
GF	0.37	0.40	0.40	0.42	0.41	0.41	0.36	0.38	0.39	0.39	0.39	0.39	0.01	0.467	0.146	0.917
FCR	2.71	2.51	2.51	2.38	2.46	2.44	2.75	2.60	2.56	2.54	2.54	2.60		0.467	0.146	0.917
Cost (€/kg gain)	0.62	0.57	0.56	0.52	0.53	0.52	0.63	0.59	0.57	0.56	0.55	0.55	0.01	0.632	0.266	0.978

Note: GF data (mean, SEM and P-value) is obtained by statistical analyses, FCR data (means and P-value) is derived from GF.

Broken-line analyses **APPENDIX IV:**

Phase 1

Note: Broken-line for barrows and gilts fitted separately due to interaction between CP level and sex. Broken-line for ADFI in barrows could not be fitted due to lack of quadratic response.

ADG Male curvilinear fit - Crude Protein level

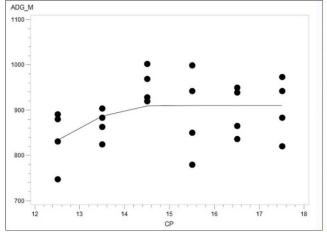
Paramet er	Estimate	Approx Std Error	Approximate 95% Confidence Limits		
L	909.900	16.937	874.700	945.100	
U	-15.407	29.094	-75.910	45.097	
R	14.731	2.039	10.490	18.972	

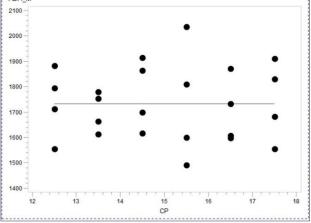
ADFI Male curvilinear fit - Crude Protein level

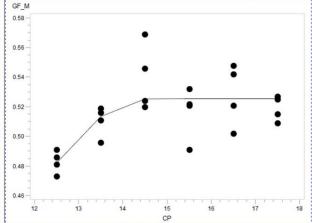
Paramet er	Estimate	Approx Std Error	Approxin Confic Lim	lence
L	1732.300	28.423	1673.500	1791.100
U	0.560			
R	11.433			

GF Male curvilinear fit - Crude Protein level

	amet er	Estimate	Approx Std Error	Confid	nate 95% dence nits
L		0.525	0.004	0.516	0.534
U		-0.010	0.009	-0.028	0.008
R		14.610	0.913	12.711	16.509







Phase 2

ADG Female curvilinear fit - Crude Protein level

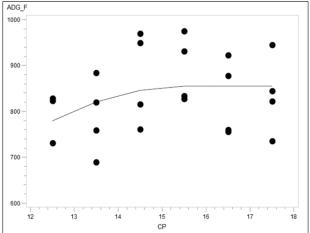
Paramet er	Estimate	Approx Std Error	Approxir Confid Lin	dence
L	855.100	24.064	804.900	905.300
U	-8.065	18.933	-47.558	31.428
R	15.562	3.348	8.578	22.547

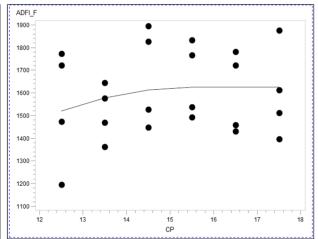
ADFI Female curvilinear fit - Crude Protein level

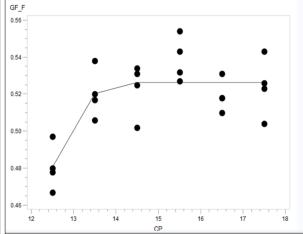
Paramet er	Estimate	Approx Std Error	Approxin Confic Lin	dence
L	1625.100	54.676	1511.400	1738.800
U	-11.433	42.224	-99.242	76.377
R	15.533	5.351	4.405	26.661

GF Female curvilinear fit - Lysine level

Paramet er	Estimate	Approx Std Error	Approximate 95% Confidence Limits				
L	0.526	0.003	0.519	0.533			
U	-0.019	0.013	-0.046	0.009			
R	14.072	0.559	12.910	15.233			







Note: Broken-line for barrows and gilts fitted combined due to lack of interaction between CP level and sex.

ADG curvilinear fit - Crude Protein level

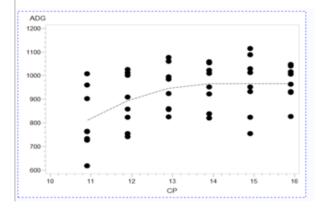
10000	no our viniteur it. Orace i rotein level												
Paramet		Approx											
er	Estimate	Std Error	Limits										
L	966.100	21.926	921.900	1010.200									
U	-16.116	15.788	-47.914	15.682									
R	14.012	1.465	11.062	16.963									

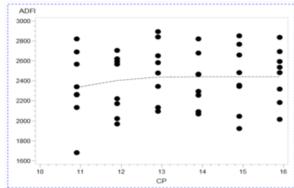
ADFI curvilinear fit - Crude Protein level

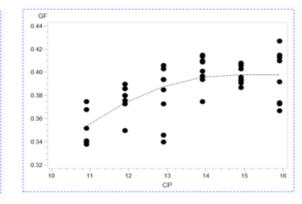
Paramet		Approx		nate 95% dence
er	Estimate	Std Error	Lin	nits
L	2441.600	58.444	2323.800	2559.300
U	-17.788	76.682	-172.200	136.700
R	13.351	5.115	3.050	23.653

GF curvilinear fit - Crude Protein level

Paramet		Approx	Approxin Confi	nate 95% dence
ег	Estimate	Std Error	Lin	nits
L	0.398	0.004	0.389	0.406
U	-0.003	0.002	-0.006	0.000
R	14.751	1.060	12.613	16.888





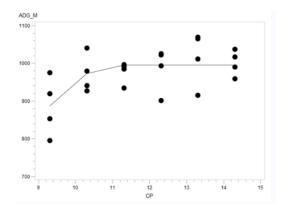


Phase 3

Note: Broken-line for barrows and gilts fitted separately due to linear response when combined. Broken-line for GF in barrows could not be fitted due to lack of quadratic response.

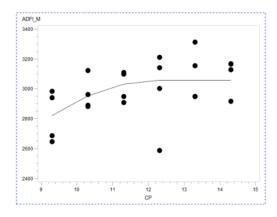
curvilinear models GF1603 Raw Data ADG_Male

Paramet		Approx	Approxin Confid	
er	Estimate	Std Error	Lin	nits
L	995.300	13.456	967.300	1023.300
U	-32.013	36.261	-107.400	43.395
R	11.140	1.008	9.044	13.237



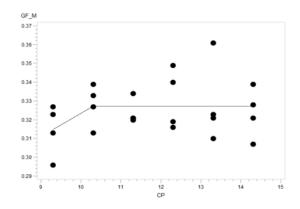
ADFI Male curvilinear fit - Crude Protein level

Paramet		Approx	Approxin Confid	
er	Estimate	Std Error	Lin	nits
L	3057.200	46.789	2959.900	3154.500
U	-26.421	37.368	-104.100	51.289
R	12.293	2.017	8.099	16.488



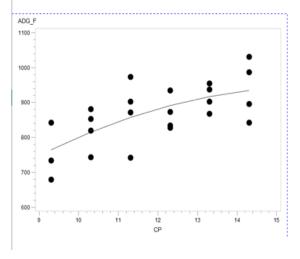
GF Male curvilinear fit - Crude Protein level

Paramet		Approx	Approxir Confi	
er	Estimate	Std Error	Lin	nits
L	0.327	0.003	0.321	0.333
U	-0.013	0.008	-0.029	0.003
R	10.279			



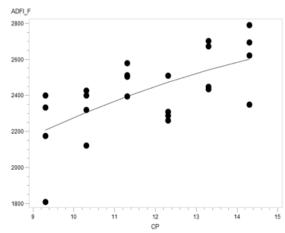
ADG Female curvilinear fit - Crude Protein level

Paramet			Approximate 95% Confidence					
er	Estimate	Std Error	Limits					
L	945.500	87.412	763.100	1127.800				
U	-4.104	5.742	-16.081	7.873				
R	15.940	5.788	3.866	28.014				



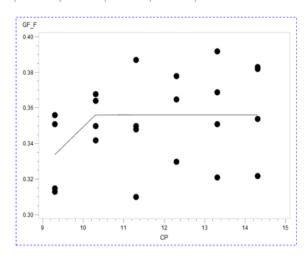
ADFI Female curvilinear fit - Crude Protein level

Paramet		Approx	Approxin Confid	
ег	Estimate	Std Error	Lin	nits
L	2754.300	896.900	889.200	4619.500
U	-4.839	13.934	-33.816	24.137
R	19.932	23.508	-28.955	68.820



GF Female curvilinear fit - Lysine level

			Approximate 95%				
Paramet		Approx	Confidence				
er	Estimate	Std Error	Limits				
L	0.356	0.006	0.343	0.369			
U	-0.019	0.161	-0.354	0.316			
R	10.384	4.623	0.741	20.026			



APPENDIX V: Carcass characteristics

Main effects

Treatment	Α	В	С	D	E	F	G	Н	- 1	J	К	L		Р	P Linear	Р
Sex			Gilt	s					Barro	ws			SEM	•	CP * Sex	Quadratic
																CP * Sex
Final BW (kg)	104.258	109.874	116.480	117.744	116.846	119.348	115.710	122.594	126.656	124.939	127.344	126.132	2.144	0.511	0.137	0.949
Slaughter weight (kg)	101.421	106.982	113.328	114.904	113.937	116.941	112.975	119.910	124.219	121.737	124.469	122.695	2.086	0.300	0.070	0.741
Carcass weight (kg)	81.737	86.633	91.670	92.566	92.524	94.086	90.280	97.169	100.773	97.119	101.302	97.949	1.789	0.207	0.086	0.565
Backfat depth (mm)	13.600	12.596	14.448	12.865	13.794	14.115	18.878	17.118	16.240	16.478	17.826	20.210	1.142	0.569	0.754	0.092
Loin depth (mm)	63.322	64.512	68.420	69.796	69.050	70.954	66.697	69.515	71.536	70.500	70.281	70.458	1.365	0.378	0.048	0.690
Lean Meat (%)	59.266	59.948	58.823	59.873	59.257	59.095	55.876	57.096	57.720	57.546	56.671	55.090	0.747	0.550	0.705	0.086
Carcass yield (%)	80.593	80.073	80.947	80.543	81.192	80.452	80.663	80.987	81.111	80.801	81.372	81.354	0.378	0.491	0.560	0.589
Expressed as a percentage of slaugh	ter weight'	k														
Backfat, % of slaughter weight	13.861	11.716	12.692	11.225	12.077	12.003	16.590	14.249	13.161	13.565	14.347	16.396	0.915	0.497	0.402	0.116
Loin depth, % of slaughter weight	61.822	60.675	60.642	61.248	60.081	60.886	59.364	58.134	57.646	58.195	56.780	57.735	1.774	1.000	0.753	0.920
Lean meat, % of slaughter weight	59.081	56.816	52.493	52.716	52.666	50.905	50.344	47.978	46.633	47.797	45.973	45.389	1.591	0.623	0.172	0.496

^{*}To correct for differences in slaughter weight

Interactions

Treatment	Α	В	С	D	E	F	G	Н	1	J	K	L		Р	P Linear	Р
Sex			Gil	ts					Barro	ws			SEM	•		Quadratic CP * Sex
Final BW (kg)	104.258	109.874	116.480	117.744	116.846	119.348	115.710	122.594	126.656	124.939	127.344	126.132	2.144	0.511	0.137	0.949
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To explore the potential of nature to improve the quality of life



Wageningen Livestock Research P.O. Box 338 6700 AH Wageningen The Netherlands T +31 (0)317 48 39 53 E info.livestockresearch@wur.nl www.wur.nl/livestock-research Wageningen Livestock Research creates science based solutions for a sustainable and profitable livestock sector. Together with our clients, we integrate scientific knowledge and practical experience to develop livestock concepts for future generations.

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