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Amino acid requirements of pigs. 2. Requirement for apparent digestible threonine of young pigs

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Abstract

Six trials involving a total of 460 young pigs were performed to study the threonine requirement during the live weight range of approximately 20 to 40 kg. To a negative control diet, containing 160 g crude protein and 5.6 g kg^{-1} threonine, four additions of L-threonine (0.0, 0.6, 1.2 and 1.8 g kg^{-1}) were tested. Results were compared with those of a positive control diet with 185 g kg^{-1} crude protein and 6.8 g kg^{-1} threonine. The negative control diet was supplemented with lysine, methionine, tryptophan, isoleucine, histidine and valine in order to be sure that no other amino acids than threonine were limiting. The positive control diet was supplemented with lysine and methionine. The experimental diets were fed ab libitum as pellets. In two separate trials, the apparent faecal and ileal digestibility of the amino acids of the two control diets was determined. The requirement for total threonine was found to be 7.1 g kg^{-1} for maximum weight gain, and 7.4 g kg^{-1} for maximum efficiency of feed utilization, in a diet containing 2290 kcal kg^{-1} net energy. These figures correspond with 6.2 and 6.5 g kg^{-1} faecal digestible and 5.7 and 6.0 g kg^{-1} ileal digestible threonine, respectively. Pig performance on the negative control diet supplemented with the first limiting amino acids, was almost similar to that on the positive control diet.

Keywords: pigs, threonine, requirement, digestibility

Introduction

In the Netherlands, nitrogen (N) pollution of the environment is becoming a major problem. A substantial part of this N is excreted by pigs and poultry, in areas with dense populations of these animals (Lenis, 1989). Nitrogen excretion can be reduced by increasing the digestibility of the feed protein and/or increasing the utilization of the absorbed nitrogen. About 75 % of the nitrogen loss is excreted via the urine.

Not only the crude protein (CP) content of the diet, but particularly the contents of the individual digestible essential amino acids determine the value of the feed protein. Normally, Dutch pig diets are composed on the basis of the requirements for faecal digestible lysine and methionine + cystine. This results in an oversupply of other amino acids. When the amino acid profile of the feed protein is close to the amino acid profile needed for maintenance and production, utilization of the nitrogen can be high. When the CP content of the diet is lowered, after lysine and methionine, in present-day feed formulations threonine and tryptophan are becoming limiting.

The contents of amino acids in diets for pigs, recommended by ARC (1981), are related to the lysine content. For threonine, the ARC (1981) recommends a dietary level of 6.5 g kg^{-1} for pigs in the weight range of 15-50 kg, which corresponds with 60 % of the lysine content. Lewis et al. (1986) estimated the threonine requirement for pigs in the weight range of 5 - 15 kg at 7.0 g kg^{-1}. The dietary lysine level was 12.8 g kg^{-1}, thus threonine requirement was 55 % of that of lysine. Rosell & Zimmerman (1985) similarly found a threonine requirement of 7.0 g kg^{-1} for pigs weighing 5-15 kg. This corresponded with 60 % of the lysine requirement. Gomez-Rojas et al. (1985) found, for pigs in the weight range of 16-33 kg, a threonine requirement of 5.5-5.7 g kg^{-1} (lysine 8.6 g kg^{-1}). Wang & Fuller (1989) advised, in their revised ideal amino acid pattern for growing pigs, a threonine level of 72 % of the lysine level.

The requirement for amino acids is strictly a requirement for ileal digestible amino acids (Herrmann et al., 1988). Differences in ileal digestibility can possibly explain the wide rang of requirement figures for total threonine. The studies reported in this paper were carried out to determine the requirement for ileal digestible threonine of young pigs in the live weight range of approximately 20 to 40 kg.

Materials and methods

The studies involved two digestibility trials, for determination of the contens of apparent faecal and ileal digestible amino acids in the basal diets used, and six growth trials. The trials were performed at two institutes: the TNO-Institute of Animal Nutrition and Physiology (ILOB), Wageningen, and at the Research Institute for Livestock Feeding and Nutrition (IVVO), Lelystad.

Diets

The composition of the two basal diets (coded as A and B) used, is presented in Table 1. Basal diet A was used as a positive control diet and analysed to contain 185 g kg^{-1} crude protein (CP), and 6.8 g kg^{-1} total threonine. Basal diet B was used as a negative control diet and analysed to contain 160 g kg^{-1} CP and 5.6 g kg^{-1} threonine. Four additions of L-threonine (0.0, 0.6, 1.2 and 1.8 g kg^{-1}) to basal diet B were tested, resulting in dietary theonine levels of 5.6, 6.2, 6.8 and 7.4 g kg^{-1}, respectively. Both diets were supplemented with the same antibiotic (40 ppm Tylosine). Both diets were calculated to contain approximately 2290 kcal net energy kg^{-1}. Basal diet B was supplemented with lysine, methionine, tryptophan, isoleucine, valine and histidine in order to be sure that no other amino acids than threo-

Table 1. Composition of the two basal diets (g kg^{-1}).

	Basal A	Basal B
Ingredients		
Tapioca	156.0	263.0
Barley	350.0	300.0
Corn	156.9	157.4
Soya flour	160.0	110.0
Corn gluten meal	40.0	40.0
Alfalfa	20.0	20.0
Skim milk powder	50.0	40.0
Cane molasses	20.0	20.0
Soya oil	19.0	14.0
Limestone	5.7	4.7
Dicalciumphosphate	14.0	16.0
Salt	3.0	3.0
Vitamin/mineral premix[1]	2.0	2.0
L-lysine HCL	3.2	5.5
DL-methionine	0.4	1.3
L-isoleucine		1.0
L-tryptophan		0.4
L-histidine		0.6
L-valine		0.5
Analysed contents		
Crude protein (CP)	185	160
Dry matter (DM)	905	899
Ash	55	54
Crude fat	33	26
Crude fibre	34	35
Nitrogen free extract (NFE)[2]	598	625
Net energy (calculated MJ kg^{-1})	9.59	9.59
Ca	7.6	8.1
P	6.0	6.0

[1] The vitamin/mineral premix supplied per 1 kg feed: 9000 IU vitamin A, 1800 IU vitamin D_3, 40 mg DL-α-tocopheryl acetate, 5 mg riboflavin, 30 mg niacin, 12 mg d-pantothenic acid, 250 mg choline-chloride, 40 μg cobalamin, 3 mg menadione, 50 mg ascorbic acid, 0.3 mg folic acid, 160 mg Cu, 80 mg Fe, 73 mg Zn, 44 mg Mn, 0.5 mg Co, 0.06 mg Se, 0.4 mg I and 40 mg Tylosin.
[2] NFE = DM - ash - CP - crude fat - crude fibre.

nine were limiting in this diet. Basal diet A was only supplemented with lysine and methionine.

Before inclusion in the diets, all feed ingredients, except for tapioca, were analysed for CP, Ca, P and amino acids. Based on these figures, the basal diets were composed. The experimental diets were mixed as one batch for all experiments. The two basal diets were analysed for the contents of CP, Ca, P and amino acids (Table 2).

Table 2. Analysed contents of amino acids in the two basal diets (in g kg^{-1}).

Amino acids	Basal A	Basal B
Isoleucine	7.8	7.2
Leucine	16.4	14.2
Lysine	10.2	10.4
Methionine	3.4	3.8
Cystine	3.0	2.7
Methionine + cystine	6.4	6.5
Phenylalanine	9.0	7.4
Tyrosine	6.8	6.0
Threonine	6.8	5.6
Tryptophan	2.2	2.2
Valine	9.3	8.2
Arginine	10.2	8.0
Histidine	4.2	4.0
Alanine	8.9	7.6
Aspartic acid	16.0	12.4
Glutamic acid	36.8	30.1
Glycine	6.6	5.4
Proline	14.4	12.2
Serine	9.4	7.8

Experimental procedures

Faecal digestibility trial at IVVO

The apparent faecal digestibility of the dry matter (DM), organic matter (OM), CP, crude fat, crude fibre (CF) and the individual amino acids of the two basal diets A and B was determined using four castrated male pigs (GY × (NL × FL)) per diet. The mean live weight of the pigs was approximately 50 kg. The pigs were individually housed in metabolism cages in an environmentally controlled barn.

The diets were fed as pellets on a restricted basis, twice a day. The amount of feed supplied was approximately 2.7 times the energy requirement for maintenance.

The faeces were collected for 10 days. From chemical composition and digestibility of Weende components, the net energy of the diets was calculated according to the Rostock method (CVB, 1988).

Ileal digestibility trial at ILOB

The apparent ileal digestibility of protein and amino acids of the two basal diets A and B was determined in four pigs (GY × (NL × FL) per diet. The pigs were provided with an ileocaecal re-entrant cannula (20 mm internal diameter, silicone rubber) according to the technique developed by Easter & Tanksley (1973). The mean weight of the animals during the experimental period was approximately 45 kg. The pigs were individually housed in metabolism cages in an environmentally controlled barn with continuous light. Air temperature varied between 18 and 21 °C. Water was administered with the meal at a ratio of 2:1. The amounts of feed given were based on live weight of the animals and energy content of the diets. The feeding level was 2.4 times the energy requirement for maintenance.

The ileal contents were collected quantitatively for 3 times 24 h on alternate days. During the collection of digesta, the cannula was opened at the center and a polyethylene tube (2 m in length and 8 cm in diameter) was connected to the proximal part of the cannula. Digesta were collected continuously through the polyethylene tube into a collection container packed in crushed ice. A perforated rubber plug, inserted into the distal part of the cannula, had to prevent the backflow of digesta, and to allow for saline (0.9 %) infusion into the colon (2400 ml per 24 h). Digesta were collected hourly, weighed and frozen (−20 °C). After the collection period, a sample of the pooled digesta was freeze -dried, ground and analysed for DM, CP and amino acids.

Growth experiments

The experimental design (Table 3) was the same for all growth experiments; two ILOB trials and four IVVO trials. The apparent faecal and ileal digestibility of the supplemented L-threonine was assumed to be 100 % (Huisman et al., 1986). The animals (GY × (NL × FL)) were group-housed in an artificially heated, ventilated and lighted pig unit. The daily temperature in the pig unit varied between 20 and 23 °C.

After an acclimatization period (17 days for the two ILOB trials, 7 days for the four IVVO trials), the animals were allotted to the five treatments, based on live weight, origin, sex and weight gain after weaning. Per ILOB trial, each experimental diet was fed to four replicate pens of 8 pigs each; two pens with barrows and two with gilts. Per IVVO trial, each diet was fed to 7 group-housed pigs (ratio barrows to gilts was 3:4 or 4:3). The experimental diets were fed as pellets ad libitum for a period of four weeks. Water was also available ad libitum. For all experiments, the mean weight of the animals at the start of the experimental periods was approximately 19 kg.

The animals were weighed individually at the end of the trials (after four weeks

Table 3. Experimental treatments of the growth trials.

Group	Diet	Content of threonine (g kg^{-1})		
		total	faecal digestible	ileal digestible
1	A	6.8	5.9	5.4
2	B	5.6	4.7	4.2
3	B + 0.6 g kg^{-1} L-threonine	6.2	5.3	4.8
4	B + 1.2 g kg^{-1} L-threonine	6.8	5.9	5.4
5	B + 1.8 g kg^{-1} L-threonine	7.4	6.5	6.0

experimental period). At this time, feed intake as well as feed conversion efficiency, calculated as kg feed kg^{-1} weight gain, were determined per replicate.

Chemical analysis

Nitrogen (N) content of the freeze-dried ileal samples was determined according to the Kjeldahl method. CP content was calculated as N 6.25. Amino acids in the basal diets, except methionine, cystine and tryptophan, were determined after acid hydrolysis (6 M HCL during 22 h, at 100 °C) according to Slump (1969). Methionine and cystine were determined as methionine sulfon and cysteic acid after oxidation with performic acid according to Moore (1963); the oxidized samples were hydrolysed in the same way as the unoxidized samples. The amino acid composition of the hydrolysates were determined with an automatic amino acid analyzer (Biotronic LC 6001). Tryptophan was determined after hydrolysis with 2.7 N $Ba(OH)_2$ (8 h at 130 °C) according to Slump & Schreuder (1969).

Statistical analysis

The variables weight gain and feed conversion efficiency were analysed according to a randomized-block design with experiment, sex (only ILOB trials) and treatment as factors. Contrasts were calculated and tested by using least significant difference tests.

To estimate requirement figures, respons curves were fitted according to a quadratic equation, and 95 % confidence intervals were calculated. This was done for the ILOB and the IVVO growth experiments separately, because of the differences in experimental design. For the IVVO experiments, the growth respons curve did not significantly differ from a linear curve, so a confidence interval could not be calculated.

Results and discussion

Goedhart (1990) reported a small, linear increase in apparent faecal dry matter digestibility for pigs in the weight range of 30 to 100 kg of 1 % per 20 kg live weight gain. It is not clear whether the apparent faecal or ileal protein digestibility show the same increase. For the digestibility trials, the weight of the pigs was somewhat higher than for the growth trials (45-50 kg versus 20-40 kg). The digestibility coefficients determined in this experiment, however, were not only used to calculate the contents of faecal and ileal digestible amino acids for pigs in the weight range of 20 to 40 kg, but also for pigs in the weight range of 45 - 105 kg (Lenis & van Diepen, 1990).

The apparent faecal digestibilites for amino acids, dry matter, organic matter, crude protein, crude fat and crude fibre are shown in Table 4. The apparent differences in faecal digestibilities of individual amino acids were small. In both basal diets the faecal (Table 4) and ileal (Table 5) digestibility of cystine and threonine was found to be somewhat lower than that of other amino acids.

Table 4. Faecal digestibility coefficients (%) of individual amino acids, dry matter, organic matter, crude protein, crude fat and crude fibre (±SD).

	Basal A	Basal B
Isoleucine	89.1±0.9	88.1±1.3
Leucine	91.1±0.6	90.6±1.1
Lysine	88.9±0.6	89.5±1.2
Methionine	89.4±1.2	90.2±1.1
Cystine	86.7±1.0	84.6±1.0
Phenylalanine	90.5±0.7	88.5±1.0
Threonine	86.3±1.1	83.3±1.8
Tryptophan		88.1±1.0
Valine	88.3±0.8	86.3±1.4
Arginine	92.1±0.5	90.4±1.0
Histidine	90.3±0.7	90.0±1.0
Dry matter	88.0±0.4	87.9±0.1
Organic matter	90.4±0.5	90.0±0.1
Crude protein	88.7±0.8	85.9±1.5
Crude fat	72.7±1.2	81.5±1.0
Crude fibre	44.1±1.8	42.1±3.9

Table 5. Ileal digestibility (%) of amino acids, dry matter and crude protein (±SD).

	Basal A	Basal B
Isoleucine	86.8±0.8	86.4±0.8
Leucine	91.2±2.6	88.6±0.7
Lysine	87.0±1.9	87.7±0.6
Methionine	90.8±0.5	91.5±0.6
Cystine	76.0±1.9	73.8±1.8
Phenylalanine	89.1±2.7	83.0±1.1
Tyrosine	81.2±1.9	79.6±1.2
Threonine	80.0±1.7	75.7±1.4
Tryptophan	78.9±0.7	80.8±1.6
Valine	84.6±0.8	82.7±1.0
Arginine	89.9±0.8	86.9±0.6
Histidine	84.3±1.0	84.6±0.9
Alanine	84.1±0.7	82.3±0.7
Aspartic acid	83.8±1.0	79.7±1.0
Glutamic acid	90.4±0.1	88.4±0.4
Glycine	77.0±1.3	70.7±1.9
Proline	87.8±1.2	86.7±1.1
Serine	83.2±1.4	81.1±0.9
Dry matter	77.9±1.5	76.6±0.8
Crude protein	83.3±1.0	80.4±0.9

Compared to the faecal digestibilities, for all indispensable amino acids, except for methionine, lower ileal values were found. The amino acids threonine, cystine and tryptophan disappeared to the largest extent in the large intestine. These findings are in agreement with those reported by Sauer & Ozimek (1986) and van Leeuwen et al. (1987). The net synthesis of methionine in the large intestine observed in our study agrees with the finding of van Weerden et al. (1980), which may partly be attributed to the conversion of cystine to methionine in the large intestine.

The results of the growth trials performed at ILOB are summarized in Table 6. In both trials no significant ($P > 0.05$) treatment × sex interaction was found, so the results for both sexes are combined. Weight gain of the pigs was quite good, being on average 675 g d^{-1} during the experimental periods of 4 weeks. The results show that weight gain was improved significantly upto a dietary threonine level of 6.8 g kg^{-1}. Above this level, weight gain was not clearly affected. Maximum efficiency of feed utilization was achieved at the highest dietary level of threonine of 7.4 g kg^{-1}. However, the improvement in feed conversion efficiency above a

Table 6. Mean combined results of the two ILOB growth experiments for weight gain (g kg^{-1}), feed conversion efficiency (kg feed kg^{-1} gain) and daily feed intake (kg d^{-1} $animal^{-1}$).

Group	Diet	Threonine content (g kg^{-1})		Weight gain[1]	Feed/ gain[1]	Feed intake
		total	ileal digestible			
1	A	6.8	5.4	707[ab]	1.91[a]	1.36
2	B	5.6	4.2	550[c]	2.27[c]	1.25
3	B	6.2	4.8	671[b]	2.05[b]	1.37
4	B	6.8	5.4	725[a]	1.91[a]	1.38
5	B	7.4	6.0	729[a]	1.89[a]	1.37

[1] Values within this column not having one letter in common differ significantly ($P < 0.05$).

Table 7. Mean combined results of the four IVVO growth experiments for weight gain (g kg^{-1}), feed conversion efficiency (kg feed kg^{-1} gain) and daily feed intake (kg d^{-1} $animal^{-1}$).

Group	Diet	Threonine content (g kg^{-1})		Weight gain[1]	Feed/ gain[1]	Feed intake
		total	ileal digestible			
1	A	6.8	5.4	696[a]	1.91[a]	1.33
2	B	5.6	4.2	604[b]	2.20[b]	1.33
3	B	6.2	4.8	650[ab]	2.01[b]	1.31
4	B	6.8	5.4	693[a]	1.95[a]	1.35
5	B	7.4	6.0	689[a]	1.91[a]	1.31

[1] Values within this column not having one letter in common differ significantly ($P < 0.05$).

dietary level of 6.8 g kg^{-1} was not significant. Daily feed intake was not affected above a dietary threonine level of 6.2 g kg^{-1}.

The results of the IVVO trials (Table 7) agree well with those of the ILOB trials. The mean daily weight gain, being 665 g per animal was almost similar to that obtained in the ILOB trials. Maximum weight gain was again obtained at a dietary threonine level of 6.8 g kg^{-1}.

In the IVVO trials also maximum efficiency of feed utilization was found at the highest (7.4 g kg^{-1}) dietary level of threonine. However, similar to the ILOB trials, the improvement in feed conversion efficiency above a dietary threonine level of 6.8 g kg^{-1} was not significant. Daily feed intake was not affected clearly in the IVVO trials by increasing the dietary threonine level from 5.6 to 7.4 g kg^{-1}.

In Figure 1 the respons curve of weight gain to increasing ileal digestible threonine level is given, and Figure 2 shows the respons curve of feed conversion efficiency to an increase in ileal digestible threonine level. From the regression curves it was estimated that for obtaining maximum weight gain, the dietary content of ileal digestible threonine should be approximately 5.7 g kg^{-1} (ILOB: 5.7 g kg^{-1}, 95 % confidence interval 5.45-6.55 g kg^{-1}, IVVO: 5.8 g kg^{-1}). This figure corresponds with 7.1 g kg^{-1} total dietary threonine and 6.2 g kg^{-1} faecal digestible threonine.

Maximum efficiency of feed utilization was calculated to be found at an ileal digestible threonine content of 6.0 g kg^{-1} (ILOB: 6.1 g kg^{-1}, 95 % confidence interval 5.74-7.24, IVVO: 5.9 g kg^{-1}, 95 % confidence interval 5.57-7.17). This corresponds with 7.4 g kg^{-1} total dietary threonine and 6.5 g kg^{-1} faecal digestible threonine.

These results indicate, that for obtaining maximum efficiency of feed utilization, threonine requirement is somewhat higher than for obtaining maximum weight gain. This is in agreement with the results of Grosbach et al. (1985) and of Rosell & Zimmerman (1985).

In literature the requirement for threonine and other essential amino acids is often expressed as a percentage of the dietary level of lysine. The requirement values of 7.1 g kg^{-1} threonine for maximum weight gain, and 7.4 g kg^{-1} threonine for maximum efficiency of feed utilization, found in our studies, correspond with 68 % and 71 % of the dietary level of lysine, respectively. These values are close to the value recommended by Wang & Fuller (1989) of 72 %. However, ileal digestibility is a better estimate than faecal digestibility for amino acid valuation. Based on the ileal digestibility values found in our studies, the requirement figures for ileal digestible threonine of 5.7 g kg^{-1} and 6.0 g kg^{-1}, correspond with approximately 63 % and 66 % of the dietary level of ileal digestible lysine, being 9.1 g kg^{-1}.

At equal threonine levels almost the same performance was achieved on both basal diets (Table 6 and 7). This means that the dietary level of protein in a pig diet can be reduced from 185 to 160 g kg^{-1}, when the first limiting amino acids are added to the low protein diet. As a result this will contribute substantially to the reduction of the N excretion. The practical potentials, however, depend on the price relations between protein bound amino acids in feed components and synthetic amino acids.

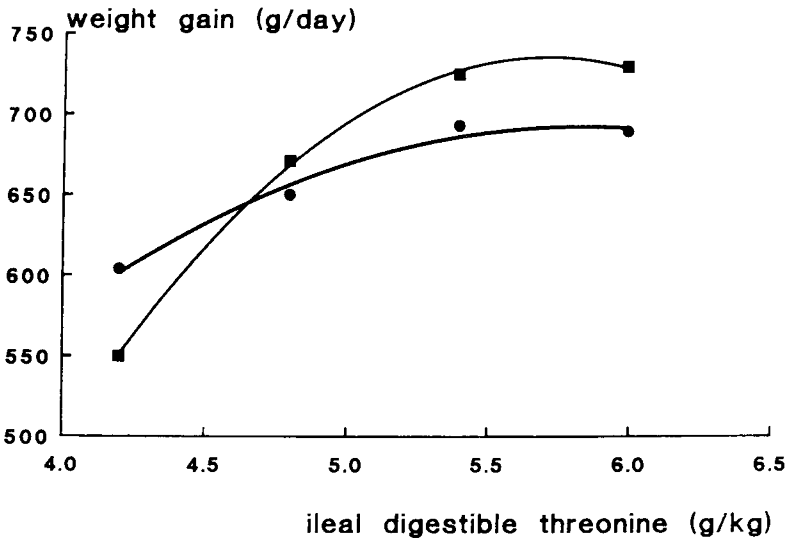

Fig. 1. Weight gain (g d^{-1}) as related to ileal digestible threonine content in the diet (g kg^{-1}). Regression curves based on the results of the ILOB (■; $Y = -1870.04 + 912.87X - 79.99X^2$) and the IVVO (●; $Y = -572.56 + 439.23X - 38.13X^2$) growth experiments.

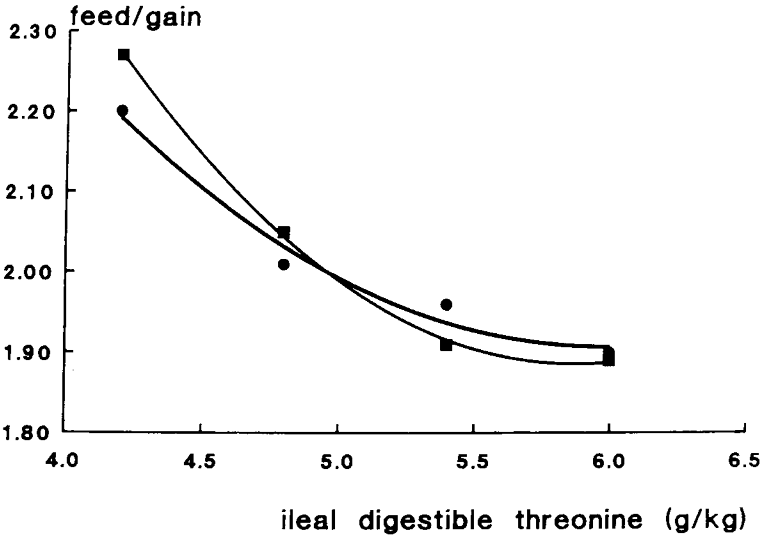

Fig. 2. Feed conversion efficiency (feed/gain) as related to ileal digestible threonine content in the diet (g kg^{-1}). Regression curves based on the results of the ILOB (■; $Y = 6.12 - 1.398X + 0.115X^2$) and the IVVO (●; $Y = 5.45 - 1.16X + 0.99X^2$) growth experiments.

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