# LEVELS OF TRUE DIGESTIBLE LYSINE AND METABOLIZABLE ENERGY FOR GROWING PIGS: EFFECT ON NITROGEN AND ENERGY BALANCE<sup>1</sup>

Níveis de lisina digestível verdadeira e energia metabolizável para suínos em crescimento: efeito sobre o balanço de nitrogênio e energético

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#### **ABSTRACT**

An experiment was conducted at the Department of Animal Husbandry of Universidade Federal de Lavras aiming to study the nitrogen and energy balance in diets with reduced crude protein rates (CP) and different levels of energy and lysine for growing pigs. 52 castrated pigs (initial weight =  $36.13 \pm 2.8$  kg) of high genetic potential were used, housed individually in metabolism cages (experimental unit). The treatments consisted of 12 diets with three levels of metabolizable energy (ME) (3060, 3230 and 3400 kcal / kg) and four of true digestible lysine (TDL) (0.7, 0.9, 1.1 and 1, 3%) with 14% CP plus a control diet with 3230 kcal ME / kg, 1.03% TDL and 18% CP. We used a DBC in time 3x4 + 1 factorial design with four replications to verify the absorbed nitrogen (AN), N retained (NR), the NR / NA (%), absorbed energy (AE), retained energy (RE) and the ratio RE /AE (%). In relation retained nitrogen / absorbed nitrogen was declining linear regression (P <0.05) when 3400 kcal / kg was used, where the lowest levels (0.7 and 0.9%) of TDL showed the best results. In the study of energy balance, for the variables retained energy and the relationship between energy there was no difference (P> 0.05) between the control treatment and other experimental diets. It is concluded that the levels 3230 kcal / kg and 1.03% of TDL can be used without affecting the utilization of nitrogen by growing pigs in diets with reduced crude protein content.

Index terms: Synthetic amino acids, nutrition, metabolism, ideal protein.

#### **RESUMO**

Foi conduzido um experimento no Departamento de Zootecnia da Universidade Federal de Lavras com o objetivo de estudar o balanço de nitrogênio e energético em rações contendo reduzido teor de proteína bruta (PB) e diferentes níveis de energia e lisina para suínos em crescimento. Foram utilizados 52 suínos machos castrados (peso inicial = 36,13 ± 2,8 kg) de alto potencial genético, alojados individualmente em gaiolas de metabolismo (unidade experimental). Os tratamentos consistiram em 12 dietas formuladas com três níveis de energia metabolizável (EM) (3060, 3230 e 3400 kcal/kg) e quatro de lisina digestível verdadeira (LISD) (0,7; 0,9; 1,1 e 1,3%) com 14% PB mais um tratamento controle, com 3230 kcal EM/kg, 1,03% LISD e 18% PB. Utilizouse um DBC no tempo em fatorial 3x4+1, com quatro repetições, para verificar o N absorvido (NA), N retido (NR), a relação NR/NA (%), energia absorvida (EA), energia retida (ER) e a relação ER/EA (%). Na relação nitrogênio retido /nitrogênio absorvido houve regressão linear decrescente (P<0,05) quando 3400 kcal/kg foi utilizado, onde os menores níveis (0,7 e 0,9%) de LISD apresentaram os melhores resultados. No estudo do balanço energético, para a variáveis energia retida e a relação entre energia não houve diferença (P>0,05) entre o tratamento controle e as demais dietas experimentais. Conclui-se que os níveis 3230 kcal EM/kg e 1,03% de LISD podem ser utilizados sem afetar o aproveitamento do nitrogênio pelos suínos em crescimento, em rações com reduzido teor de proteína bruta.

Temos para indexação: Aminoácidos sintéticos, nutrição, metabolismo, proteína ideal.

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#### INTRODUCTION

Swine production has undergone intense development in Brazil after the introduction of modern commercial hybrids with high genetic potential. However, breeding systems still face difficulty in meeting efficiently the high nutritional requirements of these animals. In this context, in order to meet these requirements and at the same time improving the efficient use of nutrients in the diets, new concepts for the formulation of rations have been investigated.

Le Bellego et al. (2001) and Le Bellego & Noblet (2002), studying diets with low crude protein and supplemented with synthetic amino acids, say the amount

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of nitrogen retention is similar to that obtained with conventional diets, provided that there is adequate addition of synthetic amino acids to avoid deficiencies of essential amino acids. However, changes in energy balance of animals can be promoted with this practice, resulting in lower feeding efficiency and poor performance and carcass conformation. In addition, the use of only a few synthetic amino acids in diets with reduced crude protein and the lysine: energy ratio, have been highlighted as points of questions for further research (Rao et al. 2006; Zangeronimo et al. 2006). The possibility of manipulation of protein levels in the diet by reducing the crude protein and amino acid supplementation and correction of industrial energy levels can be a viable alternative to improve the efficiency of nutrient use by animals.

Thus, the aim of this work was to study the nitrogen balance and energy in diets with reduced crude protein content and levels of energy and lysine, while the level of the amino acid methionine and threonine in accordance with the concept of ideal protein.

#### MATERIALAND METHODS

The metabolism assay for the balance of nitrogen and energy was conducted in the Poultry Division at the Animal Husbandry Department on Universidade Federal de Lavras, Minas Gerais.

52 castrated swine (36.13  $\pm$  2.8 kg) of high genetic potential were used, divided into four groups of 13 animals. The pigs were housed in metabolism cages (experimental unit) that allowed the collection of feces and urine separately. The cages remained in a room equipped with air conditioning, allowing the internal temperature control at 21.0  $\pm$  1.6 ° C.

A randomized block design in a factorial  $3 \times 4 + 1$  (three levels of energy and four true digestible lysine) plus a control diet (formulated in accordance with national requirements tables) with four replications, and blocks consisting of the experimental period.

The treatments consisted of 12 diets based on corn and soybean meal, formulated with three levels of metabolizable energy (ME) (3060, 3230 and 3400 kcal / kg) and four true digestible lysine (TDL) (0.7, 0.9, 1.1 and 1.3%) with 14% CP and one control treatment, with 3230 kcal ME / kg, 1.03% TDL and 18% CP, formulated according to the recommendations suggested by Rostagno et al. (2005). The values of true digestible lysine in each treatment were set based on the true ileal digestibility of each food, according to these same authors. In calculating the nutritional value of diets, were not taken into account the energy and protein levels of the amino acids added, since the purpose of this work is to study changes in the amounts

of lysine, methionine and threonine may interfere with the metabolic balance of nitrogen and energy of animals on diets containing similar levels of protein and energy.

The experimental diets can be seen in Table 1.

The experiment lasted ten days, seven for adaptation and three for total collection of feces and urine. The amount of feed was calculated based on metabolic weight of each animal (LW<sup>0.75</sup>) and given twice daily, mixed with water in the ratio 2:1 (water: feed). Five grams of ferric oxide was used to define the beginning and end of fecal collection, according to the methodology described by Fialho et al. (1979). Urine was collected in buckets containing hydrochloric acid (1:1 with distilled water) and equipped with screens (1 mm) for separation of solids. Every day, all feces and a homogenous (10% of total volume) of urine from each cage were collected twice a day and after the removal of foreign material (hair), were kept in a freezer (-10 C) for later analysis . At the end of the experiment, feces were weighed and homogenized. It was analyzed the absorbed nitrogen (AN), nitrogen retention (NR), the NR / AN, the absorbed energy (AE), the retained energy (RE) and grating ER / AE.

Statistical analysis was performed using the statistical package SISVAR (Ferreira, 2000), and the data submitted to ANOVA, the SNK test at 5% to compare levels of energy and regression analysis when lysine levels were compared. Additional treatment was compared to other treatments by Dunnett test at 5%.

#### RESULTS AND DISCUSSION

The results obtained for the nitrogen balance of pigs during the growth phase are shown in Table 2. There was interaction (P < 0.05) levels of TDL and MS for the nitrogen balance. With respect to absorbed nitrogen (AN), the levels of 1.1% and 1.3% TDL was no difference (P < 0.05) between the metabolizable energy. The level of 1.1% TDL, the value of 3230 kcal / kg was similar to the value of 3400 kcal / kg and both higher than 3060 kcal / kg as the percentage of NA. At 1.3% of TDL, the higher nitrogen uptake was obtained when use was made of 3230 kcal / kg in diets and for the values of 3060 and 3400 in ME there was a percentage of AN similar, in this level of used amino acids. Le Bellego et al. (2001) argue that the reduction of CP should be limited to the point where the supply of nitrogen is not essential to allow effective use of energy. Moreover, there is evidence that the absorption of synthetic amino acids is faster when compared to the absorption of amino acids in protein foods (Partridge et al., 1985). However, from the results obtained in this work, it is possible to infer that the energy levels present in the diet may influence the absorption of nitrogen, depending on the levels of lysine.

Table 1 – Composition of experimental diets.

Ingredients								3					
		3060				3230				3400			*
					Dig	Digestible Lysine Levels (%)	ine Levels	(%)					נ
	7,0	6,0	1,1	1,3	0,7	6,0	1,1	1,3	7,0	6,0	1,1	1,3	
Com	000.99	66.000	000.99	000.99	000.99	000.99	000.99	000.99	000,99	000.99	000.99	65.388	64.000
Soy Bran	19.00	19.000	19.000	19.000	19.000	19.000	19.000	19.000	19.000	19.000	19.000	19.000	28.000
Soybean oil	2.500	2.500	2.500	2.500	3.500	3.500	3.500	3.500	4.500	4.500	4.500	4.500	2.500
Starch	1.600	1.600	1.600	1.600	4.000	4.000	4.000	4.000	6.300	6.300	6.300	6.300	0.000
Bicalcic Phosphate	1,700	1.700	1,700	1.700	1.700	1.700	1,700	1.700	1.700	1.700	1,700	1,700	1.710
Limestone	0.380	0.380	0.380	0.380	0.380	0.380	0.380	0.380	0.380	0.380	0.380	0.380	0.385
Iodine salt	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
Sodium Bicarbonate	0.260	0.370	0.500	0.640	0.260	0.370	0.500	0.640	0.260	0.370	0.500	0.635	0.000
Px. Vita. 1	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Px. Min. <sup>2</sup>	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
L-lysine HCl 78	0.166	0.420	0.705	0.991	0.166	0.420	0.705	0.991	0.166	0.420	0.700	0.985	0.300
DL-Methionine 98	0.000	090.0	0.120	0.180	0.000	0.060	0.120	0.180	0.000	090.0	0.115	0.176	0.040
L-Threonine 99	0.000	0.120	0.250	0.380	0.000	0.120	0.250	0.380	0.000	0.120	0.245	0.376	090.0
Antibiotic <sup>3</sup>	0.050	0.050	0.050	0.050	0.050	0.050	0.50	0.050	0.050	0.050	0.050	0.050	0.050
BHT 8	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Caolim <sup>9</sup>	7.834	7.290	6.685	690.9	4.434	3.890	3.285	2.669	1.134	0.590	0.000	0.000	2.445
Total	100	100	100	100	100	100	100	100	100	100	100	100	100
					Calc	Calculated Valu	ıes						
Crude Protein (%)	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	18.0
Met.En.(kcal)	3060	3060	3060	3060	3230	3230	3230	3230	3400	3400	3400	3400	3230
Calcium (%)	0.630	0.630	0.630	0.630	0.630	0.630	0.630	0.630	0.630	0.630	0.630	0.630	0.650
Av. Phos. (%)	0.332	0.332	0.332	0.332	0.332	0.332	0.332	0.332	0.332	0.332	0.332	0.332	0.332
Dig Lysi. (%)	0.7	6.0	1.1	1.3	0.7	6,0	1.1	1.3	0.7	6.0	1.1	1.3	1.03
Methionine dig (%)	0.211	0.271	0.331	0.391	0.211	0.271	0.331	0.391	0.211	0.271	0.331	0.391	0.301
Dig.Threonine (%)	0.455	0.585	0.716	0.846	0.455	0.585	0.716	0.846	0.455	0.585	0.716	0.846	0.660
Sodium (%)	0.206	0.236	0.271	0.309	0.206	0.236	0.271	0.309	0.206	0.236	0.271	0.309	0.138

\* Control Treatment

(B2), 4000 mg, pyridoxine (B6), 2000 mg, vitamin B12, 20,000 mcg, niacin, 25,000 mg, penthothenic acid, 10 g, folic acid, 600 mg; biotin, I Premix vitamin: vitamin A, 8,000,000 IU, vitamin D3, 1,200,000 IU, vitamin E, 20 g, vit. K3, 2500 mg, vitamin B1, 1000 mg, riboflavin

50 mg, vitamin C, 50 g; antioxidant, 125 mg and excipients qs 1000g.

2 Premix mineral selenium, 500 mg, iron, 180 g, copper, 20 g, manganese, 80 g, zinc, 140 g, iodine, 4 g; cobalt, 4 g excipients qs 1000g. 3 = antibiotic Tylan ® Sulfa containing, per kg of product: tylosin (as phosphate) 100 g, 100 g sulfamethazine, and excipients qsp 1000g

Table 2 – Nitrogen balance in growing pigs receiving diets with low crude protein level and with different levels of lysine and metabolizable energy.

	Absorbed N	Nitrogen (%)		
Metabolizable Energy (kcal/kg)*		-		Aama a.a
3060 <sup>1</sup>	3230	3400		— Average
0.7 %	82.9	86.0	84.8	84.5
0.9 %	89.3	86.2	87.0	87.5
1.1 %	83.5 b	88.3 a	88.0 a	86.6
1.3 %	84.0 b	88.5 a	85.1 b	85.9
Average	84.9	86.2	87.2	
Witness	87.1			
CV (%)	2.63			
	Nitrogen r	etained (%)		
Digestible Lysine (%)	Metal	polizable Energy (kca	l/kg) *	Average
•	3060	3230	$3400^2$	
0.7 %	73.9	66.4	75.2	73.4
0.9 %	72.0	71.2	72.6	70.3
1.1 %	70.3	76.3	73.4	71.3
1.3 %	65.6 b	70.1 a	60.0 b	67.3
Average	70.5	71.0	70.3	
Witness	65.7			
CV (%)	8.30			
. ,	Nitrogen retained/ A	bsorbed Nitrogen (%)	)	
Digestible Lysine (%)	Metabolizable Energy (kcal/kg) *		Average	
	3060	3230	$3400^2$	
0.7 %	89.0	77.2	88.6	86.8
0.9 %	80.7	82.6	83.5	80.4
1.1 %	84.2	86.4	83.6	82.5
1.3 %	78.0 a	79.2 a	70.5 b	78.4
Average	83.0	81.4	81.5	
Witness	75.4			
CV (%)	8.85			

<sup>\*</sup>Average followed by different letters in the line differ by SNK test (P < 0.05)

According to Frenhani & Burini (1999), the absorption of peptides is not as influenced by levels of protein or energy rations as compared to free amino acids, i.e., the type of diet can affect the fastening absorption of amino acids, but not to di-peptides in the same proportion.

Furthermore, the lipid content of the diets may have changed the flow speed of the substrate through the digestive tract, affecting the digestion and absorption. The presence of lipids in the duodenum stimulates the production of cholecystokinin, which, in turn, stimulates the production of bile and pancreatic juice. Increased secretion of pancreatic juice, the amount of digestive enzymes in the small intestine will be greater. Thus, coupled with the fact that the lipids reduce the rate of gastric

emptying, may allow greater absorption of nutrients, especially proteins (Almeida et al., 2007).

Figueroa et al. (2002) suggest that nitrogen balance is more sensitive both to the inadequacy of amino acids on the energy level of the diets, compared to performance data of pigs. The results obtained in this study (Table 2) showed a quadratic effect (P <0.05) for AN as a function of increased levels of lysine in the diet with 3060 kcal / kg of ME, and estimated level of lysine which provided greater N uptake by the animals was 0.98%, with an absorption maximum of 86.78%. This level is close to that recommended by Rostagno et al. (2005), which is 1.03% of TDL, however for a higher level of energy (3230 kcal / kg) and PB (18%).

<sup>&</sup>lt;sup>1</sup>Quadratic regression (P < 0.05)

<sup>&</sup>lt;sup>2</sup>Linear regression (P < 0.05)

The percentage of nitrogen absorbed was greater (P < 0.05) when using 3230 kcal / kg and 1.3% of lysine, as compared to other levels of this amino acid. However, no difference (P> 0.05) between energy levels in diets when levels of 0.7% and 0.9% of lysine were tested, except when 1.1% was used in which 3060 kcal / kg diet had the lowest percentage of nitrogen absorbed. According to these results, the increase in energy level decreases the efficiency of absorption of amino acids, especially when they are unbalanced in the diet. This reinforces the proposal by Zangeronimo et al. (2007) that the energy levels of the diets could interfere in the utilization of dietary nitrogen, especially in diets with low levels of CP, where the smallest increase caloric provided by these diets could lead to a greater amount of net energy available to animals and interference in the processes of deamination and transamination of amino acids. This, somehow, can also be true in the epithelial tissue of the mucosa of the gastrointestinal tract, i.e., excess dietary energy may have reduced the use of amino acids for this purpose.

There was no difference (P> 0.05) by Dunnett's test between the control and experimental diets for nitrogen absorption. Zangeronimo et al. (2007) also found no differences in the percentages of AN by reducing the dietary crude protein percentage in four units for growing animals.

The nitrogen retention (NR) to the level of 1.3% of TDL were no differences (P < 0.05) in the levels of metabolizable energy. When the level of lysine was used, the value of 3230 kcal / kg of DM was higher than in the other, thus suggesting a better relationship between this lysine and energy level. In this case, the best ratio was 2.48 (Mcal metabolizable energy: percentage of true digestible lysine). The surplus of lysine may compromise the use of other amino acids by competing for sites of absorption or protein synthesis (Susenbeth, 1995), thus promoting greater excretion of nitrogen. For other levels of TDL was no difference (P> 0.05) between the metabolizable energy studied. Within the level of metabolizable energy of 3400 kcal, there was a negative linear regression (P < 0.05) with increasing levels of TDL studied. The results are consistent with those reported by Verstegen & Jongbloed (2002) stated that there is less nitrogen retention decreased when the crude protein and crystalline amino acids added to the diet.

The higher nitrogen retention in animals fed lower levels of TDL can be explained by the better balance in the sites of protein synthesis, demonstrating the importance of manipulating the levels of amino acids and energy in the rations. Moreover, the variation in levels of lysine ratio

of 0.7 to 1.3%, the level in 3400 kcal / kg, provided a difference in the retention of N 13.44%, indicating the importance of manipulating the levels this amino acid in the diet.

Moreira et al. (2004) observed a quadratic effect for nitrogen retention by working with diets with 3400 kcal / kg digestible energy and 14% crude protein supplemented with lysine (0.8, 1.0, 1.2 and 1.4%) for barrows from 20 kg. Similar data for reduced levels of CP are reported by Oliveira (2004).

Regarding the percentage of N retained in relation to what has been absorbed (NR / AN), there was interaction (P <0.05) levels of lysine with the levels of DM in the diet. For the level of 1.3% of TDL were no differences (P <0.05) in% / AN levels in the ME. The 3230 level of DM was similar to 3060 kcal / kg of ME and both higher than 3400 kcal / kg.

For 3400 the level of DM, there was decreased linearly (P <0.05) with the increase in TDL (Figure 1). This result differs from those found by Moreira et al. (2004) who observed a quadratic effect for the variable / AN. However, this relationship may have been affected by the addition of other non-essential amino acids in the diet, for example, tryptophan (Oliveira, 2004; Zangeronimo et al., 2007).

It is possible that other factors have limited the retention of nitrogen, for example, the relationship between essential nitrogen and total nitrogen (EN: NT) was analyzed.

The results obtained for the energy balance of pigs in the growth phase are shown in Table 3. There was no interaction ME x TDL (P> 0.05) in absorbed energy (AE), retained energy (RE) and the ratio ER / AE (%).

The level of 3060 kcal / kg had lower mean (P < 0.05) energy absorption in relation to other levels. However, treatments with higher levels of energy showed similar absorption. As for the energy absorbed, the difference between the treatments may be due to the use of starch and oil in the diet formulation because they are easy to digest food, and the extra-caloric effects promoted by oil.

Moreover, evaluating the addition of increasing levels of lysine, it is assumed that no difference observed in the absorption of energy was provided, because the amount of energy provided by amino acids is less than the energy sources of carbohydrates and lipids are included in larger amounts in feed. The synthetic amino acids lysine and threonine supply in metabolizable energy for pigs, 4410 kcal / kg and 3790 kcal / kg, respectively (Ajinomoto Animal Nutrition, 2008). In this work, the energy contribution of amino acids in the diet with the highest addition of synthetic amino acids was 67.7 kcal ME / kg.

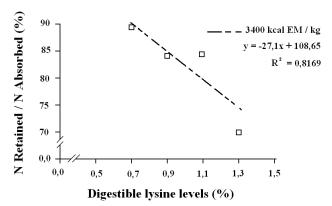


Figure 1 – Efficiency of utilization of nitrogen absorbed (%) of the 36 kg pigs fed diets with low crude protein content (14%) and different levels of lysine and metabolizable energy.

Table 3 – Balance of energy for growing pigs receiving diets with low crude protein and with different levels of lysine and metabolizable energy.

	Absorbe	ed Energy (%)		
Digestible Lysine(%)	Metabolizable Energy (kcal/kg)			Average
	3060	3230	3400	
0.7	87.9	89.8	89.4	89.0
0.9	90.3	90.6	90.4	90.4
1.1	87.4	90.2	91.0	89.5
1.3	86.6	90.1	89.2	88.6
Average	88.1 b	90.2 a	90.0 a	
Witness	87.4			
CV (%)	2.30			
	Energy	Retained (%)		
Digestible Lysine(%)	Meta	abolizable Energy (kca	ıl/kg)	Average
	3060	3230	3400	
0.7	87.1	88.9	86.8	87.6
0.9	88.6	88.3	87.9	88.3
1.1	85.0	87.6	89.9	87.5
1.3	84.3	86.5	85.6	85.5
Average	86.3	87.8	87.6	
Witness	84.7			
CV (%)	3.19			
. ,	Energy Retained	/ Absorbed Energy (%	5)	
Digestible Lysine(%)		abolizable Energy (kca		Δ
• • • •	3060	3230	3400	— Average
0.7	99.2	99.0	97.1	98.4
0.9	98.1	97.5	97.2	97.6
1.1	97.3	97.1	98.8	97.7
1.3	97.4	95.9	96.0	96.4
Average	98.0	97.4	97.3	
Witness	96.9			
CV (%)	2.38			

<sup>\*</sup> Averages followed by different letters in the line differ by SNK test (P < 0.05)

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<sup>&</sup>lt;sup>1</sup>Significant linear regression (P < 0.05)

In the case of retained energy, Kerr et al. (1995), evaluating the reduction of crude protein and amino acid supplementation in diets for growing pigs, found an increase in energy retention in animals fed diets with low crude protein, supplemented with amino acids. By reducing the levels of protein intake, may notice a decrease in deamination of amino acids, the synthesis and urinary excretion of urea in protein turnover and heat production. Thus, it seems consistent that many researchers claim that with the decrease in protein content of feed, there will be more energy available to be used (Le Bellego et al., 2001). However, there were no differences (P> 0.05) differences for the following experimental diets and control diet for the variable energy retained.

There was no difference (P> 0.05) by Dunnett's test between the experimental diets and control diet for the variables studied. This result confirms those obtained by Zangeronimo et al. (2006) by reducing the crude protein percentage in four units by adding the main essential amino acids (lysine, methionine and threonine) in the diets.

#### **CONCLUSION**

Nitrogen balance and energy can be affected in diets with different levels of metabolizable energy and true digestible lysine, containing reduced crude protein content. The level of 1.03% true digestible lysine should be kept on diets with 3230 kcal / kg metabolizable energy and reduced crude protein for growing pigs.

The level of protein in the ration can be reduced without affecting the nitrogen balance and energy of animals, provided that, if properly supplemented with synthetic amino acids.

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