UNIVERSIDADE FEDERAL DE VIÇOSA

JOYCE BARCELLOS

LYSINE REQUIREMENTS FOR *Escherichia coli* LIPOPOLYSACCHARIDE-CHALLENGED GROWING PIGS

VIÇOSA - MINAS GERAIS
2020

JOYCE BARCELLOS

LYSINE REQUIREMENTS FOR *Escherichia coli* LIPOPOLYSACCHARIDE-CHALLENGED GROWING PIGS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Zootecnia, para obtenção do título de *Magister Scientiae*.

Orientador: Melissa Izabel Hannas

Coorientadores: Paulo Henrique Reis F. Campos
Horacio Santiago Rostagno

VIÇOSA - MINAS GERAIS
2020

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
B242L
2020

Barcellos, Joyce, 1995-
Lysine requeriments for *Escherichia coli* lipolysaccharide-challenged growing pigs / Joyce Barcellos. – Viçosa, MG, 2020.
51 f. : il. (algumas color.) ; 29 cm.

Orientador: Melissa Izabel Hannas.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Referências bibliográficas: f. 30-36.

1. Lisina na nutrição animal. 2. Suínos - Crescimento. 3. Inflamação. 4. Crescimento exponencial. 5. Nitrogênio na nutrição animal. I. Universidade Federal de Viçosa. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. II. Título.

CDD 22. ed. 636.40852

JOYCE BARCELLOS

LYSINE REQUIREMENTS FOR *Escherichia coli* LIPOPOLYSACCHARIDE-CHALLENGED GROWING PIGS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Zootecnia, para obtenção do título de *Magister Scientiae*.

APROVADA: 19 de fevereiro de 2020.

Assentimento:

Joyce Barcellos
Autora

Melissa Izabel Hannas
Orientadora

AGRADECIMENTOS

À Universidade Federal de Viçosa, em especial ao Departamento de Zootecnia pela oportunidade de realização do curso.

Aos meus pais, por serem meu alicerce e porto seguro. Minha maior riqueza é tê-los ao meu lado, me apoiando a cada passo. Obrigada por todos os ensinamentos e serem a minha maior referência, amo vocês.

À minha orientadora Melissa Izabel Hannas por ter me inspirado e apoiado ao longo do mestrado, incentivando a buscar o meu melhor e estando sempre ao meu lado.

Ao Professor coorientador Paulo Henrique Reis Furtado por compartilhar sua experiência, me auxiliando e orientando em conjunto com a Professora Melissa I. Hannas.

A banca examinadora, Professora Luciana Navajas Rennó e ao Doutor Juliano Cesar de Paula Dorigam por aceitarem o convite e contribuírem grandemente na com a dissertação.

A minha equipe de trabalho: Jorge, Warley, Pedro, Carolaine, Filipe e Lucimauro. Vocês fazem parte dessa conquista.

Aos funcionários do Setor de Suinocultura da UFV, em especial ao Fernando, Dedeco, Arlindo (Lindão), Valdeir e Leandro por todo auxílio, companheirismo e por terem me proporcionado um excelente ambiente de trabalho durante a condução da pesquisa.

Aos demais professores, colegas e funcionários do Departamento de Zootecnia que de alguma forma contribuíram para a conclusão de mais uma etapa em minha vida.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

A todos vocês, muito obrigada!

RESUMO

BARCELLOS, Joyce, M.Sc., Universidade Federal de Viçosa, fevereiro de 2020. **Exigência de lisina para suínos em crescimentos desafiados com *Escherichia coli* lipopolissacarídeo.** Orientador: Melissa Izabel Hannas. Coorientadores: Paulo Henrique Reis Furtado Campos e Horacio Santiago Rostagno.

Para avaliar o efeito do desafio com *E. coli* lipopolissacarídeo (LPS) sobre a exigência de lisina digestível (Lys) para suínos em crescimento usando o modelo estabelecido na Universidade de Goettingen, foi realizado um ensaio de balanço de nitrogênio. Setenta e dois suínos machos castrados [19 ± 1,49 kg de peso corporal (PC)] foram alocados em um delineamento fatorial 2 x 6 composto por dois estados de ativação imunológica (controle e desafiado com LPS) e 6 tratamentos dietéticos com níveis de Nitrogênio (N) (0,94; 1,69; 2,09, 3,04, 3,23 e 3,97% N, na ração), sendo a Lys limitante, com seis repetições e um suíno por unidade. O desafio consistiu em uma dose inicial de LPS de 30 µg / kg PC por via intramuscular (IM) e uma dose subsequente de 33,6 µg / kg PC após 48 h. O período experimental foi de 11 dias composto 7 dias de adaptação, e subsequentes 4 dias de coleta, avaliando-se o consumo de N (NI), a excreção de N (NEX) e a deposição de N (ND). Mediadores inflamatórios e temperatura retal (TR) foram avaliados durante período de coleta de 4 dias. Uma interação tripla (N × desafio LPS × tempo) para IgG ($P < 0,05$) foi observada Adicionalmente, interações dupla (desafio × tempo) foram identificadas para IgA, ceruloplasmina, transferrina, haptoglobina, glicoproteína ácida α-1 (α1AGp), proteína total, TR e (nível N × tempo) para transferrina, albumina, haptoglobina proteína total e TR ($P < 0,05$). Suínos desafiados com LPS apresentaram menor ($P < 0,05$) consumo de ração. Interação dupla (níveis N × desafio LPS) foi observada ($P < 0,05$) para NI, NEX e ND, com dose-resposta evidente ($P < 0,05$). Suínos desafiados com LPS apresentaram menor NI e ND a 2,09% de N e 1,69 a 3,97% de N ($P < 0,05$), respectivamente, e maior NEX a 3,23% de N ($P < 0,05$). Os parâmetros obtidos pelo modelo não linear (exigência de N de mantença, NMR e deposição máxima teórica de N, $ND_{max}T$) foram 152,9 e 197,1 mg / $PC_{kg}^{0,75}$ / d para NMR e 3,524,7 e 2,077,8 mg / $PC_{kg}^{0,75}$ / d para $ND_{max}T$, nos grupos controle e desafiado com LPS, respectivamente. A exigência estimada de Lis digestível foi de 1994,83 e 949,16 mg / $PC_{kg}^{0,75}$ / d, respectivamente, para suínos controle e desafiados com LPS. A ingestão diária de Lys digestível necessária para atingir 0,68 e 0,54 pontos do valor de $NR_{max}T$ foi de 18,12 e 8,62 g / d, respectivamente, e a concentração ideal de Lys digestível na dieta pode mudar dependendo dos níveis de ingestão de ração. Com base nos parâmetros dos modelos

derivados obtidos em ensaio de balanço de N com menor custo e tempo, foi possível diferenciar a exigência de Lis digestível para suínos em condição de desafio.

Palavras-chave: Modelo exponencial. Suínos em crescimento. Resposta inflamatória. Exigência de lisina. Balanço de nitrogênio.

ABSTRACT

BARCELLOS, Joyce, M.Sc. Universidade Federal de Viçosa, February, 2020. **Lysine requirements for *Escherichia coli* lipopolysaccharide-challenged growing pigs.** Adviser: Melissa Izabel Hannas. Co-advisers: Paulo Henrique Reis Furtado Campos and Horacio Santiago Rostagno.

To evaluate the effect of an *E. coli* lipopolysaccharide (LPS) challenge on the digestible lysine (Lys) requirement for growing pigs using the model established at the University of Goettingen, a nitrogen balance assay was performed. Seventy-two castrated male pigs [19 ± 1.49 kg body weight (BW)] were allocated in a 2 x 6 factorial design composed by two immune activation states (control and LPS-challenged) and 6 dietary treatments with Nitrogen (N) levels (0.94; 1.69; 2.09, 3.04, 3.23, and 3.97 % N, as fed), with Lys being limiting in the dietary levels, with six replicates and one pig per unit. The challenge consisted of an initial LPS dose of 30 μg/kg BW via intramuscular (IM) injection and a subsequent dose of 33.6 μg/kg BW after 48 h. The experimental period lasted 11 days composed by a 7-day adaptation period, and a subsequent 4-day collection period which N intake (NI), N excretion (NEX), and N deposition (ND) evaluated. Inflammatory mediators and rectal temperature (RT) were assessed during each 4-d collection period. A 3-way interaction (N levels × LPS-challenged × time) for IgG ($P < 0.05$) was observed. Additionally, 2-way interactions (LPS-challenge × time) were verified for IgA, ceruloplasmin, transferrin, haptoglobin, α-1-acid glycoprotein (α1AGp), total protein, RT, and (N levels × time) for transferrin, albumin, haptoglobin, total protein and RT ($P < 0.05$). LPS-challenged pigs showed lower ($P < 0.05$) feed intake. A 2-way interaction (N levels × LPS-challenged) was observed ($P < 0.05$) for NI, NEX and ND, with a dose-response evident ($P < 0.05$). LPS-challenged pigs showed lower NI and ND at 2.09 % of N and 1.69 to 3.97 % of N ($P < 0.05$), respectively and higher NEX at 3.23 % of N ($P < 0.05$). The parameters obtained by the nonlinear model (N maintenance requirement, NMR and theoretical maximum N deposition, $ND_{max}T$) were 152.9 and 197.1 mg/$BW_{kg}^{0.75}$/d for NMR, and 3.524,7 and 2.077,8 mg/$BW_{kg}^{0.75}$/d for $ND_{max}T$, in the control and LPS-challenged groups, respectively. The estimated digestible Lys requirement were 1994.83 and 949.16 mg / $BW_{Kg}^{0.75}$ / d, respectively, for control and LPS-challenged pigs. The daily digestible Lys intake required to achieve 0.68 and 0.54 times the $NR_{max}T$ value were 18.12 and 8.62 g/ d, respectively, and the optimal dietary digestible Lys concentration may change depending in the feed intake levels. Based on the

derived models parameters obtain in N balance trial with lower cost and time, it was possible to differentiate the digestible Lys requirement for swine under the challenging conditions.

Keywords: Exponential model. Growing pigs. Inflammatory response. Lysine requirement. Nitrogen balance.

SUMÁRIO

Running head: Digestible lysine and LPS challenge

Assessment of digestible lysine requirement in lipopolysaccharide-challenged pigs[1]

Joyce Barcellos[*], Warley Júnior Alves[*], Pedro Riguetti Arnaut [*], Lucimauro Fonseca[*], Jorge Cunha Lima Muniz[*], Juliano César de Paula Dorigam[†], Paulo Henrique R. Furtado Campos[*], Fabyano Fonseca e Silva[*], Ryan N. Dilger[‡], Melissa Izabel Hannas[*]

[*]Department of Animal Sciences, Universidade Federal de Viçosa, Viçosa, Minas Gerais, 36570-900, Brazil

[†] Evonik Operations GmbH, Hanau, Germany

[‡] Department of Animal Sciences, University of Illinois, Urbana, Illinois, USA

[1]Acknowledgments

We appreciate the financial support from FAPEMIG, CAPES, and INCt for students and the Animal Science Department and PIC partner to support animals breeding at UEPE Swine, UFV.

Corresponding author: melissa.hannas@ufv.br

ABSTRACT

To evaluate the effect of an *E. coli* lipopolysaccharide (LPS) challenge on the digestible lysine (Lys) requirement for growing pigs using the model established at the University of Goettingen, a nitrogen balance assay was performed. Seventy-two castrated male pigs [19 ± 1.49 kg body weight (BW)] were allocated in a 2 x 6 factorial design composed by two immune activation states (control and LPS-challenged) and 6 dietary treatments with Nitrogen (N) levels (0.94; 1.69; 2.09, 3.04, 3.23, and 3.97 % N, as fed), with Lys being limiting in the dietary levels, with six replicates and one pig per unit. The challenge consisted of an initial LPS dose of 30 μg/kg BW via intramuscular (IM) injection and a subsequent dose of 33.6 μg/kg BW after 48 h. The experimental period lasted 11 days composed by a 7-day adaptation period, and a subsequent 4-day collection period which N intake (NI), N excretion (NEX), and N deposition (ND) evaluated. Inflammatory mediators and rectal temperature were assessed during each 4-d collection period. A 3-way interaction (N × LPS-challenged × time) for IgG ($P < 0.05$) was observed. Additionally, 2-way interactions between challenge × time were verified for IgA, ceruloplasmin, transferrin, haptoglobin, α-1-acid glycoprotein (α1AGp), total protein, rectal temperature, and between N level × time for transferrin, albumin, haptoglobin, total protein and rectal temperature ($P < 0.05$). LPS-challenged pigs showed lower ($P < 0.05$) feed intake. A 2-way interaction between N × LPS-challenged was observed ($P < 0.05$) for NI, NEX and ND, with a clear dose-response evident ($P < 0.05$). LPS-challenged pigs showed lower NI and ND at 2.09 % of N and 1.69 to 3.97 % of N ($P < 0.05$), respectively and higher NEX at 3.23 % of N ($P < 0.05$). The parameters obtained by the nonlinear model (N maintenance requirement, NMR and theoretical maximum N deposition, $ND_{max}T$) were 152.9 and 197.1 mg/$BW_{kg}^{0.75}$/d for NMR, and 3.524,7 and 2.077,8 mg/$BW_{kg}^{0.75}$/d for $ND_{max}T$, in the control and LPS-challenged groups, respectively. The estimated digestible Lys requirement were 1994.83 and 949.16 mg / $BW_{Kg}^{0.75}$ / d, respectively, for control and LPS-challenged pigs. The daily

digestible Lys intake required to achieve 0.68 and 0.54 times the $NR_{max}T$ value were 18.12 and 8.62 g/ d, respectively, and the optimal dietary digestible Lys concentration may change depending in the feed intake levels. Based on the derived models parameters obtain in Nitrogen balance trial with lower cost and time, it was possible to differentiate the digestible Lys requirement for swine under the challenging conditions.

Keywords: exponential model, growing pigs, inflammatory response, lysine requirement, nitrogen balance

Abbreviations:

AA, amino acids; AOAC, Association of Official Analytical Chemists; ADG, average daily gain; bc^{-1}, lysine efficiency; BW, body weight; $BW^{0.75}$, metabolic weight; CP, crude protein; DM, dry matter; FC, feed conversion; HCl, hydrochloric acid; IgA, immunoglobulin A; IgG, immunoglobulin G; IM, intramuscular; LPS, *E. coli* lipopolysaccharide; Lys, lysine; N, nitrogen; ND, nitrogen deposition; $ND_{max}T$, theoretical maximum nitrogen deposition; NEX, nitrogen excretion; NMR, nitrogen maintenance requirement; NR, nitrogen retention; SDS-PAGE, extraction and polyacrylamide gels electrophoresis analysis; α1AGp, α-1-acid glycoprotein.

INTRODUCTION

Dietary amino acid requirements depend on metabolic priorities of the animals, and therefore, can be modified by systemic changes in metabolism due to immune system activation and incidence of infectious disease. In such contexts, systemic inflammatory responses elicit adaptive reductions in feed intake and body weight (BW) gain, concomitant with a loss of muscle mass in pigs (Kampman, 2015; Le Floc'h et al., 2004). Understanding the causes and consequences of immune system activation and its effects on the animals' metabolic and energy status are essential to mitigate the negative effects of immune activation (Kvidera & Kay, 2017).

Dietary lysine (Lys) is typically the first limiting amino acid in pig diets (Htoo et al., 2016), due to the central role of this amino acid in muscle protein deposition. The relationship between use of Lys and the protein deposition capacity can be affected by genetics, sex, age, and animal health status (Ceron et al., 2013). For instance, genetic propensity for growth is a major driver of amino acid requirements (Hauschild et al., 2015), but dramatic shifts in metabolic priorities due to immune activation must also be considered. Immune system stimulation results in increased metabolic use of body protein and amino acids, which ultimately negatively affects protein deposition by animals (Williams et al., 1997).

When raised in commercial production systems pigs are often exposed to pathogens Pastorelli et al., 2012) wich, proliferation and dissemination are usually favored under high ambient temperatures and humidity conditions, such as those observed in tropical regions (Patz et al., 2000). In this sense, determining the nutritional requirements of pigs in challenging conditions is of ultmost importance for the development of feeding strategies for maximum growth rate and efficiency of nutrients use (Ceron et al., 2013). Evaluation of the nutrient retention capacity during times of immune activation has distinct advantages when estimating dietary amino acid requirements. To this end, nonlinear models associated with nitrogen balance assays have been proposed to determine amino acid requeriments, by estimating the

maximum protein deposition and the efficiency of the use of amino acids for growing animals (Samadi & Liebert, 2006a; Samadi & Liebert, 2006b). Given this context, the objective of this study were to evaluate N retention and the inflammatory response for growing pigs challenged with *E. coli* lipopolysaccharide (LPS) while modelling the digestible lysine (lys) requeriment for growing pigs. We hypothesized that the dietary Lys requirement estimated for LPS-challenged pigs would be decreased relative to control pigs due to shifts in metabolic demands for body protein (i.e., N).

MATERIALS AND METHODS

Animals care and handling were in accordance with Brazilian Legislation on Animal Experimentation and Welfare, and the experimental protocol was approved by the Ethics Committee on the Use of Farm Animals (CEUAP-UFV) of the Universidade Federal de Viçosa, (protocol 113/2018).

Animals, housing and experimental design

A nitrogen balance assay was conducted at the Swine Extension, Research and Teaching Unit of the Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Brazil. Seventy-two castrated male pigs (Sire line 337 × Dan line Camborough, PIC) with initial body weight (BW) of 19 ± 1.49 kg were allocated in a 2 × 6 factorial design with two immune activation states (control and LPS-challenged) and 6 dietary treatments increasing in Nitrogen (N) levels (0.94; 1.69; 2.09, 3.04, 3.23, and 3.97 % N, as fed basis), with 6 replicates and one animal per experimental unit.

Pigs were individually housed in adjustable metabolism cages (1.27 m × 0.56 m × 0.75 m) with drinker and stainless self-feeder.

Each experimental period was 11 days, with seven days of adaptation to the metabolic cages and experimental diets, and subsequent 4-day period in which total feces and urine

collection were performed to determine the N intake (NI), N excretion (NEX) and N deposition (ND). During the adaptation phase, average daily feed consumption was quantified and the lowest feed intake per metabolic weight ($BW^{0.75}$) in this phase was considered as standart to calculate feed intake for all animals during collection period (Sakomura & Rostagno, 2016). The mash diets were provided twice a day (0700 h and 1600 h), and water was provided *ad libitum* at all times.

The stimulus of the inflammatory response consisted of two intramuscular (IM) injections of *Escherichia coli* lipopolysaccharide (LPS; serotype O55: B5; Sigma Aldrich); the first injection administrated on the first day of the collection period was 30 µg/kg BW and the second 48-h later was 33.6 30 µg/kg BW, following the protocol of McGilvray et al. (2018). The pigs assigned to the unchallenged (i.e., control) group received two IM injections of sterile saline solution (0.9%) using the same timing as for LPS-challenged pigs.

Experimental diets

Principles of the dilution technique to diets produced were applied as described by Fisher & Morris (1970). Initially, two diets were formulated; a concentrated diet (N6) with higher N level based on corn, soybean meal, and corn gluten, with 27.0% CP, 1.59 % digestible Lys, and 3230 kcal/kg of Metabolizable nergy, following the ideal protein ratio recommended by Rostagno et al. (2017) (**Table 1**) and a protein-free diet (N0) defined as dilution diet formulated to meet the nutritional requeriments, except for protein and amino acids (AAs). According to diet dilution technique, graded mixing proportions of the diet N6 and diet N0 yielded the final diets with N ranged between 0.94 % (N1) and 3.97 % (N6). The rations between N6 and N0 diets were: N1 = 23.03:76.97; N2 = 38.42:61.58; N3 = 53.82:46.18; N4 = 69.21:30.79; N5 = 84.61:15.39 and N6 = 100:0, to obtain diets with CP concentrations of 6.22, 10.37, 14.53, 18.69, 22.84 and 27%, respectively, and 0.36, 0.61, 0.85, 1.10,1.34, 1.59 % of digestible Lys, respectively. The Lys concentration was the limiting factor in the composition

of the experimental diets, and the relationship of the other AAs to Lys was maintained (**Table 2**). The diets were formulated to be isoenergetic and supplemented with minerals and vitamins according to the recommendations of Rostagno et al. (2017).

Sample collection, analysis, and calculations

Pigs were weighed at the beginning of the adaptation period and the beginning of the total collection period. Samples from each experimental diets (N1 to N6) were collected randomly during feed manufacturing, homogenized, and stored at – 20°C for nutrient composition analysis. Blood samples were collected via sinus orbital puncture at 0700 h on the first day of collection (day 1), as well as 3 h and 96 h after the start of the challenge, as collected in tubes without anticoagulant immediately placed on ice and centrifuged at 1,900 × g at 4°C (centrifuge R/5702, Copyright © Eppendorf AG, Germany) to obtain serum samples. Rectal temperature was measured using a digital thermometer G-TESH (digital thermometer, TH186, Onbo Eletronics - Shenzhen, China), during the collection period on d 1, 3, and 5 at 0700 h (hereafter referred to as LPS-d1, LPS-d3, and LPS-d5), and also on d 1 and 3 at 1500 h, 3-h after the initial LPS injection (LPS-d1+3h, LPS-d3+3h).

The N balance study was carried out according to the methodology of the total collection of feces and urine (Sakomura and Rostagno, 2016), reducing the collection period by one day. This change was performed so that the samples were obtained in similar conditions, considering the challenge protocol with an interval between LPS injections of 48 h. The fecal collection was carried out once a day, for four consecutive days, with samples being weighed daily and stored in plastic bags at -20°C. At the end of the collection period, all fecal samples were thawed and homogenized, and sub-samples were lyophilized and stored for further analysis. Urine collection began at the time of IM injection and the total urine volume per pig was collected over four days in buckets containing 7.5-to-10 mL of HCl and positioned below the funnel coupled to each cage. After each 24-h period of urine collection, samples of 10% of the urine

volume was sampled and stored at -20°C to capture a homogenous and representative urine sample per pig over the 4-d collection period.

The diets, feces and urine subsample were used to determined chemical composition at Animal Nutrition Laboratory (Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil). Before chemical analysis, feces and diet samples were ground in a ball mill (Micro spray mill, R-TE 350, TECNAL® - São Paulo, Brazil). The samples were weighed (Analytical balance - AR2140, Adventurer™ PRO Analytical - OHAUS corp. USA) to quantify the dry matter content. The N content of the diets, feces and urine as analysed in a N distiller (N distiller, TE-036/1, TECNAL® - São Paulo, Brazil) using the Kjeldhal method (AOAC - determination of nitrogen content and calculation of crude protein content, ISO 5983-1:2005). The factor 6.25 was used to convert nitrogen content to crude protein (CP). Additionally, homogenous sub-samples of the diets were submitted to the CBO Laboratory (Valinos, SP, Brazil) for quantify the total amino acid content using high-performance liquid chromatography. The digestible AA data were calculated based on the tables of Rostagno et al. (2017).

The triplicate analysis was performed to determine the dry matter (DM) content in diets and feces (AOAC - dry matter content, ISO/CD 638-1). N determination in diets, feces, and urine samples was analyzed in duplicate; repetitions were performed for a sample variation coefficient (CV) above 5%.

The total serum protein determination was performed by the Biuret method, with the aid of a set of reagents (Bioclin, Total Protein Monoreagent K031, colorimetric test) through an automatic biochemical analyzer (Mindray BS-200E, Shenzhen Mindray Bio-Medical Electronics Co.., Shenzhen, China) at Animal Physiology and Reproduction Laboratory (Universidade Federal de Viçosa, Viçosa, MG, Brazil).

The serum proteins analysis were performed at the Departement of Clinical and Veterinary Surgery (Universidade Estadual de são Paulo, Jaboticabal, SP, Brazil). The serum

proteins were separated by polyacrylamide gel electrophoresis with a matrix containing sodium dodecyl sulfate (SDS-PAGE). Electrophoresis was performed according to the modified technique described by Laemmli (1970), using the vertical electrophoresis system (PROTEAN II XI-VERTICAL ELETROPHORESIS CELLS ® - BIO-RAD). The molecular weight and protein fractions concentrations were determined by computer densitometry (SHIMADZU CS-9301, Shimadzu Corp, Kyoto, Japan), using the samples scanner. For protein identification biomarkers were used (SIGMA MARKER™, ®Sigma-aldrich biotecnhnology LP). For densitometric evaluation of protein bands reference curves were made from the reading of the standard marker. From the proteinogram, the proteins albumin, ceruloplasmin, haptoglobin, immunoglobulins (IgA and IgG), transferrin, α-1-acid glycoprotein (α1AGp) and PM 20000 were quantified, at times 3-h and 96-h after the first application of LPS. These proteins are considered biomarkers of the inflammatory response, enabling the identification and monitoring of animal health. The N deposition (mg/$BW_{kg}^{0.75}$/d) was calculated as a result of the difference between N ingested (mg/$BW_{kg}^{0.75}$/d) (i.e., determined by the N of the feed provided minus N of the leftover feed) and N excreted [N_{feces} (mg/$BW_{kg}^{0.75}$/d) + N_{urine} (mg/$BW_{kg}^{0.75}$/d)].

Statistical analysis

All data were analyzed by ANOVA using a randomized complete block design with a 2 × 6 factorial arrangement with repeated measures over time to estimate the effects of N levels, immune activation, and collection time as fixed effects, along with all interactions (PROC MIXED, SAS Inst. Inc., version 9.4). Individual pig served as the experimental unit for all outcomes. Interactive effects were further evaluated using orthogonal contrasts for linear and quadratic responses to dietary Lys concentration. Differences between averages were determined using the "slice" function of "LSmeans" and Tukey test. For all outcomes, significance was accepted at $P < 0.05$.

Modeling

Modeling was performed to estimate amino acid requirements using parameters including N maintenance requirements (NMR), the N retention (NR), and the theoretical maximum N retention ($NR_{max}T$). Determining the NMR is part of the total N retention, and indicates the amount of N to be deposited to replace endogenous losses (feces and urine). A regression analysis between N intake and excretion was applied to estimate NMR following the exponential function, as performed in previous studies with birds and pigs (Samadi & Liebert, 2007; Samadi & Liebert, 2008; Wecke & Liebert, 2009; Pastor et al., 2013; Khan et al., 2015; Liebert, 2015, Dorigam et al., 2017):

(Equation 1) $NEX = NMR \cdot \exp^{b.NI}$;

where NMR is the N maintenance requirement (mg / $BW_{Kg}^{0.75}$ / d), NI is the N intake (mg / $BW_{Kg}^{0.75}$ / d), NEX is the N excretion (mg / $BW_{Kg}^{0.75}$ / d), b is the slope of the exponential function and exp is the base number of the natural logarithm (*ln*). NMR was estimated by calculating the curve intersection on the y-axis (NEX) when NI = 0. The exponential model (Equation 1) was adjusted to the N excretion data using the nonlinear optimization technique (Levenberg-Marquardt) with SAS (Statistical Analysis System, version 9.4). Once the NMR is estimated, it is possible to calculate N retention (NR). The N deposition (ND) was calculated as the difference between NI and NEX. N retention represents the total nitrogen utilization calculated as: NR = ND + NMR (Wecke & Liebert, 2009).

The theoretical maximum N retention ($NR_{max}T$) is represented by the exponential function's asymptote and is used to classify NR performance data. A regression analysis between NI and NR was performed to fit another exponential model:

(Equation 2) $NR = NR_{max}T \cdot (1 - \exp^{-b.NI})$;

where NR is N retention (mg / $BW_{Kg}^{0.75}$ / d), $NR_{max}T$ is the theoretical maximum N retention (mg / $BW_{Kg}^{0.75}$ / d), NI is N intake (mg / $BW_{Kg}^{0.75}$ / d), b is the slope of the NR curve

that expresses the quality of the protein in the diet and exp is the base number of the natural logarithm (*ln*). The exponential model (Equation 2) was adjusted to the NR data, also using the nonlinear optimization technique (Levenberg-Marquardt) in the SAS (Statistical Analysis System, version 9.4).

The $NR_{max}T$ is the asymptotic value in the exponential function. It was estimated by a statistical procedure, following several steps of the Levenberg-Marquardt algorithm, until the sum of the residual squares was minimized. Therefore, the adjective "theoretical" is assigned to this parameter because the estimated value is not attainable under production conditions, even if animals are raised under ideal conditions. However, this is an essential parameter in the modeling procedure to derive amino acid (AA) requirements considering gradual levels defined to make use of the theoretical maximum within the practical data scope.

The requirement for digestible Lys was calculated after a logarithmic transformation of Equation 2, according to previous studies (Samadi & Liebert, 2007; Samadi & Liebert, 2008; Wecke & Liebert, 2009; Pastor et al., 2013; Khan et al., 2015; Liebert, 2015, Dorigam et al., 2017).

(Equation 3) $LAAI = [ln\ NR_{max}T - ln\ (NR_{máx}\ T - NR)] / (16 . bc^{-1})$;

where LAAI is the required daily intake of limiting AA (mg / $BW_{Kg}^{0.75}$ / d), NR is N retention (mg / $BW_{Kg}^{0.75}$ / d), $NR_{max}T$ is the theoretical maximum retention of N (mg / $BW_{Kg}^{0.75}$ / d), c is the limiting AA concentration in dietary protein (g / 16 g N), b is the slope of the NR curve that expresses the quality of the protein in the diet and bc^{-1} is the efficiency parameter of using limiting AA in the diet (inclination between b and c). The number 16 results from the limitation of the AA concentration in the dietary protein (g / 16 g N). Lys was adjusted as the limiting AA in experimental diets. Consequently, the Lys requirement was derived according to Equation 3, and the Lys in the diet (%) was calculated as the Lys requirement (g / d) divided by the feed intake (g) multiplied by 100. Lys percentage in the diet was multiplied by the DM

content ration and divided by 100 and, afterward, it was multiplied by the Lys digestibility factor. The feed consumption used in the simulation was calculated based on the recommendations of Rostagno et al. (2017) for growing pigs with 20 kg of BW. In addition, it was consider the difference in feed intake observed in LPS-challenged group and the 0.10 times increase or decreased to simulated the feed intake in these three scenarios for control and LPS-challenged animals.

The N balance data were analyzed statistically by a 1-way ANOVA using the GLM procedure and these data were adjusted to exponential models using the PROC NLIN procedure in SAS (Statistical Analysis System, version 9.4).

RESULTS

Blood parameters

The effects of dietary N concentration, immune system stimulation, and time on blood parameters and their interactions are shown in **Fig. 1** and **2**, and **Table 3**. 3-way interaction (N levels × LPS-challenged × time) was observed for IgG ($P < 0.05$), with a linear effect for the control group at 3-h and 96-h after LPS administration (**Fig. 1**). 2- way interaction (LPS-challenged × time) was observed for IgA, ceruloplasmin, transferrin, haptoglobin, α1AGp, PM 20000, and total protein ($P < 0.05$). The effect between the control and LPS-challenged groups was observed 3-h after LPS administration for IgA, transferrin, haptoglobin, and total protein; and after 96-h for IgA, ceruloplasmin, haptoglobin, α1AGp and PM 20000 (Table 3).

Two-way interaction (N level × time) was observed for transferrin, albumin, haptoglobin, and total protein ($P < 0.05$). An effect of dietary N levels over time was observed for transferrin and albumin (2.09 to 3.97 % of N), total protein (0.94 to 3.97% of N), and haptoglobin (0.94 to 2.09, 3.29 and 3.97 % of N). Transferrin and haptoglobin protein concentrations exhibited linear increase and U-shape quadratic responses, respectively, 3-h after LPS administration. Similarly,

albumin, haptoglobin, and total protein all exhibited linear responses 96 h after LPS administration (**Fig. 2**).

Rectal Temperature

The effect of N levels, immune challenge and time, and their interactions on rectal temperature are shown in **Fig**. **3** and **4.** 2-way interaction (LPS-challenged x time) was observed ($P < 0.0001$). LPS-challenged increased the rectal temperature in LPS-d1 + 3h and LPS-d3 + 3h. Measurement time effect was observed within each group, an elevation of the rectal temperature after LPS-d1 + 3h and LPS-d3 + 3h for the LPS-challenged group.

Two-way interaction (N levels × LPS-challenged) was observed ($P = 0.0210$). The LPS-challenged raised the rectal temperature to all levels of N compared to the control group. The levels' effect was greater for the control groups and LPS-challenged as the N content in the diet increased.

Nitrogen Balance

Differences between control and LPS-challenged groups were observed for feed consumption ($P < 0.05$), with a reduction in feed intake in the LPS-challenged group. 2-way interaction (N levels × LPS-challenged) was observed ($P < 0.05$) for N intake, excreted, and deposited (mg / $BW_{Kg}^{0.75}$ / d), and the equations differed ($P < 0.05$) between groups. The linear regression for the N deposition in the control group is given by 127.86 + 246.95 N level (mg / $BW_{Kg}^{0.75}$ / d), and for the LPS-challenged group by 150.66 + 155.4 level of N (mg / $BW_{Kg}^{0.75}$ / d). The LPS-challenged negatively affected the capacity of N retention for growing pigs, where for each point of increase in the level of N in the diet, the ND reduced by 37%.

The effect of N levels was observed ($P < 0.05$) on the N intake, excreted, and deposited (mg / $BW_{Kg}^{0.75}$ / d) between the groups. There was a reduction ($P < 0.05$) of the N intake from 1.69% of N, and of the N deposited from 2.09% of N for the LPS-challenged group, however, the excreted N was higher in 3.29% of N.

Model parameters

The non linear models, used to determined NMR (Eq. 1) and and $NR_{max}T$ (Eq. 2), are shown in **Fig. 5** and **6**. In response to LPS-challenged administration, there was a 22.4% increase in NMR. The results of the non-linear regression fitting between NI and NR demonstrated that $NR_{max}T$ decreased decreased by 41.1% in the LPS-challenge group compared with control (non-LPS-challenged) pigs. Estimated requirements for digestible Lys are shown in **Table 5**, as dependent on the rate of body protein deposition, the efficiency of Lys use, and immune activation status of pigs. Based on the derived models parameters and the response of the pigs in control and LPS-challenged groups required to achieve 0.68 and 0.54 times the $NR_{max}T$ value, the estimated digestible lysine requirement were 1994.83 and 949.16 mg / $BW_{Kg}^{0.75}$ / d, respectively for control and LPS-challenged pigs. The criterion for selecting the percentage for $NR_{max}T$ value was taken from the mean ND value for each group. The daily digestible Lys intake required were 18.12 and 8.62 g/ d, respectively, for control and LPS-challenged and the optimal dietary Lys concentration may change depending in the feed intake levels. LPS-challenged animals were estimated to have a lower estimated digestible Lys requirement compared with non-challenged pig.

DISCUSSION

This study aimed to assess N retention and the inflammatory response of growing pigs challenged with *E. coli* LPS and to establish two key parameters of the nonlinear model (NMR and $NR_{max}T$). It was possible to estimate the requirement for Lys according to the rate of protein deposition, the efficiency of the use of Lys, and inflammatory response through these parameters. An established model to induce an inflammatory response through repeated doses of *E. coli* LPS was used, inducing a controlled and relatively moderate inflammatory response, thereby allowing the direct investigation of nutrient utilization (Ridder et al., 2012; Litvak et al., 2013; Rakhshandeh et al., 2014; McGilvray, 2018). The effect of this model of stimulation

was evidenced 3 h after the start of the initial LPS administration that was marked by an increase in rectal temperature and serum concentrations of acute and chronic response proteins, and a reduction in feed intake. Collectively, these clinical signs are compatible with those reported in the literature for LPS challenged pigs (Campos et al., 2014; Mc Gilvray, 2018).

Acute-phase proteins represent non-specific markers of the different inflammatory etiologies as part of routine monitoring of animal health (Petersen et al., 2004). Among them, haptoglobin has been listed as the main acute phase protein in pigs (Alava et al., 1997; Petersen et al. 2004). As expected, haptoglobin demonstrated higher sensitivity and stability than other acute-phase proteins, with pigs exhibiting a significant increase in haptoglobin within 3 h of the initial LPS administration and maintaining this inflammatory profile through 96 h after injection (Campos et al., 2014). The difference in the response pattern of the different proteins evaluated is related to individual characteristics, having a positive or negative response. The α1AGp, ceruloplasmin and haptoglobin are considered acute-phase positive proteins since their concentration in the blood increases in response to the challenge. The proteins albumin and transferrin have a reduction in their levels, being considered proteins of the acute negative phase (Gruys et al., 1994; Kaneko, 1997, Eckersall, 2008), as observed in this study.

In general, the interaction between challenge and response time demonstrates that acute-phase proteins have their concentration rapidly elevated in the blood compared to specific (late-response) immunoglobulins. Despite the increase in IgA and IgG concentration within 3 h of LPS administration, the response was accentuated at the 96 h time-point. This response pattern allows the monitoring of the progression of the inflammatory response through the concentration of immunoglobulins, such as IgA and IgG, indicating in this study that the challenge was consistent throughout the collection period.

In addition to the interaction between LPS challenge and response time, the diet also influenced the inflammatory response, as observed by the effect of experimental diets on acute

and chronic phase proteins. As the concentration of crude protein in the diet increased, the inflammatory response was more exaggerated, especially in the LPS-challenged group. According to the literature, the impact of the challenge on protein (and consequently amino acid) metabolism can generate specific nutritional requirements. In this condition, due to changes in specific metabolic pathways, the amino acids resulting from muscle catabolism are different from those required for the maintenance of the inflammatory and immune response, leading to the relative excess of non-limiting amino acids. In contrast, other amino acids become limiting for the immune response as body proteins are repartitioned while the animal experiences an inflammatory challenge (Reeds & Jahoor, 2001). In this context, the reduction in nitrogen (from 2.09% to 0.94% N) may have limited the expression of the inflammatory response, prioritizing some acute-phase proteins in detriment to others according to the importance of each one. This hypothesis highlights the relevance of haptoglobin as an essential biomarker of the inflammatory response of pigs. It also indicates that the ratio of amino acids in the ideal protein paradigm need to be changed for pigs experiencing an inflammatory challenge.

The interaction found between N levels and rectal temperature measurements for the control and LPS-challenged group can be attributed to different factors mediates by inflammation and nutrient metabolism. The increase in rectal temperature in animals challenged 3 h after LPS administration (LPS-d1 + 3h; LPS-d3 + 3h) compared with the control group was expected since the increase in rectal temperature is a consequence of signaling pro-inflammatory cytokine (Johnson, 1998; Petry et al., 2017). The effect of diets was also related to the increased metabolism of animals in this study, with increased protein metabolism due to excess N supplementation, leading to the rise in rectal temperature at the highest levels. The increase in metabolism may also be associated with body temperature, as homeothermic temperature regulation is achieved through a balance between the production of heat by

metabolism and its loss (Henken et al., 1993; Pedersen & Sallvik, 2002). The second is related to the caloric increase considering a pre and postprandial state. Due to the metabolic changes presented in a challenging condition, the ratio of protein synthesis and degradation is changed to a catabolic state, potentiating turnover.

Under these conditions, the association between increased N excretion per unit of N intake is common, resulting in less N retention (Rakhshandeh & de Lange, 2011). The same relationship was found in this work, evidenced by the interaction between the LPS challenge and N intake, excreted and deposited. According to Mc Gilvray et al. (2019), the challenge does not only reduce protein retention by changing the protein synthesis and degradation ratio, but also by lowering deposition efficiency.

Through the regression equations involving N deposition as a function of N intake, we observed that the LPS challenge negatively affected N retention capacity for growing pigs, where for each point of increase in the dietary N level reduced N deposited by 37% ($mg/BW_{kg}^{0.75}/d$). The influence of the challenge on N retention was similar to that reported in the literature, where the stimulation of the immune system through repeated doses of *E. coli* LPS influenced the reduction of protein deposition from 3% to 20% in pigs weighing approximately 20 kg (Ridder et al., 2012; Litvak et al., 2013).

The nitrogen balance data were used in the non-linear model. The effects of the different interactions between the inflammatory response and the nitrogen balance are reflected on NMR since the endogenous basal losses in feces and urine and protein turnover impact it (Moughan, 2008). The determination of NMR is described by equation 1 and represents the inevitable metabolic losses of nitrogen (Wecke & Liebert, 2009). Within the model, NMR is obtained by mathematical extrapolation; however, the 22.4% increase in LPS-challenged animals demonstrates alteration in basal metabolism, characterized by higher protein mobilization to maintain the inflammatory response, and altered nitrogen balance. Simultaneously, the impact

of the challenge on protein metabolism compromised the ability to deposit muscle protein, consequently leading to a 41.1% reduction in $NR_{max}T$ in the LPS-challenged group. In this study, the model was responsive to the change in protein metabolism caused by LPS challenge and allowed the estimation of dietary Lys requirements for challenged pigs, associating the practicality of the nitrogen balance and the segmentation of maintenance and production requirements observed by the factorial model. The data obtained through the nitrogen balance (NMR, $NR_{max}T$), are essential for supplying a database for later application of models, including modeling for estimating the amino acid requirement based on daily protein deposition and efficiency of use of the limiting amino acid in the diet (Samadi & Liebert, 2006a). Perhaps the LPS challenge did cause a increase in NMR, a concomitant decreased in $NR_{max}T$ that was responsible for reducing the digestible Lys requirement in this group.

The body mainly uses Lys to synthesize body proteins, representing approximately 80% of its use in young animals (Klasing, 2009). Given this characteristic, the high variation in its requirement in the literature can be associated with the difference in the potential for gaining lean mass due to the different genetics available on the market. In comparison, weaned pigs (5–20 kg) in the 1990s had an average daily gain (ADG) of 350 g/d. With the advancement of genetic improvement, the current literature shows the evolution on the protein deposition capacity for the same category, with gains close to 500 g/d (NRC, 1998; Dean et al., 2007), reaching 620 g/d for pigs weighing 15-30 kg (Rostagno et al. 2017). The constant evolution of animal breeding reflects the need to update the swine nutritional requirements.

The estimate of the digestible Lys requirement of 18.12 g/d and optimal dietary lysine concentration of 1.42% (consider 1000 g of feed intake) is in line with that found for healthy pigs with similar weight and genetics range as in this study, as observed by Graham et al. (2017) and Fruge et al. (2017). In these studies, the minimum requirement for ADG was 1.25%, reaching 1.40% for feed conversion (FC). In studies carried out with 7-16-kg pigs, Vier et al.

(2016) and Kahindi et al. (2017) indicate that the estimated requirement for digestible Lys for ADG is 1.29% and 1.32%, meaning that the requirement for Lys for 20 kg pigs estimated in this work consistent.

These results indicate that the non-linear model used in our work was able to adjust the requirements of healthy pigs, providing support for the estimation of Lys requirement in challenged pigs. As expected, we observed a reduction in the Lys requirement for LPS challenged pigs compared to the control group. This behavior is directly related to the use of Lys for the synthesis of muscle protein, which, due to the lower protein deposition capacity becomes less demanded (Littiere et al., 2017). For LPS-challenge pigs the reduction in protein deposition observed was 47% lower when compared with protein deposition in control groups.

Studies show that in the face of a challenging condition, the requirement for Lys decreases in relation to other amino acids (Mc Gilvray, 2018; Kampman, 2015) due to its lesser involvement with the maintenance of the immune response. The amino acid requirement for challenged animals is influenced by different factors, in this work the estimate of the requirement for digestible Lys was 8.62 g/d and optimal dietary lysine concentration of 0.76% was based on 0.54 times of $NR_{max}T$, due to the reduction in consumption (consider 893 g of feed intake), and N retention due to the challenge induced by repeated doses of LPS.

The non-linear model we employed made it possible to quantify the effect of the immune challenge on the Lys requirement for protein deposition, considering the inflammatory response in growing pigs. However, the estimated requirement for challenged pigs is not intended to achieve the same performance as control animals. Although our results are in accordance with the literature, it is necessary to consider that the recommendation of the digestible Lys requirement also depends on other factors, such as genotype, environment, feed intake levels, the ratio of the amino acids supplied, intensity and type of health challenges, among others, that can be studied in future models.

In conclusion, stimulation of the inflammatory response through repeated injections of *E. coli* LPS altered protein metabolism, thereby influencing the reduction of N intake and deposition and increase N excretion. Inflammatory mediator responses, especially haptoglobin, are useful outcoes to identify and monitor the progression of the immune response. The non-linear model adjustment associated with the nitrogen balance allowed estimating model parameters (NMR, $NR_{max}T$) for growing pigs challenged with *E. coli* LPS, indicating an increase in NMR by 22.4% and a reduction in $NR_{max}T$ of 41.1%. Also, the modelling procedure including control and LPS-challenge pigs provides a different estimates of the maximum genetic potential for nitrogen retention, 0.68 and 0.54 times of $NR_{max}T$, respectively and the digestible Lys requirement were estimated of 1994.83 and 949.16 mg / $BW_{Kg}^{0.75}$ / d, respectively, for control and LPS-challenged pigs. The daily digestible Lys intake required were 18.12 and 8.62 g/ d, respectively, for control and LPS-challenged and the optimal dietary Lys concentration may change depending in the feed intake levels.

In general, the differences in the nutritional requirements of the animals due to factors such as immunological challenge must be considered for the determination of swine feeding programs, and the applied modeling approach allows the calculation of the requirements of the first limiting amino acid for pigs according to the conditions immune challenge.

Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

LITERATURE CITED

Alava, M. A., González-Ramón, N., Heegaard, P., GUZYLACK, S., Toussaint, M. J. M., Lipperheide, C. Madec, F. Gruys, E., Echersall, P. D., Lampreave, F. Piñeiro, A. Pig-MAP, porcine acute phase proteins and standardisation of assays in Europe. Comparative Haematology International **7,** 208–213 (1997). doi: 10.1007/BF02658691

Association of official analytical chemists – AOAC. **Methods of analyses**, 16th ed. Washington, DC: 1997.

Campos, P. H. R. F., Merlot, E., Damon, M., Noblet, J., Le Floc'h, N., 2014. High ambient temperature alleviates the inflammatory response and growth depression in pigs challenged with Escherichia coli lipopolysaccharide. The Veterinary Journal, 200:404–409. doi: 10.1016/j.tvjl.2014.04.001.

Ceron, M. S., Oliveira, V., Lovatto, P. A., Vale, M. M., 2013. Maintenance requirement and deposition efficiency of lysine in pigs. Pesq. Agropec. Bras., Brasilia, 48 (9):1269-1274. doi: 10.1590/S0100-2013000900011.

Dean, D. W., Southern, L. L., Kerr, B. J., Bidner, T. D. 2007. The lysine and total sulfur amino acid requirements of six to twelve - kilogram pigs. J. Anim. Sci. 23:527–535. doi:10.1532/S1080-7446(15)31015-9.

De Ridder, K., Levesque, C. L., Htoo, J. K., De Lange, C. F. M. 2012. Immune system stimulation reduces the efficiency of tryptophan utilization for body protein deposition in growing pigs. J. Anim. Sci. 90:3485–3491. doi:10.2527/jas.2011-4830.

Detmann, E. et al. 2012. Métodos para Análise de Alimentos - INCT - Ciência Animal. 1.ed. Visconde do Rio Branco: Suprema, 214p.

Dorigam, J. C. P., Sakomura, N. K., Soares, L., Fernandes, J. B. K., Sünder, A., Liebert, F. 2017. Modelling of lysine requirement in broiler breeder hensbased on daily nitrogen retention

and efficiency of dietarylysine utilization. Animal Feed Science and Technology. 226:29–38. doi: 10.1016/j.anifeedsci.2016.12.003.

Eckersall, P. D. 2008. Proteins, Proteomics and the Dysproteinemias. In: Kaneko, J. J.; Harvey, J. W. Bruss, M. L. Clinical biochemistry of domestic animals. 6th ed.Burlington: Academic Press, p. 117-155.

Fisher, C. & Morris, T. R. 1970. The determination of the methionine requirement of laying pullets by a diet dilution technique. Br. Poult. Sci. 11: 67–82. doi:10.1080/00071667008415793.

Fruge, E. D., Gerhart, A. J., Hansen, E. L., Hansen, S. A., Coble, K. F. 2017. Effects of increasing standardized ileal digestible (SID) lysine on performance of 11 to 23 Kg nursery pigs. J. Anim. Sci. 95(suppl_2), 102–103. (Abstr.) doi:10.2527/asasmw.2017.12.213.

Graham, A., Knopf, B., Greiner, L., Goncalves, M. A. D., Orlando, U. A. D., Connor, J. 2017. Evaluation of the lysine requirement of eleven- to twenty-three-kilogram nursery pigs. J. Anim. Sci. 95 (suppl_2)146–147 (Abstr.). doi:10.2527/asasmw.2017.301.

Gruys, E., Obwolo, M. J., Tousaint, M. J. M. 1994. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. Veterinary Bulletin, Farnham Royal. 64(11):1009-1018.

Hauschild, L., Sakomura, N. K., Silva, E. P. 2015. Avinesp Model: predicting poultry growth, energy and amino acid requirements. In: Sakomura, N. K., Gous, R. M., Kyriazakis, I., Hauschild, L. (Eds.), Nutritional Modelling for Pigs and Poultry. CAB International, Wallingford, pp. 188–207. doi:10.1079/9781780644110.0188.

Htoo, J. K., Oliveira, J. P., Albino, L. F. T., Hannas, M. I., Barbosa, N. A. A., Rostagno, H. S. 2016. Bioavailability of L-lysine HCl and L-lysine sulfate as lysine sources for growing pigs. J. Anim. Sci. 94 (suppl_3), 253–256. doi:10.2527/jas.2015-9797.

Johnson, R. W. 1998. Immune and endocrine regulation of food intake in sick animals. Domest. Anim. Endocrinol. 15:309–319. doi:10.1016/S0739-7240(98)00031-9.

Kahindi, R. K., Htoo, J. K., Nyachoti, C. M. 2017. Dietary lysine requirement for 7–16 kg pigs fed wheat–corn–soybean meal-based diets. Journal of Animal Physiology and Animal Nutrition, 101:22–29. doi:10.1111/jpn.12491.

Kampman-Van De Hoek, E. 2015. Impact of health status on amino acid requirements of growing pigs: Towards feeding strategies for farms differing in health status. PhD Diss., Wageningen University, Wageningen, the Netherlands.

Kaneko, J. J. 1997. Serum proteins and the dysproteinemias, pp 117-138. In: Kaneko, J. J. Harvey, J. W. Bruss, M.L. editors. Clinical biochemistry of domestic animals. San Diego (CA): Academic Press.

Henken, A., Brandsma, M., Hel, H. A., Van Der, W., Verstegen, M. W. A. 1993. Circadian rhythm in heat production of limit-fed growing pigs of several breeds kept at and below thermal neutrality. J. Anim. Sci. 71:1434–1440.

Khan, D. R., Wecke, C., Sharifi, A. R., Liebert, F. 2015. Evaluating the age-dependent potential for protein deposition in naked neck meat type chicken. Animals, 5:56–70. doi:10.3390/ani5010056.

Klasing, K. C. 2009. Minimizing amino acid catabolism decreases amino acid requirements. Journal of Nutrition, 139:11–12. doi: 10.3945/jn.108.099341.

Kvidera, S. & Kay, S. 2017. Causes and consequences of immune activation and its effect on metabolic and energetic status in production animals. Graduate Theses and Dissertations. Iowa State University. 15344.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, London, 227 (5259):680-685.

Le Floc'h, N., Melchiora, D., Oble, C. 2004. Modifications of protein and amino acid metabolism during inflammation and immune system activation. Livestock Production Science. 87:37–45. doi:10.1016/j.livprodsci.2003.09.005.

Liebert, F. 2015. Basics and applications of an exponential nitrogen utilization model ('Goettingen approach') for assessing amino acid requirements in growing pigs and meat type chickens based on dietary amino acid efficiency. In: Sakomura, N.K., Gous, R.M., Kyriazakis, I., Hauschild, L. (Eds.). Nutritional Modelling for Pigs and Poultry. CAB International, Wallingford, pp. 73–87. doi:10.1079/9781780644110.0073.

Littiere, T. O., Campos, P. H. R. F., Merlot, E., Renaudeau, D., Noblet, J., Le Floc'h, N. 2017. Lysine, threonine and tryptophan postprandial metabolism in LPS challenged growing pigs. IN: 54ª. Reunião Anual da Sociedade Brasileira de Zootecnia. Anais da 54ª. Reunião Anual da Sociedade Brasileira de Zootecnia | Foz do Iguaçu 2017 | ISSN 1983-4357.

Litvak, N., Rakhshandeh, A., Htoo, J. K., De Lange, C. F. M. 2013. Immune system stimulation increases the optimal dietary methionine to methionine plus cysteine ratio in growing pigs. J. Anim. Sci. 91: 4188–4196. doi:10.2527/jas2012-6160.

Moughan, P. J. 2008. Efficiency of amino acid utilization in simple-stomached animals and humans – a modeling approach. IN: France, J. Kebreab, E. (Ed.). Mathematical modelling in animal nutrition. Palmerston North: Massey Univesity; London, pp. 241-253. doi: 10.1079/9781845933548.0241.

Mc Gilvray, W. D., Klein, D., Wooten, H., Dawson, J. A., Hewitt, D., Rakhshandeh, A.R., De Lange, C. F. M., Rakhshandeh, A. 2018. Immune system stimulation induced by Escherichia coli lipopolysaccharide alters plasma free amino acid flux and dietary nitrogen utilization in growing pigs. J. Anim. Sci. 97:315–326. doi:10.1093/jas/sky40.

Mc Gilvray, W. D., Johnson, B., Wooten, H., Rakhshandeh, A. R., Rakhshandeh, A. 2019. Immune System Stimulation Reduces the Effiency of Whole-Body Protein Deposition and Alters Muscle Fiber Characteristics in Growing Pigs. Animals, 9:323. doi:10.3390/ani9060323.

NRC. 1998. Nutrient Requirements of Swine, 10th edn. National Academic Press, Washington, DC, USA.

Pastor, A., Wecke, C., Liebert, F. 2013. Assessing the age-dependent optimal dietary branched-chain amino acid ratio in growing chicken by application of a nonlinear modeling procedure. Poult. Sci. 92:3184–3195. doi:10.3382/ps.2013-03340.

Pastorelli, H., van Milgen, J., Lovatto, P., Montagne, L. 2012. Meta-analysis of feed intake and growth responses of growing pigs after a sanitary challenge. Animal. 6:952-961.

Patz, J. A., Graczyk, T. K., Geller, N., Vittor, A. Y. 2000. Effects of environmental change on emerging parasitic diseases. Int. J. Parasitol. 30: 1395–1405, 2000.

Pedersen, S., Sallvik, K. 2002. 4th report of working group on climatization of animal houses. Heat and moisture production at animal and house levels. International Commission of Agricultural Engineering, Section II. Research Centre Bygholm, Danish Institute of Agricultural Sciences. www.agrisci.dk/jbt/spe/CIGReport.

Persen, H. H., Nielsen, J. P., Heegaard, P. M. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res., 35:2 (2004) 163-187. doi:10.1051/vetres:2004002.

Petry, A., Mc Gilvray, W., Rakhshandeh, A. R., Rakhshandeh, A. 2017. Technical note: Assessment of an alternative technique for measuring body temperature in pigs. J. Anim. Sci. 95:3270–3274. doi:10.2527/jas2017.1566.

Rakhshandeh, A. & De Lange, C. F. M. 2011. Immune system stimulation in the pig: effect on performance and implications for amino acid nutrition. In: R. J. Van Barnevled, editor. Manipulating pig production XIII. Australasian Pig Science Association Incorporation, Werribee, Victoria, Austrilia. pp 31–46.

Rakhshandeh, A., Htoo, J. K., Karrow, N., Miller, S. P., De Lange, C. F. M. 2014. Impact of immune system stimulation on the ileal nutrient digestibility and utilisation of methionine plus cysteine intake for whole-body protein deposition in growing pigs. Br. J. Nutr. 111:101–110. doi:10.1017/S0007114513001955.

Reeds, P. J. & Jahoor, F. 2001. The amino acid requirement of disease. Clin. Nutr. 20:15–22. doi: 10.1054/clnu.2001.0402.

Rostagno, H. S., Albino, L. F. T., Donzele, J. L. et al. 2017. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4rd edition, Viçosa: UFV. 488 p.

Sakomura, N. K. & Rostagno, H. S. 2016. Métodos de pesquisa em nutrição de monogástricos. Editor FUNEP, Jaboticabal. 2 ed.

Samadi; Liebert, F. 2006a. Estimation of nitrogen maintenance requirements and potential for nitrogen deposition in fast-growing chickens depending on age and sex. Poultry Science. 85(8):1421-1429.

Samadi; Liebert, F. 2006b. Modeling of threonine requirement in fast- growing chickens, depending on age, sex, protein deposition, and dietary threonine efficiency. Poultry Science. 85(11):1961- 1968.

Samadi; Liebert, F. 2007. Lysine requirement of fast growing chickens - effect of age, sex, level of protein deposition and dietary lysine efficiency. J. Poult. Sci. 44:63–72. doi:10.1093/ps/86.6.1140.

Samadi; Liebert, F. 2008. Modelling the optimal lysine to threonine ratio in growing chickens depending on age and efficiency of dietary amino acidutilization. Br. Poult. Sci. 49:45–54. doi:10.1080/00071660701821667.

SAS - Statistical analyses system. Statistical analisys system user's guide. Version 9.4. Cary:Statistical Analysis System Institute.

Vier, C. M., De Souza, I. B., De Jong, J. A., Goncalves, M. A. D., Jones, A. M., Goodband, R. D., Tokach, M. D., Derouchey, J. M., Woodworth, J. C., Dritz, S. S. 2016. Determining the standardized ileal digestible lysine requirement of 6.8 to 15.9 kg pigs. J. Anim. Sci. 94 (suppl_2) 191 (Abstr). doi: 10.2527/msasas2016-408.

Wecke, C., Liebert, F. 2009. Lysine requirement studies in modern genotype barrows dependent on age, protein deposition and dietary lysine efficiency. J. Anim. Physiol. Anim. Nutr. 93:295–304. doi:10.1111/j.1439-0396.2009.00923.x.

Williams, N. H. et al. 1997. Effect of chronic immune system activation on the rate, efficiency, and composition of growth and lysine needs of pigs fed from 6 to 27 kg. J. Anim.Sci. 75: 2463–2471. doi:10.2527/1997.7592463x.

Figure Legends

Figure 1. Interactive effects of nitrogen levels × challenge status × time (hours after first challenge) for serum IgG concentrations from growing pigs challenged with *E. coli* lipopolysaccharide (LPS). [1] Nitrogen levels (0.94 to 3.97), % N as fed.[2] Polynomial contrasts: Linear effect for control group in times 3 hours ($P = 0.0006$), $y = -0.4686x + 7.15$, $R^2 = 86\%$ and 96 hours ($P = 0.0059$), $y = -0.3646x + 7.466$, $R^2 = 88\%$.[3] LPS was used to induce inflammatory response in challenged groups and consist of initial dose of 30 µg / kg IM and a subsequent dose of 33.6 µg / kg IM after 48 hours. The control group received saline solution (0.9%, IM). [A-B] Different superscripts capital letters indicate a difference between times (3 hours × 96 hours) using the Tukey test ($\alpha = 5\%$).[a-b] Different superscripts letters indicate differences for challenge status (control × LPS-challenged) by the Tukey test ($\alpha = 5\%$).

Figure 2. Interactive effects of nitrogen levels × time (hours after first challenge) for albumin, haptoglobin, transferrin and total protein serum concentrations from growing pigs challenged with *E. coli* lipopolysaccharide (LPS).[1] Nitrogen levels (0.94 to 3.97), % N as fed. [2] LPS was used to induce inflammatory response in challenged groups and consist of initial dose of 30 µg / kg IM and a subsequent dose of 33.6 µg / kg IM after 48 hours. The control group received saline solution (0.9%, IM).[3] Polynomial contrasts: **A** - Linear effect for albumin at 96 hours ($P < 0.0001$), $y = 0.7882x + 29.518$, $R^2 = 67\%$; **B** - Linear effect for haptoglobin at 3 hours ($P = 0.0467$), $y = 0.03x + 0.4147$, $R^2 = 45\%$ and 96 hours ($P = 0.0002$), $y = 0.0588x + 0.523$, $R^2 = 87\%$; **C** - Linear effect for total protein at 3 hours ($P = 0.0248$), $y = 0.4849x + 45.221$, $R^2 = 53\%$ and 96 hours ($P < 0.0001$), $y = 1.008x + 46.592$, $R^2 = 78\%$; **D** - Quadratic effect for transferrin in 3 hours ($P < 0.0001$), $y = 0.0228x^2 - 0.0102x + 3.5785$, $R^2 = 91\%$.[a-b] Different superscripts letters indicate differences between nitrogen levels by the Tukey test ($\alpha = 5\%$).

Figure 3. Interactive effects of challenge status × rectal temperature (°C) for growing pigs challenged with *E. coli* lipopolysaccharide (LPS).[1] Measurements carried out at 7:00 am (LPS-d1 e LPS-d3 and LPS-d5) before the challenge and at 3:00 pm (LPS-d1+3h and LPS-d3+3h) 3 hours after LPS injections.[2] LPS was used to induce inflammatory response in challenged groups and consist of initial dose of 30 µg / kg IM and a subsequent dose of 33.6 µg / kg IM after 48 hours. The control group received saline solution (0.9%, IM). [A-B] Different superscripts capital letters can differentiate the challenge status (control and LPS-challenged) between the times (3 hours and 96 hours after first LPS challenge) using the Tukey test (α = 5%).[a-b] Different superscripts letters indicate differences between nitrogen levels within each challenge status by the Tukey test (α = 5%).

Figure 4. Interactive effects of nitrogen levels × rectal temperature (°C) for growing pigs challenged with *E. coli* lipopolysaccharide (LPS).[1] Nitrogen levels (0.94 to 3.97), % N as fed.[2] LPS was used to induce inflammatory response in challenged group. The challenge consisted of an initial LPS dose of 30 µg / kg IM and a subsequent dose of 33.6 µg / kg IM after 48 hours. The control group received saline solution (0.9%, IM).[3] Polynomial contrasts: Linear effect of N levels on the control group was observed ($P < 0.0001$), $y = 0.1243x + 37.122$, $R^2 = 79\%$.[A-B] Different superscripts capital letters indicate difference between control and challenged groups within nitrogen levels (0.94 to 3.97 %N, as fed), by the Tukey test (α = 5%).[a-b] Different superscripts letters indicate difference between nitrogen levels for each group (control and LPS-challenged) by the Tukey test (α = 5%).

Figure 5. Estimation of the nitrogen requirements for maintenance (NMR) by fitting an exponential function between the nitrogen intake (NI) and nitrogen excretion (NEX) during a gradual increase in supplied protein limited in lysine for growing pigs (Sire line 337 x Dam line Camborough, PIC) challenged with *E. coli* lipopolysaccharide (LPS). Observed (♦) and predicted (—) values for control group. Observed (□) and predicted (-----) values for challenged group.[1] Nitrogen intake (NI) and nitrogen excretion (NEX) were obtained in nitrogen balance trials of 11-days. The nitrogen balance data were used in the non-linear model established by the University of Goettingen.[2] LPS was used to induce inflammatory response in challenged group. The challenge consisted of an initial LPS dose of 30 µg / kg IM and a subsequent dose of 33.6 µg / kg IM after 48 hours. The control group received saline solution (0.9%, IM).

Figure 6. Estimation of the theoretical potential for nitrogen deposition ($ND_{max}T$) in growing pigs (Sire line 337 x Dam line, Camborough, PIC) challenged with *E. coli* lipopolysaccharide (LPS), based on the ratio of nitrogen intake (NI) and nitrogen deposition (ND). Observed (♦) and predicted (—) values for control group. Observed (□) and predicted (-----) values for challenged group.[1] Nitrogen intake (NI) and nitrogen excretion (NEX) were obtained in nitrogen balance trials of 11-days. The ND was calculated as a result of the difference between N intake and N excretion, ND = NI – NEX (mg/ $BW_{Kg}^{0.75}$ / d). The nitrogen balance data were used in the non-linear model established by the University of Goettingen.[2] LPS was used to induce inflammatory response in challenged group. The challenge consisted of an initial LPS dose of 30 µg / kg IM and a subsequent dose of 33.6 µg / kg IM after 48 hours. The control group received saline solution (0.9%, IM).

Table 1. Composition of the concentrated diet (3.97% N) and the protein-free diet (PFD), as fed-basis.

Ingredients	**27.0% CP**	**PFD - Dilution diet[1]**
Corn, 7.88 %	40.73	-
Soybean meal, 45 %	47.38	-
Corn gluten, 60 %	3.00	-
Sugar	2.00	3.00
Starch	-	82.15
Soy oil	1.909	2.500
Dicalcium phosphate	1.257	1.962
Calcitic limestone	0.791	0.661
Salt	0.389	0.434
Sodium bicarbonate	-	0.103
Potassium carbonate	-	1.767
DL-Methionine, 99 %	0.163	-
L-Lysine HCl, 79 %	0.397	-
L-Threonine, 98 %	0.155	-
L-Valine, 96.5 %	0.009	-
Choline chloride, 60	0.100	0.100
Vitamin supplement[2]	0.125	0.125
Mineral supplement[3]	0.125	0.125
BHT[4]	0.010	0.010
Inert	1.453	7.053
Total	**100.00**	**100.00**
Calculated Composition		
Metabolizable energy, kcal/kg	3,230	3,230
Crude protein, %	27.00	-
Available phosphorus, %	0.363	0.363
Calcium, %	0.733	0.733
Chlorine, %	0.259	0.259
Sodium, %	0.200	0.200
Potassium, %	0.999	0.999
dEB[5], meq/kg	270.0	270.0
Digestible amino acids, %		
Lys	1.585	-
Met	0.533	-
Met+Cys	0.888	-
Thr	0.999	-
Trp	0.300	-
Arg	1.694	-
Val	1.094	-
Ile	1.060	-
Leu	2.146	-
Phe	1.232	-
Phe+Tyr	2.058	-
His	0.641	-

[1] PFD: Protein-Free Diet.

[2] Vitamin supplement containing per kg of feed: Vit. A-6875 U.I.; Vit. D3 - 1500 U.I.; Vit. E-40.0 U.I.; Vit. B1 - 1.00 mg; Vit. B2 - 3.13 mg; Vit. B6 - 2.00 mg; Vit. B12 - 0.020 mg; Pantothenic Acid - 15.0 g; Biotin - 0.100 mg; Vit. K3 - 3.00 mg; Folic Acid - 0.300 mg; Nicotinic acid - 30.0 mg.

[3] Mineral supplement containing per kg of feed: Iron - 80.0 mg; Copper - 12.0 mg; Manganese - 40.0 mg; Zinc - 110 mg; Iodine - 1.00 mg; Selenium - 0.36 mg.
[4] BHT: butylated hydroxytoluene
[5]dEB: dietary electrolyte balance

Table 2. Total amino acid content of the diets (N1 to N6), as fed-basis

	Diets[1]					
	N1	N2	N3	N4	N5	N6
Amino acids[2], %						
Aspartic acid	0.50	0.71	1.59	1.82	2.49	2.96
Glutamic acid	1.06	1.62	2.64	3.26	4.18	4.80
Serine	0.30	0.47	0.68	0.90	1.10	1.28
Glycine	0.28	0.43	0.64	0.81	1.01	1.16
Histidine	0.15	0.28	0.40	0.49	0.63	0.71
Arginine	0.42	0.69	1.00	1.25	1.55	1.78
Threonine	0.26	0.36	0.53	0.78	0.94	1.10
Alanine	0.31	0.49	0.71	0.90	1.13	1.29
Proline	0.33	0.61	0.86	1.10	1.35	1.58
Tyrosine	0.20	0.36	0.53	0.69	0.81	0.93
Valine	0.30	0.50	0.71	0.88	1.11	1.24
Methionine	0.12	0.20	0.29	0.35	0.38	0.45
Cystine	0.09	0.19	0.28	0.47	0.41	0.45
Isoleucine	0.28	0.47	0.67	0.84	1.06	1.18
Leucine	0.57	0.94	1.31	1.68	1.98	2.24
Phenylalanine	0.32	0.54	0.79	1.00	1.24	1.39
Lysine	0.46	0.69	0.98	1.30	1.54	1.78
Tryptophan	0.07	0.14	0.26	0.25	0.32	0.32
Sum of amino acids	6.02	9.69	14.87	18.77	23.23	26.62

[1] Nitrogen levels: N1: 0.94; N2: 1.69; N3: 2.09; N4: 3.04; N5: 3.23 and N6: 3.97 % N, as fed basis.
[2] Analysed composition of the amino acid in the experimental diets.

Table 3. Interactive effects of challenge status and time for serum proteins from growing pigs challenged with *E. coli* lipopolysaccharide[1]

Item[2]	Means, mg/mL							Interactions *P*-value							
								Challenge Status			Time				
	Control (CON)			LPS-Challenged (LPS)				CON x LPS	CON x LPS	CON x LPS	COM	LPS	CON	LPS	LPS x Time
	Time (hours)														
	0	3	96	0	3	96	SEM	0	3	96	3 x 96	3 x 96	0 x 3 x 96	0 x 3 x 96	
IgA	-	1.11	0.97	-	1.28	1.41	0.077	-	0.011	<0.01	0.077	0.077	-	-	0.001
Cer	-	0.48	0.47	-	0.51	1.04	0.029	-	0.535	<0.01	0.820	<0.01	-	-	<0.01
Trans	-	4.05	4.27	-	3.73	4.21	0.132	-	0.001	0.598	0.016	<0.01	-	-	0.006
Hp	-	0.47	0.55	-	0.57	0.91	0.046	-	0.039	<0.01	0.004	<0.01	-	-	<0.01
α1AGp	-	0.04	0.04	-	0.05	0.03	0.002	-	0.125	0.024	0.512	0.001	-	-	0.006
PM20000	-	1.60	1.84	-	1.52	1.38	0.085	-	0.331	<0.01	<0.01	0.012	-	-	<0.01
TP	48.1A	47.7Ba	50.3A	48.4A	46.2Bb	49.9A	0.710	0.695	0.036	0.569	-	-	<0.01	<0.01	0.045

[1] *E. coli* lipopolysaccharide (LPS) was used to induce inflammatory response in LPS-challenged group. The challenge consisted of an initial LPS dose of 30 µg / kg IM and subsequent dose of 33.6 µg / kg IM after 48 hours. The control group received saline solution (0.9%, IM).

[2] Serum proteins Immunoglobulin A (IgA), Ceruloplasmin (Cer), Transferrin (Trans), Haptoglobin (Hp), α-1-acid glycoprotein (α1AGp), PM 20000 and Total Protein (TP) was measured using SDS-PAGE.

[A-B] Different superscripts capital letters indicate a difference between time (3 hours × 96 hours after first LPS challenge) using the Tukey test (α = 5%).

[a-b] Different superscripts letters indicate differences between the challenge status (control × LPS-challenged) by the Tukey test (α = 5%).

Table 4. Average of feed intake (g/d) body weight (kg), nitrogen intake (mg/$BW_{kg}^{0.75}$/d), nitrogen excretion (mg/$BW_{kg}^{0.75}$/d) and nitrogen deposition (mg/$BW_{kg}^{0.75}$/d) obtained in nitrogen balance trials for growing pigs challenged with *E. coli* lipopolysaccharide[1]

	Nitrogen levels, % N as fed basis									
Variables measured	0.94	1.69	2.09	3.04	3.23	3.97	Average	SEM	Linear	Quadratic
Feed Intake[1]										
Control	411.9	417.2	419.1	418.8	413.3	426.6	417.8	9.70	0.532	0.917
LPS-Challenged	373.5	375.0	384.5	370.6	362.7	371.6	373.0	9.70	0.533	0.754
Body Weight										
Control	19.6	19.3	18.7	18.7	18.4	19.2	19.0	0.66	0.409	0.272
LPS-Challenged	19.4	19.0	18.8	18.7	18.7	19.1	18.9	0.66	0.272	0.661
Nitrogen Intake [2.3]										
Control	519.7	958.5	1216.0	1773.3	1883.0	2309.7	1443.3	24.82	<0.001	0.029
LPS-Challenged	473.2	871.2	1111.0	1569.7	1628.5	1976.0	1271.6	24.82	<0.001	0.001
*P*_value	0.306	0.057	0.023	<0.001	<0.001	<0.001				
Nitrogen Excretion [2.3]										
Control	184.1	309.3	407.3	545.9	483.9	783.2	452.3	36.23	<0.001	0.683
LPS-Challenged	246.0	369.5	478.2	626.7	848.6	884.9	575.6	36.23	<0.001	0.474
*P*_value	0.249	0.262	0.188	0.135	<0.001	0.061				
Nitrogen Deposition [2.3]										
Control	335.6	649.2	808.6	1227.3	1405.9	1526.5	992.2	42.13	<0.001	0.028
LPS-Challenged	227.2	501.7	632.8	943.1	771.4	1091.1	694.6	42.13	<0.001	<0.001
*P*_value	0.079	0.018	0.005	<0.001	<0.001	<0.001				

[1] Effect of challenge (P<0.01), *E. coli* lipopolysaccharide (LPS) was used to induce inflammatory response in LPS-challenged group. The challenge consisted of an initial LPS dose of 30 µg / kg IM and subsequent dose of 33.6 µg / kg IM after 48 hours. The control group received saline solution (0.9%, IM).

[2] Effect of N levels x challenge status interaction (P<0.05).

[3] Polynomial contrasts: Linear effect of N levels (P<0.01): **NI**, control NI=215.13 + 350.91 NL and LPS-challenged NI=246.87 + 292.80 NL (mg/$BW_{kg}^{0.75}$/d); **NEX**, control NEX= 86.27 + 104.54 NL and LPS-challenged NEX= 97.27 + 136.69 NL (mg/$BW_{kg}^{0.75}$/d); **ND**, control ND= 127.86 + 246.95 NL and LPS-challenged ND= 150.66 + 155.40 NL (mg/$BW_{kg}^{0.75}$/d).

Table 5. Model calculation of the lysine requirement (Lys) for growing pigs (Sire line 337 x Dan line Camborough, PIC) challenged with *E. coli* lipopolysaccharide[1], depending on the determined efficiency of lysine utilization and different predictions for feed intake.

Item	Control		LPS-Challenged	
$ND_{max}T$, mg/ $BW_{kg}^{0.75}$ / d	3524.7		2077.8	
$ND_{max}T$, %	68		54	
Protein deposition, g/d	136		64	
Lys efficiency, bc^{-1}	0.00003703		0.00005713	
Lys requirement, mg/ $BW_{kg}^{0.75}$/d	1994.83		949.16	
Lys requirement, g/d	18.12		8.62	
Optimal dietary lysine concentration				
	Feed intake[2] g/day	Digestible Lys, %	Feed intake[2] g/day	Digestible Lys, %
	1100	1.29	981	0.69
	1000	1.42	893	0.76
	900	1.58	803	0.84
	800	1.77	714	0.94

$ND_{max}T$ is the theoretical maximum nitrogen deposition.

[1] *E. coli* lipopolysaccharide (LPS) was used to induce inflammatory response in LPS-challenged group. The challenge consisted of an initial LPS dose of 30 µg / kg IM and subsequent dose of 33.6 µg / kg IM after 48 hours. The control group received saline solution (0.9%, IM).

[2] The feed intake was calculated based on the recommendations of Rostagno et al. (2017) for pigs of 20 kg. Feed intake was adjusted for the LPS-challenged group, considering the 10.73% reduction in feed intake.

Figure 1

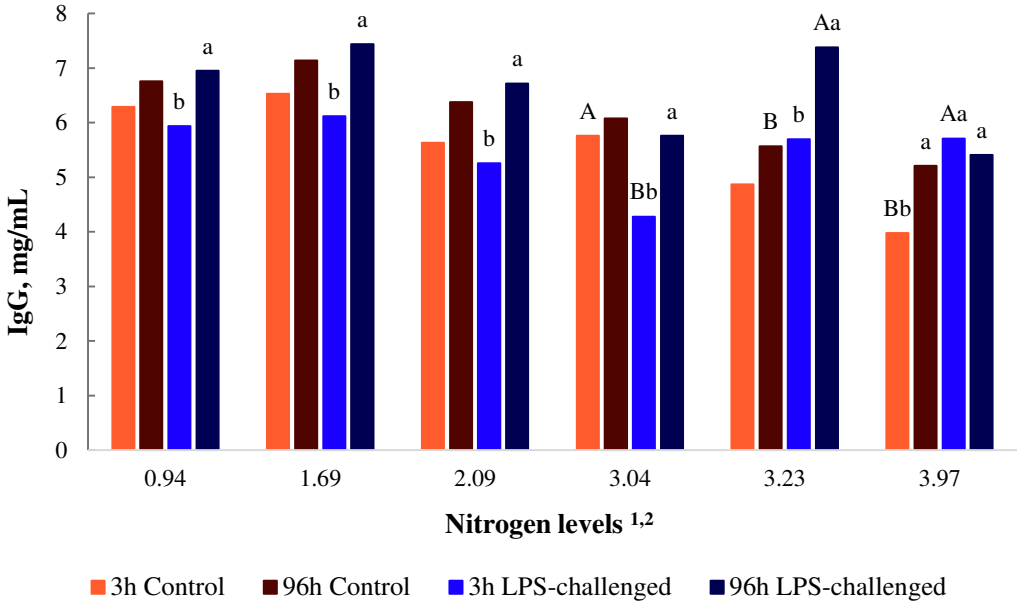

IgG, mg/mL
8
7
6
5
4
3
2
1
0
a
b
a
b
a
b
A
a
Bb
B
b
Aa
Bb
a
Aa
a
0.94
1.69
2.09
3.04
3.23
3.97
Nitrogen levels 1,2
3h Control
96h Control
3h LPS-challenged
96h LPS-challenged

Figure 2

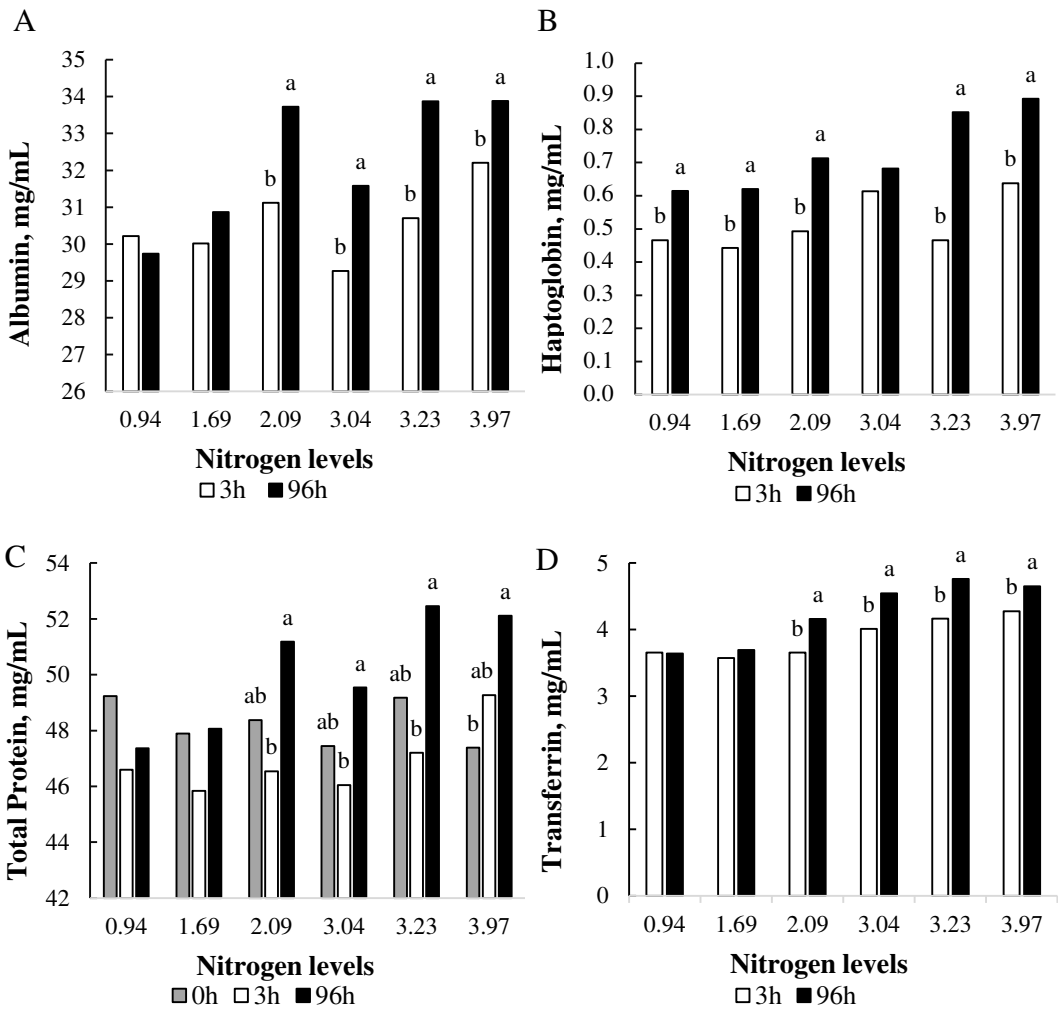

A
Albumin, mg/mL
35
34
33
32
31
30
29
28
27
26
0.94
1.69
2.09
3.04
3.23
3.97
Nitrogen levels
3h
96h
B
Haptoglobin, mg/mL
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Nitrogen levels
3h
96h
C
Total Protein, mg/mL
54
52
50
48
46
44
42
Nitrogen levels
0h
3h
96h
D
Transferrin, mg/mL
5
4
3
2
1
0
Nitrogen levels
3h
96h

Figure 3

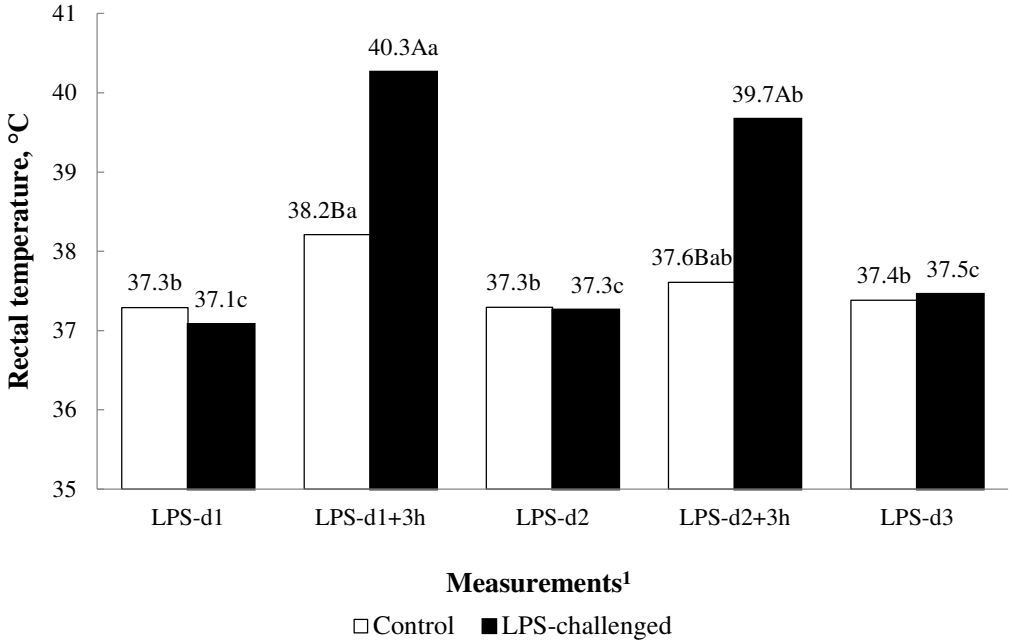

41
40
39
38
37
36
35
Rectal temperature, °C
37.3b
37.1c
38.2Ba
40.3Aa
37.3b
37.3c
37.6Bab
39.7Ab
37.4b
37.5c
LPS-d1
LPS-d1+3h
LPS-d2
LPS-d2+3h
LPS-d3
Measurements[1]
□Control ■LPS-challenged

Figure 4

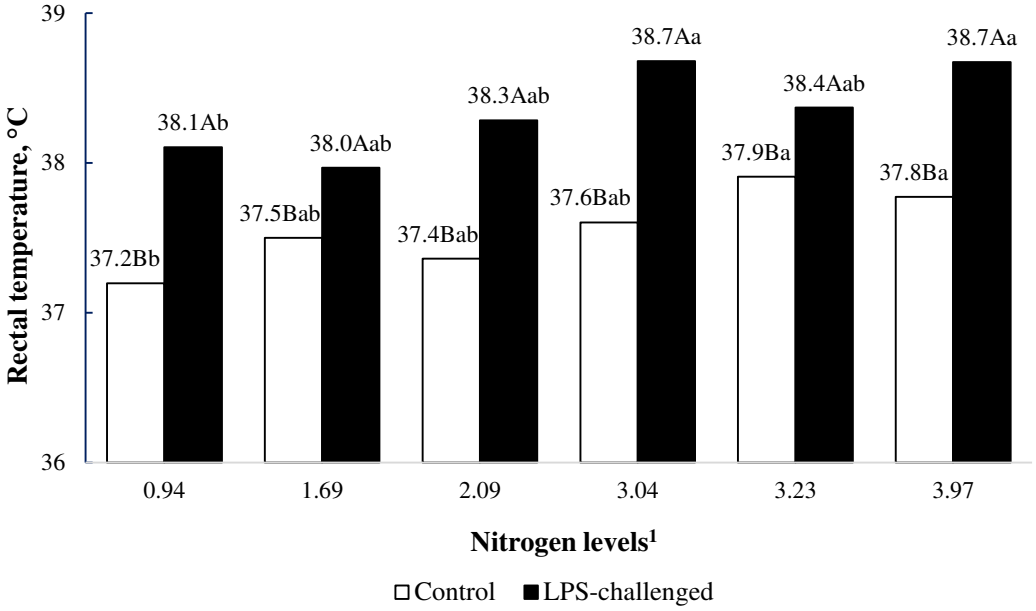

39
38
37
36
Rectal temperature, °C
37.2Bb
38.1Ab
37.5Bab
38.0Aab
37.4Bab
38.3Aab
37.6Bab
38.7Aa
37.9Ba
38.4Aab
37.8Ba
38.7Aa
0.94
1.69
2.09
3.04
3.23
3.97
Nitrogen levels[1]
Control
LPS-challenged

Figure 5

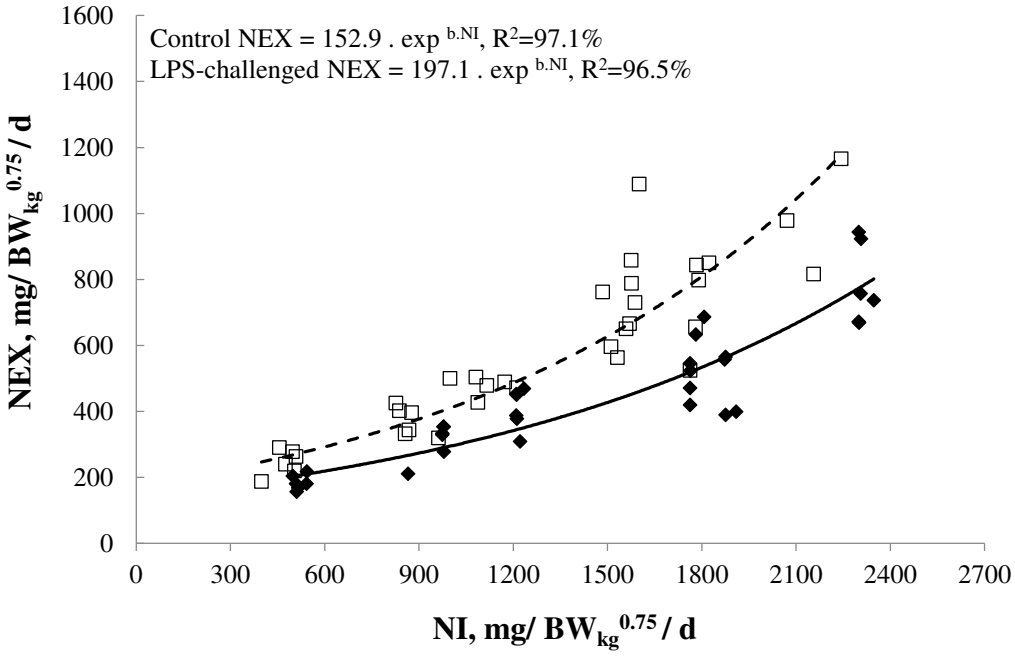
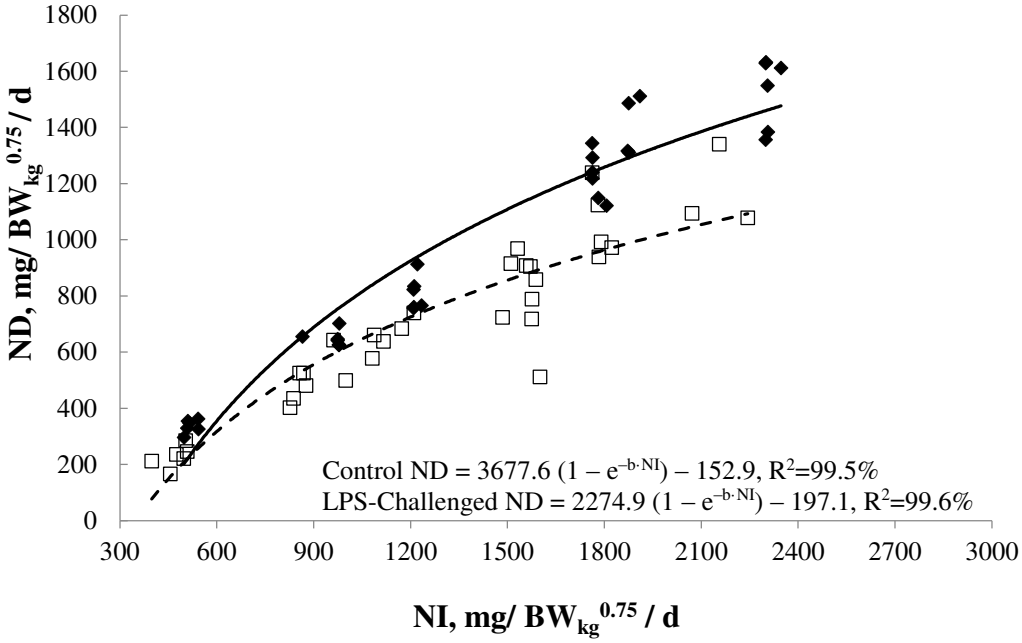

Control NEX = 152.9 . exp b.NI, R2=97.1%
LPS-challenged NEX = 197.1 . exp b.NI, R2=96.5%
NEX, mg/ BWkg 0.75 / d
NI, mg/ BWkg 0.75 / d
0
200
400
600
800
1000
1200
1400
1600
0
300
600
900
1200
1500
1800
2100
2400
2700

Figure 6


1800
1600
1400
1200
1000
800
600
400
200
0
300
600
900
1200
1500
1800
2100
2400
2700
3000
ND, mg/ BW$_{kg}^{0.75}$ / d
NI, mg/ BW$_{kg}^{0.75}$ / d
Control ND = 3677.6 (1 – e$^{-b \cdot NI}$) – 152.9, R^2=99.5%
LPS-Challenged ND = 2274.9 (1 – e$^{-b \cdot NI}$) – 197.1, R^2=99.6%