UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

AMINOÁCIDOS DE CADEIA RAMIFICADA PARA SUÍNOS NA FASE INICIAL

Autora: Laura Marcela Diaz Huepa
Orientador: Prof. Dr. Paulo Cesar Pozza
Coorientador: Prof. Dr. Ricardo Souza Vasconcellos

MARINGÁ
Estado do Paraná
Fevereiro 2017

UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

AMINOÁCIDOS DE CADEIA RAMIFICADA PARA SUÍNOS NA FASE INICIAL

Autora: Laura Marcela Diaz Huepa
Orientador: Prof. Dr. Paulo Cesar Pozza
Coorientador: Prof. Dr. Ricardo Souza Vasconcellos

"Tese apresentada como parte das exigências para obtenção do título de DOUTORA EM ZOOTECNIA, no Programa de Pós-graduação em Zootecnia da Universidade Estadual de Maringá - Área de concentração Produção Animal"

MARINGÁ
Estado do Paraná
Fevereiro 2017

Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá – PR, Brasil)

D542a

Diaz Huepa, Laura Marcela
Aminoácidos de cadeia ramificada para suínos na fase inicial / Laura Marcela Diaz Huepa. -- Maringá, PR, 2017.
86 f. : il. col. figs. tabs.

Orientador: Prof. Dr. Paulo Cesar Pozza.
Co-orientador: Prof. Dr. Ricardo Souza Vasconcellos.
Tese (doutorado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Programa de Pós-Graduação em Zootecnia, 2017.

1. Suínos - Aminoácidos. 2. Nutrição animal - Suínos. 3. Suínos - Dosagens sanguíneas. I. Pozza, Paulo Cesar, orient. II. Vasconcellos, Ricardo Souza, orient. III. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 23.ed. 636.4085

MRP-003571

UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

AMINOÁCIDOS DE CADEIA RAMIFICADA PARA SUÍNOS NA FASE INICIAL

Autora: Laura Marcela Diaz Huerpa
Orientador: Prof. Dr. Paulo César Pozza

TITULAÇÃO: Doutora em Zootecnia - Área de Concentração Produção Animal

APROVADA em 23 de fevereiro de 2017.

Prof. Dr. Paulo Levi de Oliveira Carvalho

Prof. Dr. José Mauricio Gonçalves dos Santos

Profa. Dra. Simara Márcia Marcato

Profª Drª Alice Eiko Murakami

Prof. Dr. Ricardo Souza Vasconcellos
(Co-orientador)

ALMA PERFUMADA

Tem gente que tem cheiro de colo de Deus.

De banho de mar quando a água é quente e o céu é azul.

Ao lado delas, a gente sabe que os anjos existem e que alguns são invisíveis.

Ao lado delas, a gente se sente chegando em casa e trocando o salto pelo chinelo.

Ao lado delas, a gente não acha que o amor é possível, a gente tem certeza.

Tem gente como você que nem percebe como tem a alma perfumada!

E que esse perfume é dom de Deus.

Carlos Drummond de Andrade.

A Deus e à Virgem Maria, pela vida, pela força espiritual, pelas bênçãos recebidas e por me guiarem neste caminho.

Aos meus pais, Jover Diaz Rojas e Esperanza Huepa Esquivel, pessoas que admiro e amo imensamente, pelo amor incondicional, pelo incentivo, pelo apoio nos momentos bons e difíceis, mesmo estando longe e pelas suas orações.

Ao meu irmão Andres Julian Diaz Huepa, por ser meu exemplo, pela sua incondicionalidade, pela sua preocupação e pelo apoio, e, por isso, o amo muito.

Ao meu sobrinho e afilhado, Samuel Andres Diaz Ardila, pela felicidade e amor que trouxe para nós, para mim, mesmo estando longe dele, amo muito você "mi chiquito".

À Fanny Huertas Urueña, pelo amor, seus conselhos e pelas suas orações. Obrigada, amo você.

À senhora Alice Fraga, por ser a mãe brasileira que tive a oportunidade de ter, pela companhia, conselhos e amor. Muito obrigada por tudo, sempre vou levar você no meu coração. Adoro você.

Ao meu namorado, Carlos Augusto Nantes do Castilho, pelo companheirismo, paciência e amor. Amo-te.

Ao Juan Jacobo Freyte Zarta (*In memoriam*), pelo carinho, pela amizade sempre o levaremos em nossos corações. Você sempre vai ser lembrado e querido. Saudades eternas Jacobito.

DEDICO

AGRADECIMENTOS

A Deus, pela vida, pelas experiências vividas e pela força que me permitiram chegar e me manter neste caminho.

À minha família, pela ajuda, pela confiança e pelas suas orações.

Às minhas tias, Jackeline Huepa, Lina Maria Huepa, Liliana Martinez, Blanca Huepa, Lucero Huepa, Marlene Huepa, Libaniel Huepa, pelas orações, pelo amor e carinho sempre.

Ao Programa de Pós-graduação em Zootecnia da Universidade Estadual de Maringá, pela oportunidade de realizar os meus estudos;

À embaixada do Brasil e ao Programa Estudante Convênio de Pós-graduação (PEC-PG), pela concessão da bolsa de doutorado;

Ao meu Orientador Prof. Paulo Cesar Pozza, pelo apoio e o trabalho constante.

Ao professor Ricardo Vasconcellos, pela coorientação, apoio e disposição para me ajudar;

Ao meu Orientador de mestrado Prof. Ivan Moreira, pelo apoio, pelos ensinamentos e o carinho.

Aos colegas do grupo de trabalho: Leandro Castilha, Marcelise Fachinello, Alessandra Monteiro, Silvia Leticia Ferreira, Tiago Junior Pasquetti, Lucas Esteves, Cleiton Sangali, Natalia Sitanaka, Camila de Araújo Moreira. Aos estagiários, Vinicius Cambito, Natalia Galoro, Isabella Leal, Bruno Campos, Bruno Cabrera, André Partyka, Leonardo Malavazi, Alcides Emanuel Rodrigues da Costa, Alexandre Gorte Ferreira. Pela ajuda e colaboração.

Aos funcionários da Fazenda Experimental de Iguatemi, Senhores João Salválagio, Carlos José (Huk), Toninho (Fábrica de ração), Paulo Jesus de Mello, Nelson Palmeiras e Mauro dos Santos;

Aos funcionários do Laboratório de Análise de Alimentos e Nutrição Animal (LANA): Oswaldo Tarelho Junior e Creuza de Azevedo, pelo auxílio na realização das análises.

Às minhas grandes amigas, Angélica Maria Cardona Perez, Ingrid Johanna Cardenas Rey, Edna Constanza Gomez Victoria, Paula Andrea Garzón Charry, Carolyn Diaz, Yohanna Mora, Pilar Rodriguez, Maribel Velandia, Joyce Sato, Lina Maria Peñuela Sierra, Juliana Toledo, Lina Fernanda Rodriguez, Alessandra Monteiro pela amizade, suas palavras de ânimo, pelas risadas compartilhadas por estar sempre perto, mesmo estando longe, adoro muito vocês.

Aos meus amigos Juan Camilo Guty, Orion Becker, Brian Alexander Effer, Alberto Lorente Saiz, Jairo Andres Pardo Guzman, Andres Pachón, Ana Paula Petri, João Salvalagio Rodrigues; pela amizade e pelo carinho; pela ajuda na fazenda e fora dela, pela força e apoio nos momentos difíceis, agradeço.

À Senhora Maria Carbonari Pasquetti, pelo carinho sincero, a preocupação comigo e suas atenções.

E a todos que me ajudaram a alcançar esta etapa com êxito.... Muito Obrigada!!!

BIOGRAFIA DA AUTORA

LAURA MARCELA DIAZ HUEPA, filha de Jover Diaz Rojas e Esperanza Huepa Esquivel, nasceu na cidade de Ibagué, Departamento do Tolima, Colômbia, no dia 28 de abril de 1985.

Em 2005 iniciou seus estudos em Medicina Veterinária e Zootecnia na Universidad del Tolima (Ibagué – Colômbia); formou-se no mês de dezembro do ano 2010.

Em 2011, iniciou no Programa de Pós-graduação em Zootecnia, em nível de mestrado, área de concentração Produção Animal, na Universidade Estadual de Maringá, realizando estudos na área de Nutrição de Não Ruminantes. Submeteu-se para defesa da dissertação no mês de março de 2013.

Em março de 2013 iniciou seus estudos no Programa de Pós-graduação em Zootecnia, em nível de doutorado, área de concentração Produção Animal, na Universidade Estadual de Maringá, realizando estudos na área de Nutrição de Não Ruminantes. Submeteu-se ao exame geral de qualificação da tese no mês de agosto de 2016.

Submeteu-se para defesa da tese no dia 23 de fevereiro de 2017.

ÍNDICE

LISTA DE TABELAS

LISTA DE FIGURAS

RESUMO

Foram realizados três experimentos, com o objetivo de avaliar a exigência de aminoácidos de cadeia ramificada (leucina, valina e isoleucina digestíveis) sobre as variáveis de desempenho e parâmetros sanguíneos de suínos machos castrados e fêmeas (Landrace x Large white x Pietrain), na fase inicial. No experimento I, foram avaliados cinco níveis de leucina digestível (1,10; 1,25; 1,40; 1,55 e 1,70%) sobre o desempenho, características quantitativas de carcaça *in vivo* e parâmetros sanguíneos de suínos na fase inicial. Foram utilizados 50 suínos mestiços, com peso inicial de 11,14 ± 0,24 kg, distribuídos em um delineamento experimental de blocos casualizados, com cinco repetições e dois animais por unidade experimental (UE), sendo um macho e uma fêmea. A conversão alimentar reduziu (P=0,018) em função do aumento dos níveis de leucina digestível, sem influenciar os demais parâmetros de desempenho (peso final, consumo diário de ração, ganho de peso diário, espessura de toucinho, profundidade de lombo e porcentagem de carne magra). Com relação às variáveis plasmáticas foi observado efeito quadrático para a concentração de triglicerídeos (P=0,049) e ureia (P=0,001). Ajustando o modelo quadrático associado ao platô do modelo Linear Resposta Platô (LRP) observou-se que o nível ótimo de leucina digestível para triglicerídeos e ureia foi de 1,16% e 1,24%, respectivamente. A menor concentração de ureia plasmática foi obtida ao nível de 1,24% de leucina digestível. Dietas com baixa proteína bruta e níveis de até 1,70% de leucina digestível não influenciaram o desempenho e este nivel melhorou a conversão alimentar. No experimento II, foram utilizadas 72 fêmeas suínas mestiças, com peso inicial de 15,16 ± 1,15 kg, distribuídos em um delineamento experimental de blocos casualizados, em um esquema fatorial 2 x 4, sendo dois níveis de leucina digestível (1,20 e 1,77%) e quatro níveis de valina digestível (0,58; 0,73; 0,88 e 1,03%), com nove repetições e um animal por UE. O peso final (PF) e o ganho de peso diário (GPD)

apresentaram efeito linear (P=0,017 e P=0,034, respectivamente) e quadrático (P=0,003 e P=0,0003, respectivamente). Ajustando os dados ao modelo quadrático associado ao platô do modelo Linear Response Platô (LRP), o nível ótimo de valina digestível para PF e GPD foi de 0,725% e de 0,703%. Foi observado efeito quadrático (P=0,0007) para o consumo diário de ração (CDR), sendo que o nível ótimo de valina digestível foi de 0,822%. A espessura de toucinho (ET), profundidade do músculo *Longissimus dorsi* (PL) e porcentagem de carne magra (%CM) não foram influenciadas pelos níveis de leucina ou valina digestível. Houve interação (P=0,047) entre os níveis de leucina (1,70%) e valina (1,03%) digestível sobre a ureia (11,50 mg/dL). A exigência de valina digestível para suínos na fase inicial não é influenciada pelos níveis de leucina normalmente praticados em dietas convencionais e a exigência diária é de 9,72 g de valina/dia, correspondendo a uma concentração de 0,703% na dieta, para um máximo ganho de peso diário. No experimento III, foram utilizados 56 suínos mestiços com peso inicial de 15,81 ± 1,00 kg, distribuídos em um delineamento experimental de blocos casualizados, em um esquema fatorial 2 x 4, sendo dois níveis de leucina digestível (1,10 e 1,70%) e quatro níveis de isoleucina digestível (0,45, 0,60, 0,75 e 0,90%), com sete repetições e um animal por UE. As variáveis de desempenho CDR, GPD, CA, ET e %CM não foram influenciadas pelos níveis de leucina e isoleucina digestíveis. A PL apresentou efeito linear (P=0,011) e quadrático (P=0,034). Ajustando os dados ao modelo quadrático associado ao platô do modelo (LRP), o nível ótimo de isoleucina digestível foi de 0,609%. Não houve interação entre os níveis de leucina e isoleucina digestível para as variáveis plasmáticas, no entanto, foi observado efeito da leucina sobre a concentração plasmática de creatinina (P=0,027), sendo que o nível de 1,70% apresentou a menor concentração desta variável (0,96 U/L). A exigência de isoleucina digestível para fêmeas suínas na fase inicial não é influenciada pelos níveis de leucina normalmente praticados em dietas comerciais, e o nível ótimo encontrado para a melhor profundidade do músculo *longissimus dorsi* foi de 0,609% de isoleucina digestível na dieta, correspondendo a um consumo diário de 8,94 g de isoleucina.

Palavras chave: Leucina, isoleucina, valina, consumo diário de ração, ganho de peso diário, conversão alimentar, variáveis plasmáticas.

ABSTRACT

Three experiments were conducted in order to assess the requirement of standardized ileal digestible (SID) branched chain amino acids (SID leucine, valine and isoleucine) on the performance variables and blood parameters of barrows and gilts in the initial phase. In Experiment I, five levels of SID leucine were evaluated on performance, quantitative characteristics of carcass *in vivo* and blood parameters of barrows and gilts in the initial phase. A total of 50 piglets (barrows and gilts) were used, with initial weight of 11.14 ± 0.24 kg; distributed in a randomized complete block design with five levels of SID leucine (1.10; 1.25; 1.40; 1.55 e 1.70% respectively), with five replicates and two animals per experimental unit (EU), a barrow and a gilt. The feed conversion ratio (FCR) reduced (P=0,018) due to the increase of SID leucine levels without affecting other performance parameters like final weight, average daily feed intake (ADFI), average daily gain (ADG), backfat thickness (BT), loin depth (LD) and lean mean percentage (LMP). With respect to serum parameters a quadratic effect was observed for triglyceride (P=0,049) and urea (P=0,001). Setting data to the quadratic model associated with the linear response plateu model (LRP), the optimal levels of SID leucine to triglycerides and urea were 1.16% and 1.24%, respectively. Diets with low crude protein and levels up to 1.70% SID leucine did not impair performance and this level provided the best feed conversion ratio. In Experiment II, a total of 72 gilts, with initial weight of 15.16 ± 1.15 kg; were distributed in a randomized complete block in a factorial design 2 x 4, and two levels of SID leucine (1.20 and 1.77%) and four levels of SID valine (0.58; 0.73; 0.88 and 1.03%) with nine replicates and one animal per EU. The final weight (FW) and the average daily gain (ADG) have a linear effect (P=0.017 and P=0.034) and quadratic effect (P=0,003 e P=0.0003, respectively). Setting data to the quadratic model associated with the linear response plateu model (LRP), the optimal level of SID valine for FW and ADG was 0.725% and 0.703%, respectively. A quadratic effect (P=0.0007) was observed for the

average daily feed intake (ADFI), and the optimal level of SID valine was 0.822%, without affecting other performance parameters (backfat thickness, loin depth and lean mean percentage). There was interaction (P=0,047) between the levels of SID leucine (1.70%) and SID valine (1.03%) on plasma urea (11.50 mg/dL). The requirement of SID valine for pigs in the initial phase is not influenced by the leucine levels normally practiced in conventional diets and the daily requirement is 9.72 g SID valine/day, corresponding to a concentration of 0.703% in the diet, to a maximum ADG. In Experiment III, a total of 56 pigs, with initial weight of 15.16 ± 1.15 kg; were distributed in a randomized complete block in a factorial design 2 x 4, two levels of SID leucine (1.10 and 1.70%) and four levels of SID isoleucine (0.45, 0.60, 0.75 and 0.90%) with seven replicates and one animal per experimental unit (EU). The performance variables ADFI, ADG, FCR, BT and LMP were not influenced by SID leucine and SID isoleucine. LD had linear (P=0.011) and quadratic (P=0,034) effects. Setting data to the quadratic model associated with the linear response plateu model (LRP), the optimal level of SID isoleucine was 0.609%. There was no interaction between the levels of SID leucine and SID isoleucine for the plasma variables, however, for SID leucine an effect on the plasma creatinine concentration (0.027) was observed with 1.70% presenting the lowest concentration (9.96 U/L) of creatinine. The requirement for SID isoleucine for pigs in the initial phase is not influenced by the leucine levels normally practiced in commercial diets, and the optimum level found for the best depth of the *longissimus dorsi* muscle was 0.609% SID isoleucine in the diet and the daily requirement is 8.94 g SID isoleucine/day.

Keywords: Leucine, isoleucine, valine, average daily gain, feed conversion ratio, serum parameters.

I – INTRODUÇÃO

O conhecimento das exigências nutricionais é fundamental para oferecer aos animais as quantidades adequadas de nutrientes para seu ótimo desenvolvimento e produtividade. As pesquisas realizadas nesta área permitem garantir um melhor desenvolvimento da espécie animal estudada; assim, a oferta de nutrientes deve ser planejada com o objetivo de otimizar o desempenho reprodutivo e produtivo, mantendo uma adequada condição corporal dos animais para garantir um produto de qualidade para o consumo humano.

Para alcançar estes objetivos, são necessários ajustes precisos nos requerimentos nutricionais dos animais e na estratégia de alimentação, principalmente se o foco de estudo for a exigência de aminoácidos (AA). Pesquisas demonstraram a importância da concentração de proteína bruta (PB) e dos AA industriais, indispensáveis para uma formulação mais precisa, com base no conceito da proteína ideal (Richardson et al., 1965; Oestemer et al., 1973; Gomez et al., 2002; Zangeronimo et al., 2006; Lordelo et al., 2008; Gloaguen et al., 2014).

Assim, a eficiência no aproveitamento dos ingredientes proteicos pelos não ruminantes depende da quantidade, da composição e da digestibilidade dos AA, de modo que as especificidades do aporte desses nutrientes sejam supridas.

Os AA têm grande importância fisiológica, servindo como blocos de construção para proteínas e substratos para a síntese de substâncias de baixo peso molecular. Animais em crescimento (como suínos e aves) precisam de AA para formação do tecido muscular (proteína corporal). Assim, além da construção de blocos de proteínas, os AA também são necessários para diversas funções corporais, como o desenvolvimento de órgãos e ótimo funcionamento do sistema imunológico. Além disso, o conteúdo de proteína e AA deve ser considerado na formulação de rações para melhorar a eficiência de utilização dos

nutrientes, o crescimento e o desenvolvimento, a reprodução, a lactação e o bem-estar dos animais (Wu et al., 2014).

Os aminoácidos de cadeia ramificada (ACR), valina, isoleucina e leucina (Figura 1), fazem parte do grupo de AA essenciais necessários para mantença e crescimento tecidual. Os ACR caracterizam-se por sua estrutura de cadeias laterais ramificadas, sendo oxidados como combustível principalmente pelos tecidos muscular, adiposo, renal e encefálico (Nelson & Cox, 2011). Além disso, por serem AA semelhantes em estrutura, compartilham as mesmas enzimas para suas reações de transaminação e descarboxilação oxidativa (Harper et al. 1984).

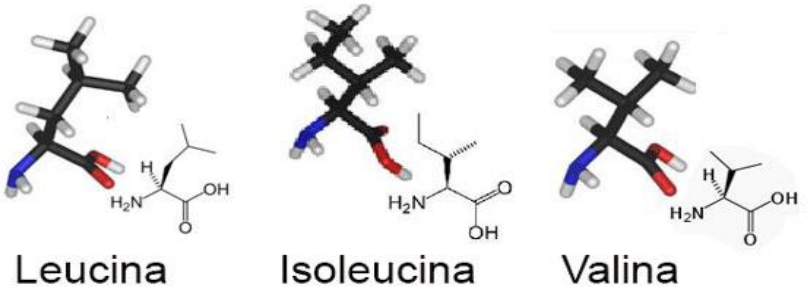


Figura 1. Estrutura dos aminoácidos de cadeia ramificada (ACR) (Adaptado de Nelson & Cox, 2011)

A primeira etapa de metabolização dos ACR ocorre no músculo esquelético (transaminação reversível), sendo comum para a leucina, valina e isoleucina. O α-cetoácido formado pode ser utilizado para a ressíntese dos ACR no fígado, ou passar por uma descarboxilação oxidativa irreversível, levando à formação de derivados da CoA (Nelson & Cox, 2011). Como os ACR compartilham as primeiras reações do seu metabolismo, vários trabalhos demonstraram que quantidades excessivas de leucina na dieta reduzem a disponibilidade de valina e isoleucina (Oestemer et al., 1973; Harper et al. 1984; Shimomura & Harris, 2006).

Apesar deste efeito da leucina sobre as concentrações de valina e isoleucina, estudos demostraram que a administração de leucina pode estimular a síntese proteica de leitões por meio da ativação da sinalização dos componentes que conduzem à tradução do mRNA (RNA mensageiro; Escobar et al., 2005), ou seja, os ACR, principalmente a leucina, têm a capacidade de iniciar as vias de tradução de sinal do mRNA em proteína, estimulando também a síntese de proteínas por meio da associação do complexo ativo

eIF4E (fator de transdução eucariótico). Os efeitos estimuladores de leucina na iniciação da tradução são mediados, em parte, através do alvo da proteína quinase da rapamicina em mamíferos (mTOR), onde tanto a sinalização da insulina e leucina convergem para promover uma resposta máxima à deposição muscular (Anthony et al., 2001).

O mecanismo pelo qual a leucina e insulina promovem a síntese e inibem a degradação proteica está relacionado ao fato do que o aminoácido aumenta sua concentração intracelular (Mata & Navarro, 2009) e aquele hormônio polipeptídico é estimulado pelo consumo de alimento, causando uma diminuição na degradação de proteínas, maior captação de AA pela célula e aumento da síntese proteica (Cabral et al., 2012).

Este fenômeno se caracteriza como o "paradoxo da leucina", pois este AA apresenta importante estímulo sobre a síntese proteica, devido a seu efeito sobre a tradução do mRNA (Nelson & Cox, 2011), ao passo que seu excesso, em dietas restritas em proteína, pode comprometer o aproveitamento dos outros ACR (Harper et al. 1984).

Os avanços obtidos nos últimos anos na área de nutrição animal proporcionaram a melhora no desempenho dos animais e a redução da contaminação ambiental, devido à formulação de rações baseadas no conceito de proteína ideal, a suplementação de AA industriais e a baixa porcentagem de PB nas dietas, diminuindo a excreção de nitrogênio (N) ao ambiente. Estima-se que a cada 1% de redução da PB em rações para suínos pode-se diminuir a excreção de N e de amônia em até 8% (NRC, 2012). Segundo Soumeh et al. (2015), a redução na porcentagem de PB na dieta e o aporte de um perfil mais equilibrado de AA ao animal, que satisfaça as exigências para mantença e crescimento, permite melhorar a utilização do N, reduzindo a sua excreção e contaminação ambiental, sem influenciar o desempenho do animal.

Apesar do benefício relacionado ao uso de dietas de baixa PB, quantidades excessivas de leucina em dietas de baixa PB têm deprimido o crescimento, a ingestão de alimento e reduzido a associação de valina e isoleucina (Harper et al., 1984). Sendo esses efeitos minimizados quando suplementadas quantidades de valina e isoleucina (D'Mello & Lewis, 1970; Gloaguen et al., 2012; Soumeh et al., 2015).

Desta forma, as pesquisas relacionadas com AA essenciais, e neste caso os ACR, são de grande importância na nutrição animal, pois eles influenciam diretamente funções fisiológicas de importância produtiva, otimizando a deposição proteica em suínos,

melhorando variáveis de produtividade e garantindo um alimento de qualidade para a sociedade.

1.1. Metabolismo dos Aminoácidos de Cadeia Ramificada

As vias de catabolismo dos AA, tomadas em conjunto, normalmente representam apenas 10 a 15% da produção de energia no organismo humano, sendo menos ativas que a glicose e a oxidação dos ácidos graxos. O fluxo ao longo das vias catabólicas também varia muito, dependendo do equilíbrio entre as necessidades para processos biossintéticos e a disponibilidade de um determinado aminoácido (Nelson & Cox, 2011).

Os AA que estão em excesso, em relação às necessidades para mantença e produção, são catabolizados, sendo o fígado o principal órgão responsável pela sua excreção. No entanto, os ACR que têm estruturas semelhantes são catabolizados principalmente no músculo esquelético e no tecido renal, compartilhando o mesmo sistema de transporte pela membrana celular e utilizando as mesmas enzimas para a degradação e, em parte, as mesmas vias metabólicas (Harper, 1984; Sakomura et al. 2014).

Apesar do fígado não catabolizar diretamente os ACR, o mesmo apresenta um sistema muito ativo para a degradação dos cetoácidos de cadeia ramificada, que são os produtos metabólicos dos ACR. A primeira etapa do catabolismo dos ACR é uma transaminação reversível pela isoenzima ATACR (aminotransferase de aminoácidos de cadeia ramificada) e a segunda etapa acontece pelo complexo enzimático desidrogenase de cetoácidos de cadeia ramificada (DCCR), responsável pela descarboxilação oxidativa dos cetoácidos de cadeia ramificada numa reação irreversível (Shimomura & Harris, 2006).

Na primeira fase do catabolismo, a isoenzima ATACR, que é dependente de piridoxal-fosfato, é responsável por aceitar os três ACR como substratos. Em células de mamíferos, duas ATACR estão presentes, sendo uma mitocondrial e outra citosólica. A partir da reação catalisada pela ATACR, os ACR são convertidos nos seus respectivos cetoácidos. A leucina é convertida em α-cetoisocaproato (KIC), a isoleucina em α-ceto-β-metilvalerato (KMV) e a valina em α-cetoisovalerato (KIV) (Rogero & Tirapegui, 2008). Ao mesmo tempo ocorre a transferência de um grupamento amino do aminoácido para o α-cetoglutarato, sendo transformado em glutamato, e a partir deste pode ocorrer a síntese de outros AA, como alanina e glutamina. Desse modo, a transaminação dos ACR

fornece mecanismos para transferir o N destes AA de acordo com a necessidade do tecido por glutamato e outros aminoácidos não-essenciais (Shimomura & Harris, 2006).

Após a ação da ATACR e a formação dos cetoácidos de cadeia ramificada, acontece uma descarboxilação oxidativa mediada pelo complexo enzimático DCCR, presente na superfície da membrana interna da mitocôndria. Por meio da reação catalisada pelo complexo DCCR, os cetoácidos de cadeia ramificada KIC, KMV e KIV são convertidos em isovaleril-CoA, 3-metilbutiril-CoA e isobutiril-CoA, respectivamente. A atividade da DCCR é maior no fígado, intermediária no rim e coração, e comparativamente baixa no músculo estriado esquelético, tecido adiposo e cérebro (Harper et al., 1984).

A função da enzima DCCR é considerada como a etapa controladora do fluxo do catabolismo dos ACR; ela é altamente regulada por um ciclo de fosforilação/desfosforilação. A enzima DCCR quinase (DCCRQ) promove a inativação da DCCR por meio da fosforilação da subunidade E1α desse complexo, enquanto a DCCR fosfatase (DCCRF) é responsável pela ativação do complexo (Figura 2) por meio da desfosforilação da subunidade E1α (Shimomura et al. 2004).

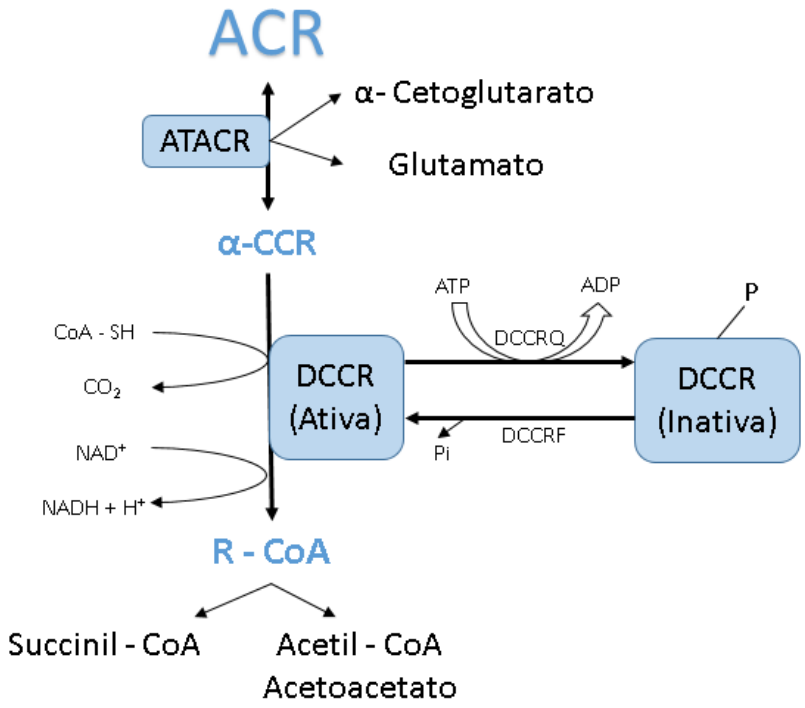


Figura 2. Regulação do complexo enzimático desidrogenase de α-cetoácidos de cadeia ramificada (DCCR). (ATACR= aminotransferase de aminoácidos de cadeia ramificada; α-CCR= α-cetoácidos de cadeia ramificada; R-CoA= acil-CoA) Adaptado de Shimomura et al. (2006).

Finalizada a segunda etapa do catabolismo, os produtos oxidados sofrem outro processo de oxidação por meio de duas diferentes desidrogenases. Assim, as vias catabólicas de cada ACR variam: a leucina é cetogênica e forma acetil-CoA e acetoacetato; a valina é glicogênica, sendo convertida em succinil-CoA; e a isoleucina é cetogênica e glicogênica, pois pode formar acetil-CoA e acetoacetato e succinil-CoA (Figura 3) (Brosnan & Brosnan, 2006).

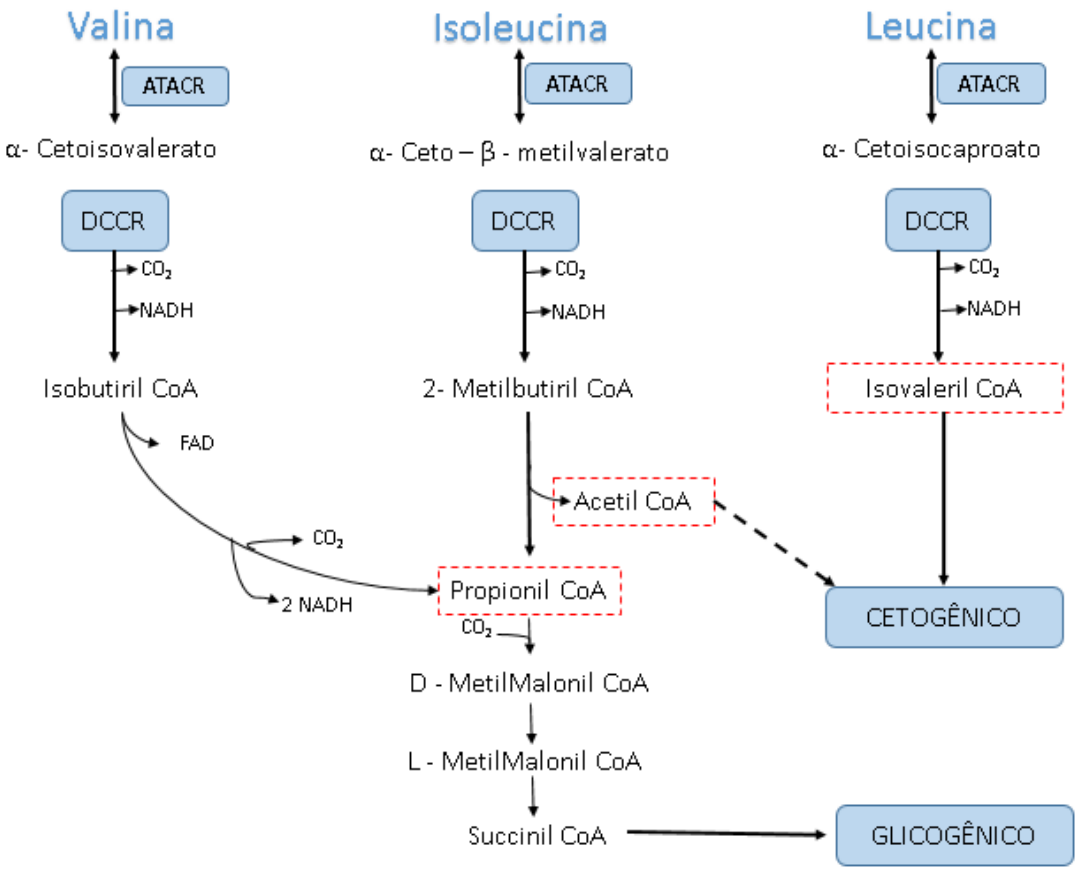


Figura 3. Catabolismo dos aminoácidos de cadeia ramificada (Adaptado de Brody, 1999)

Dessa forma, a principal razão para o antagonismo entre os ACR é o aumento da atividade da enzima DCCR, estimulado por altos níveis de KIC, que regula de maneira dose-dependente a atividade desta enzima, enquanto a valina e isoleucina, bem como seus α-cetoácidos, tem pouco ou nenhum efeito na regulação desta enzima (Harper et al., 1984). Uma vez que os ACR compartilham as primeiras reações do seu metabolismo (Figura 3), um aumento na DCCR, gerado pelo excesso de KIC, reflete-se em maior catabolismo de todos os ACR e, consequentemente, menor disponibilidade destes para a síntese proteica (Murakami et al.; 2005).

Na fase final do metabolismo dos ACR, os derivados da CoA podem ser destinados para participar no ciclo dos ácidos tricarboxílicos (TCA), por meio da formação do succinil-CoA (Figura 4), ou para a produção de corpos cetônicos, por meio da formação de acetil-CoA e acetoacetato (Nelson & Cox, 2011).

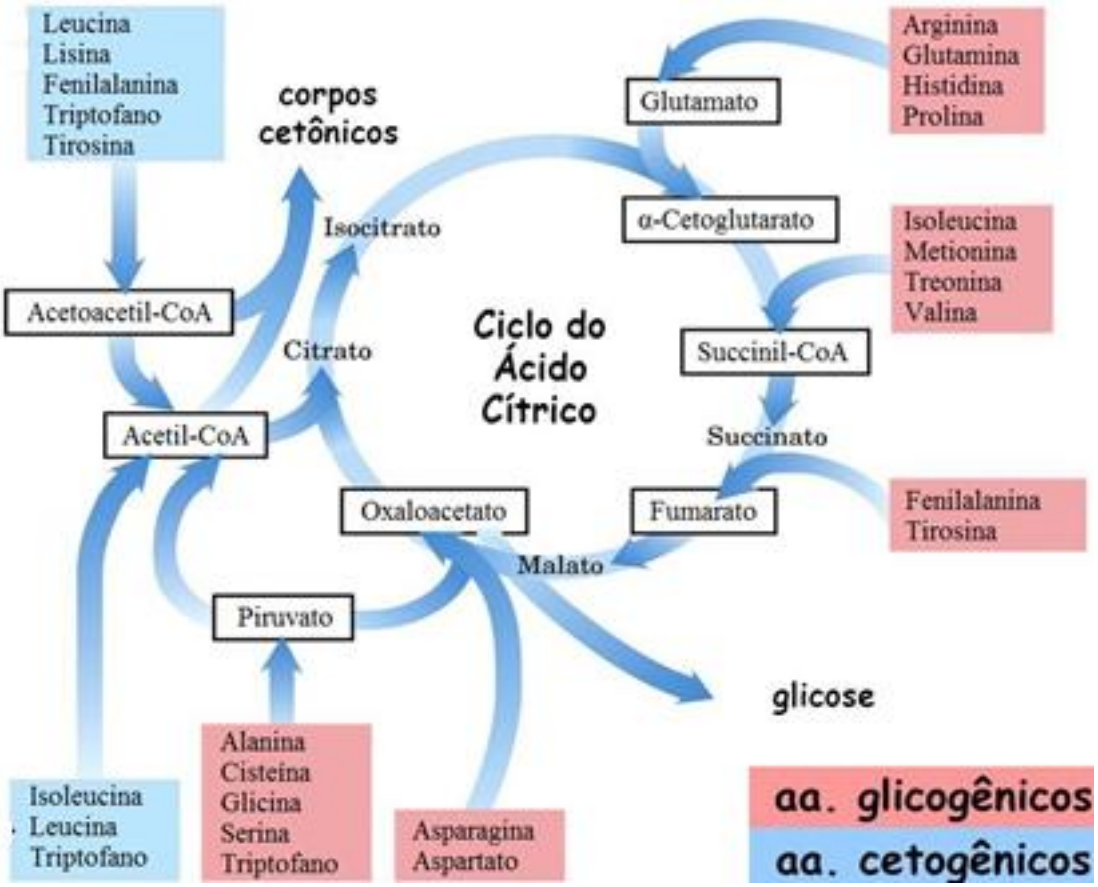


Figura 4. Vias de catabolismo dos aminoácidos (Nelson & Cox, 2011).

As características particulares do catabolismo da valina foram descritas por Shimomura et al. (2004), que relataram que este processo é único quando comparado com os outros ACR. Após a degradação do Isobutiril-CoA é formado o metacrilil-CoA (MC-CoA), um composto tóxico que pode gerar ações mutagênicas e citogênicas, pois é uma molécula reativa com tiol, sendo hidrolisado pela crotonase. Segundo Holden et al. (2001), esta superfamília é também conhecida como enoil-CoA hidratase, possuindo em comum a necessidade de estabilizar um ânion intermediário derivado de um substrato acil-CoA. A crotonase e a β-Hidroxibutiril-CoA hidrolase (HIB-CoA) são as responsáveis pela rápida eliminação do MC-CoA nas células (Figura 5).

O MC-CoA é gerado durante o catabolismo da valina no espaço da matriz mitocondrial, onde pode reagir com a glutationa e interferir no mecanismo de proteção

da mitocôndria contra as espécies reativas de oxigênio (radicais livres). Estudos realizados por Taniguchi et al. (1996) demonstraram uma alta atividade da crotonase e HIB-CoA, mesmo tendo uma atividade constante das DCCR. A alta concentração destas duas enzimas permitiu uma importante proteção fisiológica das células ao hidrolisar a atividade da MC-CoA em ratos. Da mesma forma, Ooiwa et al. (1995) verificaram uma alta atividade das enzimas crotonase e HIB-CoA nos tecidos de cães, sendo a MC-CoA rapidamente degradada. Assim, Shimomura et al. (2004) concluíram que a adição dos ACR, mesmo em alta concentração de valina, não é toxica porque o organismo promove a atividade enzimática da crotonase e HIB-CoA hidratase.

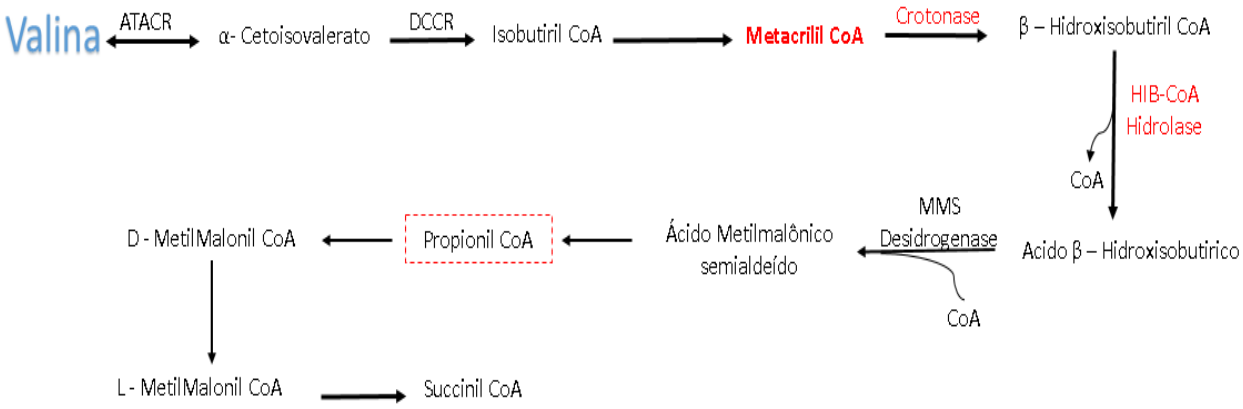


Figura 5. Via catabólica da Valina. (Adaptado de Shimomura et al. 2004)

1.2. Antagonismo dos Aminoácidos de Cadeia Ramificada

Os ACR são estruturalmente semelhantes e compartilham as mesmas vias de degradação no organismo e a sua importância fisiológica tem sido estudada por diferentes pesquisadores, evidenciando uma competição constante pelas vias catabólicas e, como consequência, seu antagonismo (Oestemer et al., 1973; Harper et al. 1984; Shimomura & Harris, 2006).

O antagonismo é uma relação específica entre AA com estrutura similar e, quando existe excesso ou deficiência de um aminoácido, eleva-se a exigência de outro aminoácido, ou seja, deve-se aumentar ou diminuir a concentração do aminoácido que este em desbalanço.

A leucina é o único aminoácido que tem efeitos potentes sobre as duas primeiras etapas do catabolismo dos ACR. A ingestão de uma refeição desequilibrada, contendo grandes quantidades de leucina, geralmente induz a uma redução acentuada na

concentração de valina e de isoleucina no organismo. Um equilíbrio positivo de N é obtido quando a leucina é fornecida na dieta, mas as concentrações dos diversos AA no interior do músculo diminuem, concluindo que há aumento na oxidação dos ACR e aumento da síntese de proteínas, podendo contribuir para o paradoxo da leucina (Shimomura & Harris, 2006).

Estudos realizados por Oestemer et al. (1973) indicaram que os excessos de leucina nas dietas de leitões reduziram consideravelmente os níveis plasmáticos de valina, isoleucina e de seus respectivos α-cetoácidos, devido ao aumento da atividade da DCCR, que incrementa a degradação desses dois ACR, ou seja, altos níveis de KIC (produto da degradação da leucina) resultaram em um maior catabolismo dos outros ACR pela estimulação da DCCR.

Além de reduzir as concentrações plasmáticas de valina e de isoleucina, o excesso de leucina na dieta provoca outras respostas. Gatnau et al. (1995) avaliaram o efeito do excesso de leucina e seus subprodutos sobre o crescimento e a resposta imunológica de leitões recém desmamados e observaram uma diminuição no ganho de peso diário (GPD) e consumo diário de ração (CDR) quando o nível de leucina foi aumentando na dieta.

A leucina é abundante na maioria dos alimentos, sendo que a exigência dos animais é facilmente atendida, portanto, torna-se difícil criar situações de deficiência severa (Franco, 2011). Assim, os ingredientes normalmente usados nas rações dos suínos podem proporcionar um possível antagonismo com os demais ACR.

O uso de farinha de sangue ou glúten de milho, com 7,70% e 9,77% de leucina digestível, respectivamente (Rostagno et al., 2011), em rações para leitões, bem como o uso de dietas com baixo nível de PB, mantendo-se o nível ótimo dos ACR, geram questionamentos sobre um possível antagonismo entre a leucina, isoleucina e valina, uma vez que as concentrações de isoleucina e valina são menores (Figura 6, Htoo & Wiltafsky, 2012).

Utilizando subproduto do processo de produção do plasma suíno (hemácias secas por pulverização) que possuem uma baixa concentração de isoleucina na sua composição, Kerr et al. (2004) observaram uma redução no CDR, GPD e conversão alimentar (CA) à medida que aumentaram o nível de inclusão de hemácias *spray-dried* na dieta. No entanto, este subproduto pode ser incluído nas dietas de leitões desmamados até o nível de 6%, sempre que a exigência de isoleucina para esta fase seja atendida. Os mesmos resultados foram encontrados por Dijk et al. (2000) trabalhando com leitões desmamados, no entanto

a inclusão de até 9,5% de hemácias *spray-dried* resultou em diminuição de até 25% no CDR e uma redução de até 70% na CA dos leitões. As particularidades de cada fase de crescimento dos suínos podem gerar respostas variadas no consumo, concluindo que a palatabilidade da ração muda quando há inclusão de hemácias secas por pulverização, e como consequência diminui o consumo de ração.

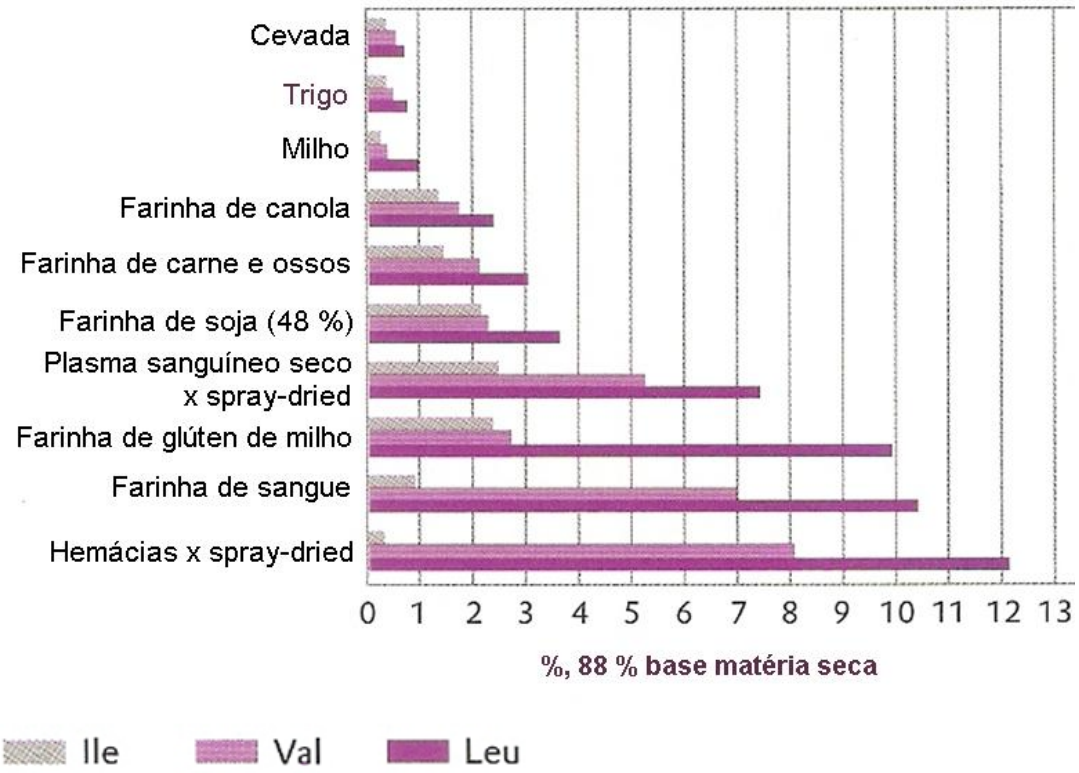


Figura 6. Conteúdo de aminoácidos de cadeia ramificada em ingredientes selecionados (Adaptado de Aminodat® 4.0, 2012)

A suplementação de L-leucina (0,27 e 0,55%) em dietas de baixa PB, (16,9%), para leitões com 21 dias de idade, foi objeto do estudo de Yin et al. (2010), os quais observaram que a inclusão de 0,55% de L-leucina, durante duas semanas, aumentou os níveis de fosforilação da proteína ribossomal S6 quinase 1 (S6K1) e da proteína 1 ligante do fator de iniciação eucariótico 4E (4E-BP1), aumentando a síntese proteica no tecido muscular esquelético, fígado, coração, rins, pâncreas, baço e estômago.

Os autores também observaram uma melhora no GPD (61%) em relação à dieta controle com a inclusão de 0,27% de L-leucina, sendo que os leitões que receberam esta dieta apresentaram aumento da síntese proteica no intestino delgado, rins e pâncreas. Estes resultados demonstraram que a adição de L-leucina na dieta pode estimular ainda

mais a síntese proteica, no entanto, deve-se ressaltar que os níveis de valina e isoleucina não devem estar em excesso, para evitar o antagonismo dos ACR.

Ainda vale ressaltar que os ACR competem com o triptofano pela ligação ao mesmo transportador de AA neutros de cadeia longa (AANCL, valina, isoleucina, leucina, tirosina e fenilalanina) na barreira hemato-encefálica (Henry et al., 1992). Desse modo, a entrada do triptofano no sistema nervoso central (SNC) é regulada pela razão plasmática triptofano livre:ACR e favorecida pela redução da concentração de ACR no sangue, decorrente do aumento da sua taxa de oxidação (Rogero & Tirapegui, 2008), que pode ser relacionada diretamente com a diminuição da produção de serotonina (produto do metabolismo do triptofano) em nível cerebral e, como consequência, apresentar uma redução no consumo de alimento (Feijó et al., 2011).

Em estudo conduzido com suínos machos castrados e fêmeas na fase de terminação, foi constatada redução no consumo de ração como consequência da baixa relação entre o triptofano e os AANCL (Henry et al., 1992), sendo este efeito relacionado à menor concentração de serotonina, e mais expressivo em fêmeas que em machos castrados (Henry et al., 1996).

Assim, pode-se concluir que o excesso de ACR pode comprometer o consumo de ração, devido à redução da entrada de triptofano no SNC, levando à redução das concentrações de serotonina. Da mesma forma, o excesso de leucina influencia a utilização de valina e isoleucina, devido ao aumento no catabolismo destes AA, e o excesso de valina e isoleucina não influencia de maneira significativa as variáveis de desempenho de suínos, quando comparados com a leucina (Htoo & Wiltafsky, 2012).

1.3. Aminoácidos de Cadeia Ramificada e regulação da síntese proteica muscular

A síntese proteica no tecido muscular é rapidamente estimulada após a ingestão dos nutrientes na dieta. Alguns benefícios promovidos pela dieta são atribuídos ao alto consumo dos ACR (Vianna et al. 2010), uma vez que estes AA representam 35% dos AA essenciais presentes na proteína do músculo (Riazi et al., 2003). Pelo fato dos ACR serem metabolizados primeiramente no músculo esquelético, serão fonte de energia e substratos para a síntese proteica muscular, sua suplementação poderia influenciar o processo de anabolismo proteico.

É fato que a insulina e AA estimulam o processo de anabolismo, atuando na transcrição genética. Ao administrar uma mistura de AA e glicose pela via endovenosa em ratos, previamente privados de alimentação, Rogero & Tirapegui (2008) observaram um aumento eficiente da síntese proteica no músculo esquelético. Contudo, Manjarrez et al. (2015) demonstraram, em pesquisas realizadas em animais e humanos, que em condições normais não há efeito anabólico, mas na presença de estresse ou trauma severo (fases de excessiva proteólise) observou-se uma ação contrária ao catabolismo proteico, sugerindo que a leucina e o seu metabólito são ativos nos períodos de estresse excessivo.

A leucina exerce os seus efeitos em nível pós-transcricional, no início da fase de tradução do mRNA em proteína (Mata & Navarro, 2009). Assim, o estímulo é dado pelo aumento da concentração deste aminoácido no interior da célula, promovendo a ativação da mTOR (Du et al. 2007).

Uma das principais funções da mTOR é a síntese proteica no metabolismo celular. A mTOR quinase existe em dois complexos estruturalmente e funcionalmente distintos, o complexo mTOR 1 (mTOR1), sensível à rapamicina; e o complexo mTOR2 (mTOR2) insensível à rapamicina. A mTOR1 é responsável pela regulação da tradução do mRNA, fosforilando a proteína ribossomal S6 quinase 1 (S6K1), o fator de iniciação eucariótico 4G (eIF4G) e a proteína 1 ligante do fator de iniciação eucariótico 4E (4E-BP1). A S6K1 é uma quinase de proteína ribossomal S6 (rpS6) e a sua ativação por S6K1 é crucial para a tradução do mRNA (Suryawan et al., 2012; Proud, 2007a). Segundo Hornberger et al. (2006), a mTOR também pode ser ativada pelo crescimento do músculo esquelético, a proteína quinase B (Akt) e fatores de crescimento.

O complexo 4E-BP1 é um inibidor do fator de iniciação da tradução proteica; quando este complexo é fosforilado, se libera o eIF4E unindo-se ao eIF4G, formando o complexo ativo eIF4G/eIF4E para participar do início da tradução (Rogero & Tirapegui, 2008). A mTOR2 é responsável por regular a ativação da proteína quinase B (Akt) (Proud, 2007b). A montagem desse complexo é necessária para a continuação da etapa de iniciação da tradução do RNA-mensageiro em proteína.

A proteína quinase S6 ribossômica (p70S6k), ativada pela mTOR, é responsável pela estimulação da iniciação dos processos de tradução e de elongação da síntese proteica por diferentes mecanismos. A p70S6k fosforila e inativa a enzima quinase (eEF2K), ativando o fator de elongação eucariótico 2 (eEF2), promovendo a elongação a nível

celular (Drummond et al. 2009) e, como resultado, a síntese proteica (Hornberger et al., 2006).

A leucina, juntamente com a insulina, tem um efeito sinérgico e uma influência sobre o controle a curto prazo da etapa de tradução da síntese proteica (Proud, 2007b). A insulina é conhecida como um hormônio anabólico, importante na manutenção da síntese proteica muscular. Atua ainda no transporte de glicose e AA para o interior das células, favorecendo a síntese de proteínas, glicogênio e triglicerídeos (Schneider et al., 2008).

O tecido muscular esquelético é responsável por aproximadamente 75% da captação de glicose estimulada pela insulina. Este processo se inicia quando a insulina se liga ao seu receptor (IR) na superfície da membrana celular do tecido muscular, estimulando a atividade do IR tirosina quinase. Quando está ativado, o IR fosforila os receptores de insulina (IRS-1 e IRS-2), permitindo que estes substratos se associem e ativem a fosfatidil-inositol 3 quinase (PI3-K), conduzindo à fosforilação da fosfatidil-inositol 2 fosfato (PIP-2) e, como consequência, existe um aumento na concentração de PI3-K no conteúdo celular (Zanchi et al., 2012).

Ao mesmo tempo, a Akt é ativada pelo teor de PI3-K, levando ao aumento do transportador de glicose 4 (GLUT4) no citosol; este aumento de GLUT4 faz com que a sua posição seja mudada do citoplasma até a membrana celular, para assim iniciar o processo de captação de glicose pela célula (Wang et al.; 1998).

Outra via ativada pela Akt, na posição da via de GLUT4, é a via mTOR/p70S6K, o que é importante na síntese de proteínas do músculo esquelético. Esta via é um regulador negativo da sinalização de insulina, levando à fosforilação de resíduos de serina no IRS-1, diminuindo desta forma sua associação com PI3-K (Figura 7) (Zanchi et al., 2012). Segundo Krebs et al. (2002), a leucina estimula a síntese proteica pela modulação de elementos que atuam na tradução da via de sinalização da insulina via fosfatidil-inositol 3 quinase (PI3-K), inibindo a sinalização da insulina e diminuindo a utilização de glicose muscular.

Estudos *in vitro*, realizados por Iwanaka et al. (2010), sobre os efeitos da leucina na estimulação do transporte de insulina nos músculos do antebraço (pronador redondo, flexor radial do carpo, palmar longo e flexor ulnar do carpo) de ratos demonstraram que a leucina tem um efeito estimulador sobre o transporte de glicose, estimulada por contração e um efeito inibitório sobre o transporte da mesma quando há presença de insulina. Da mesma forma, Nishitani et al. (2002) observaram que a administração oral

de ACR (1,5 g/kg), aumentou a concentração plasmática dos ACR em 2mM e, após 30 min, houve um aumento no transporte de glicose pela insulina livre no plasma e no músculo de ratos.

Igualmente, Yoon (2016) concluiu que a administração oral de leucina em ratos *Sprague-Dawley* aumentou a síntese proteica no tecido adiposo, músculo gastrocnêmio e rim, mas não no fígado e coração, ao contrário das refeições de carboidratos, que não alteraram a síntese proteica em nenhum tecido, mas aumentaram a concentração de insulina plasmática. Segundo Macotela et al. (2011), o suplemento dietético de leucina para ratos alimentados com dietas hipercalóricas (gordura) melhora a tolerância à glicose e sensibilidade à insulina, diminuindo problemas como a esteatose hepática e inflamação do tecido adiposo, sem afetar variáveis de desempenho como GPD e CDR.

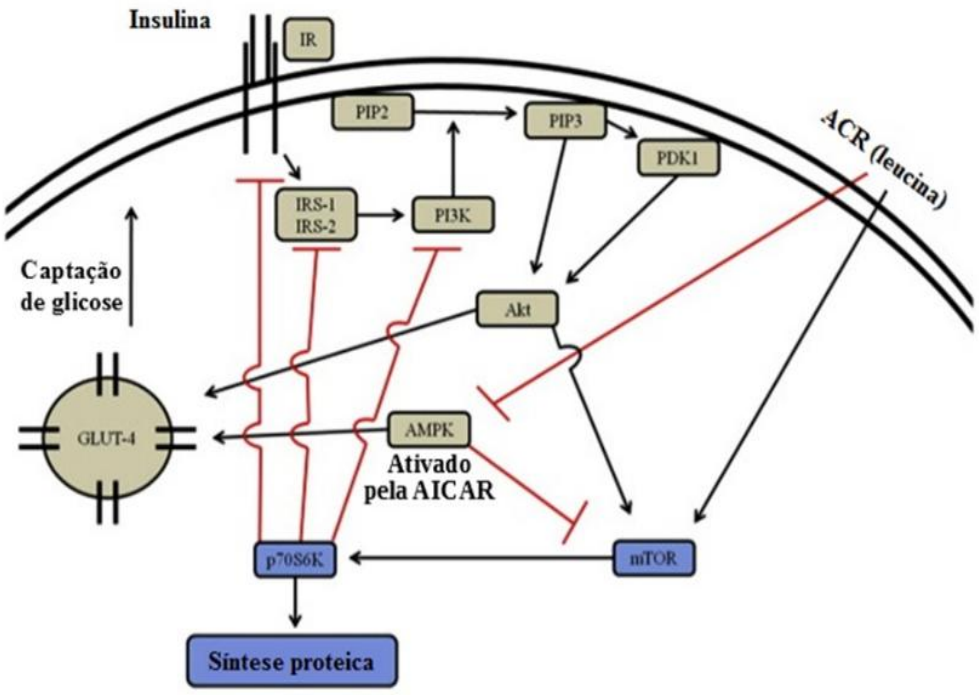


Figura 7. Visão esquemática da leucina no processo de estimulação ou inibição da via de sinalização da insulina no músculo esquelético, conduzido para a síntese de proteína e absorção de glicose (setas pretas) ou resistência à insulina (setas vermelhas) (Adaptado de Zanchi et al. 2012).

Por outro lado, Baum et al. (2005), após administrarem leucina oralmente (1,35 g/kg) para ratos, não observaram aumento da captação de glicose, nem mudanças na concentração de PI3-K no músculo gastrocnêmio. Resultados similares foram encontrados por Doi et al. (2005), quando administraram via oral uma concentração de

1,35 g de L-leucina/kg de peso em ratos, sem encontrar diferença na concentração de insulina plasmática no músculo gastrocnêmio uma hora após a administração.

1.5. Aminoácidos de Cadeia Ramificada e sua importância na nutrição de suínos

Conforme relatado anteriormente, os ACR fazem parte dos AA essenciais, os quais são fundamentais para síntese de proteínas e em outros processos fisiológicos do organismo, participando com aproximadamente um terço das proteínas musculares (Gois et al. 2015), sendo a valina o quinto e a isoleucina o sexto aminoácido limitante para suínos. Já a leucina é o ACR que dificilmente estará em deficiência, pois os alimentos convencionais apresentam altos teores deste aminoácido, garantindo altos níveis de leucina em relação à exigência dos suínos.

Os estudos sobre as exigências dos ACR têm sido constantes, pois a importância que eles apresentam nas variáveis zootécnicas faz com que sejam objeto de pesquisa, tanto com relação a seus efeitos no desempenho (CDR, GPD, CA), quanto os efeitos relacionados à fisiologia celular (captação de glicose, síntese proteica, expressão gênica, metabólitos dos ACR, corpos cetogênicos, etc).

As pesquisas realizadas por Brinegar et al. (1950) determinaram a exigência de isoleucina (Ile) para leitões recém desmamados. Os autores incluíram farinha de sangue nas rações experimentais e observaram que o menor nível de Ile (0,23%) teve uma resposta negativa para CDR e, como consequência, um menor GPD. Este resultado pode ter sido influenciado pelo problema de palatabilidade que apresenta a farinha de sangue quando usada em altos níveis (Henn et al., 2006). Por outro lado, os níveis de 0,46, 0,58 e 0,70% de L-isoleucina apresentaram os melhores GPD e CA. Os autores concluíram que o melhor nível de L-isoleucina para esta fase produtiva foi de 0,70%, ao se utilizar dietas com o conteúdo proteico de 22%.

Da mesma forma, Oestemer et al. (1973) avaliaram a relação da leucina (Leu) e Ile na alimentação de leitões recém desmamados, em que a dieta basal (0,45% de Ile e 0,70% de Leu) foi suplementada com L-Ile e L-Leu para atender os níveis de 0,45; 0,60 e 0,75% de Ile, e de 0,70; 0,78; 0,86; 0,94 e 1,02% de Leu. Os autores não observaram interação Leu x Ile, indicando que a suplementação de Ile foi ineficiente na presença de altos níveis de Leu, sem encontrar uma resposta negativa no GPD dos animais.

Por outro lado, o equilíbrio ótimo entre os ACR (Leucina:Valina:Isoleucina; 1:0,75:0,75 e 0,51:0,63 com 17% de PB) permitiu aumento no GPD em suínos na fase de crescimento, melhorado o fluxo de ácidos graxos no músculo esquelético, melhorando a qualidade da carne e seu valor nutricional (Duan et al., 2016).

De maneira geral, sempre houve um grande interesse em estudar as exigências dos ACR na nutrição animal, sendo importante ressaltar que esses estudos ajudaram a melhorar a utilização dos AA industriais na dieta. Além disso, com o passar do tempo, diferentes pesquisas mostraram a importância do conceito de proteína ideal e a relação ACR:lisina também foi melhorada.

Pesquisas recentes (Gloaguen et al., 2014; Nørgaard et al., 2015; Soumeh et al., 2015) demonstraram que a concentração da PB das rações está sendo reduzida por meio de formulações baseadas nas exigências dos AA essenciais, como os ACR, lisina e triptofano, bem como utilizando o conceito da PB (Van Milgen et al. 2015). Reduzindo a porcentagem de PB na dieta de leitões recém desmamados e avaliando diferentes níveis de valina (Val) em relação à lisina (0,58; 0,62; 0,66; 0,70; 0,74 e 0,78%), Soumeh et al. (2015) observaram uma resposta significativa na CA, CDR e GPD, assim como aumento na concentração plasmática de Val. A deficiência de Val influenciou negativamente o CDR e, como consequência, o GPD.

Pesquisas realizadas por Gaines et al. (2011) e Gloaguen et al. (2011) mostraram que os níveis ótimos de valina em relação à lisina, para um melhor desempenho, são de 63% para leitões dos 13 aos 32 kg, e de 70% para leitões dos 10 aos 20 kg, respectivamente.

Resultados similares foram encontrados por Gloaguen et al. (2012) ao avaliarem o efeito de uma dieta deficiente em valina e com excesso de leucina para leitões de 4 semanas de idade. Os autores observaram uma depressão no apetite dos leitões após a ingestão da dieta deficiente em valina e, uma hora após o consumo desta dieta, os leitões reduziram o consumo de ração em 14%, em relação ao grupo controle, além de redução das concentrações de valina e isoleucina no sangue.

A relação leucina:lisina e sua influência na expressão gênica, na concentração plasmática e no desempenho de leitões foi estudada por Garcia et al. (2015), que observaram respostas linear e quadrática para GPD; em contraste, o CDR reduziu linearmente e a CA apresentou uma resposta quadrática. Os autores relacionaram a resposta do GPD com os transportadores catiônicos da lisina e leucina no duodeno, pois

a expressão do transportador $b^{0,+}$ (transportador específico da membrana celular intestinal para lisina e leucina) foi maior no jejuno, assim como a alta concentração de lisina no sangue. Os autores concluíram que a relação ideal leucina:lisina é de 100 a 110%, podendo ser utilizada sem influenciar negativamente o desempenho dos suínos.

O desequilíbrio dos ACR pode reduzir a utilização da leucina, valina e isoleucina tendo efeito direto nas variáveis de desempenho como consumo de ração e ganho de peso diário e ainda pode influenciar a concentração de alguns parâmetros sanguíneos, como insulina e os corpos cetônicos (β-Hidroxi-β-metilButirato) (Duan et al., 2016).

1.6. REFERÊNCIAS

Anthony, J. C.; Anthony, T. G.; Kimbal, S. R. and Jefferson, L.S. 2001. Signaling Pathways Involved in Translational Control of Protein Synthesis in Skeletal Muscle by Leucine. The Journal of Nutrition 131:856S–860S.

Baum, J. I.; O'Connor, J. C.; Seyler, J. E.; Anthony, T. G.; Freund, G. G. and Layman, D. K. Leucine reduces the duration of insulin-induced PI 3-kinase activity in rat skeletal muscle. 2005. American journal of physiology. Endocrinology and metabolism 288:86-91.

Brinegar, M. J.; Loosli, J. K.; Maynaed, A. and Williams, H. H. The Isoleucine requirement for the growth of swine. 1950. The Journal of Nutrition 42:619–624.

Brody, T. 1999. Nutritional biochemistry. 2 ed. Academic Press. San Diego.

Brosnan, J. T. and Brosnan, M. E. 2006. Branched-Chain Amino Acids: Enzyme and Substrate Regulation. The Journal of Animal Science. Branched-Chain Amino Acids: Metabolism, Physiological Function, and Application 136:207S-211S.

Cabral, C. H. A.; Almeida, D. M.; Martins, L. S. and Mendes, R. K. V. 2012. Mecanismos fisiológicos e bioquímicos envolvidos no turnover proteico: deposição e degradação de proteína muscular. Enciclopédia Biosfera, Centro Científico Conhecer. 8:1185-1203.

Dijk, A. J.; Everts, H.; Nabuurs, M. J. A.; Margry, R. J.C. F. and Beynen, A. C. 2001. Growth performance of weanling pigs fed spray-dried animal plasma: a review. Livestock Production Science 68:263–274.

Doi, M.; Yamaoka, I.; Nakayama, M.; Mochizuki, S.; Sugahara, K. and Yoshizawa, F. 2005. Isoleucine, a blood glucose-lowering amino acid, increases glucose uptake in rat skeletal muscle in the absence of increases in AMP-activated protein kinase activity. The Journal of Nutrition 135:2103-2108.

Drummond, M. J.; Dreyer, H. C.; Christopher S. Fry, C. S.; Glynn, E. L. and Rasmussen, B. B. 2009. Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. Journal of Applied Physiology 106:1374–1384.

Du, M.; Shen, Q. W.; Zhu, M. J. and Ford, S. P. 2007. Leucine stimulates mammalian target of rapamycin signaling in C2C12 myoblasts in part through inhibition of adenosine monophosphate-activated protein kinase. The Journal of Animal Science 85:919-927

Duan, Y.; Duan, Y.; Li, F.; Li, Y.; Guo, Q.; Ji, Y.; Tan, B.; Li, T.; Yin, Y. 2016. Effects of supplementation with branched‐chain amino acids to low‐protein diets on

expression of genes related to lipid metabolism in skeletal muscle of growing pigs. Amino Acids. doi 10.1007/s00726-016-2223-2.

Escobar, J.; Frank, J. W.; Suryawan, A.; Nguyen, V.; Kimball, S. R.; Jefferson, L. S. and Davis, T. A. 2005. Physiological rise in plasma leucine stimulates muscle protein synthesis in neonatal pigs by enhancing translation initiation factor activation. American Journal of Physiology Endocrinology and Metabolism 228:E914-E921.

Feijó, F. M.; Bertoluci, M. C. and Reis, C. 2011. Serotonina e controle hipotalâmico da fome: uma revisão. Revista da Associação Medica Brasileira 57:74-77.

Franco, S. M. 2011. Níveis dietéticos de leucina, de histidina e de fenilalanina + tirosina para frangos de corte na fase inicial. Dissertação (M.Sc). Universidade Federal de viçosa. Minas Gerais.

Gaines, A. M.; Kendall, D. C.; Allee, G. L.; Usry, J. L. and Kerr, B. J. 2011. Estimation of the standardized ileal digestible valine-to-lysine ratio in 13 to 32 kg pigs. Journal of Animal Science 89:736–742.

Garcia, H.; Morales, A.; Araizal, A.; Htoo, J. K. and Cervantes, M. 2015. Gene expression, serum amino acid levels, and growth performance of pigs fed dietary leucine and lysine at different ratios. Genetics and Molecular Research 14:1589-1601.

Gatnau, R.; Nissen, L.; Zirnmerman, D. R.; Wannemuehlert, M. and Ewan, C. 1995. Effects of Excess Dietary Leucine and Leucine Catabolites on Growth and Immune Responses in Weanling Pigs. The Journal of Animal Science 73:159-165.

Gloaguen, M.; Le Floc'h, N.; Brossard, L.; Barea, R.; Primot, Y.; Corrent, E. and van Milgen, J. 2011. Response of piglets to the valine content in diet in combination with the supply of other branched-chain amino acids. Animal 5:1734–1742.

Gloaguen, M.; Le Floc'h, N.; Corrent, E.; Primot, Y. and Van Milgen, J. 2014. The use of free amino acids allows formulating very low crude protein diets for piglets. Journal of Animal Science 92:637-644.

Gloaguen, M.; Le Floc'h, N.; Corrent, E.; Primot, Y. and Van Milgen, J. 2012. Providing a diet deficient in valine but with excess leucine results in a rapid decrease -keto acid ain feed intake and modifies the postprandial plasma amino acid and concentrations in pigs. The Journal of Animal Science 90:3135-3142.

Gois, H. D. C.; Ferreira, K. C. G.; Oliveira, L. C. N. and Bernardo, D. N. A. 2015. Os efeitos da suplementação de ACR sobre a fadiga no exercício de endurance. Revista Odontológica de Araçatuba 36:19-23.

Gomez, R. S.; Lewis, A. J.; Miller, P. S. and Chen, H. Y. 2002. Growth performance, diet apparent digestibility, and plasma metabolite concentrations of barrows fed corn-soybean meal diets or low-protein, amino acid-supplemented diets at different feeding level. The Journal of Animal Science. v. 80. p. 644-653.

Harper, A. E. 1984. Interrelationships among the branched chain amino acids. p. 81-99. In: Branched Chain Amino and Keto Acids in Health and Disease. S. A.

Henn, J. D.; Ribeiro, A. M. L. and Kessler, A. M. 2006. Comparação do valor nutritivo de farinhas de sangue e de farinhas de vísceras para suínos utilizando-se o método da proteína e gordura digestíveis e o método de substituição. Revista Brasileira de Zootecnia 35:1366-1372.

Henry, Y.; Sève, B.; Colleaux, Y.; Ganier, P.; Saligaut, C. and Je´go, P. 1992. Interactive effects of dietary levels of tryptophan and protein on voluntary feed intake and growth performance in pigs, in relation to plasma free amino acids and hypothalamic serotonin. The Journal of Animal Science 70:1873– 1887.

Holden, H. M.; Benning, M. M.; Haller, T. and Gerlt, A. 2001. The Crotonase Superfamily: Divergently Related Enzymes That Catalyze Different Reactions

Involving Acyl Coenzyme A Thioesters. Accounts of Chemical Research 34:145-157.

Hornberger, T. A.; Chu, W. K.; Mak, Y. W.; Hsiung, J. W.; Huang, S. A. and Chien, S. 2006. The role of phospholipase D and phosphatidic acid in the mechanical activation of mTOR signaling in skeletal muscle. PNAS, Proceedings of the National Academy of Sciences 103:4741–4746. Htoo, J., M. Wiltafsky. 2012. Branched-chain amino acids in broiler nutrition. Aminonews 16:25-32.

Iwanaka, N.; Egawa, T.; Satoubu, N.; Karaike, K.; Ma, X.; Masuda, S. and Hayashi, T. 2010. Leucine modulates contraction- and insulin-stimulated glucose transport and upstream signaling events in rat skeletal muscle. Journal of Applied Physiology 108:274-282.

Kerr, B.J.; Kidd, M. T.; Cuaron, J. A.; Bryant, K. L.; Parr, T. M.; Maxwell, C. V. and Weaver, E. 2004. Utilization of spray-dried blood cells and crystalline isoleucine in nursery pig diets. The Journal of Animal Science 82:2397-2404.

Krebs, M.; Krssak, M.; Bernroider, E.; Anderwald, C.; Brehm, A.; Meyerspeer, M.; Nowotny, P.; Roth, E.; Waldhäusl, W. and Roden, M. 2002. Mechanism of amino acid-induced skeletal muscle insulin resistance in humans. Diabetes 51:599-605.

Lordelo, M. M.; Gaspar, A. M.; Le Bellego, L. and Freire, J. P. B. 2008. Isoleucine and valine supplementation of a low-protein corn-wheat-soybean meal-based diet for piglets: Growth performance and nitrogen balance. The Journal of Animal Science 86: 2936-2941.

Macotela, Y.; Brice, E.; Bang, A. M.; Espinoza, D. O.; Boucher, J.; Beebe, K.; Gall, W. and Kahn, R. 2011. Dietary Leucine - An Environmental Modifier of Insulin Resistance Acting on Multiple Levels of Metabolism. Plos One 6:1-13.

Manjarrez, R. M. O.; Torres-Vaca, M.; Gonzales-Gallego, J.and Ordones-Alvear, I. 2015. El β-hidroxi-β-metilbutirato (HMB) como suplemento nutricional (I): metabolismo y toxicidad. Nutrición Hospitalaria 2:590-596.

Mata, G. R. and Navarro, F. 2009. O Efeito da Suplementação de Leucina na Síntese proteica muscular. Revista Brasileira de Nutrição Esportiva 3:367-378.

Milgen, J. V. and Dourmad, J-Y. 2015. Concept and application of ideal protein for pigs. Journal of Animal Science and Biotechnology 6-15.

Murakami, T., Matsuo, M., Shimizu, A., Shimomura, Y. 2005. Dissociation of branched-chain alpha-keto acid dehydrogenase kinase (BDK) from branched-chain alpha-keto acid dehydrogenase complex (BCKDC) by BDK inhibitors. Journal of Nutritional Science and Vitaminology 51:48-50.

NATIONAL RESEARCH COUNCIL – NRC. 2012. Nutrients requirement of swine. 11 ed. National Academic Press. Washington DC.

Nelson, D.L. and Cox, M.M. 2011. Princípios de Bioquímica de Lehninger. 5 ed. Sarvier. São Paulo.

Nishitani, S.; Matsumura, T.; Fujitani, S.; Sonaka, I.; Miura, Y. and Yagasaki, K. 2002. Leucine promotes glucose uptake in skeletal muscles of rats. Biochemical and Biophysical Research Communications 299:693-696.

Nørgaard, J. V.; Pedersen, T. F.; Soumeh, E. A.; Blaabjerg, N.; Canibe, N.; Jensen, B. B. and Poulsen, H. D. 2015. Optimum standardized ileal digestible tryptophan to lysine ratio for pigs weighing 7–14 kg. Poulsen Livestock Science 175:90-95. 2015.

Oestemer, G. A.; Hanson, L. E. and Meade, R. 1973. Leucine-Isoleucine interrelationship in the Young pig. The Journal of Animal Science 36:674-678.

Ooiwaa, T.; Gotoa, H.; Tsukamotoa, Y.; Hayakawaa, T.; Sugiyamab, S.; Fujitsukac, N. and Shimomura, Y. 1995. Regulation of valine catabolism in canine tissues: tissue distributions of branched-chain aminotransferase and 2-oxo acid dehydrogenase

complex, methacrylyl-CoA hydratase and 3-hydroxyisobutyryl-CoA hydrolase. Biochimica et Biophysica Acta (BBA) - General Subjects 1243:216-220.

Proud, C. G. Amino acids and mTOR signalling in anabolic Function. Biochemical Society Transactions. v. 35. p. 1187-1190. 2007b.

Proud, C. G. 2007a. Signalling to translation: how signal transduction pathways control the protein synthetic machinery. Biochemical Journal 403:217-234.

Richardson, L. R.; Hale, F. and Ritchey, S. J. 1965. Effect of fasting and level of dietary protein on free amino acids in pig plasma. The Journal of Animal Science 24:368-372.

Rogero, M. M. and Tirapegui, J. 2008. Aspectos atuais sobre aminoácidos de cadeia ramificada e exercício físico. Brazilian Journal of Pharmaceutical Sciences 44:563-575.

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T.; Euclides, R. F. 2011. Tabelas Brasileiras para Aves e Suínos: Composição de alimentos e exigências nutricionais. 3rd ed. UFV, Viçosa, Minas Gerais.

Sakomura, N. K.; Da Silva, J. H. V.; Costa, F. G. P.; Fernandes, J. B. K. and Hauschild, L. 2014. In: Nutrição de Não Ruminantes. p. 116. FUNEP.

Schneider, A.; Schwegler, E.; Goulart, A. M.; Roos, T. B.; Rabassa, V. R.; Del Pino, F. A. B.; Pfeifer, L. F. M. and Correa, N. M. 2008. Efeito do jejum e da administração de insulina sobre os parâmetros metabólicos de ovelhas em confinamento. Acta Scientiae Veterinariae 36:39-42.

Shimomura, Y.; Honda, T.; Shiraki, M.; Murakami, T.; Sato, J.; Kobayashi, H.; Mawatari, K.; Obayashi, M. and Harris, R. A. 2006. Branched-Chain Amino Acid Catabolism in Exercise and Liver Disease. The Journal of Animal Science. Branched-Chain Amino Acids: Metabolism, Physiological Function, and Application 250S-253S.

Shimomura, Y. and Harris, R.A. 2006. Metabolism and physiological function of branched-chain amino acids: discussion of session 1. Journal of Nutrition 136:232–233.

Shimomura, Y.; Murakami, T.; Nakai, N.; Nagasaki, M. and Harris, R. A. 2004. Exercise Promotes BCAA Catabolism: Effects of BCAA Supplementation on Skeletal Muscle during Exercise. The Journal of Nutrition. 3rd Amino Acid Workshop. 1583S-1587S.

Soumeh, E. A.; Milgen Van, J.; Sloth, N. M.; Corrent, E.; Poulsen, H. D.; Nørgaard, J. V. 2015. Requirement of standardized ileal digestible valine to lysine ratio for 8- to 14-kg pigs. The Animal Consortium 1-7.

Suryawan, A.; Nguyen, H. V.; Almonaci, R. D. and Davis, T. A. 2012. Differential regulation of protein synthesis in skeletal muscle and liver of neonatal pigs by leucine through an mTORC1-dependent pathway. Journal of Animal Science and Biotechnology 3:1-10.

Vianna, D.; Fullin, G.; Teodoro, R.; Torres-Leal, F. L. and Tirapegui, J. 2010. Protein synthesis regulation by leucine. Brazilian Journal of Pharmaceutical Sciences 46: 29-36.

Wang, X.; Campbell, L. E.; Miller, C. M. and Proud, C. G. 1998. Amino acid availability regulates p70 S6 kinase and multiple translation factors. Biochemical Journal 34: 261-267.

Wu, G. 2014. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. Journal of Animal Science and Biotechnology 5: 34.

Yin, Y.; Yao, K.; Liu, Z.; Gong, M.; Ruan, Z.; Deng, D.; Tan B.; Liu, Z. and Wu, G. 2010. Supplementing L-leucine to a low-protein diet increases tissue protein synthesis in weanling pigs. Amino Acids 39:1477-1486.

Yoon, M. S. 2016. The Emerging Role of Branched-Chain Amino Acids in Insulin Resistance and Metabolism. Nutrients 8:1-13.

Zanchi, N. E.; Guimarães-Ferreira, L.; Siqueira-Filho, M. A.; Camporez, J. P. N.; Nicastro, H.; Chaves, D. F. S.; Campos-Ferraz, P.; Lancha, a. H. and Carvalho, C. R. O. 2012. The possible role of leucine in modulating glucose homeostasis under distinct catabolic conditions. Medical Hypotheses 79:883-888.

Zangeronimo, M. G.; Tadeu Fialho, E.; Lima, A. F.; Rodrigues, P. B. and Murgas, L. D. S. 2006. Redução do nível de proteína bruta da ração com suplementação de aminoácidos sintéticos para leitões na fase inicial. Revista Brasileira de Zootecnia 35:849-856.

II – OBJETIVO GERAL

O presente trabalho teve como objetivo determinar as exigências de aminoácidos de cadeia ramificada para suínos na fase inicial.

2.1 Objetivos específicos

- ✓ Determinar as exigências de leucina digestível para suínos machos castrados e fêmeas, na fase inicial (11 a 25 kg), alimentados com dietas com baixa proteína bruta.
- ✓ Determinar as exigências de valina digestível para fêmeas suínas na fase inicial (15 a 30 kg), alimentados com dietas contendo diferentes níveis de leucina.
- ✓ Determinar as exigências de isoleucina digestível para fêmeas suínas na fase inicial (15 a 30 kg), alimentados com dietas contendo diferentes níveis de leucina.

III - Níveis de leucina em dietas com baixa proteína bruta para suínos na fase inicial

RESUMO- O objetivo do presente trabalho foi avaliar os níveis de leucina digestível sobre o desempenho, espessura de toucinho, profundidade do músculo e parâmetros sanguíneos de suínos na fase inicial. Foram utilizados 50 suínos mestiços (Landrace x Large white x Pietrain), com peso inicial de 11,14 ± 0,24 kg; distribuídos em um delineamento experimental de blocos casualizados, com cinco tratamentos (1,10; 1,25; 1,40; 1,55 e 1,70% de leucina digestível), cinco repetições e dois animais por unidade experimental (UE), sendo um macho castrado e uma fêmea. A conversão alimentar reduziu (P=0,018) em função do aumento dos níveis de leucina digestível, sem influenciar os demais parâmetros de desempenho (peso final, consumo diário de ração, ganho de peso diário, espessura de toucinho, profundidade de lombo e porcentagem de carne magra). Com relação às variáveis plasmáticas foi observado efeito quadrático para a concentração de triglicerídeos (P=0,049) e ureia (P=0,001). Ajustando o modelo quadrático associado ao platô do modelo Linear Response Platô (LRP) observou-se que o nível ótimo de leucina digestível para triglicerídeos e ureia foi de 1,16% (26,44 mg/dL) e 1,24% (13,54 mg/dL), respectivamente. A menor concentração de ureia plasmática foi obtida ao nível de 1,24% de leucina digestível. As dietas com baixa proteína bruta e níveis de até 1,70 não prejudicaram o desempenho e o nível 1,70% de leucina digestível proporcionou a melhor conversão alimentar.

Palavras-chave: Aminoácidos de cadeia ramificada, desempenho, parâmetros sanguíneos.

III – Leucine levels in low protein diets for pigs in the initial phase

ABSTRACT – The objective of this study was to evaluate the dietary leucine levels on performance, backfat thickness, loin depth and blood parameters of pigs in the initial phase. A total of 50 pigs were used, with initial weight of 11.14±0.24 kg; distributed in randomized complete block design with five treatments (1.10; 1.25; 1.40; 1.55 and 1.70% SID leucine), five replicates and two animals per experimental unit (EU), a baroow and a gilt. The feed conversion ratio (FCR) reduced (P=0.018) due to the increase of SID leucine levels without affecting other performance parameters (final weight, average daily feed intake, average daily gain, backfat thickness, loin depth and lean mean percentage. With respect to serum parameters quadratic effect for triglyceride (P=0.049) and urea (P=0.001) were observed. Setting data to the quadratic model associated with the linear response plateu model (LRP), the optimal level of SID leucine to triglycerides and urea was 1.16% and 1.24%, respectively. Diets with low crude protein and levels up to 1.70% SID leucine did not impair performance and the 1.24% level provided the best use of dietary amino acids based on plasma urea.

Keywords: SID Leucine, performance, feed conversion ratio, plasma variables, quadratic model, linear response plateau model.

3.1 INTRODUCTION

The use of industrial amino acids (AA) in the diets aims to meet the needs of the animals and provide the formulation of more balanced diets, applying the ideal protein concept, minimizing the excretion of nitrogen in the environment and optimizing the productive parameters. Branched Chain Amino Acids (BCAA) are part of the essential AA group, and the requirements of pigs must be met due to their physiological inability to produce these AA. BCAA comprise leucine, valine and isoleucine and are characterized by sharing the same enzymes for their oxidative transamination and decarboxylation (Shimomura & Harris, 2006).

Leucine appears to antagonize other BCAA, since excess reduces the concentrations of valine, isoleucine and their keto acids in plasma and tissues (Tannous et al., 1966; Langer & Fuller, 2000). Different researches with rats (Torres et al., 1995), poultry (Calvert et al., 1982; Farran et al., 2002) and pigs (Langer et al., 2000; Wiltafsky et al., 2010) reported negative responses in average daily gain (ADG) and reduction in feed intake when there was an excess of leucine in the feed. Although leucine excess is detrimental to the productive parameters, when it is supplied in high amounts it can influence cellular processes by stimulating and increasing protein synthesis through the activation of mTOR, and reducing the losses of endogenous nitrogen through the inhibition of protein degradation through its metabolite, β-hydroxybutyrate, originated from α-ketoisocaproate (Manjarrez et al., 2015).

Although this paradox characterizes the need for research that quantifies the requirement of standardized ileal digestible (SID) leucine for pigs, a challenge to the performance of these studies is the difficulty of inducing a leucine deficiency in the diet due to its high content in the ingredients (Rostagno et al., 2011). The main source of protein used in conventional diets (soybean meal) has low levels of valine (1.93%) and isoleucine (1.88%) when compared to leucine (3.11%) (Rostagno et al., 2011). Thus, conventional diets generally have an excess of SID leucine in relation to the requirements, which may negatively influence the performance variables due to antagonism with the other BCAA.

The difficulty in working with the requirement of SID leucine led to the fact that most of the studies were directed to the study of the antagonism of leucine with the other BCAA (Langer et al., 2000; Wiltafsky et al., 2010; Gloaguen et al., 2011). This fact can also be associated with the greater importance given to valine and isoleucine, since they

are the fifth and sixth limiting AA, respectively, in diets based on corn and soybean meal for pigs (Rostagno et al., 2011).

Thus, the knowledge of the pigs responses to different levels of SID leucine becomes important as a way of quantifying the requirements of this AA, and to measure if the levels commonly found in conventional diets are harmful. The objective of this study was to evaluate levels of SID leucine in diets with low crude protein (CP) concentration for pigs in the initial phase.

3.2 MATERIAL AND METHODS

The experiment was carried out in the swine sector of the Experimental Farm of Iguatemi (FEI), which belongs to the Agrarian Science Center of the State University of Maringá (CCA/UEM) and in the Laboratory of Animal Nutrition (LANA/UEM) of the Animal Science Department. All experimental procedures were previously submitted to the Committee for Ethical Conduct on the Use of Animals under Experimentation (CEUA/UEM), and have been approved for execution (Protocol n. 8538100616).

Fifty crossbred (Landrace x Large White x Pietrain) pigs, 25 barrows and 25 gilts, with initial weight of 11.14 ± 0.240 kg were distributed on the basis of initial weight in a randomized complete block design with five treatments, five replicates and two animals per experimental unit (EU), one barrow and one gilt.

The treatments consisted of five levels of SID leucine (1.10, 1.25, 1.40, 1.55 and 1.70%). The experimental diets were formulated with corn, soybean meal, minerals, vitamins, amino acids and additives (Table 1). The content of CP was reduced in relation to that suggested by Rostagno et al. (2011), but the nitrogen level proposed by NRC (2012) was granted. L-leucine was added in the basal diet at the expense of the inert to meet SID leucine levels. Glutamic acid was used in order to maintain the same nitrogen content in the different diets.

The amino acid compositions of corn and soybean meal used to formulate the diets were analyzed in EVONIK Industries, applying the true ileal digestibility coefficients proposed by Rostagno et al. (2011), to estimate the SID amino acid values of these foods.

The animals were housed in masonry nursery, with suspended pens (1.32 m^2) and partly leaked floor, equipped with semiautomatic feeders, located in the front of the pen, and a nipple type drinker in the back.

Table 1. Chemical and energetic composition (as fed basis) of diets containing different levels of SID leucine (Leu) for pigs in the initial phase (15-30 kg) [1]

	SID leucine (%)				
Item (g Kg^{-1})	1.10	1.25	1.40	1.55	1.70
Corn	773.06	773.06	773.06	773.06	773.06
Soybean meal	146.26	146.26	146.26	146.26	146.26
Limestone	8.19	8.19	8.19	8.19	8.19
Dicalcium Phosphate	13.59	13.59	13.59	13.59	13.59
Sodium Bicarbonate	6.72	6.72	6.72	6.72	6.72
Soybean oil	19.74	19.36	18.98	18.60	18.58
Glutamic acid	7.68	5.76	3.84	1.92	0.000
L-Lysine HCl	8.94	8.94	8.94	8.94	8.94
L-Threonine	3.34	3.34	3.34	3.34	3.34
DL-Methionine	2.95	2.95	2.95	2.95	2.95
L-Tryptophan	0.08	0.08	0.08	0.08	0.08
L-Valine	2.63	2.63	2.63	2.63	2.63
L-Isoleucine	1.78	1.78	1.78	1.78	1.78
L-Leucine	0.06	1.65	3.23	4.82	6.41
L-Histidine	0.99	0.99	0.99	0.99	0.99
L-Phenylalanine	1.59	1.59	1.59	1.59	1.59
Clean sand	0.00	0.69	1.41	2.12	2.47
Vit+Min supplement[2]	1.50	1.50	1.50	1.50	1.50
Antioxidant[3]	0.10	0.10	0.10	0.10	0.10
Antibiotic[4]	0.20	0.20	0.20	0.20	0.20
Total	1000	1000	1000	1000	1000
Calculated Composition (g Kg^{-1})					
Metabolizable Energy (MJ kg^{-1})	14.00	14.00	14.00	14.00	14.00
Total nitrogen (%)	2.42	2.42	2.42	2.42	2.42
Calcium	7.00	7.00	7.00	7.00	7.00
Available phosphorus	3.30	3.30	3.30	3.30	3.30
Sodium	2.00	2.00	2.00	2.00	2.00
Potassium	4.94	4.94	4.94	4.94	4.94
Chloride	2.26	2.26	2.26	2.26	2.26
SID Lys	12.30	12.30	12.30	12.30	12.30
SID Met + Cyst	6.80	6.80	6.80	6.80	6.80
SID Threonine	7.30	7.30	7.30	7.30	7.30
SID Tryptophan	2.00	2.00	2.00	2.00	2.00
SID Arginine	7.51	7.51	7.51	7.51	7.51
SID Valine	7.80	7.80	7.80	7.80	7.80
SID Leucine	11.00	12.50	14.00	15.50	17.00
SID Isoleucine	6.30	6.30	6.30	6.30	6.30
SID Methionine	4.74	4.74	4.74	4.74	4.74
SID Histidine	4.20	4.20	4.20	4.20	4.20
SID Phen + Tyr	9.15	9.15	9.15	9.15	9.15
SID Phenylalanine	7.20	7.20	7.20	7.20	7.20

[1]-SID: standardized ileal digestible. [2]- Provide per kilogram of diet: (nutrition levels kg^{-1} of diet): Vit. A - 3000 UI; Vit. D3 - 600 UI; Vit. E – 6 UI; Vit. B1 – 0,588 mg; Vit. B2 – 1,2795 mg; Vit. B6 – 0,594 mg; Vit. B12 – 7,9995 mcg; Vit. K3 – 0,7275 mg; Calcium pantothenate – 4,749 mg; Niacin – 12,7995 mg; Folic acid – 0,159 mg; Biotin – 0,039 mg; Antioxidant – 3 mg; Zn – 0,03 g; Fe – 0,0225 g; Mn – 0,0156 mg; Cu – 3,6 mg; I – 0,2475 mg; Co – 0,0405 mg; Selênio – 0,09 mg; Veiculo Q.S.P. – 1,5 g.[3]-BHT; [4]-Tylan

Animals were weighed at the beginning and at the end of the experiment, and the diets were weighed whenever provided to the animals for the determination of average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR).

At the end of the trial period, the animals were submitted to the evaluation of backfat thickness (BT) and depth of the *longissimus dorsi* muscle (LD) through the ultrasound equipment Aloka® SSD 500 Vet, coupled to a probe of 7.5 cm and 3.5 MHz. The region P2, located at the last rib of the chest, was determined at 6 cm from the midline, where the site was cleaned and the images captured. Afterwards, the measurements of backfat thickness (BT) and depth of LD muscle were performed. Measurements were performed using Image Pro Plus® software.

For determination of the lean meat percentage (LMP) an equation was applied for the estimation of this parameter to pigs *in vivo*: Y= 60.69798 – 0.89211S + 0.10560M; (Vítek et al., 2008); where Y = the estimated percentage of lean meat; S = Backfat thickness (mm), measured at a point on the medial line, 7 cm between the penultimate and antepenultimate rib; M = depth of the muscle at the measurement point.

After a 6-hour fast, cranial vena cava blood samples (6 mL) were collected and stored in tubes containing EDTA anticoagulant (Cai et al., 1994) for triglyceride, urea, total protein, total cholesterol and creatinine further analysis. To determine glucose, 3 mL of blood samples were collected in tubes containing sodium fluoride.

After sampling, the samples were centrifuged at 3,000 RPM for 15 minutes and only the samples for glucose determination were centrifuged for 30 minutes to obtain the plasma. Then 3 mL of plasma (in duplicate) were transferred to Eppendorf® type tubes.

For the accomplishment of the biochemical analyzes, commercial reagent kits were used (Laborclin®), the quantities of each blood component being determined by reading in BIOPLUS® 2000 Spectrophotometer, following the operational procedures (POP) described in the kits. The laboratory tests were carried out in the Laboratory of Plasma Analyzes, in the swine sector of the Experimental Farm (Iguatemi district).

Data regarding performance, BT, LD, LMP and blood parameters were submitted to analysis of variance, where the initial weight of the pigs was used as covariate. The degrees of freedom related to the leucine levels were deployed in orthogonal polynomials to obtain the regression equations. The data were adjusted by the quadratic model associated with the linear response plateau model (LRP). The data were submitted to the

statistical analysis by the System of Statistical and Genetic Analysis - SAEG (Universidade Federal de Viçosa, 2009).

3.3 RESULTS AND DISCUSSION

The FCR reduced (P = 0.018) as the levels of SID leucine in the diet increased. No differences (P> 0.05) were observed for the final weight (FW), ADFI, ADG, LD, BT and LMP in relation to the leucine levels evaluated (Table 2).

Table 2. Performance (kg), Backfat thickness (BT) (cm^2), depth of the *longissimus dorsi* muscle (LD) (cm) and lean meat percentage (LMP) of pigs in the initial phase fed low crude protein diets with different levels of SID leucine

	SID leucine (%)						*P* - value	
Item[1]	1.10	1.25	1.40	1.55	1.70	SEM	Linear	Quadratic
IW	11.04	11.17	11.21	11.21	11.09	0.107	0.7077	0.2011
FW	23.81	24.85	24.95	25.44	24.86	0.590	0.1912	0.2799
ADFI	0.916	0.974	0.916	0.951	0.946	0.027	0.7834	0.9588
ADG	0.437	0.455	0.450	0.487	0.473	0.019	0.1182	0.8481
FCR[2]	2.11	2.14	2.04	1.96	2.01	0.072	0.0188	0.6039
BT	0.453	0.435	0.473	0.422	0.491	0.023	0.2587	0.2604
LD	2.353	2.378	2.380	2.290	2.276	0.070	0.2620	0.1899
LMP	60.54	60.56	60.52	60.56	60.49	0.021	0.1488	0.1312

[1]-IW= Initial weight; FW= Final weight; ADFI= Average daily feed intake; ADG= Average daily gain; FCR= Feed conversion ratio; [2]-$Y= 2.48874 – 0.331127X$ (R^2=0.46). SEM: Standard Erro of Mean.

Piglets fed diets containing 1.97% and 3.75% SID leucine presented a reduction in ADFI (0.583 and 0.490 kg), when compared to the control group (1.09% SID leucine, 0.638 kg; Wessels et al., 2016). According to the authors, this reduction in feed intake may be related to competition in the uptake of amino acids, specifically in brain tissue, since the membrane transporters are the same for BCAAs and tryptophan, reducing the concentration of the latter amino acid that is responsible for production of serotonin, which regulates appetite. In this sense, it can be inferred that the levels of SID leucine evaluated in the present study should have not provided the same effects mentioned above, since the ADFI was not influenced (P>0.05). However, the levels of SID leucine evaluated were lower (1.10 - 1.70%) than those studied by Wessels et al. (2016).

The opposite result was observed by Yin et al. (2010), when they performed the supplementation of L-leucine (1.88%) in diets with 16.9% of CP in recently weaned

piglets. The ADG (0.332 kg) was better than the animals fed the control diet (1.36% SID leucine) (ADG: 0.206 kg) without influencing the ADFI. They concluded that chronic L-leucine supplementation promotes the growth of muscle tissue, liver and intestine. Probably, these differences are due to the different phases of production evaluated.

According to Gloaguen et al. (2012), excess leucine and valine deficiency reduce the use of protein offered in the diet, and the first response of the animal is the reduction in feed intake and, therefore, reduction in growth rate. Thus, it can be inferred that the higher level of leucine evaluated did not characterize an excess, since a conventional feed for pigs at this stage of production has a percentage of SID leucine of 1.50%.

As SID leucine levels increased (1.10; 1.25; 1.40; 1.55 and 1.70%) the FCR improved. An opposite result was found by Wessels et al. (2016) who observed that FCR was not influenced by excess SID leucine in the piglets diet. However, Wiltafsky et al. (2010), when evaluating the leucine:valine ratio in which the levels of SID leucine evaluated were 1.29; 1.81; 2.09; 2.35 and 2.41% for piglets from 8 to 25 kg, observed reduction in FCR as dietary leucine levels increased. These results demonstrate that levels higher than those used in the present study may reduce FCR.

Although the levels studied by these authors present a difference of 1.12 percentage points between the lowest and highest levels of SID leucine (1.29 and 2.41%) in the present study a reduction in the FCR was also observed when evaluating a smaller amplitude between the lowest and highest level (1.10 to 1.70%), but without influencing variables such as ADG, ADFI, LD and BT.

A worse FCR was observed by Gloaguen et al. (2013) when evaluating SID ratio leucine: lysine (70; 78; 86; 94; 102 e 130%) for piglets from 10 to 15 kg. The authors concluded that leucine excess in diets (102 and 130%), as well as leucine deficiency (10% less SID leucine in the basal diet) influenced negatively the productive efficiency of the piglets, also indicating that the SID ratio leucine:lysine of 102% is not necessary for protein deposition, but may be an important factor in the competition of AA, which may increase catabolism, especially of valine and isoleucine.

The addition of L-leucine in low-protein diets may have adverse effects on animal performance variables, reducing feed intake and increasing FCR even if results are not significant (Erwan et al., 2009). However, a high variability of the performance results between the different researches is observed and, in general, it is observed that the effects

of SID leucine in rations with low CP are obtained when using very high levels of leucine in diets.

On the other hand, Garcia et al. (2015) observed a quadratic response for FCR when evaluating different levels of leucine in diets for growing pigs. These authors related this result to the increase in expression of the cationic amino acid transporter ($b^{0,+}$), responsible for the absorption of leucine and lysine at the intestinal level.

Leucine stimulates the activity of mTOR, which is responsible for protein metabolism in muscle tissue (Drummond et al., 2009). Studies have demonstrated a high activity of proteins responsible for protein synthesis at the cellular level in piglets submitted to leucine infusions after 24 hours of life (Escobar et al., 2005; Boutry et al., 2013). Similarly, Torrazza et al. (2010) observed that leucine supplementation in diets with low CP for piglets stimulated protein synthesis in the LD, gastrocnemius and masseter muscles, as well as increased the anabolic capacity of organs such as the heart, jejunum, kidneys and pancreas.

Thus, although leucine supplementation promotes muscle tissue growth (Yin et al., 2010), the levels of SID leucine evaluated in the present research did not cause the same effects mentioned above, since the LD and LMP were not influenced (P>0.05).

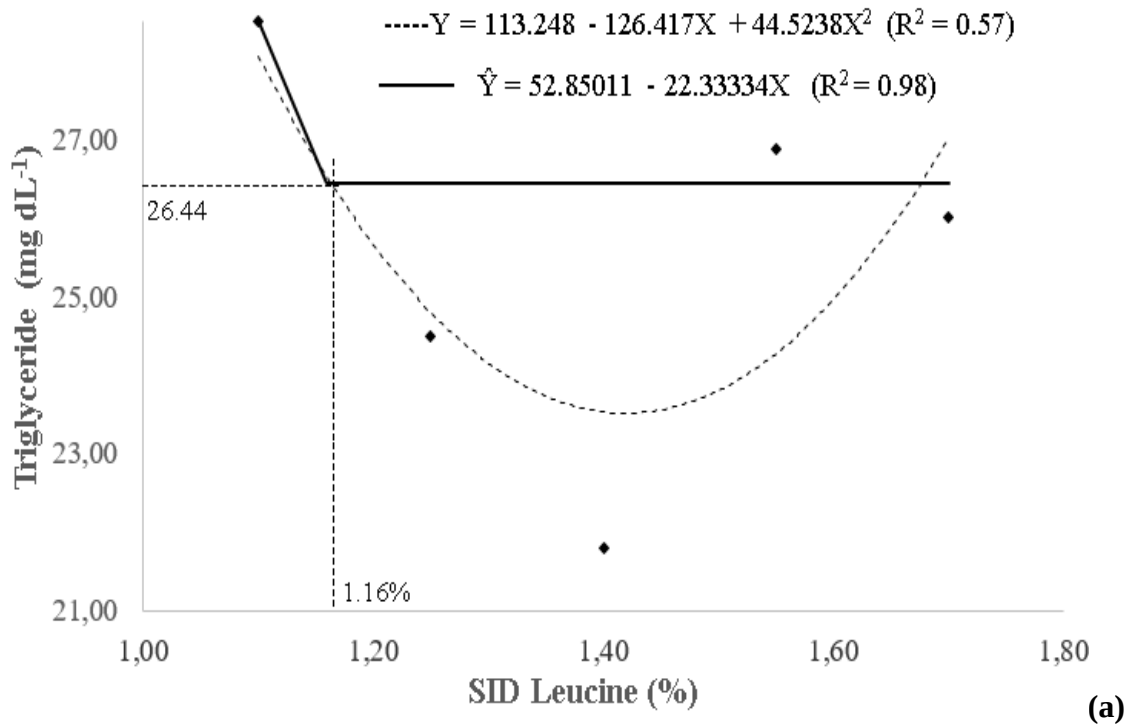
Regarding the plasma variables (Table 3), no differences (P>0.05) were observed for glucose, total proteins, total cholesterol and creatinine. However, triglycerides (P=0.0497) and urea (P=0.0014) presented a response to SID leucine levels, in which there was an adjustment of the quadratic model associated with the LRP model.

Table 3. Plasma levels of glucose, triglycerides, urea, total proteins, total cholesterol (mg dL^{-1}) and creatinine (U L^{-1}) of pigs in the initial phase fed low crude protein diets with different levels of SID leucine

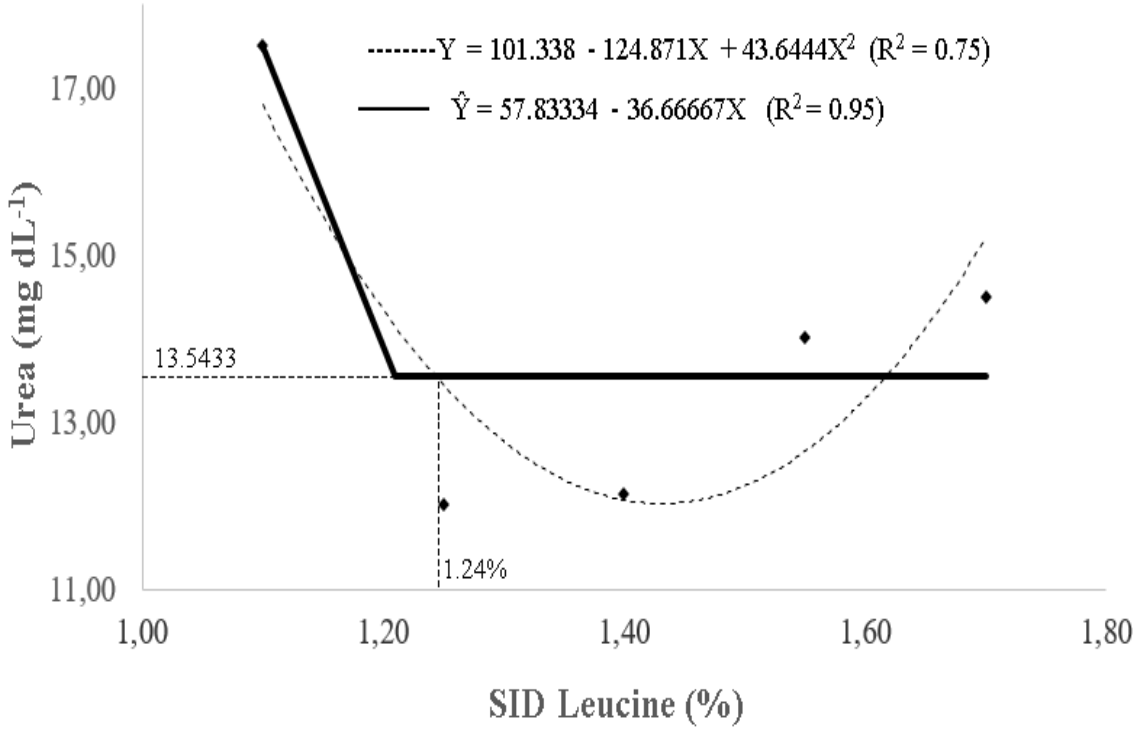
	SID leucine (%)						P - value	
Item	1.10	1.25	1.40	1.55	1.70	Stand. Erro	Linear	Quad
Glucose	75.33	74.83	76.33	75.00	74.33	0.333	0.8096	0.7242
Triglyceride[1]	28.50	24.50	21.80	26.88	26.00	1.136	0.6446	0.0497
Urea[2]	17.50	12.00	12.13	14.00	14.50	1.000	0.2061	0.0014
Total proteins	5.93	5.28	5.70	5.59	5.46	0.110	0.4014	0.5531
Total cholesterol	70.32	60.10	66.88	69.50	71.12	2.002	0.1942	0.0589
Creatinine	1.24	1.30	1.26	1.27	1.19	0.018	0.6663	0.5473

[1-]$Y= 113.248 – 126.417X + 44.5238X^2$ (R^2=0.57); [2-]$Y= 101.338 – 124.871X + 43.6444X^2$ (R^2=0.75).

The triglycerides had a lower concentration (26.44 mg dL^{-1}) at the 1.16% level of leucine (Figure 8a) and for plasma urea the optimum level was 1.24% SID leucine, with a concentration of 13.543 mg dL^{-1} urea (Figure 8b).



(a)



(b)

Figure 8. Triglyceride (a) and urea (b) in pig plasma (11 to 25 kg) as a function of different levels of SID leucine in the diet.

In the process of carbon catabolism of AA there are specific routes for each amino acid. Metabolic intermediates such as Acetyl-CoA and Acetoacetyl-CoA form fatty acids, which are derived from the metabolism of ketogenic amino acids, such as leucine, responsible for the formation of ketone bodies such as energy and cholesterol stores (Nelson & Cox, 2011). However, increasing levels of SID leucine showed a reduction in the plasma concentration of triglycerides at the level of 1.16% SID leucine, although leucine stimulated adipogenesis and lipogenesis (Melnik, 2012).

Studying the effects of BCAA granules on the accumulation of triglycerides in the tissues of obese rats, Arakawa et al. (2011) observed that rats fed a diet with a high concentration of BCAA (2%) reduced the levels of triglycerides in skeletal muscle and liver, concluding that this reduction was induced by BCAA, avoiding an increase in the concentration of triglycerides, because their excess interferes with the uptake of glucose by the GLUT4 intracellular transporter, stimulated by plasma insulin.

Contrary to what was observed in the present study, Duan et al. (2016) and Li et al. (2016) did not observe alterations in plasma triglyceride concentrations when they evaluated diets containing 17 and 20% CP with different levels of BCAA for piglets in the initial phase, concluding that diets with 17% CP and 1.44% and 2.35% SID leucine increased the concentration of intramuscular fat in the femoral biceps.

It is fact that leucine levels did not influence ADFI and ADG, but improving FCR (Table 2) seems to indicate a better use of leucine, not affecting the use of other BCAA. However, it is known that urea is the main product of protein catabolism and if the amino acids are not reused for the synthesis of new amino acids, or nitrogen products, the amino groups are directed to this final excretion product (Nelson & Cox, 2011).

This effect may have been shown to be below the 1.24% level because the reduction in plasma urea concentrations due to protein metabolism suggests that increased protein deposition is associated with increased protein synthesis, reducing the catabolism of AA (Reeds et al., 1987). This was found by Ren et al. (2015) when they observed a reduction in plasma urea concentration (98.22 nMol/mL) in piglets fed diets supplemented with BCAAs (1.38% SID leucine) and low percentage of CP (17.85%), indicating that in addition to the reduction in protein levels in the diets, BCAA supplementation further reduces plasma urea concentration because it allows better balance between the limiting AA in the diet.

On the other hand, the excess of leucine in the diet is related to the greater degradation of valine and isoleucine due to competition for the enzyme dehydrogenase of branched-chain α-ketoacid (Harris et al. 2001). This response was observed by Gatnau et al. (1995), who attributed the reduction in plasma concentrations of valine and isoleucine to excess leucine and its metabolites in piglet diets. Thus, plasma urea concentration may be a good indicator of the amino acid degradation process. According to Reeds et al. (1987), metabolic changes of excess leucine in the diet can be studied by measuring urea synthesis and by leucine turnover, as well as isoleucine and valine (Holecek, 2013).

The results of the present research suggest that levels above 1.24% of SID leucine may have contributed to the increase of BCAA catabolism and other amino acids, increasing the concentration of urea, indicating a greater degradation of the AA.

Leucine, in addition to altering the plasma levels of urea and other AA, such as valine and isoleucine (Macotela et al., 2011), stimulates the synthesis of muscle protein (Pasiakos & McClung, 2011). Although the level of 1.24% SID leucine provided the lowest catabolism (lower nitrogen excretion), no significant effect was observed for LD. Similar results were found by Madeira et al. (2014) who did not observe any influence of leucine supplementation, in diets with low CP, in the performance variables, or in the carcass characteristics.

However, the optimal level of SID leucine related to the lower concentration of plasma urea was determined by the association of the quadratic model and LRP (1.24%) and is close to that recommended by the NRC (2012), 1.23% for pigs from 10 to 25 kg.

The use of industrial AA allows a formulation of diets with low percentage of CP (Lordelo et al. 2008), as in the present study (15.16% CP), taking into account the nutritional requirements of the essential AA and allowing the increase in the efficiency of the use of nitrogen in the diet. However, according to Mansilla et al. (2015), the percentage of CP used influences the concentration of endogenous nitrogen, which is an important factor in the synthesis of non-essential AA.

Thus, emphasizing the importance of the percentage of CP in body nitrogen deposition, Langer et al. (2000) indicated that diets with 15% CP and leucine excess increased the catabolism of valine or isoleucine, reducing the nitrogen utilization of the diet and could affect the deposition of muscle protein. However, Langer & Fuller (2000) did not observe changes in the body nitrogen concentrations of females fed diets

containing 15% CP and excess leucine, concluding that even with high BCAAs levels in the diet, the concentration and retention of body nitrogen were not affected.

Likewise, the variables LD and LMP were not influenced (P>0.05) by levels of SID leucine, indicating that possibly the retention of body nitrogen was not influenced by the levels of SID leucine evaluated in the present study.

3.4 CONCLUSIONS

Diets with low crude protein and levels up to 1.70% SID leucine did not impair performance and this level provided the best feed conversion ratio.

3.5 REFERENCES

Arakawa, M.; Masaki, T.; Nishimura, Masataka, S.; Yoshimatsu, H. 2011. The effects of branched-chain amino acid granules on the accumulation of tissue triglycerides and uncoupling proteins in diet-induced obese mice. Endocrine Journal 58:161-170.

Boutry, C.; El-Kadi, S. W.; Suryawan, A.; Wheatley, S. M.; Orellana, R. A. Kimball, S. R.; Nguyen, H. V.; Davis, T. A. 2013. Leucine pulses enhance skeletal muscle protein synthesis during continuous feeding in neonatal pigs. American Journal of Physiology, Endocrynology and Metabolism 305:E620-E631.

Cai, Y.; Zimmerman, D.R.; Ewan, R.C. 1994. Diurnal variation in concentrations of plasma urea nitrogen and amino acids in pigs given free access to feed or fed twice daily. The Journal of Nutrition 124:1088-1093.

Calvert, C. C.; Klasing, K. C.; Austic, R. E. 1982. Involvement of food intake and amino acid catabolism in the branched-chain amino acid antagonism in chicks. The Journal of Nutrition 112:627–635.

Cojocaru, E. Filip, N.; Ungureanu, C.; Filip, C.; Danciu, M. 2014. Effects of Valine and Leucine on Some Antioxidant Enzymes in Hypercholesterolemic Rats. Health 6:2313-2321.

Drummond, M. J.; Dreyer, H. C.; Christopher S. Fry, C. S.; Glynn, E. L.; Rasmussen, B. B. 2009. Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. Journal of Applied Physiology 106:1374–1384.

Duan, Y.; Duan, Y.; Li, F.; Li, Y.; Guo, Q.; Ji, Y.; Tan, B.; Li, T.; Yin, Y. 2016. Effects of supplementation with branched‐chain amino acids to low‐protein diets on expression of genes related to lipid metabolism in skeletal muscle of growing pigs. Amino Acids. doi 10.1007/s00726-016-2223-2.

Erwan, E.; Alimon, A. R.; Sazili, A. Q.; Yaakub, H.; Hilmi, M. 2009. Effect of L-Leucine Supplementation on Growth Performance and Carcass Characteristics of Grower-Broiler Chickens Fed Low Protein Diets. American Journal of Animal and Veterinary Sciences 4:95-100.

Escobar, J.; Frank, J. W.; Suryawan, A.; Nguyen, H. V.; Kimball, S. R.; Jefferson, L. S.; Davis, T. A. 2005. Physiological rise in plasma leucine stimulates muscle protein synthesis in neonatal pigs by enhancing translation initiation factor activation. American Journal of Physiology, Endocrynology and Metabolism 288:E914-E921.

Farran, M. T.; Barbour, E. K.; Ashkarian, V. M. 2002. Effect of excess leucine in low protein diet on ketosis in 3-week-old male broiler chicks fed different levels of isoleucine and valine. Animal Feed Science and Technology 103:171-176

Garcia, H.; Morales, A.; Araizal, A.; Htoo, J. K.; Cervantes, M. 2015. Gene expression, serum amino acid levels, and growth performance of pigs fed dietary leucine and lysine at different ratios. Genetics and Molecular Research 14:1589-1601.

Gatnau, R.; Nissen, L.; Zimmerman, D. R.; Wannemuehlert, M. Ewan, C. 1995. Effects of Excess Dietary Leucine and Leucine Catabolites on Growth and Immune Responses in Weanling Pigs. The Journal of Animal Science 73:159-165.

Gloaguen, M.; Le Floc'h1, N.; Primot, Y.; Corrent, E.; van Milgen, J. 2013a. Response of piglets to the standardized ileal digestible isoleucine, histidine and leucine supply in cereal–soybean meal-based diets. Animal 7:901-908.

Gloaguen, M.; Le Floc'h, N.; Corrent, E.; Primot, Y.; Van Milgen, J. 2012. Providing a diet deficient in valine but with excess leucine results in a rapid decrease -keto acid ain feed intake and modifies the postprandial plasma amino acid and concentrations in pigs. The Journal of Animal Science 90:3135-3142.

Harris, R. A.; Kobayashi, R.; Murakami, T.; Shimomura, Y. 2001. Regulation of branched-chain α-keto acid dehydrogenase kinase expression in rat liver. The Journal of Nutrition 131:841–845.

Holecek, M. 2013. Branched-chain amino acids and ammonia metabolism in liver disease: Therapeutic implications. Nutrition 29:1186–1191.

Langer, S.; Fuller, M. F. 2000. Interactions among the branched-chain amino acids and their effects on methionine utilization in growing pigs: effects on nitrogen retention and amino acid utilization. The British Journal of Nutrition 83:43-48.

Langer. S; Scislowski, P. W. D.; Brown, D. S.; Dewey, P.; Fuller, M. F. 2000. Interactions among the branched-chain amino acids and their effects on methionine utilization in growing pigs: effects on plasma amino- and keto-acid concentrations and branched-chain keto-acid dehydrogenase activity. The British Journal of Nutrition 83:49-58.

Li, Y.; Wei, H.; Li, F.; Chen, S.; Duan, Y.; Guo, Q.; Liu, Y.; Yin, Y. 2016. Supplementation of branched-chain amino acids in protein-restricted diets modulates the expression levels of amino acid transporters and energy metabolism associated regulators in the adipose tissue of growing pigs. Animal Nutrition 2:24-32.

Lordelo, M. M.; Gaspar, A. M.; Le Bellego, L.; Freire, J. P. B. 2008. Isoleucine and valine supplementation of a low-protein corn-wheat-soybean meal-based diet for piglets: Growth performance and nitrogen balance. Journal of Animal Science 86:2936-2941.

Macotela, Y.; Brice, E.; Bang, A. M.; Espinoza, D. O.; Boucher, J.; Beebe, K.; Gall, W.; Kahn, R. 2011. Dietary Leucine - An Environmental Modifier of Insulin Resistance Acting on Multiple Levels of Metabolism. Plos One 6:1-13.

Madeira, M. S.; Alfaia, C. M.; Costa, P.; Lopes, P. A.; Lemos, J. P. C.; Bessa, R. J. B.; Prates, J. A. M. 2014. The combination of arginine and leucine supplementation of reduced crude protein diets for boars increases eating quality of pork. The Journal of Animal Science 92:2030-2040.

Manjarrez, R. M. O.; Torres-Vaca, M.; Gonzales-Gallego, J.; Ordones-Alvear, I. 2015. El β-hidroxi-β-metilbutirato (HMB) como suplemento nutricional (I): metabolismo y toxicidad. Nutrición Hospitalaria 2:590-596.

Mansilla, W. D.; Columbus, D. A.; Htoo, J. K.; Lange C. F. M. 2015. Nitrogen Absorbed from the Large Intestine Increases Whole-Body Nitrogen Retention in Pigs Fed a Diet Deficient in Dispensable Amino Acid Nitrogen. The Journal of Nutrition. doi:10.3945/jn.115.212316.

Melnik, B. C. 2012. Leucine signaling in the pathogenesis of type 2 diabetes and obesity. World Journal of Diabetes 3:38-53.

NATIONAL RESEARCH COUNCIL – NRC. 2012. Nutrients requirement of swine. 11 ed. National Academic Press. Washington DC.

Nelson, D.L.; Cox, M.M. Princípios de Bioquímica de Lehninger. São Paulo: SARVIER, 5Ed., 2011, 682p.

Pasiakos, M.; McClung, J. P. 2011. Supplemental dietary leucine and the skeletal muscle anabolic response to essential amino acids. Nutrition Reviews 69:550-557.

Reeds, P. J.; Fuller, M. F.; Dadenhead, A.; Hay, S. M. 1987. Urea synthesis and leucine turnover in growing pigs: changes during 2 d following the addition of carbohydrate or fat to the diet. The British Journal of Nutrition 58:301-311.

Ren, M.; Zhang, H.; Zeng, X. F.; Liu, H.; Qiao, Y. 2015. Branched-chain Amino Acids are Beneficial to Maintain Growth Performance and Intestinal Immune-related Function in Weaned Piglets Fed Protein Restricted Diet. M. The Journal of Animal Science 28:1742-1750.

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T.; Euclides, R. F. 2011. Tabelas Brasileiras para Aves e Suínos: Composição de alimentos e exigências nutricionais. 3rd ed. UFV, Viçosa, Minas Gerais, Brasil.

Shimomura, Y.; Harris, R.A. 2006. Metabolism and physiological function of branched-chain amino acids: discussion of session 1. Journal of Nutrition 136:232–233.

Tannous, R. I.; Quinton, R. R.; Harper, A. E. 1966. Effect of Leucine-lsoleucine Antagonism on the Amino Acid Pattern of Plasma and Tissues of the Rat. Archives of Biochemistry and Biophysics 113:356-351.

Torrazza, R. M.; Suryawan, A.; Gazzaneo, M. C.; Orellana, R. A.; Frank, J. W. Nguyen, H. V.; Fiorotto, M. L. El-Kadi, S.; Davis, T. A. 2010. Leucine Supplementation of a Low-Protein Meal Increases Skeletal Muscle and Visceral Tissue Protein Synthesis in Neonatal Pigs by Stimulating mTOR-Dependent Translation Initiation. The Journal of Nutrition 140:2145-2152.

Vítek, M.; Pulkrábek, J.; Vališ, L.; David, L.; Wolf, J. 2008. Improvement of accuracy in the estimation of lean meat content in pig carcasses. Czech Journal of Animal Science 53:204-211.

Wessels, A. G.; Kluge, H.; Hirche, F.; Kiowski, A.; Schutkowski, A.; Corrent, E.; Bartelt, J.; Konig, B.; Stangl, I. 2016. High Leucine Diets Stimulate Cerebral Branched-Chain Amino Acid Degradation and Modify Serotonin and Ketone Body Concentrations in a Pig Model. Plos One. doi:10.1371/journal.pone.0150376.

Wiltafsky, M. K.; Pfaffl, M. W.; Roth, F. X. 2010. The effects of branched-chain amino acid interactions on growth performance, blood metabolites, enzyme kinetics and transcriptomics in weaned pigs. British Journal of Nutrition 103:964-976.

Yin, Y.; Yao, K.; Liu, Z.; Gong, M.; Ruan, Z.; Deng, D.; Tan, B.; Liu, Z.; Wu, G. 2010. Supplementing L-leucine to a low-protein diet increases tissue protein synthesis in weanling pigs. Amino Acids 39:1477-1486.

IV – A exigência de valina para fêmeas suínas na fase inicial não é influenciada por níveis moderados de leucina

RESUMO- O objetivo deste trabalho foi avaliar os níveis de valina digestível sobre o desempenho, espessura de toucinho, profundidade do músculo e parâmetros sanguíneos de suínos na fase inicial. Foram utilizadas 72 fêmeas mestiças, com peso inicial de 15,16 ± 1,15 kg; distribuídas em um delineamento experimental de blocos casualizados, em um esquema fatorial 2 x 4, sendo dois níveis de leucina digestível (1,20 e 1,77%) e quatro níveis de valina digestível (0,58; 0,73; 0,88 e 1,03%), nove repetições e um animal por unidade experimental. O peso final e o ganho de peso diário apresentaram efeito linear (P=0,017 e P=0,034, respectivamente) e quadrático (P=0,003 e P=0,0003, respectivamente). Ajustando os dados ao modelo quadrático associado ao platô do modelo Linear Response Platô, o nível ótimo de valina digestível para PF e GPD foi de 0,725% e de 0,703%. Foi observado efeito quadrático (P=0,0007) para o consumo diário de ração, o nível ótimo de valina digestível é de 0,822%. A espessura de toucinho, profundidade do lombo *Longissimus dorsi* e porcentagem de carne magra não foram influenciadas pelos níveis de leucina ou valina digestível. Houve interação (P=0,047) entre os níveis de leucina (1,70%) e valina (1,03%) digestível sobre a ureia (11,50 mg/dL). A exigência de valina digestível para suínos, na fase inicial, não é influenciada pelos níveis de leucina normalmente praticados em dietas convencionais e a exigência diária é de 9,72 g de valina/dia, correspondendo a uma concentração de 0,703% na dieta, para um máximo ganho de peso diário.

Palavras-chave: Leucina digestível, valina digestível, ganho de peso diário, modelo quadrático.

IV – The requirement of valine for gilts in the initial phase is not influenced by moderate levels of leucine

ABSTRACT – The objective of this study was to evaluate the Standardized Ileal Digestible (SID) valine levels on performance, backfat thickness, muscle depth and blood parameters of sows in the initial phase. A total of 72 pigs, with initial weight of 15.16 ± 1.15 kg; were distributed in a randomized complete block in a factorial design 2 x 4, two levels of SID leucine (1.20 and 1.77%) and four levels of SID valine (0.58; 0.73; 0.88 and 1.03%) with nine replicates and one animal per experimental unit (EU). The final weight (FW) and the average daily gain (ADG) presented a linear effect (0.017 and 0.034) and quadratic effect (0,003 and 0.0003, respectively). Setting data to the quadratic model associated with the linear response plateu model (LRP), the optimal level of SID valine for FW and ADG was 0.725% and 0.703%. A quadratic effect (P=0.0007) for the average daily feed intake (ADFI) was observed, in which, the optimal level of SID valine was 0.822% without affecting other performance parameters (backfat thickness, loin depth and lean meat percentage). There was interaction (P=0,047) between levels of SID leucine (1.70%) and SID valine (1.03%) on plasma urea (11.50 mg/dL). The requirement of SID valine for pigs in the initial phase is not influenced by the leucine levels normally practiced in conventional diets and the daily requirement is 9.72 g valine/day, corresponding to a concentration of 0.703% in the diet, to a maximum average daily gain.

Keywords: SID Leucine, SID valine, average daily gain, quadratic model.

4.1 INTRODUCTION

The reduction crude protein (CP) reduction in pigs diets is possible through the addition of industrial amino acids (AA), reducing the inclusion of soybean meal, while providing the animals with adequate amounts of AA for a better productive performance and reduction of nitrogen excretion into the environment.

All requirements must be met in ideal quantities; AAs are building blocks for proteins and must be present in cells for the synthesis of different polypeptides. AAs are classified as non-essential AAs (NEAA) and essential AA (EAA). These latter have a carbon skeleton that is not synthesized in animal cells, being classified as nutritionally essential, and should be included in diets to maintain the physiological functions of body cells and tissues (Wu, 2014). In this class there are branched-chain amino acids (BCAA), which are represented by leucine, isoleucine and valine.

Valine in present in a lower concentration in corn (0.32%) and soybean meal (1.93%) than leucine, which represents 0.87% in corn and 3.11% in soybean meal (Rostagno et al., 2011). Valine is considered the fifth limiting amino acid in diets for pigs, and it is often necessary to include L-valine in low CP diets with to ensure compliance with their requirements at different stages of production.

Leucine is abundant in most foods, and the requirement of animals is easily met, so it becomes difficult to create severe disability (Franco, 2011). From the three BCAA, leucine has been the most studied in different animal species and is characterized by having potent effects on protein stimulation (Gaines et al., 2011; Gloaguen et al., 2012; Columbus et al., 2015; Wessels et al. 2016), increase in intramuscular fat in pigs (Duan et al., 2016), energy metabolism (Arakawa et al., 2010; Macotela et al., 2011; Ren et al., 2015; Li et al., 2016) and other physiological functions by optimizing processes such as glucose uptake, mTOR stimulation and insulin resistance.

However, excess leucine may adversely affect the viability of other BCAA and consequently reduce the productive performance of the animals (Morales et al., 2016). Productive parameters such as average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) are influenced by BCAA and, according to Langer & Fuller (2000), the excess of leucine increases the oxidation of isoleucine and valine, which are responsible for the dietary intake, affecting the performance of the

animals and reducing the growth when these two BCAA are limited, being important the study its requirements.

Excess of valine, when compared to leucine, does not affect the performance variables of the pigs. Torres et al. (1995) evaluated the uptake of valine (infusion of 0.2 μmol/L) in the presence of leucine (1 μmol/L), and found a reduction of 74% in valine absorption, in the uptake and catabolism of valine by muscle tissue (decrease from 25.2 μmol/dL to 6.6 μmol/dL), influenced directly by leucine increase; but the efficiency of the protein synthesis process was not influenced. Thus, excess leucine may influence the catabolism of valine, however the antagonism is not so severe in relation to isoleucine (Langer & Fuller 2000).

The requeriments of BCAA, as well as its catabolism and antagonism, have been studied over time (Oestemer et al., 1973; Gatnau et al., 1995; Gaines et al., 2011; Gloaguen et al., 2012; Nørgaard et al., 2015; Soumeh et al., 2015; Garcia et al., 2015), which has provided an improvement over the requirement of these AA; as well as the improvement in CP concentration in the diet when applying the concept of ideal protein, to reduce the excretion of nitrogen to the environment. However, studies are needed to evaluate the requirements of valine in diets with different concentrations of leucine for pigs in the initial phase.

The BCAA requirements are different for each stage of production and the imbalance of these in the traditional ingredients used in animal feed has been studied. The objective in this study is to determine the requirements of SID valine of gilts in the initial phase (15-30 kg), influenced by moderate levels of SID leucine and low CP diets.

4.2 MATERIAL AND METHODS

The experiment was carried out in the Swine Sector of the Experimental Farm of Iguatemi (FEI), which belongs to the Agrarian Science Center of the State University of Maringá (CCA/UEM) and in the Laboratory of Animal Nutrition (LANA/UEM) of the Animal Science Department. All experimental procedures were previously submitted to the Committee for Ethical Conduct on the Use of Animals under Experimentation (CEUA/UEM), and have been approved for execution (Protocol n. 8538100616).

Seventy-two gilts (Landrace x Large White x Pietrain), with initial weight of 15.16 ± 1.15 kg were distributed on the basis of initial weight in a randomized complete block

design in a 2 x 4 factorial scheme, consisting of two levels of SID leucine (1.20 and 1.77%), four levels of SID valine (0.58, 0.73, 0.88 and 1.03%) and nine replicates.

The experimental diets were formulated with corn, soybean meal, minerals, vitamins, amino acids and additives (Table 4). The CP content was reduced in relation to that suggested by Rostagno et al. (2011). L-leucine was added to diets at the expense of the inert to meet the levels of SID leucine. Glutamic acid was used in order to maintain the same nitrogen content in the different diets.

The amino acid compositions of corn and soybean meal used to formulate the diets were analyzed in EVONIK Industries, applying the true ileal digestibility coefficients proposed by Rostagno et al. (2011), to estimate the SID amino acid values of these foods.

The animals were housed in masonry nursery, with suspended bays (1.32 m^2) and partly leaked floor, equipped with semiautomatic feeders, located in the front of the bay, and a nipple type drinker in the back.

Animals were weighed at the beginning and at the end of the experiment, and the diets were weighed whenever provided to the animals for the determination of average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR).

At the end of the trial period, the animals were submitted to the evaluation of the backfat thickness (BT) and depth of the *longissimus dorsi* (LD) muscle through the ultrasound equipment Aloka® SSD 500 Vet, coupled to a probe of 7.5 cm and 3.5 MHz. The region P2, located at the last rib of the chest, was determined at 6 cm from the midline, where the site was cleaned and the images captured. Afterwards, the measurements of BT and depth of LD muscle were performed. Measurements were performed using Image Pro Plus® software.

For determination of the lean meat percentage (LMP) an equation was applied for the estimation of this parameter to pigs *in vivo*, as follows: Y= 60.69798 – 0.89211S + 0.10560M; (Vítek. et al., 2008); where Y = the estimated percentage of lean meat; S = Backfat thickness (mm), measured at a point on the medial line, 7 cm between the penultimate and antepenultimate rib; M = depth of the muscle at the measurement point.

Table 4. Chemical and energetic composition (as fed basis) of diets containing different levels of SID leucine (Leu) and SID valine (Val) (g kg^{-1}) for gilts from 15 to 30 kg of live weight[1]

SID leucine	1.20				1.77			
SID valine	0.58	0.73	0.88	1.03	0.58	0.73	0.88	1.03
Ingredients g kg^{-1}								
Corn	760.21	760.21	760.21	760.21	760.21	760.21	760.21	760.21
Soybean meal	179.00	179.00	179.00	179.00	179.00	179.00	179.00	179.00
Dicalcium Phosphate	16.06	16.06	16.06	16.06	16.06	16.06	16.06	16.06
Limestone	8.33	8.33	8.33	8.33	8.33	8.33	8.33	8.33
Salt	4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56
Soybean oil	4.98	4.93	4.89	4.84	3.09	2.32	1.56	0.79
Glutamic acid	7.40	5.22	3.05	0.87	1.37	0.91	0.46	0.00
L-Lysine HCl	6.45	6.45	6.45	6.45	6.45	6.45	6.45	6.45
L-Threonine	2.45	2.45	2.45	2.45	2.45	2.45	2.45	2.45
DL-Methionine	1.84	1.84	1.84	1.84	1.84	1.84	1.84	1.84
L-Tryptophan	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64
L-Valine	0.05	1.62	3.20	4.77	0.05	1.62	3.20	4.77
L-Isoleucine	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
L-Leucine	0.00	0.00	0.00	0.00	5.92	5.92	5.92	5.92
Vit+Min supplement[2]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Clean sand	2.02	2.67	3.32	3.97	4.01	3.67	3.32	2.97
Antibiotic[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total	1000	1000	1000	1000	1000	1000	1000	1000
Calculated Composition g kg^{-1}								
Metabolizable Energy (MJ kg^{-1})	13.51	13.51	13.51	13.51	13.51	13.51	13.51	13.51
Total nitrogen (%)	2.44	2.44	2.44	2.44	2.45	2.46	2.48	2.49
Calcium	0.774	0.774	0.774	0.774	0.773	0.774	0.774	0.774
Available phosphorus	3.82	3.82	3.82	3.82	3.82	3.82	3.82	3.82
Sodium	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Potassium	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Cloride	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.51
SID Lys	10.81	10.81	10.81	10.81	10.81	10.81	10.81	10.81
SID Met + Cyst	6.05	6.05	6.05	6.05	6.05	6.05	6.05	6.05
SID Threonine	6.81	6.81	6.81	6.81	6.81	6.81	6.81	6.81
SID Tryptophan	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95
SID Arginine	8.13	8.13	8.13	8.13	8.13	8.13	8.13	8.13
SID Valine	5.80	7.30	8.80	10.30	5.80	7.30	8.80	10.30
SID Leucine	12.60	12.60	12.60	12.60	17.70	17.70	17.70	17.70
SID Isoleucine	5.95	5.95	5.95	5.95	5.95	5.95	5.95	5.95
SID Methionine	3.84	3.84	3.84	3.84	3.84	3.84	3.84	3.84
SID Fen + Tir	10.62	10.62	10.62	10.62	10.62	10.62	10.62	10.62
SID Phenylalanine	6.31	6.31	6.31	6.31	6.31	6.31	6.31	6.31

[1]-SID: standardized ileal digestible. [2]-Provide per kilogram of diet: (nutrition levels kg^{-1} of diet): Vit. A - 10000 UI; Vit. D3 - 2000 UI; Vit. E – 20 UI; Vit. B1 – 1,96 mg; Vit. B2 – 4,265 mg; Vit. B6 – 1,98 mg; Vit. B12 – 26,665 mcg; Vit. K3 – 2,425 mg; Calcium pantothenate – 15,83 mg; Niacin – 42,665 mg; Folic acid – 0,53 mg; Biotin – 0,13 mg; BHT – 10 mg; Zn – 0,1 g; Fe – 0,075 g; Mn – 0,052 mg; Cu – 12 mg; I – 0,825 mg; Co – 0,135 mg; Se – 0,3 mg; Vehicle Q.S.P. – 5 g. [3]-Leucomag (Ceva Animal Health. Paulínia. Brazil).

After a 6-hour fast, cranial vena cava blood samples (6 mL) were collected and stored in tubes containing EDTA anticoagulant (Cai et al., 1994) for urea, total protein, creatinine, ALT and AST. To determine glucose, 3 mL of blood samples were collected in tubes containing sodium fluoride.

After sampling, the samples were centrifuged at 3,000 RPM for 15 minutes and only the samples for glucose determination were centrifuged for 30 minutes to obtain the plasma. Then 3 mL of plasma were transferred to Eppendorf® type tubes.

For the accomplishment of the biochemical analyzes, commercial reagent kits were used (Laborclin®), the quantities of each blood component being determined by reading in BIOPLUS® 2000 Spectrophotometer, following the operational procedures (POP) described in the kits. The laboratory tests were carried out in the Laboratory of Plasma Analyzes, in the swine sector of the Experimental Farm (Iguatemi district).

For the determination of hematocrit, 1 mL of blood was collected in tubes containing heparin. After harvest, the tubes were immediately placed in an automatic homogenizer for 5 minutes. Subsequently, the blood was transferred to capillary microtubes and centrifuged at 12,000 RPM for 5 minutes. The percentage of hematocrit was measured using a specific scale.

Data regarding performance, BT, LD, LMP and blood parameters were submitted to analysis of variance. Test F was applied to the means obtained for SID leucine levels. The degrees of freedom related to the levels of SID valine were deployed in orthogonal polynomials to obtain the regression equations. For the performance variables, the initial weight was used as a covariate. The data were adjusted by the quadratic model associated with the LRP model. The data were submitted to statistical analysis by the Statistical and Genetic Analysis System – SAEG (Universidade Federal de Viçosa, 2009).

4.3 RESULTS AND DISCUSSION

No interactions ($P>0.05$) were observed between the levels of SID leucine (1.20 and 1.77%) and SID valine (0.58, 0.73, 0.88 and 1.03%) for the variables of performance (Table 5). Thus, it can be inferred that the SID leucine levels studied did not influence the requirements of SID valine, even at the highest level of leucine (1.77%).

Table 5. Performance (kg), depth of the *longissimus dorsi* muscle (LD) (cm), Backfat thickness (BT) (cm^2) and lean meat percentage (LMP) of gilts fed low crude protein diets with different levels of SID leucine (Leu) and SID valine (Val)

Item	SID leucine (%)								SEM	P - value			
	1.20				1.77								
	SID valine (%)											Valine	
	0.58	0.73	0.88	1.03	0.58	0.73	0.88	1.03		Leu*Val[1]	Leu[2]	Linear[3]	Quadratic[4]
IW	15.16	15.07	15.01	15.14	15.41	15.21	15.12	15.19	0.407	0.9939	0.5700	0.7131	0.7242
FW[5]	28.37	30.40	30.08	30.60	28.76	30.74	30.96	29.53	0.916	0.5119	0.9498	0.0176	0.0034
ADG[6]	0.604	0.700	0.690	0.672	0.594	0.709	0.731	0.654	0.037	0.7408	0.8310	0.0342	0.0003
ADFI[7]	1.255	1.410	1.387	1.296	1.292	1.393	1.434	1.341	0.057	0.8716	0.4492	0.2824	0.0007
FCR	2.10	2.01	2.05	1.94	2.22	1.98	1.97	2.10	0.088	0.3882	0.4986	0.1043	0.1292
LD	2.82	2.71	2.71	2.91	2.77	2.79	2.80	2.78	0.092	0.4402	0.8909	0.5616	0.2806
BT	0.52	0.57	0.56	0.55	0.53	0.57	0.55	0.53	0.027	0.9375	0.8775	0.5830	0.1129
LMP	60.53	60.48	60.48	60.51	60.52	60.49	60.50	60.51	0.026	0.9129	0.9207	0.7542	0.0643

IW= Initial weight; FW= Final weight; ADG= Average daily gain; ADFI= Average daily feed intake; FCR= Feed conversion ratio; SEM: Standard Error of Mean; [1-] Interaction between SID leucine and SID valine; [2-]Effect of SID leucine; [3-]Linear effect of SID valine; [4-]Quadratic effect of SID valine;

[5-] $Y= 20.82632 + 13.34547X$ (R^2=1.00);$Y= 10.6285 + 46.9032X – 27.2980X^2$ (R^2=0.78);

[6-] $Y= 0.1931538 + 0.70039X$ (R^2=1.00); $Y= -0.486065 + 2.85809X – 1.69420X^2$ (R^2=0.96);

[7-] $Y= -0.239161 + 4.02503X – 2.44022X^2$ (R^2=0.94).

In the formulation of commercial feed there is no inclusion of L-leucine because, unlike valine, it is present in high amounts in traditional ingredients, indicating that even at levels above those normally practiced under commercial conditions, leucine (1.77%) do not interfere with the use of valine.

The findings in the present study are similar to the results by Gloaguen et al. (2012), when they studied the requirement of valine for piglets from 10 to 20 kg (a diet without addition of L-valine and a diet with 0.18% of L-valine), concluding that this requirement does not seem to depend on the intake of leucine.

On the other hand, when there is excess leucine in valine-deficient diets, the use of this latter AA is not influenced to the same extent as in isoleucine-deficient diets (Pelletier et al., 1991; Langer & Fuller, 2000). To the competition of branched-chain ketoacids, since α-keto-β-methylvalerate and α-ketoisovalerate have similar values of K_m (Michaelis-Menten constant), competing with substrates for branched-chain ketoacids dehydrogenase (Boyer & Odessey, 1990).

Quadratic effects were observed (Table 5), for FW (P=0.0034), ADG (P=0.0003) and ADFI (P=0.0007) as a function of the valine levels studied. The adjustment of the quadratic model associated with the LRP plateau estimated the optimum level of SID valine at 0.725% for FW (30.29 kg) (Figure 9a) and for the ADG the optimum level was 0.703% (0.668 g/day) (Figure 9b). The highest ADFI was observed at the level of 0.825% of SID valine (1.421 kg of feed day^{-1}).

When there is valine deficiency in diets for pigs (less than 0.74% of SID valine) there is a reduction in performance, so the first response of the animal to this imbalance is the decrease in feed intake and, as a consequence, reduction in the weight gain (Gloaguen et al., 2012). This was observed in the present study, since the improvement (0.58, 0.73, 0.88 and 1.03%) showed improvement in FW and ADG, at optimal levels of 0.725% and 0.703% of SID valine, respectively.

Similar results were observed by Nørgaard & Fernández (2009) when piglets with 28 day old were fed a diet deficient in SID valine (ratio lysine:valine 0.60), and presented a reduction in ADG. The authors attributed this result to the fact that valine is more limiting for animal performance than isoleucine.

The optimal levels of valine found in the present study were lower than those found by Mavromichalis et al. (2001), who observed improvement in piglet weight gain

(10 to 20 kg) when fed diets in which 0.5% of L-valine was included, and the optimal level of SID valine was between 0.75 and 0.80%.

Likewise, they determined the optimal level of SID valine at 0.86% for piglets from 5 to 10 kg, improving ADG and ADFI. The authors concluded that younger piglets require a higher concentration of valine in the diet when compared to piglets with a greater weight range.

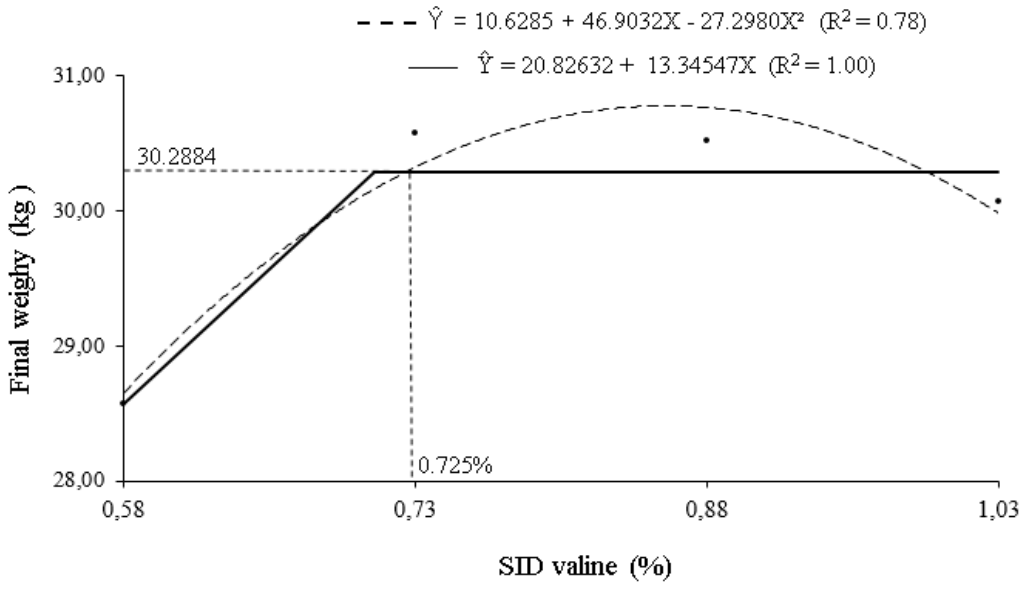


Figure 9. Final weigth (a) and average daily gain (g day^{-1}) of gilts in the initial phase as a fuction of different levels of SID valine in low CP diets.

The supplementation of L-valine in diets with low (17%) and high (20%) CP for recently post piglets was studied by Lordelo et al. (2008), where they observed improvement in ADFI (0.955 kg) and ADG for animals fed low CP and inclusion of 0.15% L-valine, but no differences were observed in the performance of piglets consuming diets with low CP compared to those that consumed the high CP diet. In addition, valine supplementation reduced the excretion of total nitrogen in the environment, demonstrating a better use of the ingested AA. This is also attributed, in parts, to the industrial AA supplemented in the diets because they are offered in ideal amounts.

The FW and ADG presented a quadratic response (Figure 9a and 9b), which was not demonstrated by Wiltafsky et al. (2010), because they observed that ADG and FW reduced linearly, attributing these results to SID valine deficiency. Although in the present study the FW and ADG variables presented a quadratic response, contrary to the response observed by Wiltafsky et al. (2010), the SID ratio valine:lysine for better FW and ADG obtained by these authors (0.60) is within the range observed in the present study, between 0.53 and 0.67. The relation between SID ratio valine:lysine for swine from 8 to 14 kg was also evaluated by Soumeh et al. (2015), in which they concluded that the 0.70 ratio was the best for the CDR and GPD, without influencing the FCR.

When evaluating the inclusion of L-valine in diets for 28-days-old piglets, Nørgaard and Fernández (2009) reported that the optimal level of SID valine was 0.72%, and that valine supplementation resulted in better ADG and FW in relation to the diet supplemented with L-isoleucine. These results corroborate those obtained in the present study, where a response was observed in FW and ADG, with optimum level of 0.725% and 0.703% of SID valine, respectively.

The results obtained by Gloaguen et al. (2012) reported that feed intake of piglets fed a valine-deficient diet rapidly decreased after ingestion. In the present study, a low ADFI was also observed by animals fed diets deficient in SID valine (Table 5); however the amount ingested by the animals resembles that presented by Rostagno et al. (2011), which is 1.200 kg per day. This effect is probably mediated by a reduction in protein retention, as the AA deficiency in the diets results in a reduction in nitrogen retention. Thus, one of the first responses to this deficiency by the animal is the reduction of feed intake, resulting in a reduction in growth rate (Gloaguen et al., 2012).

The requirements of SID valine are studied according to the production phase and, according to the Brazilian Poultry and Pork Tables (Rostagno et al., 2011); the nutritional requirements are also different depending on the sex. A study by Lohman et al. (2012), including increasing levels of SID valine (0.60, 0.67, 0.74, 0.81 and 0.88%) for castrated males, from 15 to 30 kg, observed an increasing linear response in daily valine intake (DVI), but without any variation in the performance parameters studied (ADFI, ADG and FCR). According to the authors, the optimum level of SID valine was 0.74% when the retained nitrogen: absorbed nitrogen ratio was considered. Although in the present study SID valine levels influenced the performance of gilts at the same weight, DVI was 9.72 g day^{-1} at a concentration of 0.703% of SID valine in the diet; being close to that found by Lohman et al. (2012), of 9.53 g/day at the level of 0.74% of SID valine in the diet. Thus, it can be observed that the requirement of SID valine for both barrows and gilts is similar for this weight range, as indicated by the NRC (2012).

The optimum level of SID valine for pigs in the initial phase varies among studies, presenting values of 0.70% (Soumeh et al., 2015), 0.72% (Nørgaard & Fernández, 2009; Gloaguen et al., 2012) and 0.74% (Lohman et al., 2012), which provided improvement in the performance variables (ADG, ADFI and FW), as in the present work, in which the optimal levels of SID valine for FW and ADG were 0.725 and 0.703%, respectively.

Plasma levels of glucose, total protein, creatinine, alanine aminotransferase, aspartate aminotransferase and hematocrits were not influenced ($P>0.05$) by SID leucine and valine levels. However, an interaction ($P=0.0477$) between SID leucine and valine levels on plasma urea was observed (Table 6). There was an increase in plasma urea concentration when the animals received diets with the highest concentration of leucine (1.77%) at the levels of 0.58 and 0.88% of SID valine (Table 7).

According to Langer & Fuller (2000), excess leucine increases the catabolism of valine and isoleucine in pigs, by the same routes as poultry, rats and humans, increasing nitrogen excretion. As urea is the main product of protein catabolism, animals fed diets with excess AA are supposed to have higher levels of plasma urea. This happens because if AA are not reused for the synthesis of new AA or nitrogen products, amino groups are excreted as urea (Nelson & Cox, 2011).

Table 6. Plasma levels of glucose, urea, total proteins (mg dL^{-1}), creatinine, ALT, AST (U L^{-1}) and hematocrit (%) of gilts fed low crude protein diets with different levels of SID leucine (Leu) and SID valine (Val)

Item	SID leucine (%)										SEM	P - value			
	1.20					1.77						Valine			
	SID valine (%)					SID valine (%)									
	0.58	0.73	0.88	1.03	Mean	0.58	0.73	0.88	1.03	Mean		Leu*Val[1]	Leu[2]	Linear[3]	Quadratic[4]
Glucose	82.33	78.31	71.50	80.83	78.24	85.17	82.58	89.25	87.08	86.02	3.385	0.0941	0.0013	0.9566	0.1341
Urea	11.86	10.00	11.88	12.83	11.64	15.03	12.86	13.45	11.50	13.21	0.928	0.0477	0.0132	0.3437	0.2178
Total proteins	5.12	5.17	5.40	5.37	5.26	4.90	5.34	5.40	5.22	5.22	0.186	0.6755	0.6867	0.0744	0.1523
Creatinine	1.07	0.93	1.05	1.11	1.04	1.01	0.98	1.10	1.01	1.03	0.050	0.2627	0.6499	0.2641	0.3197
ALT[7]	39.14	41.03	43.58	37.67	40.36	37.90	41.83	37.29	38.14	38.79	3.393	0.7034	0.5161	0.7912	0.2612
AST[8]	53.58	51.17	39.83	48.75	48.33	39.80	42.14	42.00	45.43	42.34	4.994	0.4487	0.1077	0.7814	0.3993
Hematocrit	34.83	38.34	35.58	34.55	35.83	30.75	33.90	32.60	35.25	33.13	1.508	0.2314	0.0766	0.3247	0.1981

SEM: Standard Error of Mean;[1-]Interaction between SID leucine and SID valine; [2-] Effect of SID leucine; [3-] Linear effect of SID valine; [4-] Quadratic effect of SID valine; [5-] $Y = -30.3628 + 160.348X - 97.478X^2$ (R^2=0.95); [7-] Alanine Aminotransferase; [8-] Aspartate Aminotransferase.

Table 7. Deployment of SID leucine vs SID valine for urea plasma (mg dL^{-1}) of gilts fed low crude protein diets with different levels of SID leucine and SID valine

Item	SID leucine (%)	SID valine (%)				SEM
		0.58	0.73	0.88	1.03	
Urea	1.10	11.86[a]	10.00[a]	11.88[a]	12.83[a]	11.64
	1.77	15.03[b]	12.86[b]	13.45[a]	11.50[a]	13.21
Mean		13.44	11.43	12.66	12.16	12.42

SEM: Standard Error of Mean;[*]Equal lowercase letters in the columns do not differ significantly by the F test a t the 5% level of significance

The low level SID leucine (1.20%) associated with 0.58 and 0.73 of SID valine provided a decreased plasma urea concentration (Table 7). This result is related to the better efficiency of use of the AA pool in the body and, consequently, there is a reduction in plasma urea concentration (Reeds et al., 1987) and a better utilization of body nitrogen (Lohman et al., 2012).

The plasma concentration of AA is a dynamic process. According to Ren et al. (2015), the restricted protein diet (17% CP) reduced plasma urea concentration in diets supplemented with BCAA (1.26% of SID leucine and 0.74% of SID valine) due to the lower absorption of AA offered in the diet or by increased peripheral absorption of circulating AA.

The excess of leucine in the diet is related to the greater degradation of valine and isoleucine due to the competition for the enzyme dehydrogenase of the branched-chain α-ketoacid (Harris et al., 2001). This response was observed by Gatnau et al. (1995), who attributed the reduction in plasma concentrations of valine and isoleucine to excess leucine and its metabolites in piglet diets. In this sense, plasma urea concentrations may be a good indicator of the amino acid degradation process. According to Reeds et al. (1987), metabolic changes of excess leucine in the diet can be studied by measuring urea synthesis and by leucine turnover, as well as isoleucine and valine (Holecek, 2013).

Changes in plasma urea concentration where influenced by valine but not by leucine and valine interaction, as found by Soumeh et al. (2015) when evaluating the requirement of valine for piglets, indicating that the SID valine:lysine ratio of 0.78 had the lowest urea concentration (51.12 mg / dL) in piglets from 8 to 14 kg.

When evaluating SID valine levels for barrows from 15 to 30 kg, Lohman et al. (2012) observed an increase in plasma creatinine concentration as the levels of SID valine increased. In contrast, no response ($P>0.05$) was observed in the plasma creatinine concentration in the present study. According to Motta (2009), creatinine is an indicator of the dietary protein quality, passing from the muscle (increase of catabolism) to the plasma, from where it is removed at a constant speed, being a high concentration of this variable in the plasma. This seems to demonstrate that there was no degradation of the muscle protein, which may be related to an optimal balance of the amino acids in the diet.

4.4 CONCLUSIONS

The requirement for SID valine for pigs in the initial phase is not influenced by the leucine levels normally practiced in conventional diets and the daily requirement is 9.72 g valine day^{-1}, corresponding to a concentration of 0.703% in the diet, to a maximum average daily gain.

4.5 REFERENCES

Arakawa, M.; Masaki, T.; Nishimura, J.; Seike, M.; Yoshimatsu, H. 2011. The effects of branched-chain amino acid granules on the accumulation of tissue triglycerides and uncoupling proteins in diet-induced obese mice. Endocrine Journal 3:161-170.

Boyer, B.; Odessey, R. 1990. Quantitative control analysis of branched-chain 2-oxo acid dehydrogenase complex activity by feedback inhibition. Biochemical Journal 271:523-528.

Cai, Y., Zimmerman, D.R., Ewan, R.C. 1994. Diurnal variation in concentrations of plasma urea nitrogen and amino acids in pigs given free access to feed or fed twice daily. Journal Nutrition 124:1088-1093.

Columbus, D. A.; Steinhoff-Wagner, J.; Suryawan, A.; Nguyen, H. V.; Hernandez-Garcia, a.; Fiorotto, M. L.; Davis, T. A. 2015. Impact of prolonged leucine supplementation on protein synthesis and lean growth in neonatal pigs. American Journal Physiology and Endocrinology Metabolism 309:E601–E610.

Franco, S. M. 2011. Níveis dietéticos de leucina, de histidina e de fenilalanina + tirosina para frangos de corte na fase inicial. Dissertação (M. Sc.). Universidade Federal de viçosa. Minas Gerais.

Gaines, A. M.; Kendall, D. C.; Allee, G. L.; Usry, J. L.; Kerr, B. J. 2011. Estimation of the standardized ileal digestible valine-to-lysine ratio in 13 to 32 kg pigs. Journal of Animal Science 89:736–742.

Gatnau, R.; Nissen, L.; Zirnmerman, D. R.; Wannemuehlert, M. Ewan, C. 1995. Effects of Excess Dietary Leucine and Leucine Catabolites on Growth and Immune Responses in Weanling Pigs. The Journal of Animal Science 73:159-165.

Gloaguen, M.; Le Floc'h, N.; Corrent, E.; Primot, Y.; Van Milgen, J. 2012. Providing a diet deficient in valine but with excess leucine results in a rapid decrease -keto acid ain feed intake and modifies the postprandial plasma amino acid and concentrations in pigs. Journal of Animal Science 90:3135-3142.

Harris, R. A.; Kobayashi, R.; Murakami, T.; Shimomura, Y. 2001. Regulation of branched-chain α-keto acid dehydrogenase kinase expression in rat liver. Journal Nutrition 131:841–845, 2001.

Holecek, M. 2013. Branched-chain amino acids and ammonia metabolism in liver disease: Therapeutic implications. Nutrition 29:1186–1191.

Langer, S.; Fuller, M. F. 2000. Interactions among the branched-chain amino acids and their effects on methionine utilization in growing pigs: effects on nitrogen retention and amino acid utilization. The British Journal of Nutrition 83:43-48.

Lohman, A. C. Pozza, P. C.; Pozza, M. S. dos S.; Nunes, R. V.; Castilha, L. D.; Possamai, M.; Bruno, L. D. G.; Lazerri, D. B. 2012. Níveis de valina digestível para suínos machos castrados dos 15 aos 30 kg. Archivos de Zootecnia 61:267-278.

Lordelo, M. M.; Gaspar, A. M.; Le Bellego, L.; Freire, J. P. B. 2008. Isoleucine and valine supplementation of a low-protein corn-wheat-soybean meal-based diet for piglets: Growth performance and nitrogen balance. Journal of Animal Science 86:2936-2941.

Macotela, Y.; Brice, E.; Bang, A. M.; Espinoza, D. O.; Boucher, J.; Beebe, K.; Gall, W.; Kahn, R. 2011. Dietary Leucine - An Environmental Modifier of Insulin Resistance Acting on Multiple Levels of Metabolism. Plos One 6:1-13.

Mavromichalis, I.; Kerr, B. J.; Parr, T. M.; Albin, D. M.; Gabert, V. M.; Baker, D. H. 2001. Valine requirement of nursery pigs. Journal of Animal Science 79:1223–1229.

Morales, A.; Arcel, N.; Cota, M.; Buenabad, L.; Avelar, E.; Htoo, J. K. Cervantes, M. 2016. Effect of dietary excess of branched-chain amino acids on performance and serum concentrations of amino acids in growing pigs. Journal of Animal Physiology and Animal Nutrition 100:39-45.

Motta, V. T. 2009. Bioquimica clínica para o laboratório. 5 Edição. Curitiba, Paraná.

NATIONAL RESEARCH COUNCIL – NRC. 2012. Nutrients requirement of swine. 11 ed. National Academic Press. Washington DC.

Nelson, D.L.; Cox, M.M. Princípios de Bioquímica de Lehninger. 2011. 5 Ed. SARVIER, São Paulo, Brasil.

Nørgaard, J. V. Fernandéz, J. A. 2009. Isoleucine and valine supplementation of crude protein-reduced diets for pigs aged 5–8 weeks. Animal Feed Science and Technology 154:248–253.

Nørgaard, J. V.; Pedersen, T. F.; Soumeh, E. A.; Blaabjerg, N.; Canibe, N.; Jensen, B. B.; Poulsen, H. D. 2015. Optimum standardized ileal digestible tryptophan to lysine ratio for pigs weighing 7–14 kg. Poulsen Livestock Science 175:90-95.

Oestemer, G. A.; Hanson, L. E.; Meade, R. 1973. Leucine-Isoleucine interrelationship in the Young pig. Journal of Animal Science 36:674-678.

Pelletier, V.; Marks, L.; Wagner, D. A.; Hoerr, R. A.; Young, V. R. 1991. Branched-chain amino acid interactions with reference to amino acid requirements in adult men: Valine metabolism at different leucine intakes. The American Journal of Clinical Nutrition 54:395-401.

Reeds, P. J.; Fuller, M. F.; Dadenhead, A.; Hay, S. M. 1987. Urea synthesis and leucine turnover in growing pigs: changes during. The British Journal of Nutrition 58:301-311.

Ren, M.; Zhang, H.; Zeng, X. F.; Liu, H.; Qiao, Y. 2015. Branched-chain Amino Acids are Beneficial to Maintain Growth Performance and Intestinal Immune-related Function in Weaned Piglets Fed Protein Restricted Diet. M. The Journal of Animal Science 28: 1742-1750.

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T.; Euclides, R. F. 2011. Tabelas Brasileiras para Aves e Suínos: Composição de alimentos e exigências nutricionais. 3rd ed. UFV, Viçosa, Minas Gerais, Brasil.

Soumeh, E. A.; Milgen Van, J.; Sloth, N. M.; Corrent, E.; Poulsen, H. D.; Nørgaard, J. V. 2015. Requirement of standardized ileal digestible valine to lysine ratio for 8- to 14-kg pigs. The Animal Consortium. 1-7.

Torres, N.; Tomar, A. R.; Harper, A. 1995. Leucine Affects the Metabolism of Valine by Isolated Perfused Rat Hearts: Relation to Branched-Chain Amino Acid Antagonism. The Journal of Nutrition 125:1884-1893.

Vítek, M.; Pulkrábek, J.; Vališ, L.; David, L.; Wolf, J. 2008. Improvement of accuracy in the estimation of lean meat content in pig carcasses. Czech Journal of Animal Science 53:204-211.

Wessels, A. G.; Kluge, H.; Hirche, F.; Kiowski, A.; Schutkowski, A.; Corrent, E.; Bartelt, J.; Konig, B.; Stangl, I. 2016. High Leucine Diets Stimulate Cerebral Branched-Chain Amino Acid Degradation and Modify Serotonin and Ketone Body Concentrations in a Pig Model. Plos One. doi:10.1371/journal.pone.0150376.

Wiltafsky, M. K.; Pfaffl, M. W.; Roth, F. X. 2010. The Effects of Branched-Chain Amino Acid Interactions on Growth Performance, Blood Metabolites, Enzyme Kinetics and Transcriptomics in Weaned Pigs. British Journal of Nutrition 103:964-976.

Wu, G. 2014. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. Journal of Animal Science and Biotechnology 5: 34.

V – Exigência de isoleucina para fêmeas suínas na fase inicial recebendo dietas com diferentes níveis de leucina

RESUMO- O objetivo deste trabalho foi avaliar os níveis de isoleucina digestível sobre o desempenho, espessura de toucinho, profundidade do músculo e parâmetros sanguíneos de suínos na fase inicial. Foram utilizados 56 suínos mestiços com peso inicial de 15,81 ± 1,00, distribuídos em um delineamento experimental de blocos casualizados, em um esquema fatorial 2 x 4, sendo dois níveis de leucina digestível (1,10 e 1,70%) e quatro níveis de isoleucina digestível (0,45, 0,60, 0,75 e 0,90%), com sete repetições e um animal por unidade experimental. As variáveis de desempenho não foram influenciadas pelos níveis de leucina e isoleucina digestíveis. A profundidade do músculo *longissimus dorsi* apresentou efeito linear (P=0,011) e quadrático (P=0,034). Ajustando os dados ao modelo quadrático associado ao platô do modelo LRP, o nível ótimo de isoleucina digestível foi de 0,609%. Não houve interação entre os níveis de leucina e isoleucina digestível para as variáveis plasmáticas, no entanto, foi observado efeito da leucina sobre a concentração plasmática de creatinina (P=0,027), sendo o nível de 1,70% que apresentou a menor concentração (0,96 U/L) de creatinina. A exigência de isoleucina digestível para fêmeas suínas na fase inicial não é influenciada pelos níveis de leucina normalmente praticados em dietas comerciais, e o nível ótimo encontrado para a melhor profundidade do músculo *longissimus dorsi* foi de 0,609% de isoleucina digestível na dieta, correspondendo a um consumo diário de 8,94 g de isoleucina.

Palavras-chave: Leucina digestível, isoleucina digestível, ganho de peso diário, modelo quadrático.

V – Requeriment of isoleucine for gilts in the initial phase fed diets with different levels of leucine

ABSTRACT – The objective in this study was to evaluate the SID isoleucine levels on performance, backfat thickness, depth of *longissimus dorsi* muscle and blood parameters of gilts in the initial phase. A total of 56 gilts, with initial weight of 15.81 ± 1.00 kg; distributed in a randomized complete block in a factorial design 2 x 4, two levels of Standardized ileal digestible (SID) leucine (1.10 and 1.70%) and four levels of SID isoleucine (0.45, 0.60, 0.75 e 0.90%) with seven replicates and one animal per experimental unit. The performance variables were not influenced by SID leucine and SID isoleucine. Depth of *longissimus dorsi* muscle have linear (P=0.011) and quadratic (P=0.034) effects. Setting data to the quadratic model associated with the linear response plateu model (LRP), the optimal level of SID isoleucine was 0.609%. There was no interaction between the levels of SID leucine and SID isoleucine for the plasma variables, however an effect of SID leucine was observed on the plasma creatinine concentration (P=0.027), with 1.70% presenting the lowest concentration (9.96 U L^{-1}) of creatinine. The requirement of SID isoleucine for pigs in the initial phase is not influenced by the leucine levels normally practiced in commercial diets, and the optimum level found for the best depth of the *longissimus dorsi* muscle was 0.609% SID isoleucine in the diet and the daily requirement is 8.94 g SID isoleucine.

Keywords: SID Leucine, SID isoleucine, average daily gain, serum parameters, quadratic model.

5.1 INTRODUCTION

The use of industrial amino acids (AA) has been established as an efficient strategy to reduce crude protein (CP) content in pork diets, besides promoting the optimal supply of essential AA and reducing nitrogen (N) excretion. The correct balance of essential AA is important to maintain the physiological functions of cells and tissues of the body, as well as functions of the immune system (Wu, 2014). Isoleucine, together with leucine and valine, is part of the essential AAs group classified as branched chain AA (BCAA) which, in addition to participating in protein synthesis, influence glucose metabolism of energy, hepatic metabolism and functions (Kawaguchi et al., 2011), being mainly metabolized in muscle, adipose, renal and encephalic tissues (Nelson & Cox, 2011).

In diets based on corn and soybean meal for pigs, BCAA represent approximately 35 to 40% of the essential AAs (Riazi et al., 2003), so it is expected that there will be an excess, not a deficiency of the same. However, isoleucine is lower in corn (0.23%) and soybean meal (1.88%) in relation to leucine (0.87% in corn and 3.11% in soybean meal, Rostagno et al., 2011), what results in leucine levels higher than those required by pigs. This excess of leucine may result in increased oxidation of isoleucine and valine, affecting animal performance and reducing growth when these two BCAA are limited (Langer & Fuller, 2000).

Thus, leucine excess may negatively affect the viability of other BCAA and consequently reduce the productive performance of the animals (Morales et al., 2016), with the effect of leucine being more evident on isoleucine when compared to valine (Langer & Fuller, 2000). Despite the interaction between leucine and isoleucine, studies that evaluated the requirement of isoleucine in diets based on corn and soybean meal for pigs (Van Milgen et al., 2012; Soumeh et al., 2014) are limited. In addition, most of the studies determined the requirement of isoleucine in diets through the use of byproducts from pig plasma production process (Kerr et al., 2004; Fu et al., 2005; Wiltafsky et al., 2009; Barea et al., 2009), which presents high levels of leucine and low isoleucine (Rostagno et al., 2011).

Considering the importance of these BCAA for the productive performance of pigs and the influence of leucine excess even at practical levels of this AA on isoleucine degradation, this study aimed to determine the requirements of standardized ileal

digestible (SID) isoleucine for gilts in the initial phase (15-30 kg), fed low crude protein (CP) diets with different levels of SID leucine.

5.2 MATERIAL AND METHODS

The experiment was carried out in the Swine Sector of the Experimental Farm of Iguatemi (FEI), which belongs to the Agrarian Science Center of the State University of Maringá (CCA/UEM) and in the Laboratory of Animal Nutrition (LANA/UEM) of the Animal Science Department. All experimental procedures were previously submitted to the Committee for Ethical Conduct on the Use of Animals under Experimentation (CEUA/UEM), and have been approved for execution (Protocol n. 8538100616).

Fifty six gilts (Landrace x Large White x Pietrain) with initial weight of 15.81 ± 1.00 kg were distributed on the basis of initial weight in a randomized complete block design in a 2 x 4 factorial scheme, consisting of two levels of SID leucine (1.10 e 1.70%) four levels of SID isoleucine (0.45; 0.60; 0.75 and 0.90%), seven replicates and one gilt as experimental unit (EU).

The experimental diets were formulated with corn, soybean meal, minerals, vitamins and additives (Table 8). The CP content was reduced in relation to that suggested by Rostagno et al. (2011). L-leucine was added to the diets at the expense of the inert to meet the levels of SID leucine. Glutamic acid was used in order to maintain the same nitrogen content in the different diets.

The amino acid compositions of corn and soybean meal were determined in EVONIK Industries, and the true ileal digestibility coefficients proposed by Rostagno et al. (2011) were applied to estimate the SID amino acid values of these foods.

The animals were housed in masonry nursery, with suspended pens (1.32 m^2) and partly leaked floor, equipped with semiautomatic feeders, located in the front of the bay, and a nipple type drinker in the back.

The animals were weighed at the beginning and at the end of the experiment, and the diets were weighed whenever provided to the animals for the determination of avegare daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR).

Table 8. Chemical and energetic composition (as fed basis) of diets containing different levels of SID leucine (Leu) and SID isoleucine (Ile) (g kg^{-1}) for gilts from 15 to 30 kg of live weight[1]

	SID Leucine %	1.10				1.70			
Item g kg^{-1}	SID Isoleucine %	0.45	0.60	0.75	0.90	0.45	0.60	0.75	0.90
Corn		785.5	785.5	785.5	785.5	785.5	785.5	785.5	785.5
Soybean meal		148.00	148.00	148.00	148.00	148.00	148.00	148.00	148.00
Dicalcium Phosphate		16.34	16.34	16.34	16.34	16.34	16.34	16.34	16.34
Limestone		8.30	8.30	8.30	8.30	8.30	8.30	8.30	8.30
Salt		4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56
Soybean oil		2.59	2.22	1.86	1.49	1.10	0.73	0.37	0.00
Glutamic acid		13.10	11.24	9.38	7.52	5.58	3.72	1.86	0.00
L-Lysine HCl		6.96	6.96	6.96	6.96	6.96	6.96	6.96	6.96
L-Threonine		2.81	2.81	2.81	2.81	2.81	2.81	2.81	2.81
DL-Methionine		2.01	2.01	2.01	2.01	2.01	2.01	2.01	2.01
L-Tryptophan		0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
L-Valine		2.17	2.17	2.17	2.17	2.17	2.17	2.17	2.17
L-Isoleucine		0.00	1.53	3.07	4.60	0.00	1.53	3.07	4.60
L-Leucine		0.00	0.00	0.00	0.00	6.23	6.23	6.23	6.23
Vit+Min supplement[2]		5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Clean sand		1.81	2.50	3.19	3.89	4.60	5.29	5.98	6.67
Antibiotic[3]		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total		1000	1000	1000	1000	1000	1000	1000	1000
Calculated Composition (g kg^{-1})									
Metabolizable Energy (MJ kg^{-1})		13.50	13.50	13.50	13.50	13.50	13.50	13.50	13.50
Total nitrogen (%)		2.39	2.39	2.39	2.39	2.39	2.39	2.39	2.39
Calcium		7.73	7.74	7.74	7.74	7.73	7.74	7.74	7.74
Available phosphorus		3.82	3.82	3.82	3.82	3.82	3.82	3.82	3.82
Sodium		2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Potassium		5.01	5.01	5.01	5.01	5.01	5.01	5.01	5.01
Cloride		4.60	4.60	4.60	4.60	4.60	4.60	4.60	4.60
SID Lys		10.81	10.81	10.81	10.81	10.81	10.81	10.81	10.81
SID Met + Cyst		6.05	6.05	6.05	6.05	6.05	6.05	6.05	6.05
SID Threonine		6.81	6.81	6.81	6.81	6.81	6.81	6.81	6.81
SID Tryptophan		1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95
SID Arginine		7.66	7.66	7.66	7.66	7.66	7.66	7.66	7.66
SID Valine		7.46	7.46	7.46	7.46	7.46	7.46	7.46	7.46
SID Leucine		11.05	11.05	11.05	11.05	17.00	17.00	17.00	17.00
SID Isoleucine		4.53	6.02	7.51	9.00	4.53	6.02	7.51	9.00
SID Methionine		3.90	3.90	3.90	3.90	3.90	3.90	3.90	3.90
SID Phen + Tyr		9.67	9.67	9.67	9.67	9.67	9.67	9.67	9.67
SID Phenylalanine		5.72	5.72	5.72	5.72	5.72	5.72	5.72	5.72

[1]-SID: standardized ileal digestible. [2]-Provide per kilogram of diet: (nutrition levels kg^{-1} of diet): Vit. A - 10000 UI; Vit. D3 - 2000 UI; Vit. E – 20 UI; Vit. B1 – 1,96 mg; Vit. B2 – 4,265 mg; Vit. B6 – 1,98 mg; Vit. B12 – 26,665 mcg; Vit. K3 – 2,425 mg; Calcium pantothenate – 15,83 mg; Niacin – 42,665 mg; Folic acid – 0,53 mg; Biotin – 0,13 mg; BHT – 10 mg; Zn – 0,1 g; Fe – 0,075 g; Mn – 0,052 mg; Cu – 12 mg; I – 0,825 mg; Co – 0,135 mg; Se – 0,3 mg; Vehicle Q.S.P. – 5 g. [3]-Leucomag (Ceva Animal Health. Paulínia. Brazil).

At the end of the trial period, the animals were submitted to the evaluation of backfat thickness (BT) and depth of the *longissimus dorsi* (LD) muscle through the ultrasound equipment Aloka® SSD 500 Vet, coupled to a probe of 7.5 cm and 3.5 MHz. The region P2, located at the last rib of the chest, was determined at 6 cm from the midline, where the site was cleaned and the images captured. Afterwards, the measurements of BT and depth of LD muscle were performed. Measurements were performed using Image Pro Plus® software.

For determination of the lean meat percentage (LMP) an equation was applied for the estimation of this parameter to pigs *in vivo,* as follows: Y= 60.69798 – 0.89211S + 0.10560M; (Vítek et al., 2008), where Y = the estimated percentage of lean meat; S = BT (mm), measured at a point on the medial line, 7 cm between the penultimate and antepenultimate rib; M = depth of the muscle at the measurement point.

After a 6-hour fast, cranial vena cava blood samples (6 mL) were collected and stored in tubes containing EDTA anticoagulant (Cai et al., 1994) for triglyceride, urea, creatinine, cholesterol total and total protein. To determine glucose, 3 mL of blood samples were collected in tubes containing sodium fluoride.

After sampling, the samples were centrifuged at 3,000 RPM for 15 minutes and only the samples for glucose determination were centrifuged for 30 minutes to obtain the plasma. Then 3 mL of plasma were transferred to Eppendorf® type tubes.

For the accomplishment of the biochemical analyzes, commercial reagent kits were used (Laborclin®), the quantities of each blood component being determined by reading in BIOPLUS® 2000 Spectrophotometer, following the operational procedures (POP) described in the kits. The laboratory tests were carried out in the Laboratory of Plasma Analyzes, in the swine sector of the Experimental Farm (Iguatemi district).

Data regarding performance, BT, LD, LMP and blood parameters were submitted to analysis of variance. Test F was applied to the means obtained for SID leucine levels. The degrees of freedom related to the levels of SID isoleucine were deployed in orthogonal polynomials to obtain the regression equations. The initial weight was used as a covariate for the performance variables. Data were adjusted by the quadratic model associated with the LRP model. The data were submitted to statistical analysis by the Statistical and Genetic Analysis System – SAEG (Universidade Federal de Viçosa, 2009).

5.3 RESULTS AND DISCUSSION

No interactions (P>0.05) were observed between the levels of SID leucine (1.10 and 1.70%) and isoleucine (0.45, 0.60, 0.75 and 0.90%) for ADG, ADFI, BT and LMP (Table 9). Although studies have shown that leucine significantly affects the use of isoleucine in relation to valine (Langer & Fuller, 2000), in the present study leucine levels did not influence the use of isoleucine for the aforementioned variables.

Similar results were found by Henry et al. (1976), who studied the levels of SID leucine (0.64 and 1.14%) and isoleucine (0.38 and 0.48%) in diets for pigs in the initial phase. The authors did not observe leucine *vs.* isoleucine interaction for the performance variables, and concluded that the increasing levels of leucine and isoleucine neither influence these parameters nor the requirement of isoleucine. According to Langer & Fuller (2000), the importance of BCAA interactions for pigs has not yet been well established and the study of moderate excesses of BCAA seems to be more important than the study of interactions as they may influence animal performance.

Still, Gloaguen et al. (2013) found that piglets from 10 to 20 kg of live weight, which received L-isoleucine supplementation in the diet, presented lower ADG than those receiving L-histidine supplementation, although feed efficiency was not affected. According to these authors, in diets based on corn and soybean meal, the isoleucine: lysine ratio should not be greater than 49%. Likewise, Van Milgen et al. (2012) found that the ADG can be maximized with SID isoleucine: lysine 50% ratio. In the present study, this ratio varied from 42, 56, 69 and 83% for the levels of 0.45, 0.60, 0.75 and 0.90% of SID isoleucine, respectively (Table 8), which may have contributed to the increase in FCR (Table 9) as levels of SID isoleucine increased in diets.

SID isoleucine:lysine ratios higher than 50% may not provide responses of the animals (Barea et al., 2009) that consume diets based on corn and soybean meal. On the other hand, in diets containing blood cells the ratio for maximum ADG is up to 59% (Wiltafsky et al., 2009).

Blood cells have high content of leucine, valine, histidine and phenylalanine (Rostagno et al., 2011) and these amino acids compete with isoleucine for transporters (Kerr et al., 2004; Barea et al., 2009). As a result, a higher level of isoleucine is required to maintain animal performance, but in the present study, leucine supplementation did not influence the use of isoleucine.

Table 9. Performance (kg), depth of the longissimus dorsi muscle (LD) (cm), Backfat thickness (BT) (cm^2) and lean meat percentage (LMP) of gilts fed low crude protein diets with different levels of SID leucine (Leu) and SID isoleucine (Ile)

Item	SID leucine (%)										SEM	*P* - Value			
	1.10					1.70						Isoleucine			
	SID isoleucine (%)														
	0.45	0.60	0.75	0.90	Mean	0.45	0.60	0.75	0.90	Mean		Leu*Ile[1]	Leu[2]	Linear[3]	Quadrática[4]
IW	16.32	15.78	15.63	15.37	15.78	15.65	15.56	16.07	16.05	15.83	0.294	0.797	0.809	0.758	0.708
ADG	0.744	0.688	0.683	0.634	0.687	0.689	0.678	0.762	0.669	0.700	0.031	0.221	0.606	0.126	0.440
ADFI	1.504	1.308	1.531	1.434	1.464	1.415	1.461	1.556	1.460	1.473	0.064	0.486	0.842	0.681	0.514
FCR[5]	2.03	2.05	2.24	2.27	2.15	2.05	2.19	2.05	2.19	2.12	0.092	0.281	0.642	0.028	0.976
LD[6]	2.65	2.77	2.84	2.80	2.77	2.48	2.74	2.84	2.74	2.70	0.090	0.209	0.263	0.011	0.034
BT	0.49	0.46	0.51	0.47	0.48	0.50	0.52	0.47	0.50	0.49	0.027	0.306	0.573	0.722	0.976
LMP	60.54	60.58	60.54	60.58	60.56	60.52	60.53	60.58	60.55	60.54	0.025	0.283	0.320	0.205	0.527

IW= Initial weight; ADG= Average daily gain; ADFI= Average daily feed intake; FCR= Feed conversion ratio; SEM: Standard error of mean [1-] Interaction between SID leucine and SID isoleucine; [2-]Effect of SID leucine; [3-]Linear effect of SID isoleucine; [4-]Quadratic effect of SID isoleucine;

[5-]$Y = 1,877 + 0,382X$ ($R^2 = 0,96$);

[6-]$Y = 2,203 + 0,867X$ ($R^2 = 0,60$); $Y = 1,278 + 4,095X - 2,708X^2$ ($R^2 = 0,91$).

Evaluating different levels of SID isoleucine (0.45, 0.52, 0.59, 0.66 and 0.73%) for gilts from 15 to 30 kg Castilha et al. (2012) also observed no effect on ADG, but observed a reduction ($P<0.05$) in ADFI as levels of isoleucine increased, being this response related to the association of BCAA and its antagonism, which reduced ADFI. Similarly, Van Milgen et al. (2012) in a meta-analysis did not observe differences in the ADG and ADFI when increased the isoleucine concentration of diet, corroborating the findings of the present research, since no influence of isoleucine levels on these variables was observed.

Conversely, Li et al. (2016) obtained optimal levels of SID leucine (1.77%) and isoleucine (0.90%) when using diets with low CP concentration (17%), showing improvement on FW, ADFI and ADG, relating these results to the stimulation of these BCAA as substrates for protein synthesis (Wu et al., 2013).

The effects of isoleucine supplementation also depend on the adequate intake of the other limiting AAs. A diet with low PB (11%) and 0.67% SID isoleucine for gilts from 20 to 50 kg provided a reduction in ADFI (1.309 g) and, as a consequence, in ADG (508 g) (Figueroa et al., 2003). However, when valine was added (0.67%) to the same diet the authors observed an increase in ADFI (1.661 g) and ADG (716 g). This result was related to the difficulty in establishing the limiting AA after valine, because according to the authors, isoleucine and histidine were considered collimating.

Animals fed diets based on corn, soybean meal and addition of blood cells, deficient in SID isoleucine, presented a reduction in performance (Parr et al., 2003). In the present study (Table 8), even at the lower levels of isoleucine, there was no decrease in ADG ($P>0.05$). Parr et al. (2003) reported that one of the reasons may be related to isoleucine deficiency, which was more severe (0.38% of the diet) than in the present study (0.45% of the diet). In addition, it has been reported that isoleucine supplementation does not provide an evident response to ADFI and ADG when diets do not contain blood cells (Van Milgen et al., 2012), as in the present study. Variables were not influenced by the levels of SID isoleucine evaluated.

Regarding the *in vivo* carcass characteristics, no differences ($P>0.05$) were observed for BT and LMP. However, a quadratic effect was observed for LD ($P=0.034$) as a function of the studied isoleucine levels (Table 9). The adjustment of the quadratic model associated with the plateau of the LRP model estimated the optimum level of SID isoleucine at 0.609% for LD (2.77 cm) (Figure 10).

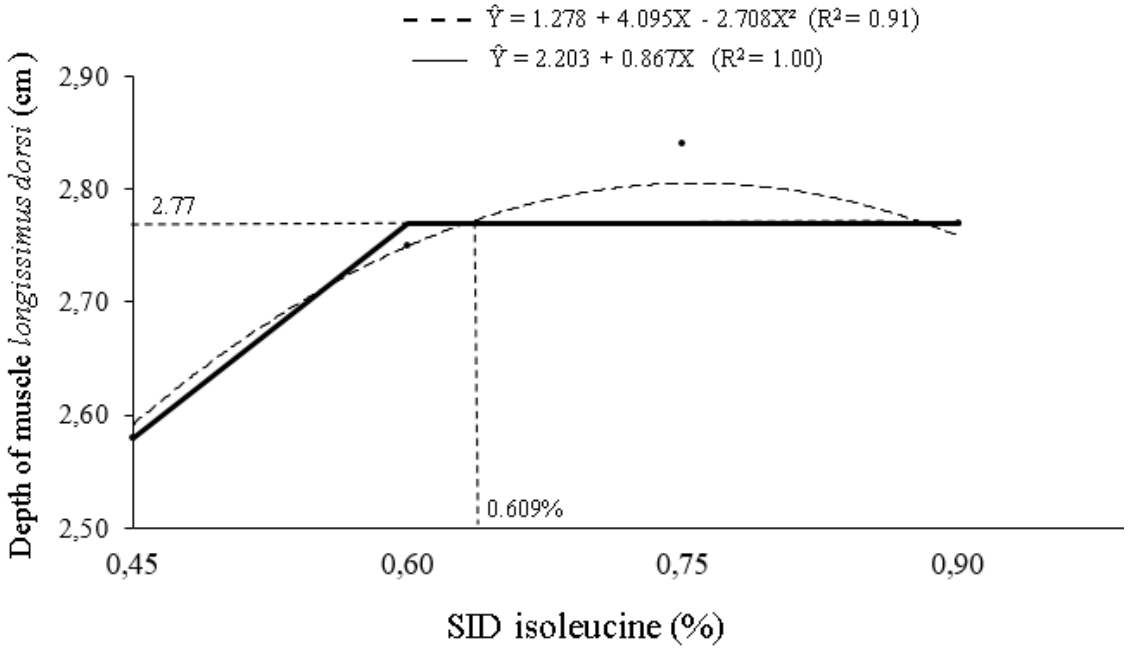


Figure 10. Depth of muscle *longissimus dorsi* (cm) of gilts in the initial phase as a fuction of different levels of SID isoleucine in the diet.

Similarly, Torraza et al. (2011) observed that the supplementation of BCAA in diets with low CP for piglets stimulated the protein synthesis in the *longissimus dorsi* muscle. BCAA are substrates for protein synthesis and are vital for the signaling of protein synthesis and energy source to regulate physiological functions of metabolism and maintenance (Wu et al., 2013). On the other hand, Madeira et al. (2014) did not find differences for loin weight in diets with different levels of BCAA supplementation.

When some AA is in excess, in relation to the requirements for maintenance and production, it is catabolized, and the liver is the main organ responsible for its excretion (Nelson & Cox, 2011). If some AA is deficient, the other AAs will not be used efficiently. In the case of leucine, its excess increases the catabolism of valine and isoleucine, and there is less availability of these BCAA for protein synthesis, and this antagonism is more evident on isoleucine than valine (Gloaguen et al., 2013).

This demonstrates that the requirement of isoleucine for maximum growth may be affected by the imbalance between BCAA, more markedly than the requirement for valine. Despite this, LMP was not influenced by the levels of leucine and isoleucine (P=0.283), even as there was no effect on the BT (P=0.306) (Table 9). This same result was found by Duan et al. (2016), which did not observe effect of different SID leucine:

isoleucine: valine ratios (from 1: 1: 1 to 1: 0.25: 0.25, respectively) on lean mass and LMP.

In studies with gilts from 20 to 50 kg, receiving diets supplemented with isoleucine, valine or both, Figueroa et al. (2003) reported lower BT and LD when providing one with 0.62% SID isoleucine. The authors attributed this result to the lighter weight of gilts at the end of the experimental period.

Plasma levels of glucose, triglycerides, urea and total proteins were not influenced (P>0.05) by levels of SID leucine and isoleucine. However, the effect of leucine on the plasma creatinine concentration (P=0.027) (Table 10) was observed, in which the increase in leucine level, from 1.10 to 1.70% in the diet, was responsible for the reduction of plasma creatinine concentration (from 1.05 to 0.96 U L^{-1}, Table 10). Creatinine is an indicator of the dietary protein quality since it is catabolized in the muscle and transported to plasma (Motta, 2009). Therefore, high plasma levels indicate high degradation of muscle proteins. According to the findings of the present study, the 1.70% level of SID leucine provided a reduction in plasma creatinine, which seems to indicate a reduction of muscle catabolism and, consequently, a better efficiency in the use of dietary AA.

Isoleucine is a glycoketogenic amino acid, so the end products of its metabolism are succinyl-CoA, acetoacetyl-CoA and acetyl-CoA (Nelson & Cox, 2011). Its ketogenic route is responsible for the formation of ketone bodies, with a function of energy reserve and cholesterol. Although it could follow this route, no statistical differences in leucine and isoleucine levels were observed on plasma concentrations of triglycerides (P=0.671) and total cholesterol (P=0.062) (Table 10). Similarly, Duan et al. (2016) also observed no effect of variations in the relationship between BCAA on triglyceride levels, despite observing a trend (P=0.07) of increase in total cholesterol in function of isoleucine and valine reduction.

Plasma urea levels reflect protein metabolism, as the reduction in the concentrations of this parameter suggests an increase in protein deposition, and it can be inferred that a lower concentration of AA is being catabolized and excreted as urea (Reeds et al., 1987). Ren et al. (2015) reported a reduction in plasma urea concentration of piglets fed diets supplemented with BCAA (1.38, 1.11 and 0.80% SID leucine, valine and isoleucine, respectively).

Table 10. Plasma levels of glucose, triglycerides, urea, total proteins, total cholesterol (mg dL^{-1}) and creatinine (U L^{-1}) of gilts fed low crude protein diets with different levels of SID leucine (Leu) and SID isoleucine (Ile)

Item	SID leucine (%)										SEM	*P* - Value			
	1.10					1.70						Isoleucine			
	SID isoleucine (%)														
	0.45	0.60	0.75	0.90	Mean	0.45	0.60	0.75	0.90	Mean		Leu*Ile[1]	Leu[2]	Linear[3]	Quadrátic[4]
Glucose	73.25	73.07	72.57	73.42	73.07	74.67	77.30	72.21	76.50	75.17	3.418	0.737	0.497	1.000	0.821
Triglyderides	43.20	34.85	38.50	38.57	38.78	41.07	29.00	35.83	31.00	34.22	3.794	0.671	0.107	0.182	0.165
Urea	5.83	5.62	5.18	3.84	5.12	5.42	6.22	5.58	5.55	5.69	0.651	0.437	0.248	0.133	0.392
Total proteins	5.94	5.61	5.86	5.44	5.71	5.39	5.47	5.57	5.59	5.50	0.167	0.230	0.094	0.658	0.757
Total cholesterol	76.80	71.64	75.64	74.50	74.64	65.64	67.50	78.21	76.50	71.96	3.187	0.124	0.241	0.062	0.967
Creatinine[5]	1.04	1.06	1.09	1.00	1.05[b]	1.02	0.95	0,95	0.91	0.96[a]	0.053	0.557	0.027	0.234	0.643

SEM: Standard Error of Mean; [1-] Interaction between SID leucine and SID isoleucine; [2-] Effect of SID leucine; [3-] Linear effect of SID isoleucine; [4-] Quadratic effect of SID isoleucine; [1-] Interaction between SID leucine and SID isoleucine; [2-] Effect of SID leucine; [3-] Linear effect of SID isoleucine; [4-] Quadratic effect of SID isoleucine; [5-] Means followed by different letters on the line differ from one another by the F-Test.

The authors indicated that this reduction was 71.8% lower when compared to the control diet (1.61, 0.97 and 0.84% SID leucine, valine and isoleucine, respectively) and 44.4% lower when compared to the diet with low protein (1.26, 0.74 and 0.60% SID leucine, valine and isoleucine, respectively), demonstrating that, in addition to the reduction in protein levels in the diets, BCAA supplementation reduces the concentration of urea (1.62 mg dL^{-1}), as it allows better balance between the limiting amino acids of the diet.

However, the concentrations of urea (P=0.437) and total proteins (P=0.124) were not affected by the leucine and isoleucine levels of the diets (Table 9), similar to the results observed by Figueroa et al. (2003), because plasma urea concentrations in the diets with isoleucine (6.46 mg dL^{-1}), valine (5.84 mg dL^{-1}) or both (4.31 mg dL^{-1}) were not influenced by these AA.

In general, BCAA influence glucose metabolism to obtain energy (Kawaguchi et al., 2011), mainly leucine, whose excess interferes with the uptake of glucose by the intracellular transporter GLUT4 (Arakawa et al., 2011). However, the increase in leucine levels (from 1.10 to 1.70% of the diet) did not influence the plasma glucose concentration (P=0.737), as well as the SID isoleucine levels evaluated (Table 9).

5.4 CONCLUSIONS

The requirement of SID isoleucine for gilts in the initial phase is not influenced by the leucine levels normally practiced in commercial diets, and the optimum level found for the best depth of *longissimus dorsi* muscle was 0.609% in the diet and the daily requirement is 8.94 g SID isoleucine day^{-1}.

5.5 REFERENCES

Arakawa, M.; Masaki, T.; Nishimura, Masataka, S.; Yoshimatsu, H. 2011. The effects of branched-chain amino acid granules on the accumulation of tissue triglycerides and uncoupling proteins in diet-induced obese mice. Endocrine Journal 58:161-170.

Barea, R., L. Brossard, N. Le Floc'h, Y. Primot, and J. and Van Milgen. 2009. The standardized ileal digestible isoleucine-to-lysine requirement ratio may be less than fi fty percent in eleven- to twenty-three-kilogram piglets. J. Anim. Sci. 87:4022-4031.

Cai, Y., Zimmerman, D.R., Ewan, R.C. 1994. Diurnal variation in concentrations of plasma urea nitrogen and amino acids in pigs given free access to feed or fed twice daily. Journal of Nutrition 124:1088-1093.

Castilha, L. D.; Pozza, P. C.; Nunes, R. V.; Lazzeri, B. D.; Somensi, M. L. and Pozza, M. S. S. 2012. Levels of digestible isoleucine on performance, carcass traits and organs weight of gilts (15 – 30 kg). Ciência e Agrotecnologia 36:446-453.

Duan, Y.; Duan, Y.; Li, F.; Li, Y.; Guo, Q.; Ji, Y.; Tan, B.; Li, T.; Yin, Y. 2016. Effects of supplementation with branched‐chain amino acids to low‐protein diets on expression of genes related to lipid metabolism in skeletal muscle of growing pigs. Amino Acids. doi 10.1007/s00726-016-2223-2.

Figueroa, J. L.; Lewis, A. J.; Miller, P. S.; Fischer, R. L.; Diedrichsen, R. M. 2003. Growth, carcass traits, and plasma amino acid concentrations of gilts fed low-protein diets supplemented with amino acids including histidine, isoleucine, and valine. Journal of Animal Science, 81: 1529-1537.

Fu, S. X., D. C. Kendall, R. W. Fent, G. L. Allee, and J. L. Usry. 2005. True ileal digestible (TID) isoleucine:lysine ratio of late finishing barrows fed corn-blood cell or corn-amino acid diets. J. Anim. Sci. 83(Suppl. 2):67.

Gloaguen, M.; Le Floc'h1, N.; Primot, Y.; Corrent, E.; Van Milgen, J. 2013. Response of piglets to the standardized ileal digestible isoleucine, histidine and leucine supply in cereal–soybean meal-based diets. Animal 7:901-908.

Henry, Y.; Due´e, P. H. and Re´rat, A. 1976. Isoleucine requirement of the growing pig and leucine–isoleucine interrelationship. The Journal of Animal Science 42:357–364.

Kawaguchi, T.; Izumi, N.; Charlton, M. R.; Sata, M. 2011. Branched-chain amino acids as pharmacological nutrients in chronic liver disease. Hepatology 54:1063-1070.

Kerr, B.J.; Kidd, M. T.; Cuaron, J. A.; Bryant, K. L.; Parr, T. M.; Maxwell, C. V. and Weaver, E. 2004. Utilization of spray-dried blood cells and crystalline isoleucine in nursery pig diets. The Journal of Animal Science 82:2397-2404.

Langer, S.; Fuller, M. F. 2000. Interactions among the branched-chain amino acids and their effects on methionine utilization in growing pigs: effects on nitrogen retention and amino acid utilization. The British Journal of Nutrition 83:43-48.

Li, Y.; Wei, H.; Li, F.; Chen, S.; Duan, Y.; Guo, Q.; Liu, Y.; Yin, Y. 2016. Supplementation of branched-chain amino acids in protein-restricted diets modulates the expression levels of amino acid transporters and energy metabolism associated regulators in the adipose tissue of growing pigs. Animal Nutrition 2:24-32.

Lohman, A. C. Pozza, P. C.; Pozza, M. S. dos S.; Nunes, R. V.; Castilha, L. D.; Possamai, M.; Bruno, L. D. G.; Lazerri, D. B. 2012. Níveis de valina digestível para suínos machos castrados dos 15 aos 30 kg. Archivos de Zootecnia 61:267-278.

Madeira, M. S.; Alfaia, C. M.; Costa, P.; Lopes, P. A.; Lemos, J. P. C.; Bessa, R. J. B.; Prates, J. A. M. 2014. The combination of arginine and leucine supplementation of reduced crude protein diets for boars increases eating quality of pork. The Journal of Animal Science 92:2030-2040.

Morales, A.; Arcel, N.; Cota, M.; Buenabad, L.; Avelar, E.; Htoo, J. K. Cervantes, M. 2016. Effect of dietary excess of branched-chain amino acids on performance and serum concentrations of amino acids in growing pigs. Journal of Animal Physiology and Animal Nutrition 100:39-45.

Motta, V. T. 2009. Bioquimica clínica para o laboratório. 5 Edição. Curitiba, Paraná.

Nelson, D.L.; Cox, M.M. Princípios de Bioquímica de Lehninger. 2011. 5 Ed. SARVIER, São Paulo, Brasil.

Parr, T. M.; Kerr, B. J.; Baker, D. H. 2003. Isoleucine requirement of growing (25 to 45 kg) pigs. Journal of Animal Science, 81: 745-752.

Reeds, P. J.; Fuller, M. F.; Dadenhead, A.; Hay, S. M. 1987. Urea synthesis and leucine turnover in growing pigs: changes during 2 d following the addition of carbohydrate or fat to the diet. The British Journal of Nutrition 58:301-311.

Ren, M.; Zhang, H.; Zeng, X. F.; Liu, H.; Qiao, Y. 2015. Branched-chain Amino Acids are Beneficial to Maintain Growth Performance and Intestinal Immune-related Function in Weaned Piglets Fed Protein Restricted Diet. M. The Journal of Animal Science 28:1742-1750.

Riazi, R.; Wykes, L. J.; Ball, R. O.; Pencharz, P. B. 2003. The total branched-chain amino acid requirement in young healthy adult men determined by indicator amino acid oxidation by use of L-[1-13C] phenylalanine. Journal of Nutrition 133:1383–1389.

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T.; Euclides, R. F. 2011. Tabelas Brasileiras para Aves e Suínos: Composição de alimentos e exigências nutricionais. 3rd ed. UFV, Viçosa, Minas Gerais.

Soumeh, E. A.; Van Milgen, J.; Sloth, N. M.; Corrent, E.; Poulsen, H. D.; Nørgaard, J. V. 2015. Requirement of standardized ileal digestible valine to lysine ratio for 8- to 14-kg pigs. The Animal Consortium 1-7.

Torrazza, R. M.; Suryawan, A.; Gazzaneo, M. C.; Orellana, R. A.; Frank, J. W. Nguyen, H. V.; Fiorotto, M. L. El-Kadi, S.; Davis, T. A. 2010. Leucine Supplementation of a Low-Protein Meal Increases Skeletal Muscle and Visceral Tissue Protein Synthesis in Neonatal Pigs by Stimulating mTOR-Dependent Translation Initiation. The Journal of Nutrition 140:2145-2152.

Van Milgen, J.; Gloaguen, M.; Le Floc´h, N.; Brossard, Y. Primot, Y. and Corrent, 2012. E. Meta-anialysis of the response of growing pigs to the isoleucine concentration in the diet. Animal 6:1601–1608.

Vítek, M.; Pulkrábek, J.; Vališ, L.; David, L.; Wolf, J. 2008. Improvement of accuracy in the estimation of lean meat content in pig carcasses. Czech Journal of Animal Science 53:204-211.

Wiltafsky, M.K., Bartelt, J., Relandeau, C., Roth, F.X., 2009. Estimation of the optimum ratio of standardized ileal digestible isoleucine to lysine for eight- totwenty-five-kilogram pigs in diets containing spray-dried blood cells or corn gluten feed as a protein source. The Journal of Animal Sciences 87:2554–2564.

Wu, G. 2014. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. Journal of Animal Science and Biotechnology 5: 34.

Wu, G.; Wu, Z.; Dai, Z.; Y, Yang.; Wang, W.; Liu, C.; Wang, B.; Wang, J. and Yin, Y. 2013. Dietary requirements of ‘‘nutritionally non-essential amino acids’’ by animals and humans. Amino Acids 44:1107–1113.

VI – CONSIDERAÇÕES FINAIS

Nos últimos anos, inúmeros estudos foram conduzidos avaliando o efeito da suplementação de aminoácidos de cadeia ramificada (ACR) na dieta de suínos. Apesar de haver um antagonismo cientificamente consolidado entre os ACR, no presente trabalho a leucina não apresentou efeitos consistentes sobre os parâmetros avaliados, bem como a suplementação de valina e isoleucina, demonstrando interação em alguns casos.

Embora exista considerável variação nas exigências de ACR digestíveis, propostos em tabelas nacionais e internacionais de exigências nutricionais para suínos, além de dados disponíveis na literatura, os níveis avaliados no presente estudo atenderam às exigências de leitões, machos castrados e fêmeas, na fase inicial (15 aos 30 kg) e foi possível determinar o melhor nível de ACR para alguns parâmetros.

Assim, em dietas com baixa proteína bruta, o nível de 1,24% de leucina digestível proporcionou a melhor utilização dos aminoácidos da dieta, com base na ureia plasmática. Para a valina, a exigência diária estimada foi de 9,72 g/dia, correspondendo a uma concentração de 0,703% na dieta, para um máximo ganho de peso diário. No caso da isoleucina, o nível ótimo encontrado para a melhor profundidade de lombo foi de 0,609% da dieta.

De maneira geral, a redução do conteúdo de proteína bruta e suplementação com os seis primeiros aminoácidos limitantes para suínos, na forma sintética, mantém o desempenho dos suínos. Entretanto, mais estudos devem ser realizados no sentido de determinar, simultaneamente, a exigência dos três ACR, devido à interação/antagonismo que pode ocorrer. Quando determinadas simultaneamente, as exigências e as relações ideais entre estes aminoácidos podem ser alteradas e, possivelmente, pode-se formular dietas com valores de exigências mais próximas às necessidades dos animais.