UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

INOSINA-5’-MONOFOSFATO NA DIETA DE SUÍNOS EM TERMINAÇÃO

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MARINGÁ
Estado do Paraná
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TITULAÇÃO: Doutor em Zootecnia - Área de Concentração Produção Animal

APROVADO em 18 de setembro de 2020.

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Profª Drª Tatiana Carlesso dos Santos

Prof. Dr. Paulo Cesar Pozza
Orientador

Construa, não destrua, a preguiça acaba com o ser humano talentoso, nos dias mais difíceis de sua vida, não deixe de colocar pelo menos um tijolo na estrutura que te suporta por maior que seja sua preguiça, busque em sua vida cumprir as responsabilidades com amor, vivendo a vida intensamente, almejando a felicidade.

Gilberto
Bonagurio

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DEDICO

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BIOGRAFIA

Lucas Pimentel Bonagurio, filho de Gilberto Bonagurio e Claudia Dias Pimentel, nasceu em Capivari-SP, no dia 24 de outubro de 1990.

Em dezembro de 2014, concluiu a graduação em Zootecnia pela Universidade Estadual de Maringá, Campus Maringá.

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RESUMO

A inosina-5’-monofosfato (5’-IMP) é precursora das principais moléculas energéticas celulares, pois é convertida em adenosina monofosfato (AMP) e guanosina monofosfato (GMP) e posteriormente, em adenosina trifosfato (ATP) e guanosina trifosfato (GTP). Veem sendo evidenciado na literatura efeitos benéficos da suplementação da 5’-IMP sobre o desempenho produtivo, qualidade de carne, sistema antioxidante, sistema imune e funcionalidade do metabolismo de leitões na fase de matermidade e no período pós-desmame. Diante disso, foram conduzidos três experimentos com o objetivo de avaliar a suplementação de diferentes níveis dietéticos de 5’-IMP na dieta de suínos machos castrados e fêmeas, dos 75 aos 100 kg PV, sobre o desempenho produtivo, características quantitativas de carcaça, qualidade de carne, perfil hepático plasmático, *status* do sistema antioxidante, funcionalidade das mitocôndrias hepáticas, concentração de creatina muscular e respostas de defesa do sistema imune. No experimento I, foram utilizados Cinquenta e quatro suínos machos castrados, mestiços, com peso inicial médio de 75,62 ± 0,96 kg e peso final médio de 102,26 ± 3,23 kg foram distribuídos em delineamento de blocos casualizados com seis tratamentos e nove repetições por tratamento. As dietas experimentais foram as seguintes: dieta controle positivo (PC, 3300 kcal EM/kg), dieta controle negativo (NC, 3200 kcal EM/kg) e quatro dietas com adição de 0,050%, 0,100%, 0,150% , ou 0,200% de 5'-IMP na dieta CN. A análise de regressão indicou que a suplementação com 0,129% de 5'-IMP promoveu um ganho de peso diário de 1,29 kg e a suplementação com 0,200% de 5'-IMP resultou na menor conversão alimentar. A espessura de toucinho e o pH45minutos aumentaram linearmente com o aumento no nível de suplementação de 5'-IMP. As dietas suplementadas com 0,050% ou 0,100% de 5'-IMP resultaram em maiores pH 24 horas ($P \leq 0,05$) do que as dietas NC e PC. A vermelhidão do m. Longissimus Lumborum (LL) aumentou linearmente com o nível de suplementação de 5'-IMP. A perda por gotejamento teve uma resposta quadrática ($P = 0,002$) à suplementação de 5'-IMP. A suplementação de dietas de suínos em terminação com 5'-IMP foi eficaz em melhorar o status antioxidante de LL. Conclui-se que dietas com menor valor energético (3200 kcal EM/kg) suplementadas com 5'-IMP não afetaram os parâmetros de desempenho em comparação com a dieta PC (3300 kcal EM/kg) e a suplementação com 0,129% de 5'-IMP proporcionou o maior ganho de peso diário (1,29 kg). Além disso, a suplementação de 5'-IMP influenciou positivamente as características da carcaça, qualidade da carne LL e estado de oxidação do plasma em machos castrados em terminação (75-100 kg). No experimento II, foi utilizado o mesmo delineamento experimental e suínos do experimento I, após ao abate, foram realizadas analises de qualidade de carcaça, análise respiração mitocondrial, concentração de creatina e análises antioxidantes. Demonstramos que a suplementação de dietas de suínos em terminação com 5'-IMP reduz o peso relativo do fígado e aumenta o consumo de oxigênio durante a respiração mitocondrial sem alterar a relação ADP/O, indicando aumento na eficiência

respiratória das mitocôndrias hepáticas. Também observamos redução da peroxidação lipídica hepática e aumento da creatina muscular. Adicionalmente, a suplementação de 5'-IMP aumentou o peso de abate, rendimento de carne magra, comprimento do sarcômero e espessura de toucinho em suínos machos castrados em fase de terminação, demonstrando infuência no metabolismo protéico e energético. Sugerimos que a suplementação de 5'-IMP aumenta a capacidade respiratória mitocondrial quando a atividade metabólica hepática é estimulada, bem como a defesa antioxidante e promove o crescimento muscular em suínos machos castrados em terminação. No experimento III, Foram utilizadas 40 fêmeas suínas, com peso inicial de 75,58 ± 0,941 kg e final de 101,13 ± 1,993 kg foram distribuídas em um design de blocos randomizados, em um design fatorial 2 x 2, sendo utilizadas 10 repetições, em que a unidade experimental foi representada por um animal. Os suínos receberam a dieta basal, contendo 3200 kcal EM/kg e adição de 0,100% de 5'-IMP ou convencional, contendo 3300 kcal EM/kg. Adicionalmente, os suínos receberam o placebo ou foram estimulados imunologicamente. As dietas e as condições de estimulação do sistema imune influenciaram o GPD ($P < 0,001$) e o CDR ($P = 0,006$), também houve efeito da dieta ($P < 0,001$) e inoculação ($P = 0,012$) sobre a CA. A dieta 5'-IMP proporcionou o maior GPD (1,12 kg) aos suínos estimulados imunologicamente e menor consumo de ração submetidos ao placebo, além da menor CA ($P < 0,001$). A estimulação do sistema imune proporcionou uma maior CA ($P = 0,012$). A dieta 5'-IMP promoveu aumento no rendimento de carne magra na carcaça ($P = 0,012$) e a interação entre dieta e condição de estimulação de sistema imune apresentou uma redução na quantidade de gordura da carcaça, refletida em menores índices de P1P2P3 ($P = 0,030$) e espessura de toucinho ($P < 0,001$). A dieta 5'-IMP proporcionou maiores valores pH mensurado 45 min ($P = 0,035$) após ao abate e vermelhidão ($P < 0,001$) no músculo longissimus lumborum (*M. LL*), houve interações entre dietas e condições do sistema imune ($P \leq 0,05$), em que a dieta 5'-IMP proporcionou o maiores valores de pH mensurado 24 horas após ao abate e menores valores para a perda de agua por gotejamento (PAG), descongelamento e cocção e força de cisalhamento do *M. LL* em suínos estimulados imunologicamente e aos submetidos ao placebo. As interações entre dietas e períodos avaliados demonstraram que a 5'-IMP proporcionou um aumento na concentração plasmática de proteínas totais ($P = 0,006$), albumina ($P < 0,001$), ácido úrico ($P < 0,001$), plaquetas ($P < 0,001$) leucócitos totais, neutrófilos, linfócitos, relação neutrófilo:linfócito ($P < 0,001$) e plaqueta:linfócito ($P = 0,017$), eosinófilos ($P < 0,001$), basófilos ($P < 0,001$), transferrina ($P < 0,001$) e IgG de cadeia pesada ($P < 0,001$), demonstrando potentes benefícios sobre o sistema imune. Em conclusão, a suplementação de 0,100 % de 5'-IMP supriu o déficit energético proveniente da estimulação do sistema imune, promovendo benefícios sobre o desempenho produtivo, parâmetros de carcaça, qualidade de carne e parâmetros bioquímicos do plasma sanguíneo correlacionados ao sistema imune. Evidenciado a possibilidade da 5'-IMP ser empregada na formulação de ração como aditivo energético, proteico e um possível substituto para o uso de antibióticos em dietas para suínos na fase de terminação.

Palavras-chave: Defesa do sistema imune, estado oxidativo, metabolismo energético, respiração mitocondrial hepática. .

ABSTRACT

Inosine-5'-monophosphate (5'-IMP) is a precursor of the main cellular energy molecules, as it is converted into adenosine monophosphate (AMP) and guanosine monophosphate (GMP) and later, into adenosine triphosphate (ATP) and guanosine triphosphate (GTP) Beneficial effects of 5'-IMP supplementation on productive performance, meat quality, antioxidant system, immune system and piglet metabolism functionality have been evidenced in the literature in the maternity phase and in the post-weaning period. Therefore, three experiments were conducted with the objective of evaluating the supplementation of different dietary levels of 5'-IMP in the diet of male castrated and specific pigs, from 75 to 100 kg BW, on the productive performance, quantitative carcass characteristics, quality meat, plasma liver profile, state of the antioxidant system, functionality of liver mitochondria, muscle creatine concentration and immune system defense responses. In experiment I, . Fifty-four crossbred castrated male pigs with a mean initial weight of 75.62 ± 0.96 kg and a mean final weight of 102.26 ± 3.23 kg were distributed in a randomized block design consisting of six treatments and nine replications per treatment. Experimental diets were as follows: positive control diet (PC, 3300 kcal ME/kg), negative control diet (NC, 3200 kcal ME/kg), and four diets prepared by supplementing the NC diet with 0.050%, 0.100%, 0.150%, or 0.200% 5′-IMP. Regression analysis indicated that supplementation with 0.129% 5′-IMP promoted a daily weight gain of 1.29 kg and supplementation with 0.200% 5′-IMP resulted in the lowest feed conversion. Backfat thickness and pH45minutes increased linearly with 5′-IMP supplementation level. Diets supplemented with 0.050% or 0.100% 5′-IMP resulted in higher ($P \leq 0.05$) pH at 24 h post-slaughter than NC and PC diets. The redness of m. *Longissimus lumborum* (LL) increased linearly with 5′-IMP supplementation level. Drip loss had a quadratic response ($P = 0.002$) to 5'-IMP supplementation. Supplementation of finishing pig diets with 5′-IMP was effective in improving LL antioxidant status. It is concluded that 5′-IMP-supplemented, low-energy (3200 kcal ME/kg) diets did not affect performance parameters as compared with the PC diet (3300 kcal ME/kg), and supplementation with 0.129% 5′-IMP provided the highest daily weight gain (1.29 kg). Furthermore, 5′-IMP supplementation positively influenced carcass characteristics, LL meat quality, and plasma oxidation status in finishing barrows (75–100 kg). In experiment II, the same experimental design was used and pigs from experiment I, after slaughter, analyzes of carcass quality, mitochondrial respiration, creatine concentration and antioxidants were performed. We demonstrated that supplementation of fnishing pig diets with 5′-IMP reduces the relative weight of the liver, and increases oxygen consumption during mitochondrial respiration without changing the ADP/O ratio, indicating an increase in the respiratory efciency of liver mitochondria. We also observed a reduction in liver lipid peroxidation and an increase in muscle creatine. Moreover, 5′IMP supplementation increases slaughter weight, lean meat yield, sarcomere length, and

backfat thickness in fnishing barrows, demonstrating infuence on protein metabolism. We suggest that 5′-IMP supplementation increase the mitochondrial respiratory capacity when the liver metabolic activity is stimulated, enhances antioxidant defense, and promotes muscle growth in fnishing barrows. Forty female pigs, with an initial weight of 75.58 ± 0.941 kg and final weight of 101.13 ± 1.993 kg were distributed in a randomized block design, in a 2 x 2 factorial design, with 10 repetitions and each animal representing an experimental unit. The gilts received the basal diet, containing 3200 kcal ME/kg and addition of 0.100% 5'-IMP or conventional diet, containing 3300 kcal ME/kg. Additionally, the gilts received a placebo or received immunostimulation. Diets and immune system stimulation conditions influenced DWG ($P < 0.001$) and DFI ($P = 0.006$). Furthermore, there was an effect of diet ($P < 0.001$) and inoculation ($P = 0.012$) on FC. The 5'-IMP diet provided the highest DWG (1.12 kg) to immune-stimulated gilts and the lowest feed intake treated with placebo, in addition to the lowest FC ($P < 0.001$). Stimulation of the immune system provided a higher FC ($P = 0.012$). The 5'-IMP diet promoted an increase in lean meat yield ($P = 0.012$) and the interaction between diet and immune system stimulation condition showed a reduction in the amount of carcass fat, reflected in lower P1P2P3 indexes ($P = 0.030$) and backfat thickness ($P < 0.001$). The 5'-IMP diet provided higher pH values measured at 45 min ($P = 0.035$) after slaughter and redness ($P < 0.001$) in longissimus lumborum muscle (LLM), there were interactions between diets and conditions of the immune system ($P \leq 0.05$), in which the 5-IMP diet provided the highest pH values measured 24 hours after slaughter and lowest values for drip loss (DL), fluid lost in thawing (FLT) and fluid lost in cooking (FLC) and shear force of LLM in immunologically stimulated gilts and those treated with placebo. The interactions between diets and evaluated periods demonstrated that 5'-IMP increased in the plasma concentration of total proteins ($P = 0.006$), albumin ($P < 0.001$), uric acid ($P < 0.001$), platelets ($P < 0.001$), total leukocytes, neutrophils, lymphocytes, neutrophil:lymphocyte ratio ($P < 0.001$), platelet:lymphocyte ratio ($P = 0.017$), eosinophils ($P < 0.001$), basophils ($P < 0.001$), transferrin ($P < 0.001$) and chain heavy IgG ($P < 0.001$), demonstrating potent benefits on the immune system. In conclusion, the supplementation of 0.100% of 5'-IMP supplied the energy deficit resulting from the stimulation of the immune system, promoting benefits on productive performance, carcass characteristics, meat quality, and biochemical parameters of blood plasma correlated to the immune system. It highlights the possibility of 5'-IMP being used in the feed formulation as an energy and protein additive and a possible substitute for the use of antibiotics in diets for finishing gilts.

Keywords: Defense of the immune system, energy metabolism, hepatic mitochondrial breathing, oxidative state.

INTRODUÇÃO

Revisão de literatura

1. Nucleotídeos

1.1. Estrutura molecular dos Nucleotídeos

Os nucleotídeos NTs armazenam e transferem energia para inúmeras atividades celulares no metabolismo, principalmente para a biossíntese das células. Atuam também como sinalizadores químicos e fatores coenzimáticos, modulando hormônios e atividades sinápticas (Carver e Walker, 1995).

Determinadas bases nitrogenadas ligam seu grupo hidroxila (OH) ao carbono 2’ da pentose, produzindo NTs ligados a ribose, que atuam na síntese de RNA. Diferentemente, as bases nitrogenadas que ligam seu hidrogênio livre (H) ao carbono 2’ da pentose, produzem NTs ligados a desoxirribose, que atuam principalmente na síntese de DNA (Carver, 1999).

Os ácidos nucleicos possuem em sua estrutura inúmeras ligações químicas entre NTs. Por sua vez, os NTs são formados por ligações entre nucleosídeos (NSs) e grupos fosfatos. Os NSs são moléculas formadas por ligações entre bases nitrogenadas, nucleobases e a ribose ou desoxirribose. As bases nitrogenadas pirimidinas e purinas diferenciam os NTs em purínicos ou pirimídicos. A adição de grupos fosfato aos NSs resulta na formação dos NTs, podendo ser adicionado um grupo fosfato (NTs monofosfato), 2 grupos fosfatos (NTs Difosfato) ou 3 grupos fosfatos (NTs trifosfato) (Rudolph, 1994).

A inosina-5’-monofosfato (5’-IMP), adenina monofosfato (AMP), guanina monofosfato (GMP), hipoxantina (HXP), Adenosina Trifosfato (ATP), Guanosina Trifosfato (GTP), Nicotinamida Adenina Dinucleotídeo (NADH) e Adenosina Monofosfato cíclica (AMPc), entre outras moléculas, são empregadas em diversas funções essenciais ao metabolismo, à exemplo da síntese do DNA, transcrição do RNA,

como componentes estruturais de diversas moléculas, co-fatores enzimáticos, etc (Carver e Walker, 1995). Os anéis da estrutura dos NTs purínicos são formados a partir de moléculas de CO_2 e de N doadas pela glicina, aspartato, glutamina e de reações químicas que envolvem o tetrahidrofolato.

Na fita dupla do DNA, as bases púricas se ligam por pontes de hidrogênio as bases pirimidinas, como a Citosina Monofosfato (CMP), Uracila Monofosfato (UMP) e Tiamina Monofosfato (TMP) (Nelson e Cox, 2009). Por sua vez, a estrutura dos anéis dos NTs pirimídicos são formados por uma molécula de bicarbonato e amônia, reação em que duas moléculas de ATP são consumidas pela carbamoil-fosfato para acoplar uma molécula de nitrogênio (N) e uma de carbono (C) em três moléculas de C e uma de N no aspartato, completando o anel pirimídico dos NTs (Nelson e Cox, 2009). Depois de formado anel, o substrato PRPP acopla uma molécula de ribose-5-fosfato formando o UTP, intermediário metabolizado a CTP para a síntese de RNA e metabolizado a TTP e DCTP para a síntese de DNA (Nelson and Cox, 2009).

1.2.1. Metabolismo dos NTs purínicos

Os NTs podem ser obtidos por meio da dieta ou sintetizados endogenamente (Cosgrove, 1998). A biossíntese endógena envolve mais de 100 diferentes reações, classificadas em quatro diferentes classes de reações, conforme proposto por Martinussen et al. (2011):

- Biossíntese de novo: IMP e UMP são formados.
- Interconversão: IMP e UMP são convertidos em nucleosídeos trifosfato (NTPs) e dinucleosídeos trifosfatos (DNTPs).
- Degradação: Moléculas de açucares e energéticas são degradadas e doam grupos de N e C para a síntese de NSs e NTs.
- Via de recuperação de NTs: Nucleosídeos (NSs) e nucleobases são convertidas em NSs e NTs.

1.2.2. Absorção dos NTs fornecidos na dieta

Os NTs presentes na dieta ao serem ingeridos são metabolizados em ácidos nucleicos, nucleosídeos e bases nitrogenadas, e posteriormente, ao de ácido úrico em

mamíferos e em ureia nas aves. Em determinadas espécies de mamíferos, os NTs são metabolizados em alantoína ao invés de ácido úrico (Maiuolo et al., 2016).

O maior sítio de absorção dos NTs, nucleosídeos e bases nitrogenadas é o duodeno. Durante o processo de absorção, as enzimas proteolíticas clivam as nucleoproteínas em ácidos nucleicos. Posteriormente, as enzimas nucleases sintetizadas pelo pâncreas clivam os ácidos nucleicos em mono, di, tri e poliNTs. A conversão dos ácidos nucleicos em monoNTs é finalizada no intestino pelas enzimas polinucleotidases ou fosfoesterases (Sauer et al., 2011).

Após a hidrólise dos ácidos nucleicos, os NTs serão hidrolisados pela enzima alcalina fosfatase em nucleosídeos, que em seguida serão clivados por nucleosidases em bases nitrogenadas purínicas ou pirimídicas. Em animais, estudos prévios demonstraram que 90% dos nucleosídeos e bases nitrogenadas são absorvidas nos enterócitos (Ho et al., 1979). Destacaram também que após absorvidos, cerca de 2 a 5 % dos NTs das dietas são encontrados no intestino delgado, fígado e tecido muscular, devido ao intenso processo de mitose que ocorre nestes tecidos (Carver e Walker, 1995).

No metabolismo não existe um sistema de transporte especifico para os NTs, em função de sua baixa permeabilidade na membrana plasmática celular. Além disso, possui um grupo fosfato com carga iônica negativa que reduz sua absorção (Sanderson e He, 1994). Por sua vez, os NSs são transportados por difusão facilitada ou mecanismos carreadores sódio dependente através da membrana plasmática das células intestinais para os enterócitos do intestino (Sauer et al., 2011).

Os metabólitos de degradação dos nucleosídeos são reabsorvidos e modificados a mononucleosídeos na via de recuperação de NTs para serem utilizados por diferentes tipos de células ou excretados pelo intestino delgado e urina na forma de ácido úrico ou alantoína em mamíferos e em ureia em aves (Maiuolo et al., 2016). A biossíntese *de novo* e a via de recuperação de NTs purínicos são as principais reguladoras da homeostase dos NTs no metabolismo (Calver and walker, 1995).

1.2.3. Biossíntese de novo de NTs púricos

Para biossintetizar uma molécula de nucleotídeo, são gastos alta quantidade de energia e tempo, sendo consumidas de sete a nove moléculas de ATP durante os 9 processos metabólicos que ocorrem para sintetizar a 5’-IMP, primeiro nucleosídeo

biossintetizado (Zhu and Thompson, 2019). Além disso, células de rápida proliferação e crescimento não biossintetizam NTs na via *de novo* em quantidade suficiente para atender à exigência de NTs das atividades destas células. É possível citar como exemplo as células de defesa do sistema imune (Hess e Greenberg, 2012), os eritrócitos (Jarvis et al., 1980) e os enterócitos (Uauy, 1994).

O fígado é o principal local de biossíntese de NTs, no citosol dos hepatócitos estão presentes todas as enzimas necessárias para a síntese e degradação das bases purínicas (Carver e Walker, 1995). No fígado, a ribose-fosfato difosfoquinase ativa a ribose-5-fosfato formando o fosforibosil pirofosfato (PRPP), fonte de ribose ativada para a biossíntese de novo e via de recuperação de NTs. O processo de síntese está apresentado abaixo, conforme descrito por Berg et al. (2002):

A PRPP, glutamina, ATP, CO_2 e outros componentes são substratos empregados na síntese do anel purínico da 5'-IMP, cuja é o primeiro NTs a ser produzido (Figura 1), conforme descrito por Berg et al. (2002):

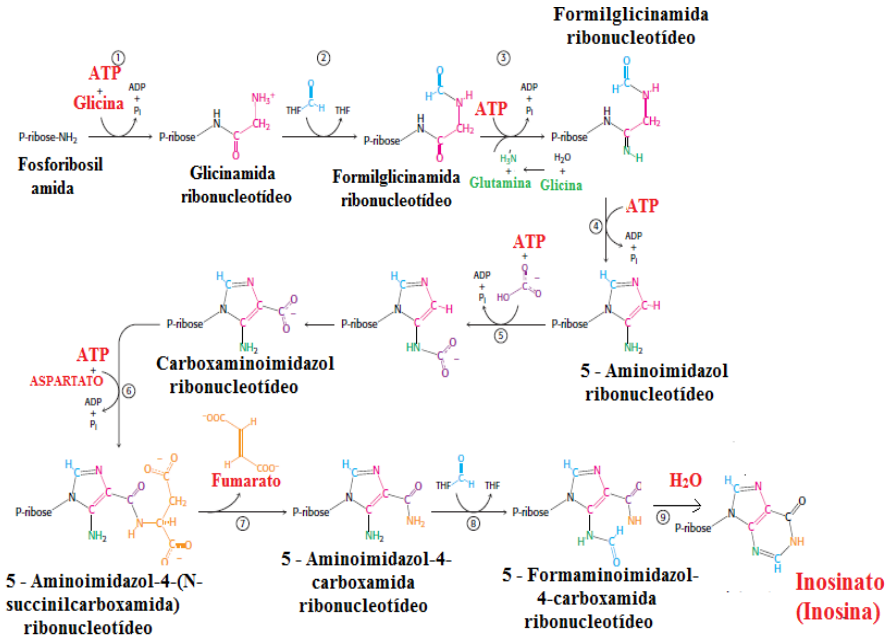

Figura 1. Biossíntese de novo de purinas. (1) Glicina é ligada ao grupo amino da fosforibosilamina. (2) N^{10}-Formiltetrahidrofolato (THF) transfere um grupo formil para o grupo amina de um resíduo de glicina. (3) O grupo amida interno é fosforilado e convertido para uma amidina com a adição de uma amônia derivada da glutamina. (4) Um conjunto de reações intramoleculares forma o anel imidazol. (5) O bicarbonato é adicionado primeiramente ao grupo amino exocíclico e depois a um átomo de C do anel amidazol. (6) O carboxilato imidazol é fosforilado, e o fosfato é deslocado pelo grupo

amino do aspartato. (7) O fumarato é liberado. (8) Um segundo grupo formil é doado pelo N^{10}-formiltetrahidrofolato (THF). (9) Um conjunto de reações intramoleculares causa a perda de H_2O do 5-formaminoimidazol-4-carboxamida ribonucleotídeo completando a síntese do inosinato, um nucleosídeo purínico. Adaptado de Berg et al. (2002).

A função do aspartato de doar um grupo amino e a liberação concomitante de fumarato são processos remanescentes da conversão da citrulina em arginina no ciclo da ureia, etapas catalisadas por enzimas da família ATP-grasp, com funções homólogas a da enzima carbamoil-fosfato demonstradas em diversas vias (Berg et al., 2002). A 5'-IMP após poucos processos metabólicos é convertida em AMP ou GMP de acordo com a necessidade do metabolismo.

1.2.4. Biossíntese de novo dos NTs adenina e guanina a partir da IMP.

A inosina sintetiza o adenilosuccinato a partir da adição de uma molécula de aspartato e GTP. O adenilosuccinato libera de sua estrutura uma molécula de fumarato, produzindo o adenilato (AMP). Na síntese do adenilosuccinato, o GTP doa seu grupo fosfato para o aspartato, a transferência do grupo fosfato é catalisada pela enzima adenilosuccinato sintetase, a qual possui estrutura molecular semelhante as proteínas G e não as enzimas da família ATP-Grasp (Berg et al., 2002; Martinussen et al., 2011).

Para biossentizar o GMP, a inosina sofre uma reação de oxidação, em que o NAD^+ recebe uma molécula de H_2O. Desta molécula de H_2O, é acoplada em sua estrutura uma molécula de hidrogênio (H), a outra molécula de H^+ é liberada no meio celular e o oxigênio é acoplado no C-5, resultando no xantilato (XMP). A adição de uma molécula de H_2O na glicina forma a glutamina e a amônia (NH_3), ambos são adicionados à estrutura do XMP, assim como é adicionada uma molécula de ATP, doador do AMP ao átomo de oxigênio para formar o grupo carbonila (Berg et al., 2002).

A hidrólise da glutamina gera uma molécula de amônia (NH_3), a qual é acoplada ao AMP, e quando a enzima GMP sintetase catalisa a ligação entre a NH_3 o AMP, origina o GMP (Berg et al., 2002; Martinussen et al., 2011).

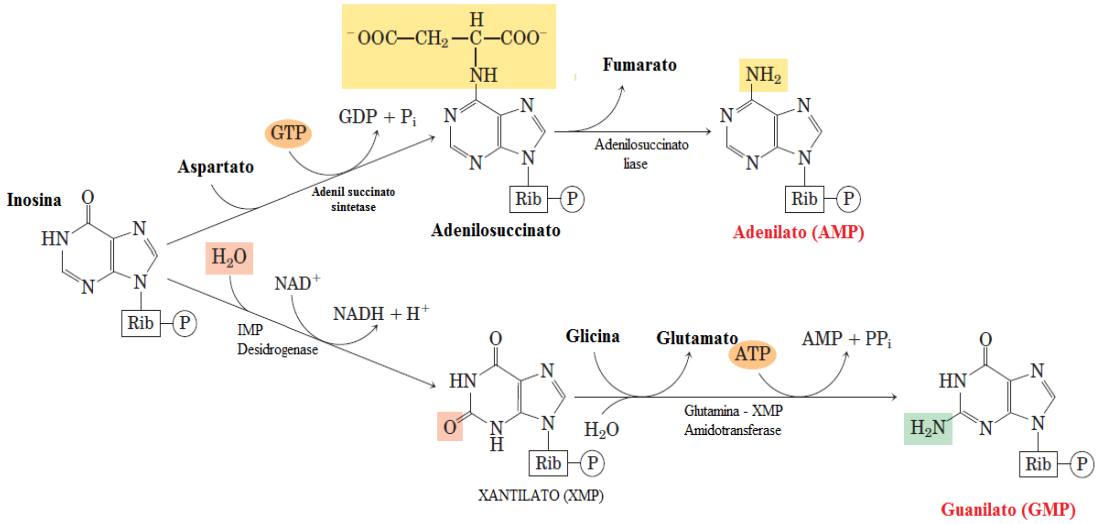

Figura 2. Síntese do adenilato (AMP) e guanilato (GMP). A inosina é precursora do AMP e GMP. O AMP é formado pela adição de aspartato, seguido da liberação do fumarato de sua estrutura. O GMP é gerado pela adição de H_2O, desidrogenação pelo NAD^+ e a reposição do átomo de oxigênio carbonil pelo NH_2 formado no processo de hidrólise da glutamina. Adaptado de Berg et al. (2002).

1.2.5. Interconversão dos NTs.

O controle alostérico da biossíntese de NTs é realizado por feed back negativo em diversos pontos chaves da biossíntese. O primeiro feed back negativo é efetuado pela IMP, AMP e GMP sobre a enzima glutamina fosforibosil amidotransferase, a fim de evitar conversão da ribose-5-fosfato em PRPP e da PRPP para a fosforibosilamina. No segundo controle de feed back negativo, a AMP limita a conversão da 5'-IMP para o adenilosuccinato. Do mesmo modo, o GTP limita a conversão das 5'-IMP em xantilato (XMP). O terceiro controle de feed back negativo é realizado pelo GTP sobre a síntese de AMP e do ATP sobre a síntese de GMP (Berg et al., 2002).

1.2.6. Via de recuperação dos NTs purínicos

A via de recuperação dos NTs purínicos modula a homeostase de NTs no metabolismo. Uma vez em maior concentração no metabolismo, os NTs estimulam a via de recuperação de NTs e reduzem a biossíntese de novo de NTs. Opostamente, a menor concentração de NTs estimula a biossíntese de novo de NTs. A principal vantagem desta via é a economia de ATP proporcionado ao metabolismo, pois para reciclar um NTs, apenas 1 molécula de ATP é consumida (Berg et al., 2002).

Praticamente 90 % das bases purínicas metabolizadas ou obtidas na dieta, são recicladas na via de recuperação de NTs. A hidrólise de ácidos nucleicos, NTs e aminoácidos, resultam em bases nitrogenadas e nucleobases livres no metabolismo (Moffatt et al., 2002). Estas são capturadas e acopladas ao PRPP, produzindo nucleosídeos monofosfatos purínicos. A enzima adenina fosforibosiltransferase (APRT) catalisa a ligação N-glicosil entre adenina e ribose, utilizando a PRPP como substrato (Martinussen et al., 2011).

$$\text{Adenina} + \text{PRPP} \xrightarrow{\text{APRT}} \text{Adenilato (AMP)} + \text{PPi}$$

A enzima hipoxantina-guanina fosforibosiltransferase (HGPRT) catalisa as reações que formam o GMP, assim como a 5'-IMP (Martinussem et al., 2011).

$$\text{Guanina} + \text{PRPP} \xrightarrow{\text{HGPRT}} \text{Guanilato} + \text{PPi}$$

$$\text{Hipoxantina} + \text{PRPP} \xrightarrow{\text{HGPRT}} \text{Inosinato (5'-IMP)} + \text{PPi}$$

Após a conversão da 5'-IMP para AMP ou GMP, o próximo processo metabólico é a conversão da AMP para adenosina difosfato (ADP) e do GMP em guanosina difosfato (GDP). Posteriormente, a ADP e GDP são convertidos em ATP e GTP, principais moléculas carreadoras de energia para os processos celulares (Harmsen et al., 1984).

Desta forma, ao fornecer NTs na dieta presume-se aumento na absorção e degradação das bases nitrogenadas e nucleobases, elevando suas concentrações no metabolismo, bem como a via de recuperação de NTs. Isso pode promover redução nas moléculas de ATP, as quais seriam consumidas na biossíntese de novo de NTs. É possível que isso reduza o déficit de NTs das células incapazes de biossintetizar NTs na via de novo, à exemplo das células de mucosa (Sauer et al., 2011) e linfócitos T (Wang et al., 2019).

A biossíntese *de novo* e a via de recuperação de NTs são processos metabólicos que ocorrem simultaneamente no metabolismo para manter em níveis ideais a concentração de NTs. Nestes processos, a 5'-IMP pode ser convertida para AMP na biossíntese de novo, mas a 5'-IMP também é sintetizada a partir da AMP na via de recuperação de NTs. Estes processos em conjunto influenciam a deaminação do aspartato em fumarato. Isto é

importante, pois em condições de rápido crescimento muscular é necessário que o metabolismo aumente as atividades do ciclo do ácido cítrico em função do aumento na síntese de NADH decorrida do aumento na síntese de ATP. Para isto, o músculo fornece o fumarato para o ciclo do ácido cítrico, garantindo o aumento de suas atividades (Mateo, 2005).

1.2.7. Degradação dos NTs purínicos

Os monoNTs purínicos são primeiramente convertidos em nucleosídeos livres pelas ações das enzimas 5'-nucleotidases citosólicas. O nitrogênio é removido da adenosina pela ação da enzima adenosina desaminase (ADA) gerando a inosina. A ribose é removida dos NTs pela ação da enzima purina nucleosídeo fosforilase (PNP), produzindo as nucleobases, hipoxantina, xantina e guanina.

A seguir, o nitrogênio é removido da guanina pela enzima guanina-desaminase produzindo xantina. A hipoxantina e a xantina são então convertidas em ácido úrico, pela enzima xantina oxidase. No fígado, o ácido úrico é degradado a alantoína pela enzima ureato oxidase e, posteriormente, o ácido glioxílico e a ureia são formados por ações de diversas enzimas. A ureia é eliminada na urina, enquanto o ácido glioxílico é utilizado na síntese da glicina (Kim, 2019).

As bases nitrogenadas livres e as nucleobases que foram liberadas no metabolismo a partir da hidrólise dos NTs, podem ser capturadas e recicladas em NTs. O excesso de NTs no metabolismo é catabolizado principalmente ao ácido úrico, no entanto, existem mamíferos que catabolizam os NTs em maior parte para a alantoína (Henderson e Paterson, 1973).

1.3. Funções dos NTs.

Os NTs são moléculas de baixo peso molecular classificados como nutrientes condicionalmente essenciais, estes participam de praticamente todos os processos celulares que ocorrem no metabolismo (Xie et al., 2019). Na síntese de RNA ou DNA, os NTs desenvolvem diversas funções intracelulares nas reações energéticas necessárias para o processo de transdução. A hidrólise do nucleotídeo adenosina trifosfato (ATP), fornece energia à maioria das reações celulares. O ATP também atua como carreador

ativos de carboidratos, lipídeos, sulfato e grupos metilos em diversas vias metabólicas (Carver, 1999; Engelking, 2015).

Os NTs são componentes estruturais de diversas coenzimas, incluindo a coenzima A (CoA.SH), dinucleotídeo de nicotinamida e adenina (NAD^+) e dinucleotídeo de flavina e adenina (FAD^+), reguladores alostéricos essenciais para o funcionamento de enzimas intracelulares. A adenosina e guanosina monofosfato cíclica (cAMP e cGMP), são os principais mensageiros secundários do metabolismo, estes modulam efeitos de diversas substâncias que se acoplam aos receptores da membrana plasmática (Engelking, 2015; Ślepokura, 2016).

Em diversos tecidos, a biossíntese de novo de NTs ocorre a partir de pequenas moléculas orgânicas disponíveis nas células ou são regenerados em vários tecidos na via de recuperação de NTs, tendo como substratos as bases nitrogenadas e nucleobases (Le et al., 2017; Mackinnon e Deller, 1973). Sob condições normais o metabolismo sintetiza endogenamente NTs em taxas suficientes. Mas em situações que aumentam a exigência de NTs (processos inflamatórios), como o rápido crescimento muscular (Grimble e Westwood, 2001), causa um déficit nutricional de NTs, limitante da eficiência de processos fisiológicos e metabólicos, a exemplo da divisão celular, ativação de coenzimas, estimulação de crescimento celular e da resposta imune humoral, pois estes processos dependem das concentrações de ATP e GTP, bem como da síntese de RNA e DNA (Muto et al., 2014; Sauer et al., 2011; Uauy et al., 1994).

1.3.1 Funções metabólicas do ATP

A manutenção da vida e a prolongação das atividades dos organismos celulares demandam de alta quantidade de energia disponível na forma de ATP. O ATP desenvolve atividades essenciais em praticamente todas as vias metabólicas, mesmo em condições fisiológicas ou patológicas (Lu et al., 2014). A regulação entre a síntese e degradação do ATP modula as atividades metabólicas de praticamente todos os organismos celulares (Rajendran et al., 2016).

O ATP tem como principal função armazenar e carrear energia para as células, energia originada de processos oxidativos e da fosforilação. A maior parte desta energia é empregada na manutenção do DNA, na síntese e replicação, na expressão de genes relacionados a síntese de RNA e proteínas, assim como no transporte de macromoléculas

e elementos químicos (Hesketh e Oliver, 2019). Além de armazenar energia, o ATP doa grupos fosfatos para a ativação de inúmeras reações metabólicas (Dzeja e Terzic, 2003). No metabolismo proteico, o ATP doa grupos fosfatos e adenililo para as modificações pós-translacionais (Rajendran et al., 2016). Adicionalmente, o ATP estimula o aumento na expressão de genes relacionados a síntese proteica (Dzeja e Terzic, 2003).

O ATP também é requerido na biossíntese da adenosina monofosfato (cAMP), mensageiro secundário essencial para os sinais de transdução e regulação hormonal, bem como para outras diversas enzimas quinases. Adicionalmente, atua como sinalizador químico em diversas reações sinápticas no metabolismo (Dzeja e Terzic, 2003).

Funções metabólicas do ATP descritas em diversas pesquisas realizadas:

1º - Disponibiliza energia para a contração do músculo esquelético durante o processo de *rigor mortis* (Wei et al., 2017).

2º - No processo de *rigor mortis*, o ácido lático em conjunto com a hidrólise do ATP, causam o acúmulo de H+, acidificando o músculo (Matarneh et al., 2017).

3º- O ATP é indispensável para o processo de fosforilação (Ren et al., 2016).

3º- A síntese mitocondrial de ATP suporta a demanda de energia gasta pelo metabolismo para sintetizar agentes imunológicos, como os linfócitos T, macrófagos, etc (Mehta et al., 2017).

4º- O ATP e a ADP extracelular induzem a ativação do inflamassoma pirina de 3 estruturas (NLRP3), para induzir a maturação proteolítica das interleucinas-1 beta (IL-1b), liberando-as para o meio extracelular (Baron et al., 2015).

5º- O ATP possui relação moduladora sobre as ações das ROS/RNS no metabolismo, pois dímeros da enzima ATP sintetase formam os poros de transição da permeabilidade mitocondrial (PTP), mas também realizam modificações pós-traducionais causadas pelas ROS / RNS afetando o metabolismo bioenergético celular, por meio da modulação da catálise realizada pela ATP sintase (Kaludercic e Giorgio, 2016).

6º - A proteína de mitocôndria interna denominada de atrofia óptica 1 (OPA1) desenvolve importantes ações nas fusões mitocondriais e estruturas que mantém a integridade da membrana. A OPA1 também desenvolve ações essenciais no sistema imune inato. Adicionalmente, a OPA1 é dependente da produção de ATP, pois requerem ATP para a manutenção das atividades da rede de microtúbulos, relacionados com a

síntese de neutrófilos. Assim, o ATP pode modular a síntese de neutrófilos (Amini et al., 2018).

1.3.2. Funções metabólicas NTs guanínicos

Os NTs guanínicos são carreadores energéticos essenciais para as atividades celulares (Carver e Walker, 1995). Suas atividades no metabolismo energético são pesquisadas com menor intensidade em relação à dos NTs adenínicos, segundo os pesquisadores Hesketh e Oliver (2019), isto ocorre devido a 3 fatores:

1º - Não é o principal carreador de energia.

2º - Suas concentrações nas células são menores se comparados as concentrações de ATP celular.

3º - Pode ser sintetizado a partir da doação de um grupo fosfato do ATP para o GDP.

A principal função do GTP é fornecer energia para a síntese proteica, em que duas moléculas de GTP são consumidas a cada molécula de aminoácido incorporada na cadeia polipeptídica. O GTP também é requerido para a funcionalidade e composição das células do retículo endoplasmático e citoesqueleto, bem como para as sinalizações intracelulares moduladoras da síntese das proteínas da família G e da biossíntese de NTs adenínicos (Hesketh e Oliver, 2019).

O GTP acopla-se ao sítio Rheb ativando o complexo mTORC1, em função da modulação sobre o sítio Rheb GTP/GDP, determinando que o complexo mTORC1 execute suas atividades para estimular a síntese proteica, bem como a ativação de células B (Benjamin e Hall, 2017; Ortega-Molina et al., 2019). O complexo mTORC1 é a maior conexão entre os níveis variáveis de energia dos nutrientes com fatores relacionados ao crescimento, reguladores do crescimento celular. Este complexo é ativado pelo processo de anabolismo, necessário para o crescimento celular, e síntese de proteínas NTs e lipídeos (Benjamin e Hall, 2017). A maior concentração de GTP no metabolismo possivelmente maximize estas atividades supracitadas.

Funções metabólicas do GTP descritas em diversas pesquisas realizadas:

1º - Precursor na síntese de RNA e DNA (Weber, 2003).

2º - O GTP reduz a biossíntese de novo da cistidina trifosfato (CTP), limitando a atividade da enzima CTP sintetase, assim como limita a biossíntese de ATP, devido reduzir a atividade catalítica da enzima adenilosuccinato sintetase, enzima que promove a conversão da IMP em AMP (Weber, 1983).

3º - O GTP ativa as enzimas ribonucleotídeo redutases, convertendo o GDP em DGTP, aumentando a biossíntese de DNA (Takeda e Weber, 1981).

4º - O GTP participa da formação de complexos lipídicos, fornecendo moléculas de manose da manose-GDP para receptores de lipídeos ligados a oligossacarídeos (Spencer e Elbein, 1980).

5º - O GTP é um cofator da síntese da enzima fosfolipase C, catalizadora do processo final de transdução, necessário para a produção de mensageiros secundários, como o diacilglicerol (DAG) e as enzimas da família IP_3 (Downes, 1989).

6º - O GTP acopla-se ao oxaloacetato livre no citosol da mitocôndria, produzindo a enzima carboxinase fosfoenolpiruvato, importante para a via de gliconeogênese hepática e renal (Hanson e Reshef, 1997).

7º - O GTP rapidamente disponibiliza energia para as atividades de células de rápido crescimento e proliferação, à exemplo dos linfócitos (Jayaram et al., 1999).

8º - O GTP atua na síntese de NTs adenínicos, biopterinas e proteínas (Bourne et al., 1990).

As evidências demonstram que a suplementação de 5'-IMP na dieta de suínos, provavelmente estimule a síntese de ATP e GTP, beneficiando a eficiência de todas as atividades supracitadas.

1.4. Inosina-5'-monofosfato(5'-IMP)

As primeiras pesquisas com a 5'-IMP ocorreram por meados de 1942. Entre a década de 50 e 2000, diversos estudos foram efetuados para avaliar a suplementação de 5'-IMP na dieta de humanos, ratos, cavalos e suínos, afim de compreender sua via metabólica e as suas funções no metabolismo. Foi relatado, que a deposição e a concentração de 5'-IMP na carne varia conforme a espécie e o músculo avaliado (Dannert and Pearson, 1967; Warrren, 1961). Apresentou efeitos benéficos sobre o rim (Fernando et al., 1976), o fígado (Watts et al., 1979) e o coração (Dobson and Schrader,1984), assim como ações moduladoras sobre o sistema imune (Carver, 1999), funções no metabolismo energético

celular (Watts et a., 1979) e ação sobre a síntese proteica, refletida no ganho de peso (Li et al., 2018).

As atividades metabólicas dos NTs inosina, adenosina e guanosina dependem de diversas enzimas, diante disto, diversas pesquisas avaliaram as atividades enzimáticas da enzima IMPDH, assim como o Dinucleotídeo de Nicotinamida de Adenina (NAD), as quais serão abordadas a seguir.

1.4.1. Atividades da enzima inosina monofosfato desidrogenase (IMPDH)

A enzima (IMPDH) é essencial tanto para o metabolismo dos NTs, quanto para a proliferação celular, pois catalisa a conversão da IMP em XMP, processo em que o NAD é oxidado a NAD+, em seguida o NAD+ é reduzido a Nicotinamida Adenina Dinucleotídeo (NADH). Após estes processos, a XMP é catalisada pela enzima GMP sintetase em GMP (Buey et al., 2017). O GTP é catalisado por várias enzimas, disponibilizando unidades estruturais para a síntese de DNA (dGTP) ou RNA (GTP) (Juvale et al., 2019).

Existem dois genes que dão origem a dois diferentes tipos da enzima IMPDH (tipo I e II). A IMPDH I é expressa continuamente nas células normais, enquanto a tipo II tem sua expressão modificada por células neoplásticas e em linfócitos ativados (Pankiewickz e Goldstein, 2003; Zimmerman et al., 1998).

A produção da IMPDH é regulada pelos níveis de IMP e GTP (Keppek et al., 2018). A IMPDH regula o pool intracelular de NTs purínicos e pirimidínicos, se sua atividade é inibida, a concentração de NTs guanínicos também reduz, incluindo a guanosina, GMP, GDP e GTP. No entanto, a concentração de PRPP, IMP e metabolitos da síntese de purinas aumentam quando a IMPDH é inibida (Gooding et al., 2015). Quando a enzima IMPDH esta inativada, os dois tipos da enzima IMPDH formam a organela denominada citofidia, esta é requerida para a sintetize de NTs guanínicos, utilizados na manutenção da proliferação celular (Keppek et al., 2018).

Em relação a proliferação celular, foi demonstrado *in vitro* que a junção entre a sangliferina A, receptor ciclofílina A e a IMPDH2, formam um complexo modulador do crescimento celular, atividade influenciada pela a interação deste complexo com o domínio cistationa-β-sintase (CBS) da IMPDH2, revelando importante atividade do CBS no estimulo da proliferação celular (Pua et al., 2017).

A IMPDH como mencionado acima é essencial para a proliferação celular. No entanto, evidências crescentes destacam sua atividade imunossupressora e seu potencial terapêutico, enfatizando que substancias inibidoras da IMPDH2 são moléculas alvos para pesquisas antivirais e antitumorais (Braun-Sand e Peetz, 2010). O desenvolvimento das pesquisas com a enzimas IMPDH2 resultaram na produção de diversos medicamentos contra tumores, vírus, parasitas, bactérias e atividades imune supressivas, incluindo o ácido micofenólico, mizoribina e ribavirina (Buey et al., 2015).

1.4.2. Complexo de redução NAD+ e NADH (NAD(H)

O complexo de redução formado entre NAD+ e NADH (NAD(H)) é indispensável para inúmeras reações bioquímicas dependentes de troca de elétrons, essencialmente para as reações redoxes, nas quais as atividades das enzimas oxidoreductases são mediadas por reações em que ocorrem transferência de hidreto. O NAD é um cofator essencial para diversas reações, realizando transferência de elétrons entre NAD+ e NADH. Além de ser utilizado como substrato na produção da poli (ADP-ribose), polimerase (PARP), sirtuína, e hidrolases de glicinas, como a CD38 e CD157 (Fang et al., 2017).

O NAD+ é a forma oxidada do NAD, este atua como aceptor de elétrons, enquanto, o NADH representa a forma reduzida de NAD, tem como principal função doar elétrons (Katsyuba et al., 2020; Yaku et al., 2019).

Foi relatado por Katsyuba et al. (2020) que o NAD+ é utilizado em reações relacionadas a processo de catabolismo, reações que demandam alta quantidade de energia para serem executadas. Destacaram também, que o NAD(H) é utilizado como cofator de diversas enzimas envolvidas nos seguintes metabolismos:

1 – Glicólise.
2 – Descarboxilação oxidativa do piruvato em acetil- CoA.
3 – Oxidação de ácidos graxos e fosforilação oxidativa (Yaku et al., 2019).
4 – Ciclo do ácido tricarboxílico.
5 – Ciclo de Cori (Lactato dehidrogenase).
6 – Síntese de ácidos graxos insaturados (Enzimas desaturases).

Evidências demonstram que a redução da concentração do NAD+ no metabolismo, tem relação com o envelhecimento e doenças que acometem frequentemente pessoas de idade mais avançada, à exemplo das doenças neurodegenerativas, diabetes e câncer (Katsyuba et al., 2017; Yoshino et al., 2018), destacando uma nova linha de pesquisa em relação as funções metabólicas do NAD+.

Diferenças estruturais entre o NAD+ e o fosfato de dinucleotídeo de nicotinamida e adenina (NADP+) são ocasionadas pelas ações de diferentes enzimas durante o processo de síntese, promovendo funções distintas para o NAD+ e o NADP+. O NAD+ atua principalmente em reações catabólicas. Enquanto, o NADP+ e sua forma reduzida (NADPH), atuam principalmente em reações anabólicas e na defesa celular contra o estresse oxidativo (Katsyuba et al., 2020; Yaku et al., 2019).

O NADP+ limita a velocidade da via metabólica pentoses-fosfato, em que quantidade significativa de NADPH é produzida. O NADPH também é produzido a partir da ligação entre enzimas e o NADP+. O NADP+ é precursor na síntese do fosfato e do Dinucleotídeo de Adenina do Ácido Nicotínico (NAADP), mensageiro secundário responsável por mobilizar Ca^{2+} (Clapper et al., 1987).

Na defesa do estresse oxidativo, a glutationa peroxidase depende do NADPH para realizar suas atividades, assim como as respostas imunes são mediadas pelas enzimas NADP oxidase, produtora de radicais livres utilizados no combate à agentes patógenos (Ogawa et al., 2008).

A conversão da 5'-IMP em ATP ou GTP, foi demonstrada em estudos *in vitro* (Faber et al.,1965; Jarvis et al.,1980; Kamatani et al., 2017; Kamatani et al., 2019). Revelando aumento na disponibilidade de ATP. A maior concentração de ATP altera a relação ATP/ADP, bem como as concentrações de NAD, NAD+, NADP e NADP+.

O aumento na concentração de ATP e dos NTs supracitados, modula diversos metabolismo, à exemplo da glicólise, processo em que estimula redução na utilização do lactato como substrato na via da gliconeogênese (Marchand et al., 1980; Neogi et al., 2014). Deste modo, a suplementação dietética de 5'-IMP pode ser uma alternativa para maximizar a eficiência do processo de glicólise, assim como de outros processos que ocorrem durante a ativação da defesa contra o estresse oxidativo, patógenos e antígenos, promovendo benefícios a saúde dos suínos, que podem ser refletidos em aumento do desempenho produtivo de suínos em terminação.

1.5. Suplementação dietética de blends de NTs ou 5'-IMP na dieta de suínos.

Evidências crescentes mostram que a suplementação de NTs na dieta de leitões promovem benefícios ao desempenho produtivo (Jang et al., 2019), utilização dos nutrientes da dieta (Waititu et al., 2016), saúde intestinal (Waititu et al., 2017, sistema imune e antioxidantes de leitões recém desmamados (Weaver e Kim, 2014).

A proliferação de enterócitos depende da concentração de NTs disponível no metabolismo, pois biossintetizam NTs na *via de novo* em quantidades insignificantes. Situações de alto nível de estresse, à exemplo do período de desmame, reduz drasticamente a concentração de NTs no metabolismo, prejudicando a proliferação de diversas células, assim como suas funções (Sauer et al., 2011).

Os autores Jang e Kim. (2019) avaliaram a suplementação de NTs sobre a saúde intestinal e o desempenho produtivo de leitões desmamados, foram utilizados 50 leitões recém-desmamados (19 dias de vida), designados a 5 tratamentos (0, 50, 150, 250 e 500 mg/kg *blend* de NTs) por 21 dias (fase 1 = 1 a 11 dias e fase 2 = 12 a 21 dias). A suplementação de NTs aumentou o consumo médio de ração diário (CDR) na fase 1, nos níveis de 50 e 150 mg/kg do *blend* de ração os leitões apresentaram maior (GPD) durante a fase 1.

Os mesmos autores descreveram que a relação entre tamanho de *villus* e profundidade de cripta apresentou uma resposta quadrática, em que o nível estimado de 247 mg/kg de blend de NTs proporcionou a melhor relação entre *villus* e cripta. A proliferação celular no jejuno foi reduzida linearmente, assim como a concentração de IL-6 também reduziu conforme houve aumento da adição do *blend* nas dietas dos leitões. A suplementação com o *blend* de NTs influenciou de forma quadrática a concentração de MDA, apresentando que o nível estimado foi de 231 mg/ kg de *blend* de NTs promoveu a menor concentração de MDA. Ao suplementar o blend de nucleotídeo ao nível de 50 a 250 mg/kg houve um aumento na digestibilidade ileal da energia e de outros compostos (Jang e Kim, 2019).

Os autores concluíram que a suplementação de 50 a 250 mg/kg do blend de NTs demonstrou ser benéfica aos leitões recém desmamados, em função de ter promovido aumento no desempenho produtivo, o qual pode ter ocorrido em função da menor inflamação intestinal e estresse oxidativo, além da melhora na estrutura dos *villus* intestinais e na digestibilidade da energia (Jang e Kim, 2019).

Na mesma linha de pesquisa, os autores Weaver e Kim. (2014) avaliaram os efeitos da suplementação de um blend de NTs enriquecido com a 5'-IMP sobre o desempenho produtivo e a saúde dos leitões na fase de creche. Em função disto, foram utilizados 120 leitões com peso inicial de 7,27 ± 0,07 kg PV até atingirem 22,1 ± 0,3 kg PV, os leitões na fase 1 (do desmame até 7 dias após o desmame) tiveram a dieta basal formulada para esta fase suplementada com 0,0, 0,2, 0,5 e 1,0 g/kg . E a fase 2, compreendeu do 7º dia após o desmame até ao 35º dia após o desmame, a dieta basal foi formulada para atender as exigências desta fase e foi suplementada com os mesmos níveis de blend de NTs utilizados na fase 1.

Os autores Weaver e Kim. (2014) observaram que durante a fase 1 o ganho de peso diário (GPD), o CDR e a conversão alimentar (CA) aumentaram conforme os níveis do blend de NTs também aumentaram na dieta, o mesmo ocorreu para o CDR na fase 2 e para o GPD e CDR em todo o período de experimento avaliado. Em relação ao sistema imune, na fase 2 foi observado que a suplementação de NTs influenciou de modo quadrático a concentração de IgA, mostrando menores concentrações com 0,2 e 0,5 g/kg do blend de NTs. A concentração de IgM também foi influenciada, entretanto de modo cúbico, apresentado menor concentração quando os leitões foram suplementados com o blend de NTs ao nível de 0,5 g/kg. Os autores concluíram que a suplementação dietética do blend de NTs enriquecido com a 5'-IMP pode aumentar o desempenho produtivo e reduzir o estresse oxidativo no período pós desmame.

Em estudo conduzido por Waititu et al. (2017), foi avaliada a suplementação de um extrato de levedura rico em NTs (NRYE) sobre o desempenho produtivo, estrutura do intestino, imunidade e microflora de leitões submetidos as salas de creche com diferentes condições de higienização (higienizada e não higienizada). Foram utilizados 84 leitões desmamados com 21 dias de vida, depois de desmamados alimentados por 14 dias com a dieta basal ou com a suplementação de 0,1 % de NRYE. Os autores concluíram que a suplementação de 0,1 % de NRYE para os leitões alojados na sala sem higienização, melhorou a resposta imune ileal, elevando a concentração de citocinas e a proliferação de bactérias intestinais benéficas e suprimiu a proliferação de bactérias nocivas nesta condição.

Os autores Jiao e Kim. (2018) avaliaram os efeitos da suplementação de NTs obre o desempenho produtivo, a digestibilidade dos nutrientes e o perfil imunológico do sangue de suínos em crescimentos submetidos ao estresse ocasionado pela vacinação contra a

febre aftosa. Utilizaram 120 suínos em crescimento com peso inicial de 25,76 ± 1,83 kg PV, divididos em três tratamentos (1- Dieta basal, 2 – Dieta basal + 0,5 % de nucleotídeo, 3 – Dieta basal + 0,100 % de nucleotídeo). Relataram redução linear na CA e na digestibilidade aparente total da matéria seca e do nitrogênio conforme a inclusão de NTs aumentou na dieta basal. Após a vacinação contra a febre aftosa, os suínos em crescimento suplementados com maiores níveis de NTs apresentaram redução nos níveis de cortisol e epinefrina. Os autores concluíram que a suplementação de NTs para suínos em crescimento vacinados contra a febre aftosa promoveu aumento no desempenho produtivo e na defesa do sistema imune, além de reduzir o estresse ocasionado pela vacinação.

A literatura sobre a suplementação de NTs ou 5'-IMP para suínos em terminação é escassa, mas assim como em leitões recém desmamados e em suínos em crescimento, espera-se que a suplementação dietética de 5'-IMP promova benefícios ao desempenho produtivo, defesa do sistema imune e antioxidante, saúde intestinal e eficiência energética da dieta.

1.6. Ações da 5'-IMP no metabolismo energético e muscular dos suínos

Em estudos conduzidos por Watts (1979) e Young et al. (1985), foi demonstrado que a 5'-IMP é a principal candidata a disponibilizar ATP e GTP para eritrócitos de suínos e outros metabolismos. Além disso, disponibiliza ao metabolismo quantidade superior de ATP em relação a adenosina.

No músculo, os processos bioquímicos dependem da biogênese mitocondrial, ou seja, o consumo de O_2, a eliminação de hidrogênio do citosol e a síntese de ATP mitocondrial, devem ocorrer sem falhas ou variações, reduzindo as disfunções mitocondriais e os prejuízos ao metabolismo em condições aeróbicas (Scheffler and Gerrad, 2007). Estes processos são regulados de acordo com a concentração de ATP, a relação entre ATP/ADP e a concentração de fosfocreatina. A fosfocreatina pode ser rapidamente utilizada pela enzima creatina kinase para refosforilar o ADP em ATP (Scheffler and Gerrad, 2007).

O ATP tem como principal função armazenar e distribuir energia para as células desenvolverem suas atividades, garantindo a vida praticamente a todos os organismos celulares (Di Virgilio, 1998). A hidrólise do ATP libera energia para ser utilizada na

contração muscular, sinalização celular, transporte ativo de íons e a biossíntese de macromoléculas. Estas atividades são desenvolvidas com alta eficiência em função de o metabolismo energético manter estável a relação entre a síntese e degradação de ATP (Matarneh et al., 2017).

Mesmo com a alta demanda metabólica de ATP, o músculo esquelético *in vivo* armazena baixa concentração de ATP, aproximadamente 5 a 8 µmol/g de tecido muscular, concentração semelhante a apresentada logo após a morte dos suínos (Bendall et al., 1963). Para compensar este déficit, a síntese de ATP no músculo acontece continuamente, em função disto carboidratos e lipídeos são catabolizados, liberando energia empregada na manutenção da síntese de ATP (Matarneh et al., 2017).

Os primeiros processos anaeróbicos ocorrem após a sangria dos suínos, quando as concentrações de oxigênio no músculo são esgotadas. Posteriormente, o glicogênio é convertido em ácido láctico até o pH do músculo reduzir a valores próximos de 5,3 a 5,6. Condições em que as atividades enzimáticas são inativadas (Matarneh et al., 2017).

Conforme o processo de glicólise segue, é iniciado o processo de *rigor mortis*, no qual, o músculo começa a ser transformado em carne "*rigor mortis*". Após a sangria dos suínos, a concentração de oxigênio disponível para manter a homeostase muscular reduz drasticamente até ser esgotado, mas a síntese e a utilização de ATP não são interrompidas instantaneamente. A fim de manter a síntese de ATP, o glicogênio muscular e composto fosfatado são clivados para liberarem energia para ser empregada na manutenção da homeostase da concentração de ATP muscular (Matarneh et al., 2017).

O músculo dispõe de três principais processos para auxiliar a manutenção da homeostase de ATP, sendo eles:

1º - Sistema de fosfagênios.
2º - Glicólise.
3º - Fosforilação oxidativa.

No início do processo de *rigor mortis*, compostos fosfatados de alta energia, como o fosfato de creatina (PCr) são degradados para fornecer energia empregada na síntese de ATP. Neste processo, a enzima creatina quinase (CK) é responsável por catalisar a transferência de um fosfato inorgânico (Pi) da PCr para a ADP, formando ATP e creatina (Matarneh et al., 2017).

A degradação de compostos fosfatados mantém a homeostase do ATP durante o início do período *post-mortem*, mas somente por um curto período de tempo. Com o fim da disponibilidade de CK, a hidrólise de ATP aumenta, produzindo ADP em excesso. A maior concentração de ADP ativa a enzima adenilato quinase (AK), responsável por converter duas moléculas de ADP em uma molécula de ATP e AMP, reduzindo a intensa redução na concentração de ATP (Matarneh et al., 2017).

Na sequência, O AMP é deaminado pela enzima adenosina monofosfato desaminase (AMPD), originando a inosina monofosfato (IMP), a qual então é armazenada no músculo (Matarneh et al., 2017).

Em pesquisas prévias, foi observado que o aumento nas concentrações de AMP e da CK no músculo no período *antemortem* está relacionado com a maior taxa de glicólise, maiores valores de pH mensurado 24 horas após ao abate e melhorias na qualidade de carne (England et al., 2015; Sheffler et al., 2013).

A partir de baixas concentrações de CK, o metabolismo utiliza principalmente a via de gliconeogênese e glicólise para sintetizar ATP. O glicogênio é utilizado como substrato para formar a glicose-6 fosfato, ADP e H+. Posteriormente à 10 reações, a glicose-6 fosfato é convertida em piruvato, na última etapa são geradas 2 moléculas de ATP a cada molécula de glicose degradada (Bender e Mayes, 2015).

Avaliando *in vitro* qual proteína mitocondrial é responsável por aumentar o fluxo glicolítico, os autores Matarneh et al. (2018) reportaram que a enzima mitocondrial F1-ATPase promove a hidrólise do ATP, degradação do glicogênio, acumulação de lactato, e redução do pH. Foi concluído que a F1-ATPase é capaz de estimular a via glicolítica, promovendo maior taxa de hidrólise do ATP em menores valores de pH.

A suplementação dietética de inosina-5’-monofosfato (5’-IMP) provavelmente aumente a concentração da mesma no metabolismo. Depois de absorvida, a 5’-IMP é convertida a AMP e GMP, e posteriormente, em ATP e GTP que podem ser depositados em maior concentração no músculo. *In vivo* a maior concentração de ATP no músculo será hidrolisado a IMP, aumentando as concentrações de 5’-IMP no músculo. Deste modo, no período *post-mortem* fontes exógenas de 5’-IMP podem aumentar a concentração de ATP, prolongando o processo de *rigor mortis* e aumentar a concentração de 5’-IMP na carne, fato relacionado com aumento na qualidade da carne (Scheffler et al., 2013).

Com a evolução do processo de *rigor mortis*, os substratos disponíveis para a síntese de ATP se esgotam e o músculo passa a degradar as moléculas de ATP, para manter a homeostase muscular, até esgotar a concentração de ATP. Sem a presença de ATP, as miosinas se conectam as actinas, formando o complexo actina-miosina, resultando na perda da capacidade de contração e relaxamento, completando a transformação do músculo em carne (Lópes-Bote, 2017).

A formação do complexo actina-miosina ocorre em duas etapas, na primeira etapa é lenta a velocidade das reações de ligações entre actina e miosina, enquanto, na segunda etapa as reações entre actina e miosina ocorrem rapidamente. O tempo para que a segunda etapa seja finalizada depende da concentração de ATP no músculo (Matarneh et al., 2017).

O sistema de contração muscular é estimulado pelos íons de Ca^{2+}, mas quando ainda há disponibilidade de ATP, o retículo sarcoplasmático exporta de forma ativa o Ca^{2+} para fora do sarcoplasma, mantendo temporariamente a capacidade de relaxamento do músculo, até novos íons entrarem no sarcoplasma e a disponibilidade de ATP ser esgotada (Matarneh et al., 2017).

A hidrólise do ATP em 5'-IMP é um processo irreversível, pois a 5'-IMP é armazenada na carne. Evidências crescentes demonstram que a concentração da 5'-IMP na carne tem relação com o aumento das qualidades sensoriais e químicas da carne (Flores et al., 1999; Jilan et al., 2004; Huijuan et al., 2018), aumentando o sabor "UMAMI" da carne. Durante o cozimento da carne, a 5'-IMP e outros compostos são degradados e liberam substâncias químicas responsáveis por aumentar o sabor e o aroma da carne (Aaslyng e Meinert, 2017). Foi relatado que a 5'-IMP é um realçador de sabor 50 vezes mais potente do que o glutamato de sódio (Yan et al., 2018).

Outra importante ação fisiológica da 5'-IMP na carne de suínos é sua capacidade de dissociar o complexo actina-miosina em concentrações salinas fisiológicas (Okitani et al., 2008). Na mesma linha de pesquisa, os autores Nakamura et al. (2012) avaliando a capacidade da 5'-IMP e do pirofosfato (KPP) em aumentar a extração de miosina e actina, utilizando homogeonatos de carne suínas com nove soluções com volumes de 0,3, 0,4 e 0,5 $_{M}$ NaCl contendo 0 a 36 mM 5'-IMP ou 0 a 9 mM KPP, reportaram que a 5'-IMP aumentou a extração de miosina e actina a partir do complexo actina-miosina presente na carne suína.

Do mesmo modo, Nakamura et al. (2013) avaliaram o mecanismo da 5'-IMP e do pirofosfato para aumentar a extração da actina e miosina do complexo actina-miosina presentes na carne suína. Foram utilizados homogeonatos de carne suína avaliados em nove soluções com volumes de 0,3, 0,4 e 0,5 $_{M}$ NaCl contendo 0 a 36 mM 5'-IMP ou 0 a 9 mM KPP, o homogeonato foi incubado a 4º C por 0 ou 12 horas. Foi reportado, que a 5'-IMP aumentou a extração tanto de miosina, quanto de actina em ambas as soluções de NaCl, conforme houve aumento no tempo de incubação. Destacaram também que a ação da 5'-IMP sobre o complexo actina-miosina dependeu do tempo e da concentração de NaCl. Além disso, a adição da 5'-IMP no lugar do pirofosfato, beneficia a qualidade do produto, pois a 5'-IMP diferentemente do pirofosfato, não causa odor de ranço conforme o tempo de armazenamento do produto aumenta (Nakamura et al., 2013).

A ação da 5'-IMP sobre as miofibrilas no período *post-mortem,* foi avaliada pelos autores Matsuishi et al. (2016), para isto foi isolado o complexo actina-miosina, e posteriormente, solubilizada em meio contento IMP e concentração de KCL variando as concentrações de 0,19 a 0,20 mol/L, em função de as miofibrilas não desassociarem em concentração de 0,2 mol/L de KCL. No entanto, foi observado a capacidade da 5'-IMP em solubilizar a actina e miosina das miofibrilas, mesmo em concentração de 0,2 mol/L. Os resultados demonstram que a 5'-IMP reagiu com as miofibrilas na presença de substâncias sarcoplasmáticas presentes no músculo, resultando no rompimento das ligações entre os filamentos finos e grossos do músculo. Sugerindo que a 5'-IMP pode reagir com miofibrilas tanto *in vivo* quanto *in vitro.* Concluindo, a 5'-IMP é um agente candidato a beneficiar o processo de *rigor mortis*, pois possui habilidade de romper as ligações do complexo actina-miosina.

Em gel produzido a partir da carne suína e induzido ao calor, utilizado na produção de salsicha, foi avaliada a capacidade de retenção de água deste gel na presença de 5'-IMP e de pirofosfato. Em função disto, foram utilizadas nove soluções com volumes de 0,3, 0,4 e 0,5 $_{M}$ NaCl contendo 0 a 36 mM 5'-IMP ou 0 a 9 mM KPP. Os pesquisadores Nakamura et al. (2014) observaram que a 5'-IMP na concentração de 36 mmol / L aumentou a capacidade de retenção de água para o mesmo nível apresentado pelo KPP. Concluindo, a 5'-IMP possivelmente substitui o pirofosfato na produção de salsicha, promovendo uma salsicha de melhor qualidade.

1.7. Ações dos NTs sobre o trato gastrointestinal de suínos

O trato gastrointestinal (TGI) é o local de absorção de digestão dos nutrientes, tendo como principais funções a manutenção da concentração de fluídos para garantir uma viscosidade adequada para o conteúdo luminal. A secreção de enzimas digestivas é outro importante processo que ocorre no TGI, à exemplo das liberações de mucinas e imunoglobulinas para garantir a funcionalidade de estruturas utilizadas como barreiras contra determinados patógenos e antígenos (Wijtten et al., 2011).

O TGI sempre está sob recorrentes processos de inflamações devido ao processo de absorção e digestão dos nutrientes, em função disto as células de mucosa do intestino e outras, demandam de alta taxa de proliferação e crescimento para garantir a menor incidência de disfunções no TGI (Uauy et al., 1994).

A proliferação celular depende da biossíntese de NTs, em função das células captarem NTs do meio extracelular para o meio intracelular em quantidade insignificante. Em contrapartida, durante a proliferação celular a exigência de NTs aumenta, devido ao aumento na síntese, duplicação do genoma para a síntese de DNA e manutenção da transcrição. Os NTs são essenciais para todos os processos energéticos celulares, uma vez que os processos responsáveis pela produção de ribose, pirimidinas e purinas são dependentes de substratos e energia oriunda da via pentose-fosfato, ciclo de uma unidade de carbono e ciclo do ácido cítrico (Zhu e Thompson, 2019).

Desta forma, o fornecimento de fontes exógenas de NTs nas dietas de suínos pode ser uma alternativa para aumentar a proliferação e o crescimento das células de mucosas presentes no TGI. Principalmente, porque o TGI não é capaz de biossintetisar NTs na via *de novo* (Carver, 1994).

Avaliando em leitões os mecanismos moleculares regulados por efeitos dos NTs fornecidos nas dietas dos leitões durante o período de desmame, os autores Lee e Kim. (2018) encontraram 748 genes diferencialmente expressos influenciados pelos NTs, dentro destes, 559 genes foram estimulados e 189 genes foram regulados negativamente.

Destacaram também, que a inclusão de NTs na dieta induziu a expressão do fator de transcrição EPS (SPDEF), responsável por regular a expressão da proteína denominada fator três do trevo (TFF3), resultando na modulação mediada pelo TFF3 sobre a cicatrização do tecido intestinal, assim como na função de barreira do intestino contra patógenos, a qual é mediada via sinalizações realizadas por PI3K/Akt, ERK1/2, p38 e JAK/STAT (Lee e Kim, 2018).

Além disso, a suplementação de 0,1 % de NTs nas dietas dos leitões proporcionou maior tamanho de villus no duodeno, jejuno e íleo em relação aqueles que receberam a dieta controle no 14º dia após o desmame, demonstrando benefícios sobre a morfologia intestinal dos leitões. Os autores Lee e Kim. (2018) concluíram que a suplementação de NTs pode ser utilizada como um aditivo na dieta de leitões.

Efeitos benéficos sobre a estrutura do TGI também foram demonstrados por meio do aumento da altura de *villus* e da redução da profundidade de cripta (Jang e Kim, 2019; Lee e Kim 2018; Weaver e Kim, 2014), assim como pela maior incidência de bactérias benéficas na microbiota intestinal dos leitões após o desmame (Jang e Kim, 2019; Lee e Kim, 2018; Waititu et al., 2017), demonstrando benefícios na absorção e digestibilidade dos nutrientes, assim como na eficiência da defesa do sistema antioxidante e imunológico do GTI, fatores que maximizam o desempenho produtivo de suínos.

1.8. Atividades de NTs no metabolismo antioxidante de suínos

As substâncias reativas ao oxigênio (ROS) e as reativas ao nitrogênio (RNS) são originadas durante o processo de respiração mitocondrial, à exemplo do processo de fosforilação oxidativa (Rigoulet et al., 2011). As ROS e RNS causam danos nas células e nos tecidos, prejudicando as importantes atividades metabólicas executadas por estes organismos durante a presença de determinadas patologias (Salobir et al., 2005). Os NTs e NSs desenvolvem importantes ações para prevenir danos no DNA induzidos por uma elevada carga oxidativa. Estes podem desenvolver mecanismos de reparação de moléculas de DNA fragmentadas (Salobir et al., 2005).

Em pesquisa conduzida por Salobir et al., 2005, foram avaliados os efeitos da suplementação de NTs sobre o estresse oxidativo induzido por uma alta inclusão de PUFAs nas dietas de suínos em crescimento. Foram avaliados a intensidade dos danos no núcleo do DNA, a concentração de MDA excretada na urina, concentração de eritrócitos presentes nas glutationas peroxidases e a capacidade antioxidante total do plasma de suínos em crescimento. Foram utilizados 24 suínos em crescimento divididos em 3 tratamentos (1 - Controle; 2 - Óleo de linhaça; 3 – Óleo de linhaça + NTs). Foi reportado que a suplementação de NTs reduziu a intensidade de danos no DNA em relação ao apresentado pelo grupo suplementado com o óleo de linhaça. Concluindo, a suplementação de NTs foi efetiva em eliminar os efeitos genotóxicos da alta ingestão de

PUFAs sobre os linfócitos presentes no sangue, demonstrando um efeito imunonutritivo dos NTs.

Diante disto, os autores Jang e Kim. (2019) avaliaram a suplementação de NTs sobre a saúde intestinal e o desempenho produtivo de leitões desmamados, 50 leitões recém-desmamados (19 dias de vida) que foram designados a 5 tratamentos (0, 50, 150, 250 e 500 mg/kg blend de NTs) por 21 dias (fase 1 = 1 a 11 dias e fase 2 = 12 a 21 dias). Foi relatado que a concentração de MDA no plasma apresentou uma tendência de resposta quadrática conforme houve aumento na suplementação de NTs, em que no nível estimado de 231mg/kg de NTs proporcionou a menor concentração de MDA (12,9 µM/mL) no plasma sanguíneo dos leitões. Foi concluído, que a suplementação de NTs variando de 50 a 250 mg/kg possivelmente reduziu o estresse oxidativo dos leitões recém desmamados.

Avaliando os efeitos da suplementação de um blend de NTs enriquecido com 5'-IMP sobre o desempenho produtivo e a saúde dos leitões na fase de creche. Foram utilizados 120 leitões com peso inicial de 7,27 ± 0,07 kg PV até atingirem 22,1 ± 0,3 kg PV, divididos em duas fases de produção (fase 1 e fase 2) e suplementados com 0,0, 0,2, 0,5 e 1,0 g/kg de NTs com 5'-IMP. Os autores Weaver e Kim. (2014) observaram houve tendência (P=0,093) em redução linear da citocina fator de necrose tumoral-α e uma tendência (P=0,064) de resposta quadrática para o 8-Oxo-2'-desoxiganosina (marcador de danos no DNA), em que a menor concentração foi identificada com a adição de 0,5 g/kg de NTs enriquecido com 5'-IMP, deste modo a adição de NTs enriquecidos com a 5'-IMP reduziu as respostas imune e o estresse oxidativo dos leitões durante o período de desmame.

Em pesquisa prévia, conduzida por Jiao e Kim. (2019), foram utilizadas 45 matrizes suínas e seus respectivos leitões para avaliar a eficiência da suplementação de NTs sobre o desempenho reprodutivo, desempenho produtivo, microflora fecal e perfil sanguíneo das matrizes e dos leitões. 15 matrizes e 15 leitões foram distribuídos em um dos três tratamentos: 1- Controle: Dieta basal; 2- Controle + 0,5 % NTs; 3- Controle + 1,0 % NTs. Sobre as análises antioxidantes, foi observado redução linear da concentração de epinefrina, norapinefrina e cortisol no plasma das matrizes no dia do desmame dos leitões, enquanto a concentração da enzima superóxido dismutase aumentou linearmente. Também foi identificado aumento linear no peso dos leitões e do GPD conforme houve aumento na adição dos níveis de NTs. Também foi identificado aumento linear no número total de leitões nascidos, nascidos vivos e na sobrevivência dos leitões. Concluindo, a

suplementação de NTs nas dietas das matrizes suínas aumentou o desempenho reprodutivo e produtivo das matrizes e dos leitões.

Evidências crescentes revelam importante função das 5'-IMP sobre reparação de danos no DNA, a qual vem sendo avaliada no processo de deaminação da adenosina em inosina, processo dependente das ações das enzimas da família adenosina desaminase sobre o RNA (Janostiak e Wajapeyee, 2019).

1.9. NTs e suas relações com o sistema imune

A ativação do sistema imune demanda de alta quantidade de energia para estimular a produção, proliferação e crescimento de diversos agentes celulares, responsáveis por garantir uma defesa eficiente contra patógenos e antígenos, os quais causam danos ao metabolismo, resultando em queda no desempenho produtivo de suínos.

Semelhante ao GTI, o sistema imune não é capaz de biossintetizar NTs na via de *novo* em quantidades significantes, quando estimulado a exigência de NTs aumenta para suprir a demanda gasta na síntese de diversos agentes imunes, como as proteínas de fase aguda, células T, linfócitos e imunoglobulinas (Gil, 2002). Diante disso, o efeito da suplementação de NTs nas dietas vem sendo pesquisadas em dietas de suínos, bem como na alimentação humana.

A eficiência das respostas imune adaptativa depende do quão rápido as células T específicas aos antígenos modificam do seu estado inativo para o estado ativo (proliferativo), assim como do período em que estas células são mantidas ativadas durante a presença dos patógenos (Wang et al., 2020).

Avaliando *in vitro* as modificações bioenergéticas, biossintéticas e nos processos redox, causadas pelas estimulações de antígenos sobre a síntese e proliferação das células T de humanos e camundongos, os autores Wang et al. (2020) reportaram que as células T na ausência de glicose, utilizam a inosina como substrato para suportar a proliferação, crescimento e funções essenciais das células T, demonstrando que a catálise da ribose presente na estrutura da inosina disponibiliza substrato para a síntese de ATP, bem como para as vias de glicólise, pentose-fosfato e ciclo do ácido cítrico. Além disso, relataram que dentre os NTs, somente a inosina apresentou esta habilidade de doar carbonos e energia para as células T, excluindo assim a possibilidade da adenosina e guanosina efetuarem atividade semelhante. Concluíram que a inosina pode substituir a glicose para promover *in vitro* o crescimento e as funções das células T.

Nesta mesma linha de pesquisa, foi conduzida uma pesquisa *in vitro* para avaliar os efeitos de NTs purínicos e pirimídicos sobre a ativação de células T de camundongos, em que os autores Shinohara e Tsukimoto. (2018) reportaram que NTs ou NSs como a guanina e a inosina podem inibir as citocinas liberadas pelas células T, sendo assim considerados como possíveis candidatos como um agente suplementar a ser utilizado no tratamento de doenças, em que a principal defesa do metabolismo contra esta doença, depende das atividades das células T.

Em estudo *in vitro* conduzido por Chen et al. (2017), foram utilizadas células de humanos como os melanomas B16F10 e os sinovitos tipo B na presença de células mortas ou morrendo para avaliar a estimulação da proliferação destas células e qual substância promove a proliferação. Os autores reportaram que as células mortas ou das que estão morrendo liberam inosina para o meio extracelular, a qual desenvolve ações sobre os receptores de adenosina para estimular a proliferação de todas as células avaliadas. Concluindo, que a inosina liberada durante o processo de apoptose celular estimula a proliferação de células remanescentes após processos de radioterapia.

Evidências crescentes revelam que a inosina desenvolve ações imunomoduladoras por meio das atividades de sua enzima IMPDH II, está é identificada como responsável por reconhecer inúmeras substâncias utilizadas em medicamentos para ativar a defesa do sistema imune (Buey et al., 2017). Sobre a IMPDH, há evidências de que suas funções como fatores de transcrição possuam relações com uma complexa rede de atividades executadas para controlarem a expressão de inúmeros genes (Kozhevnikova et al., 2012), demonstrando ações em diversos metabolismos, à exemplo do sistema imune, redox e da síntese proteica.

Outra via metabólica da inosina que influencia o sistema imune, é a catabolização da adenosina em inosina durante o processo de transcrição, causando diversas modificações do RNA, executadas pelas ações das enzimas adenosina deaminase sobre o RNA (Mellis et al., 2017; Nakahama e Kawahara, 2020). Estas modificações são responsáveis por manter praticamente todos os processos da síntese do RNA, à exemplo da estabilidade do RNA, divisão celular, exportação do núcleo e localização, assim como na recodificação de proteínas. Além disso, o processo da deaminação da adenosina em inosina e a modulação sobre o RNA foi relacionada com a prevalência de inúmeras doenças (Gatsiou et al., 2017; Nakahama e Kawahara, 2020).

Em suínos, o fato de a inosina servir como doador de carbono para a proliferação, crescimento e funções das células T é relevante, pois após 1 mês de vida, os suínos não apresentam capacidade de biossintetizar ATP a partir da glicose, assim como há ausência de transportadores essenciais para converter a glicose em ATP, reduzindo a quantidade de energia disponível para a ativação das células T (Fernando et al., 1976).

Em pesquisa conduzida por Superchi et al. (2012), foram avaliados os efeitos da suplementação de NTs na dieta de leitões incluída a partir do nono dia de vida até o desmame (21 dias) e no período pós desmame (22 até 55 dias) sobre a composição hormonal, resposta imune e desempenho produtivo dos leitões. Em função disto, foram utilizados 108 leitões, distribuídos e submetidos às seguintes dietas: controle ou 0,1% da inclusão de NTs extraídos da levedura. Foi observado maior peso vivo aos 21 ($P<0,10$), 35 e 55 dias de vida ($P<0,05$) apresentado pelos leitões suplementados com 0,1 % de NTs.

A suplementação de NTs aumentou a subpopulação de linfócitos (CD4-CD8+) no 21º e 35º dia ($P<0,05$). Além disso, apresentou menor expressão da interleucina IL-6 e IL-β e maior expressão da interleucina IL-10 no sangue periférico dos leitões durante o desmame ($P<0,05$). Aos 28 dias de vida, os leitões suplementados com NTs apresentaram maiores concentrações dos fatores de necrose tumoral (TNF-α) e menor expressão da IL-10 em relação aos suínos do grupo controle ($P<0,05$). Adicionalmente, os NTs demonstram um efeito supressivo sobre a expressão da IL-6 e IL-10 no 35º dia de vida dos leitões, mas em contrapartida, houve aumento na expressão de IFN-ẏ, TNF-α e IL-β. Os autores Superchi et al. (2012) concluíram que a suplementação de NTs na dieta de leitões antes do desmame pode aumentar a capacidade dos leitões a lidarem com a alta carga de estresse imposta pelo processo desmame, aumentando o desempenho produtivo.

Avaliando a suplementação dietética de NTs sobre o desempenho produtivo, desenvolvimento intestinal e função imune de leitões submetidos a crescimento restrito durante a fase intra uterina (IUGR). Os autores Che et al. (2017) utilizaram 28 leitões IUGR e 28 leitões de crescimento normal durante a fase intra uterina, distribuídos na dieta controle baseada na composição do leite ou na dieta controle suplementada com NTs por um período de 21 dias. Foi reportado, que a suplementação de NTs reduziu a conversão alimentar ($P<0,05$), aumentou o comprimento dos villus no duodeno ($P<0,05$), as atividades da lactase e maltase no jejuno ($P<0,05$), a contagem periférica de leucócitos ($P<0,05$), a concentração sérica de IgA e IL-β, assim como a expressão de TLR-9, TLR-4 e TOLLIP no íleo ($P<0,05$). Também houve, aumento ($P<0,05$) na expressão das

proteínas de junção no íleo (claudina-1 e ZO-1). Concluindo, a suplementação de NTs reduziu os impactos impostos aos leitões IUGR, promovendo aumento na utilização dos nutrientes da dieta, assim como as funções intestinais e imunes dos leitões.

1.10. Ações da 5'-IMP no metabolismo refletidas em diversos parâmetros sanguíneos

O perfil metabólico do plasma sanguíneo reflete as mudanças nas atividades metabólicas e fisiológicas em função das dietas avaliadas. Os parâmetros, que refletem as alterações nas concentrações dos metabolitos presentes no plasma causada por um determinado nutriente, são úteis para auxiliar na compreensão de como o nutriente modifica as atividades fisiológicas, as metabólicas e os efeitos positivos ou negativos sobre determinados parâmetros, como por exemplo, sobre o desempenho produtivo (Archer et al., 2003; Regmi et al., 2018).

O metabolismo dos lipídeos e carboidratos corresponde ao metabolismo energético, diversas funções bioquímicas são realizadas por NT (purinas ou pirimidina) no metabolismo energético. Na síntese de novo de purinas, a 5'-IMP é a primeira purina sintetizada, sua estrutura é composta por uma molécula de hipoxantina e uma ribose (He et al., 2016). A 5'-IMP é um importante componente do metabolismo energético, pois é metabolizada tanto a AMP, quanto a GMP (Harmsen et al., 1984). Estas, quando fosforiladas, formando a ATP e a GTP respectivamente, componentes responsáveis pelas reações de transferência de energia nos processos celulares. A adenosina (ADP) pode ser metabolizada em duas vias. Na primeira será metabolizada em AMP, depois em ADP e finalmente em ATP. Na segunda via, é convertida diretamente a 5'-IMP. É conhecido que a relação entre a concentração de IMP e a de ADP, interfere no controle dos processos de glicólise e de gliconeogênese (De Pinã et a., 1989; Marchand et at., 1979; Guinzberg et al., 2006). Já se sabe que a gliconeogênese é alterada pela concentração micromolar de NTs (Lund et al., 1975).

O mecanismo de ação da ADP e da IMP no processo de gliconeogênese foi demonstrado, em pesquisa realizada por De Pinã et al. (1989), avaliando in vitro, hepatócitos isolados do fígado de ratos foram incubados em um meio, contendo determinadas concentrações de IMP e ADP e ao término da incubação as concentrações de glicose foram mensuradas. Os autores reportaram que a ADP em resposta ao hormônio glucagon reduziu o processo de gliconeogênese a partir do lactato, resultando em aumento

da concentração de lactato de ureia. Enquanto, a IMP estimulou a gliconeogênese a partir do lactato em reposta ao hormônio glucagon.

Lavoinne et al. (1987) destacaram que a inibição da glicogenólise que o ADP causa, acontece porque a ADP é metabolizada ao nucleotídeo adenina, ao invés de ir a ATP, isto reduz a relação ATP/ADP, a menor disponibilidade de ATP, limita o processo de gliconeogênese.

Em pesquisa *in vitro* desenvolvida em camundongos, os hepatócitos foram isolados e submetidos a um meio contendo diferentes concentrações de ADP e IMP. Os resultados indicaram aumento na concentração de glicose nos hepatócitos logo após a exposição ao meio com 5'-IMP (Sharma et al., 1982). O mesmo efeito foi encontrado em estudo conduzido por Guinzbertg et al. (2006), no qual, foi demonstrado que a 5'-IMP, é capaz de estimular a gliconeogênese hepática, em condições de hipóxia estimula a liberação de glicose armazenada no fígado, para ser utilizada na síntese de ATP e manter a homeostase energética do metabolismo na ausência de O_2.

Na via metabólica do nucleotídeo 5'-IMP, estão presentes diversas enzimas, essenciais para sua síntese ou a degradação. Os produtos de degradação enzimática das purinas, inclusive das 5'-IMP, são a hipoxantina, a xantina e o ácido úrico (Lima et al., 2015; Ryu et al., 2016). Estudos demonstram que as enzimas, e os metabólitos da degradação das 5'-IMP, possuem atividades bioquímicas no metabolismo lipídico (Farber et al., 1965; Ryu et al., 2016; Whitehead et a., 2004).

Em pesquisa conduzida por Ryu et al. (2016) foram avaliadas as funções metabólicas da hipoxantina na síntese do colesterol e desenvolvimento de aterosclerose. Foram utilizados camundongos geneticamente modificados para não reconhecerem a alipoprotina E (Knocknouts APOE mouse). Os camundongos foram alimentados com dieta rica em colesterol (16% de gordura e 1,25% colesterol) por 12 semanas. Os grupos experimentais foram com ou sem infusão de hipoxantina, a qual foi injetada (200 mg/kg) diariamente por 8 semanas, após sacrificados, o sangue foi colhido e incubado a 37ºC, para obtenção do soro e as células HepG2 foram isoladas e incubadas por 3 a 4 dias para crescerem, posteriormente, foram incubadas em meio de crescimento que continha variadas concentrações de hipoxantina KO (1 e 2,5 mM) ou sem a sua presença (0 %). Os autores reportaram, que a hipoxanina induziu o acúmulo de colesterol e o desenvolvimento aterosclerose no soro e nas células HepG2. A degradação da hipoxantina elevou a concentração de ROS no sangue e nas células HepG2, os autores

sugeriram que a concentração de ROS tem função no controle da síntese e deposição de colesterol no metabolismo.

O colesterol é um importante componente da estrutura das células, pode ser incorporado superfície da membrana plasmática, no citosol e ou na própria estrutura molecular das células (Yeagle, 1985). É responsável por organizar os lipídeos da membrana de lipídeos e de proteínas, a fim, de estimular processos celulares dependentes da conformação das membranas, como na sinalização e divisão celular, na translocação de lipídeos e proteínas e no processo de apoptose celular.

A deposição de colesterol nas membranas das células é influenciada pelas concentrações de HDL, LDL e VLDL presentes na membrana celular (Neufeld et al., 2014). As células do sangue possuem concentração superior de colesterol em sua membrana em relação a qualquer célula de outro tecido. Devido serem essenciais para o crescimento celular, a maior concentração no sangue possivelmente estimule a proliferação e o crescimento celular (Yeagle, 1985). A IMP exerce atividade sobre o crescimento e proliferação celular, tanto por aumentar a concentração de colesterol no metabolismo, quanto por aumentar a quantidade de substratos para a síntese de serina (Pacold et al., 2016).

Na via de recuperação de NTs, as nucleobases necessitam do carbono derivado da serina para serem sintetizados novamente em NTs. Na degradação de 5’-IMP, é formado a hipoxantina, primeiro doador de carbono para a síntese dos NTs adenilato e guanilato, ambos representam a disponibilidade de serina (Diehl et al., 2019). A serina é o maior precursor de biomoléculas e doador de carbono. E, é componente essencial para o crescimento e proliferação celular (Pacold et al., 2016). Deste modo, o fornecimento exógeno de 5’-IMP, pode alterar a disponibilidade de serina e influenciar a proliferação celular.

Avaliando as mudanças no perfil de lipoproteínas no plasma de crianças alimentadas do primeiro dia de nascimento, até completarem um mês de vida, pela mãe (n=26), por leite sintético padrão (n=35) ou por leite sintético com suplementação de NTs (n= 23). Sánchez-Pozo et al. (1986) reportaram que apesar do leite humano conter maior concentração de colesterol em sua composição do que no leite sintético suplementado com NTs, a concentração de colesterol no plasma foram semelhantes entre as duas dietas, indicando que os NTs aumentaram a concentração de colesterol no plasma, ainda foi

evidenciado redução nos valores de VLDL. Os autores relatam que possivelmente os NTs tenham influenciado a síntese de lipoproteínas no fígado.

Altos níveis plasmáticos de colesterol podem prejudicar outros metabolismos, entretanto, em pesquisa realizada por Sánchez-Pozo (1995), foi avaliada a adição de NTs em fórmula láctea infantil sobre a enzima lecitina colesterol-aciltransferase (LCAT), enzima essencial no metabolismo lipídico, mas que possui baixa atividade em recém-nascidos. Os autores destacaram que os NTs aumentaram a atividade da enzima LCAT no plasma, provavelmente, porque os NTs estimularam a síntese no intestino da apoA, posteriormente carreada ao sangue onde pode ter aumentado a atividade da LCAT. Indicando que as crianças alimentadas com NTs foram mais tolerantes, a maiores concentrações lipídicas (colesterol) no plasma.

O colesterol não é o único componente lipídico influenciado pelas 5'-IMP. Estudos demonstram que a deposição de triglicerídeos é influenciada pela ação da insulina e do glucagon sobre a enzima IMPDH, o hormônio insulina induz a translocação e atividade da IMPDH para tecidos lipídicos, onde está é capaz de reduzir a deposição lipídica. Parte desta ação ocorre devido a ADP e a 5'-IMP aumentarem a síntese de ATP, evitando assim a redução da síntese proteica e do uso de ATP na síntese lipídica (Farber et al., 1965).

A concentração de triglicerídeos no plasma de peixes foi mensurada em pesquisa conduzida por Hossain et al. (2016), em que avaliaram os efeitos da suplementação de diferentes níveis (0,2, 0,4, 0,6 e 0,8 %) de inosina (INO) e de inosina monofosfato (IMP) sobre o crescimento, a resposta imune, resistência ao estresse e a morfologia intestinal em peixes (*pagrus major*). Os resultados demonstram que a adição de 0,8 % de INO e 0,2, 0,6 e 0,8 % de IMP reduziram as concentrações de triglicerídeos no plasma em comparação com a dieta controle. O efeito da redução de triglicerídeos no plasma proporcionado pela suplementação de 5'-IMP na dieta de peixes, também foi relatado em pesquisa de Hossain et al. (2017), na qual a maior redução de triglicerídeos no plasma foi proporcionada pela adição de 0,3 e 0,6 % de INO em comparação com a dieta controle.

Parte do excesso de triglicerídeos é depositada em órgãos essenciais e no músculo. O músculo será transformado em carne, aumentando os triglicerídeos na carne e quando consumida, pode aumentar as concentrações de triglicerídeos em humanos. Destacamos, que as lipoproteicas (HDL, LDL e VLDL) carrearam para o sangue tanto o colesterol quanto os triglicerídeos, entretanto, as concentrações de colesterol aumentaram com a suplementação de 5'-IMP e a de triglicerídeos, a de LDL, HDL e VLDL reduziram,

indicando que a redução das lipoproteínas no plasma ocorreu devido a menor concentração de triglicerídeos. O alto nível de triglicerídeos e de LDL estão relacionados com a incidência de doenças cárdicas e outros distúrbios metabólicos acometidos em humanos (Helgadottir et al., 2016).

A concentração de proteínas totais no plasma indica a taxa de deposição proteica no corpo. Ou seja, um aumento na concentração plasmática de proteínas totais significa que a taxa de deposição proteica também aumentou (Zeng et al., 2013). Indica também o status do sistema imune, em que a maior concentração de PT no plasma reflete em uma defesa mais robusta do sistema imune contra agentes patógenos (Kumar et al., 2005).

A concentração de ureia no plasma está relacionada com a deposição proteica no corpo e com a eficiência na utilização de nutrientes proteicos, sendo que baixas concentrações de ureia no plasma indicam maior deposição proteica e maior eficiência na absorção e o uso dos nutrientes proteicos (Zeng et al., 2013).

Com relação a biossíntese de ureia, foi realizado um estudo por Guinzberg et al. (1987) para avaliar a influência da ADP e da INO sobre a biossíntese da ureia, foram utilizadas células do fígados incubadas em um meio contendo baixa concentração de ADP ou INO para avaliar suas ações como hormônio em baixas concentrações. Os resultados mostraram que a ativação do processo de ureogênese é alterada pelas concentrações de ADP ou IMP. Sugerindo que as ações fisiológicas da ADP e IMP tenham relevância no controle da biossíntese da ureia.

2. Considerações finais

As literaturas referenciadas demonstram que os NTs são nutrientes condicionalmente essenciais. Entretanto, em condições que reduzem as concentrações de NTs no metabolismo, estes se tornam essenciais para manter a funcionalidade de importantes metabolismos, a exemplo da estrutura e morfologia do TGI, dos sistemas energético, antioxidante e imune dos suínos. Destaca também, que a suplementação dietética de NSs ao invés de NTs nas dietas de suínos em terminação pode ser mais efetiva em promover benefícios a síntese proteica, aos metabolismos antioxidantes e 77s a estrutura e morfologia do TGI, e consequentemente, ao desempenho produtivo. Devido ao fato de os NSs possuírem sistema de transporte específico no metabolismo, enquanto isto não ocorre com os NTs.

Diante das diversas funções exercidas pelos NSs no metabolismo de suínos, acreditamos que a suplementação de 5'-IMP na dieta de suínos em terminação pode ser uma nova fonte energética a ser utilizada por nutricionistas nas formulações de rações, capaz de promover redução no custo da dieta e benefícios sobre a síntese proteica e a saúde dos suínos em terminação, maximizando o desempenho produtivo, principalmente sob condições que reduzem drasticamente a concentração de NSs no metabolismo e limitam o crescimento de suínos em terminação.

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II – OBJETIVO GERAL

Avaliar o efeito da suplementação ditéticas de diferentes níveis de inosina-5'-monofosfato (IMP) associada à redução de 100 kcal EM/kg na dieta de suínos em terminação (75-100 Kg) sobre o desempenho produtivo e diferentes metabolismos.

Objetivos específicos:

- Avaliar o efeito de diferentes níveis dietéticos de 5'-IMP sobre o desempenho produtivo de suínos em terminação (75-100 kg).

- Avaliar o efeito de diferentes níveis dietéticos de 5'-IMP sobre as características da carcaça, qualidade da carne e a oxidação lipídica do músculo *longissimus lumborum* e fígado.

- Avaliar o efeito de diferentes níveis dietéticos de 5'-IMP sobre os parâmetros do plasma sanguíneo.

- Estudar a influência de diferentes níveis dietéticos de 5'-IMP sobre os parâmetros imunológicos e do plasma de suínos.

I - Efeitos da inosina-5’-monofosfato (5’-IMP) suplementada em diferentes níveis na dieta de suínos em terminação (75 aos 100 kg) sobre o desempenho produtivo e a qualidade de carne.

Resumo. Este estudo teve como objetivo avaliar os efeitos da suplementação dietética de inosina-5'-monofosfato (5'-IMP) na eficiência energética, desempenho produtivo, características de carcaça, qualidade da carne, estado oxidativo e perfil bioquímico do plasma sanguíneo de suínos em terminação. Cinquenta e quatro suínos machos castrados, mestiços, com peso inicial médio de 75,62 ± 0,96 kg e peso final médio de 102,26 ± 3,23 kg foram distribuídos em delineamento de blocos casualizados com seis tratamentos e nove repetições por tratamento. As dietas experimentais foram as seguintes: dieta controle positivo (PC, 3300 kcal EM/kg), dieta controle negativo (NC, 3200 kcal EM/kg) e quatro dietas com adição de 0,050%, 0,100%, 0,150% , ou 0,200% de 5'-IMP na dieta CN. A análise de regressão indicou que a suplementação com 0,129% de 5'-IMP promoveu um ganho de peso diário de 1,29 kg e a suplementação com 0,200% de 5'-IMP resultou na menor conversão alimentar. A espessura de toucinho e o pH45minutos aumentaram linearmente com o aumento no nível de suplementação de 5'-IMP. As dietas suplementadas com 0,050% ou 0,100% de 5'-IMP resultaram em maiores pH 24 horas ($P \leq 0,05$) do que as dietas NC e PC. A vermelhidão do m. *Longissimus Lumborum* (LL) aumentou linearmente com o nível de suplementação de 5'-IMP. A perda por gotejamento teve uma resposta quadrática ($P = 0,002$) à suplementação de 5'-IMP. A suplementação de dietas de suínos em terminação com 5'-IMP foi eficaz em melhorar o status antioxidante de LL. Conclui-se que dietas com menor valor energético (3200 kcal EM/kg) suplementadas com 5'-IMP não afetaram os parâmetros de desempenho em comparação com a dieta PC (3300 kcal EM/kg) e a suplementação com 0,129% de 5'-IMP proporcionou o maior ganho de peso diário (1,29 kg). Além disso, a suplementação de 5'-IMP influenciou positivamente as características da carcaça, qualidade da carne LL e estado de oxidação do plasma em machos castrados em terminação (75-100 kg).

Palavras chaves: Malonaldeído, nucleosídeos, nucleotídeos, qualidade de carne.

I - Effects of different levels of inosine-5′-monophosphate (5′-IMP) supplementation on the productive performance and meat quality of finishing pigs (75 to 100 kg)

Abstract. This study aimed to assess the effects of dietary supplementation of inosine-5′-monophosphate (5′-IMP) on energy efficiency, productive performance, carcass characteristics, meat quality, oxidative status, and biochemical profile of blood plasma in finishing pigs. Fifty-four crossbred castrated male pigs with a mean initial weight of 75.62 ± 0.96 kg and a mean final weight of 102.26 ± 3.23 kg were distributed in a randomized block design consisting of six treatments and nine replications per treatment. Experimental diets were as follows: positive control diet (PC, 3300 kcal ME/kg), negative control diet (NC, 3200 kcal ME/kg), and four diets prepared by supplementing the NC diet with 0.050%, 0.100%, 0.150%, or 0.200% 5′-IMP. Regression analysis indicated that supplementation with 0.129% 5′-IMP promoted a daily weight gain of 1.29 kg and supplementation with 0.200% 5′-IMP resulted in the lowest feed conversion. Backfat thickness and pH45minutes increased linearly with 5′-IMP supplementation level. Diets supplemented with 0.050% or 0.100% 5′-IMP resulted in higher ($P \leq 0.05$) pH at 24 h post-slaughter than NC and PC diets. The redness of m. *Longissimus lumborum* (LL) increased linearly with 5′-IMP supplementation level. Drip loss had a quadratic response ($P = 0.002$) to 5'-IMP supplementation. Supplementation of finishing pig diets with 5′-IMP was effective in improving LL antioxidant status. It is concluded that 5′-IMP-supplemented, low-energy (3200 kcal ME/kg) diets did not affect performance parameters as compared with the PC diet (3300 kcal ME/kg), and supplementation with 0.129% 5′-IMP provided the highest daily weight gain (1.29 kg). Furthermore, 5′-IMP supplementation positively influenced carcass characteristics, LL meat quality, and plasma oxidation status in finishing barrows (75–100 kg).

Keywords: Malondialdehyde, nucleoside, nucleotide, meat quality.

1. Introduction

Nucleotides (NTs) are essential components of cell function, having important roles in energy metabolism, acting as enzymatic cofactors, and participating in DNA and RNA synthesis and transcription (Rudolph, 1994). Despite such biological importance, there is scarce information on the effects of dietary supplementation with NTs in finishing pigs, which precludes identification of potential benefits on anabolic processes, post-mortem parameters, and pork meat quality.

The first NT formed in the de novo synthesis pathway is inosine-5′-monophosphate (5′-IMP), which can be converted to adenosine monophosphate (AMP) or guanosine monophosphate (GMP). In this process, 7 molecules of adenosine triphosphate (ATP) are consumed in 10 metabolic steps to produce 1 molecule of 5′-IMP (Harmsen et al., 1984; Zhu and Thompson, 2019).

5′-IMP is considered a conditionally essential NT. Under conditions of stress or rapid muscle growth, such as those seen in finishing male pigs, which can experience daily weight gains of more than 1.2 kg, NTs need to be consumed in greater quantity (Haskó et al., 2004; Xie et al., 2019). In these cases, there is a need to supplement 5′-IMP to meet nutritional requirements and thereby achieve maximum efficiency in cellular activities (Hess and Greenberg, 2012). However, there remains the question of which level of 5′-IMP supplementation is necessary to meet the requirements of finishing pigs.

We hypothesized that inclusion of 5′-IMP in finishing pig diets reduces the amount of energy expended in 5′-IMP biosynthesis and has the potential to enhance 5′-IMP recovery, resulting in an increase in conversion rates to AMP or GDP and ATP or GTP, which are high-energy molecules that can influence energy metabolism as well as anabolic, post-mortem, and oxidation processes (Kamatani et al., 2019). This study was conducted to investigate the effects of dietary supplementation with different levels of 5′-IMP on energy efficiency, productive performance, carcass characteristics, meat quality, and plasma oxidative status in castrated male pigs in the finishing phase (75 to 100 kg).

2. Material and methods

The experiment was carried out in the pig farming sector of the Iguatemi Experimental Farm (FEI), State University of Maringá, Paraná, Brazil. All experimental

procedures were approved by the local Animal Ethics Committee (CEUA, protocol no. 9056170220).

2.1. Animals and housing conditions

The pig shed where pigs were housed had fiber cement roof and cement floor and was divided into 40 pens (1.88 m^2 each) equipped with a semi-automatic feeder at the front and a nipple-type drinker at the back. Animals had ad libitum access to water and feed throughout the experimental period.

2.2. Experimental design and treatments

Fifty-four castrated male pigs with an initial mean weight of 75.62 ± 0.96 kg and a final mean weight of 102.26 ± 3.23 kg were distributed in a randomized block design consisting of six treatments, nine replications, and one pig per experimental unit.

Experimental diets consisted of a positive control diet formulated to meet the metabolizable energy (ME) requirements of finishing pigs (3300 kcal ME/kg), a negative control diet (3200 kcal ME/kg), and four diets prepared by supplementing the negative control diet with 0.050%, 0.100%, 0.150%, or 0.200% 5′-IMP. All experimental diets were based on corn, soybean meal, minerals, vitamins, and additives and met the nutritional requirements estimated by the National Research Council (2012), except for ME (Table 1). The net energy for the pig breed used in the study was previously determined by Araujo et al. (2020).

2.3. Productive performance

Pigs were weighed at the beginning and end of the experimental period. Feed portions were weighed before being provided to the animals. These data were used for the calculation of daily weight gain, daily feed intake, and feed conversion.

2.4. Slaughter procedures

At the end of the experiment, pigs were fasted for 24 h and weighed to obtain the fasted weight. Slaughter was carried out at the Iguatemi Experimental Farm slaughterhouse. Animals were subjected to electrical desensitization (200 W) and slaughtered by exsanguination. Carcasses were scalded in water (60 °C), waxed, singed, washed, gutted, cut in half, weighed, and stored in a cold room for 24 h (0.5 ± 1.0 °C).

2.5. Quantitative analysis of carcass characteristics

Quantitative carcass parameters were assessed according to the recommendations of Bridi and Silva (2009). The following parameters were evaluated: hot carcass yield, cold carcass yield, ham weight, ham yield, backfat thickness at the first thoracic vertebra (P1), backfat thickness at the last thoracic vertebra (P2), and backfat thickness at the point between the last and penultimate lumbar vertebra (P3). P1, P2, and P3 were used to calculate the mean backfat thickness of carcasses.

2.6. Meat quality

pH measurements were taken on the right half of carcasses at the height of the last rib in the m. *Longissimus lumborum* (LL) by using a digital pH meter (Hanna Instruments, HI98163, USA, Woonsocket). The pH was measured at 45 min (pH_{45min}) post-slaughter and after 24 h (pH_{24h}) of cold storage (Bridi and Silva, 2009). LL samples were used for color analysis, which consisted of six measurements performed using a colorimeter (Konica Minolta™ CR400; Japan, Tokyo) previously calibrated to X = 80.4, Y = 85.3, and Z = 91.5. Lightness (L*, white–black), redness–greenness (a*), and yellowness–blueness (b*) are expressed in the CIELAB system space.

LL samples were weighed, stored for 72 h at 4 °C, and weighed again to determine drip loss (Boccard et al., 1981). For determination of thawing loss, samples were weighed, frozen, thawed, weighed, and stored at 4 °C for 24 h. Cooking loss was calculated from the difference between the weight of the thawed sample and the weight of the sample cooked at 170 °C until reaching an internal temperature of 71 °C (Bridi and Silva, 2009).

After cooking, LL samples were cut longitudinally to the muscle fibers with a cylindrical tool (1.27 cm in diameter) to obtain six subsamples (Ramos and Gomide, 2012). Each subsample was placed in direction of the fibers on a Warner-Bratzler support,

and a texture analyzer (TA-XT2I, Surrey, UK) was used to measure shear strength. The speed was set at 5 mm/s during the pre-test, 2 mm/s during the test, and 5 mm/s during the post-test.

2.7. Antioxidant analysis

The radical scavenging activity of LL samples was assessed by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) assay. For this, samples were stored at 4 °C for different periods (0, 24, 48, and 72 h). Subsequently, samples were weighed, mixed (5 g) with 15 mL of methanol, and homogenized using an Ultra Turrax for 1 min. The extract was filtered through qualitative filter paper no. 42.

For determination of concentrations of thiobarbituric acid reactive substances (TBARS), loin samples were weighed, mixed (5 g) with an extract solution (15 mL) containing trichloroacetic acid (7.5%), gallic acid (0.1%), and EDTA, homogenized using an Ultra Turrax for 1 min, and filtered through qualitative filter paper no. 42. Supernatants (deproteinized tissues) were stored in Falcon tubes and frozen at −20 °C until analysis (Chrzczanowicz et al., 2008).

2.8. DPPH$^{\bullet}$ assay

The DPPH$^{\bullet}$ assay was performed according to Brand-Williams et al. (1995). Briefly, 200 µL of deproteinized supernatant was homogenized with 1.8 mL of a solution containing 0.0024 g of DPPH (catalog number D9132, Sigma-Aldrich, St. Louis, MI, USA) in 100 mL of 96.5% methanol and incubated in the dark for 30 min. Absorbance was measured spectrophotometrically (SP 22, Biospectro, Curitiba, Brazil) at 515 nm.

2.9. TBARS assay

Malondialdehyde (MDA) concentrations were determined by the TBARS method, according to Chrzczanowicz et al. (2008). Briefly, 500 µL of deproteinized supernatant was homogenized with 2.0 mL of a solution containing 15% thiobarbituric acid, 10% trichloroacetic acid, and 0.06% hydrochloric acid. Samples were then incubated in a water

bath at 100 °C for 15 min and cooled for 5 min. Absorbance was determined spectrophotometrically (SP 22, Biospectro, Curitiba, Brazil) at 532 nm.

2.10. Plasma analysis

At the end of the experiment, after animals had been fasted for 6 h, blood samples were collected from the jugular vein and transferred to two tubes, one containing sodium fluoride (used for glucose determination) and another containing EDTA (used for the other biochemical analyses). Subsequently, tubes were centrifuged at 3000 × *g* for 15 min. Plasma was collected using an automatic pipette and transferred to Eppendorf tubes. All laboratory procedures for determination of glucose, lipids, proteins, and nitrogen in plasma samples were performed in accordance with kit instructions. Globulin concentration was calculated from the difference between the concentration of total proteins and that of albumin. VLDL was calculated according to the instructions of the LDL kit (Gold Analisa).

2.11. Statistical analysis

The OUTLIER procedure of SAS (version 9.0, Cary, NC, USA) was applied to assess the presence of outliers. Subsequently, dietary supplementation data were subjected to analysis of variance (ANOVA); block effects and 5′-IMP levels were included in the model. The degrees of freedom of 5′-IMP levels were partitioned into orthogonal polynomials by using the Mixed procedure of SAS to obtain regression equations. A linear response plateau (LRP) model was used in association with a quadratic model. Differences between negative and positive control diets as well as between control diets and 5′-IMP-supplemented diets were assessed using Tukey's test with the GLM procedure of SAS.

3. Results

3.1. Productive performance

The positive control diet contained 100 kcal ME more than the negative control diet and diets supplemented with different levels of 5′-IMP (Table 1). This difference allowed

us to evaluate possible negative effects of energy reduction and investigate whether 5′-IMP supplementation can compensate for such dietary restriction by minimizing losses on productive performance, carcass parameters, meat quality, and oxidative parameters.

Experimental diets did not influence ($P > 0.05$) daily feed intake. The final weight of pigs was not influenced ($P > 0.05$) by negative or positive control diets, and the positive control group did not differ significantly from supplemented groups. Pigs assigned to the negative control diet had lower ($P = 0.041$) final weight than those fed a diet supplemented with 0.150% 5′-IMP (Tables 2 and 8).

Daily weight gain was lower ($P = 0.029$) in the negative control group than in the positive control group. The negative control diet resulted in lower ($P \leq 0.05$) daily weight gain than diets supplemented with 0.100% or 0.150% 5′-IMP. Finally, the positive control diet provided a higher ($P = 0.019$) daily weight gain than the 0.050% 5′-IMP-supplemented diet (Table 2 and 7). Daily weight gain had a quadratic response ($P < 0.001$) to 5′-IMP level, with the maximum daily weight gain (1.29 cm) estimated at 0.129 % 5′-IMP (Tables 2 and 8, Figure 1a).

Feed conversion did not differ ($P > 0.05$) between positive and negative control diets or between the positive control diet and supplemented diets. However, feed conversion was higher ($P = 0.024$) in pigs fed the negative control diet than in those supplemented with 0.150% 5′-IMP (Tables 2 and 8). Regression analysis showed a linear response ($P = 0.001$) of feed conversion to increasing 5′-IMP levels (Tables 2 and 7, Figure 1b).

3.2. Quantitative carcass parameters

Hot carcass yield did not differ ($P > 0.05$) between positive and negative control diets or between negative control and supplemented diets. The positive control diet provided a lower hot carcass yield (Table 3) than diets supplemented with 0.050%, 0.150%, or 0.200% 5′-IMP ($P = 0.025$, $P = 0.014$, and $P = 0.019$, respectively). For cold carcass yield, no difference was observed ($P = 0.825$) between control diets; however, negative and positive control groups had lower ($P = 0.028$ and $P = 0.034$, respectively) cold carcass yield than the group supplemented with 0.050% 5′-IMP (Table 3).

There was no significant difference ($P = 0.263$) in ham weight between control diets, but positive and negative control diets resulted in lower ($P = 0.037$ and $P < 0.001$, respectively) ham weight than dietary supplementation with 0.150% 5′-IMP (Table 3).

Ham yield did not differ between control groups or between control and supplemented groups, but the positive control diet resulted in a lower (P = 0.036) ham yield than the diet supplemented with 0.150% 5′-IMP (Table 3).

The negative control group had lower average backfat thickness (P1P2P3) than the positive control group (P < 0.001) and the 0.200% 5′-IMP-supplemented group (P = 0.023). Furthermore, the positive control diet provided higher backfat thickness than supplementation with 0.050% (P < 0.001), 0.100% (P = 0.002), or 0.150% (P = 0.002) 5′-IMP (Table 3). There was a linear response (P = 0.014) of backfat thickness to increasing 5′-IMP levels (Table 3 and 8, Figure 2).

3.3. Meat quality

The pH_{45min} of LL in the negative control group was lower (P = 0.004) than in the positive control group. The negative control diet resulted in a lower pH_{45min} than 5′-IMP-supplemented diets. There was no difference (P > 0.05) between the positive control and 5′-IMP-supplemented diets. (Table 4). pH_{45min} showed a linear response (P = 0.017) to 5’-IMP level (Tables 4 and 8, Figure 3a).

Analysis of pH_{24h} data revealed no difference (P = 0.865) between control diets, but this parameter was lower in the negative control group than in groups supplemented with 0.050% (P = 0.028) or 0.100% (P = 0.017) 5′-IMP (Table 4). A quadratic response was observed for pH_{24h} as a function of 5′-IMP level (Tables 4 and 8, Figure 3b). The maximum pH_{24h} (5.65) was estimated to be obtained with 5′-IMP supplementation at 0.117% (Tables 4 and 8, Figure 3b).

Values of lightness (L*), measured in LL, were similar between negative and positive control diets as well as between control and supplemented diets (Table 4). The redness of LL from pigs fed the negative control diet was lower (P = 0.010) than that of animals fed the positive control diet. LL redness was lower with the negative control treatment (P = 0.043) than with 5′-IMP supplementation at the highest level tested (0.200%). 5′-IMP supplementation at the lowest level (0.050%) resulted in low (P = 0.040) meat redness compared with the positive control diet (Table 4). LL redness increased (P = 0.031) with increasing 5′-IMP level (Tables 4 and 8, Figure 3c). The yellowness of LL samples did not differ (P = 0.729) between positive and negative control

diets. Both control diets, however, led to higher yellowness values than treatments containing 0.050% or 0.100% 5′-IMP (Table 4).

There was no difference (P = 0.133) in drip loss from LL samples between control diets, but the negative control diet resulted in lower (P = 0.022) drip loss than the diet containing 0.150% 5′-IMP. Drip loss was higher (P = 0.027) in the positive control group than in the 0.200% 5′-IMP-supplemented group (Table 4). A quadratic response (P = 0.004) was observed for drip loss as a function of 5′-IMP level (Tables 4 and 8, Figure 3d). The maximum drip loss (5.50%) was estimated to be obtained with 5′-IMP supplementation at 0.101 %. Thawing loss and cooking loss did not differ between control diets (Table 4), but both control diets led to higher losses than the diet supplemented with 0.150% 5′-IMP (P = 0.002, P < 0.001, P = 0.018, and P = 0.006, respectively).

LL samples from pigs in the negative control group had lower shear strength (P = 0.010) than those from pigs fed the positive control diet. There was no difference in muscle shear strength between the negative control diet and supplemented diets, but the positive control diet resulted in higher shear strength than all supplementation levels (Table 4).

3.4. DPPH$^{\bullet}$ inhibitory activity and TBARS values of LL samples throughout storage

DPPH$^{\bullet}$ scavenging values of LL samples before storage did not differ (P = 0.241) between positive and negative control diets, but both control groups had lower ($P \leq 0.05$) DPPH$^{\bullet}$ scavenging activity than 5′-IMP-supplemented groups (Table 5).

After 24 h of storage at 4 °C, there were no differences (P > 0.05) between control diets. However, the negative control diet resulted in lower (P = 0.042) DPPH$^{\bullet}$ inhibition than the 0.100% 5′-IMP treatment, and the positive control diet promoted higher (P = 0.012) DPPH$^{\bullet}$ inhibition than the 0.150% 5′-IMP diet (Table 5).

After a 48 h storage period, LL samples from pigs fed the negative control diet showed higher (P = 0.001) DPPH$^{\bullet}$ inhibition values than samples from animals fed the positive control diet. The negative control group had higher (P = 0.031) DPPH$^{\bullet}$ scavenging activity than the group supplemented with 0.200% 5′-IMP. The DPPH$^{\bullet}$ inhibition value of the positive control group was lower (P = 0.021) than that of the 0.050% 5′-IMP-supplemented group (Table 5).

After 72 h of cold storage, LL samples from pigs fed the negative control diet had higher (P = 0.003) DPPH$^{\bullet}$ scavenging activity than samples from pigs fed the positive control diet. No differences were observed between the negative control and 5′-IMP-supplemented diets. However, DPPH$^{\bullet}$ values were lower in the positive control group than in groups supplemented with 0.050% (P = 0.014) or 0.100% (P = 0.018) 5′-IMP (Table 5).

There was a linear interaction (P < 0.001) of storage period and 5′-IMP level on DPPH$^{\bullet}$ scavenging activity (Table 5). For analysis of the interaction within each 5′-IMP level, equations were adjusted to the storage period; this procedure revealed a linear increase in DPPH$^{\bullet}$ values for 0.0, 0.100 and 0.050 % 5'-IMP with storage and a linear reduction in DPPH values for 0.150 and 0.200 % 5'IMP. The highest DPPH$^{\bullet}$ scavenging activity was observed at 0.0 % 5′-IMP (Figure 4a).

MDA concentrations (mg/kg) in LL samples prior to storage were higher (P < 0.001) in the negative control group than in the positive control group. Furthermore, the negative control group had higher values than all supplemented groups. The positive control diet afforded higher MDA concentrations than diets supplemented with 0.050% (P < 0.001), 0.100% (P = 0.033), or 0.150% (P = 0.043) 5′-IMP (Table 5).

MDA concentrations in LL samples after 24 h of storage were higher (P < 0.001) in the negative control group than in the positive control group. The negative control diet resulted in higher (P < 0.001) MDA concentrations than the 0.200% 5′-IMP-supplemented diet. MDA concentrations in LL samples from pigs fed the positive control diet were lower (P < 0.001) than in samples from pigs fed diets supplemented with 0.050%, 0.100%, or 0.150% 5′-IMP (Table 5).

MDA concentrations in samples subjected to 48 h of storage were higher (P < 0.001) in the negative control group than in the positive control group. The negative control diet provided higher MDA levels than 5′-IMP supplementation at 0.100%, 0.150%, or 0.200% (P = 0.007, P = 0.020, and P = 0.003, respectively). The positive control diet resulted in higher MDA concentrations than diets containing 0.050% (P < 0.001), 0.100% (P = 0.018), or 0.150% (P = 0.007) 5′-IMP (Table 5).

For LL samples stored at 4 °C for 72 h, no differences (P = 0.076) in MDA concentration were observed between control diets. MDA concentrations in the negative control group were higher than in the 0.100%, 0.150%, or 0.200% 5′-IMP treatments (P < 0.001, P = 0.001, and P < 0.001, respectively). The positive control diet resulted in

lower MDA concentrations than diets supplemented with 0.100% ($P = 0.010$) or 0.200% ($P < 0.001$) 5′-IMP (Table 5).

There was a linear interaction ($P < 0.001$) between storage periods and 5′-IMP levels on MDA concentration (Table 5). Partitioning of the interaction showed a reduction in MDA concentration with increasing 5′-IMP level for storage periods of 0, 24, 48 and 72 h. The highest MDA was observed at 72 h storage period (Figure 4b).

Analysis of the interaction between 5′-IMP levels was performed by adjusting the equations to storage period. There was a linear increase in MDA concentration with increasing storage time, with the lowest concentration observed at 0.200% 5′-IMP (Figure 4c).

3.5. Biochemical profile of blood plasma

Differences between control and experimental diets and the effects of different levels of 5′-IMP supplementation on plasma biochemical parameters are presented in Tables 6, 7 and 8.

Plasma glucose concentration was lower ($P < 0.001$) in the negative control group than in the positive control group. The negative control diet resulted in lower glucose levels than the 0.150% ($P < 0.001$) and 0.200% ($P < 0.001$) 5′-IMP diets. Plasma glucose levels were higher in pigs fed the positive control diet than in those fed diets supplemented with 0.050% ($P < 0.001$), 0.100% ($P < 0.001$), or 0.200% ($P = 0.027$) 5′-IMP. There was a linear response ($P < 0.001$) of plasma glucose with increasing 5′-IMP levels (Tables 6 and 8, Figure 5a).

Plasma lactate levels did not differ ($P = 0.753$) between negative and positive control diets. Both control groups had higher lactate levels than 0.050%, 0.100%, or 0.150% 5′-IMP-supplemented groups ($P < 0.001$). Plasma lactate responded quadratically ($P < 0.001$) to 5′-IMP level (Tables 6 and 8, Figure 5b), and the lower inflection point (28.59 mg/dL) was estimated at 0.098 % 5′-IMP.

Triglyceride concentration was higher ($P = 0.001$) in pigs fed the negative control diet than in those fed the positive control diet. The negative control group had higher triglyceride levels than supplemented groups. Plasma triglyceride responded quadratically ($P = 0.001$) to 5′-IMP level (Tables 6 and 8, Figure 5c), and the lower inflection point (37.83 mg/dL) was estimated at 0.133 % 5′-IMP.

Total cholesterol levels were lower in pigs fed the negative control diet ($P = 0.028$) than in those fed the positive control diet. The negative control group had lower cholesterol levels than groups supplemented with 0.100%, 0.150%, or 0.200% 5′-IMP ($P = 0.019$, $P < 0.001$, and $P = 0.001$, respectively). There were no differences between the positive control diet and 5′-IMP-supplemented diets. Total cholesterol showed a linear response ($P < 0.001$) to 5′-IMP level (Tables 6 and 8, Figure 5d).

The negative control diet resulted in lower ($P < 0.001$) HDL concentrations than the positive control diet. No differences were observed between the negative control and 5′-IMP-supplemented groups ($P > 0.05$). HDL levels were higher ($P \leq 0.05$) in pigs fed the positive control diet than in supplemented pigs.

LDL concentrations did not differ ($P = 0.113$) between control groups. The negative control diet resulted in higher LDL levels than diets supplemented with 0.050%, 0.100%, or 0.150% 5′-IMP ($P < 0.001$, $P = 0.013$, and $P = 0.004$, respectively). The LDL concentration of pigs fed the positive control diet was higher than that of pigs fed diets supplemented with 0.050% or 0.150% 5′-IMP ($P = 0.008$ and $P = 0.030$, respectively). There was a quadratic response ($P = 0.001$) of LDL level to 5'-IMP supplementation level (Tables 6 and 8, and Figure 5e), and the lower inflection point (48.59 mg/dL) was estimated at 0.101 % 5′-IMP.

The negative control diet provided higher VLDL concentrations than the positive control diet ($P < 0.001$) and 5′-IMP-supplemented diets ($P \leq 0.05$). Pigs in the positive control group had higher ($P = 0.002$) VLDL concentrations than those fed a diet supplemented with 0.100% 5′-IMP. There was a quadratic response ($P < 0.001$) of plasma VLDL concentration to 5′-IMP level (Tables 6 and 8, Figure 5f), and the lower inflection point (7.36 mg/dL) was estimated at 0.127 % 5′-IMP.

No difference in plasma uric acid concentration was observed ($P = 0.532$) between control diets. Uric acid levels were higher in pigs fed the negative control diet than in those fed diets supplemented with 0.050% ($P = 0.022$) or 0.150% ($P < 0.001$) 5′-IMP. Plasma uric acid was higher ($P = 0.002$) in pigs fed the positive control diet than in those supplemented with 0.150% 5′-IMP.

Plasma urea levels were higher ($P = 0.038$) in pigs assigned to the negative control diet than in those fed the positive control diet. Both control diets resulted in higher ($P \leq 0.05$) urea levels than diets supplemented with 0.050%, 0.100%, or 0.150% 5′-IMP.

Plasma urea responded quadratically ($P < 0.001$) 5′-IMP level (Tables 6 and 8, Figure 5g), and the lower inflection point (25.34 mg/dL) was estimated at 0.093 % 5′-IMP.

Plasma protein levels did not differ ($P > 0.05$) between control diets or between the negative control diet and 5′-IMP-supplemented diets. Protein levels were lower in pigs fed the positive control diet than in those fed diets containing 0.050%, 0.100%, or 0.150% 5′-IMP ($P = 0.004$, $P = 0.003$, and $P = 0.001$, respectively). There was a quadratic response ($P = 0.004$) of plasma protein level to 5′-IMP supplementation level (Tables 7 and 8, Figure 6a), with the upper inflection point (9.09 g/dL) estimated at 0.095 % 5′-IMP.

Plasma albumin levels in pigs fed the negative control diet were lower ($P < 0.001$) than in those fed the positive control diet but higher ($P \leq 0.05$) than in those fed a diet supplemented with 0.050% ($P = 0.019$) or 0.200% ($P = 0.016$) 5′-IMP. The positive control group showed higher ($P \leq 0.05$) plasma albumin levels than supplemented groups.

Plasma globulin levels did not differ ($P = 0.556$) between control groups. Both negative and positive control diets resulted in lower ($P = 0.006$ and $P = 0.002$, respectively) globulin levels than the 0.050% 5′-IMP-supplemented diet. There was a quadratic response ($P = 0.003$) of plasma globulin level to 5′-IMP supplementation level (Tables 7 and 8, Figure 6b), with the upper inflection point (5.41 g/dL) estimated at 0.083 % 5′-IMP.

The albumin/globulin (A/G) ratio did not differ ($P = 0.145$) between control diets. The negative control group had a lower A/G ratio than 0.050% and 0.100% 5′-IMP groups ($P = 0.001$ and $P = 0.002$, respectively). The positive control diet resulted in a higher ($P = 0.049$) A/G ratio than the 0.050% 5′-IMP diet. Plasma A/G ratio responded quadratically ($P < 0.001$) 5′-IMP level (Tables 7 and 8, Figure 6c), and the lower inflection point (0.706 g/dL) was estimated at 0.097 % 5′-IMP.

4. Discussion

Soybean oil is the feed component with the highest ME content in finishing pig diets. Its proportion in feed formulations depends on its market price. In the case that the price of soybean oil is high, the ME content can be reduced to minimize feed costs, thereby decreasing the amount of soybean oil required (Hinson et al., 2011). However, a reduction in ME content may negatively affect the daily weight gain and feed conversion ratio of

finishing pigs (Lee et al., 2002). An alternative is to use low-cost energy additives to enhance the ME content of finishing diets.

A negative effect on daily weight gain was exerted by the negative control diet, resulting from the 100 kcal ME/kg reduction in energy level. Diets supplemented with 5′-IMP had the same ME content as the negative control diet; however, the daily weight gain of pigs supplemented with 0.100%, 0.150%, or 0.200% 5′-IMP was not impaired, being similar to that of pigs fed the positive control diet. Such a finding shows that dietary supplementation with 5′-IMP might have increased energy availability. Similar results were observed in a previous study, in which piglets supplemented with NTs had increased energy availability (Jang et al., 2019).

The influence of 5′-IMP on daily weight gain and feed conversion was assessed in other studies conducted with piglets. In the study of Weaver and Kin (2014), piglets (n = 120, initial body weight 7.3 ± 0.1 kg) were supplemented with 0.200, 0.500, or 1.00 g/kg of an additive rich in 5′-IMP. Daily weight gain and feed conversion ratio were found to increase and decrease linearly, respectively, as a function of 5′-IMP content. Increased daily weight gain was also observed in piglets fed diets supplemented with hydrolyzed yeast, which contained 1000–2000 ppm/kg NTs (Waititu et al., 2016).

The feed conversion ratio of pigs in the negative control group was higher than that of animals supplemented with 0.150% 5′-IMP. In the quadratic model, we found that a 5′-IMP content of 0.129% 5’-IMP result in the highest daily weight gain (1.29 kg) and in the linear model we found that 0.200 % 5’-IMP result in the lower feed conversion ratio, showing that provision of adequate dietary levels of 5′-IMP may increase daily weight gain and reduce feed conversion. Supplementation may also lead to an increase in energy efficiency and a reduction in formulation costs, thereby increasing farmer profitability. This information is also useful to nutritionists, demonstrating that 5′-IMP might have potential as a food additive.

It is assumed that dietary 5′-IMP supplementation can increase the concentration of 5′-IMP available for cell metabolism, where it can be converted into ATP or GTP, energy molecules that are essential to life and cell activity (Meyrat and Ballmoos, 2019). When converted to GTP, 5′-IMP benefits cell proliferation and growth (Radhika and Dhanasekaran, 2001), participating, for instance, in the synthesis, differentiation, and proliferation of pre-T cells (Gomez et al., 2000), production of T-cell-dependent antibodies (Jyonouchi et al., 1994), and activation of genes related to protein synthesis

(Hesketh and Oliver, 2019). GTP also associates with the mTOR complex to stimulate protein synthesis (Benjamin and Hall, 2017; Emmanuel et al., 2017), which may partially explain why supplementation with 0.109% 5′-IMP was estimated to afford the highest daily weight gain and supplementation with 0.112% 5′-IMP was estimated to provide the lowest feed conversion ratio.

Dietary 5′-IMP supplementation influenced feed conversion, mainly by promoting an increase in daily weight gain, given that the daily feed intake of finishing pigs was not influenced by 5′-IMP level. This result is interesting, as it shows that there were no losses in daily feed intake with supplementation of up to 0.200% 5′-IMP.

Hot carcass yield is related to the weight of the carcass after blood, hair, nails, and organs have been removed, representing weight loss after slaughter. The parameter was higher in groups supplemented with 0.050%, 0.150%, or 0.200% 5′-IMP than in the positive control group, suggesting that 5′-IMP minimized carcass weight loss after slaughter. This effect might be due to the action of 5′-IMP on lipid and protein deposition; thus, 5′-IMP supplementation might have altered the proportion of water in carcass, increasing the concentrations of glycogen, phosphate compounds, and ATP in muscles (Carver and Walker, 1995).

Muscle is transformed into meat after rigor mortis, a process that begins after bleeding and, in the absence of O_2, causes ATP to be metabolized so that biochemical processes remain active (Matsuishi et al., 2016). To reduce the rate of rigor mortis, it is common to refrigerate carcasses in a cold chamber at −0.5 °C for 24 h; the weight loss during refrigeration is used to calculate cold carcass yield (Hinson et al., 2011). The cold carcass yield of pigs fed positive or negative control diets was higher than that of pigs supplemented with 0.050% 5′-IMP.

Ham is one of the most consumed pig cuts, so it is important to evaluate leg weight. In the present study, the ham weight of pigs fed the positive and negative control diets was lower than that of pigs fed the diet supplemented with 0.150% 5′-IMP. Similarly, ham yield was lower in the positive control group than in the 0.150% 5′-IMP group.

It is possible that 5′-IMP was deposited at a higher concentration in the ham, leading to an increase in ATP; this effect is relevant because type I fibers, which predominate in the ham, are aerobic, synthesize ATP at a lower intensity than type II fibers, and store purines (e.g., 5′-IMP) in greater quantity (Essen-Gustavsson et al., 1992). Thus, a higher

contribution of ATP in tissues with a predominance of type I fibers might promote muscle development and enhance meat quality because type I fibers produce less lactate.

Backfat thickness, measured at three points on the carcass (P1, P2, and P3), represents the amount of fat deposited between muscle and skin. The negative control diet resulted in a lower backfat thickness than the positive control and 0.200% 5′-IMP-supplemented diets. The positive control diet afforded higher backfat thickness than diets supplemented with 0.050%, 0.100%, or 0.150% 5′-IMP. It is relevant to emphasize that supplementation with up to 0.150% 5′-IMP resulted in a lower backfat thickness than the positive control diet, which had a 100 kcal ME/kg higher energy level. Thus, addition of oil at 0.750% per kilogram of feed in the positive control diet, as well as addition of 0.200% 5′-IMP in the negative control diet, possibly increased the energy/protein ratio, generating surplus energy, with excess energy being deposited in fat cells and then converted into carcass fat (Hinson et al., 2011).

The influence of 5′-IMP on lipid metabolism was observed in previous studies conducted with rats. Dietary supplementation with 5′-IMP was shown to modify lipid deposition rate and fatty acid concentration in tissues such as erythrocytes, plasma, liver, and the brain (Carver and Walker, 1995). In another study, it was highlighted that 5′-IMP supplementation reduced lipid concentration in abdominal tissues of chicken carcass (Chen et al., 2008).

Meat quality depends on factors that influence rigor mortis (López, 2017), such as the rapid pH reduction seen at 45 min and 24 h after slaughter. The greater the pH reduction, the higher the production of lactate, which may result in pale, soft, exudative (PSE) meat (Scheffler and Gerrard, 2007). The pH_{45min} of the negative control group was lower than that of the positive control and 5′-IMP-supplemented groups and the supplementation of 0.200 % 5’-IMP resulted in the highest value of pH_{45min}. Carcass pH_{24h} was lower in the 0.100% 5′-IMP group than in the negative control group. The positive control diet resulted in a lower pH_{24h} than diets supplemented with 0.050% or 0.100% 5′-IMP. The highest carcass pH_{24h} (5.65%) was estimated to be achieved with 0.117% 5′-IMP supplementation, indicating that 5′-IMP might have delayed the onset of glycolytic processes, reducing the amount of lactate produced by glucose degradation, minimizing pH reduction at 45 min and 24 h after slaughter.

Meat color is the quality parameter that most influences consumer choice (Lee et al., 2016). The parameter can be used to verify the incidence of PSE or dark, firm, dry (DFD)

meat. For meat color to be considered normal, lightness must range from 49 to 60 (American Meat Science Association, AMSA, 2001). In the present study, the lightness of LL was within the limits established by AMSA (2001).

The increased redness of LL from 5′-IMP and positive control groups might have occurred due to changes in the oxygenation of oxymyoglobin toward reduced deoxygenation. The oxidation conditions of this pathway increase the concentration of metmyoglobin, a brown pigment, in meat; brown meat color is associated with inadequate storage conditions by consumers (Kennedy et al., 2004).

Diets supplemented with 0.050% or 0.100% 5′-IMP led to a reduction in LL yellowness compared with negative and positive control diets, indicating reduced intramuscular lipid deposition. Muscle yellowness did not differ between 0.150% and 0.200% 5′-IMP-supplemented groups and negative and positive control groups.

Meat is composed mainly of water (65–80%). The water content of meat can alter sensory characteristics, such as softness and juiciness (Cheng and Sun, 2008). Storage conditions, whether in a refrigerator (4 °C) or freezer (−20 °C), can influence water loss and, consequently, meat softness and juiciness before and during cooking.

The drip loss of LL from the negative control group was lower than that of LL from the 0.150% 5′-IMP group. The positive control diet afforded higher drip loss than the 0.200% 5′-IMP-supplemented diet. The highest drip loss (5.50%) was estimated to be achieved with 0.101% 5′-IMP supplementation, suggesting that dietary 5′-IMP levels may influence meat lipid composition, altering drip loss. Positive and negative control diets afforded lower thawing losses than the 0.150% 5′-IMP-supplemented diet, showing that dietary 5′-IMP may vary depending on the type of meat storage.

Cooking may alter the nutritional value of meat by promoting protein and lipid denaturation and water exudation (Fennema, 1993). The meat of finishing pigs supplemented with 0.050% 5′-IMP had lower cooking loss than that of pigs fed the negative control diet. In comparing the negative control diet and supplemented diets, it was observed that the 0.150% 5′-IMP-supplemented diet resulted in a lower cooking loss than the negative control diet. This reduced cooking loss suggests that 5′-IMP minimized exudation, thereby improving the nutritional composition, juiciness, and softness of meat.

Meat softness is highly relevant for consumer acceptance (Holman et al., 2020). Here, we observed that the positive control diet (3,300 kcal ME/kg) resulted in a higher meat shear strength than negative control and 5′-IMP-supplemented diets (3,200 kcal

ME/kg). This finding shows that addition of 0.750% soybean oil (positive control diet) might have altered LL composition, increasing hardness and decreasing juiciness. On the other hand, 5′-IMP might have altered meat structure during cooking, resulting in softer and juicier meat, sensory aspects that are desired by consumers (Lee et al., 2016).

It is believed that 5′-IMP acts on the actin–myosin complex, formed by the junction between myosins in thick filaments and actins in thin filaments (Nakamura et al., 2012). Nakamura et al. (2012), in assessing the dissociation of the actin–myosin complex in sausages, reported that 5′-IMP, as well as AMP, GMP, and pyrophosphates, can dissociate the actin–myosin complex under heat (65 °C) and low salt concentration (0.2 mM) conditions. It was also shown that 5′-IMP is more efficient in extracting myosin by disassociating the actin–myosin complex. Similarly, Nakamura et al. (2013) reported that 5′-IMP is a potential substitute of pyrophosphates for increasing actin–myosin complex dissociation, with the added benefit of not causing rancid odor when added to sausage formulations.

These findings, together with the beneficial effects of 5′-IMP supplementation on thawing loss, cooking loss, and LL shear strength, as well as increased ham weight and reduced backfat thickness, suggest that 5′-IMP may enhance both the quantity and quality of meat.

Meat, whether cut or ground, is generally sold under refrigeration at 4 °C. The composition and color of meat may change because of oxidation of lipids and proteins during storage. In this study, the DPPH$^{\bullet}$ scavenging activity of raw LL was lower in the negative and positive control groups than in the 5′-IMP-supplemented groups, suggesting that 5′-IMP positively contributed to the antioxidant system of pigs, resulting in an increased ability to scavenge free radicals. This result is important because high ROS concentrations in meat lead to increased lipid and protein oxidation (Surai et al., 2016).

The DPPH$^{\bullet}$ scavenging activity of LL after storage for 24 h was lower in the negative control group than in the 0.100% 5′-IMP group. The positive control diet afforded a higher DPPH$^{\bullet}$ scavenging activity than the 0.200% 5′-IMP-supplemented diet. These findings indicate that storage time can affect the action of 5′-IMP according to supplementation level.

After 48 h of storage, LL DPPH$^{\bullet}$ scavenging activity was higher in the negative control group than in the positive control and 0.200% 5′-IMP groups. However, the positive control diet resulted in a lower DPPH$^{\bullet}$ activity than the 0.050% 5′-IMP-

supplemented diet, suggesting that the higher energy level of the positive control diet (100 kcal ME/kg higher) affected the free radical scavenging capacity of meat. Furthermore, the effect of 5′-IMP supplementation on DPPH• activity seemed to vary as a function of storage period depending on 5′-IMP level.

In LL samples stored for 72 h, DPPH• activity was higher in the negative control group than in the positive control group. Diets supplemented with 0.050% or 0.100% 5′-IMP afforded higher DPPH• activities than the positive control diet, demonstrating that, at the end of the storage period, meat from pigs fed the positive control diet had reduced ability to scavenge free radicals and that 5′-IMP supplementation contributed to antioxidant activity, possibly minimizing oxidation in LL.

The increase in DPPH• scavenging activity provided by 5′-IMP supplementation is relevant because it minimizes the degradation of long-chain polyunsaturated fatty acids (Surai et al., 2016). The contribution of 5′-IMP to the removal of free radicals might be related to its activity in repairing the fragmented DNA of lipid molecules (Gudkov et al., 2006; Salobir et al., 2005). This mechanism is considered the last line of defense of the antioxidant system to prevent oxidation (Surai et al., 2016).

During refrigerated storage, raw and processed meat undergo lipid peroxidation, which increases MDA concentrations. Lipid peroxidation in meat causes rancid odor and browning, traits that are undesirable to consumers (Domínguez et al., 2019). MDA concentration in LL before storage was higher in the negative control group than in the positive control and 5′-IMP-supplemented groups. Furthermore, the positive control diet resulted in a lower MDA concentration than 0.050 and 0.100% 5′-IMP-supplemented diets, indicating that 5′-IMP-supplemented and positive control diets reduced MDA levels in fresh LL.

The negative control diet afforded a high MDA concentration in LL after 24 h of storage at 4 °C compared with the positive control diet and the diet supplemented with 0.200% 5′-IMP. The parameter was lower in the positive control group than in groups supplemented with 0.050%, 0.100%, or 0.150% 5′-IMP, indicating that storage time might modify the influence of 5′-IMP.

At 48 h of storage, MDA levels were higher in LL from the negative control group than in LL from the positive control and 0.050%, 0.100%, and 0.150% 5′-IMP groups. However, the positive control diet resulted in a lower MDA concentration than 0.050%, 0.100%, and 0.150% 5′-IMP-supplemented diets. This finding suggests that

supplementation with 0.750% soybean oil (positive control diet) did not increase oxidation in LL after 48 h of storage. It is important to highlight that supplementation with 0.200% 5′-IMP benefited the antioxidant defense system, resulting in reduced MDA levels after 48 h.

In LL samples stored for 72 h, MDA concentration did not differ between negative and positive control groups. The parameter was higher in the positive control group than in 0.100% and 0.200% 5′-IMP groups. This result shows that, at the end of storage, the positive control diet resulted in increased MDA levels, partly because of the inclusion of 0.750% soybean oil per kilogram of feed, which might have increased the concentration and peroxidation of intramuscular lipids, resulting in increased MDA concentration.

MDA concentration decreased with increasing 5′-IMP level after 0, 24, 48, and 72 h storage. Furthermore, MDA concentration increased with storage period, and the lowest MDA level was observed in LL from pigs supplemented with 0.200% 5′-IMP.

The reduction in MDA concentration with 5′-IMP supplementation can be attributed to the increase in DPPH$^{\bullet}$ scavenging activity, which suggests greater removal of free radicals during storage and, consequently, reduced lipid peroxidation. Therefore, it can be said that 5′-IMP exerts antioxidant effects, increasing the quality of LL during storage, possibly improving color and odor, which are relevant for consumer purchase decisions. Such a reduction in MDA levels is also important in the context of food safety, given that the consumption of meat with high levels of oxidized lipids is directly related to impairment of human mitochondria, such as dysregulation of ROS and ATP synthesis, possibly leading to higher incidence of metabolic disorders (Domínguez et al., 2020; Huang and Ahn, 2019).

Concentrations of glucose, lactate, triglycerides, cholesterol, HDL, LDL, and VLDL are indicators of the status of energy and lipid metabolisms. Protein metabolism is indicated by the levels of total proteins, albumin, globulin, A/G ratio, uric acid, and urea (Archer et al., 2003; Regmi et al., 2018). Plasma glucose concentration is related to feed intake (Guyton and Hall, 2006), ME content, and feed energy source. Plasma glucose levels were higher in pigs fed the positive control diet, resulting from the 100 kcal/kg higher energy content of the feed (provided by addition of 0.750% soybean oil/kg feed), than in pigs fed negative control and 0.050%, 0.100%, or 0.200% 5′-IMP-supplemented diets. The negative control diet afforded lower plasma glucose levels than diets supplemented with 0.150% or 0.200% 5′-IMP. The highest glucose concentration was

estimated to be achieved by supplementation with 0.200% 5′-IMP, demonstrating the positive effect of 5′-IMP on the energy metabolism of finishing pigs. It is possible that 5′-IMP stimulated gluconeogenesis during the 6 h fasting period, thereby increasing plasma glucose concentration (Guinzberg et al., 2006).

The effects of 5′-IMP on gluconeogenesis were reported in in vitro studies conducted with rats. Gluconeogenesis was found to be controlled by IMP or ADP concentrations in cells (Guinzberg et al., 2006; Marchand et al., 1979). Moreover, when ADP is metabolized to adenine rather than to ATP, the ATP/ADP ratio is altered, influencing gluconeogenesis (Lavoinne et al., 1987). Such effects were also observed in in vitro studies conducted by De Pinã et al. (1989) and Sharma et al. (1982): in response to the hormone glucagon, gluconeogenesis rate was increased by IMP and reduced by ADP, and lactate degradation was increased.

In the present study, lactate concentration did not differ between negative and positive control groups, indicating that the high plasma glucose concentration provided by the positive control diet compared with the negative control diet was not a result of lactate degradation. Dietary supplementation with 0.050%, 0.100%, or 0.150% 5′-IMP reduced plasma lactate concentration compared with positive and negative control diets. The lowest lactate concentration (28.59 mg/dL) was estimated to be achieved with 0.098% 5′-IMP supplementation. These reductions in lactate concentration provided by 5′-IMP supplementation indicate that gluconeogenesis might have been stimulated by 5′-IMP, leading to an increase in plasma glucose concentrations.

The positive control diet afforded lower triglyceride levels than the negative control diet but similar levels compared with supplemented diets, showing that the 100 kcal ME/kg increase in energy did not lead to an increase in triglycerides. It was observed that plasma triglyceride was lower in pigs fed the negative control diet than in pigs supplemented with 5′-IMP. The lowest triglyceride concentration (37.83 mg/dL) was estimated to be achieved with 0.133% 5′-IMP supplementation.This finding demonstrates that 5′-IMP might have stimulated a reduction in plasma triglycerides.

Under normal conditions, the action of 5′-IMP is modulated by the enzyme inosine monophosphate dehydrogenase. Insulin induces the translocation of inosine monophosphate dehydrogenase to lipid tissues to reduce lipid concentrations. Another factor influencing triglyceride reduction is the increase in ATP synthesis from inosine

monophosphate (Hesketh and Oliver, 2019), which stimulates protein synthesis and limits lipid synthesis (Farber et al., 1965).

The influence of 5′-IMP in reducing triglycerides was also reported by Hossain et al. (2016a). The authors investigated the effects of different levels (0.2%, 0.4%, 0.6%, and 0.8%) of inosine and inosine monophosphate and found that the addition of 0.8% inosine or 0.2%, 0.6%, or 0.8% inosine monophosphate reduced plasma triglycerides compared with the control diet. In another research, Hossain et al. (2017) reported that addition of 0.3% or 0.6% inosine reduced plasma triglyceride concentration compared with the control diet.

Cholesterol is a structural component of the plasma membrane of several cells. Its function is to modify membrane structure to stimulate or inhibit cell proliferation, lipid and protein translocation, and cell apoptosis (Yeagle, 1985). In the present study, plasma cholesterol level was higher in the positive control group than in the negative control group. The negative control diet provided a lower cholesterol concentration than diets supplemented with 0.100%, 0.150%, or 0.200% 5′-IMP.

5′-IMP level had a positive linear effect ($P < 0.001$) on total cholesterol. This result may be explained, in part, by the increased concentration of 5′-IMP in cell metabolism. When metabolized, 5′-IMP can form hypoxanthine, xanthine, or uric acid (Lima et al., 2015; Ryu et al., 2016). Ryu et al. (2016), in evaluating the metabolic function of hypoxanthine in endogenous cholesterol synthesis in rats, reported that cholesterol and ROS accumulated in blood serum and liver HepG2 cells as a result of greater hypoxanthine degradation.

Lipoproteins (HDL, LDL, and VLDL) are responsible for transporting cholesterol and triglycerides in plasma. The positive control diet increased HDL concentration compared with the negative control diet and diets supplemented with 5′-IMP, indicating that excess cholesterol resulting from consumption of the positive control diet had to be transported to the liver by HDL to be eliminated. The influence of 5′-IMP in reducing HDL concentration might be related to its action on cell growth stimulation; cholesterol is used as a substrate for cell proliferation in plasma (Ryu et al., 2016).

Plasma LDL did not differ between negative and positive control diets, but the former afforded higher LDL levels than diets supplemented with 0.050%, 0.100%, or 0.150% 5′-IMP. The positive control diet resulted in higher LDL concentrations than 0.050% and 0.150% 5′-IMP-supplemented diets.

The reduction in plasma LDL concentration promoted by 5′-IMP supplementation might indicate a lower LDL concentration in meat. This effect is relevant, as meat with reduced LDL levels is beneficial to health. The consumption of food products with high concentrations of LDL and triglycerides can lead to the accumulation of these molecules, increasing the incidence of heart diseases and other metabolic disorders in humans (Helgadottir et al., 2016).

VLDL concentration was lower in the positive control group than in the negative control group. 5′-IMP-supplemented diets afforded lower VLDL concentration than the negative control diet. A quadratic effect of 5′-IMP level on VLDL concentration was observed. The lowest concentration (7.36 mg/DL) was estimated to be achieved with 0.127% 5′-IMP supplementation. Such findings indicate that 5′-IMP exerts modulating or structural effects on VLDL synthesis or that the increase in cholesterol synthesis provided by 5′-IMP altered VLDL concentration in cell membranes (Neufeld et al., 2014).

Uric acid is a metabolite resulting from purine oxidation. It has powerful antioxidant action and is one of the major antioxidants in plasma (Ames et al., 1981; Mandal et al., 2015). In the present study, the negative control diet afforded higher uric acid concentration than diets supplemented with 0.050% or 0.150% 5′-IMP. The parameter was higher in pigs fed the positive control diet than in pigs supplemented with 0.150% 5′-IMP. The reduced concentration of uric acid in 5′-IMP-supplemented pigs suggests that uric acid is not the main metabolite of 5′-IMP. In line with these results, Settle et al. (2012) reported that control poultry did not differ in liver uric acid concentration from those supplemented with IMP.

Urea is the main metabolite of amino acid degradation (Guyton and Hall, 2006). Plasma urea was lower in the negative control group than in the positive control group, and both groups exhibited higher plasma urea than groups supplemented with 0.050%, 0.100%, or 0.150% 5′-IMP. We observed a positive linear effect of 5′-IMP level on plasma urea concentration.

The reducing effect of 5′-IMP supplementation on plasma urea concentration compared with negative and positive control diets might be related to the ability of 5′-IMP to enhance the use efficiency of amino acids, as supported by the increase in daily weight gain and final weight with 5′-IMP supplementation. This behavior may also be related to the energy supply provided by 5′-IMP, which contributed to maintaining an

energy/protein ratio that was more suitable for protein deposition, as compared with the negative control diet.

The influence of ADP and inosine or IMP on urea biosynthesis was assessed by Guinzerber et al. (1987). Rat liver cells were incubated in a medium with low levels of ADP or inosine. The authors reported that activation of ureagenesis was influenced by ADP or inosine, suggesting that the physiological actions of ADP and IMP are important in the control of urea biosynthesis.

Proteins are components of cells, tissues, and hormones that regulate various body functions; their concentration in plasma indicates the rate of protein deposition in muscle and the immune system status (Kumar et al., 2005; Zeng et al., 2013). In the current study, the positive control diet afforded lower total plasma protein than 0.050%, 0.100%, or 0.150% 5′-IMP-supplemented diet. A quadratic effect of 5′-IMP level on plasma total protein was observed. The highest concentration (9.09 mg/dL) was estimated to be achieved with 0.095% 5′-IMP supplementation. The increase in total plasma protein suggests increased protein deposition in muscles and, ultimately, meat (Zeng et al., 2013), in agreement with the results observed for final weight, daily weight gain, and ham weight.

Supplementation of fish with inosine or 5′-IMP at 0.100–1.0% resulted in an increase in daily weight gain and total protein in plasma or serum compared with control diets (Jha et al., 2007; Hossain 2016a, b, c, d; Hossain et al., 2017; Tahmasebi-Kohyani et al., 2012).

Albumin and globulins correspond to the largest fraction of total plasma protein. Albumin is the main nitrogen carrier in the body and interacts with the 5′-IMP carrier (He et al., 2016). Globulins are essential in the maintenance of the immune system (Kumar et al., 2005). The plasma A/G ratio indicates the immune system status: the higher the ratio, the higher the concentration of globulins.

Our results show that plasma albumin was higher in pigs fed the positive control diet than in pigs fed negative control or 5′-IMP-supplemented diets. The negative control diet, however, afforded higher plasma albumin levels than diets supplemented with 0.050% or 0.200% 5′-IMP. The reduction in plasma albumin promoted by 5′-IMP supplementation might be related to the reduction in lipids provided by 5′-IMP, which would decrease the concentration of fat-soluble hormones to be transported by albumin (He et al., 2016)

Negative and positive control diets afforded lower globulin concentrations than the 0.050% 5′-IMP supplemented diet. 5′-IMP level had a linear reducing effect on globulin concentration.

The increase in globulin production suggests that 5′-IMP might have improved immune defense responses. Future studies should be carried out on the effect of 5′-IMP on immune system stimulation (Kumar et al., 2005).

The A/G ratio was higher in the negative control group than in groups supplemented with 0.050 or 0.100% 5′-IMP. The positive control diet afforded a higher A/G ratio than the 0.050% 5′-IMP-supplemented diet. A/G ratio decreased quadratically with increasing 5′-IMP level, suggesting that 5′-IMP carries out essential physiological and metabolic activities in the immune system and that exogenous sources of 5′-IMP can maximize immune defenses. These results are in line with research demonstrating the positive influence of dietary inosine, 5′-IMP, or NTs on immune responses of humans, fish, and pigs (Gil, 2002; Jiao and Kin, 2018; Li et al., 2018; Song et al., 2012; Weaver and Kim, 2014).

Daily weight gain, pH24hr, drip loss, lactate, triglycerides, LDL, VLDL, urea, total plasma protein, globulins, and A/G ratio had a quadratic response to 5′-IMP level, allowing identification of the optimal supplementation level for each parameter. Previous studies also observed different optimal levels of 5′-IMP for each evaluated parameter (Hossain et al., 2017; Song et al., 2012).

The results of the current study showed that 5′-IMP supplementation positively contributed to energy and protein metabolism, productive performance, protein deposition, reduction of carcass lipid content, rigor mortis, meat color, meat quality during refrigerated storage, and biochemical profile of blood plasma in finishing pigs.

5. Conclusion

Supplementation of a lower energy diet (negative control, 3200 kcal ME/kg) with 5′-IMP, as compared with a positive control diet (3300 kcal ME/kg), did not impair performance parameters. It was estimated that supplementation with 0.129% 5′-IMP could provide the highest daily weight gain (1.29 kg). 5′-IMP supplementation positively influenced carcass traits, meat quality, LL oxidation, and plasma status of castrated male finishing pigs (75–100 kg).

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Table 1. Composition of experimental diets

Ingredients (%)	NC[5]	5′-IMP[1] (%)				PC[6]
		0.050	0.100	0.150	0.200	
Corn	79.12	79.12	79.12	79.12	79.12	80.49
Soybean Meal	16.82	16.82	16.82	16.82	16.82	16.60
Soybean oil	-	-	-	-	-	0.750
Dicalcium phosphate	0.598	0.598	0.598	0.598	0.598	0.596
Limestone	0.744	0.744	0.744	0.744	0.744	0.747
Salt	0.225	0.225	0.225	0.225	0.225	0.223
5′-IMP	-	0.050	0.100	0.150	0.200	-
Inert[2]	1.900	1.850	1.800	1.750	1.700	-
Vitamin and mineral supplement[3]	0.400	0.400	0.400	0.400	0.400	0.400
L-Lysine HCl 78.4%	0.160	0.160	0.160	0.160	0.160	0.164
Enramycin	0.020	0.020	0.020	0.020	0.020	0.020
Feed dry[4]	0.015	0.015	0.015	0.015	0.015	0.015
Calculated composition, %						
Metabolizable energy (Mcal/kg)	3,200	3,200	3,200	3,200	3,200	3,300
Crude protein	14.00	14.00	14.00	14.00	14.00	14.00
Total calcium	0.500	0.500	0.500	0.500	0.500	0.500
Available phosphorus	0.190	0.190	0.190	0.190	0.190	0.190
Potassium	0.561	0.561	0.561	0.561	0.561	0.561
Sodium	0.100	0.100	0.100	0.100	0.100	0.100
Chlorine	0.248	0.248	0.248	0.248	0.248	0.249
SID Lysine	0.690	0.690	0.690	0.690	0.690	0.690
SID Methionine	0.200	0.200	0.200	0.200	0.200	0.200
SID Methionine + Cysteine	0.425	0.425	0.425	0.425	0.425	0.425
SID Threonine	0.449	0.449	0.449	0.449	0.449	0.449
SID Tryptophan	0.135	0.135	0.135	0.135	0.135	0.135
SID Valine	0.627	0.627	0.627	0.627	0.627	0.627
SID Leucine	1.197	1.197	1.197	1.197	1.197	1.197
SID Isoleucine	0.502	0.502	0.502	0.502	0.502	0.502
SID Arginine	0.791	0.791	0.791	0.791	0.791	0.791
SID Histidine	0.350	0.350	0.350	0.350	0.350	0.350
SID Phenylalanine	0.616	0.616	0.616	0.616	0.616	0.616
SID Phenylalanine + tyrosine	1.072	1.072	1.072	1.072	1.072	1.072

[1]Inosine-5′-monophosphate.
[2]Kaolinite.
[3]Provided per kilogram: vitamin A, 30000 UI; vitamin D3, 5000 UI; vitamin E, 120 UI; vitamin K, 5 mg; vitamin B12, 120 mcg; niacin, 150 mg; calcium pantothenate, 75 mg; folic acid, 8 mg; choline chloride, 0.48 g; iron, 350 mg; copper, 15 mg; manganese, 250 mg; zinc, 0.75 g; iodine, 10 mg; selenium, 3 mg.
[4]Antioxidant.
[5]Negative control (0.00% 5′-IMP and 3200 kcal ME/kg).
[6]Positive control (0.00% 5′-IMP and 3300 kcal ME/kg).

Table 2 – Performance of barrows from 75 to 100 kg fed diets with different levels of inosine-5'-monophosphate and metabolizable energy

Itens	Initial weight, kg	Final weight, kg	ADG[3], kg	F:G[4], kg/kg	DFI[5], kg
NC[1]	75.34	101.18	1.18	2.93	3.47
PC[2]	75.96	101.72	1.28	2.78	3.55
0.050 %	75.99	101.75	1.19	2.93	3.49
0.100 %	75.11	103.10	1.32	2.67	3.50
0.150 %	75.21	104.06	1.33	2.54	3.37
0.200 %	75.12	101.60	1.26	2.64	3.32
Mean	75.44	102.34	1.26	2.74	3.43
SD	0.650	2.056	0.080	0.254	0.245
SEM	0.089	0.280	0.011	0.035	0.033
Contrasts					
NC×PC	-	0.225	0.029	0.314	0.878
NC×0.050 %	-	0.615	0.854	0.893	0.743
NC×0.100 %	-	0.161	0.004	0.107	0.870
NC×0.150 %	-	0.041	0.002	0.024	0.615
NC×0.200 %	-	0.845	0.068	0.087	0.713
PC×0.050 %	-	0.443	0.019	0.372	0.888
PC×0.100 %	-	0.992	0.356	0.658	0.757
PC×0.150 %	-	0.509	0.257	0.273	0.768
PC×0.200 %	-	0.146	0.986	0.702	0.869
Statistical analysis					
Linear	-	0.155	<0.001	0.001	0.793
Quadratic	-	0.159	0.005	0.174	0.557

[1]Negative control (0.00 % of 5'-IMP and 3,200 kcal ME/kg).
[2]Positive control (0.00 % of 5'-IMP and 3,300 kcal ME/kg).
[3]Average daily gain.
[4]Feed gain ratio.
[5]Daily feed intake.

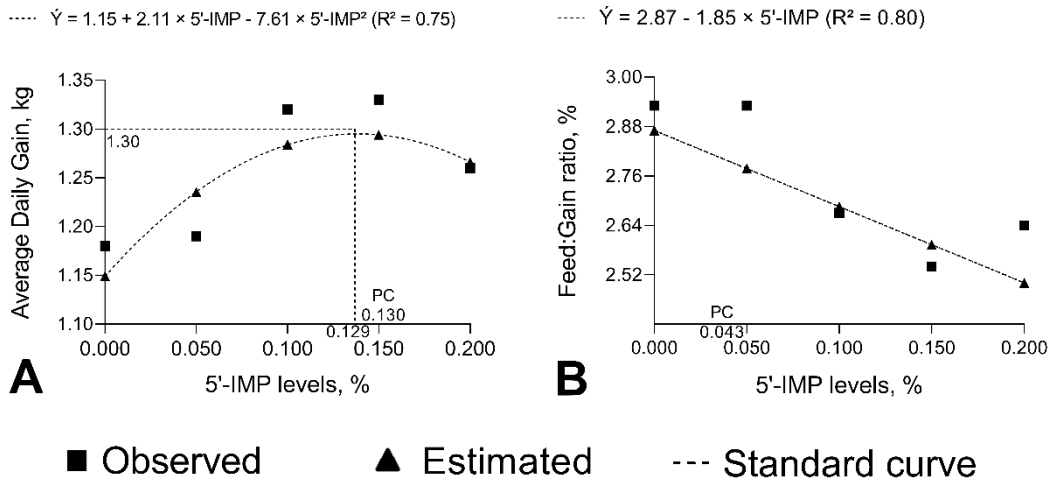

Figure 1. Effects of inosine-5'-monophosphate (5'-IMP) levels and metabolizable energy on average daily gain (A) and feed:gain ratio (B) of barrows from 75 to 100 kg fed with diets containing different levels of 5'-IMP and metabolizable energy.

Table 3 - Carcass traits of barrows from 75 to 100 kg, fed with different levels of inosine-5'-monofosfato and metabolizable energy

Item	HCY, %	CCY, %	Ham Weight, kg	Ham yield, %	P1P2P3, cm
NC[1]	80.65	78.49	10.77	27.91	2.20
PC[2]	80.22	78.59	10.55	27.19	2.43
0.050 %	81.17	79.41	10.51	27.24	2.20
0.100 %	80.60	78.26	10.58	27.61	2.24
0.150 %	81.21	79.20	11.27	28.33	2.25
0.200 %	81.22	79.00	10.73	27.35	2.35
Mean	80.83	78.84	10.76	27.64	2.24
SD	0.800	0.800	0.420	0.920	0.150
SEM	0.110	0.110	0.060	0.130	0.030
Contrasts					
NC×PC	0.336	0.825	0.263	0.300	<0.001
NC×0.050 %	0.187	0.028	0.190	0.239	0.856
NC×0.100 %	0.950	0.596	0.324	0.564	0.488
NC×0.150 %	0.134	0.090	0.037	0.459	0.447
NC×0.200 %	0.156	0.204	0.799	0.324	0.023
PC×0.050 %	0.025	0.034	0.764	0.974	<0.001
PC×0.100 %	0.382	0.450	0.902	0.492	0.002
PC×0.150 %	0.014	0.107	<0.001	0.036	0.002
PC×0.200 %	0.019	0.258	0.349	0.854	0.106
Statistical Analysis					
Linear	0.966	0.527	0.653	0.740	0.003
Quadratic	0.981	0.708	0.986	0.815	0.150

[1]Control Negative (0.00 % of 5'-IMP and 3,200 kcal ME/kg).
[2]Control Positive (0.00 % of 5'-IMP and 3,300 kcal ME/kg).
[3]Hot carcass yield.
[4]Cold carcass yield.
[5]Backfat thickness mensured at three different points on the carcass.

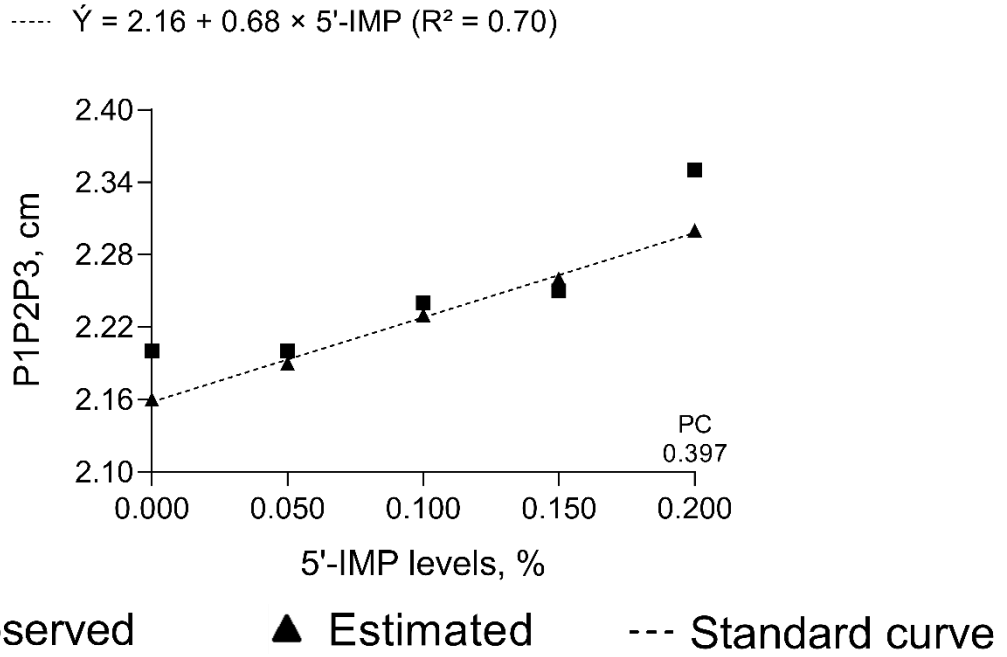

Figure 2. Effects of inosine-5’-monophosphate (5’-IMP) levels and metabolizable energy on backfat thickness measured at tree points at carcass (P1P2P3) of barrows from 75 to 100 kg fed with diets containing different levels of 5’-IMP and metabolizable energy.

Table 4 – Meat quality mensured on m. *Longissimus Lumborum* of barrows from 75 to 100 kg fed with different levels of inosine-5-monophosphate and metabolizable energy

Item	pH45min	pH24h	Luminosity	Redness	Yellowness	DL[3], %	TL[4], %	CL[5], %	SF[6], N
NC[1]	6.02	5.48	57.99	6.65	3.99	4.45	9.50	25.23	27.44
PC[2]	6.30	5.49	57.82	7.44	3.90	5.16	10.42	25.89	32.57
0.050	6.40	5.60	57.46	6.82	3.20	4.63	9.28	21.07	24.10
0.100	6.23	5.62	57.10	7.06	3.33	5.40	10.37	24.11	27.07
0.150	6.33	5.54	57.21	7.07	3.54	5.88	5.98	18.52	24.10
0.200	6.35	5.59	57.26	7.26	3.88	3.92	9.49	21.06	27.15
Mean	6.26	5.57	57.40	6.94	3.67	4.91	9.27	22.44	27.05
SD	0.150	0.060	0.820	0.240	0.330	0.650	2.420	5.200	4.300
SEM	0.060	0.020	0.330	0.100	0.130	0.260	0.350	0.740	0.620
Contrasts									
NC×PC	0.004	0.865	0.600	0.010	0.729	0.133	0.442	0.750	0.010
NC×0.050 %	<0.001	0.028	0.536	0.514	0.004	0.725	0.798	0.122	0.105
NC×0.100 %	0.026	0.017	0.301	0.116	0.018	0.055	0.469	0.533	0.926
NC×0.150 %	<0.001	0.251	0.260	0.141	0.177	0.022	0.002	0.018	0.117
NC×0.200 %	<0.001	0.062	0.750	0.043	0.346	0.340	0.654	0.317	0.971
PC×0.050 %	0.120	0.047	0.956	0.040	0.007	0.293	0.322	0.054	<0.001
PC×0.100 %	0.463	0.029	0.641	0.185	0.032	0.622	0.963	0.349	0.003
PC×0.150 %	0.532	0.344	0.576	0.267	0.293	0.371	<0.001	0.006	<0.001
PC×0.200 %	0.293	0.098	0.882	0.566	0.534	0.027	0.280	0.184	0.005
Statistical analysis									
Linear	0.017	0.028	0.297	0.031	0.327	0.004	0.478	0.229	0.219
Quadratic	0.094	0.050	0.420	0.457	0.287	0.004	0.677	0.417	0.193

[1]Negative control (0.00 % of 5'-IMP and 3,200 kcal ME/kg).
[2]Positive control (0.00 % of 5'-IMP and 3,300 kcal ME/kg).
[3]Drip loss.
[4]Thawing loss.
[5]Cooking loss.
[6]Shear force.

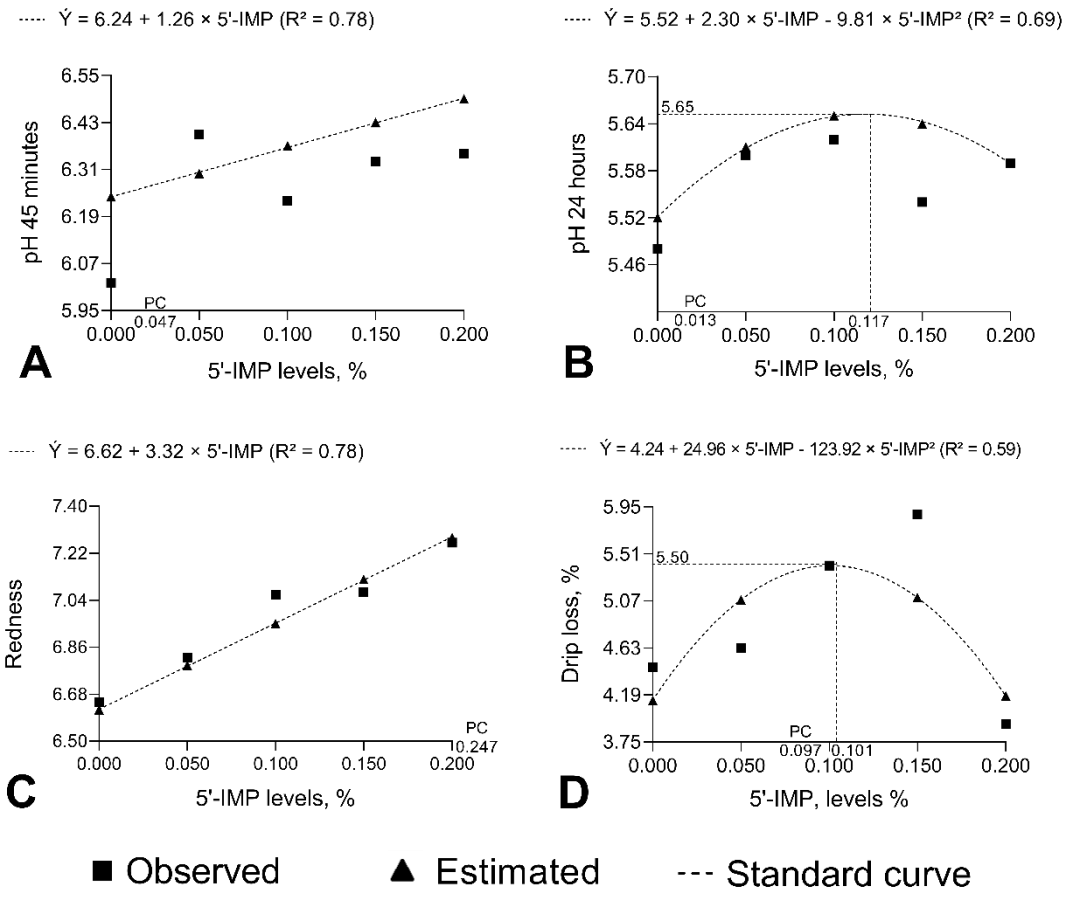

Figure 3. Effects of inosine-5'-monophosphate (5'-IMP) levels and metabolizable energy on pH45 minutes (A), pH24 hours (B), redness of LL (C), and drip loss of LL (D) of barrows from 75 to 100 kg fed with diets containing different levels of 5'-IMP and metabolizable energy.

Table 5. Free radical scavenging assay (DPPH %) and MDA (mg/kg) concentration in the *m. Longissimus Lumborum* subject to different storage periods of barrows from 75 to 100 kg, fed with different levels of inosine-5'-monophosphate and metabolizable energy

Item	DPPH (%)[3]				MDA (mg/kg)[4]			
	0	24	48	72	0	24	48	72
NC[1]	19.10	20.76	21.51	21.93	0.604	0.598	0.642	0.789
PC[2]	19.66	21.85	19.95	19.56	0.492	0.529	0.565	0.750
0.050 %	22.55	20.82	21.36	21.55	0.547	0.593	0.663	0.771
0.100 %	20.78	22.15	20.84	21.46	0.549	0.612	0.601	0.692
0.150 %	20.72	20.14	20.69	21.03	0.526	0.586	0.607	0.714
0.200 %	21.10	21.44	20.21	20.85	0.504	0.539	0.584	0.657
Mean	20.60	21.27	20.56	21.19	0.537	0.576	0.610	0.729
SD	1.410	1.920	1.720	1.820	0.043	0.038	0.043	0.062
SEM	0.200	0.280	0.250	0.260	0.006	0.006	0.006	0.009
Contrasts								
NC×PC	0.276	0.105	0.001	0.003	<0.001	<0.001	<0.001	0.076
NC×0.050 %	<0.001	0.924	0.792	0.495	<0.001	0.698	0.159	0.406
NC×0.100 %	0.005	0.042	0.252	0.436	<0.001	0.194	0.007	<0.001
NC×0.150 %	0.005	0.349	0.168	0.211	<0.001	0.305	0.020	0.001
NC×0.200 %	<0.001	0.307	0.031	0.149	<0.001	<0.001	0.003	<0.001
PC×0.050 %	<0.001	0.126	0.021	0.014	<0.001	<0.001	<0.001	0.333
PC×0.100 %	0.033	0.657	0.140	0.018	<0.001	<0.001	0.018	0.010
PC×0.150 %	0.043	0.012	0.213	0.065	0.009	<0.001	0.007	0.109
PC×0.200 %	0.007	0.536	0.666	0.104	0.353	0.394	0.193	<0.001
Statistical analysis	5'IMP	Periods	5'IMP × Periods		5'IMP	Periods	5'IMP × Periods	
Linear	0.009	0.054	0.009		0.020	<0.001	0.002	
Quadratic	0.034	0.659	0.119		0.335	0.102	0.022	

[1]Negative control (0.00 % of 5'-IMP and 3,200 kcal ME/kg).
[2]Positive control (0.00 % of 5'-IMP and 3,300 kcal ME/kg).
[3]Free radical scavenging assay at *longissimus lumborum* with different storage periods.
[4]Mensurement of malondialdehyde at *longissimus lumborum* with different storage periods.

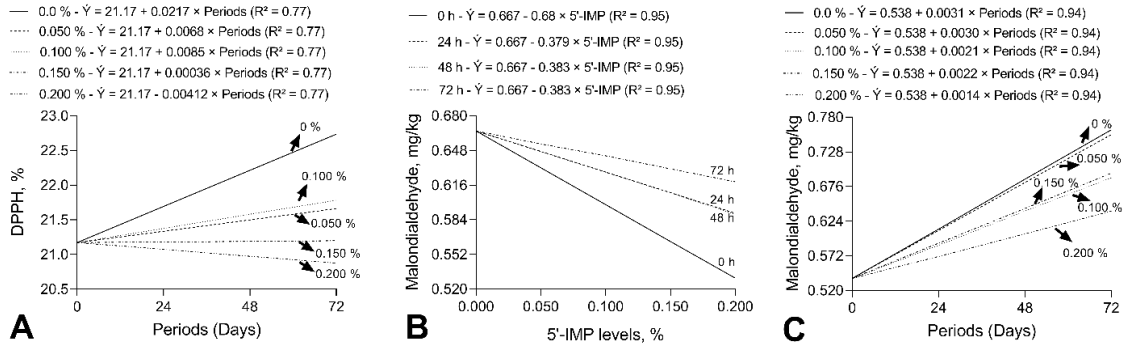

Figure 4. Effects of inosine-5'-monophosphate (5'-IMP) levels, metabolizable energy, and different storage periods on DPPH % (A) and MDA concentration (B, C) in the *longissimus lumborum* of barrows from 75 to 100 kg fed with diets containing different levels of 5'-IMP and metabolizabe energy.

Table 6. Blood plasma analysis of barrows from 75 to 100 kg fed with differnt levels of inosine-5'-monophosphate and metabolizable energy

Item	Glucose mg/dL	Lactate mg/dL	Triglycerides mg/dL	Cholesterol mg/dL	HDL, mg/dL	LDL, mg/dL	VLDL, mg/dL	Acid Uric, mg/dL	Urea, mg/dL
NC[1]	73.40	59.38	47.70	122.50	40.00	69.32	9.54	0.76	34.25
PC[2]	94.13	58.80	40.20	135.50	53.70	64.00	8.04	0.73	40.20
0.050 %	68.60	26.40	41.42	124.10	37.25	48.75	8.28	0.65	26.50
0.100 %	77.60	30.75	34.50	139.60	39.40	61.30	6.76	0.73	28.10
0.150 %	92.07	29.33	39.60	147.00	36.10	55.00	7.92	0.57	29.40
0.200 %	85.53	53.60	38.41	146.13	39.57	67.30	8.02	0.74	35.60
Mean	81.51	34.88	38.76	138.54	38.16	58.65	7.79	0.70	29.90
SD	10.280	12.720	4.060	13.990	3.470	10.030	0.837	0.120	4.740
SEM	1.713	2.120	0.677	2.331	0.578	1.672	0.139	0.020	0.791
Contrasts									
NC×PC	<0.001	0.753	0.001	0.028	<0.001	0.113	<0.001	0.532	0.038
NC×0.050 %	0.085	<0.001	0.002	0.723	0.145	<0.001	0.001	0.022	<0.001
NC×0.100 %	0.154	<0.001	<0.001	0.019	0.598	0.013	<0.001	0.533	0.003
NC×0.150 %	<0.001	<0.001	0.002	<0.001	0.054	0.004	<0.001	<0.001	0.015
NC×0.200 %	<0.001	0.196	<0.001	0.001	0.677	0.243	0.001	0.684	0.541
PC×0.050 %	<0.001	<0.001	0.106	0.058	<0.001	0.008	0.497	0.087	0.048
PC×0.100 %	<0.001	<0.001	0.569	0.612	<0.001	0.386	0.002	0.998	0.009
PC×0.150 %	0.478	<0.001	0.106	0.094	<0.001	0.030	0.734	0.002	0.006
PC×0.200 %	0.027	0.305	0.134	0.122	<0.001	0.557	0.699	0.826	0.626
Statistical analysis									
Linear	<0.001	<0.001	<0.001	<0.001	0.169	0.002	<0.001	0.947	<0.001
Quadratic	0.966	<0.001	0.001	0.461	0.173	0.001	<0.001	0.470	<0.001

[1]Negative control (0.00 % of 5'-IMP and 3,200 kcal ME/kg).
[2]Positive control (0.00 % of 5'-IMP and 3,300 kcal ME/kg).
[3]High density lipoprotein.
[4]Low density lipoprotein.
[5]Very low density lipoprotein.

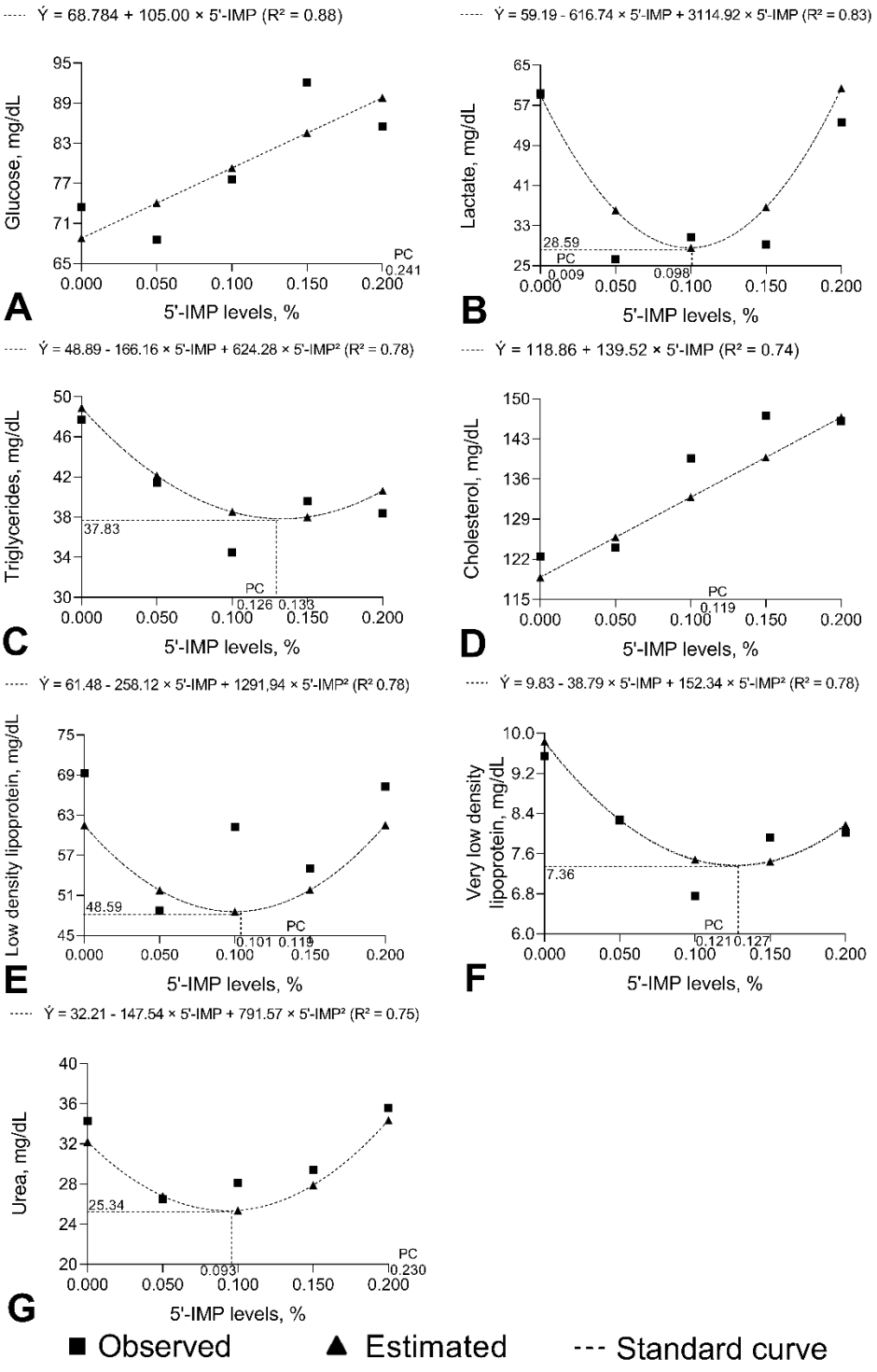

Figure 5. Effects of inosine-5'-monophosphate (5'-IMP) levels and metabolizable energy on blood plasma concentration of glucose (A), lactate (B), triglycerides (C), cholesterol (D), low density lipoprotein (E), very low density lipoprotein (F), and Urea (G) of barrows from 75 to 100 kg fed with diets containing different levels of 5'-IMP and metabolizable energy.

Table 7. Blood plasma analysis of barrows from 75 to 100 kg fed with differnt levels of inosine-5'-monophosphate and metabolizable energy

Item	Total proteins, g/dL	Albumin, g/dL	Globulin, g/dL	Albumin/Globulin[3], %
NC[1]	8.23	3.99	4.60	0.99
PC[2]	7.74	4.26	3.10	0.86
0.050 %	8.55	3.53	5.49	0.67
0.100 %	8.62	3.79	4.99	0.69
0.150 %	8.82	3.97	4.71	0.90
0.200 %	7.98	3.52	4.15	0.91
Mean	8.47	3.70	4.86	0.79
SD	0.591	0.372	0.732	0.161
SEM	0.098	0.062	0.122	0.027
Contrasts				
NC×PC	0.088	<0.001	0.556	0.145
NC×0.050 %	0.148	0.019	0.006	0.001
NC×0.100 %	0.137	0.287	0.146	0.002
NC×0.150 %	0.054	0.916	0.499	0.342
NC×0.200 %	0.433	0.016	0.350	0.379
PC×0.050 %	0.004	0.029	0.002	0.049
PC×0.100 %	0.003	0.001	0.053	0.086
PC×0.150 %	0.001	<0.001	0.222	0.615
PC×0.200 %	0.327	0.034	0.756	0.523
Statistical analysis				
Linear	0.008	0.847	0.017	0.019
Quadratic	0.004	0.917	0.003	<0.001

[1]Negative control (0.00 % of 5'-IMP and 3,200 kcal ME/kg).
[2]Positive control (0.00 % of 5'-IMP and 3,300 kcal ME/kg).
[3]Albumin:Globulin ratio.

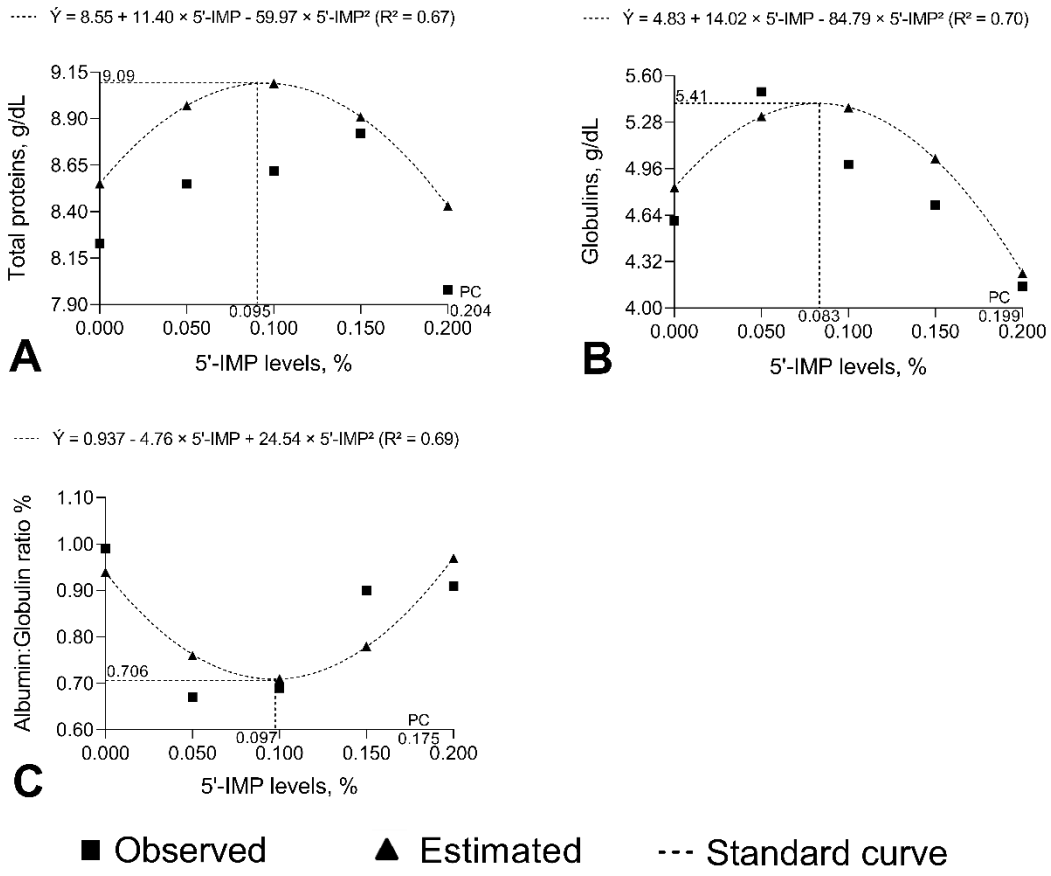

Figure 6. Effects of inosine-5-monophosphate (5'-IMP) and metabolizable energy on blood plasma concentration of total proteins (A), globulins (B), and albumin:globulin ratio (C) of barrows from 75 to 100 kg fed with diets containing different levels of 5'-IMP and metabolizabe energy.

Table 8 – Equation regressions estimated

Item	Equation quadratic estimated	R^2	IP[7]	5'-IMP
ADG, kg[1]	$\dot{Y} = 1.15 + 2.11 \times 5'\text{-IMP} - 7.61 \times 5'\text{-IMP}^2$	0.75	1.30	0.139
pH 24 hou5rs	$\dot{Y} = 5.52 + 2.30 \times 5'\text{-IMP} - 9.81 \times 5'\text{-IMP}^2$	0.69	5.65	0.117
Drip loss, %	$\dot{Y} = 4.24 + 24.96 \times 5'\text{-IMP} - 123.92 \times 5'\text{-IMP}^2$	0.59	5.50	0.101
Lactate, mg/dL	$\dot{Y} = 59.19 - 616.74 \times 5'\text{-IMP} + 3114.92 \times 5'\text{-IMP}^2$	0.78	28.59	0.098
Triglycerides, mg/dL	$\dot{Y} = 48.89 - 166.16 \times 5'\text{-IMP} + 624.98 \times 5'\text{-IMP}^2$	0.88	37.83	0.133
LDL, mg/dL[2]	$\dot{Y} = 61.48 - 258.12 \times 5'\text{-IMP} - 1291.94 \times 5'\text{-IMP}^2$	0.78	48.59	0.101
VLDL, mg/dL[3]	$\dot{Y} = 9.83 - 38.79 \times 5'\text{-IMP} + 152.34 \times 5'\text{-IMP}^2$	0.78	7.36	0.127
Urea, mg/dL	$\dot{Y} = 32.21 + 147.54 \times 5'\text{-IMP} - 791.57 \times 5'\text{-IMP}^2$	0.75	25.34	0.093
Total protein, g/dL	$\dot{Y} = 8.55 + 11.40 \times 5'\text{-IMP} - 59.97 \times 5'\text{-IMP}^2$	0.67	9.09	0.095
Globulin, g/dL	$\dot{Y} = 4.83 + 14.02 \times 5'\text{-IMP} - 84.79 \times 5'\text{-IMP}^2$	0.70	5.41	0.083
Alb/Glob, %[4]	$\dot{Y} = 0.937 - 4.76 \times 5'\text{-IMP} + 24.54 \times 5'\text{-IMP}^2$	0.69	0.706	0.097
	Equations linear estimated			
F:G, kg/kg[5]	$\dot{Y} = 2.87 - 1.85 \times 5'\text{-IMP}$	0.80	-	-
BF (P1P2P3), cm[6]	$\dot{Y} = 2.16 - 0.68 \times 5'\text{-IMP}$	0.70	-	-
pH 45 minutes	$\dot{Y} = 6.24 + 1.26 \times 5'\text{-IMP}$	0.78	-	-
Redness	$\dot{Y} = 6.62 + 3.32 \times 5'\text{-IMP}$	0.78	-	-
Glucose, mg/dL	$\dot{Y} = 68.784 + 105.00 \times 5'\text{-IMP}$	0.68	-	-
Cholesterol, mg/dL	$\dot{Y} = 118.86 + 139.52 \times 5'\text{-IMP}$	0.74	-	-

[1]Average daily gain.
[2]Low density lipoprotein.
[3]Very low density lipoprotein.
[4]Albumin/Globulin ratio.
[5]Feed:Gain ratio.
[6]Backfat thickness measured at three different points (P1P2P3) at carcass of barrows.
[7]Inflection point.

II - A suplementação dietética de inosina-5’-monofosfato melhora o *status* funcional, energético e antioxidante do fígado e a disponibilidade de creatina muscular de suínos.

Resumo. A inosina 5'-monofosfato (5'-IMP) é um nucleotídeo essencial para a biossíntese de novo de nucleotídeos, metabolismo de energia, proteínas e antioxidantes. Os nucleotídeos são condicionalmente essenciais, pois não podem ser produzidos em taxas suficiente para atender às necessidades do corpo em situações de estresse oxidativo ou rápido crescimento muscular. Uma ingestão deficiente de nucleotídeos pode resultar na redução da síntese de ATP e GTP e prejudicar a eficiência metabólica. Demonstramos que a suplementação de dietas de suínos em terminação com 5'-IMP reduz o peso relativo do fígado e aumenta o consumo de oxigênio durante a respiração mitocondrial sem alterar a relação ADP/O, indicando aumento na eficiência respiratória das mitocôndrias hepáticas. Também observamos redução da peroxidação lipídica hepática e aumento da creatina muscular. Adicionalmente, a suplementação de 5'-IMP aumentou o peso de abate, rendimento de carne magra, comprimento do sarcômero e espessura de toucinho em suínos machos castrados em fase de terminação, demonstrando infuência no metabolismo protéico e energético. Sugerimos que a suplementação de 5'-IMP aumenta a capacidade respiratória mitocondrial quando a atividade metabólica hepática é estimulada, bem como a defesa antioxidante e promove o crescimento muscular em suínos machos castrados em terminação.

II - Dietary supplementation with inosine-5′-monophosphate improves the functional, energetic, and antioxidant status of liver and muscle growth in pigs

Abstract. Inosine 5′-monophosphate (5′-IMP) is an essential nucleotide for de novo nucleotide biosynthesis and metabolism of energy, proteins, and antioxidants. Nucleotides are conditionally essential, as they cannot be produced sufciently rapidly to meet the needs of the body in situations of oxidative stress or rapid muscle growth. A defcient intake of nucleotides can result in decreased ATP and GTP synthesis and impaired metabolism. We demonstrated that supplementation of fnishing pig diets with 5′-IMP reduces the relative weight of the liver, and increases oxygen consumption during mitochondrial respiration without changing the ADP/O ratio, indicating an increase in the respiratory efciency of liver mitochondria. We also observed a reduction in liver lipid peroxidation and an increase in muscle creatine. Moreover, 5′IMP supplementation increases slaughter weight, lean meat yield, sarcomere length, and backfat thickness in fnishing barrows, demonstrating infuence on protein metabolism. We suggest that 5′-IMP supplementation increase the mitochondrial respiratory capacity when the liver metabolic activity is stimulated, enhances antioxidant defense, and promotes muscle growth in fnishing barrows.

1. Introduction

Oxidative phosphorylation, the main pathway for the synthesis of energy in the form of ATP, takes place in the inner membrane of mitochondria via the electron transport chain[1,2]. Complexes I, III, IV, and V (ATP synthetase) are essential for oxidative phosphorylation. The electrons of complexes I and III react with oxygen molecules to form superoxide radicals, which are responsible for the generation of reactive oxygen species (ROS). In excess, ROS are known to cause damage to mitochondrial DNA[3].

Mitochondrial DNA is susceptible to oxidation because of the frequent exposure to ROS produced during oxidative phosphorylation and the deficiency of antioxidant molecules in the inner membrane of mitochondria[4,5]. Thus, the presence of antioxidant molecules that can be converted into ADP and subsequently into ATP may promote benefits to oxidative phosphorylation and help repair ROS-induced damage to mitochondrial DNA. An important molecule with such characteristics is the nucleotide inosine 5′-monophosphate (5′-IMP). 5′-IMP can be converted to ADP or GDP and, subsequently, to ATP or GTP[6,7]. There is increasing evidence that 5′-IMP plays an important role in DNA repair, as observed in the deamination of adenosine to inosine, which depends on the actions of the adenosine deaminase family of enzymes on RNA[8]. Another important function of 5′-IMP is in mTORC1 complex that serves as a link between energy, nutrient levels and anabolic processes[9]

Given the importance of 5′-IMP to metabolism, it is possible that dietary supplementation of finishing barrows with 5′-IMP may influence several metabolisms. We hypothesized that diets supplemented with 5′-IMP may promote beneficial additive effects on antioxidant, protein and energy metabolism of finishing barrows. This study aimed to investigate the effects of low- (3200 kcal ME/kg) and high-energy (3300 kcal ME/kg) diets without 5′-IMP with those of low-energy diets (3200 kcal ME/kg) containing varying levels of 5′-IMP on liver antioxidant status, respiratory activity of isolated liver mitochondria, plasma concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), plasma and muscle levels of creatine, carcass traits, and sarcomere length in *longissimus lumborum* muscle in finishing pigs (75–100 kg).

2. Results

2.1. Relative weight of the liver.

Tables 2 and 6 describe the effects of control (negative and positive) and 5′-IMP-supplemented diets on relative weight of liver. Dietary supplementation with 5′-IMP influenced the relative weight of the liver ($p < 0.05$) (Table 5). Orthogonal contrast analysis of relative liver weights showed that supplementation with 0.150 and 0.200% 5′-IMP decreased ($p \leq 0.05$) the variable compared with CN and PC diets. Furthermore, we observed that the relative weight of the liver decreased linearly ($p < 0.001$) with increasing 5′-IMP supplementation levels (Fig 2A).

2.2. Mitochondrial respiratory activity.

Tables 2 and 6 describe the effects of control (negative and positive) and 5′-IMP-supplemented diets on basal, III, and IV state respiration rates, respiratory control, and ADP/O ratio of liver mitochondria incubated with succinate or α-ketoglutarate. Fig. 1 depicts the experimental approach used to assess the respiratory activity of liver mitochondria.

The basal respiration rate of liver mitochondria incubated with succinate did not differ significantly ($P > 0.05$) between treatments (Table 2). By contrast, in medium containing α-ketoglutarate, basal respiration rate was higher in mitochondria from pigs fed 0.050% 5′-IMP than in those from pigs fed negative control ($P = 0.015$) or positive control ($P = 0.005$) diets, but no differences were observed between control treatments ($P = 0.625$), as shown in Table 2.

As depicted in Fig. 2B, the relationship between basal oxygen consumption in α-ketoglutarate medium and 5′-IMP level was quadratic ($P = 0.022$). The highest basal oxygen consumption (6.27) was estimated to be achieved by supplementation with 0.083% 5′-IMP.

No differences in state III respiration rates were observed between control treatments for mitochondria incubated with succinate ($P = 0.721$) or α-ketoglutarate ($P = 0.295$). However, the respiration rate of liver mitochondria from pigs supplemented with 0.200% 5′-IMP was higher than that of mitochondria from negative ($P = 0.003$) and positive ($P < 0.001$) control pigs (Table 2). Compared with the negative control diet, dietary 5′-IMP supplementation increased state III respiration rate ($P = 0.038$) in mitochondria incubated with α-ketoglutarate (Table 2).

The relationship between mitochondrial state III respiration rate in succinate medium and dietary 5′-IMP level was well explained by a quadratic model ($P = 0.020$) (Fig. 2C). The lowest respiration rate was estimated to be achieved by supplementation with 0.055% 5′-IMP and the highest rate (66.77%), with 0.200% 5′-IMP. Similarly, in α-ketoglutarate medium the state III respiration rate of liver mitochondria had a quadratic response ($P < 0.001$) to 5′-IMP supplementation (Fig. 2D). The lowest respiration rate was estimated to be achieved by supplementation with 0.075% 5′-IMP and the highest rate (34.37%), with 0.200% 5′-IMP

No differences ($P > 0.05$) in state IV respiration rate were observed between treatments, regardless of incubation medium (Table 2). In succinate medium, respiration rate did not differ between negative and positive control diets ($P = 0.161$) but was higher ($P < 0.05$) with 0.050% and 0.200% 5′-IMP supplementation (Table 2). Only 0.200% 5′-IMP supplementation differed from the negative control diet ($P = 0.005$) with regard to state IV respiration rate in α-ketoglutarate medium.

As depicted in Fig. 2E and 2F, the respiratory control in succinate and in α-ketoglutarate medium increased linearly ($P < 0.050$) with 5′-IMP supplementation level. Thus, the highest respiratory control in the presence of succinate or α-ketoglutarate was also achieved with 0.200% 5′-IMP supplementation. ADP/O ratios did not differ between treatments ($P > 0.05$), regardless of incubation medium (Table 2).

2.3. Liver antioxidant activity.

Tables 3 and 6 show the differences between experimental diets and the effects of 5′-IMP supplementation level on 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging activity and malondialdehyde (MDA) concentration in liver tissues. The liver $DPPH^{\bullet}$ scavenging activity of pigs fed the negative control diet was higher ($P < 0.001$) than that of pigs fed the positive control diet ($P \leq 0.05$) or diets supplemented with 0.050%, 0.100%, and 0.150% 5′-IMP. The positive control diet resulted in a lower ($P < 0.001$) $DPPH^{\bullet}$ scavenging activity than the 0.200% 5′-IMP-supplemented diet. $DPPH^{\bullet}$ activity was shown to have a quadratic relationship with 5′-IMP level ($P < 0.001$): the lowest activity (23.10%) was predicted to occur with 0.101% 5′-IMP supplementation (Fig. 3A).

MDA concentration in the liver of pigs fed the negative control diet was lower ($P < 0.001$) than in pigs fed the positive control diet. However, the negative control diet

resulted in a higher ($P \leq 0.05$) MDA level than 5′-IMP-supplemented diets, except for the diet containing 0.200% 5′-IMP ($P = 0.666$). Pigs fed the positive control diet showed higher liver MDA concentrations ($P < 0.001$) than those supplemented with 5′-IMP, except for those supplemented with 0.200% 5′-IMP ($P = 0.289$). MDA concentration in the liver had a quadratic response ($P < 0.001$) to 5′-IMP supplementation (Fig. 3B). The lowest MDA concentration (1.29 mg/kg) was estimated to be reached with 0.097% 5′-IMP supplementation.

2.4. AST and ALT levels in plasma and creatine levels in plasma and m. longissimus lumborum.

No differences ($P > 0.05$) were observed in AST, ALT, or creatine levels (in plasma or muscle) between pigs fed negative and positive control diets (Table 4). Plasma ALT was lower ($P \leq 0.05$) in pigs fed control diets than in pigs supplemented with 5′-IMP. Similarly, plasma AST was lower ($P = 0.012$) in pigs fed the negative control diet than in animals supplemented with 0.200% 5′-IMP, but there were no differences ($P > 0.05$) between the positive control and 5′-IMP-supplemented diets.

The negative control diet resulted in lower ($P = 0.037$) plasma creatine levels than the diet supplemented with 0.050% 5′-IMP. There were no differences ($P > 0.05$) in plasma ALT between positive control and 5′-IMP-supplemented diets. All levels of 5′-IMP supplementation increased muscle creatine levels compared with control diets ($P \leq 0.05$). Furthermore, as shown in Fig. 4, muscle creatine increased linearly ($P < 0.001$) with 5′-IMP supplementation level. Thus, the highest muscle creatine concentration was achieved with 0.200% 5′-IMP supplementation.

2.5. Carcass traits.

No differences ($P > 0.05$) in slaughter weight were observed between pigs fed negative and positive control diets (Table 5). The slaughter weight of pigs fed negative or positive control diets was lower ($P = 0.004$ and $P = 0.037$, respectively) than that of pigs fed the diet supplemented with 0.150% 5′-IMP. Slaughter weight had a quadratic response ($P < 0.015$) to 5′-IMP supplementation (Fig. 5A). The highest slaughter weight (100.09 kg) was estimated to be reached with 0.138% 5′-IMP supplementation.

The lean meat yield of pigs fed the negative control diet was lower ($P < 0.001$) than that of pigs fed the positive control diet or 5′-IMP-supplemented diets ($P \leq 0.05$). The positive control diet resulted in a lower ($P < 0.020$) lean meat yield than the 0.150% 5′-IMP-supplemented diet (Table 5). Lean meat yield was shown to have a quadratic relationship with 5′-IMP level ($P < 0.001$): the highest yield (58.79%) was predicted to occur with 0.148% 5′-IMP supplementation (Fig. 5B).

The *m. longissimus lumborum* depth of pigs did not differ significantly ($P > 0.05$) between treatments (Table 5). The backfat thickness of pigs fed the negative control diet was lower ($P = 0.002$) than that of pigs fed the positive control diet or diets supplemented with 0.050%, 0.100%, or 0.150% 5′-IMP ($P = 0.003$, $P = 0.009$, and $P = 0.025$, respectively). There were no differences ($P > 0.05$) in backfat thickness between positive control and 5′-IMP-supplemented diets (Table 5). Furthermore, as shown in Fig. 5D, backfat thickness had a quadratic response ($P < 0.001$) to 5′-IMP supplementation. The highest backfat thickness (1.37 cm) was estimated to be reached with 0.108% 5′-IMP supplementation.

2.6. Sarcomere length in m. longissimus lumborum.

Sarcomere length in *longissimus lumborum* was measured at 45 min and 24 h after slaughter. Pigs fed the negative control diet showed a higher sarcomere length at 45 min after slaughter ($P = 0.045$) than pigs fed the positive control diet (Table 5). However, the negative control diet resulted in a lower ($P \leq 0.05$) sarcomere length at 45 min than diets supplemented with 0.150% or 0.200% 5′-IMP. Similarly, sarcomere length was lower in pigs fed the positive control diet ($P \leq 0.05$) than in animals fed 5′-IMP-supplemented diets. Sarcomere length at 45 min increased linearly ($P = 0.004$) with 5′-IMP supplementation level (Fig. 5C). The highest sarcomere length at 45 min after slaughter was estimated to be reached with 0.200% 5′-IMP supplementation.

At 24 h after slaughter, sarcomere length in *longissimus lumborum* was higher in pigs fed the negative control diet ($P < 0.001$) than in pigs fed the positive control or the 0.200% 5′-IMP-supplemented diet ($P = 0.025$). However, the positive control diet resulted in a lower ($P \leq 0.05$) sarcomere length at 24 h than 0.050%, 0.100%, and 0.150% 5′-IMP-supplemented diets (Table 5).

3. Discussion

The largest de novo biosynthesis site of 5′-IMP is the liver. In this organ, 5′-IMP participates in several biochemical processes, such as ischemia[6], cell growth and proliferation[7,18] and regulation of insulin and glucose metabolism[10,41].

Previous studies have shown that 5′-IMP supplementation influences the relative weight of the liver. In assessing dietary supplementation of newborn rats with NT, Novak et al.[11] observed a reduction in the relative liver weight of rats fed the NT diet compared with unsupplemented animals. The same effect on the relative weight of the liver was reported in broilers fed diets supplemented with 5′-IMP and allopurinol[12].

The reduction in the relative weight of the liver in different species corroborates the linear reduction in this parameter with increasing 5′-IMP supplementation levels observed in the current study in finishing pigs. These findings suggest that 5′-IMP supplementation enhances liver functionality and minimizes metabolic disorders that could increase mitochondrial oxidation and apoptosis in hepatic tissues.

Oxidative phosphorylation, the main ATP synthesis pathway, takes place in the electron transport chain located in the inner membrane of mitochondria[1,2]. The process depends on the energy released by oxidation of carbohydrates, lipids, and peptides, which generates a proton gradient throughout the inner mitochondrial membrane, used for ATP synthesis[13]. The respiration rate of liver mitochondria is associated with the rate of ATP synthesis[14]. During oxidative phosphorylation, ROS are generated by complexes I and III, leading to excess production of these substances. Thus, mitochondria and mitochondrial DNA are exposed to oxidation, causing metabolic dysfunction and further increasing ROS synthesis[15,16].

5′-IMP, a precursor of other nucleotides, can be metabolized to adenosine monophosphate (AMP) or guanosine monophosphate (GMP) and subsequently converted to ATP or GTP, respectively, according to the metabolic needs of the body[17,18]. ATP is the main energy carrier for metabolic activities; various metabolic processes are benefited by high concentrations of ATP[19–21]. GTP is the second most important energy molecule for cellular activities. High GTP concentrations are associated with gene expression and enzymatic activities responsible for the growth and proliferation of immune cells, protein synthesis, and DNA synthesis[22–24].

5′-IMP supplementation is important in situations of rapid muscle growth and oxidative stress, such as occurs in finishing pigs. Under these conditions, the amount of 5′-IMP synthesized by the body is not sufficient to meet metabolic requirements[7]. We

hypothesized that dietary supplementation of an exogenous 5′-IMP source could reduce ATP and GTP consumption in de novo biosynthesis of 5′-IMP (seven molecules of ATP and one of GTP are needed to synthesize one molecule of 5′-IMP) and stimulate the recovery pathway of 5′-IMP and other nucleotides, such as ATP and GTP[25,26]. These effects are relevant, given that intracellular concentrations of ATP and GTP control major metabolic activities and may influence oxidative phosphorylation, ROS-mediated oxidation of mitochondrial DNA, and anabolism.

In this study, oxidative phosphorylation of liver mitochondria was assessed by determining mitochondrial respiration rate, respiratory control, and ADP/O ratio. Supplementation of finishing pig diets with 5′-IMP, particularly at 0.200%, increased state III (after ADP addition) mitochondrial respiration rate in the presence of either substrate (succinate or α-ketoglutarate) compared with positive and negative control diets.

State III respiration rate in succinate medium was influenced by 5′-IMP level: the highest rate (66.77 nmol/mg) was estimated to be achieved by using 0.200% 5′-IMP. Similarly, in the α-ketoglutarate medium, state III respiration rate the highest rate (34.37 nmol/mg) was estimated to be achieved by using 0.200% 5′-IMP, thus 0.200% 5’-IMP supplementation provided the highest liver mitochondrial respiration rate regardless of incubation medium.

The fact that the ADP/O ratio was not altered in state III indicates that there was an increase in the respiratory capacity of liver mitochondria. It is likely that ADP stimulated liver metabolic activity, thereby increasing ATP production from ADP and, consequently, oxygen consumption[27]. Thus, finishing pigs supplemented with 5′-IMP exhibited higher ADP-stimulated mitochondrial respiration efficiency.

Mitochondrial respiration was associated with higher efficiency of oxidative phosphorylation when ADP was present at higher concentrations, that is, in the face of work overload. Such a condition is similar to that occurring in finishing pigs from new genetic lines. The results suggest that dietary supplementation of 5′-IMP might have benefited oxidative phosphorylation, an important route for ATP synthesis.

ROS are formed in mitochondria, mainly in the basal complex and complex III of the electron transport chain[28]. ROS are well known to be involved in oxidative stress and lipid peroxidation[29]. In assessing the antioxidant status in the liver of finishing pigs, we found that diets containing 0.050, 0.100, and 0.150% 5′-IMP were less effective in promoting the removal of free radicals compared with the negative control diet and resulted in lower liver MDA concentrations than negative and positive control diets.

DPPH• scavenging activity and MDA concentration decreased quadratically as a function of 5′-IMP supplementation level. The minimum inflection points were estimated to be reached by supplementation with 0.101% (DPPH• activity) and 0.097% 5′-IMP (MDA level). The fact that the lowest values of both variables were estimated at similar 5′-IMP concentrations is interesting, given that a reduction in DPPH• scavenging activity is expected to lead to an increase in ROS-mediated lipid peroxidation, affording degradation metabolites such as MDA. The simultaneous decrease in MDA concentration with 5′-IMP supplementation suggests that ROS were formed in smaller amounts during mitochondrial respiration, particularly during state III respiration. Such a reduction in ROS production might be related to the higher liver mitochondrial respiration rate and improved respiratory control, when the ADP is added, i.e., to higher oxygen uptake to convert ADP in ATP, a condition that dissipates the mitochondrial proton gradient, increases the mitochondrial flow of electrons and prevent the ROS generation.

This hypothesis was tested in previous research. Jing et al.[30] used genetically modified mice lacking the sirtuin-3 gene to investigate oxygen consumption and oxidative stress. The authors reported that genetically modified mice showed lower oxygen consumption and increased oxidative stress. In a similar line of research, Heise et al.[20] found that ROS production correlated negatively with respiratory control.

The reduction in MDA concentration and DPPH• scavenging activity in the liver of finishing pigs may also be partially explained by the fact that mitochondria and mitochondrial DNA are exposed to oxidation by ROS, which are generated by complexes I and III. Supplementation with 5′-IMP might have contributed to antioxidant defense against ROS, as 5′-IMP and other nucleotides act directly in DNA repair and replication[31].

The higher state III respiration rate and respiratory control of liver mitochondria might be associated with the altered antioxidant status of the liver. We measured plasma concentrations of ALT and AST as indicators of liver functional status[32]. Plasma ALT and AST are tests for the detection of hepatocellular injury in most animal species, but in non-rodents as pigs, ALT and AST also are associated with metabolic adaptation[33]. ALT is more specific and sensitive biomarker than AST for hepatocellular injury, when aminotransferase activities are increased as a consequence of hepatotoxicity the concentration of the ALT increase is usually greater than AST[33]. In the present study, diets 5'-IMP-supplemented showed higher ($P \leq 0.05$) plasma ALT concentration than pigs fed with control diets, but the differences between concentrations were too low to be related to hepatocellular damage, even more, that the plasma ALT was lower than AST,

indicating a normal liver functionality and possible interaction between plasma ALT with the metabolic adaption to 5'-IMP supplementation in diets of finishing pigs.

Creatine is a metabolite used as an energy substrate for muscle growth[34]. In the current study, plasma creatine levels were higher with 0.050% 5′-IMP supplementation than with the negative control diet. Supplementation of diets with 0.100%, 0.150%, and 0.200% 5′-IMP increased muscle creatine level compared with the negative diet, and all 5′-IMP-supplemented diets enhanced muscle creatine compared with the positive control diet.

Creatine concentration increased linearly with 5′-IMP supplementation level; the highest creatine level was estimated to be reached by supplementation with 0.200% 5′-IMP. These findings indicate that 5′-IMP influenced protein synthesis, promoting an increase in muscle mass and slaughter weight (as observed in pigs supplemented with 0.150% 5′-IMP compared with control diets). Muscle creatine levels are proportional to muscle mass and lean meat yield[35].

In this study, the increase in muscle creatine concentration might have directly contributed to the increase in lean meat yield in pigs fed 5′-IMP-supplemented diets compared with pigs fed the negative control diet. Furthermore, pigs supplemented with 0.150% 5′-IMP showed a higher lean meat yield than pigs fed the positive control diet, even though the supplemented diet had a 100 kcal ME/kg lower energy level than the positive control diet. These results suggest that 5′-IMP supplementation promotes an increase in energy and protein synthesis. The increase in muscle creatine level also reflected on sarcomere length at 45 min after slaughter. Pigs fed diets supplemented with 0.150 and 0.200% 5′-IMP had higher sarcomere lengths than pigs fed negative or positive control diets.

The increase in muscle and plasma creatine levels can be explained by the activity of 5′-IMP on the mTOR complex. A previous study showed that 5′-IMP and other purines acted on this complex, stimulating anabolism[9,18]. Another possible explanation is the improvement in oxidative phosphorylation efficiency promoted by supplementation with 0.200% 5′-IMP, which might have increased creatine availability for metabolic pathways.

In this study, we evaluated the following quantitative carcass and *m. longissimus lumborum* quality parameters: slaughter weight, lean meat yield, muscle depth, sarcomere length in *m. longissimus lumborum* at 45 min and 24 h after slaughter, and backfat thickness. Lean meat yield is the main and most practical quantitative carcass parameter; it is an important index to assess the amount of lean meat in carcasses, contributing to the

development of new swine genotypes[36]. *M. longissimus lumborum* is one of the most appreciated cuts by consumers, having high commercial value. Backfat thickness is measured in the P2 region and represents the amount of fat between *m. longissimus lumborum* and the skin. Sarcomere length in *longissimus lumborum* has been associated with muscle tenderness in several studies[37,38,39]. The shorter the sarcomere length, the less tender the meat, a characteristic that indicates low muscle quality.

In comparing negative and positive control diets, we observed that, although there were no differences in slaughter weight, the lean meat yield of pigs fed the positive control diet was higher than that of pigs fed the negative control diet. This finding demonstrated that the lower ME level of the negative control diet negatively influenced carcass lean meat deposition. The negative control diet (3200 kcal ME/kg) resulted in longer sarcomere lengths at 45 min and 24 h after slaughter compared with the positive control diet (3300 kcal ME/kg); however, the negative control diet afforded a lower backfat thickness than the positive control diet. The positive control diet, which had a 100 kcal ME/kg higher energy level, provided greater energy input, part of which was used to increase protein and muscle deposition, thereby increasing lean meat yield. Surplus energy was stored in the form of lipids in adipocytes, promoting an increase in backfat thickness.

5′-IMP-supplemented diets had a lower level of ME (3200 kcal ME/kg). Pigs fed the 0.150% 5′-IMP-supplemented diet had the highest slaughter weight, higher than that of pigs fed control diets. Slaughter weight showed a quadratic response to 5′-IMP level; the highest yield (100.09 kg) was estimated to be reached with 0.138% 5′-IMP. Similarly, pigs fed 5′-IMP-supplemented diets had a higher lean meat yield than pigs fed the negative control diet, and pigs supplemented with 0.150% 5′-IMP had higher lean meat yield than pigs fed the positive control diet (3300 kcal ME/kg). Lean meat yield showed a quadratic response to 5′-IMP level; the highest yield (58.79 %) was estimated to be reached with 0.148% 5′-IMP.

The higher slaughter weight and lean meat yield of pigs supplemented with 0.150% 5′-IMP can be explained by the increase in muscle creatine concentration promoted by supplementation. Another possible explanation is the conversion of 5′-IMP to ATP and GTP, the main energy sources for cellular activities, influencing protein synthesis through the action of ATP and ATPases[6,7] or by the relationship between GTP and the mTORC complex[19].

Sarcomere length at 45 min after slaughter was higher in pigs supplemented with 0.150% and 0.200% 5′-IMP than in pigs fed the negative control diet. Similarly, the parameter was higher in pigs fed 5′-IMP-supplemented diets than in pigs fed the positive control diet. A diet containing 0.200% 5′-IMP was estimated to result in the longest sarcomere length (1.769 μm). Similarly, sarcomere length at 24 h after slaughter increased with 0.050%, 0.100%, and 0.150% 5′-IMP supplementation compared with the positive control diet.

The greater sarcomere lengths at 45 min and 24 h after slaughter in pigs supplemented with 0.150% and 0.200% 5′-IMP can be explained in part by the higher muscle creatine concentration proportionated by 5'-IMP supplementation. The highest availability of creatine may stimulates muscle growth, which, during postmortem aging, increases the availability of energetic substrates, thereby prolonging the time until ATP begins to be consumed.

It is also known that 5′-IMP participates in the dissociation of the actin–myosin complex during the first 24 h of postmortem aging, an effect previously observed in processed[40] and fresh[41] pork meat. According to Miller[42], the overlap between actin and myosin is smaller in longer sarcomeres, resulting in reduced resistance when cutting the fibers of the *m. longissimus lumborum*.

Backfat thickness results showed that dietary 5′-IMP supplementation combined with low ME level provided an increase in energy input, given that pigs supplemented with 0.050%, 0.100%, and 0.150% 5′-IMP showed greater backfat thickness than pigs fed the negative control diet. Such a greater energy input was evidenced by the lack of differences in backfat thickness between pigs fed 5′-IMP diets and animals fed the positive control diet, which had a higher energy level (3200 vs 3300 kcal ME/kg). Furthermore, we observed a quadratic response in backfat thickness with 5′-IMP level supplementation. The increase in energy input with 5′-IMP supplementation might be associated with the conversion of 5′-IMP to ATP and GTP, the main energetic molecules for cellular activities[6,7]. The reduction in backfat thickness after the supplementation with 0.108% 5'-IMP demonstrated by the quadratic response, on the other hand, might have occurred as a result of the activity of IMP dehydrogenase (IMPDH) in lipid deposition.

According to Whitehead et al.[43], insulin stimulates phosphorylation and translocation of IMPDH to adipose tissues; both processes are blocked by inhibition of the insulin substrate receptor phosphatidylinositol 3-kinase. Oleic acids stimulate IMPDH translocation only, and inhibition translocation of IMPDH to adipose tissues results in

lower amounts of lipids. Thus, the authors concluded that IMPDH exerts regulatory and dynamic roles in lipid deposition and fatty acid metabolism.

The results of this study demonstrated that dietary 5′-IMP supplementation promoted benefits to oxidative phosphorylation by increasing mitochondrial respiration rate without altering the ADP/O ratio. Demonstrating that with the liver metabolic activity stimulated, process in which the energy is required to supply an increased metabolism, the animals supplemented with 5´-IMP presented a higher efficiency of mitochondrial oxidative phosphorylation to support the energy requirement. Supplementation also reduced liver tissue oxidation and increased plasma and muscle creatine levels, slaughter weight, lean meat yield, sarcomere length at 45 min and 24 h after slaughter, and backfat thickness in finishing barrows.

The influence of 5′-IMP supplementation on slaughter weight, lean meat yield and backfat thickness as compared with negative and positive control diets allowed us to infer that dietary 5′-IMP supplementation increased energy availability and protein synthesis through the possible use of 5'-IMP as an energy and protein additive. Further studies are needed to identify the mechanisms by which 5′-IMP produced these effects.

4. Conclusion

Supplementation of finishing pig diets with different levels of 5′-IMP increased the respiratory efficiency of liver mitochondria, reduced lipid peroxidation, and enhanced muscle creatine levels, slaughter weight, lean meat yield, sarcomere length at 45 min and 24 h after slaughter, and backfat thickness, demonstrating that 5′-IMP supplementation contributes to several metabolic processes, mainly those of energy and protein synthesis.

5. Material and methods

5.1. Animal ethics statement.

The experiment was conducted at the Pig Farming Section of the Iguatemi Experimental Farm, State University of Maringá, Brazil. All animal experimental procedures were approved by the Animal Ethics Committee of the State University of Maringá (protocol No. 9056170220).

Animal care and use standards were based on the National Council for the Control of Animal Experimentation (https://antigo.mctic.gov.br/mctic/opencms/institucional/concea/paginas/legislação.html). Study design, animal experiments, and reporting followed the ARRIVE guidelines (https://arriveguidelines.org/arrive-guidelines).

5.2. Facilities, animals, and experimental design.

Pigs were housed in a barn covered with fiber cement tiles and divided into 40 pens (1.88 m^2 each) with cement floor. Each pen was equipped with a semi-automatic feeder at the front and a nipple drinker at the back. The animals had ad libitum access to water and feed throughout the experimental period.

A total of 54 castrated male pigs (mean initial weight of 75.62 ± 0.96 kg and mean final weight of 102.26 ± 3.23 kg) were distributed in a randomized complete block design throughout the trial period (75 to 100 kg) with nine blocks and six treatments. Each block the animal are randomly assigned to the treatment and was considered an experimental unit.

5.3. Diet.

Pigs were fed one of the following six experimental diets: a positive control diet containing 3300 kcal metabolizable energy (ME)/kg, a negative control diet containing 3200 kcal ME/kg, or the negative control diet supplemented with 0.050, 0.100, 0.150, or 0.200% 5′-IMP. Experimental diets were composed of corn, soybean meal, minerals, vitamins, and additives (Table 1) and were formulated to meet the nutritional requirements of pigs according to National Research Council guidelines[44], except for ME.

5.4. Slaughter procedures.

At the end of the experiment, pigs were fasted for 24 h and weighed to obtain the slaughter weight. Slaughter was carried out at the slaughterhouse of the Iguatemi Experimental Farm. The animals were slaughtered by exsanguination after electrical stunning (200 W). Pig carcasses were scalded in water (60 °C), dehaired, singed, washed, eviscerated, split into two, weighed, and stored in a cold room (0.5 ± 1.0 °C) for 24 h.

5.5. Relative weight of the liver.

Following evisceration, nine livers per treatment (n = 56) were weighed and used to calculate the relative weight of the liver by the following equation: Relative weight of the liver = Liver weight × 100/Body weight after fasting.

5.6. Collection of liver samples.

After slaughter, specimens (15 g) from the medial segment of the liver were excised from six pigs per treatment (n = 36) and promptly analyzed for mitochondrial respiration. For determination of oxidative stress, specimens (15 g) from the medial segment of the liver were collected from nine pigs per treatment (n = 54), frozen at −20 °C until the end of the slaughter process, and taken to the laboratory for extraction and analysis.

5.7. Isolation of liver mitochondria.

Liver specimens were immediately immersed in cold buffer containing 200 mM mannitol, 75 mM sucrose, 0.2 mM ethylene glycol tetraacetic acid, 2 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl, pH 7.4), and 50 mg bovine serum albumin. A Dounce-type homogenizer was used to lyse cells, and mitochondria were isolated by differential centrifugation[45].

5.8. Mitochondrial respiratory activity.

Mitochondrial oxygen consumption was measured polarographically by using a Teflon-coated platinum electrode[45]. Mitochondria were incubated in a closed-chamber oxygraph in medium (2.0 mL) containing 0.25 M mannitol, 5 mM sodium diphosphate, 10 mM KCl, 0.2 mM EDTA, and 10 mM Tris-HCl (pH 7.4). Succinate and α-ketoglutarate (both at 10 mM) were used as electron donor substrates for complexes I and II, respectively, of the mitochondrial electron transport chain. ADP (final concentration of 0.125 mM) was added at predetermined times. Oxygen consumption rates were calculated from the slope of oxygen consumption plots generated on paper by the recording system. Results are expressed in nmol min^{-1} mg^{-1} protein. Oxygen

consumption was measured under three conditions: (i) before ADP addition (basal or substrate respiration), (ii) shortly after ADP addition (state III respiration), and (iii) after cessation of ADP stimulation (state IV respiration). Respiratory control was calculated as the ratio of oxygen consumption in state III to state IV. The ADP/O ratio was determined as described by Chance and Williams[46].

5.9. Sample preparation for antioxidant analysis.

For antioxidant analysis by DPPH• assay, liver samples (5 g) were mixed with methanol (15 mL), homogenized with an Ultra Turrax for 1 min, and filtered through qualitative filter paper No. 42. For the thiobarbituric acid-reactive substances (TBARS) assay, liver samples (5 g) were mixed with 15 mL of extraction solution (7.5% trichloroacetic acid, 0.1% gallic acid, and 0.1 % EDTA), homogenized with an Ultra Turrax for 1 min, and filtered through qualitative filter paper No. 42. Supernatants (deproteinized liver tissues) were stored in Falcon tubes at −20 °C until use[47].

5.10. DPPH assay.

The ability of liver tissues to scavenge DPPH• (D9132, Sigma–Aldrich, St. Louis, MI, USA) was determined according to the method described by Brand-Williams et al.[46] Deproteinized liver tissue samples (200 µL) were homogenized with 1.8 mL of DPPH• solution (0.0024 g of DPPH• in 100 mL of 96.5% methanol) and incubated in the dark for 30 min. The absorbance was measured at 515 nm on a spectrophotometer (Sp 22, Biospectro, Curitiba, PR, Brazil).

5.11. TBARS assay.

MDA concentrations were determined by the TBARS method[47]. Deproteinized liver tissue samples (500 µL) were homogenized with 2.0 mL of a solution consisting of 15% thiobarbituric acid, 10% trichloroacetic acid, and 0.06% HCl. Subsequently, the mixtures were incubated in a water bath at 100 °C for 15 min and allowed to cool for 5 min. Absorbance was determined spectrophotometrically (SP 22, Biospectro, Curitiba, PR, Brazil) at 532 nm.

5.12. AST and ALT levels in plasma and creatine levels in plasma and m. longissimus lumborum.

Pigs weighing on average 100 kg live weight (n = 54) were fasted for 6 h before sample collection. Blood samples were collected from the jugular vein into tubes containing EDTA and centrifuged at 3000 × g for 15 min. The plasma was withdrawn with an automatic pipette and added to Eppendorf tubes. Plasma levels of AST, ALT, and creatine were determined by using test kits (Gold Analisa, Belo Horizonte, MG, Brazil). All laboratory procedures were performed according to kit instructions.

After slaughter of pigs, 15 g of m. *longissimus lumborum* was excised from each animal and stored on ice until extraction at the laboratory. Creatine extraction was performed as proposed by Chamruspollert et al.[49] Creatine concentrations were measured by reading the absorbance (SP 22, Biospectro, Curitiba, PR, Brazil) of the resulting supernatants at 450 nm.

5.13. Carcass traits.

Carcasses were chilled (0–1 °C) for 24 h and then subjected to quantitative evaluation, according to the Brazilian Method of Swine Carcass Classification[50]. Carcass traits were evaluated by measuring slaughter weight, lean meat yield (LMY, %), *longissimus lumborum* depth (LLD, mm), and backfat thickness (BF, mm). BF and LLD were measured between the last thoracic vertebra and the first lumbar vertebra, 6 cm away from the vertebral column, using a digital caliper (precision of 0.02 mm; Digimess, King tools, Sheffield, England) after 24 h postmortem. Carcass LMY was determined using the equation proposed by Irgang et al.[51], as follows: LMY = 60 – [(BF × 0.58) + (LLD × 0.10)].

5.14. Sarcomere length in m. longissimus lumborum.

The method for determining sarcomere length was similar to that previously described by Cross, West, and Dutson[52]. Samples of *longissimus lumborum*, located between the last thoracic vertebra and the first lumbar vertebra, were collected from eight animals per treatment at two different periods (45 min and 24 h after slaughter).

To measure sarcomere length, we collected and fixed chilled samples of *longissimus lumborum* in 10% buffered formalin (pH 7.0–7.2) for 24 h. Subsequently, samples were processed for paraffin embedding. Semi-serial 6 µm thick longitudinal histological sections were obtained using a microtome. The cuts were distended in a histological water bath at 45 °C and transferred to slides. Slides were placed on a wood support and incubated at 60 °C for 24 h to allow for greater adherence between the paraffin and the slide. After deparaffinization in an oven at 60 °C, samples were washed in running water for 2 min, treated with an aqueous solution of 0.25% potassium permanganate for 10 min, and then washed in running water for another 3 min. Subsequently, samples were immersed in oxalic acid for 5 min, washed in running water for 3 min, and stained with 10% Mallory's phosphotungstic acid-hematoxylin 0.5 g of hematoxylin, and 1 mL of hydrogen peroxide in a total volume of 500 mL for 24 h. The stain was placed in an amber vial and stabilized for 2 to 3 days before use.

Sections were analyzed under an optical microscope (Olympus BX40 equipped with a Nikon DS-Fi1 camera connected to a Nikon Digital Mira DS-43, Tokyo, Japan) and photographed using an attached camera with oil immersion lenses (100× objective and 10× eyepiece). Processing of images, scales, and measurements was performed using Nikon Elements software version 3.22 (http:// https://www.nikon.com/products/microscope-solutions/support/download/software/imgsfw/nis-f_v4600064.htm)

Eight histological slides were obtained per treatment and period (45 min and 24 h after slaughter), using three histological sections per slide. The average sarcomere length (µm) was calculated by determining the length (µm) of 10 sarcomeres in the histological section of each slide, totaling 30 distinct fibers chosen at random per treatment, resulting in a total of 300 observations per animal and period.

5.15. Statistical analyses.

The OUTLIER procedure of SAS version 9.0 (Cary, NC, USA, available from http:// https://www.sas.com/en_us/software/on-demand-for-academics.html)[53] was applied to detect the presence of outliers. Subsequently, data on 5′-IMP levels were subjected to analysis of variance; block effects and 5′-IMP level effects were included in the model. For regression analysis, the degrees of freedom of 0.0, 0.050, 0.100, 0.150 and 0.200% 5′-IMP levels were partitioned in orthogonal polynomials with the PROC MIXED

procedure of SAS. Then, a linear response plateau model was used to assess associations with a quadratic model. Comparisons between positive and negative control diets and 5′-IMP levels were performed by Tukey's test using orthogonal contrast analysis (PROC GLM procedure of SAS).

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7. Author contributions

L.P.B. and P.C.P analyzed the data. L.P.B. wrote the main manuscript text. C.A.M. contributed to the laboratory work. J.F.C. conducted the liver mitochondrial oxygen consumption analyses and statistical analyses. P.C.P and A.E.M supervised the experiment. All authors contributed to manuscript revisions and have read and approved the final version of the manuscript.

8. Competing interests

The authors declare no competing interests.

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Table 1. Composition of experimental diets

Ingredients (%)	NC[5]	5′-IMP[1] (%)				PC[6]
		0.050	0.100	0.150	0.200	
Corn	79.12	79.12	79.12	79.12	79.12	80.49
Soybean Meal	16.82	16.82	16.82	16.82	16.82	16.60
Soybean oil	-	-	-	-	-	0.750
Dicalcium phosphate	0.598	0.598	0.598	0.598	0.598	0.596
Limestone	0.744	0.744	0.744	0.744	0.744	0.747
Salt	0.225	0.225	0.225	0.225	0.225	0.223
5′-IMP	-	0.050	0.100	0.150	0.200	-
Inert[2]	1.900	1.850	1.800	1.750	1.700	-
Vitamin and mineral supplement[3]	0.400	0.400	0.400	0.400	0.400	0.400
L-Lysine HCl 78.4%	0.160	0.160	0.160	0.160	0.160	0.164
Enramycin	0.020	0.020	0.020	0.020	0.020	0.020
Feed dry[4]	0.015	0.015	0.015	0.015	0.015	0.015
Calculated composition, %						
Metabolizable energy (Mcal/kg)	3,200	3,200	3,200	3,200	3,200	3,300
Crude protein	14.00	14.00	14.00	14.00	14.00	14.00
Total calcium	0.500	0.500	0.500	0.500	0.500	0.500
Available phosphorus	0.190	0.190	0.190	0.190	0.190	0.190
Potassium	0.561	0.561	0.561	0.561	0.561	0.561
Sodium	0.100	0.100	0.100	0.100	0.100	0.100
Chlorine	0.248	0.248	0.248	0.248	0.248	0.249
SID Lysine	0.690	0.690	0.690	0.690	0.690	0.690
SID Methionine	0.200	0.200	0.200	0.200	0.200	0.200
SID Methionine + Cysteine	0.425	0.425	0.425	0.425	0.425	0.425
SID Threonine	0.449	0.449	0.449	0.449	0.449	0.449
SID Tryptophan	0.135	0.135	0.135	0.135	0.135	0.135
SID Valine	0.627	0.627	0.627	0.627	0.627	0.627
SID Leucine	1.197	1.197	1.197	1.197	1.197	1.197
SID Isoleucine	0.502	0.502	0.502	0.502	0.502	0.502
SID Arginine	0.791	0.791	0.791	0.791	0.791	0.791
SID Histidine	0.350	0.350	0.350	0.350	0.350	0.350
SID Phenylalanine	0.616	0.616	0.616	0.616	0.616	0.616
SID Phenylalanine + tyrosine	1.072	1.072	1.072	1.072	1.072	1.072

[1]Inosine-5′-monophosphate.
[2]Kaolinite.
[3]Provided per kilogram: vitamin A, 30000 UI; vitamin D3, 5000 UI; vitamin E, 120 UI; vitamin K, 5 mg; vitamin B12, 120 mcg; niacin, 150 mg; calcium pantothenate, 75 mg; folic acid, 8 mg; choline chloride, 0.48 g; iron, 350 mg; copper, 15 mg; manganese, 250 mg; zinc, 0.75 g; iodine, 10 mg; selenium, 3 mg.
[4]Antioxidant.
[5]Negative control (0.00% 5′-IMP and 3200 kcal ME/kg).
[6]Positive control (0.00% 5′-IMP and 3300 kcal ME/kg).

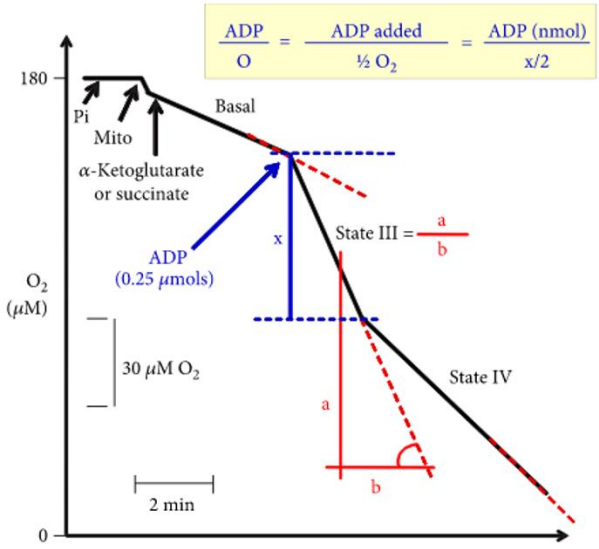

Figure 1. Experimental protocol and calculation procedures.

Table 2. Oxygen consumption at different states of respiration in mitochondria isolated from the liver of 75–100 kg barrows fed diets containing different levels of inosine-5-monophosphate (5′-IMP) and metabolizable energy (ME), using succinate or α-ketoglutarate as respiratory substrate and stimulated by adenosine monophosphate (ADP)

Item	Liver relative weight, %	Succinate					α-Ketoglutarate				
		Basal[3]	III[4]	IV[5]	RC[6]	ADP/O[7]	Basal	III	IV	RC	ADP/O
NC[1]	1.708	14.47	43.55	16.69	2.68	2.00	5.44	28.09	11.60	2.23	2.57
PC[2]	1.753	13.92	45.19	15.99	2.89	1.84	5.17	25.99	10.25	2.56	2.21
0.050%	1.773	13.00	49.10	15.02	3.28	1.73	6.83	23.80	10.27	2.48	2.42
0.100%	1.758	14.75	47.75	14.79	3.22	1.97	5.73	25.59	10.34	2.44	2.26
0.150%	1.601	12.98	44.65	16.62	2.70	1.74	5.69	27.30	11.78	2.42	2.21
0.200%	1.560	14.68	69.20	18.76	3.82	2.03	5.26	34.48	11.90	2.93	2.36
Mean	1.687	13.97	49.90	16.31	3.09	1.89	5.68	27.54	10.99	2.51	2.33
SD	0.132	2.132	11.521	2.996	0.656	0.304	1.016	4.407	1.630	0.384	0.348
SEM	0.047	0.360	1.947	0.506	0.111	0.051	0.172	0.745	0.276	0.065	0.059
NC × PC	0.336	0.671	0.721	0.683	0.517	0.349	0.625	0.295	0.206	0.161	0.116
NC × 0.050%	0.152	0.255	0.232	0.336	0.072	0.119	0.015	0.038	0.216	0.290	0.509
NC × 0.100%	0.216	0.823	0.363	0.274	0.105	0.883	0.612	0.211	0.240	0.347	0.152
NC × 0.150%	0.039	0.248	0.810	0.967	0.947	0.142	0.647	0.690	0.829	0.413	0.102
NC × 0.200%	0.004	0.865	<0.001	0.234	0.001	0.875	0.738	0.003	0.740	0.005	0.347
PC × 0.050%	0.560	0.671	0.396	0.683	0.236	0.565	0.005	0.273	0.975	0.717	0.346
PC × 0.100%	0.837	0.471	0.577	0.576	0.316	0.424	0.333	0.828	0.917	0.635	0.825
PC × 0.150%	0.004	0.518	0.907	0.713	0.560	0.598	0.347	0.512	0.102	0.544	0.997
PC × 0.200%	<0.001	0.553	<0.001	0.114	0.007	0.279	0.878	<0.001	0.079	0.113	0.508
Linear	<0.001	0.476	<0.001	0.503	0.050	0.257	0.063	0.002	0.315	0.008	0.225
Quadratic	0.214	0.734	0.020	0.272	0.056	0.215	0.022	<0.001	0.151	0.325	0.244

[1]Negative control (0.00% 5′-IMP and 3200 kcal ME/kg).
[2]Positive control (0.00% 5′-IMP and 3300 kcal ME/kg).
[3]Oxygen consumption before ADP addition (substrate or basal).
[4]Oxygen consumption shortly after ADP addition (state III).
[5]Oxygen consumption after stopping ADP stimulation (state IV)
[6]Respiratory control (RC) calculated by the relationship between states III/IV.
[7]ADP/O ratio determined as proposed by Chance and Williams (1955).

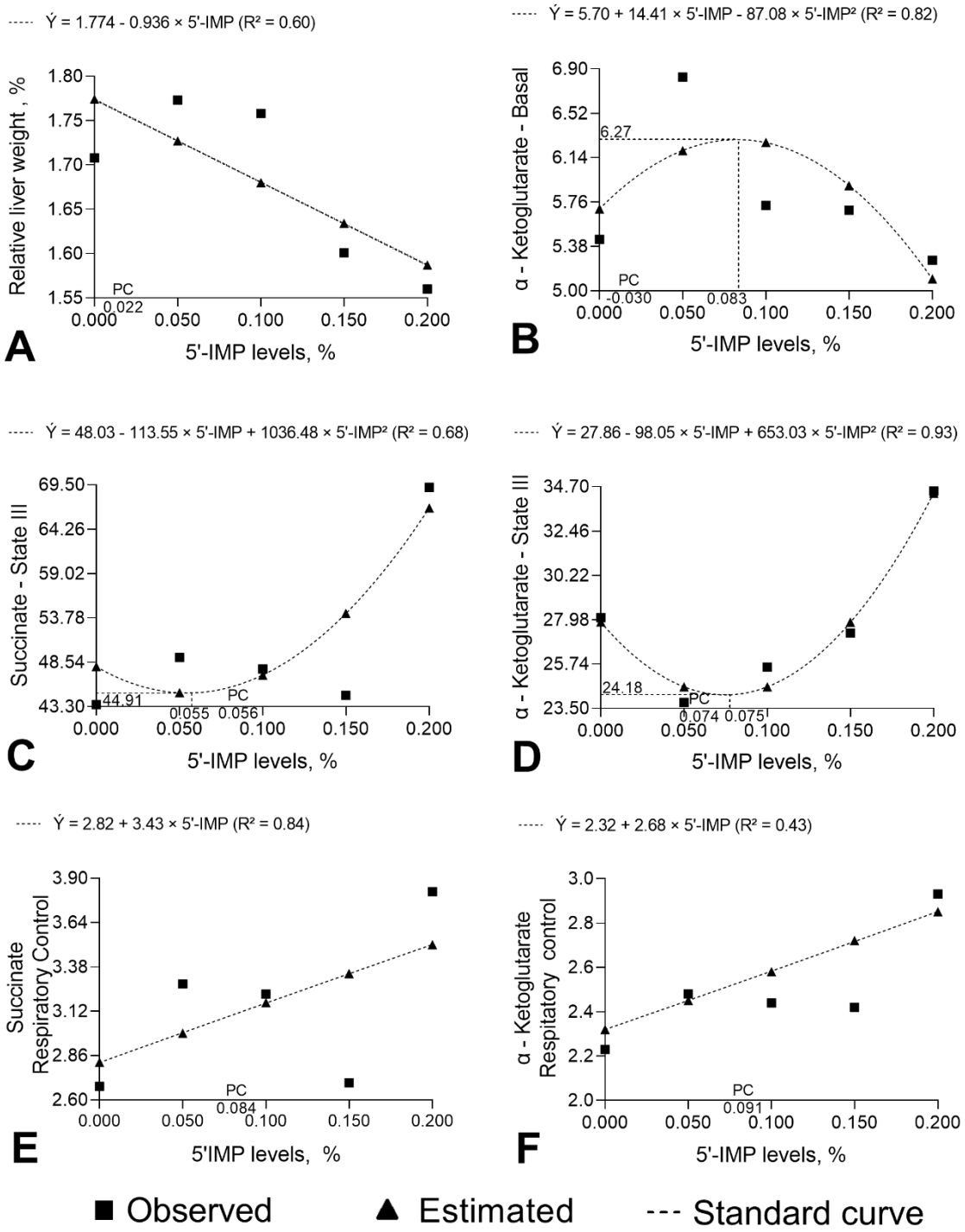

Figure 2. Relative liver weight and oxygen consumption at different states of respiration in mitochondria isolated from the liver of 75–100 kg barrows fed diets containing different levels of inosine-5′-monophosphate (5′-IMP) and a 100 kcal/kg lower metabolizable energy level than the positive control (high-energy) diet.

Table 3. Free radical scavenging assay (DPPH %) and malondialdehyde concentration (MDA) in the liver of 75–100 kg barrows fed diets containing different levels of inosine-5-monophosphate (5′-IMP) and metabolizable energy (ME)

Item	DPPH, %	MDA, mg/kg
NC[1]	30.340	1.540
PC[2]	22.640	1.689
0.050%	24.600	1.350
0.100%	23.740	1.375
0.150%	24.040	1.256
0.200%	30.170	1.625
Mean	25.920	1.472
SD	3.854	0.194
SEM	0.556	0.028
Contrasts		
NC × PC	<0.001	0.017
NC × 0.050%	<0.001	0.003
NC × 0.100%	<0.001	0.009
NC × 0.150%	<0.001	<0.001
NC × 0.200%	0.889	0.666
PC × 0.050%	0.108	<0.001
PC × 0.100%	0.316	<0.001
PC × 0.150%	0.242	<0.001
PC × 0.200%	<0.001	0.289
Polynomial regression		
Linear	<0.001	<0.001
Quadratic	<0.001	<0.001

[1]Negative control (0.00% 5′-IMP and 3200 kcal ME/kg).
[2]Positive control (0.00% 5′-IMP and 3300 kcal ME/kg).

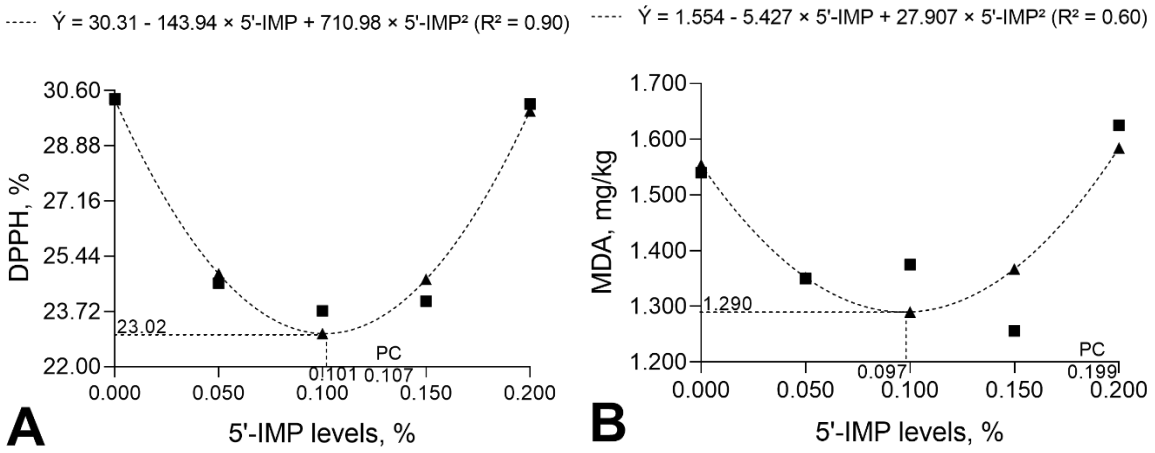

Figure 3. Free radical scavenging assay (A) and malondialdehyde (MDA) concentration (B) in the liver of 75–100 kg barrows fed diets containing different levels of inosine-5′-monophosphate (5′-IMP) and a 100 kcal/kg lower metabolizable energy level than the positive control (high-energy) diet.

Table 4. Concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine in blood plasma, as well as creatine concentration in *longissimus lumborum* muscle of 75–100 kg barrows fed diets containing different levels of inosine-5-monophosphate (5′-IMP) and metabolizable energy (ME)

Item	Blood plasma			Muscle
	ALT, mg/dL	AST, mg/dL	Creatine, mg/dL	Creatine, mg/dL
NC[1]	30.25	38.54	1.36	5.60
PC[2]	30.58	43.39	1.38	5.33
0.050%	37.88	43.66	1.58	5.91
0.100%	36.36	44.70	1.37	6.04
0.150%	36.61	44.43	1.47	6.06
0.200%	36.53	48.65	1.30	6.23
Mean	34.51	43.66	1.41	5.86
SD	5.290	7.190	0.183	0.433
SEM	0.720	0.978	0.020	0.059
Contrasts				
NC × PC	0.882	0.191	0.790	0.105
NC × 0.050%	0.003	0.180	0.037	0.066
NC × 0.100%	0.014	0.090	0.929	0.010
NC × 0.150%	0.014	0.124	0.282	0.007
NC × 0.200%	0.010	0.012	0.575	<0.001
PC × 0.050%	0.004	0.926	0.048	0.001
PC × 0.100%	0.000	0.725	0.856	<0.001
PC × 0.150%	0.020	0.765	0.376	<0.001
PC × 0.200%	0.009	0.192	0.381	<0.001
Polynomial regression				
Linear	0.112	0.459	0.186	<0.001
Quadratic	0.076	0.715	0.142	0.212

[1]Negative control (0.00% 5′-IMP and 3200 kcal ME/kg).

[2]Positive control (0.00% 5′-IMP and 3300 kcal ME/kg).

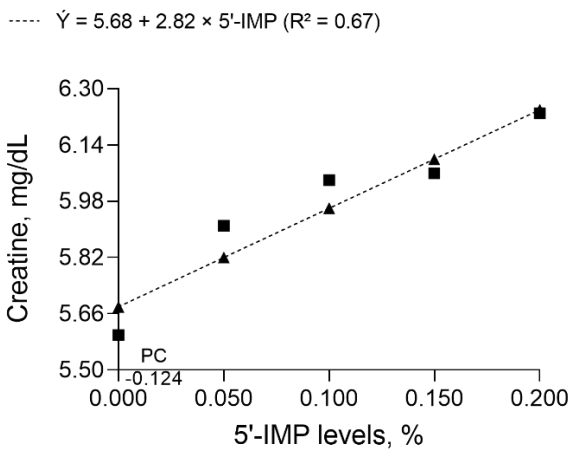

Figure 4. Creatine concentration in the *longissimus lumborum* muscle of 75–100 kg barrows fed diets containing different levels of inosine-5′-monophosphate (5′-IMP) and a 100 kcal/kg lower metabolizable energy level than the positive control (high-energy) diet.

Table 5. Carcass traits and sarcomere length in *longissimus lumborum* muscle of 75–100 kg barrows fed diets containing different levels of inosine-5-monophosphate (5′-IMP) and metabolizable energy (ME)

Item	Slaughter weight, kg	LMY[3], %	LLD[4], cm	SL45 min[5], µm	SL24 hr[6], µm	BT[7], cm
NC[1]	97.03	58.05	6.03	1.729	1.693	0.97
PC[2]	97.94	58.69	6.08	1.706	1.673	1.44
0.050%	98.24	58.49	6.28	1.731	1.695	1.44
0.100%	98.48	58.65	6.06	1.743	1.688	1.31
0.150%	100.55	58.90	6.01	1.790	1.694	1.23
0.200%	98.56	58.69	5.91	1.750	1.680	1.21
Mean	98.47	58.56	6.07	1.740	1.690	1.27
SD	2.380	0.350	0.380	0.032	0.013	0.230
SEM	0.320	0.050	0.050	0.005	0.002	0.030
Contrasts						
NC × PC	0.353	<0.001	0.627	0.045	0.001	0.002
NC × 0.050%	0.262	<0.001	0.183	0.855	0.701	0.003
NC × 0.100%	0.156	<0.001	0.763	0.169	0.386	0.009
NC × 0.150%	0.004	<0.001	0.986	<0.001	0.898	0.025
NC × 0.200%	0.155	<0.001	0.751	0.052	0.025	0.098
PC × 0.050%	0.758	0.270	0.401	0.030	0.003	0.866
PC × 0.100%	0.556	0.835	0.951	0.013	0.010	0.590
PC × 0.150%	0.037	0.020	0.622	<0.001	<0.001	0.298
PC × 0.200%	0.589	0.590	0.451	0.002	0.224	0.168
Statistical analysis						
Linear	0.002	<0.001	0.495	0.004	0.654	0.326
Quadratic	0.015	<0.001	0.324	0.077	0.299	<0.001

[1]Negative control (0.00% 5′-IMP and 3200 kcal ME/kg).
[2]Positive control (0.00% 5′-IMP and 3300 kcal ME/kg).
[3]Lean meat yield.
[4]*Longissimus lumborum* depth.
[5]Sarcomere length at 45 min after slaughter.
[6]Sarcomere length at 24 h after slaughter.
[7]Backfat thickness.

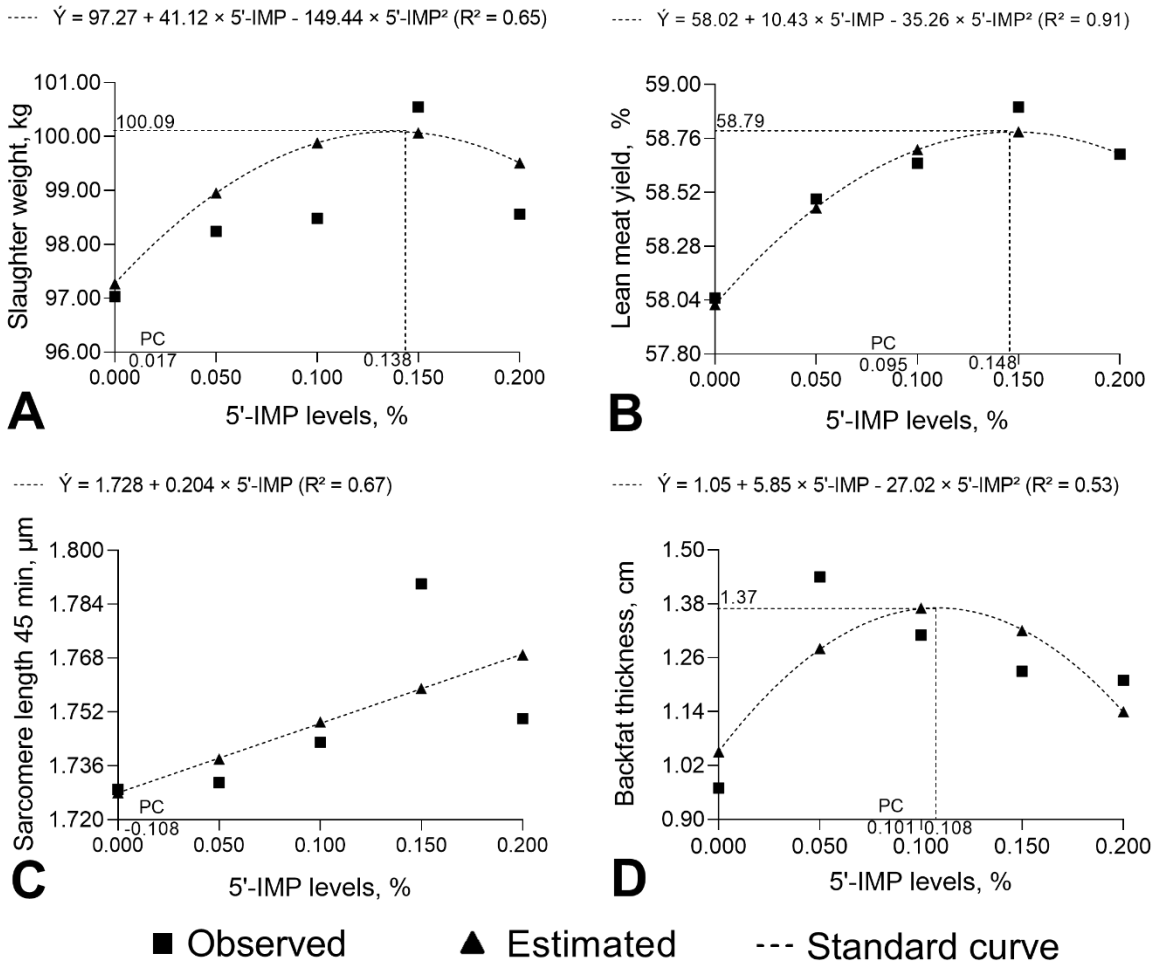

Figure 5. Slaughter weight (A), lean meat yield (B), sarcomere length 45 min after slaughter (C) and backfat thickness (D) of 75–100 kg barrows fed diets containing different levels of inosine-5′-monophosphate (5′-IMP) and a 100 kcal/kg lower metabolizable energy level than the positive control (high-energy) diet.

Table 6 – Fitted regression equations for the studied parameters as a function of dietary inosine-5′-monophosphate (5′-IMP) level

Item	Quadratic equations	R^2	IP[11]	5′-IMP	PC[12]
Basal (k) [1]	$\dot{Y} = 5.70 + 14.41 \times 5'\text{-IMP} - 87.08 \times 5'\text{-IMP}^2$	0.82	6.27	0.083	-0.030
State III(s)[2]	$\dot{Y} = 48.03 - 113.55 \times 5'\text{-IMP} + 1036.48 \times 5'\text{-IMP}^2$	0.68	44.91	0.055	0.056
State III(k)[3]	$\dot{Y} = 27.86 - 98.05 \times 5'\text{-IMP} + 653.03 \times 5'\text{-IMP}^2$	0.93	24.18	0.075	0.074
DPPH, %[4]	$\dot{Y} = 30.31 - 143.94 \times 5'\text{-IMP} + 710.98 \times 5'\text{-IMP}^2$	0.90	23.02	0.101	0.107
MDA, mg/dL[5]	$\dot{Y} = 1.554 - 5.427 \times 5'\text{-IMP} + 27.907 \times 5'\text{-IMP}^2$	0.60	1.29	0.097	0.199
Slaughter weight, kg	$\dot{Y} = 97.27 + 41.12 \times 5'\text{-IMP} - 149.44 \times 5'\text{-IMP}^2$	0.65	100.09	0.138	0.017
LMY, %[6]	$\dot{Y} = 58.02 + 10.43 \times 5'\text{-IMP} - 35.26 \times 5'\text{-IMP}^2$	0.91	58.79	0.148	0.095
BT, cm[7]	$\dot{Y} = 1.05 + 5.85 \times 5'\text{-IMP} - 27.02 \times 5'\text{-IMP}^2$	0.53	1.37	0.108	0.101
Linear equations					
Relative liver weight	$\acute{Y} = 1.744 - 0.936 \times 5'\text{-IMP}$	0.60	-	-	-
RC (s)[8]	$\dot{Y} = 2.82 - 3.43 \times 5'\text{-IMP}$	0.84	-	-	0.084
RC (k)[9]	$\dot{Y} = 2.32 + 2.68 \times 5'\text{-IMP}$	0.43	-	-	0.091
Creatine mg/dL	$\dot{Y} = 5.68 + 2.82 \times 5'\text{-IMP}$	0.67	-	-	-0.124
SL45min, µm[10]	$\dot{Y} = 1.728 + 0.204 \times 5'\text{-IMP}$	0.67	-	-	-0.108

[1]Basal state respiration rate in liver mitochondria incubated with α-ketoglutarate.
[2]State III respiration rate in liver mitochondria incubated with succinate, stimulated by ADP.
[3]State III respiration rate in liver mitochondria incubated with α-ketoglutarate and stimulated by ADP.
[4]Free radical scavenging assay.
[5]Malondialdehyde concentration.
[6]Lean meat yield.
[7]Backfat thickness.
[8]Respiratory control of liver mitochondria incubated with succinate.
[9]Respiratory control of liver mitochondria incubated with α-ketoglutarate.
[10]Sarcomere length at 45 min after slaughter.
[11]Inflection point.
[12]Positive control diet (0.00% 5′-IMP and 3300 kcal ME/kg).

III - A suplementação dietética de inosina-5'-monofosfato melhora o desempenho da produção por meio da redução do custo energético proveniente da estimulação do sistema imunológico de femêas na fase terminação (75-100kg)

Resumo: O objetivo deste estudo foi de avaliar a suplementação de 0,100% de 5'-IMP em dietas com redução de 100 kcal EM/kg para suínos em terminação, sob diferentes condições imunes sobre o desempenho produtivo, parâmetros de carcaça, qualidade de carne, homeostase e concentração de células vermelhas e brancas e a concentração das proteínas de fase aguda no soro sanguíneo. Foram utilizadas 40 fêmeas suínas, com peso inicial de 75,58 ± 0,941 kg e final de 101,13 ± 1,993 kg foram distribuídas em um design de blocos randomizados, em um design fatorial 2 x 2, sendo utilizadas 10 repetições, em que a unidade experimental foi representada por um animal. Os suínos receberam a dieta basal, contendo 3200 kcal EM/kg e adição de 0,100% de 5'-IMP ou convencional, contendo 3300 kcal EM/kg. Adicionalmente, os suínos receberam o placebo ou foram estimulados imunologicamente. As dietas e as condições de estimulação do sistema imune influenciaram o GPD ($P < 0,001$) e o CDR ($P = 0,006$), também houve efeito da dieta ($P < 0,001$) e inoculação ($P = 0,012$) sobre a CA. A dieta 5'-IMP proporcionou o maior GPD (1,12 kg) aos suínos estimulados imunologicamente e menor consumo de ração submetidos ao placebo, além da menor CA ($P < 0,001$). A estimulação do sistema imune proporcionou uma maior CA ($P = 0,012$). A dieta 5'-IMP promoveu aumento no rendimento de carne magra na carcaça ($P = 0,012$) e a interação entre dieta e condição de estimulação de sistema imune apresentou uma redução na quantidade de gordura da carcaça, refletida em menores índices de P1P2P3 ($P = 0,030$) e espessura de toucinho ($P < 0,001$). A dieta 5'-IMP proporcionou maiores valores pH mensurado 45 min ($P = 0,035$) após ao abate e vermelhidão ($P < 0,001$) no músculo longissimus lumborum (*M. LL*), houve interações entre dietas e condições do sistema imune ($P \leq 0,05$), em que a dieta 5'-IMP proporcionou o maiores valores de pH mensurado 24 horas após ao abate e menores valores para a perda de agua por gotejamento (PAG), descongelamento e cocção e força de cisalhamento do *M. LL* em suínos estimulados imunologicamente e aos submetidos ao placebo. As interações entre dietas e períodos avaliados demonstraram que a 5'-IMP proporcionou um aumento na concentração plasmática de proteínas totais ($P = 0,006$), albumina ($P < 0,001$), ácido úrico ($P < 0,001$), plaquetas ($P < 0,001$) leucócitos totais, neutrófilos, linfócitos, relação neutrófilo:linfócito ($P < 0,001$) e plaqueta:linfócito ($P = 0,017$), eosinófilos ($P < 0,001$), basófilos ($P < 0,001$), transferrina ($P < 0,001$) e IgG de cadeia pesada ($P < 0,001$), demonstrando potentes benefícios sobre o sistema imune. Em conclusão, a suplementação de 0,100 % de 5'-IMP supriu o déficit energético proveniente da estimulação do sistema imune, promovendo benefícios sobre o desempenho produtivo, parâmetros de carcaça, qualidade de carne e parâmetros bioquímicos do plasma sanguíneo correlacionados ao sistema imune. Evidenciado a possibilidade da 5'-IMP ser empregada na formulação de ração como aditivo energético, proteico e um possível substituto para o uso de antibióticos em dietas para suínos na fase de terminação.

Palavras chaves: Defesa imune, energia, leucócitos diferenciados, NTs.

III - Dietary supplementation of inosine-5'-monophosphate improves production performance by reducing the energy cost of stimulating the immune system of finishing gilts (75 to 100 kg).

Abstract: This study aimed to evaluate the supplementation of 0.100% 5'-IMP in diets with a reduction of 100 kcal ME/kg for finishing gilts, under different immune conditions on productive performance, carcass characteristics, meat quality, homeostasis, and concentration of red and white cells, and the concentration of acute-phase proteins in blood serum. Forty female pigs, with an initial weight of 75.58 ± 0.941 kg and final weight of 101.13 ± 1.993 kg were distributed in a randomized block design, in a 2 x 2 factorial design, with 10 repetitions and each animal representing an experimental unit. The gilts received the basal diet, containing 3200 kcal ME/kg and addition of 0.100% 5'-IMP or conventional diet, containing 3300 kcal ME/kg. Additionally, the gilts received a placebo or received immunostimulation. Diets and immune system stimulation conditions influenced DWG ($P < 0.001$) and DFI ($P = 0.006$). Furthermore, there was an effect of diet ($P < 0.001$) and inoculation ($P = 0.012$) on FC. The 5'-IMP diet provided the highest DWG (1.12 kg) to immune-stimulated gilts and the lowest feed intake treated with placebo, in addition to the lowest FC ($P < 0.001$). Stimulation of the immune system provided a higher FC ($P = 0.012$). The 5'-IMP diet promoted an increase in lean meat yield ($P = 0.012$) and the interaction between diet and immune system stimulation condition showed a reduction in the amount of carcass fat, reflected in lower P1P2P3 indexes ($P = 0.030$) and backfat thickness ($P < 0.001$). The 5'-IMP diet provided higher pH values measured at 45 min ($P = 0.035$) after slaughter and redness ($P < 0.001$) in longissimus lumborum muscle (LLM), there were interactions between diets and conditions of the immune system ($P \leq 0.05$), in which the 5-IMP diet provided the highest pH values measured 24 hours after slaughter and lowest values for drip loss (DL), fluid lost in thawing (FLT) and fluid lost in cooking (FLC) and shear force of LLM in immunologically stimulated gilts and those treated with placebo. The interactions between diets and evaluated periods demonstrated that 5'-IMP increased in the plasma concentration of total proteins ($P = 0.006$), albumin ($P < 0.001$), uric acid ($P < 0.001$), platelets ($P < 0.001$), total leukocytes, neutrophils, lymphocytes, neutrophil:lymphocyte ratio ($P < 0.001$), platelet:lymphocyte ratio ($P = 0.017$), eosinophils ($P < 0.001$), basophils ($P < 0.001$), transferrin ($P < 0.001$) and chain heavy IgG ($P < 0.001$), demonstrating potent benefits on the immune system. In conclusion, the supplementation of 0.100% of 5'-IMP supplied the energy deficit resulting from the stimulation of the immune system, promoting benefits on productive performance, carcass characteristics, meat quality, and biochemical parameters of blood plasma correlated to the immune system. It highlights the possibility of 5'-IMP being used in the feed formulation as an energy and protein additive and a possible substitute for the use of antibiotics in diets for finishing gilts.

Keywords: Immune Defense, Metabolic Energy, Leukocytes, Nucleotides.

1. Introduction

Among purine nucleosides, the first nucleoside to be synthesized in de novo biosynthesis is 5'-IMP, a precursor of the nucleosides adenine monophosphate (AMP) and guanosine monophosphate (GMP). They will be metabolized to adenosine triphosphate (ATP) and guanosine triphosphate (GTP), energy molecules essential for the maintenance and regulation of cellular activities (Harmsen et al., 1984; Kamatani et al., 2019). However, few studies are show the effects of 5'-IMP supplementation on the productive performance and immune system of finishing gilts.

When metabolism needs to stimulate the proliferation and growth of immune system cells (leukocytes, lymphocytes, neutrophils, monocytes, eosinophils, and basophils) and their secretions, such as acute-phase proteins (immunoglobulins A and G, ceruloplasmin, transferrin, albumin, haptoglobin, and 23,000 Dalton) to make the immune defense more robust, 5'-IMP is converted to GTP at a higher rate. When metabolism needs to stimulate protein synthesis, promote cell growth related to muscle growth or increase energy availability to cells, 5'-IMP is metabolized at a higher rate to ATP (Hara and Kondo, 2015).

The importance of the metabolic pathway and the activities of 5'-IMP in metabolism, as well as of NTs, have been demonstrated in vivo in previous research, in which 5'-IMP or NT blends were supplemented at levels ranging from 0.1 to 1.0 g/kg in the diets of suckling and weaned piglets. The main results indicate that 5'-IMP increases nutrient availability, energy, improve diet efficiency (Jang et al., 2019) and DWG (Weaver and Kim, 2014), as well as reduces FC (Weaver and Kim, 2014), modulates the intestinal microbiota (Jang and Kim, 2019) and provides a more robust immune system defense response to piglets (Jiao and Kim, 2019).

Therefore, we hypothesize that in finishing gilts subjected to immunological stress, supplementation of 5'-IMP at the level of 0.100% in diets with a reduction of 100 kcal ME/kg can increase the concentration of ATP and GTP to supply the increase in energy expenditure caused by immune stress, providing an adequate productive performance, carcass characteristics, LLM quality, and an adequate modulation of the immune system.

This study aimed to evaluate the supplementation of 0.100% 5'-IMP in diets with a reduction of 100 kcal ME/kg for finishing gilts, under different immune conditions on productive performance, carcass characteristics, meat quality, homeostasis and

concentration of red and white blood cells, and the concentration of acute-phase proteins in blood serum.

2. Materials and methods

The experiment was carried out at the Pig Farming Sector of the Iguatemi Experimental Farm - FEI of Universidade Estadual de Maringá. All experimental procedures were approved by the Ethics and Conduct Committee for the Use of Animals in Scientific Experiments (Protocol No. 9056170220).

2.1 Facilities and Animals

The experimental shed was covered with fiber cement tiles, containing 40 bays measuring 1.88 m² each, with cement floor, equipped with a semi-automatic feeder on the front and a nipple drinker on the opposite side of the feeder. The gilts had free access to water and feed during the entire experimental period.

2.2 Experimental design

A total of 40 female pigs, with an initial average weight of 75.58 ± 0.941 kg and a final weight of 101.13 ± 1.993 kg, were distributed in a randomized block experimental design, in a 2 x 2 factorial design, using 10 replications, in which each experimental unit is represented by an animal. The gilts were submitted to one of two diets (5'-IMP - 3200 kcal ME/kg supplemented with 0.100% 5'-IMP and Conventional diet - 3300 kcal ME/kg), and to one of two different immune system conditions (1st - Placebo and 2nd - Immunological stimulation).

Exceptionally, the parameters of the biochemical profile of blood plasma, blood count and white blood cell count, and the concentration of proteins in blood serum were analyzed in a 2 x 2 x 3 factorial scheme, including three evaluation periods (0, 12, and 24 days).

The experimental rations (Table 1) consisted of corn, soybean meal, minerals, vitamins, and additives to meet the nutritional requirements proposed by the National Research Council - NRC (2012), except for ME.

2.3 Blood collection and immunization

Blood samples were obtained at three different periods (0, 12, and 24 days) during the experiment, through a puncture in the jugular vein (Oliveira et al., 2014). Figure 1 depicts the immunization and blood collection protocol.

At the beginning of the experimental period (day 0), after blood collection, the gilts were inoculated with 1 mL of saline solution (placebo) or had the immune system stimulated, as described by (Li et al. 1999) and repeated by Fachinello et al. (2017). We used 0.5 mL of a solution composed of 1 mg of bovine serum albumin (BSA) (A3912, Sigma-Aldrich, St. Louis, MO, USA) which was diluted in 0.5 mL of phosphate-buffered saline (PBS) in association with 0.5 ml of Freund's complete adjuvant (F5881, Sigma-Aldrich, St. Louis, MO, USA). The same procedure was adopted on the 12th day of the experiment (Figure 1), except that the adjuvant used was Freund's incomplete adjuvant (F5506 Sigma-Aldrich, St. Louis, MO, USA).

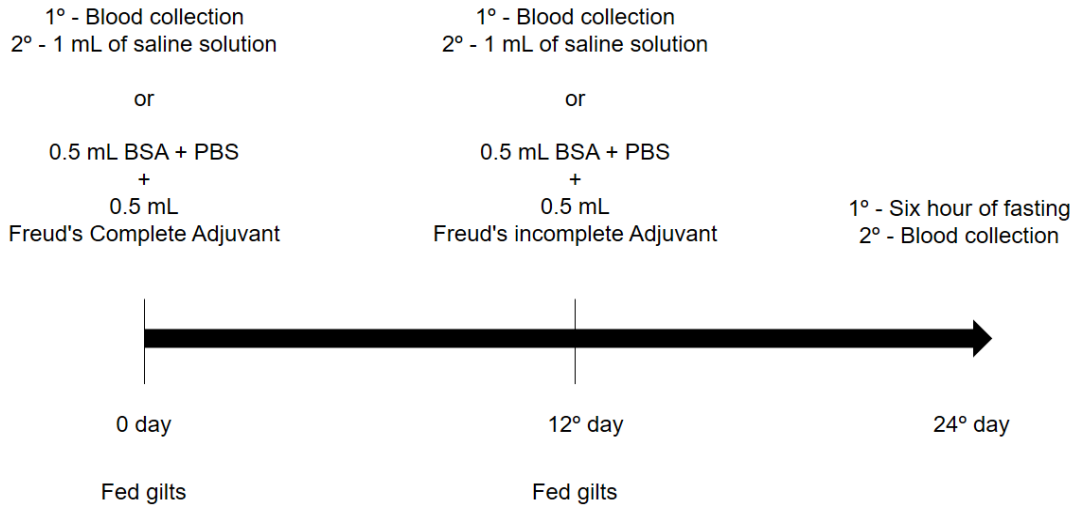

Figure 1. Gilt blood collection and immunization protocol. Adapted from Fachinello et al. (2017).

2.4 Productive performance

The gilts were weighed at the beginning and the end of the experimental period, and the rations were weighed whenever fed to the animals, to determine the daily weight gain (DWG) and daily feed intake (DFI), as well as the calculated feed conversion (FC).

2.5 Sow slaughter

At the end of the experiment, the gilts were submitted to a 24-hour fasting period and, later, they were again weighed to obtain the slaughter weight (SW). The slaughter was carried out at the abattoir of the Experimental Farm of Iguatemi – FEI/UEM. The gilts were subjected to electrical stunning (200W) and sacrificed by exsanguination. The carcasses were scalded in water (60º C), depilated, washed, eviscerated, cut in half, weighed, and stored in a cold chamber for 24 hours (0.5 ± 1.0º C).

2.6 Quantitative carcass characteristics

Quantitative carcass characteristics were evaluated following the recommendations proposed by Bridi and Silva (2009), evaluating the SW, the hot carcass yield (HCY), the cold carcass yield (CCW), the carcass lean meat yield (LMY), the ham weight (HW), the ham yield (HY), the LLM depth (MD), the backfat thickness measured at different points on the LLM (P1P2P3) and the backfat thickness measured on the LLM (BT).

BT and MD were measured 24 hours post-mortem using a digital caliper with 0.02 mm accuracy (Digimess, England, Sheffield). Measurements were taken at the insertion site between the last thoracic vertebra and the first lumbar vertebra, 6 cm away from the spine (P2). The LMY was determined using the equation proposed by Irgang et al. (1998), as follows:

$$LMY\ (\%) = 60 - ((BT\ mm \times 0.58) + (MD\ mm \times 0.10))$$

2.7 LLM Quality

To obtain the pH values, we used the right carcasses and LLM measurements were performed using a portable pH meter (Hanna Instruments, HI98163, USA, Woonsocket) at the height of the last rib, 45 minutes after slaughter (45-minutes pH). and 24 hours after the start of cooling (24-hour pH) (Bridi and Silva, 2009).

The LLM samples were used for color assessment, in which six measurements were performed using a previously calibrated colorimeter (Konica Minolta™ Model CR400, Japan, Tokyo) (X = 80.4; Y = 85, 3; Z = 91.5). The luminosity L * (white – black) and the color range between red (+) and green (-) (Minolta a*; Redness) and yellow (+) and blue (-) (Minolta b*; Yellowing) were expressed according to the CIELAB system.

The samples of LLM were weighed, packaged, stored for 72 hours at 4 º C and weighed again, thus obtaining the loss of water by dripping (Boccard et al., 1981). To determine the water loss during thawing, the samples were weighed and frozen, then thawed, weighed. and stored for 24 hours at 4º C. The cooking water loss was obtained by the difference between the thawed sample weight and the weight of the sample after cooking, at a temperature of 170ºC, until reaching an internal temperature of 71ºC (Bridi and Silva, 2009).

After cooking, six subsamples were taken in the longitudinal direction of the fibers with a cylindrical tool (1.27 cm in diameter) (Ramos & Gomide, 2012). The subsamples were placed, perpendicular to the fibers, on a Warner-Bratzler support used in a texturometer (TA-XT2i, UK, Surrey) to measure the shear force. The speed established in the pre-test was 5mm/s, during the test it was 2 mm/s, and after the test 5 mm/s.

2.8 Blood serum and plasma analysis

Blood samples were fractionated and stored in glass tubes without anticoagulants to obtain blood serum (protein electrophoresis) or in tubes containing EDTA. Then, they were automatically homogenized for 5 minutes and duly processed according to the procedures for plasma analysis of total protein (TP), albumin, globulins, albumin/globulin ratio, uric acid, Aspartate Amino Transferase (AST), and Alanine Amino Transferase (ALT), for complete blood count and protein electrophoresis analysis.

For the complete blood count and protein electrophoresis, on day 0 blood plasma samples from 20 gilts (Baseline) were used, and in the other periods (12th and 24th day) samples were collected from all animals.

After collection, the blood was homogenized and, later, the tubes were centrifuged at 3000 × g for 15 minutes. The plasma was extracted with an automatic pipette and placed in Eppendorf tubes. Measurements of TP, albumin, uric acid, AST, and ALT were performed using specific kits (Gold Analyze®) following specific standard operating procedures (SOP).

Parameter concentrations were measured by reading their absorbances in a spectrophotometer (SP 22, Bioplus 2000, Curitiba, Brazil). Globulin concentration was calculated by the difference between TP and albumin concentrations, and the albumin:globulin ratio was also calculated by dividing albumin by globulin concentrations.

2.9 Complete blood count of gilts

Blood samples were submitted to an automatic homogenizer for 5 minutes and sent to the veterinary laboratory of the São Camilo group (Maringá, Paraná, Brazil), where the samples were analyzed by fluorescent flow cytometry and impedance in a hematology analyzer (XE -2100- Sysmex, Sysmex Corporation, Tokyo, Japan), to measure concentrations of erythrocytes, hemoglobin, hematocrits, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets, total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, and basophils. We also calculated the relationships between neutrophils and lymphocytes and platelets and lymphocytes by dividing neutrophils and platelets concentration by the lymphocyte concentration.

2.10 Sow blood plasma protein electrophoresis

The blood samples remained at rest for 24 hours, then the blood plasma was extracted with the aid of an automatic pipette, stored in Eppendorf tubes, and frozen at -20º C until they were sent to the veterinary laboratory of the Universidade Estadual Paulista (UNESP, Jaboticabal, São Paulo, Brazil), where blood plasma samples were processed and protein fractionation was determined using the technique of polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE), according to the technique described by Laemmli (1970). The concentrations of TP, immunoglobulin A (IgA), ceruloplasmin, transferrin, albumin, haptoglobin, α-1 glycoprotein, heavy chain, and light chain immunoglobulin G (IgG) of molecular weight 23,000 Da were determined using computerized densitometry (Shimadzy CS9301, Tokyo, Japan). A solution with specific markers with different molecular weights was used as standard, in addition to the purified IgG protein (Sigma, St Louis, MO, USA). The percentage of these proteins was multiplied by the values of total proteins present in the blood plasma of each sample to obtain the final concentration.

2.11 Statistical analysis

We used the OUTLIER method of SAS (Statistical Analysis System, version 9.0, Cary, NC, USA) to assess the presence of outliers. Subsequently, we performed an analysis of variance (ANOVA) and the least-squares method to differentiate the means (LSMEANS) by the "Tukey" test. Next, we used TDERR, PDIFF, and ADJUST in the SAS Mixed procedure to maximize test power.

For the analysis of productive performance, carcass characteristics, and meat quality, the model evaluated the effects of blocks, diets, and immune system stimulation, as well as the interaction between diets × stimulation. The initial weight was included as a covariate and was used to explain the data when it had a significant effect ($P < 0.005$).

In the analysis of the biochemical profile of blood plasma, complete blood count, and protein electrophoresis, the effects included in the model were the experimental blocks, diets, stimulation, and periods, as well as the interaction between diets × periods and stimulation × periods. The interactions were unfolded and the effect of the periods on the parameters, both for the different diets and the stimulation of the immune system, were submitted to linear regression analysis.

The evaluation periods (0, 12, and 24 days) were fixed and evaluated within the diets and immune system stimulation. A significance level of 5% was adopted for all statistical analyses.

3. Results

3.1 Productive performance

No interactions between diets and immune system stimulations were observed for FC ($P = 0.301$), but interactions were identified for DWG ($P < 0.001$) and DFI ($P = 0.008$) (Table 2), in which the gilts treated with placebo had similar DWG between dietary treatments. However, the immunologically stimulated gilts fed the 5'-IMP diet showed a higher DWG than gilts fed the conventional diet, and gilts that received placebo fed the same diet. Additionally, gilts that received the placebo and consuming the conventional diet had higher DWG compared to immunologically stimulated gilts that consumed the same diet.

Gilts on placebo and the 5'-IMP diet had a lower DFI compared to gilts on the conventional diet, but immunologically stimulated gilts had similar DFI between dietary

treatments. Additionally, gilts subjected to placebo and consuming the 5'-IMP diet had lower DFI than immune-stimulated gilts feeding on the same diet (Table 2).

The consumption of the 5'-IMP diet promoted better FC ($P < 0.001$) compared to that presented by the gilts that consumed the conventional diet. Additionally, gilts treated with placebo had lower FC ($P = 0.012$) compared to immunologically stimulated gilts (Table 2).

3.2 Quantitative carcass characteristics

Carcass parameters can be important and value-added measures in commercial situations. For example, MD and HW are commercially valued and appreciated by the consumer market (Masferrer et al., 2018). The LMY and the length of pig carcasses are used in genetic selection when developing a new lineage of pigs (Miar et al., 2014), while the BT measurements at the points P1P2P3 and BT on the LLM are used to assess lipid deposition in pig carcasses.

No interactions between diets and immune system stimulation were observed for WHR ($P = 0.817$), RCF ($P = 0.925$), RP ($P = 0.492$), LMY ($P = 0.455$) and carcass length ($P = 0.157$), but interactions were observed (Table 3) for MD ($P = 0.004$), PP ($P = 0.024$), P1P2P3 ($P = 0.030$) and BT ($P < 0.001$).

The gilts that consumed the independent 5I-IMP diet WHR ($P = 0.022$), RCF ($P = 0.011$), and LMY ($P = 0.012$) were higher than those required by the gilts that received the conventional diet. Additionally, immunologically stimulated gilts had shorter carcass length ($P = 0.014$) compared to gilts that received placebo (Table 3).

The interaction observed for MD ($P = 0.004$) shows that gilts treated with placebo showed similarity between dietary treatments, but in immunologically stimulated gilts, consumption of the 5'-IMP diet provided a higher MD compared to gilts that received the conventional diet, which, in turn, also had lower MD than those treated with placebo and who received the same diet (Table 3).

Gilts treated with placebo had similar PP and, on the other hand, the immunologically stimulated gilts receiving the 5'-IMP diet had a higher PP than the gilts that consumed the conventional diet, which, in turn, had lower PP compared to gilts treated with placebo and that consumed the same diet (Table 3).

The interaction observed for P1P2P3 ($P = 0.03$) showed that gilts subjected to placebo and consuming the 5'-IMP diet had lower P1P2P3 than gilts receiving the

conventional diet, but immunologically stimulated gilts had similar P1P2P3 but gilts that were immune-stimulated and consuming the conventional diet had lower P1P2P3 compared to gilts that were not immune-stimulated and that consumed the same diet (Table 3). However, immunologically stimulated gilts had similar P1P2P3. Immunologically stimulated gilts consuming the conventional diet had lower P1P2P3 compared to non-immunologically stimulated gilts consuming the same diet (Table 3).

Gilts that received placebo and the 5'-IMP diet had a lower BT than the gilts that were fed the conventional diet. On the other hand, the immunologically stimulated gilts consuming the 5'-IMP diet had higher BT than those who consumed the conventional diet and the gilts receiving placebo who also received the 5'-IMP diet. In contrast, gilts that were immune-stimulated and consumed the conventional diet showed a lower BT than gilts that were not immune-stimulated but received the same diet (Table 3).

3.3 LL M Quality

Measurements of pH, color, water loss, and shear force are the main parameters used to assess the quality of LLM, directly influencing consumer purchase preference (Holman., 2020).

Dietary treatments and immune system stimulation showed no interaction on pH at 45 min ($P = 0.498$), redness ($P = 0.633$) and yellowness ($P = 0.377$) of LLM. However, interactions were identified on 24-hour pH ($P = 0.037$), luminosity ($P = 0.008$), DL ($P < 0.001$), FLT ($P = 0.030$), FLC ($P = 0.025$) and SF ($P = 0.006$) (Table 4).

The consumption of the 5’-IMP diet provided higher pH at 45 min of LLM ($P = 0.035$) compared to gilts fed the conventional diet (Table 4). However, Likewise, this diet provided higher pH values at 24h of LLM compared to gilts fed the conventional diet in both conditions of immune system stimulation. However, pigs treated with placebo that consumed the 5-IMP diet presented pH at 24h of LLM higher than those fed the same diet and received immunostimulation (Table 4).

The L* of LLM presented by the gilts treated with placebo were similar between the dietary treatments, but in immunologically stimulated gilts that consumed the 5'-IMP diet presented higher L*, as well as it was higher than the L* presented by gilts consuming the same diet but treated with placebo (Table 4).

Gilts fed a diet with a reduction of 100 kcal ME/kg and supplemented with 0.100% 5’-IMP showed greater ($P < 0.001$) redness in the LLM compared to those fed the

conventional diet. Additionally, immunologically stimulated gilts had less redness ($P < 0.001$) and yellowness ($P < 0.001$) in LLM (Table 4).

The DL for gilts treated with placebo was similar for both dietary treatments. However, the immunologically stimulated gilts that consumed the 5'-IMP diet had a lower DL than the pigs that consumed the conventional diet and DL higher than gilts that received the same diet that did not receive immune stimulation (Table 4).

The gilts that consumed the 5'-IMP diet still had lower FLT than the gilts fed the conventional diet, in both conditions of stimulation of the immune system. However, immunologically stimulated gilts that received the 5'-IMP diet had higher FLT compared to those that received the same diet and were treated with placebo (Table 4).

The CAP and SF presented by the gilts fed the diet supplemented with 0.100% 5'-IMP was lower than that of the gilts that consumed the conventional diet in both conditions of immune system stimulation (Table 4).

3.4 Blood plasma analysis

In plasma or blood serum, albumin (55%) and globulin proteins present the highest concentrations (Chung et a. 2020). Plasma albumin can be used as an indicator of nutritional status and responses to inflammation (Yang et al., 2019; Zhang et al., 2020). Globulin can bind to cortisol, modulating its levels, in addition to developing essential functions in the immune system, especially in inflammatory processes caused by pathogens or antigens (Hill et al., 2016). In turn, the relationship between albumin and globulin indicates nutritional conditions with the immune system's defense response against inflammatory processes (Chen et al., 2017).

For plasma AST concentration, no interactions were observed between diets and evaluated periods, as well as for immune stimulation and evaluated periods ($P > 0.05$, Table 5). However, AST showed a linear response over the experimental period ($P < 0.001$). Interactions between diets and evaluated periods were observed for plasma concentrations of TP ($P = 0.006$), albumin ($P < 0.001$), uric acid ($P < 0.001$) and ALT ($P = 0.011$). There were also interactions between conditions of immune system stimulation and assessment periods for plasma concentrations of TP ($P = 0.035$), albumins ($P = 0.006$), globulins ($P = 0.038$) and A:G ratio ($P < 0.001$).

Gilts fed the 5'-IMP diet showed a linear increase in plasma total protein concentration, while those fed the conventional diet showed a linear reduction response

as a function of the evaluated periods (Figure 1A). Similarly, gilts treated with placebo showed a linear increase response, but those receiving immunostimulation showed a linear decrease response as there was an increase in the evaluated periods (Figure 1B).

The plasma albumin concentration of the gilts fed the 5'-IMP diet showed a linear increase as a function of the periods, and the gilts fed the conventional diet showed the same response but provided a lower concentration of albumin compared to those fed the diet 5 '-IMP (Figure 1C). Similarly, placebo gilts showed a linearly increasing albumin concentration response, but this effect was not observed in albumin concentrations relative to immunologically stimulated gilts (Figure 1D).

The plasma globulin concentration of the gilts that received the 5'-IMP diet showed a linear increase response with the increase in the periods evaluated, and the gilts fed the conventional diet showed the same response but had a lower concentration of globulins compared to those fed the 5'-IMP diet (Figure 1E).

Gilts consuming the 5'-IMP diet showed a linear increase response for the A:G ratio as a function of the periods evaluated, but this effect was not observed in the albumin concentrations for immunologically stimulated gilts (Figure 1F).

The plasma uric acid concentration of the gilts that consumed the 5'-IMP diet increased linearly with the evolution of the experimental period, as well as the animals that received the conventional diet (Figure 1G). However, there is an evident increase in the concentration of uric acid in the plasma of animals that received the 5'-IMP diet compared to those that received the conventional diet.

The non-immunologically stimulated gilts showed a linear increase in plasma ALT concentration as a function of the periods evaluated, which was not observed in the immunologically stimulated gilts (Figure 1H).

3.5 Blood count

Erythrocytes, hemoglobin, hematocrit, and MCHC showed no significative interaction ($P > 0.05$) between diets and periods, and immune stimulation and periods (Table 6). However, we found significative interactions between diets and periods evaluated for MCV ($P = 0.024$) and platelets ($P < 0.001$), as well as interaction ($P = 0.035$) between conditions of immune system stimulation and periods evaluated for platelets (Table 6).

The diets influenced the hematocrit concentration (P = 0.016), in which the gilts fed the 5'-IMP diet had the lowest concentration (Table 6). Additionally, the periods evaluated influenced (Table 6) the plasma concentrations of hemoglobin (P = 0.037) and MCHC (P < 0.001).

Gilts fed the 5'-IMP diet showed a linear reduction in plasma MCV concentration, while those fed the conventional diet showed an inversely proportional response, with a linear increase for MCV as the experimental period progressed (Figure 2A).

The 5'-IMP diet increased in plasma platelet concentration over the evaluated periods, while those fed the conventional diet showed a reduction (Figure 2B). Gilts treated with placebo and immunologically stimulated showed a reduction in the concentration of platelets throughout the evaluated periods, but immunologically stimulated animals showed a less significant reduction during the experimental period (Figure 2C).

3.6 Leukogram

Differentiated leukocytes are the immune system's first line of defense against pathogens. This subclass includes neutrophils, eosinophils, and basophils, responsible for phagocytizing or removing pathogens from metabolism (Mortara et al., 2015). The second subclass of leukocytes includes monocytes and lymphocytes (B and T cells and natural killer cells). Monocytes remove dead or damaged cells from metabolism. In turn, lymphocytes identify external pathogens and signal to metabolism the need to increase the synthesis of specific cells and antibodies (Desai et al., 2018).

We found significative interactions (Table 7) between diets and periods for total leukocytes (P = 0.001), neutrophils (P < 0.001), lymphocytes (P = 0.014), neutrophil:lymphocyte ratio (P < 0.001) and platelet:lymphocyte (P = 0.017), eosinophils (P < 0.001) and basophils (P < 0.001). We also found interactions between immune system stimulation and time periods for total leukocytes (P = 0.048), lymphocytes (P < 0.001), platelet:lymphocyte ratio (P < 0.001), monocytes (P = 0.001), eosinophils (P = 0.009) and basophils (P < 0.001).

The consumption of the 5'-IMP or conventional diet increased in the concentrations of total leukocytes over the evaluated periods, and the 5'-IMP diet provided a more expressive increase (Figure 3A). Both conditions of immune stimulation also increased

total leukocytes during the experimental period, but the concentration presented by the immune-stimulated gilts was lower than in those who received the placebo (Figure 3B).

The plasmatic concentration of neutrophils referring only to the gilts that consumed the conventional diet showed a linear reduction over the evaluated periods (Figure 3C).

Consumption of the 5'-IMP or conventional diet increased in plasma lymphocyte concentrations as the periods increased, and the increase in lymphocyte concentration in gilts fed the 5'-IMP diet was more pronounced (Figure 3D).

The plasma concentration of lymphocytes in gilts treated with placebo or immunological stimulation also increased during the evaluated periods. In turn, the concentration of lymphocytes from immunologically stimulated gilts showed the lowest rate of increase (Figure 3E).

Only the conventional diet reduced the relationship between neutrophils and lymphocytes over the evaluated periods (Figure 3F), and the 5'-IMP diet provided the same response during the evaluated period.

Gilts that were fed the 5'-IMP or conventional diet also showed distinct linear reductions in the platelet-lymphocyte ratio, with a less marked reduction in animals that consumed the conventional diet (Figure 3G).

The relationship between platelets and lymphocytes referring to gilts that received the placebo or were immunologically stimulated showed a distinct and decreasing response over the evaluated periods. The relationship between platelets and lymphocytes shown by immunologically stimulated gilts was higher than in those treated with placebo (Figure 3H).

The consumption of the 5'-IMP or conventional diet increased in monocyte concentrations over the evaluated periods, fitting different curves, and the monocyte concentration demonstrated by the gilts fed the 5'-IMP diet showed a higher rate of increase compared to those who consumed the conventional diet (Figure 3I).

The gilts that consumed the 5'-IMP diet showed a linear increase response for the plasma eosinophil concentration, while the gilts that were fed the conventional diet showed a linear decrease for the eosinophil concentration as the evaluated periods increased (Figure 3J).

The plasma eosinophil concentration presented by the gilts that received the placebo or the immunological stimulation showed an increase over the evaluated periods, in which the eosinophil concentration presented by the immunologically stimulated gilts was lower than in those that received the placebo (Figure 3K).

The gilts that consumed the 5'-IMP diet showed an increase in the plasma concentration of basophils, while the gilts that were fed the conventional diet showed a reduction as the experiment progressed (Figure 3L). The plasma concentration of basophils presented by gilts that received placebo or immunological stimulation also increased over the evaluated periods, with distinct responses, in which the basophil concentration of immunologically stimulated gilts showed a less expressive increase (Figure 3M).

3.7 Sow blood serum protein electrophoresis

Blood acute-phase proteins are products of the secretion of differentiated leukocytes, which integrate the adaptive immune system in response to infections caused by pathogens (Marco-Ramell et al., 2014).

Similar to the results observed for differentiated leukocytes, interactions ($P < 0.05$) were observed between diets and periods evaluated, as well as stimulation of the immune system and periods for acute-phase protein concentrations in the blood serum of finishing gilts (Table 8). Interactions between diets and time periods were observed for serum transferrin concentration ($P < 0.001$), heavy chain IgG ($P = 0.026$) and light chain IgG ($P < 0.001$). Between the conditions of immune system stimulation and evaluated periods, interactions were observed on the serum concentration of IgA ($P < 0.001$), ceruloplasmin ($P < 0.001$), albumin ($P < 0.001$), haptoglobin ($P = 0.029$) and IgG light chain ($P = 0.008$).

Additionally, the periods influenced the serum concentration of TP ($P = 0.033$), α-1 acid glycoprotein ($P = 0.032$) and 23,000 Dalton protein ($P = 0.008$).

The IgA serum concentration in gilts that were immunologically stimulated showed a reduction over the evaluated periods (Figure 4A).

The immunologically stimulated gilts showed an increase in the serum concentration of ceruloplasmin as a function of the evolution of the experimental period (Figure 4B).

The consumption of the 5'-IMP diet also increased in the serum concentration of transferrin with the increase of the evaluated periods (Figure 4C).

The serum albumin concentration, presented by the gilts that received the placebo, showed an increase, while the immunologically stimulated gilts showed a linear reduction over the evaluated periods (Figure 4D).

The gilts showed different responses to the serum haptoglobin concentration as a function of the immunological condition, in which the animals treated with placebo

showed a reduction and the immunologically stimulated gilts showed an increase in the haptoglobin concentration as a function of the experimental period (Figure 4E).

The consumption of the 5'-IMP diet provided increased serum concentrations of heavy chain IgG throughout the evaluated periods (Figure 4F). However, consumption of the conventional diet provided a linear increase in serum IgG light chain concentrations (Figure 4G).

Gilts that received placebo or immunostimulation showed increased serum IgG light chain concentration, whereas immunologically stimulated gilts exhibited lower concentrations of light chain IgG throughout the period evaluated (Figure 4H).

4. Discussion

Because 5'-IMP is converted into ATP and GTP (Kamatani et al., 2019), the main energy molecules for cellular activities, we assume that dietary supplementation of 5'-IMP for finishing gilts can mainly influence productive performance and the immune system, because the increase in the concentrations of ATP and GTP in metabolism can stimulate protein synthesis (Heskt and Oliver, 2019) and the proliferation and growth of defense cells of the immune system since these cells synthesize ATP at low concentrations via the de novo biosynthesis pathway.

The benefits of including NT blends on productive performance parameters (DWG and DFI) were observed for suckling and nursery-phase piglets that received diets with the inclusion of 0.05% and 1.1% of NT blends, enriched or not enriched with 5'-IMP (Jang and Kim, 2019; Weaver and Kim, 2014).

In this study, the similarity in the DWG of gilts that were not immunologically stimulated highlights that the addition of 0.100% of 5'-IMP supplied the energy deficit of 100 kcal ME/kg, which may have occurred due to the conversion of 5'-IMP in ADP and GMP, and later in ATP and GTP, important energy molecules capable of increasing protein synthesis (Hesketh and Oliver, 2019), suggesting the possibility of using 5’-IMP as an alternative source of energy for feed formulations for finishing gilts.

Possibly, the addition of 0.100% 5'-IMP provided the energy to increase ATP synthesis, promoting actions on food intake control and reducing DFI, but without harming the DWG of finishing gilts that were not immunologically stimulated. This suggests that 5’-IMP may have provided energy and acted together with protein synthesis,

minimizing the effects of energy expenditure caused by stimulation of the immune system.

The effect of dietary supplementation of 5'-IMP on the availability of dietary protein and energy nutrients was evaluated by Jang and Kim (2019), by adding 0.5 and 1.0 % 5'-IMP to the diet of weaned pigs. The authors reported that the 1.0% supplementation increased the productive parameters, as well as the total apparent digestibility of dry matter and nitrogen.

In immunologically stimulated pigs, the consumption of the 100 kcal ME reduced diet, supplemented with 0.100% 5'-IMP, promoted the highest DWG, which in part may have occurred due to the beneficial metabolic activities developed by the 5'-IMP in the process stimulation of the immune system, such as the metabolic conversion of 5'-IMP into ADP or GMP, later converted to ATP or GTP (Kamatani et al., 2019). In vitro studies have shown that 5'-IMP may be the molecule responsible for supplying this energy deficit in pig red blood cells (Jarvis et al., 1979; Kim and McManus, 1971; Watts et al., 1979).

This higher concentration of ATP increases energy availability for cellular activities and stimulates the growth and proliferation of cells linked to protein synthesis and muscle growth (Goron et al., 2019, Hara and Kondo, 2015, Hesketh et al., 2019, Liddicoat et al., 2016). Likewise, the higher concentration of GTP and the activity of the IMPDH enzyme stimulate the synthesis and proliferation of immune system defense cells (Buey et al., 2017).

The immune system's defense cells do not synthesize NTs in sufficient quantity to guarantee the maintenance of these cells. Stimulation of the immune system reduces the availability of NTs and, thus, the dietary source of 5'-IMP may have been essential to assisting in the efficiency of essential metabolic activities of 5'-IMP in several metabolic processes, mainly protein and immune. Hess and Greenberg (2012) also reported that some situations can reduce the concentration of NTs in metabolism, making it essential to include them in diets to ensure the maintenance and increase the efficiency of various metabolic activities.

Stimulation of the immune system is a process that consumes high amounts of energy (ATP and GTP) to increase cytokine concentrations in response to inflammatory processes and reduce the DFI, reducing the availability of energy in the form of ATP or GTP for metabolism, as well as the concentration of NTs, which develop important functions in the immune system, impairing the functionality of the immune system and combating inflammatory processes and pathogens (Mehta et al., 2017).

Similarly, in gilts that were immunologically stimulated and consumed the conventional diet (3,300 kcal ME/kg), the energy that would be destined for protein synthesis and muscle growth may have been used to stimulate the immune system, and may even explain the lower DWG observed for gilts that did not receive the diet supplemented with 0.100% of 5'-IMP and that had the immune system stimulated.

In general, the gilts that consumed the diet with a reduction of 100 kcal of ME and supplemented with 0.100% of 5'-IMP had lower FC than the gilts fed a conventional diet. This reduction in FC may be related to the increase in the energy efficiency of the diet and also due to the metabolic activities of 5'-IMP, both in protein synthesis (Benjamin and Hall, 2017) and in the immune system (Shinohara and Tsukimoto, 2018).

Stimulation of the immune system causes an increase in the energy expenditure of ATP and GTP due to the increase in the synthesis of defense agents in the immune system. Another effect is the increase in the concentrations of oxytocins in metabolism, which produces fever and a reduction in animal consumption, further impairing energy availability in metabolism. These actions may partially explain the lower DWG, and the higher DFI and FC presented by immunologically stimulated gilts that consumed the conventional diet.

Pigs can incorporate 2 to 5% of the 5'-IMP provided in the diet into various muscles. In the rigor mortis process, ATP undergoes catalytic actions by ATPase and myokinase enzymes, which remove two phosphate groups from ATP, converting it into adenosine-5-monophosphate (5'-AMP), then rapidly deaminated by the enzyme adenosine deaminase to 5'-IMP, which is then converted to inosine (INO) and hypoxanthine (Battle et al., 2000, Matsuishi et al., 2016, Tsai et al., 1972)

In this study, the supplementation of 0.100% of 5'-IMP promoted the highest HCY, suggesting that, possibly, the dietary supplementation of 5'-IMP increased its muscle concentrations and during the rigor mortis process this higher concentration possibly influenced the formation of the actin-myosin complex, modifying carcass water loss rates during the first hour after animal sacrifice (Nakamura et al., 2014).

The higher CCY may be related to the fact that the storage of pig carcasses at -0º C decreases the enzymatic activities responsible for the conversion of ATP to IMP, but at this temperature, the microorganisms can grow and increase the degradation of IMP in the meat (Yoshioka et al., 2019). Therefore, the dietary supply of 5'-IMP may increase the muscle concentration of 5'-IMP, providing a higher concentration of 5'-IMP in the sow carcass. Thus, the higher concentration of 5'-IMP can ease the reduction in the

concentrations of 5'-IMP and ATP, prolonging the end of the rigor mortis process and reducing the formation of the actin-myosin complex, factors that may also be related to higher CCY (Hwang et al., 2019, Kuo et al., 2005). Additionally, 5'-IMP can act on lipid metabolism, modifying the lipid concentration in the carcass, which can also influence the HCY and CCY (Farber et al., 1965, Zhang et al., 2008).

The similarity between MD and PP was shown between gilts treated with placebo and immunologically stimulated gilts that were fed the 5'-IMP diet, suggesting that the 5'-IMP may have supplied the energy deficit arising from the immunological stimulation. Furthermore, stimulation of the immune system caused detrimental effects on MD and HW of gilts fed the conventional diet since these parameters were reduced when stimulating the immune system (Table 3).

In part, the increase in energy from dietary supplementation of 5'-IMP may be related to the conversion of 5'-IMP into the main energy molecules (ATP and GTP) used in the cellular activities of the immune system, such as T lymphocytes (Wang et al., 2019). Another effect of 5'-IMP is to stimulate protein synthesis in the mTOR complex, where the higher concentration of purines in metabolism can provide an increase in pyrimidine biosynthesis and mTOR activity (Benjamin and Hall, 2017). In contrast, high concentrations of purines can reduce mTOR stimulation, maximizing the therapeutic effects of purines on metabolism (Emmanuel et al., 2017).

The higher LMY presented by the gilts, promoted by the supplementation of 0.100% of 5'-IMP and even with a reduction of 100 kcal ME/kg, may be related to the action of 5'-IMP in increasing the protein deposition in the carcass of finishing gilts, such as the action of purine bases on the mTOR complex (Benjamin and Hall, 2017), the conversion of 5'-IMP into ATP and GTP (Harmsen et al., 1984) and in the control of protein synthesis (Hesketh and Oliver, 2019).

The reduction in carcass length due to the stimulation of the immune system (Table 3) also demonstrates the damage caused by this stimulation on the expression of the maximum genetic potential of gilts, as well as showing an action of 5'IMP on the immune system, in addition to of the energy input provided by it due to the reduction of 100 kcal ME/kg of feed. A higher concentration of 5'-IMP in dietary metabolism may increase the activities of the IMPDH enzyme (Pua et al., 2017), responsible for metabolic activities aimed at increasing or reducing lipid deposition (Harmsen et al., 1984).

The inclusion of 1.069% of soybean oil in the conventional diet was necessary to provide the 3300 kcal ME/kg of feed, providing an amount of energy in the metabolism

that resulted in greater deposition of this energy in the fat layer of gilts subjected to placebo, since they presented higher P1P2P3 when compared to gilts fed the 5'-IMP diet. On the other hand, stimulation of the immune system may have increased energy expenditure in gilts that consumed the conventional diet, caused by the increase in the synthesis of various immune system defense agents, making P1P2P3 lower than in gilts treated with placebo that received the same diet.

Similar to the results of P1P2P3, the conventional diet (3300 kcal ME/kg ration) promoted an increase in BT in gilts compared to those who received the 5'-IMP diet without stimulating the immune system. However, stimulation of the immune system may have increased energy expenditure in gilts that consumed the conventional diet, making the BT lower than those presented by gilts treated with placebo that received the same diet, highlighting that the immunological stimulation process reduced the BT of the carcass of finishing gilts, possibly due to increased energy demand for the immune system (Shattuck-Heidorn et al., 2017).

On the other hand, immunologically stimulated gilts receiving a diet with a reduction of 100 kcal of ME and 0.100% of 5'-IMP had higher BT compared to those who consumed the same diet but were treated with placebo and also to immunologically stimulated gilts who received the conventional diet. This response may be partially related to the actions of 5'-IMP on the homeostasis of NTs in metabolism (Carver and Walker, 1995) and the synthesis and proliferation of immune system defense agents (Wang et al., 2020), which can minimize the amount of energy expended on the immune stimulation process.

The 5'-IMP can also influence the activities of the enzyme IMPDH in the translocation and oxidation of lipids (Whitehead et al., 2004) and the conversion of 5'-IMP into GTP, increasing the concentrations of GTP mannose, responsible for transferring the mannose group to the lipid-linked oligosaccharide (Spencer and Elbein, 1980).

The concentration of 5’-IMP in muscles has been directly related to the quality of the meat, mainly with its effect on the “Umami” flavor that this additive implements in the meat (Aaslyng and Meinert, 2017).

The higher pH values at 45 min and 24 hours of LLM, presented by the gilts that consumed the 5'-IMP diet, may be related to the increase in the concentration of 5'-IMP in the tissue. In the rigor mortis process, 5'-IMP can be converted into ATP (Harmsen et al., 1984), in addition to releasing ribose molecules that are converted to phosphorylated

glycolytic intermediates in the pentose-phosphate pathway, used for the synthesis of ATP (Jurkowitz et al., 1998).

Additionally, dead cells or cells undergoing apoptosis can release 5'-IMP into the extracellular medium (Chen et al., 2017), which can increase its concentration in the tissue and its conversion into ATP, prolonging the rigor mortis process and increasing the pH. The higher pH values provided by the 5'-IMP diet, both 45 minutes and 24 hours after slaughter, is a key factor when considering the use of this diet. This characteristic is related to redder, softer, and more succulent meat, aspects of great appeal to the consumer market (Holman et al., 2020).

Controversially, the increased demand for ATP and GTP from stimulation of the sow's immune system probably reduced muscle ATP concentration, accelerating glycolysis and ATP depletion, increasing tissue hydrolysis and acidification (Edwards et al. al., 2010). This may explain, in part, the lower pH values at 45 min and 24 hours presented by gilts fed the conventional diet and treated with immunostimulation that received the same diet (Table 4), showing that immune stimulation can cause damage to the chemical characteristics (water retention) and physical (color) of the meat (color), causing damage to meat quality.

In this study, all pH and L values of LLM obtained are within the standard referenced by AMSA (2001) as good quality meat (pH 24 hours after slaughter, 5.7 > and < 5.3) and normal color (>49 and <60). The immunologically stimulated gilts that consumed the conventional diet had a 24-hour pH (5.37) close to the established limit since meat with pH after 24-hour lower than 5.3 is classified as PSE.

When assessing the coloration of LLM by spectral measurements using a colorimeter, we found that the luminosity presented by the LLM of immunologically stimulated gilts that consumed the 5'-IMP diet was superior to the luminosity of the other groups of gilts, resulting in a darker flesh, probably associated with greater redness of LLM. However, all LLM luminosity values presented by gilts are within the standard referenced by AMSA (2001) as normal coloration (>49 and <60). In general, the redness of LLM referring to gilts treated with placebo was higher than in immunologically stimulated gilts, as well as animals that received the 5'-IMP diet showed higher values compared to those that received the conventional diet. These results show the effect of 5'-IMP in increasing the concentration of myoglobin in LLM, a pigment responsible for increasing redness in LLM, associated by consumers with adequate storage periods and conditions, resulting in better meat quality and food safety for the consumer.

On the other hand, the process of stimulation of the immune system reduced the redness of LLM, which can be explained by the increase in the conversion of myoglobins into metmyoglobin, responsible for the increase in brown tone. Consumers consider that this characteristic refers to inadequate storage periods and conditions and also to losses in meat quality and safety (Kennedy et al., 2004).

The greater yellowness of LLM can be related to the concentration of intramuscular lipids (Saricoban and Yilmaz, 2010), noting that the immunologically stimulated pigs showed lower yellowness of LLM than the color presented by pigs treated with placebo (Table 4). These results also corroborate the reduction in BT presented by immunologically stimulated pigs that received the conventional diet, confirming a probable reduction in the concentration of the lipid in the adipose layer and in LLM, promoted by the stimulation of the immune system of finishing gilts.

It is well known that meat contains 60-80% water in its composition (Cheng and Sun, 2008). Most of the muscle water is located within the myofibrils, where a part is found in its free form and the rest is bound to proteins, mainly myosins, and actins. The water retention capacity of LLM is directly related to the pH value at the end of rigor mortis, the LMY, the concentration of intramuscular lipids, and the composition of fibers in muscle tissues (De Vries et al., 1994, Toldrá, 2003)

The lower DL of LLM presented by immunologically stimulated gilts that consumed the 5'-IMP diet, compared to those who consumed the conventional diet, demonstrate metabolic activities of the 5'-IMP on the processes that ensure water retention in the M LL, like the action of 5'-IMP in decomposing the actin-myosin complex, releasing myosins and actins which bind to water molecules in their free form, thus increasing water retention in LLM (Matsuishi et al., 2016). Additionally, the benefits of LLM`s DL in immunologically stimulated gilts, provided by the 5'-IMP diet, may be related to the higher 24-hour pH values observed in the same gilts. This indicates a lower concentration of lactic acid and the activity of muscle enzymes, responsible for the proteolysis and lipolysis processes that occur during the storage of LLM, thus minimizing water loss (Toldrá, 2003; Kim et al., 2016).

The FLT, FLC, and SF of LLM of the animals that consumed the 5'-IMP diet were lower than that of the gilts fed the conventional diet, in both conditions of stimulation of the immune system. These results evidence the metabolic activities of 5'-IMP to reduce water loss from LLM in the thawing and cooking process.

In meats frozen at -2 ºC the concentration of 5'-IMP was maintained for a long period, while when the meat was stored at 0 º C the concentration of 5'-IMP was stable for 10 days, drastically reducing after this period and minimizing their metabolic activities on the rigor mortis process (Yoshioka et al., 2019). This process is associated with the ability of 5'-IMP to increase the extraction of myosins and actins from pork (Nakamura et al., 2012). This answer exposes the possibility of 5'-IMP being a candidate to help extend the rigor mortis process, due to its ability to dissociate the connections between the thin and thick filaments (Matsuishi et al., 2016).

Impairments of the stimulation of the immune system on DL, FLT, FLC, and SF of LLM were observed in gilts fed the conventional diet, possibly related to the reduction in the amount of ATP caused by the stimulation of the immune system, reducing synthesis and deposition of lipids, both in the carcass and intramuscularly. This partly explains the reduction in the yellowness values of LLM, directly related to the reduction in the deposition of intramuscular lipids and the increase in water loss of LLM (Huang et al., 2020).

Shear force assesses meat tenderness (Jiang et al., 2017). In this study, the 5'-IMP diet provided SF lower than that of the gilts that consumed the conventional diet, suggesting an influence of the 5'-IMP on meat tenderness. The lower SF of gilts fed the diet supplemented with 5'-IMP may be due to the higher concentration of 5'-IMP in metabolism, acting to increase water retention in LLM, as it is able to dissociate the actin-complex myosin (Nakamura et al., 2014). The lower SF presented by the gilts that consumed the 5'-IMP diet agrees with Zhang et al. (2008), in which the supplementation of 5'-IMP (0, 0.25, 0.50, and 0.75%) in the diet of broiler chickens (n=240) was evaluated, with a lower FC of M. pectoralis major and M. from the thigh of broiler chickens, from the supplementation of 0.25% of 5'-IMP, in relation to broilers fed a basal diet.

The higher concentration of TP presented by gilts, both fed the 5-IMP diet and those treated with placebo, suggests that the 5'-IMP, when converted into ATP or GTP, acts by stimulating the synthesis of proteins present in the blood, which are important defensive agents for the immune system (Zhu and Thompson, 2019).

The proteins present in the blood are components of cells, tissues, and hormones that regulate various bodily functions, their concentration in plasma indicates the rate of protein deposition in muscle and the status of the immune system (Kumar et al., 2005; Zeng et al., 2013).

The significant increase in the concentration of plasma albumin provided by the consumption of the 5'-IMP diet, as a function of the periods evaluated, indicates that the dietary supplementation of 5'-IMP can benefit the nutritional status and the defense responses against inflammatory processes in finishing gilts. This may be related to reduced energy expenditure due to reduced 5'-IMP biosynthesis and increased concentration of 5'-IMP metabolism, such as immune system defense cells, which have low concentration and are not capable of biosynthesize NTs in de novo biosynthesis (Gil, 2002).

The stimulation of the pigs' immune system did not influence the albumin concentration over the evaluated periods, but it increased the plasma concentration of globulins and a reduction in the A:G ratio. The higher concentration of globulins, a negative acute-phase protein, is consistent with the higher demand arising from the need to activate the immune system's defense response, related to higher levels of cortisol and inflammatory processes (Hill et al., 2016), consequently increasing the demand for other acute-phase proteins (Chung et al., 2020). This shows that pigs whose immune system was stimulated had losses on energy metabolism and increased incidence of inflammatory processes.

The 5'-IMP diet increased in the concentration of plasma uric acid in the blood of pigs, indicating benefits on the antioxidant system, as uric acid is the most potent antioxidant present in the blood, being responsible for 50% of the antioxidant actions to fight ROS in serum or blood plasma (Hassan et al., 2012). Additionally, this increase may also be related to greater degradation of dietary 5'-IMP, which is partly converted to uric acid (Ames et al., 1981; Mandal et al., 2015).

The conventional diet increased in the concentration of ALT during the evaluated periods, and its accumulation may be related to metabolic disorders of liver activities (Villafranca et al., 2017). These disorders are caused by the intoxication of certain substances, initiating the oxidation process cascade, responsible for breaking the liver cell membrane leading to apoptosis, releasing ALT and other components into the bloodstream. When these components accumulate, they can reduce the hepatoprotective capacity, causing several liver dysfunctions (Abou-Elkhair et al., 2018).

The increase in hematocrit provided by the conventional diet indicates that these pigs had a higher concentration of red blood cells, however, both diets had a concentration within the normal range.

MCV is a parameter used to assess the size and volume of red blood cells, with higher values of MCV being indicative of the incidence of anemia and a higher incidence of infections that can lead to death (Kor et al., 2018; Wu et al., 2018). In the present study, the conventional diet increased in MCV (Figure 3A), possibly indicating greater oxidative stress in the metabolism of these animals, responsible for impairing DNA biosynthesis and increasing the concentration of MCV (Iacopetta et al., 2001).

Additionally, the higher MCV observed in gilts that consumed the conventional diet may also be related to the lower concentration of platelets presented by this group of gilts during the evaluated periods (Figure 3B) since it is associated with a lower iron concentration in the metabolism and the condition of anemia (Dratch et al., 2019).

The increase in the concentration of platelets presented by gilts fed the 5'-IMP diet over the periods shown demonstrates that the 5'-IMP benefited the defense of the immune system, as platelets develop pro-inflammatory actions and are an indicator of systemic inflammation conditions (Liaw et al., 2017).

Stimulation of the immune system also reduced the concentration of platelets (Figure 3C), probably because platelets participate in responses to inflammatory processes and act in tissue regeneration (Kia et al., 2018), explaining the lower concentration of platelets found.

The main effects of NTs on metabolism occur on the immune system (Gil, 2002; Haskó et al., 2004; Hess and Greenberg, 2012). Among NTs, 5'-IMP and inosine have shown, in several studies, numerous beneficial effects on metabolism, such as the effects of inosine in stimulating the proliferation of T lymphocytes, in addition to being used as a carbon source to maintain functions of C8+-T cells under glucose restriction (Wang et al., 2020), develop potent anti-inflammatory and antinociceptive actions, as well as modulate adenosinergic receptors, especially the A2AR, essential for the defense of the immune system (Junqueira et al., 2017).

The immune system's defense response starts with the increase in macrophage activities. When these cells identify pathogens associated with standard immune molecules (PAMPs) and toll-like receptors, they signal to metabolism the need to increase the synthesis of cytokines and prostaglandins to initiate the inflammatory response, recruiting circulating immune cells and soluble immune molecules (Patience, 2011).

Since extracellular adenosine, adenosine (A2A), and adenosine (A3) are receptors that identify anti-inflammatory signals and indicate tissue damage caused by excess inflammation, they become important regulators of inflammatory processes. These

receptors are activated from the moment inosine binds to them; thus inosine can influence these receptors and their actions on inflammatory processes (Liddicoat et al., 2016).

The results of this study demonstrated that the supplementation of 0.100% of 5'-IMP, even with a reduction of 100 kcal ME/kg, provided a significant increase over time, compared to the conventional diet, in the concentration of total leukocytes, lymphocytes, monocytes, eosinophils and basophils (Figure 3), showing that 5'-IMP acts to increase the synthesis of these defense agents in the immune system, promoting benefits to the immune system of finishing gilts and the possibility of using 5'-IMP for this purpose.

The increase in the concentration of these defense agents promoted by dietary supplementation of 5'-IMP to finishing gilts may be partially related to the inefficiency of immune system defense cells in biosynthesizing NTs in the de novo synthetic pathway. Thus, dietary supplementation of 5'-IMP may have increased their concentration in immune system defense cells, where they can be converted to other NSs and NTs, such as ATP and GTP, energetic molecules that are essential to increase the synthesis and proliferation of immune system defense cells.

The increase in the concentration of these defense agents promoted by the 5'-IMP diet demonstrates an important action of the 5'-IMP, and may also be related to the benefits promoted by this diet on productive performance, meat yield, meat quality, and biochemical parameters of the blood plasma of gilts that received the placebo, as well as in the several studied variables. Furthermore, immunologically stimulated animals also showed a positive response when receiving 5'-IMP in the diet. It is also worth noting that, in addition to the greater energy input provided by 5’-IMP, it can also provide an energy rescue in situations of immune system stimulation, which may support future studies that can elucidate this partition of the 5’-IMP functions.

The concentration of these immune system defensive agents reduced with the stimulation of the immune system, except for neutrophils. This demonstrates that the energy demand of metabolism increases, causing an energy deficit that probably caused the reduction in the concentration of total leukocytes, lymphocytes, monocytes, eosinophils, and basophils.

Stimulation of the immune system increased the relationship between platelets and lymphocytes in finishing gilts, suggesting possible losses in protein deposition in these pigs, since the relationship between platelets and lymphocytes is an important biomarker of inflammatory processes (Liaw et al. , 2017), in which the greatest relationship is directly related to the loss of muscle mass (Schaap et al., 2006). The higher relationship

between platelet and lymphocytes, observed in immunologically stimulated pigs that consumed the conventional diet, agrees with the lower DWG, MD, and ham weight observed for these animals.

The energy deficit resulting from the stimulation of the immune system, associated with the increase in the concentration of these defense agents promoted by the 5'-IMP diet, suggests that the 5'-IMP can be an important additive to be used in the formulation of feed for finishing gilts, which can be used as an additive with more than one function. The 5'-IMP enhances the defense of the immune system against pathogens, antigens, and inflammatory processes, that is, when exposed to pathological situations, the loss observed on the productive performance of these pigs will probably be smaller.

Studies carried out in maternity and post-weaning piglets have shown the beneficial effects of supplementing 5'-IMP or NTs on the immune system (Li et al., 2015; Jiao and Kim, 2018; Waititu et al., 2015; Weaver and Kim, 2013), which corroborate the results observed in this study.

Supplementation of 0.100% NTs-rich soy extract in a diet without the use of antibiotics increased the productive performance of piglets in the post-weaning period (Waititu et al., 2015). The authors also reported that these effects can benefit producers and nutritionists who need to meet the market demand not to use antibiotics in their diets.

NTs are not synthesized in biosynthesis de novo in many cells of the immune system (Hess and Greenberg, 2012). Therefore, studies have shown that supplementation from exogenous NT sources can promote a more robust defense response of the immune system (Muto et al., 2014, Superchi et al., 2012, Wang et al., 2019). In the present study, by stimulating the immune system's defense response, it is assumed that the endogenous concentration of NTs reduced, as well as increased energy expenditure with the synthesis of immune system cells. Therefore, an exogenous supply of NTs, such as 5'-IMP, can improve the defense efficiency of the immune system when stimulated.

In addition to possibly immune stimulation to reduce the concentration of NTs in pig metabolism, especially in immune system defense cells, there is also a significant increase in ATP consumption from leukocyte proliferation, increased antibody production, or rapid increase in concentrations of acute-phase proteins (Shattuck-Heidorn et al., 2017). Inflammatory processes are suppressed by the action of transferrin receptors, possibly by mechanisms that are controlled by proteins such as interferon-gamma, which decreases the expression of this receptor in phagocytic cells (Byrd and Horwitz, 1983). Additionally, transferrins and their receptors can influence the growth of immune cells

due to their high iron requirement (Seligman et al., 1992). Likewise, iron deficiency and the absence of stimuli on ferritin and its receptors have been shown to reduce T cell proliferation, impairing immunity in mice (Omara and Blakley, 1994).

In this study, the consumption of the 5'-IMP diet increased in serum transferrin concentrations. This increase is relevant because transferrin is a negative acute-phase protein, that is, during inflammatory processes its concentration increases only up to 25% compared to the condition without immunological stimuli (Gabay and Kushner, 1999), thus greater availability of transferrin can enhance its actions on the immune system.

There is evidence that one of the mechanisms of 5'-IMP on the growth and proliferation of white cells in metabolism is that under conditions of glucose restriction, 5'-IMP, when catabolized, donates the ribose molecule present in its structure, aiding the proliferation, growth, and activities of CD8+ T cells (Wang et al. 2019).

IgG is considered the main and most abundant blood immunoglobulin and the most potent antibody against pathogens, antigens, and inflammatory processes (Chu and Song, 2013; Schwab and Nimmerjahn, 2013). IgG acts on the immune system interconnecting and modulating the innate and adaptive immune systems (Schwab and Nimmerjahn, 2008), in which numerous IgG molecules bind to their respective target antigens, activating and triggering effector responses of pro-inflammatory actions (Carrol, 2004; Nimmerjahn and Ravetch, 2008). Additionally, IgG acts on the humoral immune response by activating the C1q-dependent domain, as well as stimulating the binding of Fcy family receptors to innate defense immune cells, promoting the activation of these cells and benefiting the innate immune system (Schwab and Nimmerjahn, 2008).

Two heavy immunoglobulins (Ig) chains associated with two light Ig chains form the antibody. Heavy chains have a specific binding domain to their respective antigen. It can bind to light chains and move to the plasma membrane, allowing the B cell to produce another light chain (Haas and Wabl, 1983; Mårtensson and Ceredig, 2000).

IgG is considered the main and most abundant blood immunoglobulin and the most potent antibody against pathogens, antigens, and anti-inflammatory processes (Chu and Song, 2013, Schwab and Nimmerjan, 2013).

In the present study, the finishing gilts that consumed the 5'-IMP diet showed an increase in the serum concentration of heavy chain IgG, demonstrating benefits on the defense of the immune system, since IgG is the main and most effective molecule against pathogens and antigen. Conversely, the serum concentration of light chain IgG presented by gilts fed the conventional diet showed a linear increase over the evaluated periods,

which can be explained by the lower concentration of heavy chain IgG in these animals, assuming that there was less binding between B cells and heavy chain IgG, resulting in increased concentration of light chain IgG.

IgA is the immunoglobulin responsible for protecting the intestinal mucosa against pathogens by binding to pathogens and is carried out of the intestine, avoiding the damage that would otherwise be caused (Mantis et al., 2011; Woof and Burton, 2004). Previous studies have shown that stimulation of the immune system reduces IgA concentrations (Steffen et al., 2020), and, in agreement with these studies, we observed a linear reduction in the serum IgA concentration in the blood of finishing gilts that were stimulated immunologically, showing damage to the defense of the intestinal mucosa of these animals, since IgA is responsible for binding to pathogens and removing them from metabolism.

Ceruloplasmin and haptoglobin are considered positive acute-phase proteins, which increase above 25% during an inflammatory process (Gabay and Kushner, 1999). These reports support the increase in the serum concentration of ceruloplasmin and haptoglobin over the evaluated periods presented by gilts that were immunologically stimulated. The increase in serum concentrations of these acute-phase proteins shows that the defense of the immune system is more efficient in combating pathogens and antigens (Charlie-Silva et al., 2019), reducing the damage caused by them in metabolism.

Acute-phase proteins are essential for the innate immune system response to infections, stress, and trauma. Their serum levels reflect the conditions of the immune system's defense responses against inflammatory processes, and the serum concentrations of these proteins may increase or decrease during the acute-phase response of the immune system's defense (Charlie-Silva et al., 2019; Janmohammadi et al., 2020).

Changes in the serum concentration of acute-phase proteins can be beneficial to the body, as they prevent the growth of harmful micro-organisms and help to maintain homeostasis. Some acute-phase proteins have the main function of limiting the growth of harmful microorganisms, while others are responsible for removing remnant compounds from cell degradation and free radicals, in addition to neutralizing proteolytic enzymes (Gruys et al., 2005).

In general, the supplementation of 0.100% of 5'-IMP in the finishing gilts diet, with the respective reduction of 100 kcal ME/kg, showed that it can be used as an alternative energy source, mainly in conditions that stimulate the immune system and, consequently, increase the energy cost of metabolism and reduce the concentration of endogenous NTs,

impairing energy, protein, and immune metabolism, restricting the functions of molecules from these systems or from other tissues that are also unable to biosynthesize NTs in the de novo pathway, limiting the maximum growth of gilts, that reflects in productive losses.

The results demonstrate that the dietary supplementation of 0.100% of 5'-IMP, with the respective reduction of 100 kcal ME/kg, can supply this energy reduction (100 kcal) and the energy demand coming from the stimulation of the immune system, due to the need to increase the synthesis of differentiated leukocytes and acute-phase proteins. These effects were reflected in productive performance, carcass characteristics, meat quality, and biochemical parameters related to the immune system.

5. Conclusions

The supplementation of 0.100% of 5'-IMP with the respective reduction of 100 kcal ME/kg of feed was efficient in supplying the energy restriction in the diet and also supplied the energy deficit resulting from the stimulation of the immune system, promoting benefits on the productive performance, carcass characteristics, meat quality and biochemical parameters of blood plasma correlated to the immune system, showing the possibility of 5'-IMP being used in the feed formulation as an additive with multiple uses.

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Table 1. Composition of experimental diets

Ingredients (%)	5'-IMP	Conventional
Corn	79.152	79.920
Soybean meal, 45 %	16.531	16.458
Soy oil	-	1.069
Dicalcium phosphate	0.763	0.762
Limestone	0.799	0.800
Salt	0.224	0.224
5'-IMP[1]	0.100	-
Inert[2]	1.661	-
Vitamin and mineral supplement[3]	0.400	0.400
L-Lysine HCl 78,4%	0.273	0.274
DL-Methionine 99,0%	0.019	0.019
L-Threonine 98,5%	-	0.039
Enramycin	0.020	0.020
Feed Dry[4]	0.020	0.020
Calculated composition, %		
Metabolizable energy (Mcal/kg)	3.20	3.30
Crude protein	14.000	14.000
Total calcium	0.560	0.560
Available phosphorus	0.220	0.220
Potassium	0.556	0.556
Sodium	0.100	0.100
Chlorine	0.271	0.272
SID Lysine	0.770	0.770
SID Methionine	0.222	0.222
SID Methionine + cysteine	0.440	0.440
SID Threonine	0.480	0.480
SID Tryptophan	0.134	0.134
SID Valine	0.620	0.620
SID Leucine	1.189	1.190
SID Isoleucine	0.496	0.496
SID Arginine	0.782	0.781
SID Histidine	0.346	0.347
SID Phenylalanine	0.610	0.610
SID Phenylalanine + tirosine	1.062	1.062

[1]Inosine-5'-monophosphate.
[2] Kaolinite.
[3]Content kg^{-1}: vit. A: 30000 UI, vit. D3: 5000 UI, vit. E: 120 UI, vit. K: 5 mg, vit. B12: 120 mcg, Niacin: 150 mg, Calcium Pantotenate: 75 mg, Folic Acid: 8 mg, Choline Chloride: 0.48 g, Iron: 350 mg, Cooper 15 mg, Manganese: 250 mg, Zinc: 0.75 g, Iodine: 10 mg, Selenium: 3 mg.
[4]Antioxidant.

Table 2. Performance of 75- to 100- kg gilts submitted or not to immune stimulation and fed diets with reduced metabolizable energy (ME) supplemented with 0.100 % inosine-5'-monophosphate (3.20 Mcal/kg) or a conventional diet without reducing ME (3.30 Mcal/kg)

Immune stimulation	Diets	ADG[1], kg	DFI[2], kg	F:G[3], kg/kg
Placebo	3.20 Mcal/kg + 5'-IMP	1.07b	2.58a	2.47
	3.30 Mcal/kg	1.07b	2.80b	2.67
Stimulated	3.20 Mcal/kg + 5'-IMP	1.12c	2.77b	2,52
	3.30 Mcal/kg	0.95a	2.72b	2.83
Mean		1.04	2.72	2.61
SD		0.067	0.136	0.159
SEM		0.010	0.021	0.024
Diets				
3.20 Mcal/kg + 5'-IMP		1.09	2.68	2.50a
3.30 Mcal/kg		1.01	2.76	2.75b
Immune stimulation				
Placebo		1.07	2.69	2.57a
Stimulated		1.03	2.75	2.67b
Variation sources				
Diets		<0.001	0.044	<0.001
Immune stimulation		0.020	0.029	0.012
Diets x Immune stimulation		<0.001	0.006	0.112

[1]Average daily gain.
[2]Daily feed intake.
[3]Feed gain ratio.

Table 3. Carcass traits of 75- to 100- kg gilts submitted or not to immune stimulation and fed diets with reduced metabolizable energy (ME) supplemented with 0.100 % inosine-5'-monophosphate (3.20 Mcal/kg) or a conventional diet without reducing ME (3.30 Mcal/kg)

Immune stimulation	Diets	HCY[1], %	CCY[2], %	LLD[3], mm	HW[4], kg	HY[5], %	LMY[6], %	CL[7], %	P1P2P3[8], cm	BF[9], cm
Placebo	3.20 Mcal/kg + 5'-IMP	81.66	79.53	69.59b	11.29b	29.21	62.48	100.47	1.733a	0.819a
	3.30 Mcal/kg	80.69	78.46	68.30b	11.13b	29.03	61.46	99.79	1.989b	0.951c
Stimulated	3.20 Mcal/kg + 5'-IMP	81.23	79.00	71.49b	11.45b	29.07	61.93	97.68	1.782a	0.935d
	3.30 Mcal/kg	80.40	77.86	63.44a	10.74a	28.99	61.31	97.87	1.872a	0.811b
Mean		81.09	78.69	67.53	11.14	29.14	61.90	98.96	1.884	0.885
SD		1.034	1.025	3.545	0.381	0.600	0.819	2.192	0.121	0.080
SEM		0.166	0.162	0.560	0.060	0.095	0.130	0.347	0.019	0.013
Diets										
3.20 Mcal/kg + 5'-IMP		81.44b	79.27b	70.54	11.37	29.14	62.21b	99.08	1.758	0.877
3.30 Mcal/kg		80.55a	78.16a	65.87	10.94	29.01	61.38a	98.83	1.930	0.881
Immune stimulation										
Placebo		81.17	78.99	68.94	11.21	29.12	61.97	100.13b	1.861	0.885
Stimulated		80.81	78.43	67.46	11.09	29.03	61.62	97.78a	1.827	0.873
Variation sources										
Diets		0.022	0.011	0.001	<0.001	0.261	0.012	0.436	0.001	0.858
Immune stimulation		0.434	0.122	0.165	0.317	0.431	0.376	0.014	0.428	0.019
Diets x Immune stimulation		0.817	0.925	0.004	0.024	0.492	0.455	0.185	0.030	<0.001

[1]Hot carcass yield.
[2]Cold carcass yield.
[3]*Longissimus lumborum* depht.
[4]Ham weight.
[5]Ham yield.
[6]Lean meat yield.
[7]Carcass length.
[8]Mean measurement of the backfat thickness at three different points on the carcass.
[9]Backfat thickness.

Table 4. Meat quality evaluated on *Longissimus lumborum* muscle of 75- to 100- kg gilts submitted or not to immune stimulation and fed diets with reduced metabolizable energy (ME) supplemented with 0.100 % inosine-5'-monophosphate (3.20 Mcal/kg) or a conventional diet without reducing ME (3.30 Mcal/kg)

Immune stimulation	Diets	pH45	pH24	L*	Redness	Yellowness	DL[1], %	TL[2], %	CL[3], %	SF[4], N
Placebo	3.20 Mcal/kg + 5'-IMP	6.32	5.77c	57.81a	7.54	3.96	3.40a	8.29a	28.80a	24.12a
	3.30 Mcal/kg	6.22	5.41a	57.82a	7.16	4.03	3.25a	11.12c	33.08b	31.74b
Stimulated	3.20 Mcal/kg + 5'-IMP	6.29	5.58b	58.50b	7.04	3.73	4.04b	10.55b	30.04a	25.54a
	3.30 Mcal/kg	6.24	5.38a	57.60a	6.69	3.69	5.48c	12.07c	32.15b	36.40b
Mean		6.28	5.55	57.97	7.11	3.86	4.14	10.52	30.89	29.45
SD		0.147	0.195	0.636	0.383	0.226	0.943	1.626	2.242	5.251
SEM		0.022	0.042	0.096	0.057	0.034	0.143	0.248	0.440	0.825
Diets										
3.20 Mcal/kg + 5'-IMP		6.31b	5.68	58.15	7.29b	3.85	3.72	9.42	29.42	24.83
3.30 Mcal/kg		6.23a	5.39	57.71	6.93a	3.86	4.37	11.59	32.62	34.07
Immune Stimulation										
Placebo		6.27	5.59	57.81	7.35b	4.00b	3.33	9.71	30.94	27.93
Stimulated		6.26	5.48	58.05	6.87a	3.71a	4.76	11.31	31.10	30.97
Variation sources										
Diets		0.035	<0.001	0.010	<0.001	0.929	<0.001	<0.001	<0.001	<0.001
Immune stimulation		0.848	0.004	0.234	<0.001	<0.001	<0.001	<0.001	0.709	<0.001
Diets x Immune stimulation		0.498	0.037	0.008	0.836	0.377	<0.001	0.030	0.025	0.006

[1]Drip loss.
[2]Thawing loss.
[3]Cooking loss.
[4]Shear force.

Table 5. Blood plasma analysis of 75- to 100- kg gilts submitted or not to immune stimulation and fed diets with reduced metabolizable energy (ME) supplemented with 0.100 % inosine-5'-monophosphate (3.20 Mcal/kg) or a conventional diet without reducing ME (3.30 Mcal/kg)

Immune stimulation	Diets	Days	TP[1], g/dL	Alb[2], g/dL	Glo[3], g/dL	A:G[4], %	UA[5], mg/dL	AST[6], mg/dL	ALT[7], mg/dL
Placebo	3.20 Mcal/kg + 5'-IMP	0	7.36	3.07	4.29	0.716	0.550	27.42	27.02
		12	7.38	3.36	4.01	0.822	0.570	36.76	25.16
		24	7.22	3.24	3.93	0.809	0.585	40.75	30.62
	3.30 Mcal/kg	0	7.36	3.07	4.29	0.716	0.550	27.42	27.02
		12	7.95	3.38	4.56	0.779	0.729	32.37	28.69
		24	7.62	3.43	4.19	0.789	0.609	33.45	29.36
Stimulated	3.20 Mcal/kg + 5'-IMP	0	7.36	3.07	4.29	0.716	0.550	27.42	27.02
		12	7.77	3.45	4.34	0.750	0.598	32.74	23.27
		24	8.04	3.45	4.59	0.720	0.632	39.07	32.50
	3.30 Mcal/kg	0	7.36	3.07	4.29	0.716	0.550	27.42	27.02
		12	6.75	2.98	3.78	0.740	0.740	32.41	26.70
		24	6.63	2.76	3.86	0.702	0.477	37.86	29.44
Mean			7.41	3.18	4.23	0.741	0.586	31.62	27.69
SD			0.465	0.247	0.332	0.046	0.081	5.940	3.430
SEM			0.044	0.023	0.032	0.005	0.007	0.542	0.313
Diets									
3.20 Mcal/kg + 5'-IMP			7.52	3.27	4.24	0.756	0.581	34.03	27.60
3.30 Mcal/kg			7.28	3.12	4.16	0.740	0.609	31.82	28.04
Immune stimulation									
Placebo			7.48	3.26	4.21	0.772	0.599	33.02	27.98
Stimulated			7.32	3.13	4.19	0.724	0.591	32.82	27.66
Periods									
0			7.36	3.07	4.29	0.716	0.550	27.42a	27.02a
12			7.46	3.29	4.17	0.773	0.659	33.58b	25.95a
24			7.38	3.22	4.14	0.755	0.576	37.78c	30.48b
Variation sources									
Diets			<0.001	<0.001	0.176	0.026	0.007	0.094	0.526
Immune Stimulation			0.020	0.008	0.730	<0.001	0.465	0.870	0.647
Period			0.378	<0.001	0.065	<0.001	<0.001	<0.001	<0.001
Diets × Period			0.006	<0.001	0.167	0.193	<0.001	0.326	0.011
Immune stimulation × Period			0.035	0.006	0.038	<0.001	0.063	0.555	0.262

[1]Total proteins. [2]Albumin. [3]Globulin. [4]Calculated albumin:globulin ratio. [5]Uric acid, [6]Aspartate aminotransferase, [7]Alanine Aminotransferase.

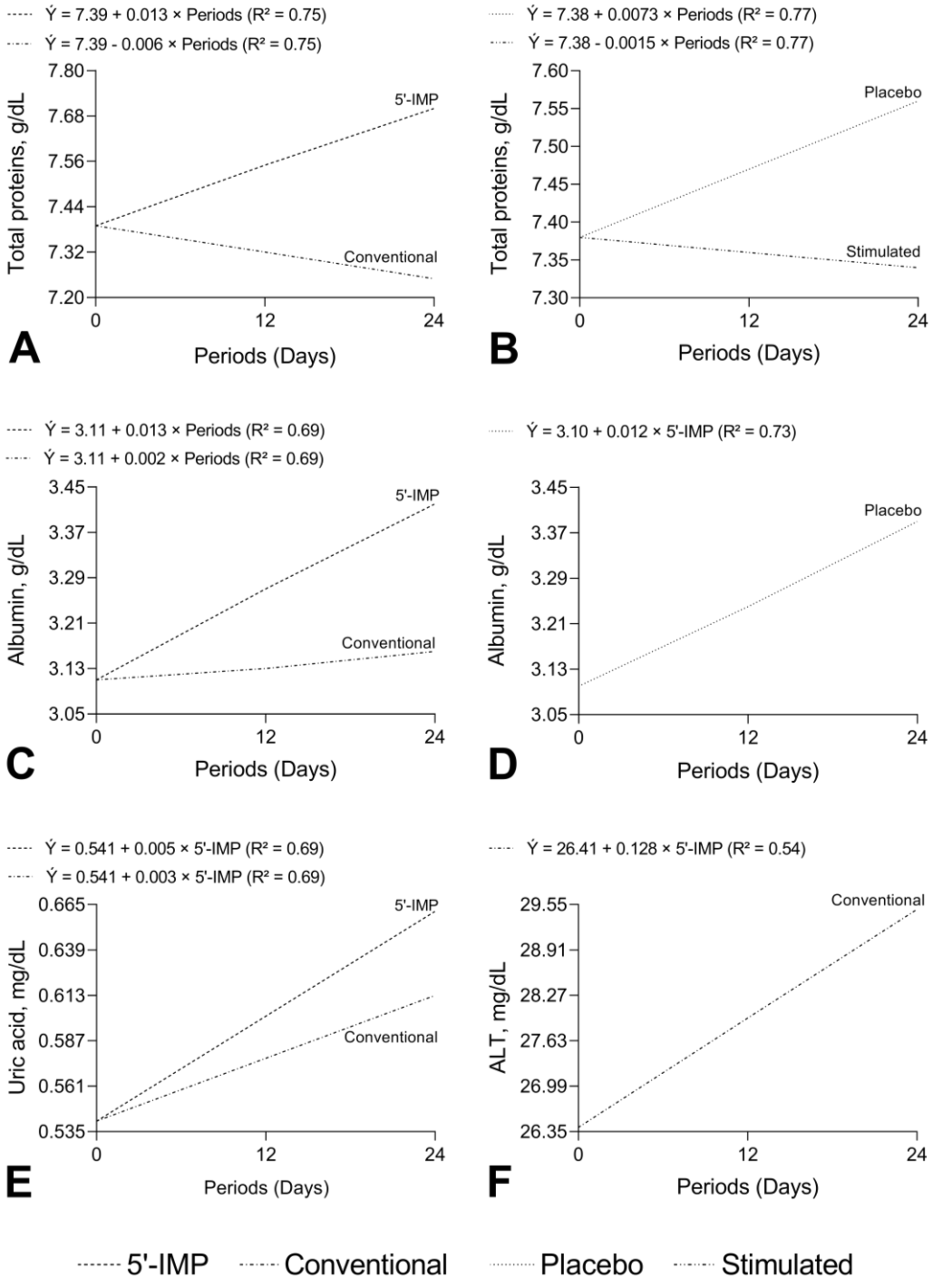

Figure 2. Unfolding the diets and evaluated periods for the concentrations of total proteins (A), albumin (C) and uric acid (E). Interactions between immune stimulations and evaluated periods for the concentrations of total proteins (B), albumin (D), and Alanine aminotransferase (F).

Table 6. Blood count of 75- to 100- kg gilts submitted or not to immune stimulation and fed diets with reduced metabolizable energy (ME) supplemented with 0.100 % inosine-5'-monophosphate (3.20 Mcal/kg + 5'-IMP) or a conventional diet without reducing ME (3.30 Mcal/kg)

Immune stimulation	Diet	Period, h	Erythrocytes, milion/μL	Hemoglobin, g/dL	Hematócrit, %	MCV[1], %	MCHC[2], %	Platelets, μL
Placebo	3.20 Mcal/kg + 5'-IMP	0	7.74	11.74	36.73	48.28	31.38	382.80
		12	7.57	11.58	35.44	47.62	32.32	353.18
		24	7.48	11.52	35.06	48.25	31.92	296.73
	3.30 Mcal/kg	0	7.74	11.74	36.73	48.28	31.38	382.80
		12	7.84	12.27	38.01	48.96	32.23	356.86
		24	7.97	12.46	39.43	47.92	32.63	294.73
Estimulated	3.20 Mcal/kg + 5'-IMP	0	7.74	11.74	36.73	48.28	31.38	382.80
		12	7.78	12.30	38.44	47.45	32.17	408.72
		24	7.91	12.54	38.05	47.79	32.47	427.89
	3.30 Mcal/kg	0	7.74	11.74	36.73	48.28	31.38	382.80
		12	7.75	11.81	37.34	49.29	32.09	316.95
		24	7.52	11.84	37.29	49.76	31.79	265.93
Mean			7.73	11.92	37.12	48.33	31.89	358.82
SD			0.334	0.609	1.842	1.410	0.666	51.36
SEM			0.037	0.069	0.203	0.164	0.071	5.467
Diets								
3.20 Mcal/kg + 5'-IMP			7.70	11.90	36.74a	47.95	31.90	375.35
3.30 Mcal/kg			7.76	11.98	37.59b	48.75	31.88	333.34
Immune stimulation								
Placebo			7.72	11.88	36.90	48.22	31.95	344.52
Stimulated			7.74	12.00	37.44	48.47	31.84	364.18
Period								
0			7.74	11.74	36.73	48.28	31.38	382.80
12			7.72	11.99	37.31	48.33	32.20	358.93
24			7.73	12.09	37.46	48.43	32.25	321.32
Variation sources								
Diets			0.404	0.533	0.016	0.002	0.882	<0.001
Immune stimulation			0.821	0.350	0.123	0.306	0.362	0.016
Period			0.969	0.037	0.148	0.869	<0.001	<0.001
Diets × Period			0.758	0.888	0.090	0.024	0.926	<0.001
Immune stimulations × Period			0.922	0.752	0.333	0.450	0.789	0.035

[1]Mean corpuscular volume. [2]Mean corpuscular hemoglobin concentration.

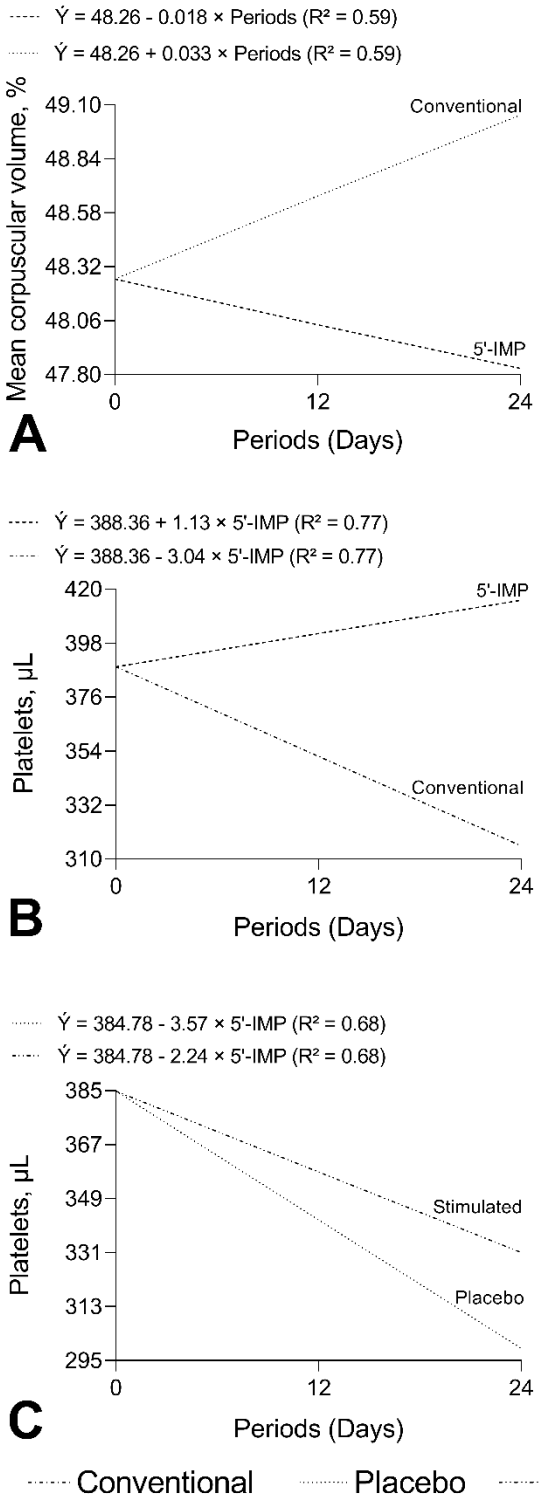

Figure 3. Unfolding the diets and evaluated periods for the mean corpuscular volume (A) and platelets (B), as the immune stimulation and evaluated periods for the platelets concentration (C).

Table 7. Leukogram of 75- to 100- kg gilts submitted or not to immune stimulation and fed diets with reduced metabolizable energy (ME) supplemented with 0.100 % inosine-5'-monophosphate (3.20 Mcal/kg) or a conventional diet without reducing ME (3.30 Mcal/kg)

Item	Diets	Period h	Leu[1], µL	Neu[2], µL	Lin[3], µL	N:L[4], %	P:L[5], %	Mon[6], µL	Eosi[7], µL	Bas[8], µL
		0	21.60	6.68	12.41	0.538	30.85	0.700	0.530	0.360
Placebo	3.20 Mcal/kg +5'-IMP	12	28.08	8.16	16.75	0.510	20.27	1.588	1.406	0.636
		24	28.04	7.98	16.93	0.520	17.47	1.185	1.287	0.816
		0	21.60	6.68	12.41	0.538	30.85	0.700	0.530	0.360
Placebo	3.30 Mcal/kg	12	27.67	5.86	15.35	0.383	22.43	1.690	0.655	0.214
		24	26.21	6.07	17.30	0.363	17.57	1.736	0.271	0.365
		0	21.60	6.68	12.41	0.538	30.85	0.700	0.530	0.360
Immune-stimulated	3.20 Mcal/kg +5'-IMP	12	30.08	10.07	15.28	0.660	27.00	1.362	0.973	0.612
		24	27.27	6.79	14.19	0.473	30.60	1.358	0.793	0.765
		0	21.60	6.68	12.41	0.538	30.85	0.700	0.530	0.360
Immune-stimulated	3.30 Mcal/kg	12	23.62	4.95	13.31	0.378	24.79	0.993	0.739	0.315
		24	20.94	5.85	12.61	0.479	21.87	0.640	0.292	0.266
Mean			24.04	6.83	13.80	0.509	27.04	0.997	0.665	0.425
SD			3.52	1.40	1.97	0.092	5.35	0.452	0.327	0.167
SEM			0.357	0.169	0.213	0.008	0.488	0.047	0.040	0.016
Diets										
3.20 Mcal/kg + 5'-IMP			26.11	7.73	14.66	0.540	26.17	1.148	0.920	0.592
3.30 Mcal/kg			23.61	6.01	13.90	0.448	24.72	1.078	0.503	0.314
Immune stimulation										
Placebo			25.53	6.90	15.19	0.475	23.24	1.266	0.780	0.459
Stimulated			24.19	6.84	13.37	0.513	27.66	0.960	0.643	0.446
Period										
0			21.60	6.68	12.41	0.538	30.85	0.700	0.530	0.360
12			27.36	7.26	15.17	0.483	23.62	1.411	0.943	0.444
24			26.21	6.67	15.26	0.461	21.88	1.229	0.661	0.553
Variation sources										
Diets			<0.001	<0.001	0.006	<0.001	0.005	0.271	<0.001	<0.001
Stimulations			0.013	0.798	<0.001	0.020	<0.001	<0.001	<0.001	0.121
Periods			<0.001	0.114	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
Diets × Period			0.001	<0.001	0.014	<0.001	0.017	0.580	<0.001	<0.001
Stimulation × Period			0.048	0.247	<0.001	0.108	<0.001	0.001	0.009	<0.001

[1]Total leukocytes. [2]Neutrophils. [3]Lymphocytes. [4]Calculated neutrophils:lymphocytes ratio. [5]Calculated platelets:lymphocytes ratio. [6]Monocytes. [7]Eosinophils. [8]Basophils.

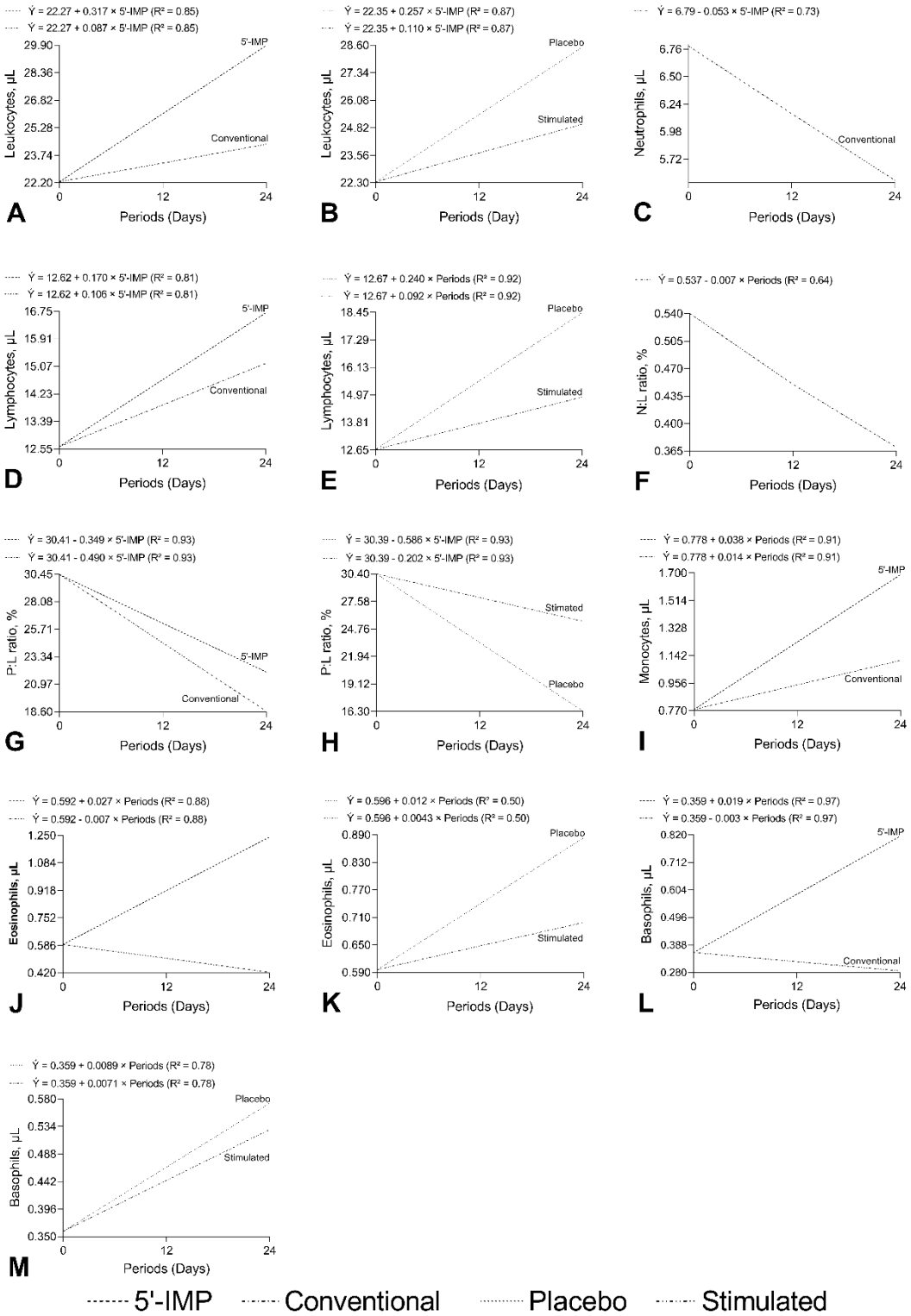

Figure 4. Unfolding the diets and evaluated periods for the concentration of leukocytes (A), neutrophils (C), lymphocytes (D), neutrophils:lymphocytes ratio (F), platelets:lymphocytes ratio (G), eosinophils (J) and basophils (L). Interactions between immune stimulations and evaluated periods for the concentration of leukocytes (B), lymphocytes (E), platelets:lymphocytes ratio (H), monocytes (I), eosinophils (K) and basophils (M).

Table 8. Concentration of acute phase proteins in the blood serum of 75- to 100- kg gilts submitted or not to immune stimulation and fed diets with reduced metabolizable energy (ME) supplemented with 0.100 % inosine-5'-monophosphate (3.20 Mcal/kg) or a conventional diet without reducing ME (3.30 Mcal/kg)

Immune stimulation	Diets	Period h	TP[1], g/dL	IgA[2], mg/dL	Cer[3], mg/dL	Tra[4], mg/dL	Alb[5], g/dL	Hap[6], mg/dL	Glic[7], mg/dL	IgGP[8], mg/dL	IgGL[9], mg/dL	Pm[10], mg/dL
Placebo	3.20 Mcal/kg + 5'-IMP	0	7.21	0.058	0.076	0.683	4.33	0.071	0.0061	1.049	0.360	0.140
		12	7.35	0.076	0.071	0.829	4.37	0.079	0.0054	1.133	0.368	0.172
		24	7.10	0.050	0.079	0.822	4.08	0.093	0.0060	1.195	0.488	0.129
	3.30 Mcal/kg	0	7.21	0.058	0.076	0.683	4.33	0.071	0.0061	1.049	0.360	0.140
		12	7.70	0.073	0.085	0.705	4.80	0.062	0.0085	1.219	0.457	0.162
		24	7.57	0.062	0.076	0.685	4.72	0.056	0.0077	1.085	0.480	0.152
Stimulated	3.20 Mcal/kg + 5'-IMP	0	7.21	0.058	0.076	0.683	4.33	0.071	0.0061	1.049	0.360	0.140
		12	7.85	0.057	0.109	0.835	4.35	0.102	0.0078	1.156	0.393	0.140
		24	7.86	0.056	0.077	0.837	4.40	0.063	0.0079	1.149	0.477	0.147
	3.30 Mcal/kg l	0	7.21	0.058	0.076	0.683	4.33	0.071	0.0061	1.049	0.360	0.140
		12	7.03	0.064	0.079	0.715	3.70	0.081	0.0067	0.999	0.412	0.159
		24	6.81	0.035	0.077	0.710	3.74	0.078	0.0061	1.009	0.392	0.149
Mean			7.34	0.059	0.079	0.733	4.31	0.074	0.007	1.087	0.401	0.147
SD			0.481	0.012	0.011	0.081	0.344	0.018	0.002	0.098	0.054	0.022
SEM			0.015	0.003	0.001	0.009	0.047	0.003	0.000	0.011	0.009	0.003
Diets												
3.20 Mcal/kg + 5'-IMP			7.43	0.059	0.081	0.782	4.31	0.079	0.0066	1.12	0.408	0.145
3.30 Mcal/kg			7.25	0.058	0.078	0.697	4.27	0.070	0.0069	1.07	0.410	0.150
Immune stimulation												
Placebo			7.36	0.063	0.077	0.735	4.44	0.072	0.0066	1.12	0.419	0.149
Stimulated			7.33	0.055	0.082	0.744	4.14	0.077	0.0068	1.07	0.399	0.146
Period, h												
0			7.21	0.058	0.076	0.683	4.33	0.071	0.0061a	1.049	0.360	0.140
12			7.49	0.068	0.086	0.771	4.30	0.081	0.0071b	1.127	0.408	0.158
24			7.34	0.051	0.077	0.764	4.24	0.072	0.0069b	1.110	0.459	0.144
Variation sources												
Diets			0.098	0.744	0.139	<0.001	0.462	0.011	0.383	0.008	0.671	0,241
Immune stimulation			0.782	0.003	0.017	0.497	<0.001	0.144	0.675	0.008	0.003	0.467
Period			0.033	<0.001	<0.001	<0.001	0.319	0.082	0.032	0.003	<0.001	0.008
Diets × Period			0.330	0.437	0.151	<0.001	0.680	0.108	0.382	0.026	<0.001	0.551
Immune stimulation × Periods			0.894	0.010	<0.001	0.799	<0.001	0.029	0.935	0.106	0.008	0.117

[1]Total proteins. [2]Immunoglobulin A. [3]Ceruloplasmin. [4]Transferrin. [5]Albumin. [6]Haptoglobin. [7]α-1 acid glycoprotein. [8]Immunoglobulin heavy chain G. [9]Immunoglobulin light chain G. [10]PM 23.000 dalton.

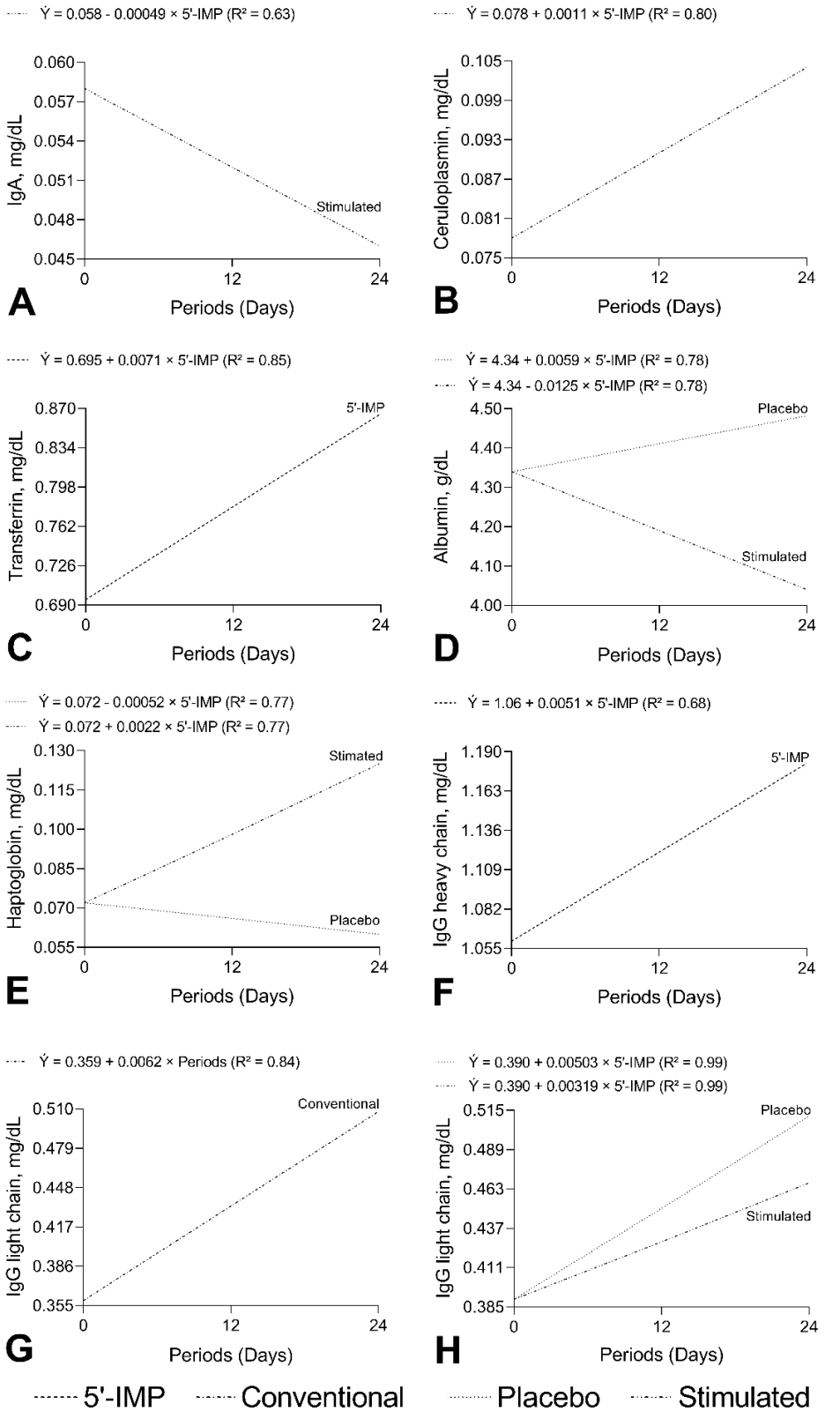

Figure 5. Unfolding the diets and evaluated periods for the concentration of transferrin (C), IgG heavy chain (F) e IgG ligth chain (G). Interactions between immune stimulations and evaluated periods for the concentration of IgA (A), ceruloplasmin (B), albumin (D), haptoglobin (E) and IgG ligth chain (H).