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UTILIZAÇÃO DE SUBPRODUTOS DA INDÚSTRIA DO ÓLEO DE SOJA E BIODIESEL E SUAS MISTURAS EM DIFERENTES PROPORÇÕES NA ALIMENTAÇÃO DE FRANGOS DE CORTE

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TESE

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UTILIZAÇÃO DE SUBPRODUTOS DA INDÚSTRIA DO ÓLEO DE SOJA E BIODIESEL E SUAS MISTURAS EM DIFERENTES PROPORÇÕES NA ALIMENTAÇÃO DE FRANGOS DE CORTE[1]

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RESUMO - A presente tese foi desenvolvida com objetivo de avaliar a utilização dos subprodutos do processamento do óleo de soja (OS) (óleo ácido de soja - OAS, lecitina - LEC) e do biodiesel (glicerol - GLI) na alimentação de frangos de corte. Dois estudos foram conduzidos para determinar EMA destes subprodutos e suas combinações. Foram usados 390 frangos de corte machos com 21 d para cada experimento distribuídos em um delineamento experimental inteiramente casualizado com arranjo fatorial com 4 fontes de gordura e 3 níveis de inclusão mais uma dieta basal sem suplementação de gordura. Cada experimento foi composto por 13 tratamentos dietéticos com 6 repetições de 5 aves por repetição. No primeiro ensaio foi usado: OAS, GLI e LEC, bem como uma mistura contendo 85% OAS, 10% GLI e 5% LEC (MIS). As proporções das fontes de energia na mistura foram semelhantes à encontrada no óleo de soja bruto. No segundo ensaio foi usado quatro diferentes misturas destas fontes de energia sendo elas: 85% OAS e 10% GLI; 80% OAS e 15% GLI; 75% OAS e 20% GLI e 70% OAS e 25% GLI, em todas as misturas estabeleceu-se 5% LEC. Os tratamentos dietéticos foram formados pela adição de cada fonte energética suplementar aos níveis de 0% (100% da dieta basal (DB), 2% (98% DB + 2% fonte de energia), 4% (96% DB + 4% fonte de energia) ou 6% (94% DB + 6% fonte de energia). Nos dois ensaios foi realizado coleta total de excretas por 72h. Aos 28 dias de idade no final do período experimental um frango por repetição foi abatido para coleta do fígado e coleta de sangue para análise de glicerol e triglicérides plasmático e enzima hepática glicerol quinase. Para os dois experimentos houve diferença estatística entre as fontes energéticas ($P<0,05$). No primeiro ensaio a mistura das três fontes (85:10:5) mostrou melhor valor de EMA, no entanto no segundo experimento a mistura de 80% OAS 15% GLI e 5% LEC apresentou melhor valor quando comparado as demais misturas. Para a concentração de glicerol e triglicerídeos plasmático, assim como para a atividade da enzima glicerol quinase, houve interação entre as fontes e o nível de inclusão ($P<0,05$). A medida que aumentou o nível de inclusão aumentou-se a concentração de glicerol no plasma e diminuiu a atividade da enzima, no entanto, isto não foi suficiente para saturar a enzima. Em conclusão, os tratamentos mostraram que a mistura de subprodutos do óleo de soja e do biodiesel podem ser usadas como fontes energéticas em dietas de frangos de corte sem acarretar problemas no metabolismo normal do glicerol.
Palavras-chave: Energia Metabolizável; Glicerol; Lecitina; Óleo ácido de soja

[1] Tese de Doutorado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (80p.) Março, 2016

UTILIZATION BY-PRODUCTS OF SOY OIL INDUSTRY AND BIODIESEL AND THEIR MIXTURE IN DIFFERENT PROPORTIONS IN BROILER FEED[2]

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ABSTRACT This thesis was carried out to evaluate the utilization of soybean oil (SO) (acidulated soap stock - ASS, lecithin - LEC) and biodiesel (glycerol - GLY) in broiler feeds. Two studies were conducted to determine AME these byproducts and their combinations. At total of 390 21-d-old male broilers were utilized for each experiment distributed in a completely randomized design with factorial arrangement of 4 fat sources and 3 levels of inclusion plus the basal diet without any fat source. Each study contained 13 dietary treatments with 6 replicates of 5 chickens per replicate. In the first experiment, the fat sources used were OAS, GLY and, LEC as well as a mixture (MIX) containing 85%, 10% and 5% respectively. The proportions of energy sources in the mix were similar to that found in crude soybean oil. In the second experiment, it was used four different mixtures of these energy sources, which are, 85% ASS: 10% GLY; 80% ASS: 15% GLY; 75% ASS: 20% GLY and 70% ASS: 25% GLY in all mixtures was fixed 5% LEC. The experimental treatments consisted of addition of each supplemental fat source at the levels of 0% (100% of basal diet (BD)), 2% (98% BD + 2% fat source), 4% (96% BD + 4% fat source) or 6% (94% BD + 6% fat source). The total excreta collection was conducted for 72h. At 28 days of age, the end of the study period one chicken by repetition was slaughtered to collect the liver and blood collection for analysis of plasmatic glycerol and triglycerides and the liver was used for analysis of glycerol kinase enzyme. For both experiments was observed statistical difference between energy sources ($P < 0.05$) in the first experiment the mixture of the three sources (85:10: 5) showed best value of AME, however, in the second experiment the mixture of 80% OAS GLI 15% and 5% LEC showed better value when compared to the other mixtures. For the concentration of plasma glycerol and triglycerides, as well as the activity of the enzyme glycerol kinase, there was an interaction between the sources and the inclusion level ($P < 0.05$). As we increased the level of inclusion increased the concentration of glycerol in plasma and decreased enzyme activity, however, this was not enough to saturate the enzyme. In conclusion, the treatment showed that the mixture of soybean oil and biodiesel by-products could be used as energy sources in broiler diets without causing problems in the normal metabolism of the glycerol.

Keywords: Metabolizable energy; acidulated soybean soapstock; glycerol; lecithin

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RELAÇÃO DE ABREVIATURAS

AGL	ácidos graxos livres
EB	energia bruta
ED	energia digestível
EM	energia metabolizável
EMA	energia metabolizável aparente
EMA_n	energia metabolizável aparente corrigida para nitrogênio
EMV	energia metabolizável verdadeira
EL	energia líquida
GLI	Glicerol
G	Gramas
IC	incremento calórico
Kg	Quilograma
kcal	Quilocaloria
LEC	Lecitina
OAS	óleo ácido de soja
ODS	óleo degomado de soja
OS	óleo de soja
ºC	graus Celsius

CAPÍTULO I

1.0 INTRODUÇÃO

Com a intensa produção no setor avícola e o aumento substancial do potencial produtivo dos frangos de corte, tornou-se indispensável nas formulações de dietas o uso de óleos e gorduras para atender as exigências nutricionais de frangos, o que tem despertado grande interesse por parte de produtores e pesquisadores.

A produção de frangos de corte possibilitou ao Brasil oferecer aos consumidores uma fonte proteica saudável e com custo mais baixo (Sousa, 2006) se consolidando como uma das mais importantes fontes de proteína animal para a população. O fornecimento de energia é importante para qualquer espécie animal, sendo essencial para a manutenção, crescimento e reprodução. Para as aves, o teor de energia da ração pode influenciar significativamente no crescimento e na utilização dos alimentos. A energia dietética, de modo geral, provém do uso dos carboidratos, proteína e lipídios, sendo os lipídios as melhores fontes de energia a serem utilizadas pelos animais, pois além de fornecer energia com baixo incremento metabólico, são fontes de ácidos graxos essenciais para manutenção da estrutura e função da membrana celular.

A incorporação de óleos na alimentação das aves, pode ser considerada um avanço na nutrição, recebendo muita atenção por parte de todos os segmentos da estrutura do setor avícola. Dentre os aspectos mais importantes na decisão do tipo de gordura a ser utilizada para a formulação de rações para frangos de corte, estão o custo e a qualidade das respectivas fontes, e quais os seus efeitos sobre o desempenho e o metabolismo do animal (Zollistsch et al., 1997). Algumas vantagens podem ser obtidas com o uso de óleos na alimentação de aves, como a elevação da densidade energética, diminuição da pulverulência das dietas, a diminuição da taxa de passagem do alimento no trato gastrintestinal, o aumento de consumo, a redução no incremento calórico e melhora na conversão alimentar (Morita, 1992).

A utilização de fontes alternativas de energia é uma das prioridades atuais, que pode contribuir significativamente para contornar os graves problemas ocasionados pelo desenvolvimento tecnológico. A preocupação atual pela redução da poluição e a crise energética têm estimulado o mercado mundial de biocombustíveis. A economia global mantém-se em crescimento e a demanda por energia limpa e recursos renováveis encontra-se em contínuo aumento (Bilgen et al., 2006).

A produção de óleo vegetal de soja para consumo humano demanda a neutralização do produto comercializado. No curso deste processo são gerados subprodutos em quantidades expressivas, tais como o óleo ácido e a lecitina, já, o processamento para geração de biodiesel gera grande volume de glicerol (Vieira et al., 2002). Para cada litro de biodiesel produzido são gerados entre 79 e 100 g de glicerina que contém em média 85% de glicerol (Thompson & He, 2006). Portanto, são esperadas quantidades crescentes de glicerina no mercado o que pode amenizar os preços da energia suplementar para a avicultura.

A incorporação de subprodutos da indústria do óleo de soja refinado tem grande potencial de uso em rações por seu valor energético, no entanto,

hoje sua utilizados é limitada pela indústria da avícola, sendo que estes podem servir como uma alternativa para redução de custos das rações animais, reduzirá a demanda biológica para tratamento de efluentes e representará também um estímulo ao crescimento ou surgimento de plantas de reprocessamento de óleo ácido de soja, lecitina e glicerol.

Em muitas situações práticas, óleos e gorduras de várias origens são misturados previamente a sua incorporação em rações para aves. Essa mistura é, em muitos casos, uma necessidade estratégica da indústria, mas pode ser benéfica, com ganhos de digestibilidade das frações com alto grau de saturação, caso da mistura do sebo bovino com gorduras de menor saturação (Artman, 1964; Ketels & DeGroote,1989; Zumbado et al., 1999).

A demanda de glicerina para alimentação animal no Brasil é quase inexistente gerando um grande acúmulo de material que está sendo estocado a alto custo, podendo gerar impactos ambientais se depositado em condições inapropriadas. Limitações de ordem prática, como a injeção destes produtos líquidos diretamente nos misturadores de ração, bem como, temores quanto à presença de metanol residual na glicerina podem ser superadas com a aplicação conjunta dos resíduos da soja que potencialmente permitem uma única aplicação. Da mesma forma, a utilização conjunta do óleo ácido se soja, glicerol e da lecitina em proporções similares aos triglicerídeos predominante no óleo de soja degomado possibilitará o surgimento de produtos de valor agregado que permitam geração de valor para a indústria deste segmento.

Desta forma o presente estudo foi desenvolvido com o intuito de avaliar de forma isolada a utilização de cada subproduto da indústria do óleo de soja e do biodiesel, assim como suas misturas em diferentes proporções de forma que suas diferentes quantidades não afetassem o metabolismo do normal de frangos de corte.

2.0 REVISÃO BIBLIOGRÁFICA

2.1 Importância da energia na produção de frangos de corte

A energia não é um nutriente, mas o resultado da oxidação de nutrientes durante o metabolismo animal (NRC, 1994). A dieta de frango de corte é composta de proteínas, carboidratos, gorduras, vitaminas e minerais e todos esses ingredientes representam uma energia química em potencial que será utilizada pelo organismo do animal, muito embora, as vitaminas, os micro e macro elementos minerais, como o cálcio e fósforo, representam meios de viabilização desta energia (Reece, 1993).

A energia química armazenada em um alimento é liberada pelo processo oxidativo no organismo, o que proporciona a capacidade de atender as demandas de energia gastas nos processos metabólicos de manutenção e de trabalho e na produção dos animais (Andriguetto et al., 1988).

De forma geral as aves necessitam de energia para mantença e produção e isso exige dietas com uma maior concentração energética para desenvolver seu potencial genético, para tal, é comum a adição de óleos ou gorduras (Englert, 1998).

Um dos aspectos mais importantes na formulação de rações para aves é o conhecimento preciso do conteúdo energético dos alimentos, o que possibilita o fornecimento adequado de energia para cada fase de produção, pois a deficiência ou a carência energética proporciona às aves crescimento retardado, incapacidade no ganho de peso e consequentemente queda na produtividade em geral (Albino et al., 1992).

A energia presente nos alimentos é dividida biologicamente em energia bruta (EB), energia digestível (ED), energia metabolizável (EM) e energia líquida (EL) (figura 1). A EB é a quantidade de energia liberada por um ingrediente quando queimado em bomba calorimétrica. No entanto, nem toda energia produzida pela oxidação dos nutrientes pode ser aproveitada pelos animais, sendo necessária a realização de ensaios biológicos para medir a EM dos alimentos (Scott et al., 1998).

A ED é determinada pela diferença entre a energia bruta consumida e a energia bruta contida nas fezes, como no caso das aves, as fezes e a urina são excretadas juntas, a utilização desse método se torna inviável. A EM é a que melhor quantifica a energia disponível dos alimentos para as aves e pode ser expressa tanto na forma de energia metabolizável aparente (EMA), como energia metabolizável verdadeira (EMV), dependendo da metodologia utilizada para determinação (Albino, 1991).

A energia da excreta é composta da energia proveniente de uma fração não assimilada dos alimentos e de uma fração de material endógeno. Quando essa última fração não é considerada nos cálculos, denomina-se EMA, entretanto quando a mesma passa a ser considerada, define-se como EMV (Sakomura & Rostagno, 2007).

A energia obtida da EM subtraindo o incremento calórico (IC), sendo este a quantidade de energia produzida pelo organismo na metabolização do

alimento, é chamada de energia líquida (EL), que é a energia do alimento efetivamente utilizada pelo organismo, podendo ser fracionada em energia para manutenção e energia para produção (Ewan, 2001).

Quando a ave ingere energia acima de suas necessidades metabólicas ocorre deposição de gordura na carcaça, sendo que a grande proporção desta gordura ocorre na área abdominal. Esta deposição pode ser resultado da alta relação energia-proteína da dieta, do desbalanço de aminoácidos ou de uma ação específica de gordura da alimentação sobre a composição da carcaça (Duarte et al., 2006).

O nível de energia metabolizável da ração limita a ingestão do alimento, visto que as aves comem para satisfazer suas exigências energéticas e, por isso, quando o nível de energia é aumentado, os demais nutrientes devem manter uma relação constante para que a alimentação não fique deficiente nos demais nutrientes, neste caso constitui um aumento na densidade energética e nutricional da ração (Mendonça, et al., 2008).

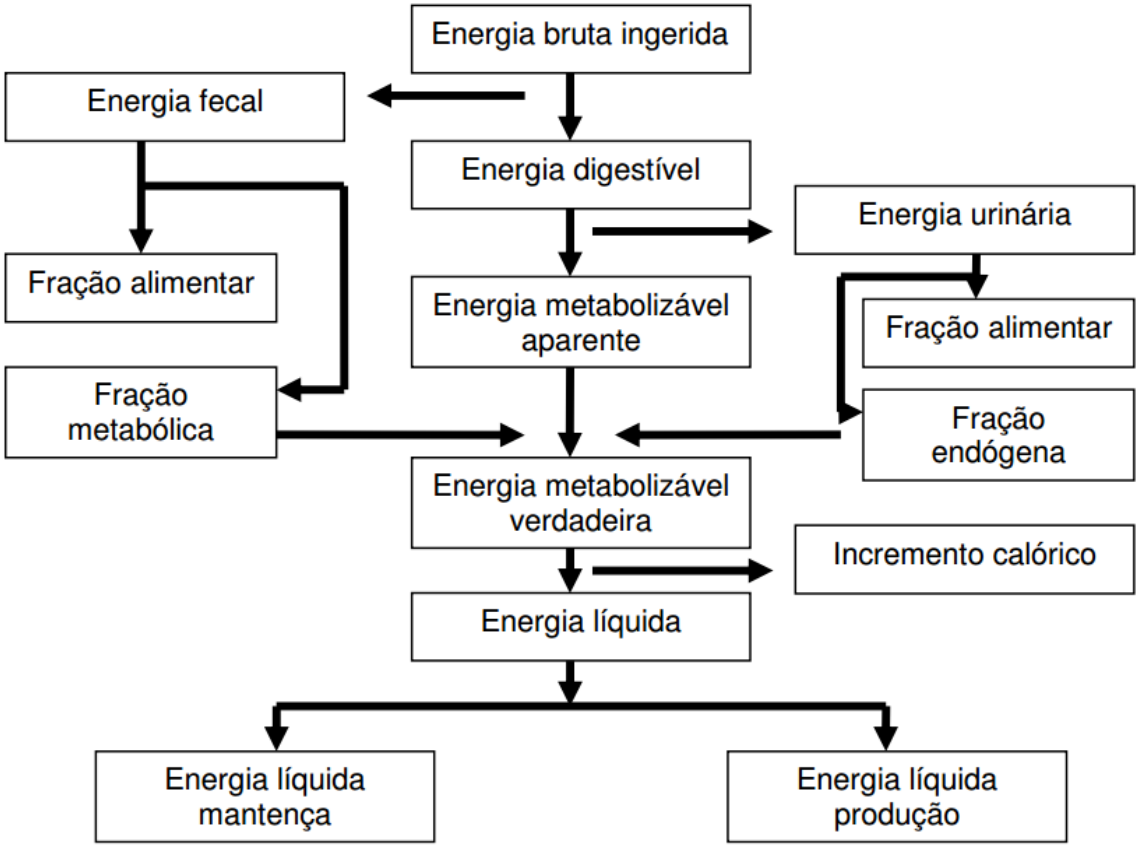

Figura 1: Utilização da energia consumida pelos animais monogástricos

Sakomura & Rostagno, (2007)

2.2 Lipídios: Digestão e absorção em aves

Os lipídios são macronutrientes existentes nos alimentos, constituídos por diversos compostos e quimicamente diferentes entre si, compõem as

estruturas das membranas biológicas e são utilizados como forma de armazenamento de energia, portanto são constituintes importantes da dieta, possuem elevados valores energéticos e são fonte de ácidos graxos essenciais, que o organismo é incapaz de sintetizar (Oliveira, 2009).

O sistema digestório é responsável por transformar os lipídios da dieta tornando-os disponíveis para serem absorvidos e aproveitados para as necessidades de manutenção, crescimento e reprodução do animal (Júnior, 2009).

O aproveitamento das fontes de lipídios por frangos de corte está diretamente relacionado com a digestibilidade do mesmo, que é dependente do comprimento da cadeia carbônica, do grau de saturação e da posição dos ácidos graxos na molécula de glicerol (Andreotti et al., 2004).

Fatores como idade da ave, a forma da gordura (como triglicerídeo ou como ácido graxo livre), a relação dos ácidos graxos saturados e insaturados na mistura de ácidos graxos livres (AGL), a flora intestinal e a composição da dieta são fatores que segundo Lesson & Summers (2001) interferem na digestão das gorduras.

Após a ingestão do alimento, pelas aves o mesmo sofre a ação mecânica na moela, onde é triturado até uma fina emulsão com grande aumento na área de superfície. No proventrículo o alimento sofre ação proteolítica e o baixo pH provocam a agregação da gordura do alimento ingerido. Nestas condições os ácidos graxos e fosfolipídios formam uma mistura com ponto de fusão mais baixo, que normalmente torna-se líquida à temperatura corporal e favorece a digestão (Moran Jr., 1994).

Quando o alimento chega ao intestino delgado, previamente digerido, e alcança o jejuno, ocorre a maior parte do processo de absorção dos nutrientes, entre eles, os lipídios (Swenson & Reece, 1996).

Os lipídios fornecidos na dieta, embora lipossolúveis, são digeridos e transportados em meio aquoso, entretanto, para que isso ocorra é necessário a presença de emulsificantes que tornem a gordura disponível à ação de enzimas digestivas sendo assim, para que ocorra a digestão e absorção de lipídios no lúmen intestinal, é necessário a presença de secreções biliares e pancreáticas (Bueno, 2006).

A bile é responsável pela emulsificação, hidrólise e solubilização da gordura no intestino, sendo que suas funções digestivas são executadas principalmente pela ação de seus maiores compostos, os sais biliares e fosfolipídios (Bruss, 1997).

Os sais biliares são moléculas anfipáticas, com extremidades hidrofílicas e hidrofóbicas, com capacidade de se associarem a moléculas polares (água) e apolares (lipídios). Tem como função permitir a dispersão dos lipídios pela fase aquosa onde atuam as enzimas, formando uma emulsão. A emulsificação permite que as enzimas envolvidas na digestão cheguem a seus substratos lipídicos. Após esse processo os sais biliares são reabsorvidos pela circulação êntero-hepática de volta para o fígado. A eficiência desse processo depende da taxa de absorção intestinal, da captação hepática e secreção de ácidos biliares visto que não são produzidas quantidade suficiente de sais para suprir a demanda do organismo (Gonzalez & Silva, 2006).

Através da emulsificação dos lipídios pelos sais biliares e a hidrolise dos triglicerídeos pela lipase pancreática ocorre a lipólise que gera gliceróis, monoglicerídios e AGL (Hornbuckle & Tennant, 1997). Os ácidos graxos de cadeias curtas e o glicerol livre solúvel em água podem ser absorvidos diretamente nos enterócitos através da corrente sanguínea. Os ácidos graxos de cadeias longas e médias, monoglicerídios e moléculas de colesterol interagem com os ácidos biliares conjugados para formar micelas que possuem a capacidade de dissolver solutos relativamente apolares em solução aquosa, pois possuem uma parte hidrofóbicas e outra hidrofílicas (Garrett & Young, 1975).

Após a absorção, já no citosol dos enterócitos, os monoglicerídios e AGL são reconvertidos em triglicerídeos, e juntamente com o colesterol e proteínas específicas formam agregados lipoprotéicos chamados quilomícrons. Os quilomícrons formados dentro do enterócitos contêm um núcleo central de triglicerídeos reesterificados envolto por uma estrutura tipo membrana composta de proteínas, colesterol e fosfolipídios. É nessa forma que os triglicerídeos reesterificados são transportados das células intestinais para o sistema circulatório da ave (Lehninger et al., 2007; Leeson & Summers, 2001).

As aves possuem uma particularidade na absorção dos lipídios, pois elas absorvem a gordura pelos capilares sanguíneos das vilosidades e aproximadamente 80 a 95% dos ácidos graxos presentes no intestino de frangos adultos são absorvidos (Reece, 1993).

Uma vez no plasma sanguíneo os lipídios ingeridos mobilizam-se para a formação de reserva no tecido adiposo. Alguns fatores influenciam as concentrações de lipídios plasmáticos tais como a quantidade e o tipo de lipídeo dietético, o tempo após consumo de alimento, a saúde e a idade do animal e o equilíbrio hormonal, sendo o glucagon um importante regulador da lipólise em aves (Dukes, 1993).

2.3 Óleo de soja

Os óleos vegetais são utilizados em larga escala na indústria animal, na avicultura seu uso é mais interessante sob o ponto de vista metabólico, uma vez que possui maior quantidade de ácidos graxos insaturados que são melhor assimilados pelas aves nas diferentes fases de criação. No óleo de soja, os ácidos graxos são predominantemente poliinsaturados de cadeia longa (Li et al., 1990). O óleo vegetal é um lipídeo extraído de plantas formado por triglicerídeos (Sanz et al.,2000).

A soja, em sua constituição, possui elevada quantidade de energia, por isso seu óleo é bastante utilizado na alimentação de frangos de corte visto que estas aves passaram, nos últimos anos, por considerável melhoramento genético o que resultou em animais de rápido crescimento e consequentemente alta exigência em energia (Bellaver & Snizek, 1999).

A obtenção do óleo de soja inicia-se com a preparação dos grãos de soja através da quebra dos mesmos que pode ser por processo mecânico no qual são esmagados sob altas temperaturas (extrusão) ou processo químico com o uso de solventes que após o processo é separado para ser reutilizado e

o material resultante passa por prensagem, resultando em soja semi-integral e óleo de soja bruto (Serrato, 1981, Liu, 1999).

A degomação consiste na retirada dos fosfolipídios do óleo de soja bruto, por polaridade, a partir da adição de 1 a 3% de água à 70ºC, e tem como produto final o óleo degomado e a lecitina (LEC). A LEC constitui 1,5 a 3,0% do óleo bruto e é separada por hidratação e centrifugação do óleo. Para a obtenção do óleo de soja refinado comercial, o óleo de soja degomado passa sequencialmente por processos de neutralização onde ocorre a retirada dos AGL por saponificação, a partir da adição de hidróxido de sódio, em seguida passa por centrifugação, clareamento e desodorização, resultando então no óleo de soja refinado (Kato, 2005).

Embora os óleos vegetais tenham maior disponibilidade no mercado, estes possuem valor elevado e sobre o ponto de vista nutricional é importante analisar o custo da energia em relação ao preço e qualidade para se medir o real benefício, uma vez que são fatores variáveis com relativa frequência (Barrera-Arellano, 1989).

2.4 Óleo ácido de soja

O “óleo ácido de soja” (OAS), conhecido genericamente como “ácidos graxos livres de soja”, é obtido após a acidificação da borra resultante do processo de refino do óleo de soja bruto para consumo humano. A borra é gerada a partir do processo de refino por meio da neutralização alcalina do óleo bruto. Esta representa cerca de 2 a 3% do óleo de soja bruto, e apresenta em torno de 70% de ácidos graxos na forma livre, enquanto que no óleo de soja refinado essa proporção é de apenas 1% (Lipstein & Bornstein, 1968).

Os óleos ácidos apresentam de 75% a 95% dos ácidos graxos presentes nos óleos de que se originam e se encontram, predominantemente, na forma de AGL em pequena proporção, na forma de triglicerídeos, mono e diglicerídeos (Vieira et al., 2002). Em razão dessa característica, os óleos ácidos têm menor digestibilidade e valor energético do que os óleos neutros dos quais procedem (Vilá & Esteve-Garcia, 1996).

O OAS é rico, também, em fosfolipídios (12,6%) e em pigmentos, principalmente carotenoides e xantofilas (Pardío et al., 2005). Este óleo tem sido estudado como fonte de energia alternativa ao óleo de soja degomado, devido ao seu baixo custo em relação a outros óleos vegetais, e tem sido utilizado como suplemento energético pela indústria de rações para animais (Raber et al., 2008).

Na Tabela 1 são apresentados o perfil de ácidos graxos do óleo de soja (OS) e do OAS segundo Vieira et al. (2002).

Tabela 1. Perfil de ácidos graxos do óleo de soja (OS) e do óleo ácido de soja (OAS), %

AG[1]	16:0	18:0	18:1	18:2	18:3	20:1	22:0	Sat.[2]	Mono[3]	Poli[4]
OS	12,6	4,4	22,7	52,8	6,8	0,3	0,4	17,4	23,0	29.6
AS	13,0	3,6	25,6	48,1	0,2	5,0	0,5	20,6	26,1	53,1

[1] Ácidos Graxos; [2] Saturados, [3] Monoinsaturado, [4] Poliinsaturado

Vários questionamentos cercam a validade e a eficiência da utilização do óleo ácido de soja na alimentação animal como, a falta de consistência da composição de ácidos graxos nos produtos comerciais disponíveis, concentração e tipo de agente neutralizante usado, a presença de impurezas na forma de sulfatos capazes de gerar desgastes em equipamentos usados em fábricas de rações, e também a presença de umidade excessiva. Sob o ponto de vista nutricional, a maior incerteza relacionada ao óleo ácido de soja diz respeito ao seu valor energético (Vieira et al. 2002).

Estes mesmos autores avaliando a utilização de energia para frangos de corte verificaram que o OAS é uma fonte energética alternativa de alto potencial econômico para uso em dietas comerciais para frangos de corte, apresentando valor energético de 8,114 kcal EMAn/kg de matéria seca, valor apenas 5% inferior ao determinado para o óleo degomado de soja (ODS). Resultados semelhantes foram obtidos por Machado et al. (2003) que avaliou a EMA do OS e o OAS com frangos de corte de uma e cinco semanas de idade e observaram valores de 8,135 e 7,701 kcal EMAn/kg para a primeira semana de idade e 9,314 e 8,559 kcal EMAn/kg para a quinta semana de idade, respectivamente.

No entanto, Freitas et al. (2005) verificaram valores menores quando avaliaram o valor energético do óleo ácido de soja para aves com 21 dias de idade, utilizando ração referência e ração teste, composta por 10% de óleo ácido de soja e 90% de ração de referência. A energia metabolizável aparente corrigida foi de 7.488 kcal/kg de matéria seca para pintos.

Wiseman & Salvador (1991) trabalharam com dietas para frangos de corte com diversas fontes de óleo e seus respectivos óleos ácidos, entre eles OAS, os autores observaram que a redução nos valores EM das gorduras com maiores conteúdos de AGL tende a ser mais elevada quanto maior for a inclusão das gorduras, e que essa redução é maior quanto maior for a saturação da gordura. Quando o OS é substituído por quantidades iguais de OAS, foi observada perda de desempenho de frangos de corte, o que é um indicativo de que este possui menor valor energético que o OS (Gaiotto et al., 2000).

2.5 Lecitina De Soja

As lecitinas são componentes naturais do grão de soja e são considerados um complexo natural de fosfolipídios, sendo composto principalmente pela fosfatidilcolina (Canty & Zeisel, 1994), motivo pelo qual muitos pesquisadores empregam os termos lecitina e fosfatidilcolina para este composto. Podem variar muito em sua composição, sendo que na avaliação das diferentes lecitinas, muitas variáveis devem ser consideradas, tais como: porcentagem de fosfolipídios, colina, nível de impurezas aceitável, coloração desejada e características funcionais (Bellaver & Snizek, 1999).

A lecitina de soja é removida do óleo de soja bruto através da hidratação da micela a uma temperatura elevada, após esta hidratação, é obtida uma goma, que contem cerca de 50% de fosfolipídios e 25% de óleo. Esta goma deve então ser aquecida a vácuo até que seja obtido cerca de 65% de fosfolipídios e filtrada a seguir para obtenção do produto refinado (Russett, 1997). Esta mistura de fosfolipídios é composta por fosfatidilcolina (16-26%), fosfatidiletanolamina (14-20%), fosfatidilinositol (10-14%), fitoglicolipídios (13%) e fosfatidilserina (4%) (Woerfel, 1981; Attia et al., 2008). O perfil químico da lecitina de soja pode variar consideravelmente. Esta variação pode ser devido à genética da planta, a qualidade da semente da soja, as variações no processamento do óleo ou mesmo no aperfeiçoamento de detecção dos diversos componentes (Cherry & Kramer, 1989).

Os fosfolipídios têm várias funções importantes no organismo como a de aumentar a emulsificação dos lipídios permitindo um aumento na digestão e absorção dos mesmos no intestino delgado, preparar a atividade da lípase pancreática e promover a incorporação dos AG principalmente dos altamente saturados, especialmente, em aves jovens que possuem ineficiente síntese e recirculação entero-hepática dos sais biliares (Overland et al., 1994; Al-marzooqi & Leeson, 1999). A molécula de LEC é composta por dois ácidos graxos esterificados a um glicerol-3-fosfato e uma extremidade colina.

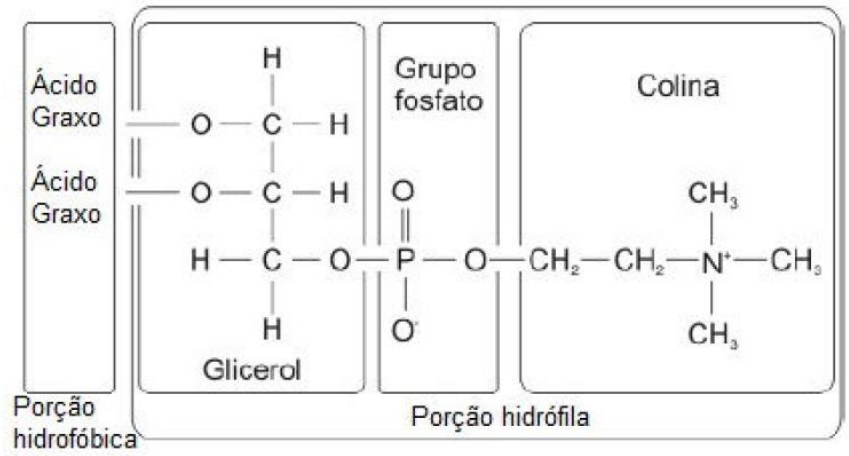

Figura 2: Molécula de Lecitina

O uso de lecitina como emulsificante nas dietas animais baseia-se no fato de que essa ação aumenta a superfície ativa nas gorduras alimentares para a ação da lipase, facilitando a hidrolise das moléculas de triglicerídeos em ácidos graxos e monoglicerídios, além de favorecer a formação de micelas de produtos da lipólise, potencializando a absorção (Raber, et. al.,2008).

A superfície ativa da LEC, ou seja, a porção dos fosfolipídios, confere ao composto uma estrutura molecular ambifílica: a parte hidrofílica, composta pelo ácido fosfórico e a parte hidrofóbica, formada pela cadeia de ácidos graxos. O tamanho desta cadeia de ácidos graxos, confere à estrutura o caráter mais ou menos hidrofóbico. Este comportamento lhe atribui uma de suas principais aplicações que é o uso como emulsificantes para substâncias que têm superfícies ativas diferentes, como a água e óleo, permitindo que eles se misturem facilmente (Mendes, 2000).

Muitos autores relaram que a utilização de lecitina em substituição a gordura da dieta contribui para o melhor desempenho de frangos de corte, Cox et al., (2000) em estudo sobre a substituição da gordura da dieta com adição suplementar de 0, 25, 50 e 100% de lecitina em relação a quantidade da gordura, relataram que a conversão alimentar foi significativamente melhor para os grupos que continham lecitina na composição da dieta, destacando os grupos com maior quantidade suplementada. Da mesma forma Neto (2005) analisando a ação do uso de emulsificante em dietas com OS, OAS e mistura de ambas as partes para frangos de corte, percebeu que os parâmetros de ganho de peso e conversão alimentar foram melhores com o uso do emulsificante a base de lecitina. Também, Raber et al. (2009), compararam a suplementação de lecitina e de glicerol em diferentes níveis de ácidos graxos, os resultados mostraram que com a suplementação de lecitina, não de glicerol, independentemente do nível de AGL usado, proporcionou melhor aproveitamento da gordura bruta adicionada a dieta.

Entretanto, Rocha et al., (2007) trabalhando com frangos de corte no período pré inicial, não observaram diferença significativa sobre consumo de ração, ganho de peso e conversão alimentar de aves suplementadas na dieta com diferentes níveis de lecitina de soja (0, 3, 6, 12 g lecitina/kg de ração). Da mesma forma que Blanch et al., (1995) estudando o efeito de diferentes fontes lipídicas, onde usaram uma inclusão de 5% de lecitina, não observaram diferença significativa no ganho de peso das aves que receberam fosfolipídio na dieta.

A variação de resultados encontrados em estudos utilizando lecitina pode estar relacionada à composição de produtos utilizados, o nível e período de suplementação, composição de ácidos graxos, grau de refinamento e tipo de gordura utilizada, pois lipídios de origem vegetal são melhor digeridos do que os de origem animal, limitando deste modo o efeito da lecitina Overland et al. (1994)

2.6 Biodiesel

De um modo geral, o biodiesel foi definido pela “National Biodiesel Board” dos Estados Unidos como o derivado monoalquil éster de ácidos graxos de cadeia longa, proveniente de fontes renováveis como óleos vegetais ou

gordura animal, cuja utilização está associada à substituição de combustíveis fósseis em motores de ignição por compressão (National Biodiesel Board, 1998).

Desde 2009 a Agência Nacional do Petróleo através da Resolução nº 6/2009 do Conselho Nacional de Política Energética (CNPE) estabeleceu o uso obrigatório de bicombustíveis com a inclusão de 5% de óleo vegetal no diesel combustível (ANP, 2011).

No entanto, com mudança ocorrida na legislação, que determina participação de 7% de biodiesel no volume total de diesel comercializado no Brasil a produção nacional de biodiesel alcançou 3,9 bilhões de litros em 2015, um crescimento de 15% em relação a 2014 sendo que, 91,5% da produção total de biodiesel foi originaria do óleo de soja (ANP, 2016).

Óleos vegetais, geralmente apresentam ácidos graxos livres, fosfolipídios, água e outras impurezas. Suas características físico-químicas os impedem de serem utilizados diretamente como combustível. Para superar estes problemas o óleo requer uma modificação química gerada por reações de esterificação, transesterificação ou craqueamento. Entre estas, atualmente a mais utilizada é a transesterificação que é uma reação de um lipídeo com um álcool, gerando um éster e um subproduto, a glicerina (Clements e Hanna, 1998).

Apesar dos aparentes benefícios ambientais em função da preocupação com a sustentabilidade e com a busca por fontes de energia renováveis, esta imensa demanda na utilização de bicombustíveis gera uma grande quantidade do subproduto, a glicerina além da pressão extra nos preços dos insumos energéticos para a produção animal. Considerando que o preço do alimento representa a maior parte dos custos de produção, os nutricionistas continuam a procura por fontes alternativas de energia no intento de reduzir o custo das rações produzidas. O uso de glicerina bruta na alimentação animal já foi objeto de estudos no passado, especialmente na Europa. Porém, com o recente impulso na produção do biodiesel e a disponibilidade de grande quantidade de glicerina bruta, houve novo interesse no seu uso em rações para animais.

2.7 Glicerol

Segundo Barcelo (1959), Howard e Neal (1992) e Ashfor (1994), a palavra glicerina é sinônimo de glicerol. Entretanto, na prática, classicamente a palavra glicerina é empregada para fazer referência de que não se trata de uma substância pura, que só tem glicerol, e o glicerol é o produto puro. Como normalmente a glicerina contém mais do que 95% de glicerol em sua composição, muitas vezes ela é tratada como sinônimo de glicerol.

Embora o glicerol não seja um nutriente específico, ele é uma fonte de energia semelhante aos carboidratos (François, 1994). Possui em sua fórmula estrutural carbono, hidrogênio e oxigênio (Brisson et al., 2001).

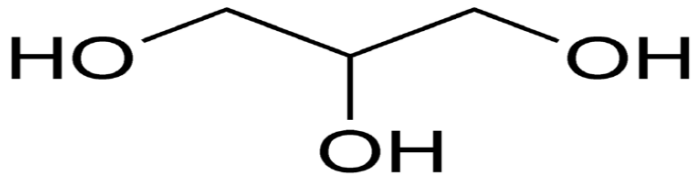

Figura 3: Fórmula estrutural plana do glicerol

O glicerol ou 1,2,3-propanotriol é um álcool simples e apresenta-se, na sua forma pura, como um líquido viscoso, incolor, inodoro e higroscópico, com sabor doce, solúvel em água e álcool, insolúvel em éter e clorofórmio (Wang et al., 2001). É derivado tanto dos triglicerídeos das gorduras animais, dos óleos vegetais e subprodutos da indústria petroquímica que é obtida de triglicerídeos a partir do processo de produção de sabões, do isolamento dos ácidos graxos e, atualmente, pela transesterificação durante a produção do biodiesel (Rivaldi, 2008).

O glicerol, reconhecido como aditivo alimentar tem sua inclusão permitida em vários alimentos e também tem sido empregado na alimentação de diferentes espécies animais. Em setembro de 2010, o Ministério da Agricultura Pecuária e Abastecimento (MAPA), estabeleceu os padrões de qualidade para a utilização da glicerina bruta e loira na alimentação animal. Estas devem conter no mínimo 80% de glicerol; no máximo 13% de umidade; e 150 mg/kg de metanol; enquanto os teores mínimos de sódio e matéria mineral, devem ser garantidos pelo fabricante, podendo variar, no entanto, de acordo como o processo produtivo.

Porém, para usá-lo é imprescindível conhecer o seu metabolismo e a capacidade máxima de uso para cada espécie, em suas diferentes fases de produção. Muitas pesquisas de metabolismo foram desenvolvidas com ratos e humanos e poucas com aves (Paule, 2010).

2.7.1 Metabolismo e Absorção do Glicerol

O glicerol é um componente encontrado normalmente no organismo animal, tanto na circulação como nas células. Ele é derivado da lipólise no tecido adiposo quando os triglicerídeos são hidrolisados liberando ácidos graxos e glicerol (Lin, 1977).

Pelo seu pequeno tamanho molecular, o glicerol pode ser facilmente absorvido no intestino dos animais passivamente, ao invés de formar micela, como os ácidos graxos de cadeia média e longa, com sais biliares (Nelson & Cox, 2000). De acordo com Lin (1977) o glicerol é bem absorvido no intestino de ratos, em menor velocidade do que a glicose.

Em estudo utilizando intestinos de ratos foi demonstrado que o glicerol possui dois sistemas de absorção intestinal - um sistema de transporte ativo e outro sistema de transporte passivo, sendo que o primeiro é responsável por 70% do transporte em baixas concentrações (Kato et al., 2004).

O fígado, rins e músculos, são os órgãos responsáveis pela metabolização do glicerol sendo que o fígado é responsável por 3/4 de toda capacidade. Os rins, além de metabolizarem o glicerol, também são responsáveis pela sua reabsorção, evitando perdas através da urina. Quando os níveis séricos de glicerol ultrapassam 1mM a utilização não é total e estes são então excretados (Lin, 1977).

Após ser absorvido, pelo organismo animal (figura 4) o glicerol é metabolizado pela enzima glicerol quinase que é a primeira a participar da metabolização do glicerol, sendo encontrada em grande parte no fígado e rins, podendo realizar a oxidação do glicerol para obtenção de energia ou convertê-lo em glicose (Robergs & Griffin, 1998). Esta enzima é considerada uma fosfotransferase, pois catalisa a transferência de um grupo fosfato do ATP para o glicerol, formando o glicerol-3-fosfato. A enzima glicerol-3-fosfato desidrogenase citosólica, oxida NADH, reduzindo dihidroxiacetona a glicerol-3-fosfato e a enzima glicerol-3-fosfato desidrogenase localizada na superfície externa da membrana mitocondrial reduz FAD, que é utilizado pela cadeia de transporte de elétrons mitocondrial, assim, a quantidade de glicose ou outros metabólitos gerados dependem da quantidade de glicerol consumido (Robergs & Griffin, 1998).

O destino metabólico do glicerol pode ser dirigido, dependendo do tecido e do estado nutricional do animal, para o fornecimento de esqueleto carbônico para a gliconeogênese, para a transferência de equivalentes redutores do citosol para a mitocôndria – com a geração de 22 ATP, ou como precursor da síntese de triglicerídeos, síntese *de novo* de ácidos graxos ou como constituinte da molécula do triacilglicerol.

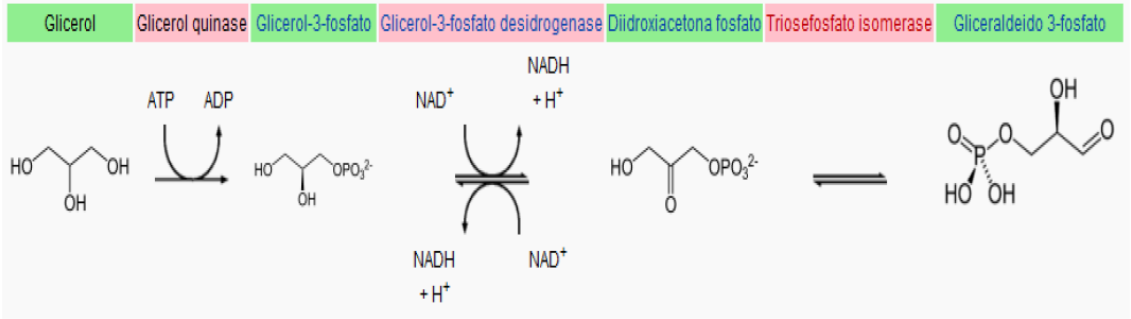

Figura 4: Conversão do glicerol a gliceraldeído-3-fosfato

Fonte: Silva (2010)

Os níveis normais de glicerol plasmático para ratos são de 0,1 mM e para humanos é 0,05-0,1 mM (Lin, 1977). Em frangos, Simon et al. (1996) encontraram nível de 0,65 mM de glicerol plasmático. Entretanto a concentração aumentou para 4,36 mM com o fornecimento de 5% de glicerol na dieta, e variou de 11 a 54 mM com a suplementação de 10% de glicerol na dieta.

Com altas taxas de inclusão de glicerol na dieta, as aves aparentemente não são capazes de metabolizar todo o glicerol absorvido, contudo, alguns autores acreditam que o fígado e rins podem sofrer adaptações

anatômicas, fisiológicas e bioquímicas quando ocorre excesso de glicerol na dieta, aumentando sua capacidade de metabolização (Cryer & Hartley, 1973).

Essa capacidade de metabolização deve-se possivelmente, à falta de ativação enzimática do glicerol, através da enzima glicerol quinase, para assim formar glicerol-3-fosfato, substrato esse necessário para o metabolismo do glicerol, o que pode vir a limitar sua absorção (Min et al., 2010).

Em estudos recentes, não foi encontrada saturação da enzima glicerol quinase do fígado, mesmo quando foram utilizados níveis de 7% de glicerina na dieta, tanto em aves mais jovens (22 a 35 dias de idade) como em aves mais velhas (33 a 42 dias de idade) (Bernardino et al., 2014).

O efeito do glicerol da dieta na atividade lipogênica foi estudado de forma comparativa em ratos e frangos demonstrando diferentes resultados. Para ratos a adição de glicerol causou aumento no peso vivo e na atividade de enzimas lipogênicas, sem aumentar a síntese de ácidos graxos do fígado. Por outro lado, em frangos não houve alteração no peso vivo e ocorreu queda na atividade de enzimas lipogênicas e na taxa de síntese de ácidos graxos de fígado (Lin et al., 1976).

Com base nisso, evidencia-se que a alimentação de animais não ruminantes com glicerol provoca respostas espécie-específicas e também órgão-específicas, que precisam ser estudadas com novas metodologias, para assegurar que os ingredientes que possuem glicerol na sua composição possam ser considerados ingredientes de uso generalizado nas rações.

3.0 HIPÓTESES E OBJETIVOS

Hipóteses

Frangos de corte consumindo dietas com diferentes níveis de inclusões de óleo ácido de soja (OAS), glicerol (GLI) e lecitina (LEC) assim como, suas misturas em diferentes proporções, aproveitem a energia disponibilizada desses produtos de maneira satisfatória podendo assim serem incorporadas em formulações.

Objetivos

Investigar a utilização dos três principais subprodutos do processamento do óleo de soja para consumo humano e do biodiesel (óleo ácido de soja, glicerol e lecitina), a combinação destes, e seus efeitos para a alimentação de frangos de corte de forma a estabelecer impactos sobre o metabolismo do glicerol.

Determinar valores de Energia Metabolizável aparente do óleo ácido de soja, glicerol e lecitina em níveis crescentes de inclusão bem como em uso conjunto nas proporções encontradas no triglicerídeo do óleo de soja (85%, 10% e 5%, respectivamente) para frangos de corte;

Determinar valores de Energia Metabolizável aparente para diferentes misturas composta por redução do óleo ácido de soja e aumento da quantidade de glicerol.

Estabelecer quantidades adequados da mistura desses três subprodutos de forma a não afetar o metabolismo normal de frangos de corte.

CAPÍTULO II[3]

[3] Artigo escrito conforme as normas da Revista Poultry Science

Energy utilization of soybean oil industry by-products: acidulated soapstock, glycerin, lecithin and their mixture

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ABSTRACT A study was conducted to estimate AME_n of fat by-products from the soybean oil industry. A total of 390 male broilers, 21 d of age was distributed in a completely randomized design of 4 fat sources and 3 levels of dietary inclusion. The fat sources used were: acidulated soybean soapstock (**ASS**), glycerol (**GLY**) and lecithin (**LEC**) as well as a mixture containing 85% ASS, 10% GLY and 5% LEC (**MIX**). Corn-soy diets were formulated totaling 13 dietary treatments consisting of a basal diet without supplemental fat and twelve diets having the addition of each supplemental fat source at the levels of 2% (98% basal diet + 2% fat source), 4% (96% basal diet + 4% fat source) or 6% (94% basal diet + 6% fat source). Total excreta were collected twice a day for 72 h starting at 25 d following a 5 d adaptation period. Intake of AME_n was regressed against feed intake and the slope was used to estimate AME_n from each fat source. Regression equations for each energy source were as follow: 7,153X - 451.9 ($P < 0.0001$) for ASS; 3,916X - 68.2 ($P < 0.0001$) for GLY; 7,051X - 448.3 ($P < 0.0001$) for LEC and 8,515X - 622.3 ($P < 0.0001$) for MIX. Results from this study present useful AME_n information for the poultry industry. The 3 fat sources present in MIX showed to synergistically react leading to a higher AME_n value when combined in the proportions utilized in MIX.

Key words: acidulated soybean soapstock, AME_n, broiler, glycerol, lecithin

INTRODUCTION

Fat is routinely added to broiler feeds to increase dietary energy contents, but it is also a source of essential fatty acids and fat-soluble vitamins (Abawi, et al., 1985; Hossain and Das, 2014). The utilization of energy from dietary fat by poultry is affected by age as well as fatty acid chain length and degree of saturation (Renner & Hill, 1960; Wiseman and Salvador, 1989). Regardless of the diverse existing sources, fats have been increasing in cost following a trend with the growth of the biodiesel industry in the recent years (Hill et al., 2006).

Soybean oil is largely used in poultry feeding, mainly in the form of degummed soy oil. However, several by-products of the soybean oil industry may result from the processing of soil oil (**SO**) for human consumption as well as from the trans esterification from biodiesel production. These low cost by-products include acidulated soybean soapstock (**ASS**), glycerol (**GLY**) and lecithin (**LEC**), which have been widely used in poultry feeding worldwide.

In most markets, SO sold for human consumption requires previous neutralization. This is usually done by chemically reacting SO with alkali such that a mixture of free fatty acid salts, mono and diglycerides results in a volume close to 6% of the original source (Vieira et al., 2002). Biodiesel processing leads to residual GLY corresponding to around 8% of the biodiesel produced (Min et al., 2010). As a precursor of gliceraldehyde 3-phosphate, GLY yields energy through the glycolytic and tricarboxylic-acid pathways in animals and, therefore, can also be used as an energy source in poultry diets (Nelson and Cox, 2002). Centrifuging crude soy oil generates a variable proportion (1.5 to 3.1 % of the original source) of LEC in an usual process conducted prior to oil refining

(Overland et al., 1994). As a feed ingredient, LEC can be a source of energy as well as P and choline (Woerfel et al., 1981; Menten et al, 1997).

Benefits of mixing sources of fats that have different degrees of saturation have been demonstrated when the proportion of unsaturated to saturated fats increases (Renner and Hill, 1960; Dvorin et al., 1998). Also, synergistic effects in energy utilization were shown for broilers when GLY and ASS were added into the same feed (Sklan, 1979). However, using LEC, ASS and GLY simultaneously in the same feed is not usual in the poultry industry.

Despite the increasing availability of the by-products from SO processing for human consumption and biodiesel production, their use as poultry feed ingredients is still not widely spread possibly because literature providing adequate information, such as AME_n values, is limited. The objective of this study was to determine the AME_n of LEC, ASS and GLY as well as in a mixture proportionally constituted by proportions that resemble their individual contribution in the original crude soy oil for broiler chickens (Overland et al., 1994; Nelson and Cox, 2002).

MATERIALS AND METHODS

Bird Husbandry

All procedures in this study were approved by the Ethics and Research Committee of Universidad Federal do Rio Grande do Sul, Brazil. A total of 390 one-day-old slow feathering Cobb *vs*. Cobb 500 male broiler chicks, vaccinated for Marek's disease, was obtained from a local hatchery (BRF, Lajeado, Brazil). Broilers were placed and grown to 20 d of age in floor pens being then randomly

allocated into 78 steel battery cages (0.40 m x 0.90 m, equipped with one feeder and one drinker), 5 per cage. Room temperature was controlled to maintain comfort throughout the study. A 16L:8D lighting program was used.

Experimental Diets

A basal corn-soybean meal mash diet without fat supplementation was formulated (Table 1). The fats used in this study were ASS, GLY and LEC alone or a combination of 85% ASS, 10% GLY and 5% LEC (**MIX**). The MIX diet was done to create a source with a profile similar to the crude soybean oil. The 4 fat sources were added to the basal diet at levels of 2, 4 and 6% to create the experimental diets as follow: control diet (100% basal diet), 2% inclusion (98% basal diet + 2% fat), 4% inclusion (96% basal diet + 4% fat), and 6% inclusion (94% basal diet + 6% fat). Feeds were provided at 94, 96, 98 and 100% of an *ad libitum* consumption previously determined. The strategy utilized to feed varying proportions of *ad libitum* intake intends for each treatment group to consume the same amount of basal diet. Therefore, differences in AME_n consumption were due to the fat added. Consumed AME_n was then regressed against feed intake with the slope representing the determined AME_n of each supplemental fat (Adeola, 2001). This approach has been suggested to provide a more consistent AME_n value because of the multiple inclusions of fats.

Broilers were distributed in a completely randomized design in a 4 x 3 + 1 factorial arrangement (4 sources of fat and 3 levels of inclusion plus the basal diet) with 6 replicates of 5 chickens per treatment. Fats used were commercially obtained (ASS and LEC from Meridional, Londrina, Brazil whereas GLY-99%

from INAQUIM Indústria e Comercio Ltda., Canoas, Brazil). All fat sources previously analyzed according to AOAC and ASTM methods (AOAC, 1995; AOCS, 2000; AOAC, 2005; ASTM, 2006) detailed in Table 2.

Excreta Collection and AME_n Determination

Total excreta were collected during a 72 h period after 5 d of adaptation to the experimental diets. Total amount of excreta voided at the end of the collection was weighed on an as is basis. Multiple subsamples were collected from the total amount of excreta and homogenized, and then 250 g representative samples were placed in plastic bags and frozen at -18°C being subsequently dried at 60°C for 72 h using standard methods (AOAC, 1990). Dried samples were then ground using a 1 mm sieve (Tecnal TE-631/2, São Paulo, SP, Brazil) to ensure homogeneous mixtures. Gross energy content of feed and excreta were determined on a 2-g sample using an isoperbol oxygen bomb calorimeter (IKA C-2000, IKA Werke, Parr Instruments, Staufen, Germany) in duplicate. Total feed consumption was calculated from the difference obtained between offered and leftover feeds per cage. Estimation of AME_n was done regressing AME_n intake against feed intake at the various replacement levels for each fat source with the slope representing the AME_n of fat source. Compared to other determinations, this method is advantageous because the slope estimation involves multiple inclusion levels (Adeola, 2001).

Statistical Analysis

Data were analyzed using the ANOVA by GLM procedure of SAS (2009). Means were compared using Tukey test at 5% of significance. Regression

analysis was done using the REG procedure of SAS (2009) with AME_n intake being regressed against feed intake to determine AME_n of the fat sources. Regressions were estimated individually for each fat source considering the basal diet as 0% of inclusion. This assumption makes the four regressions to have an intersection point (i.e. the basal diet) but with different intercepts (i.e. the point where the regression crosses the Y axis).

RESULTS AND DISCUSSION

Basal diet composition is presented in Table 1. Corresponding gross energy was 9,286, 4,165, and 9,329 kcal/kg for ASS, GLY, and LEC. These values as well as other chemical parameters used to characterize the commercial fat sources in the present study are shown in Table 3. These parameters are within acceptable parameters for fats as feed grade ingredients (Sell et al., 1998).

Balance of feed an energy were presented on Table 3. Excreta production was increased ($P < 0.05$) with high addition of energy sources, except with glycerol ($P > 0.05$) where adding this product did not increase the amount of excreta. The intake and excretion of GE follow the same pattern of feed intake, where high intake of the energy sources lead to high intake and excretion of GE ($P < 0.05$), again the exception was the glycerol ($P > 0.05$), where highest intake of GE was obtained with 2% of glycerol inclusion ($P < 0.05$) and no difference was found in GE excretion ($P > 0.05$). The intake and excretion of AME_n follow a linear pattern according level of energy source addition ($P < 0.05$).

The AME_n determined for the basal diet corresponded to 3,344 ±38 kcal/kg. Linear equations obtained when AME_n intake were regressed against

feed intake as well as the resulting AME_n for each fat sources are presented in Table 4. The slope was higher for MIX when compared to the other treatments (Figure 1).

A considerable number of AME_n values for ASS and GLY have been reported; however, data on LEC are scarce. Values obtained in the present study are somewhat similar to those previously reported for ASS (Freitas, et al., 2005; Peña et al., 2014), GLY (Cerrate, et al., 2006; Dozier, et al., 2008) and LEC (NRC, 1994; Rostagno et al., 2011). The estimated AME_n value for MIX, however, was higher than what would be obtained using proportional additions of the other individual fat sources. Proportions of the different fats in the MIX were originally thought to resemble the original composition of crude SO with ASS, GLY and LEC as building “blocks” to be assembled during absorption and metabolism (Lechowski et al., 1999). Polyunsaturated fatty acids can increase the solubility of the micellar phase when mixed with lipids predominantly saturated (Freeman, 1984). This mechanism is responsible for the phenomenon observed when combining unsaturated with saturated sources (Sibbald et al., 1961; Artman, 1964; Wiseman et al., 1986). On the other hand, free fatty acids supplied as a dominant source of lipids lack monoglycerides to maximize their absorption (Garrett & Young, 1975; Blanch et al, 1996). A possible esterification of free fatty acids with glycerol may improve the nutritive value of acid oils (Vilarrasa et al., 2015).

In addition to the length of the carbonic chain, other factors can directly influence the digestibility of fats and oils, and therefore their AME_n. These factors are the triglyceride or free fatty acid the specific arrangement of the saturated and

unsaturated fatty acids on the glycerol backbone, the composition of the free fatty acid, the composition of the diet, the type and quantity of triglycerides supplemented in the diet, the intestinal flora, the sex and age of the birds (Renner and Hill, 1960; Leeson and Summers, 2001; Nascif et al., 2004).

The present study showed that the ASS, GLY and LEC can be used in broiler feeds as long as adequate quality parameters are respected ensuring adequate performance. In conclusion, the results showing the highest AME_n value for the MIX indicates that mixing ASS, GLY and LEC in similar proportions present in the original crude soybean oil are indicative of a better utilization of these 3 fat sources when used together.

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Table 1. Ingredient and nutrient composition of the basal diets (as-is basis)

Item	Basal diet
Ingredients %	
Corn	61.15
Soybean meal (44% CP)	35.09
Limestone	0.92
Dicalcium phosphate	1.79
Salt	0.48
L-Lysine HCl 78%	0.11
DL-Methionine 99%	0.25
Choline chloride 60%	0.05
Vitamin and mineral mix[1]	0.16
Calculated nutrient composition, (%) unless noted	
AME_n[2], kcal/kg	3,344 ± 38
CP	21.00
Ca	0.90
Available P	0.45
Na	0.20
Dig. Lys	1.10
Dig. TSAA	0.82
Dig. Thr	0.69
Dig. Val	0.88

[1]Added per kg of feed: vitamin A, 8,000 UI; vitamin D_3 2,000 UI; vitamin E, 30 UI; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; monensin sodium, 275 mg (Elanco Animal Health, Greenfield, IN).

[2]Value between parentheses was obtaining following the methodology proposed by Adeola (2001).

Table 2. Chemical characterization of the energy sources utilized to determine AME_n values

Analysis	Acidulated soybean soapstock[1]	Glycerol[1]	Lecithin	Analytical method
Moisture Karl Fischer, %	0.53	0.17	0.23	AOAC 984.20
Moisture and volatiles, %	1.06	0.90	5.28	AOAC 926.12
Crude protein, %	0.12	0.00	3.42	AOAC 990.03
Ether extract, %	99.90	0.41	99.94	AOAC 920.39
Peroxide value, mEq/kg	0.00	0.00	0.00	AOAC 965.33
Acidity in oleic acid, %	77.30	9.30	10.50	AOAC Cd 3d-63
Iodine value, g/100g	11.47	-	94.82	AOAC 993.20
pH in aqueous solution	2.90	6.90	3.70	AOCS G 7-56
Ash, %	1.27	0.03	8.09	AOAC 942.05
Phosphorus, %	0.03	-	1.93	AOAC 956.01
Sodium, %	0.003	0.28	0.02	AOAC 956.01
Glycerol, %	-	99.98	-	ASTM 2006
Methanol, mg/L	-	9.50	-	AOAC 958.04
Gross energy, kcal/kg	9,286	4,165	9,329	Calorimeter

[1]Glycerol 99%.

Table 3. Balance of feed and energy of bird feed different energy sources[1]

Item	Feed intake, kg	Excreta, kg	GE[2] feed, kcal/kg	GE excreta, kcal/kg	EMA_n, kcal/kg	EMA_n intake, kcal
Acidulated soybean soapstock						
0%	0.600^{d}	0.079^{b}	3,833^{d}	3,707^{b}	3,345^{b}	2,007^{c}
2%	0.610^{c}	0.075^{b}	3,951^{c}	3736^{b}	3,493^{a}	2,131^{b}
4%	0.620^{b}	0.088^{ab}	4,076^{b}	3,856^{a}	3,530^{a}	2,189^{b}
6%	0.635^{a}	0.099^{a}	4,179^{a}	3,907^{a}	3,570^{a}	2,267^{a}
SEM	0.003	0.003	27	21	23	22
P-value	< 0.001	0.011	< 0.001	< 0.001	< 0.001	< 0.001
Glycerol						
0%	0.600^{d}	0.079	3,833^{b}	3,707	3,345^{c}	2,007^{c}
2%	0.610^{c}	0.080	3,852^{a}	3,721	3,363^{b}	2,051^{b}
4%	0.620^{b}	0.075	3,809^{d}	3,702	3,364^{b}	2,085^{b}
6%	0.635^{a}	0.074	3,815^{c}	3,748	3,379^{a}	2,146^{a}
SEM	0.003	0.001	4	12	7	11
P-value	< 0.001	0.115	< 0.001	0.524	< 0.001	< 0.001
Lecithin						
0%	0.600^{d}	0.079^{b}	3,833^{d}	3,707^{b}	3,345^{b}	2,007^{c}
2%	0.610^{c}	0.081^{b}	3,849^{c}	3,626^{c}	3,368^{b}	2,055^{bc}
4%	0.620^{b}	0.094^{a}	3,957^{b}	3,776^{a}	3,381^{b}	2,096^{b}
6%	0.635^{a}	0.086^{ab}	4,059^{a}	3,710^{b}	3,553^{a}	2,256^{a}
SEM	0.003	0.002	19	13	20	20
P-value	< 0.001	0.015	< 0.001	< 0.001	< 0.001	< 0.001
MIX[3]						
0%	0.600^{d}	0.079^{b}	3,833^{d}	3,707^{b}	3,345^{c}	2,007^{d}
2%	0.610^{c}	0.079^{b}	3,879^{c}	3,702^{b}	3,402^{c}	2,075^{c}
4%	0.620^{b}	0.090^{a}	4,040^{b}	3,830^{a}	3,482^{b}	2,159^{b}
6%	0.635^{a}	0.081^{ab}	4,122^{a}	3,864^{a}	3,627^{a}	2,303^{a}
SEM	0.003	0.002	24	18	24	24
P-value	< 0.001	0.038	< 0.001	< 0.001	< 0.001	< 0.001

[1]Means represent 6 replicates of 5 chickens.
[2]Gross energy.
[3]Mixture of 85% acidulated soybean soapstock, 10% glycerol and 5% lecithin.

Table 4. Linear regression equations used to estimate AME_n values of different fat sources

Item	Intercept		Slope		r^2	*P*-value
	Coefficient[1]	SE	Coefficient[2]	SE		
Fat sources						
ASS[4]	-451.9	97.7	7,153	793	0.787	< 0.0001
Glycerol	-68.2	39.6	3,916	322	0.871	< 0.0001
Lecithin	-448.3	72.2	7,051	585	0.868	< 0.0001
MIX[3]	-622.3	53.8	8,515	437	0.945	< 0.0001

[1]Intercepts were different ($P < 0.001$) of zero.
[2]Slopes were different ($P < 0.001$) of one.
[3]Mixture of 85% acidulated soybean soapstock, 10% glycerol and 5% lecithin.

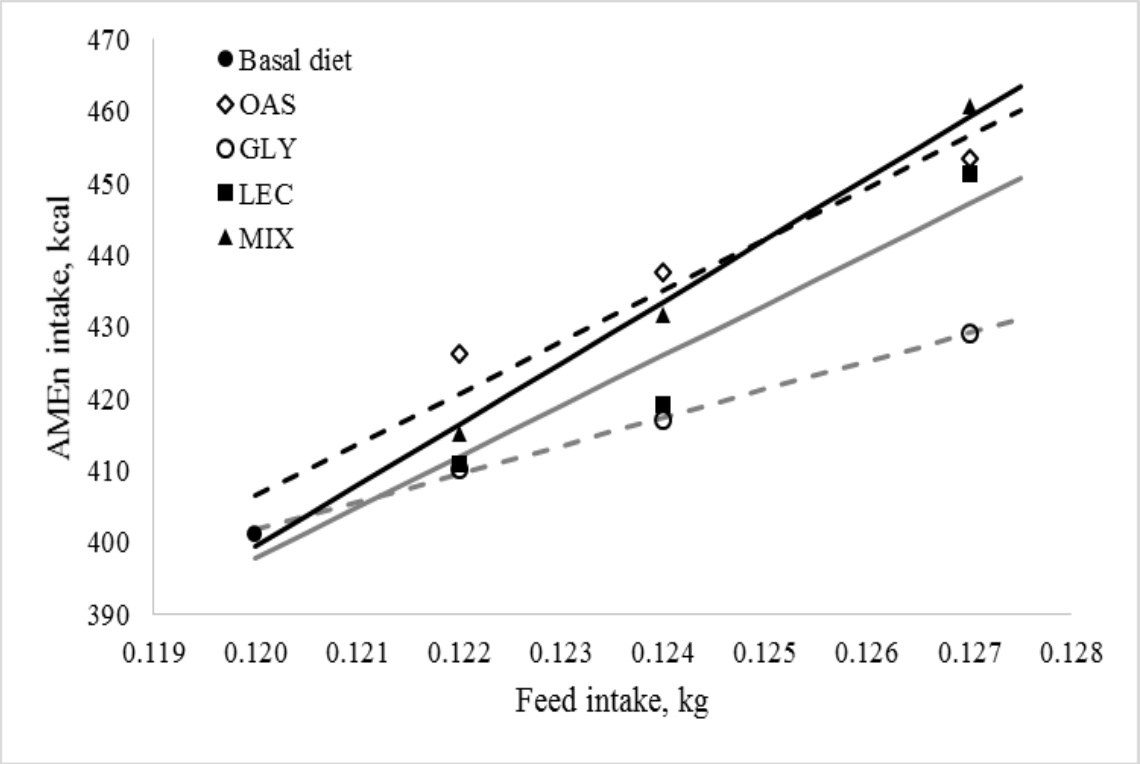

Figure 1. Regression of AME intake vs. feed intake from 25 to 28 d of age. Dietary fat source addition of 0% = 0.120 kg of feed intake; dietary fat source addition of 2% = 0.122 kg of feed intake; dietary fat source addition of 4% = 0.124 kg of feed intake; dietary fat source addition of 6% = 0.128 kg of feed intake. Dots represent observed means. Solid black line represent Mixture (MIX) regression, Y = 8,515X -622.3. Dashed black line represent acidulated soybean soapstock (OAS) regression, Y = 7,153X -451.9. Solid grey line represent lecithin (LEC) regression, Y = 7,051X -448.3. Dashed grey line represent glycerol (GLY) regression, Y = 3,916X -68.2.

CAPÍTULO III[4]

[4] Artigo escrito conforme as normas da Revista Poultry Science

METABOLISM AND NUTRITION

Energetic values, plasmatic levels of triglycerides, free glycerol and activity hepatic glycerol kinase in broilers fed by-products of soybean oil industry in different combination

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ABSTRACT A study was conducted to determine AME, plasmatic triglycerides and free glycerol, and activity of hepatic glycerol kinase in mixtures of soybean oil and biodiesel by-products. A total 390 21-d-old male broilers were distributed in a completely randomized design in a 4 x 3 factorial arrangement of 4 combination with 3 levels of inclusion (2, 4 or 6%) plus the basal diet without additional fat in total thirteen dietary treatments with 6 replicates of 5 broilers each. Each mixture were formed varying acidulated soybean soapstock (ASS) and glycerol (GLY) while the amount of lecithin (LEC) remained at 5% per mixture. The combinations used were, 85% (ASS), 10% (GLY); 80% ASS, 15% GLY; 75% ASS, 20% GLY and 70% ASS, 25% GLY. Each mixture was added to the basal mash diet. With 20 d broilers were weighed and distributed in the experimental units. Broilers went through 5 d of adaptation to dietary treatments and at 26 d of age started a period of total excreta collection for 72 h. The end in collection period of excreta blood and liver were collected from one broiler per replicate using heparinized tubes for analysis of plasmatic triglycerides, free glycerol and the activity of the hepatic enzyme glycerol kinase. The mixture 85:15 showed the best AME value ($P<0.05$) when compared to mixture 80:10, however did not differ of mixture 75:20 and 70:25. Was observed interaction ($P<0.05$) between mixture utilized and level of inclusion for plasmatic triglycerides, the same interaction was observed ($P<0.001$) to plasmatic glycerol and glycerol kinase activity of the enzyme. In conclusion, when used the mixture of 80% ASS and 15% GLI, based on the absence of saturation of the glycerol kinase activity and the high value AME, showed the best values for this study.

Key words: soybean fat, triglyceride, free glycerol, broilers

INTRODUCTION

The use of supplemental fats and oils in broiler diets as energy yielding ingredients has become a common practice in the feed industry. In soybean oil **(SO)** processing and during the transesterification required for biodiesel production, byproducts that can be used in poultry feed are usually generated at a low cost. These byproducts include lecithin **(LEC),** acidulated soybean soapstock **(ASS)** and glycerol **(GLY).**

The poultry industry has a great demand for high-energy feed ingredients. Currently there is a large availability of crude glycerin on the world market, and an increase in its use is expected (Dozier et al., 2008). Therefore, the use of this available byproduct as an energy ingredient on a large scale could mitigate the costs of food, especially of corn.

Fat is insoluble in water and difficult to digest in an aqueous medium, making it necessary fat emulsification. Since lecithin could potentially improve fat emulsion, adding phospholipids in the form of vegetable lecithin to poultry diets could be an optimal solution for improving fat digestion (Minh Siu et al. 1998, Soares et al. 2002).

To evaluate the effects of different dietary levels of free fatty acids (FFA) on the digestion and absorption of fats Sklan (1979) demonstrated that the presence of monoglycerides is important for efficient solubilization and absorption of FFA. Deficiency of monoglycerides may also result in a reduction in the secretion of bile. Therefore, glycerol is important for fat digestive processes due to its presence in molecules of mono and diglycerides that are required for micelle formation and subsequent absorption (Dvorin et al, 1998). Similary this, the

soybean lecithin apart from being a source of energy, also serves as an emulsifier and has the potential to facilitate fat absorption (Lechowski et al., 1999).

Although there are many studies on the energy value of the glycerin and its effect on broiler performance there are few related scientific about glycerol levels in broiler blood fed diets with glycerin. Such studies may contribute to a better understanding of the use of this ingredient in poultry nutrition (Bernardino et al., 2012).

Another important factor is biochemically understand the metabolism glycerol and as it is exploited by broilers and of the enzymes that need to be assessed is the glycerol kinase (EC 2.7.1.30) that catalyzes the transfer of the terminal phosphate group of ATP to glycerol molecule resulting in glycerol-3-product phosphate and ADP (FOSSATI; Prencipe, 1982). This is a key enzyme in glycerol metabolism because if it is not phosphorylated by glycerol kinase the bird representing a double nutritional prejudice, because in addition to not have been oxidized to generate energy the body will still have to spend energy to promote their excretion, will excrete it (Robergs and Griffin, 1998).

Thus understanding the glycerol metabolism in broilers will formulate diets with glycerin inclusions with a better physiological and nutritional balance. Consequently, this experiment was conducted to determine the AME of broiler diets containing mixture of ASS, GLY and LEC. This experiment also aimed to evaluate plasmatic triglycerides, free glycerol levels and activity of the hepatic enzyme glycerol kinase in broilers.

MATERIALS AND METHODS

Bird Husbandry

All procedures in this study were approved by the Ethics and Research Committee of Universidad Federal do Rio Grande do Sul, Brazil. A total of 390 one-day-old slow feathering Cobb *vs*. Cobb 500 male broiler chicks that had been vaccinated for Marek's disease were obtained from a local hatchery (Frangosul S.A, Montenegro, Brazil). Broilers were placed and raised to 20 d of age in floor pens and then randomly allocated into 78 steel battery cages (0.40 m x 0.90 m) that were equipped with one feeder and one drinker each at 21 d. The experimental facility was a solid-sided house with temperature control. Room temperature was controlled to maintain comfort throughout the study, and a 15L:8D lighting program was used. Feed and water were provided *ad libitum*.

Experimental Diets

A basal corn-soybean meal diet without fat supplementation was formulated to meet NRC (1994) nutrient recommendations for broilers (Table 1). Experimental diets were composed of this basal diet and the addition of mixtures of ASS, GLY and LEC. In all four mixtures 5% LEC was added. The mixtures of by-products were: 85% ASS, 10% GLY (80:10); 80% ASS, 15% GLY (85:15); 75% ASS, 20% GLY (75:20)**;** and 70% ASS, 25% GLY (70:25). Experimental diets were created by replacing the basal diet with each fat source at levels of 0% (100% basal diet), 2% (98% basal diet + 2% fat source), 4% (96% basal diet + 4% fat source) or 6% (94% basal diet + 6% fat source) inclusion. These diets were fed at 94, 96, 98 and 100% of *ad libitum* intake determined with a group of

broilers that consumed the basal diet. The energy value of these mixtures were estimated by regression analysis.

The ASS and LEC used in this study were obtained from a commercial company (Meridional, Londrina, Brazil) whereas the GLY (99%) was obtained from INAQUIM (Indústria e Comercio Ltda., Canoas, Brazil). All fat sources were analyzed prior to mixing according to AOAC and ASTM methods (AOAC, 1995; AOAC, 2005, AOCS, 2000; ASTM, 2006). The results of these analyses are shown in Table 2.

Excreta Collection and AME Determination

A 72 h total excreta collection period was conducted to evaluate AME for each fat source. After a 5 d acclimation period to the experimental diets, feed refusal from the control group and feed allocation were weighed daily throughout the 72 h collection period. The total amount of excreta at the end of the collection time was weighed on an as is basis. Multiple subsamples were collected from the total amount of excreta and homogenized, and then 250 g of these representative samples were placed in plastic bags and frozen at -18°C. Later, these samples were dried at 60°C for 72 h using standard methods (AOAC, 1990). Dried samples were then ground through a 1 mm sieve (Tecnal TE-631/2, Sao Paulo, SP, Brazil) to ensure homogeneous mixtures. Gross energy content of feed and excreta were determined with a 2 g sample using an isoperbol oxygen bomb calorimeter (Ika C-2000, Sao Paulo, SP, Brazil) in duplicate analyses. Was calculated AME of the basal diet and test diets using the following formula with appropriate corrections for in DM content.

$$\text{AME (kcal/kg)} = \frac{(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})}{\text{Feed intake}}$$

At 28 d after 2 h of fasting, blood and liver were collected from one broiler per replicate for analysis of plasmatic free glycerol and triglycerides. Blood was collected in tubes containing EDTA as an anticoagulant and centrifuged at 2000 g for 15 min, after centrifugation the plasma was collected.

The concentration of free glycerol in the plasma was measured using a commercial kit (Abcam, Code: ab65337, Cambridge) that was based on the generation of a colored product during the enzymatic oxidation of glycerol. In this, the color intensity of the end product was proportional to the concentration of glycerol present in the sample and could be monitored colorimetrically at 570 nm (PowerWaveTM XS Microplate Scanning Spectrophotometer, Bio-Tek Instruments, Potton Bedfordshire, UK). A standard curve was made by adding increasing concentrations of pure glycerol (0, 2, 4, 6, 8 and 10 nmol of glycerol from a 1 mM stock solution/well parting) to 50 µl of the working reagent. A final volume of 50 µl/well of glycerol was maintained with the use of adequate volumes of buffer. Next, the plates were incubated at room temperature for 30 min and absorbance was measured at 570 nm. A linear standard curve was created by plotting the values of absorption capacity on the y-axis and concentration of glycerol (nmol) on the x-axis. Glycerol content of the samples was measured in 96-well microplates with each well containing 50 µl of plasma and 50 µl of the working reagent (composed of 46 µl of buffer, 2 µl of the enzymatic reagent and 2 µl of the color reagent). Free glycerol level was expressed as nmol of glycerol/mL of plasma.

Plasmatic triglyceride level was analyzed using a commercial kit based on an enzymatic reaction with lipase, and levels were quantified by reading the reaction in a spectrophotometer (Wiener Lab, CM 200, São Paulo, Brasil) with absorbance at 505 nm.

Preparation of the hepatic extract and determination of the glycerol kinase activity

Livers were collected and immediately frozen in liquid nitrogen. For the extraction of the enzyme glycerol kinase, 1.5 g of frozen liver was macerated in a porcelain mortar in the presence of liquid nitrogen and 3 ml of Tris-HCl buffer (0.05 M, pH 8.0). After the complete maceration and homogenization of the tissue, the samples were centrifuged at 15 000 g for 20 min at 5 °C, and the supernatants were collected. The enzymatic activity in the supernatants was determined according to the method cited by Kihara et al. (2009) which is based on a series of sequential biochemical reactions. Initially, the glycerol kinase present in the sample phosphorylates the glycerol to glycerol-3 phosphate and ADP in the presence of ATP. After two sequential reactions catalysed by glycerol-3-phosphate oxidase (EC 1.1.3.21) and peroxidase (EC 1.11.1.7), the glycerol-3-phosphate is converted to quinoneimine, which is a reddishcoloured compound that can be colorimetrically monitored at 500 nm. The kinetic assay was conducted in 96-well microplates and each well contained 880 µl of Tris-HCl buffer (50 mM, pH 8.0), 10 µl of 400 mM ATP (pH 7.0, Amresco, code: 0220-25G), 10 µl of 200 mM MgCl2, 10 µl of glycerol-3-phosphate oxidase from Pediococcus sp. (100 U of enzyme/500 µl of buffer; Sigma-Aldrich, code: G9637-100UN), 10 µl of horseradish peroxidase (450 U of enzyme/ml of buffer; Amresco,

code: 0343-25,000U), 10 µl of 150 mM 4-aminoantipyrine (Sigma-Aldrich, code: A4382- 100G), 10 ll of 150 mM phenol and 50 µl of the supernatant. To start the sequence of reactions, 10 µl of 1-M glycerol was added to each well, and the plates were then incubated at 25 °C in an automatic microplate reader (PowerWaveTM XS, Bio-Tek Instruments) programmed to read the absorption at 500 nm every minute for a maximum period of 15 min. Blank samples were included to measure the background absorbance of the samples and reagents used in the kinetic assay. Subsequently, the data from the enzymatic assay were used to generate an increasing linear curve with the values of the absorption capacity plotted on the y-axis of the graph and the reaction time (in min) on the x-axis. The glycerol kinase activity was calculated according to the following equation:

$$\text{Volumetric activity (µmol of glycerol–3–P/min/ml or U/ml)} = \frac{(SLS - SLBS) \times RV \times D}{\varepsilon \times EV}$$

SLS – slope of the line of the kinetic assay with the sample (absorbance/minute)

SLBS – slope of the line of the blank sample (absorbance/minute)

RV – total reaction volume in ml (1 ml)

D – dilution of the sample (10 times)

Ɛ – molar extinction coefficient of the 4-aminoantipyrine (6.65 9 10^3 M/cm as described by Aragon et al., 2008); and

EV – hepatic extract volume in ml (0.05 ml).

The protein content in the samples (µg of protein/ml of hepatic extract) was measured according to the method of Bradford (1976) using bovine serum

albumin as a standard to allow the calculation of the specific activity of the glycerol kinase as follows:

Specific activity (U/µg of protein) = volumetric activity ÷ protein content in the sample.

Therefore, the glycerol kinase activity was expressed in U, defined as the quantity of enzyme that generates 1 µmol of glycerol per minute of reaction in a solution with pH = 8.0 and 25 °C.

Statistical Analysis

Was used a completely randomized design with a 4 X 3 factorial arrangement of treatments (4 sources of energy with 3 levels of inclusion each). There were 6 replicates of 5 chickens per treatment combination. Data were analyzed using the ANOVA procedure of SAS (2009). Tukey's test (Tukey, 1991) was used for mean separation when significant effects were detected at $P \leq 0.05$. Regression analysis was done using the PROC REG procedure of SAS (2004). Observations were removed when the response criteria exceeded 2 SD from the mean.

RESULTS AND DISCUSSION

Resulting data for AME from the assay along with the linear equations utilized in their determination are presented in Table 3. The AME values determined per regression analysis was higher for the mixture with 85:15. The AME values for 75:20 and 70:25 not differ from each other, and mixture 85:10 showed the smallest value.

Through regression, analysis yielded the following equations: 3,108 + 73.16x ($P \leq 0.0001$; $R^2 = 0.83$) for mixture 85:15; 3,151 + 84.61x ($P \leq 0.0001$; R^2

= 0.84) for 80:15; 3,125 + 75.25x ($P \leq 0.0001$; $R^2 = 0.87$) for 75:20 and 3,112 + 75.50x ($P \leq 0.0001$; $R^2 = 0.93$) for the mixture 70:25. An estimate to the AME of Mixtures was obtained by extrapolation of the equation where 100% of mixture in the diet correspond the AME kcal/kg in value as feed (Rodriguez et al., 2005). The estimated values of AME for different sources were: 7,316, 8,460, 7,520 and 7,540 kcal/kg and gross energy were analyzed to contain 9,201, 9,220, 9,246 and 9,231 kcal/kg for mixture 85:10; 80:15; 75:20 and 70:25 respectively.

The use of combinations of fat source can show higher AMEn values, because of a synergism when the different sources are consumed together by broilers Sklan (1979). Saturated fatty acid utilization, however, may be improved by the presence of unsaturated fatty acids in the fat blend Young, 1965; Garrett and Young, 1975; Leeson and Summers, 1976).This synergism is caused by the excellent emulsifying capacities of the latter Sibbald *et al.* (1961) attributed this effect to the presence of lecithin.

However, this synergistic answer seems to be dependent on an optimal balance between existing fatty acids in the diet present in the micellar phase (Ketels and DeGroote, 1989).

Villarasa et al. (2015) observed how, in general, the fat degree of saturation exerted a greater impact on FA apparent absorption than did the fat molecular structure. For this reason, the combination of re-esterified oils, differing in their degree of saturation, could be beneficial in terms of fat utilization, as occurs with native oils (Sibbald et al., 1960; Lewis and Payne, 1966).

The plasmatic glycerol level in plasma not showed differ ($P>0.099$) values for the mixtures (Table 4). However, independent of the sources used, increasing

inclusion level of mixture in the diet resulted in an increase linearly in the concentration of plasmatic glycerol in the plasma of the broilers (*P<0.001*) in comparison with the levels detected in the birds fed the control diet without fat source.

An average concentration of 8.0 nmol of free glycerol/ml of plasma was measured in the broilers that received the control diet without fat source this concentration is likely the result of the digestion of lipids present in the diet and of the catabolism of lipids in the adipose tissue. During digestion the triacylglycerol of the diet are enzymatically hydrolyzed to free fatty acids and glycerol (Champe et al., 2009) and part of this glycerol is converted again into triacylglycerol inside of the enterocytes and is then released into the blood as lipoprotein, but the other part of the free glycerol can enter by the portal circulation (Brody,1994).

However, the hydrolysis of triacylglycerol in the adipose tissue also generates free glycerol that is released into the bloodstream, because as they lack glycerol kinase, the adipocytes are unable to use the glycerol (Champe et al., 2009). During the experiment the broilers received restricted feed so it is likely that the lipolysis rate in these animals was high because it was physiologically necessary stimulate this kind of lipid catabolism. Therefore, most of the free glycerol determined in the plasma of birds that received control diet was likely originated from the digestion of the lipids present in the feed.

In addition, all experimental mixtures containing glycerol resulted in increased plasma levels of free glycerol in comparison with the broiler fed the control diet without any fat source this confirms that the glycerol present in the

diets is absorbed by the intestine and can be transported to the liver through the bloodstream.

According Lin (1977) normal plasmatic levels of glycerol to rats is 0.1 mM for humans is 0.05-0.1 mM. Simon et al. (1996) evaluated the concentration of glycerol in the blood of broilers with 2 hours of fasting and found that animals fed the control diet had levels of 0.65 mM plasma glycerol, and the concentration increased to 4.36 mM with providing 5% glycerol in the diet and ranged from 11.24 to 54.17 mM supplementation with 20% glycerol in the diet.

In this study we used mixtures of ASS, GLY and LEC with the amount of glycerol increases from 10% to 25% of inclusion to each mixture and this were included 2, 4 and 6% in the diet and it was observed that concentration plasmatic glycerol in broilers is lower that the value found by the above author, because this value was around 13 nmol of glycerol/ml (1.3×10^{-5} mM) for the different mixtures. The concentration plasmatic glycerol increases as it increased the inclusion level in the diet resulting in different values ($P<0.05$) for each level of inclusion with 13.5, 15.9 and 17.0 nmol glycerol/mL for 2, 4 and 6%, respectively.

For plasmatic glycerol values is need consider the degree of purity of this that it was 99.98%.

For triglyceride values, there was interaction ($P<0.01$) between sources of fat and inclusion levels. The mixture of 85:10 showed a higher value for triglycerides (71.2 mg/dL), this mixture contained larger amount of ASS is composed of FA to be metabolized by the body and the surplus stored. As to the proportion of decrease ASS decrease triglyceride levels which did not differ from each other.

When the mixture includes 2% to 85% ASS: 10% GLY there is an increase in the concentration of triglycerides (98.0 mg/dL), but when the inclusion becomes 4 and 6% decrease plasmatic triglycerides, as these can have been retained in the liver, since during the absorptive period which is two to four hours after normal feed, have an increase in plasma triglycerides, especially as components of chylomicron synthesized in intestinal mucosal cells (Lehninger et al., 2005).

When using the mixture 80% ASS and 15% GLY with 2 and 4% inclusion there is no difference between them (59.5 and 48.0 mg/dl respectively), but when the inclusion becomes 6% the amount FA back increase in plasma (71.4 mg/dL), therefore the proportion of ASS in the mixture decreases but the highest level show greater.

In this study, the chickens fed diet without adding fat based on corn and soybean showed plasmatic triglycerides concentration of 75.5 mg/dL (Maciel et al., 2007; Rotava et al., 2008; Souza et al., 2010). This value is in the average found by other authors that found value between 76 to 93 mg / dl for broilers at 21 days of age and 73 to 89 mg/dL for broilers at 42 days of age.

There was interaction ($P<0.01$) on the enzymatic activity of the glycerol kinase (Table 4). As the inclusion of glycerol increases in the mixture is decreased at the enzyme glycerol kinase activity, the same is observed when the mixture increases inclusion level in the diet, however, this reduction did not occur to such an extent that could saturate the enzyme, because the saturation is directly related to the amount plasmatic glycerol.

In glycerol metabolism, glycerol kinase converts glycerol to glycerol-3-phosphate (Lehninger et al., 2005). Therefore, the increase in the activity of this

enzyme demonstrates physiological self-regulation by the organism to allow better use of the glycerol from the diet. This supports the use of glycerol in the nutrition of broilers, because the glycerol-3-phosphate produced by glycerol kinase can be converted to dihydroxyacetone-phosphate (DHAP) by the catalytic action of another enzyme, glycerol-3-phosphate dehydrogenase, also present in the liver.

The DHAP can be converted to glyceraldehyde-3-phosphate by triosephosphate isomerase that is then used metabolically for the synthesis of glucose (gluconeogenesis), fatty acids (lipogenesis) or is completely oxidized for the production of energy via glycolysis and the Krebs cycle (Champe et al., 2009).

Glycerol, G3P, ATP, ADP, and Mg2+ interact in a complex manner to determine the activity of the enzyme glycerol kinase. As revealed by double-reciprocal plots, glycerol is inhibitory at high concentrations when ATP concentration is low (0.02 mM), but not when high (1 mM). When glycerol concentration is high (0.33 mM), ATP activates the enzyme glycerol kinase and when glycerol concentration is low (0.01 mM), ATP does not activate. The kinetic features of this enzyme suggest that phosphorylation of glycerol occurs when the cell is not energy-starved and that over phosphorylation in an affluent state is counteracted by the accumulation of G3P.

As has been increasing inclusion of mixtures in the diet, there was a reduction in the enzyme glycerol kinase activity however, this reduction was not enough to saturate the enzyme. The saturation would be nutritionally undesirable because although the glycerol would not be oxidized to generate energy, the

organism would still have to expend energy to promote its excretion (Doppenberg and Van der Aar, 2007).

In conclusion, glycerol inclusion levels in mixtures, in diet of chicken did not affect normal metabolism of glycerol by the glycerol kinase enzyme, showing that animals efficiently metabolized glycerol implemented in this study without causing saturation of the enzyme. Triglyceride values showed that as the inclusion of ASS decreases in the mixture lowers the concentration of plasmatic triglyceride. However, the mixture with 80% ASS, 15% GLY and 5% LEC had improved the amount of AME and the mixture indicated as energetic source for broiler. Further studies needed to evaluate the performance of the broilers to confirm this inclusion of glycerol in the diet.

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Table 1. Ingredients and nutrients composition of the basal diets (as-is basis)

Item	Basal diet
Ingredients %	
Corn	61.15
Soybean meal (44% CP)	35.09
Limestone	0.92
Dicalcium phosphate	1.79
Salt	0.48
L-Lysine HCl 78%	0.11
DL-Methionine 99%	0.25
Choline chloride 60%	0.05
Vitamin and mineral mix[1]	0.16
Calculated nutrient composition, (%) unless noted	
AME_n[2], kcal/kg	3,344 ±38
CP	21.00
Ca	0.90
Available P	0.45
Na	0.20
Dig. Lys	1.10
Dig. TSAA	0.82
Dig. Thr	0.69
Dig. Val	0.88

[1]Added per kg of feed: vitamin A, 8,000 UI; vitamin D_3 2,000 UI; vitamin E, 30 UI; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; monensin sodium, 275 mg (Elanco Animal Health, Greenfield, IN)

Table 2. Chemical characterization of the energy sources utilized to determine AME values.

Analysis	Acidulated soybean soapstock[1]	Glycerol	Lecithin	Analytical method
Moisture Karl Fischer, %	0.53	0.17	0.23	AOAC 984.20
Moisture and volatiles, %	1.06	0.90	5.28	AOAC 926.12
Crude protein, %	0.12	0.00	3.42	AOAC 990.03
Ether extract, %	99.90	0.41	99.94	AOAC 920.39
Peroxide value, mEq/kg	0.00	0.00	0.00	AOAC 965.33
Acidity in oleic acid, %	77.30	9.30	10.50	AOAC Cd 3d-63
Iodine value, g/100g	11.47	-	94.82	AOAC 993.20
pH in aqueous solution	2.90	6.90	3.70	AOCS G 7-56
Ash, %	1.27	0.03	8.09	AOAC 942.05
Phosphorus, %	0.03	-	1.93	AOAC 956.01
Sodium, %	0.003	0.28	0.02	AOAC 956.01
Glycerol, %	-	99.98	-	ASTM 2006
Methanol, mg/L	-	9.50	-	AOAC 958.04
Gross energy, kcal/kg	9,286	4,165	9,329	Calorimeter

[1]Glycerol 99 (%)

Table 3. Linear regression equations used to estimate AME of different combination fat sources.

Mixture (%)			Intercept	Slope	R^2	AME kcal/kg	P-value
ASS	LEC	GLY					
85	5	10	3,108 ± 36	73.16 ± 9.4	0.75	7,316 [b] ± 32	<0.001
80	5	15	3,151 ± 50	84.64 ± 13.4	0.64	8,460 [a] ± 49	<0.001
75	5	20	3,125 ± 35	75.17 ± 9.4	0.74	7,520 [b] ± 41	<0.001
70	5	25	3,112 ± 38	75.41 ± 10.0	0.71	7,540 [b] ± 42	<0.001

[a-b]Mean values within a column with no common letters are significantly different (P ≤ 0.05) as determined by least significant difference comparison.
[1]Values are least squares means of 6 replicate pens each pen having 5 broilers.
[2]AME (kcal/kg) = (Feed intake × GEdiet) − (Excreta output × GEexcreta) ÷ Feed intake, where GEdiet = gross energy diet; GEe = gross energy output in the excreta;

Table 4. Level of glycerol and triglycerides in the plasma and activity of glycerol kinase in the livers of broiler chickens fed diets containing different concentration of acidulated soybean soapstock (ASS) and of glycerol (GLY) in the period of 21–30 days of age

Item ASS:GLY* Ratio		Free glycerol (nmol Gly/mL)	Triglycerides (mg/dL)	Glycerol kinase activity[1] (U x 10^{-4}/µg of Protein)
85:10		13.4	71.2^{a}	5.15^{a}
80:15		13.5	63.6^{b}	5.19^{a}
75:20		13.5	65.2^{b}	4.77^{b}
70:25		14.1	65.5^{b}	4.87^{b}
Inclusion Level, (%)				
0		8.0^{d}	75.5^{a}	5.62^{a}
2		13.5^{c}	71.4^{a}	5.23^{b}
4		15.9^{b}	56.9^{c}	4.78^{c}
6		17.0^{a}	61.8^{b}	4.36^{d}
Treatment x Level (%)				
Basal		8.0^{g}	75.5^{b}	5.62^{a}
85:10	2	13.5^{ef}	98.0^{a}	5.54^{ab}
85:10	4	15.7^{bcd}	55.9^{de}	4.98^{cde}
85:10	6	16.3^{abc}	55.5^{de}	4.46^{f}
80:15	2	13.7^{def}	59.5^{de}	5.26^{bc}
80:15	4	15.6^{bcde}	48.0^{e}	5.12^{cd}
80:15	6	16.6^{abc}	71.4^{bc}	4.77e
75:20	2	13.7^{def}	66.6^{bcd}	5.24^{bc}
75:20	4	15.0^{cdef}	57.0^{de}	4.24^{fg}
75:20	6	17.2^{ab}	61.7^{cd}	3.99^{g}
70:25	2	13.0^{f}	61.5^{cd}	4.86^{de}
70:25	4	17.2^{ab}	66.6^{bcd}	4.77^{e}
70:25	6	18.0^{a}	58.5^{de}	4.23^{fg}
P-value				
Treatment		0.099	0.001	0.001
Level		0.001	0.001	0.001
Treatment x Level		0.043	0.001	0.001

[1] The activity of the glycerol kinase was expressed in U, where U is defined as the quantity of enzyme that generates 1 lmol of glycerol per minute of reaction in a solution with pH = 8.0 at 25 °C.

CAPÍTULO IV

4.0 CONSIDERAÇÕES FINAIS

Na produção animal, a nutrição é o que mais onera custo dentro do ciclo de produção e grande parte desse custo é ocasionado por alimentos ou ingredientes que fornecem energia. Diante desse fato, muitas são as pesquisas desenvolvidas de forma a viabilizar e esclarecer a utilização de subprodutos de fontes energéticas hoje consumida na cadeia de produção animal, como por exemplo, os subprodutos resultantes do processamento industrial. No entanto um dos principais problemas na viabilidade de utilização desses produtos é a falta de padronização destas novas matérias-primas, para que dessa forma os nutricionistas tenham confiabilidade na hora de formular a dieta para que essa seja nutricionalmente eficaz e economicamente eficiente.

Desta forma com a realização desse estudo foram calculados os valores de energia metabolizável aparente (EMA) dos subprodutos do processamento do óleo de soja e do biodiesel assim como a suas misturas. Na indústria de frangos de corte, existe hoje uma boa perspectiva na utilização do glicerol (GLI) e do óleo ácido de soja (OAS) como ingredientes na formulação de rações para animais, além de seu valor energético, seu valor comercial os torna cada vez desejável.

Com os resultados obtidos, foi observado que os valores de energia metabolizável para o óleo ácido de soja (OAS), lecitina (LEC) e glicerol (GLI) estão entre os valores encontrado na literatura, além disso a energia da mistura (MIS) 80% OAS, 15% GLI e 5% LEC foi superior à energia de cada uma das fontes utilizadas de forma individual. Através de avaliações de triglicerídeos e glicerol plasmático e atividade da enzima glicerol quinase, que é responsável pela metabolização do glicerol, contatou-se que essa mistura não interfere no metabolismo normal do animal não comprometendo seu desempenho.

Sendo assim estes resultados indicam que a MIS desses subprodutos além de gerar valor agregado a estes produtos pode ser também utilizado como fonte de energia na alimentação de frangos de corte. Todavia, existem algumas diferenças nos valores de energia destes subprodutos relacionadas principalmente com os processos industriais de refino durante a obtenção do óleo ácido e do biodiesel, além disso, a idade das aves deve ser considerada, pois, podem influenciar na diferença de valores. No caso do GLI, é fundamental que seja conhecido o percentual de glicerol, metanol e as quantidades de sais de sódio e potássio, presente nesse subproduto, pois esses parâmetros devem ser considerados na formulação para não trazer consequências para a saúde e desempenho dos animais.

Diante dos resultados desse estudo é possível sugerir a utilização desses subprodutos OAS, LEC, GLI e MIS na formulação de rações para frangos. No entanto, novos estudos com relação as MIS devem ser conduzidos levando em consideração a homogeneização e a estabilidade da mesma em condições industriais, pois, sua utilização em tanques de armazenagem sem aquecimento e agitação poderá ocasionar uma composição variável.

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6.0 Vita

Liliane Borsatti filha de Lenir Farias dos Santos e Ildo Borsatti (*in memoriam*), nasceu em Sorriso, MT, em 30 de julho de 1985. Cursou o ensino fundamental na Escola Municipal Estrelinha na cidade de Guarantã do Norte. Concluiu o ensino médio na Escola Estadual Premem na cidade de Pato Branco, Paraná. Em 2004 ingressou no Curso de Zootecnia da Universidade Estadual do Oeste do Paraná em Marechal Cândido Rondon, PR, obtendo o Grau de Zootecnista em dezembro de 2008. Em março de 2010 iniciou o curso de mestrado na área de Nutrição Animal, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual do Oeste do Paraná, sob a orientação do professor Ricardo Vianna Nunes desenvolvendo trabalho de dissertação sobre utilização de promotores de crescimento em ração de frangos de corte. Obteve o título de mestre em Zootecnia em fevereiro de 2012. Neste mesmo ano ingressou no Doutorado em Produção Animal pelo Programa de Pós-Graduação em Zootecnia pela Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, desenvolvendo o trabalho de tese sobre utilização de subprodutos da indústria do óleo de soja e biodiesel e suas misturas em diferentes proporções na alimentação de frangos de corte. Realizou pesquisas durante 12 meses no Departamento de Poultry Science na North Carolina State University, EUA, através do programa doutorado-sanduíche da CAPES, no período de setembro de 2014 a setembro de 2015, trabalhando com temperatura e umidade na incubação de frangos de corte. Foi submetido à banca de defesa de Tese em março de 2016 pela Universidade Federal do Rio Grande do Sul em Porto Alegre, RS.

7.0 APÊNDICES

Apêndice 1 - Instruções para publicação na revista Poultry Science

POULTRY SCIENCE INSTRUCTIONS TO AUTHORS

Editorial Policies and Procedures

Poultry Science publishes the results of fundamental and applied research concerning poultry, poultry products, and avian species in general. Submitted manuscripts shall provide new facts or confirmatory data. Papers dealing with experimental design, teaching, extension endeavors, or those of historical or biographical interest may also be appropriate. A limited number of review papers will be considered for publication if they contribute significant additional knowledge, or synthesis of knowledge, to a subject area. Papers that have been, or are scheduled to be, published elsewhere will not be accepted. Publication of a preliminary report, such as an abstract, does not preclude consideration of a complete report for publication as long as it has not been published in full in a proceedings or similar scientific publication; appropriate identification of previously published preliminary reports should be provided in a title page footnote. Translation of an article into other languages for publication requires approval by the editor-in-chief. Opinions or views expressed in papers published by *Poultry Science* are those of the author(s) and do not necessarily represent the opinion of the Poultry Science Association or the editor-in-chief.

Contact Information for Journal Staff

For information on the scientific content of the journal, contact the editor-in-chief, Dr. Tom Porter, Department of Animal and Avian Sciences, University of Maryland, College Park, Building 142, College Park, MD 20742; e-mail: ps-editor@umd.edu.

For assistance with Scholar One Manuscripts, manuscript submission, supplemental files, copyright forms, or other information, contact Nes Diaz, Oxford University Press, 198 Madison Ave., New York, NY 10016 (nes.diaz@oup.com).

Care and Use of Animals

Authors must make it clear that experiments were con- ducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and lab- oratory techniques. Experiments shall be conducted in accordance with the principles and specific guidelines presented in *Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 3rd edition, 2010 (Association Headquarters, Champaign, IL 61820); and, if applicable, *Guide for the Care and Use of Laboratory Animals* (United States Department of Human Health and Services, National Institutes of Health, Publication Number ISBN 0-309-05377-3, 1996); or *Guide to the Care and Use of Experimental Animals,* 2nd ed. Volume 1, 1993 (Canadian Council on Animal Care). Methods of killing experimental animals must be described in the text. In describing surgical procedures, the type and dosage of the anesthetic agent must be specified. Intra-

abdominal and intrathoracic invasive surgery requires anesthesia. This includes caponization. The editor-in-chief of *Poultry Science* may refuse to publish manuscripts that are not compatible with these guides. If rejected solely on that basis, however, the paper may be resubmitted for reconsideration when accompanied by a written verification that a committee on animal care in research has approved the experimental design and procedures involved.

Types of Articles

Full-Length Articles. The majority of papers published in *Poultry Science* are full-length articles. The journal emphasizes the importance of good scientific writing and clarity in presentation of the concepts, apparatus, and sufficient background information that would be required for thorough understanding by scientists in other disciplines. One of the hallmarks for experimental evidence is repeatability. The results of experiments published in *Poultry Science* must be replicated, either by replicating treatments within experiments or by repeating experiments. Care should be taken to ensure that experiments are adequately replicated.

Research Notes. Research Notes are short notes giving the results of complete experiments but are less comprehensive than full-length articles. Preliminary or progress reports will not be accepted. The running head shall be “RESEARCH NOTE.” Research Notes will be published as a subsection of the scientific section in which they were reviewed. Research Notes are limited to five printed pages including tables and figures. Manuscripts should be prepared according to the guidelines for full- length articles.

Symposium Papers. The symposium organizer or chair must present the proposal and tentative budget to the Board of Directors at the summer meeting one full year before the symposium is to be scheduled. The symposium chair must then develop detailed symposium plans, including a formal outline of the talks approved and full budgetary expectations, which must be brought to the Board of Directors at the January meeting prior to the meeting at which the symposium is scheduled. The symposium chair must decide whether the symposium is to be published and will inform the editor-in-chief of this decision at the January meeting. If the decision is not to publish the symposium, the individual authors retain the right to submit their papers for consideration for the journal as ordinary manuscripts. If publication is decided upon, all manuscript style and form, guidelines of the journal shall be followed. Manuscripts must be prepared electronically, including figures and tables, and then uploaded onto the *Poultry Science* Manuscript Central site within 2 weeks after the annual meeting. The symposium chair will review the papers and, if necessary, return them to the authors for revision. The symposium chair then forwards the re- vised manuscript to the editor-in-chief for final review. Final revisions by the author and recommendations for acceptance or rejection by the chair must be completed by December 31 of the year in which the symposium was presented. Manuscripts not meeting this deadline will not be included in the published symposium proceedings. Symposium papers must be prepared in accordance with the guidelines for full-length articles and are subject to review. Offprints and costs of pages are the responsibility of the author.

Invited Papers. Invited papers, such as the World's Poultry Science Association lecture, should be submitted online; the editorial office will then make these papers available to the editor-in-chief. These papers are subject to review, and all manuscript style and form guidelines of the journal shall be followed. Invited papers are exempt from page charges but not offprint charges.

Review Papers. Review papers are accepted only if they provide new knowledge or a high-caliber synthesis of important knowledge. Reviews are not exempt from pages charges. All *Poultry Science* guidelines for style and form apply.

Invited Reviews. Invited Reviews will be approximately 10 published pages and in review format. The editor-in-chief will send invitations to the authors and then review these contributions when they are submitted. Nominations or suggestions for potential timely reviews are welcomed and should be sent directly to the editor- in-chief.

Contemporary Issues. Contemporary Issues in *Poultry Science* will address critical issues facing poultry scientists and the poultry industry. As such, submissions to this section should be of interest to any poultry scientist, to the industry, to instructors and faculty teaching contemporary issues classes, and to undergraduate and graduate students. The section will consist of short papers (approximately 2 published pages) written in essay format and will include an abstract, appropriate subheadings, and references.

Rapid Communications. We aim for receipt to decision times of a month or less, and accepted papers will have priority for publication in the next available issue of *Poultry Science.* These papers will present informative and significant new findings, such as tissue-specific gene expression profile data with full-length cDNA and genomic gene structure characterization. These papers will be short (2 to 4 published pages), adhere to journal format, and include references and an abstract. Rapid Communications should **not** be preliminary reports or incomplete studies. Authors will select Rapid Communications as the paper type when submitting the paper.

Book Reviews. *Poultry Science* publishes reviews of books considered to be of interest to the readers. The editor-in-chief ordinarily solicits reviews. Unsolicited reviews must be sent directly to the editor-in-chief for approval. Book reviews shall be prepared in accordance to the style and form requirements of the journal, and they are subject to editorial revision. No page charges will be assessed.

Letters to the Editor. The purpose of letters will be to discuss, critique, or expand on scientific points made in articles recently published in *Poultry Science*. Introduction of unpublished data will not be allowed, nor will material based on conjecture or speculation. Letters must be received within 6 months of an article's publication. Letters will be limited to 400 words and 5 references (approximately 3 double-spaced, typed pages including references). Letters shall have a title. Author name(s) and affiliation(s) shall be placed between the end of the text and list of references. Letters will be sent electronically directly to the editor-in-chief for consideration. The author(s) of the original paper(s) will be provided a copy of the letter and offered the opportunity to submit for consideration a reply within 30 days. Replies will have the same page restrictions and format as letters, and the titles shall end with "—Reply." Letters and replies will be published together. Acceptability of letters will be decided by the editor-in-chief. Letters and replies shall follow appropriate *Poultry Science* format and may be edited by the editor-

in-chief and a technical editor. If multiple letters on the same topic are received, a representative letter concerning a specific article will be published. All letters may not be published. Letters and replies will be published as space permits.

SUBMISSION OF ELECTRONIC MANUSCRIPTS

Authors should submit their papers electronically (http://mc.manuscriptcentral.com/ps). Detailed instructions for submitting electronically are provided online at that site. Authors who are unable to submit electronically should contact the editorial office (nes.diaz@oup.com) for assistance.

Copyright Agreement

Authors shall complete the Manuscript Submission and Copyright Transfer form for each new manuscript submission; faxed copies are acceptable. The form is published in *Poultry Science* as space permits and is avail- able online (http://ps.oxfordjournals.org). The copyright agreement is included in the Manuscript Submission and Copyright Transfer Form and must be completed by all authors before publication can proceed. The corresponding author is responsible for obtaining the signatures of coauthors. Persons unable to sign copyright agreements, such as federal employees, must indicate the reason for exemption on the form.

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REVIEW OF MANUSCRIPTS

After a manuscript is submitted electronically, the editorial office checks the manuscript. If a manuscript does not conform to the format for *Poultry Science*, it will be returned to the author (rejected) without review. Manu- scripts that pass initial screening will be forwarded to the appropriate section editor, who pre-reviews the manuscript and may suggest rejection at this early stage for fatal design flaw, inappropriate replications, lack of novelty, deviation from the Instructions for Authors, or other major concerns.

The section editor assigns two reviewers, at least one of whom is an associate editor. Each reviewer has 3 weeks to review the manuscript, after which his or her comments are forwarded to the section editor. The section editor may recommend rejection or acceptance at this point, after which the manuscript and reviewer comments are made available to the editor-in-chief for a final decision.

More commonly, the manuscript will be sent back to the corresponding author for revision according to the guidelines of the reviewers. Authors have 6 weeks to complete the revision, which shall be returned to the section editor. Failure to return the manuscript within 6 weeks will cause the paper to be purged from the files. Purged manuscripts may be reconsidered, but they will have to be processed as new manuscripts. Section editors handle all initial correspondence with authors during the review process. The editor-in-chief will notify the author of the final decision to accept or reject. Rejected manuscripts can be resubmitted only with an invitation from the section editor or editor-in-chief. Revised versions of previously rejected manuscripts are treated as new sub- missions. Therefore, authors must complete a new Manu- script Submission and Copyright Transfer Form.

PRODUCTION OF PROOFS

Accepted manuscripts are forwarded by the editor-in- chief to the editorial office for technical editing and type- setting. At this point the technical editor may contact the authors for missing information or figure revisions. The manuscript is then typeset, figures reproduced, and author proofs prepared.

Proofs

Author proofs of all manuscripts will be provided to the corresponding author. Author proofs should be read care- fully and checked against the typed manuscript, because the responsibility for proofreading is with the author(s). Corrections may be returned by fax (217-378-4083), mail, or e-mail. For faxed or mailed corrections, changes to the proof should be made neatly and clearly in the margins of the proof. If extensive editing is required, corrections should be provided on a separate sheet of paper with a symbol indicating location on the proof. Changes sent by e-mail to the technical editor must indicate page, column, and line numbers for each correction to be made on the proof. Corrections can also be marked using the note and highlight tools to indicate necessary changes. Author alterations to copy exceeding 10% of the cost of composition will be charged to the author.

Editor queries should be answered on the galley proofs; failure to do so may delay publication. Proof corrections should be made and returned to the technical editor within 48 hours of receipt. The publication charge form should be returned with proof corrections so as not to delay publication of the article.

Publication Charges and Offprints

Poultry Science has two options available for the publication of articles: conventional page charges and Open Access (**OA**).

OA. For authors who wish to publish their papers OA (available to everyone when the issue is posted online), authors will pay the OA fee when proofs are returned to the editorial office. Charges for OA are $1,500 if at least one author is a current professional member of PSA; the charge is $2,000 when no author is a professional member of PSA.

Conventional Page Charges. The current charge for publication is $100 per printed page (or fraction thereof) in the journal if at least one author is a

professional member of PSA. If no author is a member of PSA, the publication charge is $170 per journal page.

Offprints. Offprints may be ordered at an additional charge. When the galley proof is sent, the author is asked to complete an offprint order requesting the number of offprints desired and the name of the institution, agency, or individual responsible for publication charges.

Color Charges. The cost to publish in color in the print journal is $600 per color image; a surcharge for off- prints will also be assessed. At the time of submission on Scholar One Manuscripts, authors will be asked to approve color charges for figures that they wish to have published in color in the print journal. Color versions of figures will be included in the online PDF and full-text article at no charge.

MANUSCRIPT PREPARATION: STYLE AND FORM

General

Papers must be written in English. The text and all sup- porting materials must use American spelling and usage as given in *The American Heritage Dictionary, Webster's Third New International Dictionary,* or the *Oxford American English Dictionary.* Authors should follow the style and form recommended in *Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers.* 2006. 7th ed. Style Manual Committee, Council of Science Editors, Reston, VA.

Authors should prepare their manuscripts with Microbold face and italic. Text that follows a first subheading should be in a new paragraph.

Second Subheadings. Second subheadings begin the first line of a paragraph. They are indented, boldface, italic, and followed by a period. The first letter of each important word should be capitalized. The text follows immediately after the final period of the subheading.

Title Page

The title page shall begin with a running head (short title) of not more than 45 characters. The running head is centered, is in all capital letters, and shall appear on the top of the title page. No abbreviations should be used.

The title of the paper must be in boldface; the first letter of the article title and proper names are capitalized, and the remainder of the title is lowercase. The title must not have abbreviations.

Under the title, names of authors should be typed (first name or initial, middle initial, last name). Affiliations will be footnoted using the following symbols:

*, †, ‡, §, #, ‖, and be placed below the author names. Do not give authors' titles, positions, or degrees. Numbered footnotes may be used to provide supplementary information, such as present address, acknowledgment of grants, and experiment station or journal series number. The corresponding author should be indicated with 1 soft Word and upload them using the fewest files pos a numbered footnote (e.g., Corresponding author: my sible to facilitate the review and editing process.

Authors whose primary language is not English are strongly encouraged to use an English-language service to facilitate the preparation of their manuscript. A partial list of services can be found in the *Poultry Science* Manuscript checklist.

Preparing the Manuscript File

Manuscripts should be typed double-spaced, with lines and pages numbered consecutively, using Times New Roman font at 12 points. All special characters (e.g., Greek, math, symbols) should be inserted using the symbols palette available in this font. Complex math should be entered using Math Type from Design Science (http:// www.dessci.com). Tables and figures should be placed in separate sections at the end of the manuscript (not placed within the text). Failure to follow these instructions may result in an immediate rejection of the manuscript.

Headings

Major Headings. Major headings are centered (except ABSTRACT), all capitals, boldface, and consist of ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION (or RESULTS AND DISCUSSION), ACKNOWLEDGMENTS (optional), AP- PENDIX (optional), and REFERENCES.

First Subheadings. First subheadings are placed on a separate line, begin at the left margin, the first letter of all important words is capitalized, and the headings are name@university.edu). Note that there is no period after the corresponding author's e-mail address.

The title page shall include the name and full address of the corresponding author. Telephone and FAX numbers and e-mail address must also be provided. The title page must indicate the appropriate scientific section for the paper (i.e., Education and Production; Environment, Well-Being, and Behavior; Genetics; Immunology, Health, and Disease; Metabolism and Nutrition; Molecular, Cellular, and Developmental Biology; Physiology, Endocrinology, and Reproduction; or Processing, Products, and Food Safety).

Authors may create a full title page as a one-page document, in a file separate from the rest of the paper. This file can be uploaded and marked "not for review." Authors who choose to upload manuscripts with a full title page at the beginning will have their papers forwarded to reviewers as is.

Abbreviations

Author-derived abbreviations should be defined at first use in the abstract and again in the body of the manuscript. The abbreviation will be shown in bold type at first use in the body of the manuscript. Refer to the Miscellaneous Usage Notes for more information on abbreviations.

Abstract

The Abstract disseminates scientific information through abstracting journals and through convenience for the readers. The Abstract, consisting of not more than 325 words, appears at the beginning of the manuscript with the word ABSTRACT without a following period. It must summarize the major objectives, methods, results, conclusions, and practical applications of the research. The Abstract must consist of complete sentences and use of abbreviations should be limited. References to other work and footnotes are not permitted. The Abstract and Key Words must be on a separate sheet of paper.

Key Words

The Abstract shall be followed by a maximum of five key words or phrases to be used for subject indexing. These should include important words from the title and the running head and should be singular, not plural, terms (e.g., broiler, not broilers). Key words should be formatted as follows: **Key words:** . . .

Introduction

The Introduction, while brief, should provide the reader with information necessary for understanding research presented in the paper. Previous work on the topic should be summarized, and the objectives of the current research must be clearly stated.

Materials and Methods

All sources of products, equipment, and chemicals used in the experiments must be specified parenthetically at first mention in text, tables, and figures [i.e., (model 123, ABC Corp., Provo, UT)]. Model and catalog numbers should be included. Information shall include the full corporate name (including division, branch, or other subordinate part of the corporation, if applicable), city, and state (country if outside the United States), or Web address. Street addresses need not be given unless the reader would not be able to determine the full address for mailing purposes easily by consulting standard references.

Age, sex, breed, and strain or genetic stock of animals used in the experiments shall be specified. Animal care guidelines should be referenced if appropriate. Papers must contain analyzed values for those dietary ingredients that are crucial to the experiment. Papers dealing with the effects of feed additives or graded levels of a specific nutrient must give analyzed values for the relevant additive or nutrient in the diet(s). If products were used that contain different potentially active compounds, then analyzed values for these compounds must be given for the diet(s). Exceptions can only be made if appropriate methods are not available. In other papers, authors should state whether experimental diets meet or exceed the National Research Council (1994) requirements as appropriate. If not, crude protein and metabolizable energy levels should be stated. For layer diets, calcium and phosphorus contents should also be specified.

When describing the composition of diets and vitamin premixes, the concentration of vitamins A and E should be expressed as IU/kg on the basis of the following equivalents:

Vitamin A

1 IU = 0.3 µg of all-*trans* retinol
1 IU = 0.344 µg of retinyl acetate
1 IU = 0.552 µg of retinyl palmitate
1 IU = 0.60 µg of β-carotene

Vitamin E

1 IU = 1 mg of dl-α-tocopheryl acetate
1 IU = 0.91 mg of dl-α-tocopherol
1 IU = 0.67 mg of d-α-tocopherol

In the instance of vitamin D3, cholecalciferol is the acceptable term on the basis that 1 IU of vitamin D3 = 0.025

μg of cholecalciferol.

The sources of vitamins A and E must be specified in parentheses immediately following the stated concentrations.

Statistical Analysis. Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statistical methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical analysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments separately.

The experimental unit is the smallest unit to which an individual treatment is imposed. For group-fed animals, the group of animals in the pen is the experimental unit; therefore, groups must be replicated. Repeated chemical analyses of the same sample usually do not constitute independent experimental units. Measurements on the same experimental unit over time also are not independent and must not be considered as independent experimental units. For analysis of time effects, use time- sequence analysis.

Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to deviations from these assumptions, but occasionally data transformations or other techniques are helpful. For ex- ample, it is recommended that percentage data between 0 and 20 and between 80 and 100 be subjected to arc sin transformation prior to analysis. Most statistical procedures are based on the assumption that experimental units have been assigned to treatments at random. If animals are stratified by ancestry or weight or if some other initial measurement should be accounted for, the model should include a blocking factor, or the initial measurement should be included as a covariate.

A parameter [mean (μ), variance (σ2)], which defines or describes a population, is estimated by a statistic (x, s2). The term **parameter** is not appropriate to describe a variable, observation, trait, characteristic, or measurement taken in an experiment.

Standard designs are adequately described by name and size (e.g., “a randomized complete block design with 6 treatments in 5 blocks”). For a factorial set of treatments, an adequate description might be as follows: “Total sulfur amino acids at 0.70 or 0.80% of the diet and Lys at 1.10, 1.20, or 1.30% of the diet were used in a 2 × 3 factorial arrangement in 5 randomized complete blocks consisting of initial BW.” Note that **a factorial arrangement is not a de- sign**; the term “design” refers to the method of grouping experimental units into homogeneous groups or blocks (i.e., the way in which the randomization is restricted).

Standard deviation refers to the variability in a sample or a population. The standard error (calculated from error variance) is the estimated sampling error of

a statistic such as the sample mean. When a standard deviation or standard error is given, the number of degrees of freedom on which it rests should be specified. When any statistical value (as mean or difference of 2 means) is mentioned, its standard error or confidence limit should be given. The fact that differences are not “statistically significant” is no reason for omitting standard errors. They are of value when results from several experiments are combined in the future. They also are useful to the reader as measures of efficiency of experimental techniques. A value attached by “±” to a number implies that the second value is its standard error (not its standard deviation). Adequate re- porting may require only 1) the number of observations, 2) arithmetic treatment means, and 3) an estimate of experimental error. The pooled standard error of the mean is the preferred estimate of experimental error. Standard errors need not be presented separately for each mean unless the means are based on different numbers of observations or the heterogeneity of the error variance is to be emphasized. Presenting individual standard errors clutters the presentation and can mislead readers.

For more complex experiments, tables of subclass means and tables of analyses of variance or covariance may be included. When the analysis of variance contains several error terms, such as in split-plot and repeated measures designs, the text should indicate clearly which mean square was used for the denominator of each *F* statistic. Unbalanced factorial data can present special problems. Accordingly, it is well to state how the computing was done and how the parameters were estimated. Approximations should be accompanied by cautions concerning possible biases.

Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was de- signed; they should form the basis for comparing treatment means. No orthogonal contrasts may be evaluated by Bonferroni *t* statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally arranged. Fixed-range, pairwise, multiple-comparison tests should be used only to compare means of treatments that are unstructured or not related. Least squares means are the correct means to use for all data, but arithmetic means are identical to least squares means unless the design is unbalanced or contains missing values or an adjustment is being made for a covariate. In factorial treatment arrangements, means for main effects should be presented when important interactions are not present. However, means for individual treatment combinations also should be provided in table or text so that future researchers may combine data from several experiments to detect important interactions. An interaction may not be detected in a given experiment because of a limitation in the number of observations.

The terms significant and highly significant tradition- ally have been reserved for $P < 0.05$ and $P < 0.01$, respectively; however, reporting the *P*-value is preferred to the use of these terms. For example, use “. . . there was a difference ($P < 0.05$) between control and treated samples” rather than “. . . there was a significant ($P < 0.05$) difference between control and treated samples.” When available, the observed significance level (e.g., $P = 0.027$) should be presented rather than merely $P < 0.05$ or $P < 0.01$, thereby allowing the reader to decide what to reject. Other probability (α) levels may be discussed if properly

qualified so that the reader is not misled. Do not report *P*-values to more than 3 places after the decimal. Regardless of the probability level used, failure to reject a hypothesis should be based on the relative con- sequences of type I and II errors. A “nonsignificant” relationship should not be interpreted to suggest the absence of a relationship. An inadequate number of experimental units or insufficient control of variation limits the power to detect relationships. Avoid the ambiguous use of $P > 0.05$ to declare no significance, such as indicating that a difference is not significant at $P > 0.05$ and subsequently declaring another difference significant (or a tendency) at $P < 0.09$. In addition, readers may incorrectly interpret the use of $P > 0.05$ as the probability of a β error, not an α error.

Present only meaningful digits. A practical rule is to round values so that the change caused by rounding is less than one-tenth of the standard error. Such rounding increases the variance of the reported value by less than 1%, so that less than 1% of the relevant information contained in the data is sacrificed. Significant digits in data reported should be restricted to 3 beyond the decimal point, unless warranted by the use of specific methods.

Results and Discussion

Results and Discussion sections may be combined, or they may appear in separate sections. If separate, the Results section shall contain only the results and summary of the author’s experiments; there should be no literature comparisons. Those comparisons should appear in the Discussion section. Manuscripts reporting sequence data must have GenBank accession numbers prior to submit- ting. One of the hallmarks for experimental evidence is repeatability. Care should be taken to ensure that experiments are adequately replicated. The results of experiments must be replicated, either by replicating treatments within experiments or by repeating experiments.

Acknowledgments

An Acknowledgments section, if desired, shall follow the Discussion section. Acknowledgments of individuals should include affiliations but not titles, such as Dr., Mr., or Ms. Affiliations shall include institution, city, and state.

Appendix

A technical Appendix, if desired, shall follow the Discussion section or Acknowledgments, if present. The Appendix may contain supplementary material, explanations, and elaborations that are not essential to other major sections but are helpful to the reader. Novel computer programs or mathematical computations would be appropriate. The Appendix will not be a repository for raw data.

References

Citations in Text. In the body of the manuscript, refer to authors as follows: Smith and Jones (1992) or Smith and Jones (1990, 1992). If the sentence structure requires that the authors’ names be included in parentheses, the proper format is (Smith and Jones, 1982; Jones, 1988a,b; Jones et al., 1993). Where there are more than two authors of one article, the first author’s name is followed

by the abbreviation et al. More than one article listed in the same sentence of text must be in chronological order first, and alphabetical order for two publications in the same year. Work that has not been accepted for publication shall be listed in the text as: “J. E. Jones (institution, city, and state, personal communication).” The author’s own un- published work should be listed in the text as “(J. Smith, unpublished data).” Personal communications and un- published data must not be included in the References section.

References Section. To be listed in the References section, papers must be published or accepted for publication. Manuscripts submitted for publication can be cited as “personal communication” or “unpublished data” in the text.

Citation of abstracts, conference proceedings, and other works that have not been peer reviewed is strongly discouraged unless essential to the paper. Abstract and proceedings references are not appropriate citations in the Materials and Methods section of a paper.

In the References section, references shall first be listed alphabetically by author(s)’ last name(s), and then chronologically. The year of publication follows the authors’ names. As with text citations, two or more publications by the same author or set of authors in the same year shall be differentiated by adding lowercase letters

after the date. The dates for papers with the same first author that would be abbreviated in the text as et al., even though the second and subsequent authors differ, shall also be differentiated by letters. All authors’ names must appear in the Reference section. Journals shall be abbreviated according to the conventional ISO abbreviations given in journals database of the National Library of Medicine (http://www.ncbi.nlm.nih.gov/entrez/que- ry.fcgi?db=journals). One-word titles must be spelled out. Inclusive page numbers must be provided. Sample references are given below. Consult recent issues of *Poultry Science* for examples not included below.

Article:

Bagley, L. G., and V. L. Christensen. 1991. Hatchability and physiology of turkey embryos incubated at sea level with in- creased eggshell permeability. Poult. Sci. 70:1412–1418.

Bagley, L. G., V. L. Christensen, and R. P. Gilder sleeve. 1990.
Hematological indices of turkey embryos incubated at high
altitude as affected by oxygen and shell permeability. Poult.
Sci. 69:2035–2039.

Witter, R. L., and I. M. Gimeno. 2006. Susceptibility of adult chickens, with and without prior vaccination, to chal- lenge with Marek’s disease virus. Avian Dis. 50:354–365. doi:10.1637/7498-010306R.1

Book:

Metcalfe, J., M. K. Stock, and R. L. Ingermann. 1984. The effects of oxygen on growth and development of the chick embryo. Pages 205-219 in Respiration and Metabolism of Embryonic Vertebrates. R. S. Seymour, ed. Dr. W. Junk, Dordrecht, the Netherlands.

National Research Council. 1994. Nutrient Requirements of
Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Federal Register:

Department of Agriculture, Plant and Animal Health Inspection Service. 2004. Blood and tissue collection at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. Reg- ist. 69:10137–10151.
Other:
Choct, M., and R. J. Hughes. 1996. Long-chain hydrocarbons as a marker for digestibility studies in poultry. Proc. Aust. Poult. Sci. Symp. 8:186. (Abstr.)
Dyro, F. M. 2005. Arsenic. WebMD. Accessed Feb. 2006. http:// www.emedicine.com/neuro/topic20.htm.
El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, as- signee. US Pat. No. 6,766,767.
Hruby, M., J. C. Remus, and E. E. M. Pierson. 2004. Nutritional strategies to meet the challenge of feeding poultry without antibiotic growth promotants. Proc. 2nd Mid-Atlantic Nutr. Conf., Timonium, MD. Univ. Maryland, College Park.
Luzuriaga, D. A. 1999. Application of computer vision and elec- tronic nose technologies for quality assessment of color and odor of shrimp and salmon. PhD Diss. Univ. Florida, Gaines- ville.
Peak, S. D., and J. Brake. 2000. The influence of feeding program on broiler breeder male mortality. Poult. Sci. 79(Suppl. 1):2. (Abstr.)

Tables

Tables must be created using the MS Word table feature and inserted in the manuscript after the references section. When possible, tables should be organized to fit across the page without running broadside. Be aware of the dimensions of the printed page when planning tables (use of more than 15 columns will create layout problems). Place the table number and title on the same line above the table. The table title does not require a period. Do not use vertical lines and use few horizontal lines. Use of bold and italic typefaces in the table body should be done sparingly; such use must be defined in a footnote. Each table must be on a separate page. To facilitate placement of all tables into the manuscript file (just after the references) authors should use “section breaks” rather than “page breaks” at the end of the manuscript (before the tables) and between tables.

Units of measure for each variable must be indicated. Papers with several tables must use consistent format. All columns must have appropriate headings. Abbreviations not found on the inside front cover of the journal must be defined in each table and must match those used in the text. Footnotes to tables should be marked by superscript numbers. Each footnote should begin a new line.
Superscript letters shall be used for the separation of means in the body of the table and explanatory footnotes must be provided [i.e., “Means within a row lacking a common superscript differ ($P < 0.05$).”]; other significant *P*-values may be specified. Comparison of means within rows and columns should be indicated by different series of superscripts (e.g., a,b, . . . in rows; x–z . . . in columns) The first alphabetical letter in the series (e.g., a or A) shall be used to indicate the largest mean. Lowercase super- scripts indicate $P \leq 0.05$. Uppercase letters indicate $P \leq 0.01$ or less.

Probability values may be indicated as follows: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, and $\dagger P \leq 0.10$. Consult a recent issue of *Poultry Science* for examples of tables.

Figures

To facilitate review, figures should be placed at the end of the manuscript (separated by section breaks). Each figure should be placed on a separate page, and identified by the manuscript number and the figure number. A figure with multiple panels or parts should appear on one page (e.g., if Figure 1 has parts a, b, and c, place all of these on the same page). Figure captions should be typed (double spaced) on a separate page.

• ***Figure Size.*** Prepare figures at final size for publication. Figures should be prepared to fit one column (8.9 cm wide), 2 columns (14 cm wide), or full-page width (19 cm wide).

• ***Font Size.*** Ensure that all type within the figure and axis labels are readable at final publication size. A minimum type size of 8 points (after reduction) should be used.

• ***Fonts.*** Use Helvetica or Times New Roman. Symbols may be inserted using the Symbol palette in Times New Roman.

• ***Line Weight.*** For line graphs, use a minimum stroke weight of 1 point for all lines. If multiple lines are to be distinguished, use solid, long-dash, short-dash, and dotted lines. Avoid the use of color, gray, or shaded lines, as these will not reproduce well. Lines with different symbols for the data points may also be used to distinguish curves.

• ***Axis Labels.*** Each axis should have a description and a unit. Units may be separated from the descriptor by a comma or parentheses, and should be consistent within a manuscript.

• ***Shading and Fill Patterns.*** For bar charts, use different fill patterns if needed (e.g., black, white, gray, diagonal stripes). Avoid the use of multiple shades of gray, as they will not be easily distinguishable in print.

• ***Symbols.*** Identify curves and data points using the following symbols only: ■, ○, ●, ▲, ▼, n, e, r, +, or ×. Symbols should be defined in a key on the figure if possible.

• ***File Formats.*** Figures can be submitted in Word, PDF, EPS, TIFF, and JPEG. Avoid PowerPoint files and other formats. For the best printed quality, line art should be prepared at 600 ppi. Grayscale and color images and photomicrographs should be at least 300 ppi.

• ***Grayscale Figures.*** If figures are to be reproduced in grayscale (black and white), submit in grayscale. Often color will mask contrast problems that are apparent only when the figure is reproduced in gray- scale.

• ***Color Figures.*** If figures are to appear in color in the print journal, files must be submitted in CMYK color (not RGB).

• ***Photomicrographs.*** Photomicrographs must have their unmagnified size designated, either in the caption or with a scale bar on the figure. Reduction for publication can make a magnification power designation (e.g., 100×) inappropriate.

• ***Caption.*** The caption should provide sufficient in- formation that the figure can be understood with excessive reference to the text. All author-derived abbreviations used in the figure should be defined in the caption.

• ***General Tips.*** Avoid the use of three-dimensional bar charts, unless essential to the presentation of the data. Use the simplest shading scheme possible to

present the data clearly. Ensure that data, symbols, axis labels, lines, and key are clear and easily read- able at final publication size.

Color Figures. Submitted color images should be at least 300 ppi. The cost to publish each color figure is $600; a surcharge for color reprints ordered will be assessed. Authors must agree in writing to bear the costs of color production after acceptance and prior to publication of the paper.

Miscellaneous Usage Notes

Abbreviations. Abbreviations shall not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by abbreviation (e.g., IgG, CD). A helpful criterion for use of abbreviation is whether it has been accepted into thesauri and indexes widely used for searching major bibliographic databases in the scientific field. Abbreviations may be used in heads within the paper, if they have been first defined within the text. The inside back cover of every issue of the journal lists abbreviations that can be used without definition. The list is subject to revision at any time, so authors should always consult the most recent issue of the journal for relevant information. Abbreviations are allowed when they help the flow of the manuscript; however, excessive use of abbreviations can confuse the reader.

The suitability of abbreviations will be evaluated by the reviewers and editors during the review process and by the technical editor during editing. As a rule, author-derived abbreviations should be in all capital letters. Terms used less than three times must be spelled out in full rather than abbreviated. All terms are to be spelled out in full with the abbreviation following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbreviations shall be used consistently thereafter, rather than the full term.

The abstract, text, each table, and each figure must be understood independently of each other. Therefore, abbreviations shall be defined within each of these units of the manuscript.

EST expressed sequence tag g gram
g gravity
G guanine
GAT glutamic acid-alanine-tyrosine
G:F gain-to-feed ratio
GLM general linear model
h hour
HEPES *N*-2-hydroxyethyl piperazine-*N*′-ethane-sulfonic acid
HPLC high-performance (high-pressure) liquid chromatography
ICU international chick units
Ig immunoglobulin
IL interleukin
IU international units
kb kilobase pairs
kDa kilodalton
L liter*
L:D hours light:hours darkness in a photoperiod (e.g., 23L:1D)
m meter
µ micro

M molar
MAS marker-assisted selection
ME metabolizable energy
MEn nitrogen-corrected metabolizable energy
MHC major histocompatibility complex
mRNA messenger ribonucleic acid
min minute
mo month
MS mean square
n number of observations
N normal
NAD nicotinamide adenine dinucleotide
NADH reduced nicotinamide adenine dinucleotide
NRC National Research Council
NS not significant
PAGE polyacrylamide gel electrophoresis
PBS phosphate-buffered saline
PCR polymerase chain reaction
pfu plaque-forming units
QTL quantitative trait loci
r correlation coefficient
r2 coefficient of determination, simple 2
R coefficient of determination, multiple

Plural abbreviations do not require “s.” Chemical symbols and three-letter abbreviations for amino acids do not need definition. Units of measure, except those in the standard *Poultry Science* abbreviation list, should be abbreviated as listed in the *CRC Handbook for Chemistry and Physics* (CRC Press, 2000 Corporate Blvd., Boca Raton, FL 33431) and do not need to be defined.

The following abbreviations may be used without definition in *Poultry Science.*

A adenine
ADG average daily gain
ADFI average daily feed intake
AME apparent metabolizable energy
AMEn nitrogen-corrected apparent metabolizable energy
ANOVA analysis of variance
B cell bursal-derived, bursal-equivalent derived cell
bp base pairs
BSA bovine serum albumin
BW body weight
C cytosine
cDNA complementary DNA
cfu colony-forming units
CI confidence interval
CP crude protein
cpm counts per minute

CV coefficient of variation
d day
df degrees of freedom
DM dry matter
DNA deoxyribonucleic acid
EDTA ethylenediaminetetraacetate
ELISA enzyme-linked immunosorbent antibody assay
RFLP restriction fragment length polymorphism
RH relative humidity
RIA radioimmunoassay
RNA ribonucleic acid
rpm revolutions per minute
s second
SD standard deviation
SDS sodium dodecyl sulfate
SE standard error
SEM standard error of the mean
SRBC sheep red blood cells
SNP single nucleotide polymorphism
T thymine
TBA thiobarbituric acid
T cell thymic-derived cell
TME true metabolizable energy
TMEn nitrogen-corrected true metabolizable energy
Tris tris(hydroxymethyl)aminomethane
TSAA total sulfur amino acids
U uridine
USDA United States Department of Agriculture
UV ultraviolet
vol/vol volume to volume
vs. versus
wt/vol weight to volume
wt/wt weight to weight
wk week
yr year
*Also capitalized with any combination, e.g., mL.

International Words and Phrases. Non-English words in common usage (defined in recent editions of standard dictionaries) will not appear in italics (e.g., in vitro, in vivo, in situ, a priori). However, genus and species of plants, animals, or bacteria and viruses should be italicized. Authors must indicate accent marks and other diacritical on international names and institutions. German nouns shall begin with capital letters.

Capitalization. Breed and variety names are to be capitalized (e.g., Single Comb White Leghorn).

Number Style. Numbers less than 1 shall be written with preceding zeros (e.g., 0.75). All numbers shall be written as digits. Measures must be in the metric system; however, US equivalents may be given in parentheses. *Poultry Science* requires that measures of energy be given in calories rather than joules, but the

equivalent in joules may be shown in parentheses or in a footnote to tables. Units of measure not preceded by numbers must be writ- ten out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically. Measures of variation must be defined in the Abstract and in the body of the paper at first use. Units of measure for feed conversion or feed efficiency shall be provided (i.e., g:g).

Nucleotide Sequences. Nucleotide sequence data must relate to poultry or poultry pathogens and must complement biological data published in the same or a companion paper. If sequences are excessively long, it is suggested that the most relevant sections of the data be published in *Poultry Science* and the remaining sequences be submitted to one of the sequence databases. Acceptance for publication is contingent on the submission of sequence data to one of the databases. The following statement should appear as a footnote to the title on the title page of the manuscript. “The nucleotide sequence data reported in this paper have been submitted to GenBank Submission (Mail Stop K710, Los Alamos National Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN.”

Publication of the description of molecular clones is assumed by the editors to place them in the public sector. Therefore, they shall be made available to other scientists for research purposes.

Nucleotide sequences must be submitted as camera- ready figures no larger than 21.6 × 27.9 cm in standard (portrait) orientation. Abbreviations should follow *Poultry Science* guidelines.

Gene and Protein Nomenclature. Authors are required to use only approved gene and protein names and symbols. For poultry, full gene names should not be italicized. Gene symbols should be in uppercase letters and should be in italics. A protein symbol should be in the same format as its gee except the protein symbol should not be in italics.

General Usage. Note that “and/or” is not permitted; choose the more appropriate meaning or use “x or y or both.”

Use the slant line only when it means “per” with numbered units of measure or “divided by” in equations. Use only one slant line in a given expression (e.g., g/d per chick). The slant line may not be used to indicate ratios or mixtures.

Use “to” instead of a hyphen to indicate a range.

Insert spaces around all signs (except slant lines) of operation (=, –, +, ×, >, or <, etc.) when these signs occur between two items.

Items in a series should be separated by commas (e.g., a, b, and c).

Restrict the use of “while” and “since” to meanings related to time. Appropriate substitutes include “and,” “but,” or “whereas” for “while” and “because” or “although” for “since.”

Leading (initial) zeros should be used with numbers less than 1 (e.g., 0.01).

Commas should be used in numbers greater than 999.

Registered (®) and trademark (™) symbols should not be used, unless as part of an article title in the References section. Trademarked product names should be capitalized.

Supplemental Information

The following information is available online and up- dated regularly. Please refer to these pages when preparing a manuscript for submission.

Journal Title Abbreviations. A list of standard abbreviations for common journal titles is available online: http://www.oxfordjournals.org/our_journals/ps/for _authors/index.html

SI Units. The following site (National Institute of Standards and Technology) provides a comprehensive guide to SI units and usage: http://physics.nist.gov/Pubs/SP811/ contents.html