LEVY DO VALE TEIXEIRA

USO DE EMULSIFICANTE EM DIETAS PARA FRANGOS DE CORTE

LAVRAS – MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Não Ruminantes, para a obtenção do título de Doutor.

Prof. Dr. Antônio Gilberto Bertechini

Orientador

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USE OF EMULSIFIER IN BROILER CHICKENS DIETS

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APROVADA em 24 de março de 2017.

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RESUMO GERAL

Foram realizados 2 experimentos para avaliar os efeitos do aditivo emulsificante sobre a digestibilidade de nutrientes e energia metabolizável aparente, corrigida para balanço de nitrogênio (EMAn) em frangos de corte, recebendo dietas com diferentes níveis de inclusão de óleo de soja na fase inicial (14-21d) e final (35-42d) de criação das aves. Os experimentos 1 e 2 foram conduzidos utilizando-se dietas fareladas a base de milho, farelo de soja e/ou farinha de carne e ossos, com ou sem a inclusão de emulsificante (0.35g/kg of ração), e cinco níveis de óleo de soja (0; 15; 30; 45; 60g/kg). Um total de 600 frangos de corte macho, Cobb 500 na fase inicial, e 360 na fase final, foram distribuídos em gaiolas metabólicas em delineamento inteiramente casualizado (DIC), com 10 tratamentos e 6 repetições cada. O método de coleta total de excretas foi realizado para determinar EMAn e os coeficientes de digestibilidade aparente para matéria seca, proteína bruta e extrato etéreo. Os dados foram submetidos à ANOVA e ao teste de contraste entre os tratamentos. Nos experimentos 1 e 2, não houve efeito ($P>0.05$) do emulsificante sobre a proteína bruta nas duas idades avaliadas. De forma geral, em todos os ensaios metabólicos, quando se compara todos os tratamentos com e sem emulsificante, houve uma melhora significativa ($P<0.05$) na EMAn e nas digestibilidade do extrato etéreo e matéria seca, nos tratamentos suplementados com emulsificante. No experimento 2, utilizando-se dietas com farinha de carne e ossos, o emulsificante obteve melhor efeito ($P<0.05$) sobre o extrato etéreo em dietas formuladas com 30, 45 e 60g/kg de óleo de soja na fase final. O emulsificante teve efeito significativo ($P<0.05$) sobre a EMAn em dietas formuladas com 30, 45 e 60 g/kg de óleo de soja no experimento 1 e 2. Concluindo, o aditivo emulsificante pode melhorar a digestibilidade de nutrientes e a EMAn de dietas formuladas com 30, 45 e 60g/kg de óleo de soja.

Palavras-chave: Emulsificante. Digestibilidade de nutrients. Energia. Frangos de corte.

GENERAL ABSTRACT

Two experiments were carried out to evaluate the effects of an emulsifier-additive on nutrient digestibility and apparent metabolizable energy corrected for nitrogen (AMEn) to broilers chickens receiving diets with different levels of soybean oil in the starter (14-21d) and finisher (35-42d) phase of bird's age. The experiments 1 and 2 were conducted using mash corn/soybean and/or meat bone meal-based diets with or without inclusion of emulsifier (0.35g/kg of feed) and five levels of soybean oil (0; 15; 30; 45; 60g/kg). A total of 600 male broilers Cobb 500 in the starter and 360 broilers in the finisher phase were allocated (metabolic cages) in a complete randomized design (CRD) with 10 treatments and 6 reps each. Total excreta method was carried out to determine AMEn and coefficient of total tract apparent digestibility for dry matter, crude protein and ether extract. Data were submitted to ANOVA and contraste test among treatments. Experiments 1 and 2, the emulsifier had no effect (P>0.05) on crude protein in the different ages evaluated. Overall, in the all-metabolic trials, when all treatments with and without emulsifier were compared, there were significant (P<0.05) improvements on EMAn, ether extract and dry matter digestibility in the treatments supplemented with emulsifier. In the experiment 2, using diets based on meat bone meal, the emulsifier had a better effect (P<0.05) on ether extract in diets formulated 30, 45 and 60g/kg of soybean oil in the finisher phase. The emulsifier had significant effect (P<0.05) on AMEn in diets with 30, 45 and 60g/kg of soybean oil inclusion in the experiment 1 and 2. In conclusion, the emulsifier-additive can improve nutrient digestibility and AMEn in diets formulated with 30, 45 and 60g/kg of soybean oil.

Keywords: Emulsifier. Nutrient digestibility. Energy. Broilers.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

A energia é o principal componente em rações para monogástricos. O conteúdo de energia das dietas das aves tem grande importância na definição do desempenho e dos custos de produção de carne de frango de corte. Nos últimos anos, com a crescente alta nos custos dos alimentos energéticos, aumentou o interesse da indústria avícola em maximizar o uso de óleos e gorduras, que são importantes fontes de energia na formulação. Energia esta, que é utilizada pelos nutricionistas para aumentar a densidade energética e, assim, atingir as exigências de animais de alta performance (ROVERS, 2014).

Algumas pesquisas sugerem uma inclusão mínima de 1% de lipídeo em dietas para aves, no entanto, 2 a 5% de lipídeos são geralmente adicionados em dietas comerciais, dependendo dos preços da fonte de lipídeo e dos ingredientes utilizados (LEESON; SUMMERS, 2005). Uma estratégia para incrementar a utilização de óleos e gorduras, e reduzir os custos de rações, se baseia na utilização de um aditivo emulsificante.

O emulsificante dietético age, principalmente, aumentando a superfície de contato dos lipídeos, permitindo a ação da enzima lipase, no qual hidroliza as moléculas de trigliacilgliceróis em monoacilglicerol, para formação de micelas. Essas são as etapas essenciais para a absorção dos lipídeos, no qual irá criar um gradiente de difusão para aumentar a absorção de outros nutrientes (GUERREIRO NETO et al., 2011; MELEGY et al., 2010).

Os lipídeos são insolúveis em água e não solubilizam na fase aquosa do trato gastrointestinal, precisando ser emulsificados, antes de serem hidrolisados pela enzima lipase. A emulsificação depende das características do lipídeo, como por exemplo, o comprimento da cadeia carbônica, posição do ácido graxo no triacilglicerol e o grau de saturação do ácido graxo. O emulsificante é uma molécula anfipática, com propriedade hidrofílica e hidrofóbica, conferindo à molécula uma característica de formar ponte ligando água e substâncias lipídicas solúveis e, assim, melhorar a utilização de lipídeos pelos animais (RAVINDRAN et al., 2016).

Comparados a outros emulsificantes disponíveis no mercado, como por exemplo, as lecitinas e lisolecitinas, o emulsificante a base de gliceril polietilenoglicol ricinoleato mostrou ser mais hidrofílico e eficiente em dissolver ácidos graxos livres, os quais qual são largamente insolúveis em sais biliares, durante a digestão de lipídeos (DIERICK; DECUYPERES, 2004). Algumas pesquisas relatam melhor desempenho de aves (KACZMAREK et al., 2015; ROY et al., 2010) e suínos (UDOMPRASERT; RUKKWAMSUK, 2006), quando as dietas são suplementadas com emulsificante a base de gliceril polietilenoglicol ricinoleato.

Porém, o uso de emulsificante dietético em associação com óleos vegetais em dietas avícolas, não tem sido completamente explorado, no entanto, o interesse no uso de emulsificante tem aumentado na última década (BOMTEMPO, 2015).

Objetiva-se com esses experimentos, avaliar os efeitos de um aditivo emulsificante sobre o desempenho e a digestibilidade de nutrientes e energia metabolizável aparente, corrigida para balanço de nitrogênio, em dietas formuladas com diferentes níveis de inclusão de óleo de soja nas fases inicial e final de criação de frangos de corte.

2 REFERENCIAL TEÓRICO

2.1 Digestão e absorção de lipídeos pelas aves

Após a ingestão do lipídeo pela ave, o mesmo tem a sua estrutura física modificada por meio de trituração da moela, modificando o lipídeo presente em pequenas partículas, transformando-os em uma fina emulsão com grande aumento da área de superfície, colocando-as a disposição das enzimas responsáveis pela hidrólise. Os ácidos graxos e fosfolipídeos presentes estão parcialmente ionizados e agem como elementos anfifíticos (MACARI; FURLAN; GONZALES, 2008).

A emulsão das gorduras oriundas da dieta, ingressam no intestino delgado, encontrando um ambiente ligeiramente ácido (pH 5,8-6,0), estimulando a atuação da colecistocinina (CCK), a incrementar a produção e excreção da bílis pelo figado. A função da bile é emulsificar os lipídeos, aumentando a superfície dos mesmos com a formação de microgotículas de gordura (LUBISCO, 2007). A bile é composta por colesterol, fosfolipídeos, e sais biliares que correspondem a 97% dos seus componentes, e o restante, correspondem à bilirrubina e proteínas. A maior parte dos fosfolipídeos encontrados na bile corresponde à fosfatidilcolina ou lecitina (WANG; COHEN; CAREY, 2009). A lecitina e colesterol, conjuntamente aos sais biliares, atuam para aumentar a área superficial e melhorar a ação das lipases na interface lipídeo-água (MACARI; FURLAN; GONZALES, 2008).

A lipase e a colipase pancreáticas, ligam-se à superfície das partículas emulsificadas e hidrolisam as ligações éster de triacilglicerol. Os lipídeos emulsificados têm a parte polar dos sais biliares conjugados e lecitinas na superfície, enquanto que os triacilgliceróis ocupam a porção central da micela. Dessa forma, a água penetra apenas superficialmente na emulsão triglicerídica, e essa pequena área, ou interface lipídeo-água, cria um local único para a ação das lípases sobre as moléculas de triglicerídeos, e também da colipase, que auxilia nesse processo (MACARI; FURLAN; GONZALES, 2008).

O processo de formação da emulsão desencadeado pela bile, também recebe o auxílio de fontes externas de surfactantes naturais, como a própria fosfatidilcolina e a lisofosfatidilcolina. Estes surfactantes estão presentes nos sucos digestivos e no próprio alimento, e também são formados pela ação de enzimas específicas no lúmen intestinal. Neste caso, a enzima fosfolipase A_2 produzida pelo pâncreas, cataliza a hidrólise da ligação sn-2 do ácido graxo da fosfatidilcolina, formando ácidos graxos livres e lisofosfatidilcolina (DENNIS, 1994; SILVA JÚNIOR, 2009).

A absorção dos triacilgliceróis é dependente da formação de micelas mistas e do contínuo movimento de gotículas lipídicas para a micela, no lúmen intestinal. Na presença dos sais biliares, os ácidos graxos livres e monoacilgliceróis produzidos por ação da lipase pancreática, são agregados às micelas, enquanto que a lisolecitina produzida por fosfolipídeos bilares e dietéticos, tem papel chave na formação e estabilização de micelas (DRACKLEY, 2000). Outro fator importante para absorção lipídica é a característica do ácido graxo. Leeson (1995) reportou que os ácidos graxos insaturados são mais bem digeridos e absorvidos do que os saturados, especialmente em aves jovens, embora haja um sinergismo entre a mistura dessas gorduras. Isto implica em dizer que não basta ter o processo de digestão eficiente, pois dependendo do tipo de ácido graxo, a absorção pode ser diferenciada e, por consequência, o aproveitamento poderá ser comprometido.

A absorção dos ácidos graxos de cadeia curta e o glicerol livre solúvel em água, pode se dar diretamente nos enterócitos, porém, os ácidos graxos de cadeias longas e médias devem ser agregados em micelas, sob a influência de agentes anfipáticos, sendo representados principalmente pela bile e monoacilglicerois em menor proporção (KROGDAHL, 1985).

Presume-se que a maior parte da absorção do conteúdo micelar pelo enterócito, se dá através de difusão passiva. A difusão passiva é o movimento de compostos a partir do lúmem intestinal, através da membrana celular do enterócito para dentro da célula, a fim de igualar a concentração de substratos em ambos os lados da membrana. Ao contrário dos ácidos graxos simples, os ácidos graxos incorporados às micelas são capazes de criar um gradiente maior de difusão na parede intestinal (SOEDE, 2005). O íleo proximal é o local mais importante para absorção dos produtos finais das gorduras digeridas. Aparentemente, a quantidade de triacilglicerol digerido e absorvido pelas aves e mamíferos é semelhante, diferindo apenas nos processos de absorção. Nos mamíferos, o triacilglicerol é absorvido nos vasos quilíferos das vilosidades, enquanto que nas aves, os mesmos são absorvidos diretamente no sangue. As aves absorvem cerca de 80 a 95% dos ácidos graxos ingeridos, porém, os pintos recém-nascidos os absorvem com menos eficiência (SOUZA-SOARES; SIEWERDT, 2005).

A maior parte dos triacilgliceróis, molécula formada por três ácidos graxos ligados ao glicerol, tem que ser degradada a ácidos graxos para absorção através do epitélio intestinal. A enzima colesterol-esterase secretada pelo pâncreas, hidrolisa os ésteres de ácido graxo em colesterol e ácidos graxos livres. Já dentro do enterócito, os graxos livres e monoacilgliceróis são transformados novamente em triacilgliceróis (reesterificação) e são embalados em partículas de lipoproteínas de transporte, chamadas de quilomícrons, partículas estáveis, de aproximadamente 200nm de diâmetro, que também transportam vitaminas lipossolúveis e colesterol (FREEMAN, 1984).

Estas partículas são formadas por uma capa hidrofílica constituída de fosfolipídeos, colesterol livre e proteínas, envolvendo um núcleo hidrofóbico que contém triacilgliceróis e, que além da função estrutural, interage com receptores da membrana celular e/ou atuam como cofatores enzimáticos. São liberadas para o sistema linfático e daí para o sangue (BERG; TYMOCZKO; STRYER, 2008). Ao entrarem na corrente sanguínea, os quilomícrons sofrem a ação da enzima lipase lipoproteica que hidroliza os triacilgliceróis, retirando os ácidos graxos e reduzindo o tamanho da lipoproteína que recebe o nome de portomícrons. Os portomícrons são removidos da circulação por receptores localizados nas células hepáticas, sendo então metabolizados (FRASER et al., 1986).

Os portomícrons são lipoproteínas que transportam os lipídeos do trato gastrointestinal para o fígado via circulação hepática. Os portomícrons passam por transformação a nível hepático e são liberados como VLDL, a qual inclui tanto portomícrons modificados que contêm lipídeos exógenos, e lipoproteínas contendo lipídeos endógenos. A grande maioria dos lipídeos absorvidos entra na veia porta e é enviada diretamente para o fígado, onde o hepatócito tem a oportunidade para anabolizar ou catabolizar esses substratos (BENSADOUN; ROTHFELD, 1972; FRASER et al., 1986).

2.2 Modo de ação dos emulsificantes

Os emulsificantes podem melhorar a utilização dos triacilgliceróis, por meio do aumento da formação de gotículas de emulsão, estimulando a formação de micelas, aumentando assim, a concentração de monoacilgliceróis no intestino, facilitando o transporte de nutrientes por meio da membrana. Este processo leva a uma melhor absorção de nutrientes lipossolúveis e utilização de energia. Os emulsificantes também podem desempenhar função de superação das insuficiências de baixa produção de bile, principalmente em aves jovens (GUERREIRO NETO et al., 2011; MELEGY et al., 2010).

Os agentes emulsificantes têm como principal característica a sua natureza anfipálica, com a parte hidrofóbica (ou lipofílica) da molécula, preferindo um ambiente lipídico (não polar) e a parte hidrofílica em um ambiente aquoso (polar). A parte lipofílica, geralmente, é uma cadeia carbônica longa de ácidos graxos obtidos a partir de uma gordura ou óleo (SOEDE, 2005). Portanto, a qualidade do emulsificante dependerá do comprimento da cauda hidrofílica, que possui a característica de fazer o emulsificante mais solúvel meio aquoso intestinal, levando-o a ter contato com um grande número de partículas gordurosas, facilitando a digestão e a absorção das mesmas (ROVERS et al., 2014).

Ao escolher um emulsificante, o princípio do Balanço Hidrófílico-Lipofílico (BHL) é muito importante. O HLB fornece um valor de como o emulsificante é solúvel em água ou gordura. A escala varia de 0 a 20. Quanto mais baixo, o HLB, mais lipofílico ou solúvel em gordura o emulsionante se torna. Quanto maior o HLB, mais hidrossolúvel ou hidrofílico será o emulsificante. O objetivo da utilização de um emulsificante determina se um HLB baixo ou um HLB mais elevado é mais adequado. De maneira ideal, o emulsionante deve ser solúvel na fase contínua (regra de Bancroft). Quando uma pequena quantidade de água é misturada em um ambiente rico em gordura, um menor HLB é aconselhado (emulsificante lipossolúvel). Se uma pequena quantidade de gordura é misturada num ambiente aquoso, recomenda-se um emulsificante com um HLB mais elevado (emulsificante solúvel em água). No caso de um emulsificante nutricional, uma quantidade limitada de gordura é adicionada ao ambiente aquoso do intestino (RAVINDRAN, 2016; ROVERS, 2013, 2014).

É importante ressaltar, que a ave consome 1,5-2,0 vezes mais água do que ração, e a ração tem apenas certa quantidade de gordura, de modo que a quantidade de água é muito maior do que a quantidade de gordura no intestino, neste caso, um EHL elevado é o mais adequado (ROVERS, 2013; SOEDE, 2005).

2.3 Emulsificantes utilizados na nutrição de aves

Os emulsificantes são normalmente utilizados na indústria de alimentos e podem ser classificados em dois grupos: emulsificantes naturais e sintéticos. Emulsificantes naturais incluem aqueles produzidos pelo corpo do animal, tais como a bile e fosfolípideos, e aqueles a partir de alimentos, tais como lecitina de soja (SOARES; LOPEZ-BOTE, 2002). Emulsificantes sintéticos são lecitina e lisolecitina ou lisofosfatilcolina modificada (ZHANG et al., 2011), e o gliceril polietilenoglicol ricinoleato, derivado do óleo de rícino (ROY et al., 2010).

A bile é um fluido excretado pelo fígado, que desempenha função importante na digestão das gorduras. A bile é formada nos hepatócitos e, em seguida, transportada para o armazenamento na vesícula biliar. A bílis contém pigmentos, sais biliares, colesterol, eletrólitos e algumas proteínas (KROGDAHL, 1985). Os sais biliares atuam como emulsificante reduzindo a tensão da interação óleo-água e ativam a lipase pancreática, bem como impedem a desnaturação desta enzima quando deixa a superfície de gotículas de gordura emulsionadas. Os sais biliares presente na bílis são moléculas anfipáticas planas, com superfície hidrofóbica de um lado, que interage com a fase de óleo da emulsão e uma superfície hidrofílica sobre o outro, que interage com água (CHEN et al., 1975). A secreção de bile é limitada em aves jovens, especialmente durante a primeira semana de vida, o que resulta numa redução na digestão e absorção de gordura (KROGDAHL, 1985; NOY; SKLAN, 1995). Além disso, as aves jovens são incapazes de repor sais biliares como as aves mais velhas, e diminuição do tamanho do *pool* de sais biliares também podem contribuir para a má digestão e absorção de lipídeos em aves jovens.

A lecitina, um subproduto do processamento do óleo de soja, foi avaliada para melhorar a digestão de gordura em dietas para suínos e aves (OVERLAND et al., 1993; SOARES; LOPEZ-BOTE, 2002). A lecitina (um fosfolipídeo) é uma mistura de agentes ativos de superfície, constituídas por uma porção hidrofóbica com afinidade para as gorduras, e uma porção hidrofóbica com afinidade para água (GU; LI, 2003). É um subproduto da linha de produção do óleo de soja, sendo extraída do mesmo, pelo processo denominado de degomagem. Este processo consiste na extração dos fosfolipídeos pela adição de água à temperatura de 60 a 90 °C e pressão constante (CASTEJON; FINZER, 2007).

Os principais fosfolipídeos são: fosfatidilcolinas, etanolaminafosfatídeos e fosfatidilinositóis, sendo acompanhados em menores proporções por outros fosfolipídeos. As principais fontes de lecitinas comerciais são óleo de soja (3,2%), óleo de semente de canola (2,5%) e óleo de semente de girassol (1,5%), contendo as proporções de fosfolipídeos totais expressas entre parênteses (GUNSTONE, 2005).

A lecitina é uma excelente fonte de fosfatidilcolina, um fosfolipídeo surfactante natural (biosurfactante), que auxilia na emulsificação das gorduras, formação da micela e, consequente hidrólise lipídica. Antes de atuar na emulsificação, a fosfatidilcolina sofre a ação da enzima fosfolipase A_2 liberada pelo pâncreas e se transforma na lisofosfatidilcolina, que é a principal responsável pela manutenção da micela, hidrólise dos lipídios na micela e posterior absorção pelos enterócitos (MORGADO et al., 1995).

A lisolecitina de soja, rica em lisofosfatidilcolina pode ser produzida industrialmente a partir da lecitina de soja, por um processo patenteado com a enzima fosfolipase A_2 imobilizada. A adição de lisofosfatidilcolina, pelos seus efeitos positivos como componente fundamental da digestão, apresenta benefícios no desempenho técnico e econômico das dietas animais e permite a formulação de dietas de menor densidade nutricional, mais econômicas, sem perda de desempenho (SILVA JÚNIOR, 2009).

Os lisofosfolipídeos produzidos a partir da hidrólise enzimática atuam como intensificadores da absorção de lipídeos. A hidrólise ocorre na porção sn-2 e, industrialmente, é realizada pela fosfolipase A_2 (origem microbiana), porém, a mera hidrólise do fosfolipídeo não serve ao propósito de promotores de absorção, pois a hidrólise enzimática tem que ser padronizada para gerar quantidades consistentes de fosfolipídeos e lisofosfolipídeos no produto final (MORGADO et al., 1995). Estes lisofosfolipídeos têm atividades emulsificantes superiores aos fosfolipídeos devido à formação de micelas muito menores, que têm uma área de superfície maior, portanto, maior é o efeito da emulsificação. Além dessas características, a suplementação externa de lisofosfolipídeos aumenta a produção de lisolecitina pelo fígado/ducto biliar, aumentando ainda mais, a emulsificação e absorção de gorduras (SABIHA, 2009).

O emulsificante a base de gliceril polietilenoglicol ricinoleato é um éster de óxido de etileno e óleo de rícino, onde é composto de uma mistura complexa de 100 ou mais componentes de estrutura química similar: o polyol é a molécula inicial, seguida por uma cadeia média de unidades de polietilenoglicol e terminação em éster de ácido graxo. O precursor da molécula de ricinoleato é o óleo de mamona, sendo a composição dos ácidos graxos feita predominantemente de ácido rinoléico, no entanto, estão presentes pequenas quantidades de ácidos graxos saturados, bem como os ácidos oleico e linoleico (DIEHL, 2011).

O gliceril polietilenoglicol ricinoleato é de natureza predominante hidrofílica, e dissolve melhor na fase aquosa do intestino delgado, e se torna dissolvido por agitação mecânica no intestino, critério no qual o qualifica como emulsificante nutricional (ROVERS et al., 2014).

O uso de suplementação de bile ou emulsificantes naturais para melhorar a utilização de gordura, não é, atualmente, economicamente viável. Emulsificantes sintéticos são mais baratos e apenas alguns produtos comerciais estão disponíveis. Exemplos de produtos sintéticos são misturas de lecitina hidrolisada (Avilac E, Nutrifeed, Netherlands), lisofosfatidilcolina (LysoforteTM, Kemin Industries, Singapura) e Ricinoleato de gliceril-polietilenoglicol (Volamel extra, Nukamel Inc., Olen, Bélgica).

Zhang et al. (2011) avaliaram o efeito da lisofosfatidilcolina comercial (LysoforteTM) sobre o desempenho e EMA de frangos, recebendo dietas com três fontes de lipídeos (óleo de soja, sebo e gorduras de aves), em 30 g/kg em rações iniciais e em 40 g/kg em dietas de crescimento. Verificou-se que a suplementação de lisofosfatidilcolina aumentou o ganho de peso das aves alimentadas com as três fontes de lipídeos durante a fase inicial, mas não foram observadas diferenças durante a fase de crescimento. A suplementação de lisofosfatidilcolina incrementou numericamente a EMA durante a fase inicial e aumentou significamente a EMA durante a fase de crescimento, com a maior EMA sendo determinada para as aves alimentadas com dietas contendo gordura de aves mais emulsificante, comparadas com aquelas aves alimentadas com rações contendo óleo de soja ou sebo mais emulsificante (3110, 3088 e 3050 Kcal, respectivamente). O emulsificante a base de gliceril polietilenoglicol ricinoleato, foi testada por Roy et al. (2010). Estes pesquisadores forneceram dietas à base de milho e farelo de soja, que continham óleo de palma em nível de inclusão de 35 g/kg no período inicial, e de 28 g/kg durante o período de crescimento, suplementado com três níveis de gliceril polietilenoglicol ricinoleato (0, 10 e 20 g/kg). A adição de 10 g/kg de emulsificante resultou em maior peso vivo (5%) e melhor conversão alimentar em relação à adição de 0 e 20 g/kg. Foram observadas melhorias na digestibilidade de extrato etéreo, matéria seca, proteína bruta e energia, porém, não foram observado efeitos sobre o rendimento de carcaça e a digestibilidade de minerais (Fe, Zn Co, Mn).

Guerreiro Neto et al. (2011), avaliando o efeito da adição 50g/kg de um emulsificante com princípio ativo a base de lecitina de soja, em dietas contendo óleo de soja, gordura de aves e/ou suas misturas para frangos de corte, não observaram influência do emulsificante sobre os parâmetros de desempenho, rendimento de carcaça e perfil lipídico do sangue das aves aos 42 dias de idade, no entanto, propiciou aumento na digestibilidade do extrato etéreo da dieta quando usado com óleo de vísceras.

Attia et al. (2008) observaram que a suplementação de 3 e 6% de lecitina em rações de poedeiras aumentou a massa e peso do ovo. Nesse mesmo estudo, verificou-se que a suplementação de 6% de lecitina aumentou as unidades Haugh e os ovos apresentaram uma cor mais intensa. Isto é explicado pelo aumento na absorção e o transporte de lipídeos e, portanto, de substâncias lipossolúveis como os pigmentos.

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). Para Azman e Ciftci (2004), que avaliaram o efeito de diferentes fontes lipídicas com adição de lecitina de soja (1 e 2%) sobre o desempenho de frangos de corte, até os 35 dias de idade, o acréscimo de lecitina não apresentou diferença significativa entre as médias dos tratamentos que continham lecitina e o grupo controle (sem lecitina).

O uso de emulsificantes em aves e rações para suínos tem aumentado a absorção de lipídios, melhorado o desempenho, a eficiência alimentar, e também tem modificado os lipídios no sangue (UDOMPRASERT; RUKKWAMSUK, 2006). No entanto, outros autores relataram que a suplementação de emulsificantes aumenta a digestibilidade dos nutrientes, mas tem menos efeito sobre o desempenho e rendimentos de carcaça (JONES et al., 1992; XING et al., 2004).

2.4 Óleo de soja e de vísceras em dieta para frangos de corte

O uso de óleos e gorduras em dietas de frangos de corte como fonte de energia, tornou-se uma prática generalizada. Em termos de Brasil, a gordura de origem animal mais utilizada é a gordura de aves ou óleo de vísceras, enquanto que o óleo vegetal comumente utilizado, é o óleo de soja.

O óleo de vísceras de aves é resultante do tratamento que se dá aos coprodutos de abatedouros de aves na graxaria, onde o óleo é o produto resultante de tecidos adiposos das aves, extraído a partir de prensagem ou solvente após a cocção, filtrada ou não, contendo no mínimo 90% de ácidos graxos totais e no máximo 3% de impurezas e insaponificáveis (BELLAVER, 2001). Já o óleo de soja é obtido pela prensagem mecânica e/ou extração por solvente dos grãos de soja, isentos de misturas de outros óleos, gorduras ou outras matérias estranhas ao produto. O óleo de soja pode ser classificado em três etapas: bruto ou cru, degomado ou purificado e refinado. O óleo bruto ou cru é extraído por esmagamento mecânico do grão de soja. Este óleo torna-se purificado ou degomado após a extração dos fosfolipídios e refinado após ser neutralizado, clarificado e desodorizado (BRASIL, 1993).

Alguns trabalhos tentam definir qual seria o melhor de nível de inclusão de óleo de soja, Dahlke et al. (2001) estudando a preferência alimentar em frangos de 1 a 21 dias de idade, alimentados com dietas isoenergéticas e diferentes inclusões de óleo de soja (0, 1, 2 e 3%), observaram que os frangos apresentaram maior consumo quanto maior foi o nível de inclusão de óleo. Pucci et al. (2003) observaram o efeito linear crescente ($P<0,01$) no consumo de ração e no ganho de peso, no período de 1 a 21 dias de idade, em frangos de corte alimentados com rações contendo 2,5; 5,0 e 7,5% de óleo refinado de soja. Raber et al. (2008), utilizando níveis crescentes de óleo degomado de soja e óleo ácido de soja (2%, 3%, 4% e 5%), observaram que o ganho de peso melhorou conforme aumentava os níveis de óleo. Alguns autores, fornecendo óleos de origem animal nas dietas de aves, como por exemplo, o óleo de vísceras, encontraram resultados similares à inclusão de outros óleos. Fornecendo dietas para frangos de corte contendo 0, 3, 6 e 9% de inclusão de diferentes fontes lipídicas (óleo de milho, óleo de vísceras de aves e mistura de gordura animal/vegetal), Griffiths et al. (1977), observaram menor peso médio para as aves que receberam ração sem adição de fonte lipídica. Testando diferentes níveis de inclusão (0, 2, 4, 6, 8 e 10%) de óleo refinado de palma, óleo de semente de palma, óleo de milho e óleo de vísceras de aves em dietas para frangos de corte, Valencia et al. (1993), não observaram diferenças de desempenho entre as diferentes fontes lipídicas, mas observaram maiores pesos e melhores conversões à medida que se aumentava o nível de inclusão dos óleos.

Por outro lado, as misturas de gorduras animais e óleos vegetais podem representam uma opção alternativa para a indústria de ração para aves. As gorduras animais contêm uma elevada proporção de ácidos graxos saturados de cadeia longa, enquanto os óleos vegetais têm uma elevada proporção de ácidos graxos insaturados (LEESON; SUMMERS, 2005). Os ácidos graxos saturados são de difícil digestão quando comparados com os ácidos graxos insaturados. Ketels e De Groote (1989) relataram que o aumento das relações I: S aumentou a utilização de ácidos graxos saturados, mas não houve qualquer efeito sobre os ácidos graxos insaturados. Leeson e Summers (2005), sugeriram que uma proporção de 3:1 (I:S) é uma razão ótima para melhorar a digestibilidade da gordura em aves de todas as idades. Alguns autores relatam que a mistura de gorduras animais com óleos vegetais podem produzir um efeito sinérgico que pode melhorar a utilização de gorduras saturadas (GUERREIRO NETO et al., 2011; POORGHASEMI et al., 2013).

O uso de emulsificantes em dietas para aves é um assunto recente, ainda com poucos trabalhos de pesquisa, porém, com resultados promissores. O emulsificante a base gliceril polietilenoglicol ricinoleato, tem características diferenciadas, e poderá contribuir para elucidar os efeitos em diferentes condições de formulação e tipos de gorduras utilizadas.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1 - *EMULSIFIER-ADDITIVE IMPROVES ENERGY UTILIZATION IN CORN/SOYBEAN MEAL DIETS FOR BROILERS*

Formatado de acordo com a norma do periódico *Animal Feed Science and Technology.*

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ABSTRACT

A completely randomized design study was conducted to evaluate the effects of five levels of soybean oil (0, 1.5, 3.0, 4.5, 6.0 g/kg) and with or without emulsifier supplementation on performance, coefficient of total tract apparent digestibility (CTTAD) of dry matter (DM), crude protein (CP), ether extract (EE) and apparent metabolizable energy corrected for nitrogen balance (AMEn) in broiler chickens. A total of 600 fourteen-day-old male Cobb 500 in the starter phase (14-21d) and 360 thirty-five-day-old male broilers in the finisher phase (35-42d) were randomly divided into 10 treatments of 6 replications each. Total excreta were collected from d 19 to 21 and from d 40 to 42. Data were analyzed using ANOVA and contrast test. During the starter and finisher phases, birds fed diets supplemented with emulsifier were characterized by higher ($P<0.05$) body weight gain (BWG) and better feed conversion ratio (FCR) compared to birds receiving diets without emulsifier. In the starter phase, emulsifier had no effect ($P>0.05$) on CP. Emulsifier increased ($P<0.05$) DM digestibility, treatments with 30 and 60g/kg of oil had higher values (0.747 *vs* 0.729; 0.740 *vs* 0.722), respectively, when compared to treatments without emulsifier supplementation. AMEn was improved ($P<0.05$) by 0.26, 0.27 and 0.29 MJ/kg when emulsifier was added in the diets with 30, 45 and 60g/kg of soybean oil, respectively. In the finisher phase, CP was not affected ($P>0.05$). Emulsifier increased ($P<0.05$) DM digestibility in the diets with 45 and 60g/kg of soybean oil (0.763 *vs* 0.741;

0.743 *vs* 0.724). Emulsifier also improved ($P<0.05$) AMEn by 0.34, 0.36 and 0.42 MJ/kg in the diets with 30, 45 and 60g/kg of soybean oil. The emulsifier can significantly improve overall performance and coefficient digestibility of DM and AMEn in the diets formulated 30, 45 and 60g/kg of soybean oil.

Key words: Feed additive, poultry, nutrient digestibility, AMEn, oil level

1. INTRODUCTION

Energy is the main cost component in diets for monogastric animals. Due to its high energy density, animal fats and vegetable oils are important energy sources in diets formulation. Improving energy efficiency of these feed ingredients has a great interest from economical point of view of poultry industry (Rovers, 2014).

Fats are insoluble in water, do not solubilize in the aqueous phase of the gastrointestinal tract and need to be emulsified before they can be hydrolyzed by lipase. The ease of emulsification depends on the characteristics of the fat such as chain length, position of fatty acid on the triglyceride and degree of saturation. As a polar amphipathic molecule, consisting of both hydrophilic and hydrophobic properties, an emulsifier (more accurately termed surfactant) is able to form a bridge between water- and fat-soluble materials, and improve fat utilization (Ravindran et al., 2016).

When selecting an emulsifier, the Hydrophilic-Lipophilic Balance (HLB) is a key indicator showing how fat or water soluble an emulsifier is and, it is assigned on an scale ranging from 0 to 20 (0=very lipophilic and 20=very hydrophilic). The lower the HLB, the more fat soluble the emulsifier becomes. On the other hand, the higher the HLB, the more water soluble the emulsifier will be. It is known birds consume almost 2 times more water than feed and the feed contains only a small amount of fat, the water amount is much higher than the fat amount in the birds's intestine. In this case, an emulsifier with a high HLB is more suitable (Ravindran et al., 2016; Rovers, 2014; Soede, 2005).

The nutritional emulsifiers act as bile salts, a natural emulsifier, which increase the active surface of fat, allowing the action of the enzyme lipase, which is produced by pancreas and breaks down the fat into fatty acids and monoglycerides and favor the formation of micelles consisting of lipolysis products. This is an important step for lipid absorption, as its creates a diffusion gradient that increases nutrient absorption (Guerreiro Neto et al., 2011).

According to Roy et al., (2010), a dietary addition of glyceryl polyethyleneglycol ricinoleate (GPGR) at 1% of added fat improved chickens' live weight by approximately 5% and, thus, improved feed conversion ratio. Kaczmarek et al., (2015) have recently shown that the inclusion of 0.04% of GPGR in broilers diets improved body weight gain and fat digestibility and improve numerically the dietary AMEn. An exogenous emulsifier based on GPGR improved average daily gain and feed conversion ratio of weanling pigs (Udomprasert & Rukkwamsuk, 2006). However, the use of nutritional emulsifiers in association with vegetable oils in poultry feed has not yet been thoroughly explored, even though the interest in using emulsifiers has increased in the last decade (Bontempo et al., 2016).

Therefore, the objective of this study was to evaluate the effects of an emulsifier-additive on performance and nutrient digestibility and apparent metabolizable energy corrected for nitrogen balance (AMEn) in broilers receiving diets with different soybean oil levels in the starter (14-21d) and finisher (35-42d) phases.

2. MATERIALS AND METHODS

The institutional animal care and use committee of the Purdue University approved all the procedures used in this experiment.

2.1 Birds and housing

A total of 600 one-day-old Cobb 500 male broiler, vaccinated for Marek's disease at the hatchery were randomly placed in 60 wired cages (0.70 x 0.50 x 0.35m). Each cage was equipped with one feeder and one drinker. Birds had *ad libitum* access to water and mash feeds. At 14 d of age, chickens were individually weight and placed into 10 treatments with 6 replicates of 10 birds each. To the second phase, 360 one-day-old birds were reared in wood shavings floor pens until 34 days receiving a standard-maize-SBM based diets. At 35 d of age, the birds were individually weighted and distributed into 10 treatments with 6 replicates of 6 birds each. The temperature of the room was 25±2°C in the starter and 23±2°C in the finisher phase. Lighting was continuous all the trial.

2.2 Experimental diets

A completely randomized design study with 10 treatments was conducted to evaluate five inclusion levels of soybean oil (0, 15, 30, 45 and 60 g/kg of diet) and with or without emulsifier supplementation (0 or 0.35g/kg of diet). The emulsifier was based on glyceryl polyethylene glycol ricinoleate and it has a high hydrophilic-lipophilic balance *(*Excential Energy Plus, Orffa, Werkendam, The Netherlands). A standard maize-SBM-based broiler pre-starter diet was fed from 1 to 13d (12.56 MJ/kg AME, 218 g/kg CP, 8.8 g/kg Ca, and 4.4 g/kg available P) and grower diet was fed from 22 to 34 d (13.18MJ/kg AME, 198 g/kg CP, 7.6 g/kg Ca, and 3.5 g/kg available P) between the trial evaluation. The dietary phases consisted of starter (14-21d) and finisher (35-42d) are shown in Table 1 and Table 2, respectively.

2.3 Performance parameters

The birds were weighed and feed intake (FI) was recorded from the beginning to the end of starter (14-21d) and finisher phase (35-42d) to calculate body weight gain (BWG) and feed conversion ratio (FCR).

2.4 Total excreta collection and chemical analysis

After 5 days of diet adaptation, total excreta were collected twice daily on plastic tray from 19 to 21d and 40 to 42d being mixed and pooled by cage and stored at -20°C until analysis. Feathers and scales were removed to avoid contamination. Excreta samples were dried in a forced air oven at 55°C for 72 hours and ground to pass through a 0.5-mm screen. Dry matter (DM) analysis was performed after oven drying the samples at 105°C for 16h (method 934.01; AOAC, 2006). Ether extract was determined by the petroleum ether extraction method using soxhlet system (method 920.39; AOAC, 2006). Gross energy was determined by adiabatic bomb calorimetry standardized with benzoic acid (IKA Werke Gmbh & Co. KG, Staufen, Germany). Crude protein (CP) was determined (N x 6.25) by combustion method (Method 968.06; AOAC, 2006) using FP-528 N analyser (Leco Corporation, St Joseph, MI, USA).

2.5 Calculation

Coefficients of total tract apparent digestibility (CTTAD) and apparent metabolizable energy corrected for nitrogen (AMEn) were calculated using the following equations as described:

$$CTTAD (\%) = [nutrient_{diet} - (^{excreta}/_{feed} \times nutrient_{excreta})] / [nutrient_{diet}] \times 100$$

$$AME (kcal/kg\ diet) = GE_{diet} - (GE_{excreta} \times {}^{excreta}/_{feed})$$

Where GE_{diet} and $GE_{excreta}$ are the analyzed gross energy values of the diet and excreta samples respectively. The N-corrected AME (AMEn) values were calculated by correcting for N retention by using a factor of 0.034 MJ/g N retained in the body (Hill & Anderson, 1958).

2.6 Statistical analysis

The data were analyzed by ANOVA using the General Linear Model (GLM) procedure of SAS (SAS Institute, Inc.; Version 9.3). Orthogonal contrasts were performed to compare the effect of emulsifier on each soybean oil levels in the diets. Significant differences were determined at $P<0.05$.

3. RESULTS

3.1 Growth performance

Results for FI, BWG and FCR are shown in Table 3. The FI values for the diets in the starter and finisher phases were not affected ($P>0.05$) by the emulsifier supplementation on different soybean oil levels. However, the contrast comparing the supplementation with or without emulsifier of diets with different soybean oil levels, indicated that broilers receiving emulsifier presented better BWG (P<0.05) and FCR (P<0.05), which improved 3.2 and 2.9% in the starter; 4.5 and 3.9% in the finisher phases, respectively. Emulsifier increased BWG (P<0.05) by 8.6 and 7.2% when were compared T2 *vs* T7 and T4 *vs* T9 in the finisher phase. Treatments with 0 and 45kg/diet of soybean oil supplemented with emulsifier had better FCR (P<0.05) by 5.5 and 5.2% (T1 *vs* T6; T4 *vs* T9).

3.2 Coefficient of total tract apparent digestibility and AMEn

Results for CTTAD of nutrients and AMEn are shown in table 4. The CP values for the diets in both ages evaluated were not affected (P>0.05) by emulsifier on different oil levels. Nevertheless, the contrast comparing the supplementation with or without emulsifier on different soybean oil levels, indicated that broilers receiving emulsifier increased (P<0.05) digestibility for DM and, EE, and AMEn**,** which improved 2.0, 2.5, 1.7% in the starter; 2.2, 2.6 and 2.2% in the finisher diets, respectively. DM were improved (P<0.05) by 2.5% when were compared the contrasts T3 *vs* T8 and T5 *vs* T10 in the starter; 3.0 and 2.6% in the contrasts T4 *vs* T9 and T5 *vs* T10 in the finisher diets. Birds fed with 30, 45 and 60g/kg of diet of soybean oil with added emulsifier had higher values (P<0.05) of AMEn than those fed without emulsifier in both dietary phases. AMEn were improved (P<0.05) in 1.9, 1.9, 2.1% in the starter and 2.4, 2.6, and 2.9% in the finisher diets, respectively.

4. DISCUSSION

4.1 Growth performance

In this study, FI was not affected by the inclusion of emulsifier on each level of soybean oil. Findings of present research are supported by Abbas et al. (2016) who reported no effect of exogenous emulsifier on FI in the diets with 10, 20 and 30g/kg of soybean oil. Also, previous studies have shown that supplementation of emulsifier in broilers diets did not decrease or stimulate feed intake (Aguilar et al., 2013; Guerreiro Neto et al., 2011; Roy et al., 2010; Zampiga et al., 2016). Overall, the emulsifier presented a better BWG and FCR in both phases when all treatments are compared. These results showed that emulsifier has effect in the diets with increasing levels of soybean oil or even in diets without vegetable oil inclusion. The improved performance after emulsifier addition could be related to the overall improvement of fat digestibility. Roy et al. (2010) found that an exogenous emulsifier improved the performance of birds during the starter and grower periods, but Zhang et al. (2011) found such a positive effect only during the starter and Kaczmarek et al. (2015) only in the grower and the whole trial.

4.2 Coefficient of total tract apparent digestibility and AMEn

Nitrogen was not affected at any feeding phase, this results were also reported by Zhang et al. (2011), who did not find significant effect of emulsifier on digestibility of nitrogen in broiler chickens from 14 to 17 and from 35 to 38 days old. Similar results were also reported by Zampiga et al. (2016), while other authors observed significant improvement on N due to the use of emulsifiers (Jansen et al., 2015; Roy et al., 2010). Abbas et al. (2016), found higher values of DM in diets with emulsifier and increasing soybean oil levels from 10 to 30g/kg. As a consequence, the improved emulsification of fats could not only enhance the digestion of the lipids but also of other nutrients (e.g. liposoluble vitamins) and energy (Dierick and Decuypere, 2004; Jansen et al., 2015;

Roy et al., 2010; Zaefarian et al., 2015). In the present study, the ether extract retention of birds were not significantly different throughout the experimental period when treatments were compared individually. It could be explained by the less pronounced effect of emulsifier in the high unsaturated lipid source (Tan et al., 2016). Jansen et al. (2015) reported only numerical improvements in the soybean oil (53 g/kg of diet) treatment supplemented with emulsifier on fat retention. Additionally, researches with broilers (Zampiga et al., 2016), ducks (Zosangpuii et al., 2015) and pigs (Soares and Lopez-Bote, 2002) also did not find significant results of emulsifiers on diets formulated with soybean oil. However, the overall ether extraction retention was significantly improved by the emulsifier when all treatments are compared. Independently of soybean oil level in the diets, the emulsifier showed effect. It could be speculated that the emulsifier worked even in the diets with none or low level of soybean oil, improving fat digestibility of other feed ingredients, such as maize. The exogenous emulsifier acts on dietary fat and for other feed ingredients along with higher degree of lipolysis of triglycerides resulting in more micelle formation, digestion and absorption of fats (Abbas et al., 2016; Ho Cho et al., 2012). During the starter and finisher phases were found improvements at AMEn in the diets supplemented with emulsifier and formulated with 30, 45 and 60 g/kg of soybean oil. These results show to contradict those obtained by Al-Marzooqi and Leeson (1999), who found that the emulsifier supplementation in the broiler's diets was not proved to be an effective method to improve fat digestibility and energy level of diet. On the other hand, our findings corroborate with the data of Zhang et al. (2011), who showed that emulsifier addition improved energy level of diets. It is known that the capacity to digest fat increases with age. Young birds have limited digestion and absorption of dietary fat because of insufficient secretion of bile salts rather than lipase (Krogdahl, 1985), which give a room to exogenous emulsifier acts. However, birds at finisher age, where they possibly have their digestive system developed and large capacity of feed intake, showed even better energy utilization with the action of emulsifier on nutrients absorption. The emulsifier evaluated has high HLB (Tan et al., 2016), which is an indicative of high water solubility. Broilers drink twice the amount of water than feed and the feed contains only a small amount of fat, then the amount of water is much higher than the amount of fat in the intestine. In this case a high HLB is more suitable

(Rovers, 2014). The exogenous emulsifier manufactured from glyceryl polyethyleneglycol ricinoleate act as bile salts, a natural emulsifier, which increase the active surface of fat, allowing the action of the enzyme lipase, which is produced by pancreas and breaks down the fat into fatty acids and monoglycerides and favor the formation of micelles consisting of lipolysis products. This is an important step for lipid absorption, as its creates a diffusion gradient that increases nutrient absorption (Guerreiro Neto et al., 2011).

5. CONCLUSION

To conclude, the emulsifier was found to be effective to improve overall performance and coefficient of nutrients retention and AMEn in the diets formulated with 30, 45 and 60 g/kg of soybean oil.

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Table 1 Ingredient and nutrient composition of starter diets.

Ingredients (g/kg)	Starter diets (14-21d)									
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Corn	668.99	558.09	536.32	512.05	473.96	668.99	558.09	536.32	512.05	473.96
Soybean meal	294.17	394.01	398.80	407.85	433.88	294.17	394.01	398.80	407.85	433.88
Soybean oil	0.00	15.00	30.00	45.00	60.00	0.00	15.00	30.00	45.00	60.00
Dicalcium phosphate	11.52	10.89	10.93	12.04	10.80	11.52	10.89	10.93	12.04	10.80
Limestone	8.88	8.68	8.65	8.00	8.55	8.88	8.68	8.65	8.00	8.55
NaCl	4.30	4.31	4.31	4.32	4.33	4.30	4.31	4.31	4.32	4.33
DL-Methionine	2.94	2.38	2.60	2.78	2.81	2.94	2.38	2.60	2.78	2.81
L-Lysine	3.36	0.82	1.08	1.21	0.83	3.36	0.82	1.08	1.21	0.83
Threonine	1.50	1.47	2.96	2.39	0.49	1.50	1.47	2.96	2.39	0.49
Vitamin premix[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Zinc Bacitracin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salynomicin 12%	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Phytase[c]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Antioxidant[d]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Emulsifier[e]	-	-	-	-	-	0.35	0.35	0.35	0.35	0.35
Inert (kaolin)	1.00	1.00	1.00	1.00	1.00	0.65	0.65	0.65	0.65	0.65
Calculated										
AME, MJ/kg	12.46	12.53	12.76	13.06	13.33	12.46	12.53	12.76	13.06	13.33
CP	190.00	224.00	226.00	228.00	235.00	190.00	224.00	226.00	228.00	235.00
Ether extract[f], g/kg	34.17	47.45	61.82	74.58	93.10	34.17	47.45	61.82	74.58	93.10
Met, g/kg	5.63	5.44	5.65	5.84	5.96	5.63	5.44	5.65	5.84	5.96
Met/Cys, g/kg	8.20	8.40	8.60	8.80	9.00	8.20	8.40	8.60	8.80	9.00
Lys, g/kg	11.45	11.72	12.00	12.28	12.55	11.45	11.72	12.00	12.28	12.55

Ingredients (g/kg)	Starter diets (14-21d)									
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Ca, g/kg	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70
Available P, g/kg	4.30	4.30	4.30	4.30	4.30	4.30	4.30	4.30	4.30	4.30
Na, g/kg	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90

[a]Composition per kg of feed: vitamin A, 9000UI; vitamin D3, 2500UI; vitamin E, 20UI; vitamin K3, 2500mg; vitamin B1, 1500mg; vitamin B2, 6000mg; vitamin B6, 3000mg; vitamin B12, 12000mcg; biotin, 60mg; folic acid, 800mg; nicotinic acid, 25000mg; pantothenic acid, 12000mg; selenium, 250mg.

[b]Composition per kg of feed: manganese, 160mg; iron, 100mg; zinc, 100 mg; copper, 20mg; cobalt 2mg; iodine, 2mg.

[c]Ronozyme Hiphos with 10,000 fungal phytase units/g (Novozymes A/S, Bagsvaerd, Denmark).

[d]Endox® 5X Concentrate Dry (Kemin Ltda, Indaiatuba, Brazil).

[e]Excential Energy Plus dosage at 0.35g/kg of feed (Orffa, Werkendam, the Netherlands).

[f]Analyzed.

Table 2 Ingredient and nutrient composition of finisher diets.

Ingredients (g/kg)	Finisher diets (35-42d)									
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Corn	646.09	614.85	585.87	557.12	528.39	646.09	614.85	585.87	557.12	528.39
Soybean meal	314.44	330.89	344.92	358.74	372.54	314.44	330.89	344.92	358.74	372.54
Soybean oil	0.00	15.00	30.00	45.00	60.00	0.00	15.00	30.00	45.00	60.00
Dicalcium phosphate	1.39	1.34	1.30	1.27	1.24	1.39	1.34	1.30	1.27	1.24
Limestone	8.58	8.53	8.48	8.44	8.39	8.58	8.53	8.48	8.44	8.39
NaCl	4.05	4.06	4.06	4.07	4.08	4.05	4.06	4.06	4.07	4.08
DL-Methionine	1.50	1.58	1.68	1.78	1.87	1.50	1.58	1.68	1.78	1.87
L-Lysine	0.54	0.40	0.33	0.23	0.15	0.54	0.40	0.33	0.23	0.15
Threonine	0.06	0.00	0.01	0.01	0.00	0.06	0.00	0.01	0.01	0.00
Vitamin premix[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Zinc Bacitracin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salynomicin 12%	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride 60%	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Phytase[c]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Antioxidant[d]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Emulsifier[e]	-	-	-	-	-	0.35	0.35	0.35	0.35	0.35
Inert (kaolin)	20.00	20.00	20.00	20.00	20.00	19.65	19.65	19.65	19.65	19.65
Calculated composition										
AME, MJ/kg	12.33	12.60	12.87	13.17	13.46	12.33	12.60	12.87	13.17	13.46
CP	193.00	198.00	202.00	206.00	210.00	193.00	198.00	202.00	206.00	210.00
Ether extract[f], g/kg	36.25	48.28	64.97	72.68	92.95	36.25	48.28	64.97	72.68	92.95
Met, g/kg	4.28	4.40	4.52	4.65	4.77	4.28	4.40	4.52	4.65	4.77
Met/Cys, g/kg	6.94	7.10	7.26	7.42	7.57	6.94	7.10	7.26	7.42	7.57

Ingredients (g/kg)	Finisher diets (35-42d)									
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Lys, g/kg	9.69	9.93	10.16	10.37	10.59	9.69	9.93	10.16	10.37	10.59
Ca, g/kg	6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50
Available P, g/kg	3.70	3.70	3.70	3.70	3.70	3.70	3.70	3.70	3.70	3.70
Na, g/kg	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80

[a]Composition per kg of feed: vitamin A, 9000UI; vitamin D3, 2500UI; vitamin E, 20UI; vitamin K3, 2500mg; vitamin B1, 1500mg; vitamin B2, 6000mg; vitamin B6, 3000mg; vitamin B12, 12000mcg; biotin, 60mg; folic acid, 800mg; nicotinic acid, 25000mg; pantothenic acid, 12000mg; selenium, 250mg.
[b]Composition per kg of feed: manganese, 160mg; iron, 100mg; zinc, 100 mg; copper, 20mg; cobalt 2mg; iodine, 2mg.
[c]Ronozyme Hiphos with 10,000 fungal phytase units/g (Novozymes A/S, Bagsvaerd, Denmark).
[d]Endox® 5X Concentrate Dry (Kemin Ltda, Indaiatuba, Brazil).
[e]Excential Energy Plus dosage at 0.35g/kg of feed (Orffa, Werkendam, the Netherlands).
[f]Analyzed.

Table 3 Growth performance of broilers fed corn-soybean meal diets with five levels of soybean oil and supplemented or not with emulsifier[a].

Oil Level (g/kg)	Emulsifier	Treatments	Day 14-21			Day 35-42		
			FI,g	BWG,g	FCR,g/g	FI,g	BWG,g	FCR,g/g
0	-[b]	T1	557	385	1.45	1043	498	2.10
	+[c]	T6	573	405	1.41	1016	508	1.99
15	-	T2	576	404	1.43	1020	533	1.91
	+	T7	581	407	1.42	1065	579	1.84
30	-	T3	576	409	1.41	1051	541	1.95
	+	T8	581	425	1.37	1039	540	1.93
45	-	T4	580	431	1.34	1038	570	1.82
	+	T9	579	437	1.33	1054	611	1.73
60	-	T5	581	401	1.46	1096	611	1.79
	+	T10	578	419	1.38	1106	643	1.72
CV(%)			3.65	4.74	3.71	5.52	5.27	4.26
Orthogonal contrasts			*P*-value					
T1 *vs* T6			0.1982	0.0750	0.2360	0.4216	0.5300	0.0377
T2 *vs* T7			0.7024	0.7800	0.7032	0.1793	0.0106	0.1265
T3 *vs* T8			0.7125	0.1456	0.1535	0.7293	0.9460	0.6260
T4 *vs* T9			0.9564	0.5970	0.5188	0.6282	0.0200	0.0430
T5 *vs* T10			0.7744	0.1167	0.0186	0.7630	0.0690	0.1344
T1,T2,T3,T4,T5 vs T6,T7,T8,T9,T10			0.4465	0.0138	0.0086	0.6585	0.0016	0.0011

[a]Means were obtained from 6 replicate cages of 10 birds each in the starter and 6 replicates of 6 birds each in the finisher phase.
[b] – Represents without emulsifier supplementation
[c] + Represents supplementation with 0.35g/kg diet of emulsifier

Table 4 Coefficient of total tract retention of dry matter, crude protein, ether extract and AMEn of broilers fed corn-soybean meal diets

Oil Level (g/kg)	Emulsifier	Treatments	Day 14-21				Day 35-42			
			DM	CP	EE	AMEn,MJ/kg	DM	CP	EE	AMEn,MJ/kg
0	-[b]	T1	0.725	0.633	0.699	12.79	0.733	0.626	0.778	13.23
	+[c]	T6	0.739	0.635	0.705	12.92	0.739	0.616	0.784	13.40
15	-	T2	0.732	0.644	0.749	13.10	0.743	0.641	0.834	13.83
	+	T7	0.741	0.656	0.754	13.28	0.758	0.660	0.845	14.06
30	-	T3	0.729	0.639	0.791	13.46	0.740	0.656	0.858	14.07
	+	T8	0.747	0.655	0.806	13.72	0.758	0.652	0.876	14.41
45	-	T4	0.733	0.662	0.817	13.94	0.741	0.663	0.876	14.12
	+	T9	0.745	0.667	0.836	14.21	0.763	0.679	0.892	14.48
60	-	T5	0.722	0.639	0.842	13.90	0.724	0.640	0.891	14.28
	+	T10	0.740	0.653	0.857	14.19	0.743	0.641	0.910	14.70
CV(%)			1.94	3.46	2.25	1.44	2.12	2.98	2.11	1.71
Orthogonal contrasts			*P*-value							
T1 *vs* T6			0.1133	0.8426	0.5591	0.2356	0.5063	0.4035	0.5698	0.2362
T2 *vs* T7			0.2418	0.3499	0.5970	0.1304	0.0974	0.0877	0.3130	0.1027
T3 *vs* T8			0.0354	0.2353	0.1486	0.0270	0.0535	0.7743	0.0818	0.0180
T4 *vs* T9			0.1462	0.6524	0.0577	0.0196	0.0210	0.1591	0.1303	0.0111
T5 *vs* T10			0.0339	0.2858	0.1548	0.0123	0.0355	0.9242	0.0803	0.0043
T1,T2,T3,T4,T5 *vs* T6,T7,T8,T9,T10			0.0003	0.0891	0.0102	<0.0001	0.0002	0.3440	0.0043	<0.0001

diets with five levels of soybean oil and supplemented or not with emulsifier[a].

[a]Means were obtained from 6 replicate cages of 10 birds each in the starter and 6 replicates of 6 birds each in the finisher phase.

[b] – Represents without emulsifier supplementation

[c] + Represents supplementation with 0.35g/kg diet of emulsifier

ARTIGO 2 - *EMULSIFIER IMPROVES ENERGY UTILIZATION IN CORN/SOYBEAN/MEAT BONE MEAL DIETS FOR BROILERS*

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ABSTRACT

A completely randomized design study was conducted to evaluate the effects of five levels of soybean oil (0, 15, 30, 45, 60g/kg) with or without emulsifier supplementation on performance, coefficient of total tract apparent digestibility (CTTAD) of dry matter (DM), crude protein (CP), ether extract (EE) and apparent metabolizable energy corrected for nitrogen (AMEn) in broiler chickens. A total of 600 fourteen-day-old male Cobb 500 in the starter (14-21d) and 360 thirty-five-day-old male broilers in the finisher phase (35-42d) were randomly divided into 10 treatments of 6 replications each. Total excreta were collected from d 19 to 21 and from d 40 to 42. Data were analyzed by ANOVA and contrast test. During the starter and finisher phase, birds fed diets supplemented with emulsifier had better feed conversion ratio (FCR) compared to birds receiving diets without emulsifier ($P<0.05$). In the starter phase, there were no effects ($P>0.05$) on CP and DM. Emulsifier improved ($P<0.05$) AMEn by 0.25, 0.28 and 0.30MJ/kg in the diets with 30, 45 and 60g/kg of soy oil. In the finisher phase, there was no effect ($P>0.05$) for crude protein. However, emulsifier increased ($P<0.05$) DM in the treatments with 30 and 60g/kg of oil (76.48 *vs* 74.33; 76.47 *vs* 73.61). The AMEn was improved ($P<0.05$) by 0.37, 0.32 and 0.38MJ/kg when emulsifier was added in the diets with 30, 45, and 60g/kg of soybean oil. Results from this experiment shows that the emulsifier can significantly improve apparent digestibility coefficient of DM and AMEn in

broilers fed 30, 45 and 60g/kg of soybean oil in the diets at the starter and finisher phases.

Key words: Feed additive, poultry, nutrient digestibility, AMEn, soybean oil

1. INTRODUCTION

In the last few years, because of the ever-increasing energy costs, there is an interest in maximizing the use fats by nutritionists to increase the dietary energy density to meet the requirements of high-performing animals. Leeson and Summers (2005) has suggested a minimum inclusion level of 10 g/kg fat in poultry diets, however, 20 to 50 g/kg fat is usually added in commercial poultry diets depending on the relative prices of fat and cereal grains.

Besides corn, soybean meal and soybean oil, meat bone meal is a feed ingredient commonly used in poultry diets. At the same time, it supplies proteins and minerals (e.g. P and Ca); meat bone meal is also a good source of animal fat (Rostagno et al., 2017). The animal fat content could be also a substrate to the emulsifier action and improving fat utilization in poultry diets.

The mode of action of exogenous emulsifiers is to improve the active surface of fats, allowing the action of the enzyme lipase, which hydrolyze triglyceride molecules into fatty acids and monoglycerides and favor the formation of micelles consisting of lipolysis products. Those are the essential steps for lipid absorption, which creates a diffusion gradient that increases nutrients absorption (Aguilar et al., 2013; Guerreiro Neto et al., 2011; Melegy et al., 2010).

When choosing an emulsifier, the principal of Hydrophilic-Lipophilic Balance (HLB) is a major indicator showing how fat or water soluble an emulsifier is and, it is assigned on an scale ranging from 0 to 20 (0=very lipophilic and 20=very hydrophilic). The lower the HLB, the more fat soluble the emulsifier becomes. On the other hand, the higher the HLB, the more water soluble the emulsifier will be (Ravindran et al., 2016; Rovers, 2014; Soede, 2005). Birds drinks almost 2 times more water than feed and the feed contains a small amount of fat, the water amount is much higher than the fat amount in the birds's gut. In this case, an emulsifier with a high HLB is more suitable. Compared with other emulsifiers such as lecithins and lysolecithins, glyceryl polyethylene glycol ricinoleate (GPGR) is more hydrophilic and dissolves free fatty acids, which are largely insoluble in bile salts during fat digestion (Dierick & Decuypere, 2004).

An nutritional emulsifier based on GPGR improved average daily gain and feed conversion ratio of weanling pigs (Udomprasert & Rukkwamsuk, 2006). Dietary addition of GPGR at 1% of added fat in the diet improved chickens' live weight by approximately 5% and, thus, improved feed conversion ratio (Roy et al., 2010). Kaczmarek et al. (2015) have shown that the inclusion of 0.04% of GPGR in broilers diets improved body weight gain and fat digestibility and tended to improve AMEn. However, the requirements of using emulsifiers in broiler diets must be investigate because feeding of nutrient dense diets containing added fat is essencial to explore the full growth potential of the high performing broiler strains (Roy et al., 2010).

Therefore, the aim of this trial was to evaluate the effects of an emulsifier on performance and nutrient digestibility and apparent metabolizable energy corrected for nitrogen (AMEn) in broilers diets formulated with meat bone meal and different soybean oil levels in the starter and finisher phases.

2. MATERIALS AND METHODS

All animal procedures were approved and conducted under the guidelines of Purdue University.

2.1 Birds and housing

A total of 600 one-day-old Cobb 500 male broiler, vaccinated for Marek's disease at the hatchery were randomly placed in 60 wired cages (0.70 x 0.50 x 0.35m). Each cage was equipped with one feeder and one drinker. Birds had ad libitum access to water and mash feeds. At 14 d of age, chickens were individually weight and placed into 10 treatments with 6 replicates of 10 birds per cage each. To the second phase, 360 one-day-old birds were reared in wood shavings floor pens until 34 days receiving a standard-maize-SBM based diets. At 35 days of age, the birds were individually weighted and distributed into 10 treatments with 6 replicates of 6 birds per cage each. The temperature of the room was 25-27°C in the starter and 22-24°C in the finisher phase with 24-h constant light.

2.2 Experimental diets

A completely randomized design study with 10 treatments was conducted to evaluate five inclusion levels of soybean oil (0, 15, 30, 45 and 60 g/kg of diet) and with or without emulsifier supplementation (0 or 0.35g/kg of diet). The emulsifier was based on glyceryl polyethylene glycol ricinoleate and it has a high hydrophilic-lipophilic balance *(*Excential Energy Plus, Orffa, Werkendam, The Netherlands). A standard maize-SBM-based broiler starter diet was fed from 1 to 13d (12.56 MJ/kg AME, 218 g/kg CP, 8.8 g/kg Ca, and 4.4 g/kg available P) and grower diet was fed from 22 to 34 d (13.18MJ/kg AME, 198 g/kg CP, 7.6 g/kg Ca, and 3.5 g/kg available P). The dietary phases consisted of starter (14-21d) and finisher (35-42d) and are shown in Table 1 and Table 2 respectively.

2.3 Total excreta collection and chemical analysis

After 5 days of diet adaptation, total excreta were collected twice daily on plastic tray from 19 to 21d and 40 to 42d being mixed and pooled by cage and stored at -20°C until analysis. Feathers and scales were removed to avoid contamination. Excreta samples were dried in a forced air oven at

55°C for 72 hours and ground to pass through a 0.5-mm screen. Dry matter (DM) analysis was performed after oven drying the samples at 105°C for 16h (method 934.01; AOAC, 2006). Ether extraction was determined by the petroleum ether extraction method using soxhlet system (method 920.39; AOAC, 2006). Gross energy was determined by adiabatic bomb calorimetry standardized with benzoic acid (IKA Werke Gmbh & Co. KG, Staufen, Germany). Crude protein (CP) was determined (N x 6.25) by combustion method (Method 968.06; AOAC, 2006) using FP-528 N analyser (Leco Corporation, St Joseph, MI, USA).

2.4 Calculations

Coefficients of total tract apparent digestibility (CTTAD) and apparent metabolizable energy corrected for nitrogen (AMEn) were calculated using the following equations as described:

$$\text{CTTAD (\%)} = [\text{nutrient}_{\text{diet}} - ({}^{\text{excreta}}/_{\text{feed}} \times \text{nutrient}_{\text{excreta}})] / [\text{nutrient}_{\text{diet}}] \times 100$$

$$\text{AME (kcal/kg diet)} = \text{GE}_{\text{diet}} - (\text{GE}_{\text{excreta}} \times {}^{\text{excreta}}/_{\text{feed}})$$

Where GE_{diet} and $\text{GE}_{\text{excreta}}$ are the analyzed gross energy values of the diet and excreta samples respectively. The N-corrected AME (AMEn) values were calculated by correcting for N retention by using a factor of 0.034 MJ/g N retained in the body (Hill and Anderson, 1958).

2.5 Statistical analysis

All experimental data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, Inc.; Version 9.3). Orthogonal contrasts were performed to compare the effect of emulsifier in each soybean oil levels in the diets. Statistical significance was accepted at P<0.05.

3. RESULTS

3.1 Growth performance

Results for FI, BWG and FCR are presented in Table 3. The FI values for the diets in the starter and finisher phases were not affected (P>0.05) by emulsifier on different soybean oil levels. BWG was not affect (P>0.05) by the treatments in the starter phase. However, the contrast comparing the supplementation with or not emulsifier in the diets with different soybean oil levels, indicated that broilers receiving emulsifier presented better FCR (P<0.05), which improved 2.4% in the starter and 3.7% in the finisher diets, respectively. FCR was improved significantly (P<0.05) by

5.5% on birds receiving diets supplemented with emulsifier and without soybean oil (T1 *vs* T6). Emulsifier increased BWG (P<0.05) by 7.7% when were compared T3 *vs* T8 and 4.5% when all treatments were contrasted in the finisher phase. Treatments with 15 and 30kg/diet of soybean oil supplemented with emulsifier had better FCR (P<0.05) by 5.0 and 4.5% (T2 *vs* T7; T3 *vs* T8) in the finisher phase.

3.2 Coefficient of total tract apparent digestibility and AMEn

Results for CTTAD of nutrients and AMEn are shown in Table 4. The CP values for the diets in both phases were not affected (P>0.05) by the emulsifier supplementation on each soybean oil levels. However, the contrast comparing the supplementation with or not emulsifier in the diets with different soybean oil levels, showed that broilers receiving the feed additive increased (P<0.05) DM, EE, and AMEn**,** which improved 2.1, 1.9, 1.7% in the starter; 2.3, 1.8 and 2.2% in the finisher diets, respectively. EE were improved (P<0.05) by 1.8 % when were compared the contrasts T4 *vs* T9, T5 *vs* T10 and a (P=0.0719) at T3 *vs* T8 in the finisher age. EE retention also had higher values (P=0.0539; P=0.0649; P=0.0879) at the contrasts T3 *vs* T8, T4 *vs* T9 and T5 *vs* T10 in the starter age, respectively. Birds fed diets with 30, 45 and 60g/kg of soybean oil supplemented with emulsifier had higher values ($P<0.05$) of AMEn than those fed without emulsifier in both dietary phases. AMEn was improved by 2.8, 2.3, 2.7% in starter and finisher diets, respectively.

4. DISCUSSION

4.1 Growth performance

Feed intake was not affected by the supplementation of emulsifier on each level of soybean oil throughout the trial. Several studies indicated that emulsifier has not effect on feed intake (Abbas et al., 2016; Aguilar et al., 2013; Guerreiro Neto et al., 2011; Melegy et al., 2010b; Roy et al., 2010; Zampiga et al., 2016; Zhang et al., 2011a; Zhao et al., 2015). The results are related to the nutrient digestibility improvement with the emulsifier which could resulted in fulfilment of the energy requirements of the birds and hence, the birds did not intake more feed. (Abbas et al., 2016). The emulsifier showed only positive effect on BWG when T3 *vs* T8 and all treatments were compared at finisher phase. However, our results disagree with the findings of Zhang et al. 2011 and Guerreiro Neto et al. 2011 who found better BWG in diets formulated with 30 and 37.9g/kg of soybean oil supplemented with emulsifier compared with other fat sources only at starter age. These results could be attributed to no lipase activity in starter phase, while increased weight gain in finisher phase could be attributed to increased lipase activity resulting in higher fat digestibility along with emulsification. Lipase activity in chickens reaches the peak from 40 to 56 days of age (Krogdahl and Sell, 1989). Starter and finisher overall FCRs had better values when supplemented with emulsifier. Roy et al. (2010) found that an exogenous emulsifier improved numerically around 5% the FCR of birds during the starter and grower periods, but Zhang et al. (2011) found 2,7% of FCR improvement only during the starter and Kaczmarek et al. (2015) only in the grower (3.7%) and the whole trial (2.9%). These researchers conclude that the improved performance after supplementation of emulsifier may be related to the improvement of fat retention or of selected fatty acids.

4.2 Coefficient of total tract apparent digestibility and AMEn

During the two ages evaluated of experiment, there was no effect of emulsifier on the N digestibility. Similar results were found by Zampiga et al. (2016) and Zhang et al. (2011) who evaluated birds from 19 to 23d, and from 14 to 17d and 35 to 38d, respectively. On the contrary, Jansen et al. (2015) and Roy et al. (2010) observed emulsifier effect on N digestibility. DM were improved by emulsifier when all treatments were compared in the starter and finisher diets, and also the contrasts T3 *vs* T8 and T5 *vs* T10 in the finisher diets, showing that DM was better in diets formulated from 30 to 60g/kg of soybean oil. Abbas et al. (2016) found significantly higher values of DM in diets supplemented with emulsifier on increasing soybean oil levels from 10 to 30g/kg of diet. The improvement of CTTAD of nutrients by supplementation of an emulsifier to the broilers diets may occurs because non-fat nutrients become less separated by fat droplets and hence more nutrients would be available for digestive enzymes and the absorptive brush border of the small intestine (Alzawqari et al., 2010) . In the current research, the emulsifier showed some effect on fat retention of birds receiving diets formulated above 30g/kg of soybean oil. Significant effect were observed in the finisher diets. On the other hand, Tan et al., (2016) found a less pronounced effect of emulsifier in the unsaturated oil source. Jansen et al. (2015) reported only numerical improvements of emulsifier on fat retention in diets with 53 g/kg of soybean oil. Additionally, researches with broilers (Zampiga et al., 2016), ducks (Zosangpuii et al., 2015) and pigs (Soares and Lopez-Bote, 2002) also did not find significant results of emulsifiers on diets formulated with vegetable oil. Nevertheless, the overall fat retention was significantly improved by the emulsifier when all treatments with or without emulsifier were compared. It could be related that the emulsifier worked also on meat bone meal, which is rich in saturated fatty acids, improving fat digestibility of this feed ingredient. The exogenous emulsifier acts on dietary fat and other feed ingredients along with higher degree of lipolysis of triglycerides resulting in more micelle formation, digestion and absorption of fat acids (Abbas et al., 2016; Ho Cho et al., 2012).This could explain the improvements on AMEn. The capacity of fat digestion increases with bird's age (Ravindran et al., 2016; Tancharoenrat et al., 2014, 2013). Young birds have limited digestion and absorption of dietary fat because of insufficient secretion of bile salts rather than lipase (Krogdahl, 1985; Noy and Sklan, 1995), which could give a room to a nutritional emulsifier acts. However, old birds, where they possibly have their digestive system developed, showed even better energy utilization with the action of emulsifier on nutrients absorption. The modern broilers are characterized by hyperphagia (Richards, 2003), which has influenced the digestion of nutrients due to the difficulties of these birds to produce sufficient secretions (e.g. enzymes, bile salts) for the efficient use of the nutrients intake. These results disagree with Kaczmarek et al. (2015) who did not find dietary energy improvement on day 35 in broilers receiving diets supplemented with glyceryl polyethylene glycol ricinoleate. The mode of action of exogenous emulsifiers is to improve the active surface of fats, allowing the action of the enzyme lipase, which hydrolyze triglyceride molecules into fatty acids and monoglycerides and favor the formation of micelles consisting of lipolysis products. Those are the essential steps for lipid absorption, which creates a diffusion gradient that increases nutrients absorption (Aguilar et al., 2013; Guerreiro Neto et al., 2011; Melegy et al., 2010). The emulsifier glyceryl polyethylene glycol ricinoleate has high HLB (Tan et al., 2016) and it is an amphipatic molecule (consisting out of a hydrophilic and a hydrophobic part) which is able to bridge between water- and fat-soluble materials. Choosing an emulsifier with high HLB means that it has more capacity to be soluble in the water environment, which is similar to bird's intestine. Normally fat and water do not mix and therefore bile salts help this process as natural emulsifier. From that, the emulsifier can assist fat digestion, emulsifying fat droplets and helping micelles formation (Rovers, 2014; Soede, 2005).

5. CONCLUSION

In conclusion, results from the current study shows that the emulsifier manufactured from glyceryl polyethylene glycol ricinoleate can be used as a feeding strategy in poultry starter and finisher diets. Overall improvements in FCR may be related with improvements in the apparent digestibility coefficients of DM, EE, and AMEn due to emulsifier action.

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Table 1 Ingredient and nutrient composition of starter diets.

Ingredients (g/kg)	Finisher diets (14-21d)									
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Corn	680.11	649.57	616.92	585.36	552.56	680.11	649.57	616.92	585.36	552.56
Soybean meal	264.05	280.27	297.81	314.62	334.43	264.05	280.27	297.81	314.62	334.43
Soybean oil	0.00	15.00	30.00	45.00	60.00	0.00	15.00	30.00	45.00	60.00
Meat bone meal	38.07	37.22	37.74	37.58	34.83	38.07	37.22	37.74	37.58	34.83
Limestone	1.88	2.09	1.83	1.80	2.64	1.88	2.09	1.83	1.80	2.64
NaCl	3.69	3.71	3.71	3.72	3.77	3.69	3.71	3.71	3.72	3.77
DL-Methionine	2.98	3.06	3.15	3.24	3.31	2.98	3.06	3.15	3.24	3.31
L-Lysine	3.36	3.24	3.05	2.91	2.74	3.36	3.24	3.05	2.91	2.74
Threonine	1.52	1.49	1.44	1.42	1.38	1.52	1.49	1.44	1.42	1.38
Vitamin premix[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Zinc Bacitracin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salynomicin 12%	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride 60%	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Phytase[c]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Antioxidant[d]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Emulsifier[e]	0.00	0.00	0.00	0.00	0.00	0.35	0.35	0.35	0.35	0.35
Inert (kaolin)	1.00	1.00	1.00	1.00	1.00	0.65	0.65	0.65	0.65	0.65
Calculated composition										
AME, MJ/kg	12.82	13.09	13.36	13.64	13.89	12.82	13.09	13.36	13.64	13.89
Crude protein g/kg	195.00	199.50	205.00	210.00	215.00	195.00	199.50	205.00	210.00	215.00
Crude fat[f], g/kg	35.72	50.99	62.90	75.38	92.33	35.72	50.99	62.90	75.38	92.33
Met, g/kg	5.72	5.84	5.97	6.10	6.22	5.72	5.84	5.97	6.10	6.22
Met/Cys, g/kg	8.20	8.36	8.54	8.71	8.88	8.20	8.36	8.54	8.71	8.88

Ingredients (g/kg)	Finisher diets (14-21d)									
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Lys, g/kg	11.45	11.68	11.91	12.15	12.39	11.45	11.68	11.91	12.15	12.39
Ca, g/kg	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
Available P, g/kg	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50
Na, g/kg	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90

[a]Composition per kg of feed: vitamin A, 9000UI; vitamin D3, 2500UI; vitamin E, 20UI; vitamin K3, 2500mg; vitamin B1, 1500mg; vitamin B2, 6000mg; vitamin B6, 3000mg; vitamin B12, 12000mcg; biotin, 60mg; folic acid, 800mg; nicotinic acid, 25000mg; pantothenic acid, 12000mg; selenium, 250mg.

[b]Composition per kg of feed: manganese, 160mg; iron, 100mg; zinc, 100 mg; copper, 20mg; cobalt 2mg; iodine, 2mg.

[c]Ronozyme Hiphos with 10,000 fungal phytase units/g (Novozymes A/S, Bagsvaerd, Denmark).

[d]Endox® 5X Concentrate Dry (Kemin Ltda, Indaiatuba, Brazil).

[e]Excential Energy Plus dosage at 0.35g/kg of feed (Orffa, Werkendam, the Netherlands).

[f]Analyzed.

Table 2 Ingredient and nutrient composition of finisher diets.

Ingredients (g/kg)	Finisher diets (35-42d)									
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Corn	679.27	648.63	619.92	590.60	561.84	679.27	648.63	619.92	590.60	561.84
Soybean meal	253.41	268.90	282.83	296.86	310.77	253.41	268.90	282.83	296.86	310.77
Soybean oil	0.00	15.00	30.00	45.00	60.00	0.00	15.00	30.00	45.00	60.00
Meat bone meal	35.67	36.64	36.54	36.44	36.33	35.67	36.64	36.54	36.44	36.33
Limestone	0.92	0.19	0.06	0.46	0.39	0.92	0.19	0.06	0.46	0.39
NaCl	3.48	3.47	3.48	3.49	3.50	3.48	3.47	3.48	3.49	3.50
DL-Methionine	1.83	1.92	2.01	2.11	2.20	1.83	1.92	2.01	2.11	2.20
L-Lysine	1.53	1.40	1.30	1.21	1.12	1.53	1.40	1.30	1.21	1.12
Threonine	0.54	0.50	0.50	0.50	0.49	0.54	0.50	0.50	0.50	0.49
Vitamin premix[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Zinc Bacitracin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salynomicin 12%	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride 60%	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Phytase[c]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Antioxidant[d]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Emulsifier[e]	0.00	0.00	0.00	0.00	0.00	0.35	0.35	0.35	0.35	0.35
Inert (kaolin)	20.00	20.00	20.00	20.00	20.00	19.65	19.65	19.65	19.65	19.65
Calculated composition										
AME, MJ/kg	12.62	12.90	13.19	13.46	13.75	12.62	12.90	13.19	13.46	13.75
CP	186.00	191.00	195.00	199.00	203.00	186.00	191.00	195.00	199.00	203.00
Crude fat[f], g/kg	35.28	47.43	62.17	75.30	95.30	35.28	47.43	62.17	75.30	95.30
Met, g/kg	4.51	4.64	4.76	4.89	5.01	4.51	4.64	4.76	4.89	5.01
Met/Cys, g/kg	6.94	7.11	7.26	7.42	7.57	6.94	7.11	7.26	7.42	7.57
Lys, g/kg	9.69	9.93	10.14	10.35	10.57	9.69	9.93	10.14	10.35	10.57

Ingredients (g/kg)	Finisher diets (35-42d)									
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Ca, g/kg	6.74	6.74	6.74	6.74	6.74	6.74	6.74	6.74	6.74	6.74
Available P, g/kg	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40
Na, g/kg	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80

[a]Composition per kg of feed: vitamin A, 9000UI; vitamin D3, 2500UI; vitamin E, 20UI; vitamin K3, 2500mg; vitamin B1, 1500mg; vitamin B2, 6000mg; vitamin B6, 3000mg; vitamin B12, 12000mcg; biotin, 60mg; folic acid, 800mg; nicotinic acid, 25000mg; pantothenic acid, 12000mg; selenium, 250mg.

[b]Composition per kg of feed: manganese, 160mg; iron, 100mg; zinc, 100 mg; copper, 20mg; cobalt 2mg; iodine, 2mg.

[c]Ronozyme Hiphos with 10,000 fungal phytase units/g (Novozymes A/S, Bagsvaerd, Denmark).

[d]Endox® 5X Concentrate Dry (Kemin Ltda, Indaiatuba, Brazil).

[e]Excential Energy Plus dosage at 0.35g/kg of feed (Orffa, Werkendam, the Netherlands).

[f]Analyzed.

Table 2 Growth performance of broilers fed corn-SBM-MBM meal diets with five levels of soybean oil and supplemented or not with emulsifier[a].

Oil Level (g/kg)	Emulsifier	Treatments	Day 14-21			Day 35-42		
			FI,g	BWG,g	FCR,g/g	FI,g	BWG,g	FCR,g/g
0	-[b]	T1	505	331	1.53	1087	566	1.92
	+[c]	T6	498	344	1.45	1076	575	1.87
15	-	T2	496	347	1.43	1099	584	1.88
	+	T7	492	353	1.39	1079	602	1.79
30	-	T3	502	366	1.37	1060	575	1.85
	+	T8	490	364	1.35	1096	619	1.77
45	-	T4	490	370	1.32	1068	639	1.68
	+	T9	496	376	1.32	1116	678	1.65
60	-	T5	480	376	1.28	1122	672	1.67
	+	T10	479	381	1.26	1122	700	1.60
	CV		3.16	3,31	2.31	4.74	5.83	3.51
Contrasts			*P*-value					
T1 vs T6			0.4285	0.0757	0.0001	0.7019	0.6745	0.1976
T2 vs T7			0.6315	0.3752	0.0546	0.4958	0.3978	0.0237
T3 vs T8			0.1591	0.7546	0.1641	0.2339	0.0434	0.0421
T4 vs T9			0.4954	0.4427	0.8420	0.1132	0.0625	0.4199
T5 vs T10			0.9411	0.4570	0.3167	0.9911	0.1840	0.0601
T1,T2,T3,T4,T5 vs T6,T7,T8,T9,T10			0.3527	0.0859	0.0003	0.4415	0.0048	0.0004

[a]Means were obtained from 6 replicate cages of 10 birds each
[b] – Represents without emulsifier supplementation
[c] + Represents supplementation with 0.35g/kg diet of emulsifier

Table 3 Coefficient of total tract apparent digestibility of dry matter, crude protein, ether extract and AMEn by broilers fed corn-SBM-MBM diets with five levels of soybean oil and supplemented or not with emulsifier[a].

Oil Level (g/kg)	Emulsifier	Treatments	Day 14-21				Day 35-42			
			DM	CP	EE	AMEn,MJ/kg	DM	CP	EE	AMEn,MJ/kg
0	-[b]	T1	0.718	0.637	0.706	12.56	0.738	0.618	0.733	12.79
	+[c]	T6	0.724	0.641	0.704	12.70	0.748	0.617	0.738	13.01
15	-	T2	0.724	0.633	0.759	13.32	0.742	0.628	0.815	13.11
	+	T7	0.732	0.645	0.769	13.50	0.753	0.639	0.813	13.32
30	-	T3	0.714	0.639	0.782	13.32	0.743	0.631	0.845	13.45
	+	T8	0.723	0.649	0.799	13.57	0.765	0.652	0.859	13.82
45	-	T4	0.717	0.629	0.810	13.57	0.746	0.650	0.866	13.85
	+	T9	0.729	0.649	0.826	13.85	0.760	0.648	0.882	14.17
60	-	T5	0.721	0.652	0.843	13.77	0.736	0.646	0.884	14.14
	+	T10	0.731	0.653	0.858	14.07	0.765	0.654	0.900	14.52
CV			1.87	3.37	1.87	1.59	2.02	3.44	1.66	1.75
Contrasts			*P*-value							
T1 vs T6			0.4017	0.7296	0.8774	0.1834	0.2731	0.9540	0.5013	0.1135
T2 vs T7			0.2890	0.3449	0.1986	0.1368	0.2112	0.3750	0.8475	0.1336
T3 vs T8			0.2589	0.4935	0.0539	0.0480	0.0171	0.1000	0.0719	0.0113
T4 vs T9			0.1020	0.1157	0.0646	0.0263	0.1097	0.8937	0.0490	0.0242
T5 vs T10			0.2154	0.9567	0.0879	0.0186	0.0019	0.5450	0.0459	0.0074
T1,T2,T3,T4,T5 vs T6,T7,T8,T9,T10			0.0101	0.1092	0.0040	<0.0001	<0.0001	0.1875	0.0062	<0.0001

[a]Means were obtained from 6 replicate cages of 10 birds each
[b] – Represents without emulsifier supplementation
[c] + Represents supplementation with 0.35g/kg diet of emulsifier