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## Effects of monoglycerides and lecithin on metabolizable energy and apparent total tract digestibility of diets in Hy-Line chicks

AliAkbar Salari<sup>1\*</sup>, Javad Ameri<sup>1</sup>, Ali Almamury<sup>2</sup>, Mohsen Teimouri<sup>1</sup>, Seyedmohammad Reza Salavati<sup>1</sup>, Seyedmohsen Nouri Hosseini<sup>1</sup>

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

<sup>2</sup>Department of Animal Production, College of Agriculture, Al-Qasim Green University, Babylon 51013, Iraq

\*Corresponding author,  
E-mail address:  
Salari.aliakbar@gmail.com

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### ORCID

AliAkbar Salari  
0009-0005-2963-5039  
Javad Ameri  
0009-0002-9254-3178  
Ali Almamury  
0009-0004-5854-0234  
Mohsen Teimouri  
0000-0001-5096-7613  
Seyedmohammad Reza Salavati  
0009-0000-5276-0448  
Seyedmohsen Nouri Hosseini  
0009-0000-7250-4388

**Abstract** Most emulsifiers are molecules with both hydrophilic and lipophilic properties that can interact with both oil and water, thereby stabilizing the mixtures and preventing their separation. Common emulsifiers used in the animal feed industry include lecithin, lysolecithin, mono- and diglycerides, and carrageenan. The objective of this investigation was to assess the effects of emulsifiers on gross energy (GE), apparent metabolizable energy (AME), nitrogen-corrected apparent metabolizable energy (AMEn) and apparent total tract digestibility (ATTD) in chick diets. A total of six hundred Hy-Line W-80 chicks at 12 weeks old was randomly assigned to ten test groups. Each group was composed of six replicates, with ten birds per cage. The study employed a completely randomized design with a 2×5 factorial arrangements, encompassing ten treatments. The treatments consisted of two levels of monoglycerides (0% and 0.05%, referred to as emulsifier A) and five levels of lecithin (0%, 0.03%, 0.04%, 0.05%, and 0.06%, referred to as emulsifier B). Hy-Line chicks fed diets supplemented with emulsifier B had significantly higher AME and AMEn compared with chicks offered the control diet. The levels of 0.04, 0.05 and 0.06% of emulsifier B improved the AME and AMEn. Linear and quadratic effects and the orthogonal contrast between the diets without and with emulsifiers B showed that addition of emulsifiers increased AME and AMEn in the diet. The interaction between emulsifiers A and B were observed on ATTD of Ca, P and ether extract (EE). The addition of emulsifiers A and B resulted in an increase in the ATTD of EE. In conclusion, the addition of lecithin at the 0.04% level can improve metabolizable energy levels by increasing fat digestibility.

**Keywords:** emulsifier, fat digestibility, lecithin, monoglycerides, chicks

## Introduction

Poultry serve as a major supplier of animal protein, significantly supporting public health for a continually expanding population. It functions as an inexpensive primary protein source for developing nations (Riaz et al., 2014). Alarming rising populations, disease outbreaks and quality ingredient scarcity are major obstacles to Iran's poultry industry, therefore, using alternate feeds and additives are imperative to boost poultry growth

(Bhatti, 2011).

Lipids in the form of oils and fats constitute highly energy-dense constituents of any feed, possessing the greatest magnitude of energy-yielding chemical bonds per unit mass (Leeson, 1993). The fat-soluble vitamins A, D, E, and K are decomposed, transported, and absorbed contingent on the presence of dietary lipids (Leeson, 1993). Moreover, bodily lipids safeguard against mechanical trauma, sustain body thermoregulation, biosynthesize hormones, and facilitate

accurate operation of the central neurological apparatus and muscle catabolism/anabolism (Bjorntorp, 1991).

Dietary lipids serve as the primary provider of crucial fatty acids that cannot be synthesized endogenously in the avian species. The inclusion of fat supplements in broiler diets has been recognized as a valuable strategy to enhance performance and achieve additional caloric benefits, enabling the fulfillment of requirements in rapidly growing chicks within a shorter time frame. This is attributed to the accelerated passage rate, as well as improved nutrient digestion and absorption in the intestinal tract (NRC, 1994).

Fat usage in poultry diets presents challenges in terms of the levels and digestibility. Digestibility varies with bird age, fat type, and source (Leeson, 1993). Higher fat levels can cause indigestion and the formation of insoluble calcium soaps, leading to calcium deficiency despite supplementation (Fedde et al., 1960; Whitehead et al., 1971; Whitehead and Fisher, 1975). Excessive fat inclusion results in reduced feed intake, lower weight gain, and economic losses, jeopardizing the bird health (Leeson, 1993). The expense associated with feed constituents, particularly fats and oils, indicates that supplying adequate energy in the diet can be quite demanding. This situation could lead to disregarding the inclusion of oil in diets, which can lead to reduced broiler performance (Classen, 2013). Thus, there is a need for a solution that enables efficient utilization of low rates of fats and easy digestion of higher rates without negatively impacting the bird performance. Fat emulsifiers can improve fat utilization in birds by overcoming the physiological constraints in the gastrointestinal tract, especially for the lipid digestibility (Zhao and Kim, 2017; Vinado et al., 2019). In poultry, fat digestion and/or absorption can be increased by emulsifiers such as bile salts, casein, sodium stearyl-2-lactylate, lecithin from soy (phospholipids), lysophospholipids, and glycerol polyethylene glycol ricinoleate (Siyal et al., 2017; Alagawany et al., 2018).

Phospholipids facilitate fat emulsification, optimize the action of lipases and stabilize the connection of fatty acids in micelles. This can lead to increased fat digestion and absorption, consequently improving the broiler growth performance (Dierick and Decuypere, 2004; Zavareie and Toghyani, 2018). Phospholipids in soy lecithin possess significant emulsifying and antioxidant features. Soy lecithin comprises 60% phospholipids (Araújo, 2008) which make it a surfactant and/or emulsifier (Huang et al., 2007; An et al., 2020; Robert et al., 2020). Phospholipids can improve the availability of AME and AMEn, when they are added to the broiler diets. In broilers, Zhang et al. (2011) showed that the addition of emulsifiers to the diet resulted in an improvement in fat digestion. Therefore, the inclusion of emulsifiers has emerged as a strategy to enhance the digestibility of lipid sources and enhance metabolizable energy, as well as maintaining optimal animal performance (Liu et al., 2020a; Liu et al., 2020b; Haetinger et al., 2021). When the oil concentration is

reduced with diet.

It is suggested that lecithin inclusion may enhance fatty acid (FA) digestibility without adversely affecting performance (Haetinger et al., 2021), but there is limited information on the impact of incorporating lecithin in Hy-Line chick diets. Hence, the primary objective of this study was to assess the impact of adding emulsifiers to the diet on GE, AME, AMEn, dry matter (DM), nitrogen retention (NR), acid insoluble ash (AIA), Ca, P, and ether extract (EE).

## Materials and methods

The experimental protocols were approved in accordance with the guidelines certified by the Iranian Council of Animal Care (1995).

### *Birds and housing*

Initially, 600 one-day-old Hy-Line chicks ( $35.1 \pm 9.2$  g) were sourced from a commercial hatchery (Morghak Co, Tehran, Bursa, Iran). Beak-trimming and vaccinated against Newcastle and Marek diseases had been performed at the hatchery. The chicks were housed in an environmentally controlled room with 10 chicks per cage ( $65 \times 123 \times 45$  cm); the cages were equipped with feeders and 3 drinker nipples. The room temperature was gradually reduced from  $34.0^\circ\text{C}$  to  $22^\circ\text{C}$  by the fifth week of age and afterwards kept at  $21^\circ\text{C}$ . The lighting schedule was 23 hours of light per day and gradually reduced to 12 hours by the sixth week. Standard commercial practices were followed in administering vaccinations against various diseases including infectious bronchitis disease, infectious bursal disease, Newcastle disease, coryza and fowl pox.

### *Experimental design*

The feeding regimen consisted of 3 conventional diets (based on the age category) consisting of cereals and soybean meal, provided at 1 to 21, 22 to 42, and 43 to 84 days of age, respectively. The diet for each period was formulated to meet the recommendations of the Hy-Line W-80 strain, as outlined in the management guide (Hy-Line, W-80., 2019). The experimental treatments were arranged as a  $2 \times 5$  factorial arrangements in a completely randomized design. There were two levels of monoglycerides (emulsifier A; 0% and 0.05%, monoglyceride powder, min.90% purity, Behin Kalaye Avid Co, Iran) and five levels of lecithin (emulsifier B; 0%, 0.03%, 0.04%, 0.05% and 0.06%, min 94.3% purity, Lipoid Co. Ltd., Ludwigshafen, Germany). There were six replicated cages containing ten birds each allocated for each experimental treatment. Feed and water were available *ad libitum*.

### *Apparent total tract digestibility (ATTD) of nutrients*

To determine the ATTD of nutrients, chromic oxide ( $\text{Cr}_2\text{O}_3$ ) at a level of 2 g/kg, was added to the experimental diets (Table 1). The experiment from days 84 to 91 consisted of a 4-day pre-experimental adaptation phase for the emulsifiers. Following the adaptation phase, a 3-day phase of excreta collection was conducted, during which data and samples were gathered for analysis. Excreta, free from feed and feather, were collected twice per day and stored at  $-20^\circ\text{C}$  for subsequent analysis. The excreta collected during the 3-day period were pooled resulting in six samples for each of the ten groups. The ATTD of nutrients was calculated using the formula outlined by Ege et al. (2019):

$$\text{ATTD (\%)} = 100 - [(\text{dietCr}_2\text{O}_3/\text{excretaCr}_2\text{O}_3) \times (\text{nutrient in excreta/nutrient in diet})] \times 100$$

The following formula used for computation of AME (Zavareie et al., 2018):

$$\text{AME (kilocalories per kilogram of diet)} = \text{GE}_{\text{diet}} - [\text{GE}_{\text{excreta}} \times (\text{marker}_{\text{diet}}/\text{marker}_{\text{excreta}})]$$

The AMEn was computed using the formula proposed by Huang et al. (2020) and applying a N-correction factor of 8.73 (Rochell et al., 2011):

$$\text{AMEn} = [\text{GE}_{\text{diet}} - \text{GE}_{\text{excreta}}] - [8.73 \times (\text{dietary N}_{\text{intake}} - \text{N}_{\text{excreta}})] / \text{feed intake}$$

**Table 1.** Ingredients chemical composition of corn-soybean meal

Ingredients	%
Corn	62.82
Soybean meal (CP 44%)	27.39
Soybean oil	3.00
Sand	2.60
Dicalcium phosphate	1.91
Limestone	1.14
Salt	0.20
DL- methionine	0.14
L- lysine hydrochloride	0.02
L-threonine	0.01
Vitamin premix <sup>1</sup>	0.25
Mineral premix <sup>1</sup>	0.25
$\text{NaHCO}_3$	0.27
<b>Calculated composition (%)</b>	
Metabolizable energy (kcal/kg)	2981
Crude protein	17.5
Calcium	0.95
Available phosphorous	0.45
Lysine	0.92
Methionine	0.42
Methionine + cystine	0.72
Threonine	0.67
Cystine	0.29
Tryptophan	0.24
Sodium	0.17
Chlorine	0.17
Potassium	0.73
DCAD (mEq)	215.62

<sup>1</sup>Provided per kg of diet: vitamin A, 4,403 IU; vitamin D3, 1,457 IU; vitamin E, 1.10 IU; menadione, 0.77 mg; vitamin B12, 4.40 µg; choline, 254.79 mg; niacin, 13.21 mg; pantothenic acid, 4.05 mg; riboflavin, 2.75 mg; Cu, 2.70 mg; Fe, 33.75 mg; I, 0.67 mg; Mn, 42.90 mg; Zn, 32.50 mg; Co, 0.17 mg.

## Chemical analysis

The amounts of DM (method 930.15, AOAC, 2007), ash and AIA (942.05, AOAC, 2007), N (method 990.03), Ca and P (method 985.01; AOAC, 2007) in samples of feed and excreta were quantified. The Fenton and Fenton (1979) method was used to quantify the amount of chromic oxide present in the samples of diet and excreta. The GE content in samples of feed and excreta was measured by an adiabatic bomb calorimeter (Parr 1266, Parr Instruments Co., and Moline, Illinois, US).

## Statistical analysis

The statistical model was as follows:

$$Y_{ijk} = \mu + A_i + B_j + (A \times B)_{ij} + e_{ijk}$$

in which

$Y_{ijk}$  is the individual observation;

$\mu$  is the experimental mean;

$A_i$  is the monoglycerides effect;

$B_j$  is the lecithin effect;

$(A \times B)_{ij}$  is the interaction effect between monoglycerides and lecithin effect;

$e_{ijk}$  is the error term with mean 0 and variance  $\sigma^2e$ .

Data were analyzed using the Proc GLM (SAS, 2002), and mean separation was performed using the Duncan's multiple range test at  $P < 0.01$ .

## Results

### Metabolizable energy

The influence of exogenous emulsifier supplementation on GE, AME and AMEn of the diet is shown in Table 2. The GE was not influenced by treatments in the study. Hy-Line chicks fed the diet containing emulsifier B (0.04, 0.05 and 0.06% lecithin) had significantly higher AME and AMEn ( $P < 0.01$ ) compared to the chicks fed the diet without emulsifier. The interaction effect of emulsifier blend ( $A \times B$ ) was not significant on AME and AMEn ( $P > 0.01$ ). The orthogonal contrast analysis between the diets without and with emulsifier B revealed that the addition of the emulsifier increased AME and AMEn. In addition, AME and AMEn levels were linearly and quadratically increased ( $P = 0.0001$ ,  $P = 0.0011$  and  $P = 0.0001$ ,  $P = 0.0021$  respectively) by addition of emulsifiers to diet.

### Nutrient digestibility

The ATTD of DM, N, ash and AIA was not affected by emulsifiers in chicks (Table 3). There was no interaction between emulsifiers A and B on ATTD of nitrogen retention, total ash and AIA. Supplementation of emulsifiers increased ( $P < 0.01$ ) the ATTD of EE, Ca and P. An interaction between emulsifiers A and B was observed on ATTD of Ca, P and EE.

Emulsifier supplementation did not significantly affect the ATTD of DM, N, AIA, and Ca. However, orthogonal comparisons revealed that the addition of emulsifiers resulted in a significant increase in EE digestibility

compared to diets without emulsifiers (A:  $P < 0.0001$ , B:  $P = 0.0003$ ). Furthermore, the comparison between the diets without emulsifier and emulsifier (B) showed improved P digestibility ( $P = 0.044$ ) at 88 to 91 days.

Linear increase in EE digestibility ( $P = 0.018$ ) and quadratic relationships for ATTD of DM, ash, P, and EE ( $P = 0.010$ ,  $P = 0.042$ ,  $P = 0.014$ ,  $P = 0.0005$ ) were observed at 88 to 91 days of age.

**Table 2.** Effects of monoglycerides and lecithin on metabolizable energy of the diet in young Hy-Line chicks

Items (%)	Observations	GE (kcal/kg)	AME (kcal/kg)	AMEn (kcal/kg)
<b>Emulsifier (A)</b>				
0	30	3382.12	2889.10	2855.04
0.05	30	3369.76	2885.49	2849.22
SEM		33.14	10.18	10.21
<b>Emulsifier (B)</b>				
0	12	3378.83	2815.80 <sup>b</sup>	2783.63 <sup>b</sup>
0.03	12	3357.94	2872.29 <sup>ab</sup>	2837.59 <sup>ab</sup>
0.04	12	3370.91	2928.10 <sup>a</sup>	2891.27 <sup>a</sup>
0.05	12	3386.80	2917.30 <sup>a</sup>	2879.83 <sup>a</sup>
0.06	12	3385.21	2902.12 <sup>a</sup>	2868.41 <sup>a</sup>
SEM		52.41	16.10	16.14
<b>Emulsifier (A) × Emulsifier (B)</b>				
0	6	3375.83	2798.50	2766.99
0.03	6	3378.66	2869.53	2836.68
0.04	6	3379.59	2902.43	2866.84
0.05	6	3386.83	2948.64	2912.10
0.06	6	3389.66	2926.56	2892.55
0	6	3381.83	2833.10	2800.12
0.03	6	3337.21	2875.23	2838.48
0.05	6	3362.21	2953.75	2915.69
0.05	6	3386.77	2885.94	2847.54
0.06	6	3380.75	2879.39	2844.27
SEM		74.12	22.77	22.83
			P-value	
Emulsifier (A)		0.793	0.803	0.689
Emulsifier (B)		0.995	0.0001	0.0001
Emulsifier (A) × Emulsifier (B)		0.998	0.062	0.066
Contrast 0 vs Emulsifier (A)		0.793	0.803	0.689
Contrast 0 vs Emulsifier (B)		0.951	0.0001	0.0001
Linear		0.802	0.0001	0.0001
Quadratic		0.833	0.0011	0.0021

<sup>a-b</sup> Within columns, means with common superscript(s) are not different ( $P > 0.05$ ).

Emulsifier (A): monoglycerides, Emulsifier (B): Lecithin

GE: Gross energy, AME: Apparent metabolizable energy, AMEn: Nitrogen-corrected apparent metabolizable energy

SEM: Standard error of the mean.

## Discussion

### Metabolizable energy

Lipids are included in animal feeds to serve as an energy source and also to aid in the regulation of feed moving through the gastrointestinal tract. This regulation helps improve the absorption of nutrients in animals. Fat digestibility is restricted by high levels of fatty acids. Emulsifiers aid in better emulsification and utilization of lipids, particularly animal fats (Upadhaya et al., 2017). Studies have shown that emulsifiers may directly modulate the interaction between lipid substrates and lipases, therefore, improved the digestion of lipids (Mun et al., 2007; Liang et al., 2018). An et al. (2020) showed that broilers fed with 0.1% or 0.2% exogenous emulsifiers compared to broilers fed the control diet exhibited a significant increase in GE digestibility. Also, other authors reported that emulsifiers improved the energy digestibility in broiler feed (Zhao and Kim, 2017). An emulsifier, such as glyceryl polyethylene glycol ricinoleate in broiler feed improved the GE digestibility and enhanced the broiler performance (Kaczmarek et

al., 2015). Some emulsifiers like lecithin are more unsaturated and contain high phospholipid molecules. Therefore, addition of lecithin may improve lipid utilization in the diet. Similar to the previous experiments, the results of this study also indicated that the metabolizable energy of Hy-Line chicks was increased by lecithin supplementation in the diet. Certain researchers reported that the improvement in energy digestibility through addition of emulsifiers was influenced by factors such as the structure, composition, and proportion of the fat source present in the diet (Zhang et al., 2011; Zaefarian et al., 2015; Zhao et al., 2015).

### Nutrient digestibility

Emulsifiers enhance the effectiveness of lipase function, aiding in the digestion and absorption of lipids (Maldonado-Valderrama et al., 2011; Majdolhosseini et al., 2019) contributed to increases in AME and AMEn. Lipids are better emulsified in the intestinal tract by oil-in-water emulsion, and increase their absorption level (Zhao and Kim, 2017). Lecithin is a strong surfactant,

which decreases the size of the emulsion droplets, and helps in more efficient mixing of the digesta with lipases in the intestine.

The findings of this study are consistent with previous research conducted by Ohtani et al. (2002), Maisonnier et al. (2003), Parsaie et al. (2007), Firman et al. (2008), Kil et al. (2010), and Cho et al. (2012), who reported that diets containing fat emulsifiers facilitated easier digestion of EE. However, Ferreira et al. (2005), Andreotti et al. (2004) and Guerreiro Neto et al. (2011) reported no significant changes in fat digestion in response to emulsifiers. These inconsistencies may be assigned to the specific types of emulsifiers incorporated into diets with varying fat content. Polin (1980) found that lecithin facilitated the fat digestion in the digestive tract. Also, Jansen (2015) showed that dietary supplementation with emulsifiers improved crude fat, DM, and AME digestion in birds. Huang et al. (2007) showed that lecithin (5 g/kg) added to a diet containing

15 g of soy oil facilitated the digestion of EE in 21-day-old birds, without affecting the nitrogen retention. The findings from that study are in line with our results that supplementation of emulsifiers increased the Ca, P and EE digestibility in chicks.

Our findings are consistent with the observations found by Huang et al. (2007), who reported that the utilization of calcium and phosphorus was significantly improved by 2% lecithin. Elevated levels of fats in poultry diets can lead to the formation of insoluble calcium soaps in the gut. This not only results in calcium deficiency but also reduces its availability for absorption by the bird (Abbas et al., 2016). Soap is formed by binding Ca with fatty acid molecules, the presence of undigested and unabsorbed fat in the gut can lead to the waste of both fatty acids and Ca (Tabeidian et al., 2010). It can be assumed that calcium and phosphorus digestibility were improved by preventing soap formation and increasing fat digestion.

**Table 3.** Effects of monoglycerides and lecithin on the apparent total tract digestibility of diet in young Hy-Line chicks

Items (%)	Observations	DM	N retention	Total ash	AIA	Ca	P	EE
Emulsifier (A)								
0	30	68.52	34.77	25.20	23.43	35.75	28.36	79.45
0.05	30	69.02	35.42	26.07	22.76	36.05	29.43	81.61
SEM		0.26	0.64	0.54	0.73	0.84	0.60	0.32
Emulsifier (B)								
0	12	68.14	34.35	24.40	23.19	34.52	27.12	78.74 <sup>b</sup>
0.03	12	69.02	35.02	24.82	22.40	34.54	29.19	80.54 <sup>ab</sup>
0.04	12	68.99	35.59	26.41	23.35	37.30	29.40	81.66 <sup>a</sup>
0.05	12	69.59	36.62	27.81	23.45	37.46	30.95	81.39 <sup>a</sup>
0.06	12	68.10	33.92	24.72	23.10	35.68	27.81	80.31 <sup>ab</sup>
SEM		0.41	1.02	0.92	1.16	1.33	0.95	0.51
Emulsifier (A) × Emulsifier (B)								
0	6	68.24	35.01	24.02	24.04	33.10 <sup>b</sup>	26.36 <sup>b</sup>	78.79 <sup>c</sup>
0.03	6	68.17	33.76	23.71	21.52	33.35 <sup>b</sup>	26.93 <sup>ab</sup>	78.99 <sup>c</sup>
0	6	68.33	34.79	24.52	21.97	40.15 <sup>ab</sup>	27.60 <sup>ab</sup>	79.17 <sup>c</sup>
0.05	6	69.73	37.01	28.07	26.42	33.88 <sup>b</sup>	31.68 <sup>a</sup>	80.51 <sup>bc</sup>
0.06	6	68.12	33.19	25.65	23.20	38.27 <sup>ab</sup>	29.20 <sup>ab</sup>	79.79 <sup>bc</sup>
0	6	68.05	33.69	24.78	22.34	35.95 <sup>ab</sup>	27.88 <sup>ab</sup>	78.69 <sup>c</sup>
0.03	6	69.86	36.27	25.93	23.28	35.72 <sup>ab</sup>	31.44 <sup>ab</sup>	82.08 <sup>ab</sup>
0.05	6	69.66	36.28	28.30	24.74	34.46 <sup>ab</sup>	31.19 <sup>ab</sup>	84.15 <sup>a</sup>
0.05	6	69.45	36.23	27.55	20.47	41.04 <sup>a</sup>	30.22 <sup>ab</sup>	82.27 <sup>ab</sup>
0.06	6	68.09	34.64	23.78	22.99	33.09 <sup>b</sup>	26.41 <sup>b</sup>	80.83 <sup>bc</sup>
SEM		0.58	1.44	1.30	1.64	1.88	1.35	0.73
P-value								
Emulsifier (A)		0.177	0.481	0.295	0.524	0.802	0.218	<0.0001
Emulsifier (B)		0.062	0.377	0.058	0.972	0.341	0.061	0.002
Emulsifier (A) × Emulsifier (B)		0.291	0.642	0.233	0.085	0.004	0.041	0.011
Contrast 0 vs Emulsifier (A)		0.177	0.481	0.295	0.524	0.802	0.218	<0.0001
Contrast 0 vs Emulsifier (B)		0.094	0.418	0.143	0.929	0.253	0.044	0.0003
Linear		0.705	0.821	0.221	0.815	0.219	0.306	0.018
Quadratic		0.010	0.107	0.042	0.996	0.219	0.014	0.0005

<sup>a-c</sup> Within columns, means with common superscript(s) are not different ( $P > 0.05$ ).

Emulsifier (A): monoglycerides, Emulsifier (B): Lecithin

DM: Dry matter, N retention: nitrogen Retention; AIA: acid insoluble ash; EE: Ether extract

SEM: Standard error of the mean.

## Conclusions

Lecithin supplementation has the potential to enhance the level of metabolizable energy in the bird by increasing fat digestibility. Lecithin supplementation (0.04%) increased the level of AME up 112 kcal/kg of the diet.

## Disclosure statement

The author(s) of this study reported no potential conflict of interest.

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