



# Emulsifiers in diets with energy reduction for laying hens

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**ABSTRACT.** Emulsifiers in animal diets are an interesting and effective strategy for nutritionists to improve the digestion of dietary lipids and, consequently, provide better performance. This study aimed to evaluate the effect of the supplementation of diets with emulsifier based on mono- and diglycerides (120 g t<sup>-1</sup> of feed) associated with different energy levels (2,775; 2,725; 2,675 and 2,625 kcal of metabolizable energy kg<sup>-1</sup> of feed) on performance and egg quality of Hisex Brown laying hens. The laying hens were distributed in 50 experimental plots with eight birds each. A completely randomized design with five treatments and ten replicates/treatments was used. The treatments in this study were: control diet (C) with 2,775 kcal of metabolizable energy (ME) kg<sup>-1</sup> of feed; C + E: diet C supplemented with an emulsifier (E) at 120 g t<sup>-1</sup> of feed; diet C with reduction of 50 kcal ME and supplemented with E (NC50 + E); diet C with reduction of 100 kcal ME and supplemented with E (NC100 + E); and diet C with reduction of 150 kcal ME and supplemented with E (NC150 + E). All diets had the same inclusion level of degummed soybean oil (2%). There was no influence of the treatments on the performance and quality of the eggs (p >0.05). The current study has shown that diets supplemented with emulsifiers based on mono- and diglycerides (120 g t<sup>-1</sup>) allows reducing the metabolizable energy level up to 150 kcal kg<sup>-1</sup> of feed, without impairment on performance and egg quality of Hisex Brown laying hens.

**Keywords:** additives; egg production; energy; poultry nutrition.

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## Introduction

The increase in population leads to a greater need for egg, milk, and meat production, which must meet the food safety of the consumer. Besides, these foods must be efficiently produced. There are several nutritional strategies to improve the production efficiency and performance of animals associated with sustainable production, in which the use of food additives is highlighted. In poultry feed, the use of additives is responsible for significant gains in productivity in the industry (Araújo, Silva, Lima Amâncio, Lima, & Lima, 2007; Moraleco et al., 2019). These products may refer to substances, microorganisms, or formulated products that belong to different categories, such as absorption promoters, prophylactic substances, auxiliary substances, and exogenous enzymes (Bertechini, 2012).

The fat emulsifier is an additive defined as a surface-active agent containing amphiphilic compounds of medium molecular weight (Araújo, 2008; Valentim et al., 2020). It has an amphiphilic nature, which facilitates the incorporation of free fatty acids released into the pancreatic digestion of dietary triglycerides into micelles, thus mediating the entire digestive process (Mandalawi, Mallo, Menoyo, Lázaro, & Mateos, 2017; Valentim et al., 2018).

In feed formulation, fats and oils are usually used as energy sources due to their high energy density. Energy is one of the most expensive components in diets for high-performing animals. Thereby, a diet formulated with a lower energy content implies in lower-cost price (Rovers & Excentials, 2014).

Emulsifiers increase the absorption and utilization of fats, improving fat digestibility in livestock and poultry, and consequently, energy efficiency. As a result, they contribute to a better growth performance (Zhao & Kim, 2017). Therefore, the use of nutritional emulsifiers may compensate for the reduction in dietary energy. In other words, low-density diets supplemented with emulsifiers seem to be a good strategy from an economical point of view, since they may reduce the food cost. Additionally, this strategy can also contribute to sustainable animal production.

Due to the improvement in the digestive process of dietary lipid sources by emulsifiers, the objective of this research was to evaluate the effect of supplementation of emulsifiers based on mono- and diglycerides in the diet of Hisex Brown laying hens with different energy levels on their performance and egg quality.

## Material and methods

The experiment was conducted in the Aviculture Sector of the Federal Institute of Minas Gerais – Bambuí Campus (IFMG – Campus Bambuí), with the approval of the Committee on Ethics in Animal Use, of the José do Rosário Vellano University (UNIFENAS), 13A/2015. A total of 400 commercial Hisex Brown hens at 25 weeks of age were distributed in 50 experimental units with 8 birds each, with an experimental period of 84 days (4 periods of 21 days).

A completely randomized design with 5 treatments and 10 replicates was used. The treatments were: control diet (C) with 2.775 kcal metabolizable energy (ME) kg<sup>-1</sup> of feed; diet C supplemented with an emulsifier (E) at 120 g t<sup>-1</sup> of feed (C + E); diet C with reduction of 50 kcal ME and supplemented with E with 2.725 kcal ME and 120 g t<sup>-1</sup> of feed of E (NC50 + E); diet C with reduction of 100 kcal ME and supplemented with E, with 2.675 kcal ME kg<sup>-1</sup> and 120 g t<sup>-1</sup> of feed of E (NC100 + E); diet C with reduction of 150 kcal ME and supplemented with E, with 2.625 kcal ME kg<sup>-1</sup> and 120 g t<sup>-1</sup> of feed of E (NC150 + E). The emulsifier was composed of mono- and diglycerides of fatty acids with guaranteed levels (minimum) of 100 g kg<sup>-1</sup>.

The diets were formulated according to the nutritional requirements of the Hisex Brown strain (Interaves, 2006), and are presented in Table 1.

**Table 1.** Percentage and estimated composition of the experimental rations for laying hens of the Hisex Brown commercial strain, in the period from 25 to 37 weeks of age, supplemented with emulsifier and energy reduction.

Ingredients (%)	Treatments				
	C	C+E	NC50+E	NC100+E	NC150+E
Corn	58.300	58.208	55.063	51.888	48.768
Soybean meal	27.500	27.600	26.600	25.600	24.800
Wheat bran	-	-	4.100	8.300	12.200
Degummed soybean oil	2.000	2.000	2.000	2.000	2.000
Limestone powder	4.350	4.350	4.400	4.400	4.450
Granulated limestone	4.600	4.600	4.600	4.600	4.600
Salt	0.430	0.430	0.430	0.420	0.420
Dicalcium phosphate	1.950	1.950	1.910	1.870	1.830
Emulsifier <sup>1</sup>	-	0.012	0.012	0.012	0.012
Inert	0.500	0.480	0.500	0.500	0.500
DL-Methionine	0.140	0.140	0.145	0.150	0.150
L-Lysine	0.030	0.030	0.040	0.060	0.070
Mineral Premix <sup>2</sup>	0.100	0.100	0.100	0.100	0.100
Vitamin Premix <sup>3</sup>	0.100	0.100	0.100	0.100	0.100
	Estimated composition (%)				
CP, %	17.000	17.016	17.000	17.000	17.068
Crude fiber, %	2.766	2.768	3.016	3.267	3.516
Ca, %	4.000	4.000	4.000	4.000	4.000
Total P, %	0.663	0.664	0.681	0.699	0.717
Available phosphorus	0.460	0.460	0.460	0.460	0.460
Na, %	0.180	0.180	0.180	0.180	0.180
Cl, %	0.295	0.295	0.294	0.294	0.293
ME, kcal kg <sup>-1</sup>	2,775	2,775	2,725	2,675	2,625
SID Lys <sup>4</sup> , %	0.840	0.840	0.840	0.840	0.840
SID Met, %	0.375	0.375	0.375	0.375	0.375
SID Met+Cis, %	0.612	0.612	0.612	0.611	0.612
SID Trp, %	0.191	0.191	0.190	0.189	0.189
SID Thr, %	0.581	0.582	0.573	0.564	0.558

<sup>1</sup> Supplementation of the emulsifier 120g t<sup>-1</sup> of feed. Composition: Mono- and diglycerides of fatty acids 10%, Silicon Dioxide 90%. <sup>2</sup> Mineral premix supplied (per kg of product): Copper (min) 7,000.0 mg; Iron (min) 50.0 g; Iodine (min) 1,500.0 mg; Manganese (min) 67.5 g; Zinc (min) 45.6 g. <sup>3</sup> Vitamin premix supplied (per kg of product): Folic acid (min) 145.0 mg; Pantothenic acid (min) 5,950.0 mg; Choline (min) 120.0 g; Niacin (min) 12.0 g; Selenium (min) 500.0 mg; Vitamin A (min) 5,000,000.0 IU; Vitamin B12 (min) 6,500.0 mcg; Vitamin B2 (min) 2,000.0 mg; Vitamin B6 (min) 250.0 mg; Vitamin D3 (min) 1,850,000.0 IU; Vitamin E (min) 4,500.0 IU; Vitamin K3 (min) 918.0 mg. <sup>4</sup> SID = standardized ileal digestibility.

All diets were isoproteic, isoaminoacidic, isocalcic, and isophosphoric and had the same inclusion level of degummed soybean oil (equal to 2%). Birds were fed *ad libitum* with a mashed feed containing corn and soybean meal.

The lighting was performed according to the recommendations of the strain manual, with delivery from 16h day<sup>-1</sup>. The maximum (late afternoon) and minimum (early morning) temperatures of the shed were daily recorded using a mercury thermometer and written down, generating the averages at the end of the study.

The percentage of egg production (EP) was estimated weekly, by two daily collections with all intact, cracked, broken, and thin bark eggs. The feed intake (FI) was measured weekly, as well as the average egg weight (AEW) weighing all the whole eggs. The percentage of viable eggs (PVE) was defined by the ratio of intact eggs (suitable for commercialization) over all eggs produced weekly. In the case of the death of one or more birds, a correction was made for the consumption and production of eggs, according to the methodology proposed by Sakomura and Rostagno (2007).

Feed conversion was assessed according to Valentim et al., (2018). The feed conversion by mass (FC m) was obtained by dividing the variable FI by the AEW multiplied by the EP. The feed conversion, per dozen (FC dz), was obtained by dividing the FI by the number of dozens produced during the week. The initial weight of the birds (IW) and the final weight of the birds (FW) were measured by weighing 50% of the birds of each plot, weighing three days before the start of the experiment and on the last day of the experiment.

The specific gravity (SG) was obtained according to Freitas Sakomura, Gonzalez, and Barbosa (2004) considering all intact eggs produced at the last two days every 21 days. A total of 8 evaluations were carried out throughout the study and the average of all these assessments was considered for statistical analysis. The assessment of the external and internal quality of the eggs was performed according to Valentim et al., (2018) at the last two days every 21 days, from two whole eggs collected per plot on each evaluation day (a total of 16 eggs evaluated per plot) during the 84 experimental days. The average of all these assessments was considered for statistical analysis.

The yolk color was made by a single evaluator in the laboratory under the same lighting conditions, from the comparison with the standard scale of the Yolk Color Fan colorimetric disc.

The egg quality was assessed according to Suk and Park (2001). Yolk percentage (YP) was obtained by the relation between the yolk weight (removing the chalazae) and the total egg weight. The shell percentage (SP) was obtained through the relation between the weight of the washed and air-dried shell, and the weight of the egg. The albumin percentage (AP) was obtained by the difference between the components of the heavy egg through the formula: AP: 100 - YP - SP. Shell thickness (ST) was assessed in the washed and dried shells, using a precision micrometer of 0.001 mm at three distinct points in the middle region of the eggshell, resulting in average thickness. The Haugh Unit (UH) was obtained according to Alleoni and Antunes (2001).

The homogeneity of the variances and normality of the studentized residuals of the data was verified previously. Data were submitted to analysis of variance and the means were compared by the Tukey test at 5% probability using the SISVAR statistical package (Ferreira, 2000).

## Results and discussion

During the experiment, the ambient temperature varied substantially, from 19.7 to 33.2°C characterizing the condition of daily ambient temperature variation that which laying hens are usually exposed in tropical climate areas.

There was no significant difference ( $p > 0.05$ ) for the analyzed performance variables, described in Table 2.

Taking into account the value of 94.91% production/hen/day for the production of eggs, in the current study, all treatments had a higher average of production than that indicated in the strain manual (Institut de Sélection Animale [Isa], 2016). The feed intake average of each of the treatments was lower than the consumption average indicated by the strain manual, for the evaluation period (108.30 and 112.38 g hen<sup>-1</sup> day<sup>-1</sup> for the consumption of the treatments and indicated in Isa (2016), respectively). ME intake was also lower when considering the values available at the Interaves strain manual (2006) with 297 and 314 kcal hen<sup>-1</sup> day<sup>-1</sup>, respectively. The reduction in energy consumption probably occurred due to the high temperatures during the experimental period, reaching an average maximum value of 31.7°C.

The maximum average temperature value is above the thermal comfort zone indicated by Yanagi, Xin, and Gates (2002), from 21 to 25°C. Thus, animals decrease their consumption, regulating energy intake. Energy reductions in the rations supplemented with emulsifiers did not affect the feed intake ( $p > 0.05$ ). According to Raber, Ribeiro, Kessler, and Arnaiz, (2009), monoglycerides are essential for the incorporation of insoluble fatty acids into the micellar complex. The emulsifier used in this research is based on this glyceride, which suggests an enhanced absorption of lipids from the diet and better use of fatty acids (Fonseca, Silva, Valentim, & Geraldo, 2018). Thus, there were no losses on the performance

of the hens and the egg quality. Also, the energy reduction levels of the diets may be insufficient to affect the consumption of the birds.

**Table 2.** Performance of laying hens Hisex Brown commercial strain submitted to diets with different energy levels and supplemented with commercial fat emulsifier at different levels.

Variables	Treatment					CV (%)	p - value
	C	C+E	NC50+E	NC100+E	NC150+E		
EP, % hen <sup>-1</sup> day <sup>-1</sup>	96.78	96.82	96.92	96.88	96.10	0.46	0.069
FI, g hen <sup>-1</sup> day <sup>-1</sup>	108.65	107.23	108.08	108.22	109.32	3.19	0.103
FCm, kg kg <sup>-1</sup>	1.91	1.90	1.88	1.910	1.950	5.64	0.170
FCdz, kg Dz <sup>-1</sup>	1.35	1.33	1.340	1.34	1.37	0.47	0.098
IW, g	1,929.90	1,929.10	1,894.80	1,932.50	1,894.40	4.30	0.083
FW, g	1,970.80	2,022.00	1,981.50	1,967.10	1,948.90	5.15	0.235

CV: Coefficient of variation; (p >0.05). Percentage of egg production (EP), feed intake (FI), feed conversion per egg mass (FC m), feed conversion per dozen eggs (FC dz), initial weight (IW), and final weight (FW).

FC m and FC dz were not affected by treatments (p >0.05) since they were estimated using the relationship between other variables, which showed no significant differences. The average body weight of birds was above the values of 1.722 g and 1.876 g at 25 and 37 weeks of age, respectively, according to Isa (2016). The energy reduction did not affect the final weight of the birds, indicating a better use of energy, possibly due to the addition of the emulsifier in the diets.

There were no significant differences (p >0.05) for any of the analyzed internal and external quality variables as shown in Table 3.

**Table 3.** Egg quality of semi-heavy Hisex Brown commercial strain fed diets with different energy levels, supplemented with commercial fat emulsifier at different levels.

Variables	Treatment					CV (%)	p - Value
	C	C+E	NC50+E	NC100+E	NC150+E		
AEW, g	58.74	58.42	59.31	58.52	58.43	2.52	0.153
PVE, %	99.65	99.65	99.72	99.54	99.66	0.06	0.226
SP, g mL <sup>-1</sup>	1.0925	1.0925	1.0919	1.0915	1.0915	0.13	0.360
ST, mm	0.398	0.398	0.398	0.399	0.393	2.46	0.177
YC	4.19	4.17	4.02	4.11	4.10	4.72	0.109
YP, %	23.62	24.08	23.63	24.06	24.18	2.6	0.258
SP, %	10.38	10.27	10.20	10.37	10.19	2.45	0.089
AP, %	66.00	65.66	66.17	65.64	65.62	1.02	0.096
HU	97.00	97.70	96.68	96.48	96.00	2.25	0.148

CV: Coefficient of variation; (p >0.05). Average weight of eggs (AWE), percentage of viable eggs (PVE), specific gravity (SG), shell thickness (ST), yolk color (YC), yolk percentage (YP), shell percentage (SP), albumen percentage (AP) and Haugh Unit (HU).

The egg weight did not differ statistically (p >0.05) among birds receiving different treatments. These results corroborate with the results found by D'Alfonso, Manbeck, and Roush (1996), in which a comparison between three levels of energy (2.580; 2.814, and 3.009 kcal kg<sup>-1</sup>) for a commercial strain of laying hens (Dekalb-XL) from 23 to 31 weeks of age did not find significant differences on egg weight.

On the other hand, the egg weight in the current study differs from the results found by Klementavičiūtė et al., (2016) and Silva, Fonseca, Valentim, and Geraldo, (2018). These authors evaluated a medium-chain fatty acid supplementation associated with soy lecithin-based emulsifier and observed a significant reduction in the average egg weight. The lack of effect on feed intake would justify the same egg weight in all treatments. Sulfur amino acids are directly related to egg mass.

According to Bertechini (2012), the reduction of methionine and cystine intake may affect egg weight. Considering the better use of fatty acids provided by the emulsifier supplementation and the same amino acid intake, the egg mass was similar among treatments, as expected, despite the reduction in ME. The high percentage of viable eggs indicated that the energetic reduction of diets supplemented with emulsifiers at the levels evaluated in this study did not affect the eggshell quality, considering the laying hens' daily nutritional requirement. Besides, adequate management of egg collection may be related to the high number of viable eggs obtained during the trial, since the egg collection was performed twice a day.

The specific gravity remained stable over the experiment. There was no significant difference (p >0.05) among treatments. These results agree with Silva et al., (2007), who evaluated three ME levels (280, 300, and

320 kcal bird<sup>-1</sup> day<sup>-1</sup>) and three oil consumptions per bird/day (0.00, 0.75, and 1.50 g), and did not find significant differences in the egg specific gravity, even at the daily intake level of 280 kcal hen<sup>-1</sup> day<sup>-1</sup>, which was lower than the average of the current experiment (297 kcal hen<sup>-1</sup> day<sup>-1</sup>).

Eggshell thickness is also related to its quality. This variable is highly dependent on the calcium and phosphorus balance of the diet and these minerals need to be well defined since they are indicative of the correct formulation of the diet. Yolk color did not vary among eggs of different treatments ( $p > 0.05$ ). We suggested that this fact occurred due to the supplementation with the emulsifier since the inclusion of corn was reduced as a consequence of the energy decrease. As this ingredient is rich in carotenoids and the natural pigmentation of the yolk is a reflection of the deposition of these carotenoids (Kljak, Drdić, Karolyi, & Grbeša, 2012), we may infer that they were better used with the supplementation of the emulsifying additive, in addition to their positive effect in digestion and lipid absorption.

Absorbed carotenoids are precursors of vitamin A, and the conversion occurs in the intestinal mucosa (Surai, Speake, & Sparks, 2001). A small intact amount of  $\beta$ -carotene escaping from the conversion is packed by the portomicrons into the enterocytes and released through the portal vein into the liver. In the liver, the metabolism of part of these portomicrons that form the modified lipoprotein for the deposition in the yolk (VLDL g) is directed to the oocyte in formation, pigmenting it (Rocha et al., 2013).

The components of eggs, yolk, eggshell, and albumen percentage did not differ ( $p > 0.05$ ) among treatments due to the average egg weight since they were not influenced by the different treatments. These variables are within the normal percentage of egg components that are approximately 65.82% albumen, 10.28% shell, 23.90% yolk.

The Haugh Unit was on average 96.77, with no difference ( $p > 0.05$ ) among treatments. The supplementation with emulsifiers associated with different levels of energy was not sufficient to affect the height of the dense albumen and egg weight. Since these variables are correlated to determine the Haugh Unit, the results obtained for Haugh Unit were above the minimum value of 72 recommended by *Egg - Grading manual* (United States Department of Agriculture [USDA], 2000),

Outcomes of the current study indicate that it is possible to reduce the metabolizable energy of the diet of Hisex Brown Laying hens when a nutritional emulsifier is provided. It was supported by the excellent egg quality and no impairment on bird's performance over the study. However, the decision of either supply or not supply diets should be based on economic criteria which include both the prices of feed ingredients and emulsifiers.

The nutritionist is responsible to analyze the food cost and make decisions to help producers to improve their profits. Due to the high price of feed ingredients, nutritional emulsifiers seem to help producers in reducing feed costs. A favorable scenario may occur when a low-density diet supplemented with emulsifiers has a lower price than a diet without reducing ME and with no emulsifier supplementation. Research investigating the effect of emulsifiers in low-density diets on the performance of laying hens is limited, so further studies are necessary.

## Conclusion

This study has shown that diets supplemented with emulsifiers based on mono- and diglycerides (120 g t<sup>-1</sup>) allows reducing the metabolizable energy level up to 150 kcal kg<sup>-1</sup> of feed, without impairment on performance and egg quality of Hisex Brown laying hens.

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