

# Increased metabolisable energy from glycerine or soybean oil in broilers<sup>1</sup>

## Energia metabolizável incrementada com glicerina ou óleo de soja para frangos

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**ABSTRACT** - The effect of increased levels of metabolisable energy with glycerine or soybean oil was evaluated in the performance of broilers at four growth stages (1 to 7, 8 to 21, 22 to 33 and 34 to 42 days), in addition to the carcass and cut yield. A total of 2,400 birds were used in a completely randomised design in a 2 x 4 factorial scheme (two sources x four energy levels), with five replications. The treatments were determined by including glycerine or soybean oil at intervals of 75 kcal/kg of metabolisable energy in the diet: the first level with 75 kcal less than the requirement, the second corresponding to the requirement for each stage (2925, 2980, 3050 and 3100 Kcal), and the third and fourth levels with 75 kcal and 150 kcal more than the required value for the stage under evaluation. The metabolisable energy requirement for broilers, irrespective of the energy source (glycerine or soybean oil), was estimated at 3,075, 3.130, 3,200 and 3,250 kcal ME/kg feed, for stages 1 to 7, 8 to 21, 22 to 33 and 34 to 42 days respectively. However, the use of glycerine as a source of increased energy promoted better performance efficiency during the first two stages (1 to 7 and 8 to 21 days of age).

**Key words:** Poultry farming. Fat. Energy sources. Performance.

**RESUMO** - Avaliou-se o efeito dos níveis de energia metabolizável incrementados com glicerina ou óleo de soja sobre o desempenho de frangos de corte em quatro fases (1 a 7; 8 a 21; 22 a 33 e 34 a 42 dias), e o rendimento de carcaça e cortes. Foram utilizadas 2400 aves com delineamento inteiramente casualizado, em esquema fatorial (duas fontes x quatro níveis de energia), com cinco repetições. Os tratamentos foram estabelecidos por meio da inclusão da glicerina ou do óleo de soja, em intervalos de 75 kcal/kg de energia metabolizável da dieta, o primeiro nível com 75 kcal abaixo da exigência, o segundo corresponde à exigência de cada fase (2925, 2980, 3050 e 3100 Kcal) o terceiro e o quarto nível com 75 kcal e 150 kcal, acima do valor da exigência para a fase avaliada. A exigência de energia metabolizável para frangos de corte, independente da fonte de incremento energético (glicerina ou óleo de soja), foi estimada em 3.075; 3.130; 3.200 e 3.250 kcal de EM/kg de ração, para as fases de 1 a 7; 8 a 21; 22 a 33; 34 a 42 dias, respectivamente. Entretanto, a utilização de glicerina como fonte de incremento energético, promoveu melhor eficiência no desempenho nas duas primeiras fases (1 a 7 e 8 a 21 dias de idades).

**Palavras-chave:** Avicultura. Gordura. Fontes de energia. Desempenho.

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## INTRODUCTION

The chemical process of converting vegetable oils into biodiesel involves methyl or ethyl transesterification reactions of triacylglycerides, which generate methyl or ethyl esters as products, and glycerol as residue (QUINTELLA *et al.*, 2009). Considering that the glycerine purification process is quite expensive, and that any surplus from biodiesel production is not fully utilised, animal production would appear to be a promising field for the use of glycerine as an energy source in diets (SUCHÝ *et al.*, 2011). Crude glycerine from the biodiesel process includes glycerol in its composition, a molecule with well-established glycogenic properties (MIN *et al.*, 2010).

Food intake increases metabolism, raising the amount of body heat, since digestion and nutrient absorption generate energy, which is released in the form of heat. In addition, broilers submitted to temperatures above the thermoneutral zone lower their capacity for dissipating heat, and use such strategies as reducing their feed intake to reduce internal heat production caused by the ingestion of energy present in the feed (COSTA *et al.*, 2012). Authors such as Silva *et al.* (2015) found that broilers subjected to thermal discomfort (38-40 °C) reduced their feed intake, weight gain and feed conversion by on average 11.3, 4.6 and 1.4% respectively.

A practical solution for meeting the energy needs of animals, and for minimising the production of heat generated by food intake, is related to the inclusion of ingredients in the diet that result in a low caloric increase (energy spent on digestion and metabolic processes). From this perspective, the use of oils and fats with a caloric increase coefficient of 0.16%, less than carbohydrates and proteins, is important (BERTECHINI, 2012). The low molecular weight of glycerol allows it to be absorbed more easily by diffusion in the enterocytes, where it is oxidised for energy production via glycolysis and the Krebs cycle (BERNARDINO *et al.*, 2013). Although no studies have been found on the actual caloric increase from glycerol, it is believed that glycerol can be included in diets as an ingredient for this purpose, since besides being easily absorbed, it presents a high coefficient of metabolisable energy, as found by Rostagno *et al.* (2017).

As such, the aim of this study was to evaluate the effect of two different sources of energy (glycerine and soybean oil), different levels of ME in the diet, and their interactions (source x energy level of the diet) on performance, and carcass and main cut yield in broilers.

## MATERIAL AND METHODS

The experiment was conducted in the poultry sector of the Bom Jesus Agricultural College (CABJ),

on the Professor Cinobelina Elvas Campus (CPCE) of the Federal University of Piauí (UFPI), in Bom Jesus in the State of Piauí (PI), with the approval of the Ethics Committee for Animal Experimentation of UFPI, under protocol no. 22/2009-CEEA/UFPI. A total of 600 male Cobb broilers were used for each stage of the evaluation (1 to 7, 8 to 21, 22 to 33 and 34 to 42 days of age), giving a total of 2,400 birds. At the beginning of each stage the birds were weighed and divided into experimental units; animals that were not being used were kept in a separate shed, where they were raised and fed following the recommendations of the breed handbook.

The design was completely randomised in a 2 x 4 factorial scheme (two sources x four energy levels), with five replications of 15 birds per experimental unit (coop). The birds were housed in coops of 2 m<sup>2</sup>, containing tubular feeders and hanging drinkers. The four energy levels of each stage were determined by the addition of glycerine or soybean oil at intervals of 75 kcal/kg of metabolisable energy in the diet: the first level being 75 kcal below the requirement recommended by Rostagno *et al.* (2011), the second corresponding to the requirement for each stage (2,925, 2,980, 3,050 and 3,100 kcal/kg feed for stages 1 to 7, 8 to 21, 22 to 33 and 34 to 42 days respectively), and the third and fourth levels with 75 kcal and 150 kcal respectively more than the value required by the birds for the stage under evaluation.

The experimental diets were formulated to meet the nutritional requirements of the chickens at each stage of the study, as per the recommendation of Rostagno *et al.* (2011) (Tables 1 and 2), except for the metabolisable energy, that varied according to the treatments being evaluated, and for the level of sodium, that was 2.91% and 4.87% higher than required during the 3rd and 4th stage respectively; as the higher levels of glycerine increased the sodium content of the feed, the same value was maintained for each of the other diets.

The glycerine used was supplied by ADM, and comprised 80.95% glycerol, 30 ppm methanol, 2.29% sodium, 10.65% moisture and 3,585 kcal ME/kg feed. The composition of the ingredients used in the formulations were as presented by Rostagno *et al.* (2011), with the Na and GE content of the glycerol being analysed. The ME value of the glycerine was obtained based on the metabolisable energy coefficient of the gross energy recommended by Rostagno *et al.* (2011), multiplied by the value for gross energy analysed in the animal nutrition laboratory of CCA-UFPI.

The temperature and relative humidity of the sheds were monitored by means of a maximum and minimum thermometer kept in the centre of the shed, where the thermometer was read twice a day (08:00 and 16:00).

Feed intake (FI) was evaluated for each stage, calculated from the difference between the amount of feed supplied and the leftovers of the experimental diets. Weight gain (WG) was determined from the difference in

the weight of the birds at the start and at end of each growth stage. From the data for feed intake and weight gain, the feed conversion was calculated. At the end of the third and fourth stages (at 33 and 44 days of age), two birds from

**Table 1** - Percentage and calculated composition of the experimental diets used in stages 1 to 7 and 8 to 21 days of age

Ingredient	Stages							
	1 to 7 days of age				8 to 21 days of age			
	Glycerine		Soybean oil		Glycerine		Soybean oil	
	2850	3075	2850	3075	2.905	3.130	2.905	3.130
Maize grain	55.486	42.278	56.092	52.213	61.297	48.324	61.287	58.178
Soybean meal, 45%	36.889	19.238	37.721	32.559	32.607	15.270	32.620	28.481
Extruded whole soybean	1.155	25.949	-	7.268	-	24.355	-	5.827
Soybean oil	-	-	0.853	3.412	-	-	0.853	3.412
Glycerine	2.090	8.360	-	-	2.090	8.360	-	-
Dicalcium phosphate	1.910	1.908	1.910	1.910	1.490	1.488	1.489	1.489
Calcitic limestone	0.913	0.886	0.914	0.907	1.058	1.030	1.058	1.050
Sand	-	-	0.773	-	0.071	-	1.128	-
Methionine MHA	0.417	0.435	0.417	0.421	0.328	0.345	0.328	0.332
L-Lysine, 79%	0.289	0.255	0.290	0.280	0.237	0.204	0.238	0.230
Common salt	0.248	-	0.248	0.253	0.229	0.009	0.229	0.234
Mineral vitamin supplement <sup>1</sup>	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Ammonia chloride	-	0.240	-	-	-	0.215	-	-
Sodium bicarbonate	0.203	0.051	0.382	0.377	0.193	-	0.370	0.367
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Nutritional Composition								
AME (Mcal/Kg)	2.850	3.075	2.850	3.075	2.905	3.130	2.905	3.130
Crude Protein (%)	22.000	22.000	22.000	22.000	20.000	20.000	20.000	20.000
Lysine Dig. (%)	1.304	1.304	1.304	1.304	1.141	1.141	1.141	1.141
Met.+Cyst. Dig. (%)	0.939	0.939	0.939	0.939	0.822	0.822	0.822	0.822
Threonine Dig. (%)	0.743	0.747	0.743	0.744	0.677	0.679	0.677	0.678
Calcium (%)	0.920	0.920	0.920	0.920	0.860	0.860	0.860	0.860
Available P (%)	0.470	0.470	0.470	0.470	0.384	0.384	0.384	0.384
Potassium (%)	0.854	0.900	0.853	0.866	0.774	0.819	0.774	0.785
Sodium (%)	0.220	0.220	0.220	0.200	0.210	0.210	0.210	0.210
Chlorine (%)	0.200	0.200	0.200	0.200	0.190	0.190	0.190	0.190
Linoleic Acid (%)	1.499	3.488	1.867	3.832	1.461	3.414	1.921	3.770
Electrolytic balance (mEq/Kg)	262	274	262	256	240	251	240	243

Pre-initial stage: <sup>1</sup>Guarantee levels per kg of product: folic acid 200 (mg); pantothenic acid 3,120 (mg); antioxidant additive 25,000 (mg); biotin 10,000 (mg); copper 2,000 (mg); choline 78,102.01 (mg); iron 11,250 (mg); halquinol 7,500 (mg); iodine 187.50 (mg); manganese 18,738.60 (mg); monensin 25,000 (mg); niacin 8,400 (mg); selenium 75 (mg); vitamin A 1,680,000 (IU); vitamin B1 436.50 (mg); vitamin B2 1,200 (mg); vitamin B6 624 (mg); vitamin B12 2,400 (mg); vitamin D3 400,000 (IU); vitamin E 3,500 (mg); vitamin K3 360 (mg); zinc 17,500 (mg); Initial stage: <sup>1</sup>Guarantee levels per kg of product: folic acid 162.50 (mg); pantothenic acid 2,600 (mg); antioxidant additive 25,000 (mg); copper 2,000 (mg); choline 71,593.49 (mg); iron 11,250 (mg); halquinol 7,500 (mg); iodine 187.50 (mg); manganese 18,750 (mg); niacin 7000 (mg); selenium 75 (mg); vitamin A 1,400,000 (IU); vitamin B1 388 (mg); vitamin B2 1,000 (mg); vitamin B6 520 (mg); vitamin B12 2,000 (mg); vitamin D3 300,000 (IU); vitamin E 2,500 (mg); vitamin K3 300 (mg); zinc 17,500 (mg)

**Table 2** - Percentage and calculated composition of the experimental diets used in stages 22 to 33 and 34 to 42 days of age

Ingredient	Stages							
	22 to 33 days of age				34 to 42 days of age			
	Glycerine		Soybean oil		Glycerine		Soybean oil	
	2.975	3.200	2.975	3.200	3.025	3.250	3.025	3.250
Maize grain	63.762	49.908	65.237	60.488	67.700	50.500	68.934	64.427
Soybean meal, 45%	27.939	9.570	29.927	23.610	25.000	2.000	26.667	20.672
Extruded whole soybean	2.781	28.700	-	8.896	2.329	34.650	-	8.442
Soybean oil	-	-	0.853	3.412	-	-	0.853	3.412
Glycerine	2.090	8.360	-	-	2.090	8.36	-	-
Dicalcium phosphate	1.243	1.240	1.243	1.243	1.027	1.025	1.027	1.026
Calcitic limestone	0.936	0.908	0.939	0.930	0.830	0.794	0.832	0.823
Sand	-	0.295	0.200	-	-	1.895	0.484	-
DL-Methionine	0.242	0.257	0.240	0.246	0.211	0.231	0.210	0.215
L-Lysine, 79 %	0.190	0.152	0.194	0.180	0.192	0.147	0.195	0.183
Common salt	0.213	-	0.085	0.218	0.195	-	0.193	0.200
Mineral vitamin supplement <sup>1</sup>	0.400	0.400	0.400	0.400	0.200	0.200	0.200	0.200
Ammonia chloride	-	0.210	0.113	-	-	0.198	-	-
Sodium bicarbonate	0.204	-	0.569	0.377	0.226	-	0.405	0.400
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Nutritional Composition								
AME (Mcal/Kg)	2.975	3.200	2.975	3.200	3.025	3.250	3.025	3.250
Crude Protein (%)	19.00	19.028	19.000	19.000	17.800	17.798	17.800	17.800
Lysine Dig. (%)	1.045	1.045	1.045	1.045	0.969	0.969	0.969	0.969
Met.+Cyst. Dig. (%)	0.763	0.763	0.763	0.763	0.707	0.707	0.707	0.707
Threonine Dig. (%)	0.646	0.649	0.646	0.647	0.604	0.607	0.604	0.605
Calcium (%)	0.750	0.750	0.750	0.750	0.650	0.650	0.650	0.650
Available P (%)	0.335	0.334	0.335	0.335	0.290	0.290	0.290	0.290
Potassium (%)	0.741	0.790	0.736	0.753	0.692	0.751	0.687	0.703
Sodium (%)	0.206	0.206	0.206	0.206	0.205	0.205	0.205	0.205
Chlorine (%)	0.180	0.180	0.180	0.180	0.170	0.170	0.170	0.170
Linoleic Acid (%)	1.735	3.813	1.973	4.068	1.740	4.333	2.014	4.073
Electrolytic balance (mEq/Kg)	232	245	231	235	222	237	221	225

Growth Stage; <sup>1</sup>Guarantee levels per kg of product: folic acid 162.50 (mg); pantothenic acid 2,600 (mg); antioxidant additive 25,000 (mg); copper 2,000 (mg); choline 71,593.49 (mg); iron 11,250 (mg); halquinol 7,500 (mg); iodine 187.50 (mg); manganese 18,750 (mg); niacine 7,000 (mg); selenium 75 (mg); vitamin A 1,400,000 (UI); vitamin B1 388 (mg); vitamin B2 1,000 (mg); vitamin B6 520 (mg); vitamin B12 2,000 (mg); vitamin D3 300,000 (UI); vitamin E 2,500 (mg); vitamin K3 300 (mg); zinc 17,500 (mg); Slaughter:<sup>1</sup> Guarantee levels per kg of product: pantothenic acid 3,536.00 (mg); copper 3,250.00 (mg); choline 117,15 (mg); iron 20.00 (g); iodine 400 (mg); manganese 30.00 (g); niacine 10.21 (g); selenium 100.00 (mg); vitamin A 982,500.00 (UI); vitamin B12 2,357.00 (mg); vitamin B2 1,212.00 (mg); vitamin D3 25,000.00 (UI); vitamin E 2,750.00 (UI); vitamin K3 275.00 (mg); zinc 25.00 g

each coop (mean weight  $\pm$  10%) were slaughtered after a 12-hour fast to evaluate the carcass and cut yields.

The data were evaluated for homoscedasticity and normality, and identified outliers were removed. The data

were then submitted to analysis of variance by the GLM procedure of the SAS (Statistical Analysis System, 9.2) software (SAS INSTITUTE, 2002). The mean values were compared by the SNK test at 5% significance, and polynomial regression models were used to explore the

effects of the increasing levels of AME from the feed, considering a significance level of 5%.

## RESULTS AND DISCUSSION

According to Abreu and Abreu (2011), the mean values for temperature and relative humidity (Table 3) indicate that the birds underwent thermal discomfort during the growth stages. Under such conditions, the physiological effort of the animals to maintain their body temperature within the thermoneutral zone is greater, altering their behaviour, and causing metabolic changes and changes to the heat-dissipation mechanism (SHINI *et al.*, 2008), resulting in energy usage being directed away from productive development to maintaining temperature.

For the variable of weight gain (WG) during the 1 to 7-day stage, there was an interaction ( $P < 0.05$ ) between the source of increased energy and the levels of metabolisable energy (ME). WG in the birds consuming diets with added glycerine at 2,850 kcal ME/kg feed was lower than the WG provided by the diet with added soybean oil at the same energy level, the opposite occurring at the level of 3,075 kcal ME/kg feed (Table 4). Xavier *et al.* (2008), working with increasing levels of ME during the 1 to 7-day stage, did not observe any significant effects from the pre-initial feed on WG. The differences seen between sources may be related to metabolism of the glycerine and oil. Dozier *et al.* (2008) stated that young birds metabolise the energy from glycerol better than do older birds. However, in the bird organism, the oil may display the extra-caloric effect that reflects the supply and utilisation of fatty acids for non-energy functions, with an improvement in the absorption of some fat-soluble vitamins; this does not happen with glycerine, which consists mainly of glycerol.

Feed conversion (FC) during the 1 to 7-day stage showed a linear effect ( $Y = 1.86 - 0.0003ME$ ,  $r^2 = 0.55$ ). For each unit of ME added to the feed a potential reduction of 0.0003 g feed/g weight gain was found for FC. The best feed conversion can be attributed to an increase in caloric density, the extra caloric and metabolic effect of fat, which results in an improvement in energy efficiency

by increasing the net energy of the feed (SAKOMURA *et al.*, 2004).

In relation to the effect of the sources of increased metabolisable energy in the diet, a difference ( $P < 0.05$ ) was seen for FC only (Table 4), where the birds that received diets with added glycerine had a 3.5% better FC than those fed on soybean oil.

During the pre-initial stage of the broiler, feed intake is low compared with the other stages, accounting for 3.5% of the total (DALLMANN *et al.*, 2010). The low intake justifies the use of ingredients of high digestibility in formulating diets to favour the use of nutrients, because at this stage of life, the birds are not fully adapted to ingesting carbohydrates or lipids (OLIVEIRA *et al.*, 2009).

A quadratic effect was seen for FI on the levels of metabolisable energy during the 8 to 21-day stage ( $P < 0.05$ ) ( $FI = 16051 - 9.8058 ME + 0.0016 ME^2$ ,  $r^2 = 0.98$ ), with the lowest intake achieved using feed containing 3.064 kcal ME/kg (Table 5). These results are similar to those found by Alvarenga *et al.* (2011), who found a reduction in the intake ( $P < 0.05$ ) of birds from 8 to 21 days with increases in the energy level of the diet, whereas Bernardino *et al.* (2014) found no interaction between the levels and sources of glycerine for feed intake.

An interaction ( $P < 0.05$ ) was seen between the sources of increased energy and the levels of metabolisable energy (ME) for the variables WG and FC during the evaluation stage (8 to 21 days). WG in the birds fed diets with added glycerine was better than in the diets with soybean oil at a level of 3.130 kcal ME/kg feed.

When evaluating the increase in metabolisable energy of the diets separately (by source), a linear increase was found for WG according to the equations for glycerine ( $WG_g = -285.53 + 0.2816 ME$ ,  $r^2 = 0.91$ ) and soybean oil ( $WG_o = 224.95 + 0.1079 ME$ ,  $r^2 = 0.62$ ), where for each additional unit of ME in the feed an increase of 0.2816 g in weight gain/bird was seen for glycerine and 0.1079 g in weight gain/bird for soybean oil.

The rise in the levels of ME in the diets for both sources of increased energy (glycerine x soybean oil) resulted in a linear decrease in FC according to the equations for

**Table 3** - Maximum and minimum ambient temperature and relative humidity (mean) for the different growth stages

Stage	Max. Temp. (°C)	Min. Temp. (°C)	Relative Humidity (%)
1-7 days	35.43	23.39	65.18
8-21 days	33.40	23.62	70.54
22-33 days	34.80	22.86	69.42
34-42 days	35.04	22.64	69.11

**Table 4** - Effect of energy levels with added glycerine or soybean oil (Soy Oil) on feed intake (FI), weight gain (WG) and feed conversion (FC) during the 1 to 7-day stage

Variable	Source	ME Level (kcal/kg)				Mean	CV (%)	P>F		
		2850	2925	3000	3075			Source	ME	SxME
FI (g)	Glycerine	146.16	142.15	141.49	136.90	141.67	4.51	0.231	0.057	0.982
	Soy Oil	148.10	143.74	144.33	140.66	144.20				
	Mean	147.13	142.94	142.75	138.78					
WG (g)	Glycerine	123.9 b	130.2 a	131.8 a	128.9 a	128.75	3.68	0.073	0.048	0.033
	Soy Oil	128.2 a	124.5 a	130.2 a	120.8 b	125.73				
	Mean	126.11	127.41	131.10	124.89					
FC (g)	Glycerine	1.17	1.09	1.07	1.06	1.10 a	4.60	0.012	0.024	0.063
	Soy Oil	1.15	1.15	1.10	1.16	1.14 b				
	Mean <sup>1</sup>	1.16	1.12	1.08	1.11					

Mean values with the same lowercase letter in a column do not differ statistically by the SNK test ( $P < 0.05$ ).<sup>1</sup>Linear effect ( $FC = 1.86 - 0.0003ME$ ,  $r^2 = 0.55$ )

glycerine ( $FC_g = 5.44 - 0.0013 ME$ ,  $r^2 = 0.91$ ) and soybean oil ( $FC_o = 3.87 - 0.0008 ME$ ,  $r^2 = 0.93$ ) (Table 5), where each additional unit of ME in the feed corresponded to a reduction of 0.0013 g of feed/g of weight gain for glycerine and 0.0008 g feed/g weight gain for the soybean oil.

Nascimento *et al.* (2004) evaluated different energy levels (2,850, 3,000 and 3,150 kcal AME/kg feed) and ME to CP ratios (125, 136.9 and 151.5 kcal/% CP) during the initial stage of broilers, and found that a level of 3,150 kcal ME/kg feed gave the best results for feed conversion, similar to the values found in this study. The gradual increase in net energy of the feed due to the reduction in caloric increase explains the results for feed conversion (OLIVEIRA *et al.*, 2000).

An interaction ( $P < 0.05$ ) between the sources of increased energy and the levels of metabolisable energy (ME) was seen for the 22 to 33-day stage for feed intake (FI) (Table 6). The birds that ingested the diet with glycerine showed a higher FI than those on the diet with soybean oil at levels of 3,050 and 3,125 kcal ME/kg feed.

When considering energy levels, a quadratic effect was seen for the sources of increase of the metabolisable energy in the diet, as per the equations for glycerine ( $CR_g = -42006 + 28.673 ME - 0.0047 ME^2$ ,  $r^2 = 0.91$ ), with the maximum value for intake occurring at the level of 3,050 kcal ME/kg feed; this value was close to the lowest evaluated energy level for the stage. There was also a

**Table 5** - Effect of energy levels with added glycerine or soybean oil on feed intake (FI), weight gain (WG) and feed conversion (FC) during the 8 to 21-day stage

Variable	Source	ME Level (kcal/kg)				Mean	CV (%)	P>F		
		2905	2980	3055	3130			Source	ME	SxME
FI (g)	Glycerine	906.00	872.69	847.92	841.38	867.00 a	2.47	0.031	<0.0001	0.707
	Soy Oil	888.96	858.10	821.40	837.83	853.16 b				
	Mean <sup>1</sup>	897.48	865.40	836.14	837.61					
WG (g)	Glycerine <sup>2</sup>	524.80 a	564.40 a	576.40 a	591.20 a	564.20	3.22	0.025	<0.0001	0.014
	Soy Oil <sup>3</sup>	546.00 a	537.80 a	549.75 a	569.00 a	550.68				
	Mean	535.40	551.10	564.55	580.10					
FC (g)	Glycerine <sup>4</sup>	1.72 a	1.54 a	1.47 a	1.42 a	1.54	3.69	0.758	<0.0001	0.021
	Soy Oil <sup>5</sup>	1.63 a	1.59 a	1.49 a	1.47 a	1.55				
	Mean	1.67	1.57	1.48	1.44					

Mean values with the same lowercase letter in a column do not differ statistically by the SNK test ( $P < 0.05$ ).<sup>1</sup>Quadratic effect ( $FI = 16051 - 9.8058ME + 0.0016 ME^2$ ,  $r^2 = 0.98$ ); <sup>2</sup>Linear effect ( $WG_g = -285.53 + 0.2816ME$ ,  $r^2 = 0.91$ ); <sup>3</sup>Linear effect ( $WG_o = 224.95 + 0.1079ME$ ,  $r^2 = 0.62$ ); <sup>4</sup>Linear effect ( $FC_g = 5.44 - 0.0013ME$ ,  $r^2 = 0.91$ ); <sup>5</sup>Linear effect ( $FC_o = 3.87 - 0.0008ME$ ,  $r^2 = 0.93$ )

**Table 6** - Effect of energy levels with added glycerine or soybean oil on feed intake (FI), weight gain (WG) and feed conversion (FC) during the 22 to 33-day stage

Variable	Source	ME Level (kcal/kg)				Mean	CV (%)	P>F		
		2975	3050	3125	3200			Source	ME	SxME
FI (g)	Glycer. <sup>1</sup>	1673.50 a	1720.60 a	1661.20 a	1602.50 a	1667.39	2.23	0.023	0.002	0.002
	Soy Oil <sup>2</sup>	1683.80 a	1623.60 b	1604.60 b	1631.20 a	1635.80				
	Mean	1679.22	1672.10	1632.90	1618.44					
WG (g)	Glycerine.	919.25	998.00	990.80	993.25	977.44	3.91	0.133	0.059	0.168
	Soy Oil	950.80	963.60	955.20	955.60	956.30				
	Mean	936.78	980.80	973.00	972.33					
FC (g)	Glycerine	1.82	1.72	1.67	1.61	1.70	3.94	0.970	0.001	0.118
	Soy Oil	1.77	1.68	1.67	1.71	1.71				
	Mean <sup>3</sup>	1.79	1.70	1.67	1.66					

Mean values with the same lowercase letter in a column do not differ statistically by the SNK test ( $P < 0.05$ ). <sup>1</sup>Quadratic effect ( $FI_g = -42006 + 28.673 ME - 0.0047 ME^2$ ,  $r^2 = 0.91$ ); <sup>2</sup>Quadratic effect ( $FI_g = 39111 - 24.058 ME + 0.0039 ME^2$ ,  $r^2 = 0.99$ ); <sup>3</sup>Linear effect ( $FC = 3.43 - 0.0006 ME$ ,  $r^2 = 0.84$ )

quadratic effect for the soybean oil, as per the equation ( $FI_g = 39111 - 24,058 ME + 0.0039 ME^2$ ,  $r^2 = 0.99$ ), with the lowest value seen at 3,084 kcal ME/kg feed (Table 6). These results characterise a reduction in feed intake for increasing levels of ME in the diets with added glycerine, which may be related to the energy theory of feed intake (GONZALES, 2008); this states that under suitable conditions, birds tend to regulate their feed intake according to the energy level of the diet as a function of their daily energy needs. However, in the present work the birds were under cyclic thermal stress (Table 3) and theoretically would not be able to properly adjust their intake as a function of the energy level. In this case, according to the ionostatic theory proposed by Felix *et al.* (2014), they might be adjusting their intake as a function of the electrolyte content, since the electrolytic balance of the diet with 3050 kcal/kg was higher (245 mEq/kg) than in the other diets, which could favour the best acid base balance for the body to improve heat dissipation. Thus, among the several existing theories, the organism may present different responses for feed intake as a function of physiological, environmental and genetic factors; however, most of the responses are regulated by the hypothalamus, which has the role of mediating stimuli that are directly connected to the behaviour of an individual in the presence of food (ABDELQADER; AL-FALAFTAH, 2014).

A decreasing linear effect was seen for FC ( $FC = 3.43 - 0.0006ME$ ,  $r^2 = 0.84$ ), where for each additional unit of ME in the feed there was a reduction of 0.0006g feed/g weight gain. Barbosa *et al.* (2008) found no significant effect for the variables WG and FC in broiler chickens fed with increasing levels of metabolisable energy at the 22 to 35-day stage.

During the 34 to 42-day stage, no interaction ( $P > 0.05$ ) was seen between the sources of increased energy and the levels of metabolisable energy (ME) for the variables FI, WG or FC (Table 7), demonstrating that the response to ME levels does not depend on the source at this stage. However, according to the equations for FI ( $FI = 2718 - 0.351 ME$ ,  $r^2 = 0.92$ ) and FC ( $FC = 5.25 - 0.001 ME$ ,  $r^2 = 0.96$ ), a decreasing linear effect was seen on the variables FI and FC for the levels of metabolisable energy, where for each additional unit of ME in the feed there was a reduction of 0.0351 g/bird for feed intake and 0.001 g feed/g weight gain for feed conversion.

This research confirms the findings of Guerra *et al.* (2011), who observed a decreasing linear effect ( $P < 0.05$ ) on feed intake in broilers from 21 to 42 days of age as the levels of glycerine in the diet were increased. This differed from authors such as Barbosa *et al.* (2008), who found no significant effect on the variables WG and FC in broiler chickens fed with increasing levels of metabolisable energy during the 36 to 42-day stage.

There was an increasing linear effect ( $WG = 25.13 \pm 0.251 ME$ ,  $r^2 = 0.77$ ) on weight gain (WG) during the 34 to 42-day stage (Table 7), where an increase of 0.251 g in weight gain/bird was seen for each additional unit of ME in the feed.

The diets with added glycerine resulted in greater feed intake than those with soybean oil, which may be related to the metabolisable coefficient of the diets with glycerine, since Dozier *et al.* (2008) found lower values for metabolisable energy from the glycerine at more-advanced ages, whereas in this study, the value for metabolisable energy from the glycerine used was the

same at all stages. There was no significant difference for WG or FC, ( $P>0.05$ ).

No significant interaction ( $P>0.05$ ) was seen between the sources of increased energy and the levels of metabolisable energy (ME) for any of the carcass or cut yield variables (Table 8). However, a linear decrease ( $P<0.05$ ) in liver yield (RF) was seen for the levels of metabolisable energy from the feed as per the equation ( $LY = 6.77 - 0.00013ME$ ,  $r^2 = 0.72$ ), with a further decrease of 0.00013g in liver weight/bird for each additional unit of ME in the feed.

Moreira *et al.* (2001) state that in general, yield can be affected by the energy level. Research carried out with the addition of increasing levels of glycerine in the feed of broilers found no changes in liver yield (TOPAL, 2013). This effect may be related to the reduction in FI and increase in WG at this stage (Table 7), since a reduction in feed intake may reduce the heat produced by the animal (which represents the ingested metabolisable energy subtracted from the retained energy) and promote a lower metabolic load for the liver, with a consequent reduction in its relative weight.

**Table 7** - Effect of energy levels with added glycerine or soybean oil on feed intake (FI), weight gain (WG) and feed conversion (FC) during the 34 to 42-day stage

Variable	Source	ME Level (kcal/kg)				Mean	CV (%)	P>F		
		3025	3100	3175	3250			Source	ME	SxME
FI (g)	Glycer.	1664.28	1642.41	1646.22	1597.42	1637.59	3.03	0.015	0.0051	0.264
	Soy oil	1667.48	1593.16	1562.00	1567.99	1597.66				
	Mean <sup>1</sup>	1665.89	1617.79	1604.11	1582.71					
WG (g)	Glycer.	786.00	794.20	859.00	857.20	824.10	5.59	0.220	0.0226	0.347
	Soy oil	807.20	779.80	805.80	831.40	806.05				
	Mean <sup>2</sup>	796.60	787.00	832.40	844.30					
FC (g)	Glycer.	2.13	2.06	1.91	1.87	1.99	5.51	0.732	0.0002	0.783
	Soy oil	2.06	2.04	1.94	1.88	1.98				
	Mean <sup>3</sup>	2.10	2.05	1.93	1.88					

Mean values with the same lowercase letter in a column do not differ statistically by the SNK test ( $P<0.05$ ). <sup>1</sup>Linear effect ( $FI = 2718 - 0.351 ME$ ,  $r^2 = 0.92$ ); <sup>2</sup>Linear effect ( $WG = 26.51 + 0.251 ME$ ,  $r^2 = 0.77$ ); <sup>3</sup>Linear effect ( $FC = 5.25 - 0.001 ME$ ,  $r^2 = 0.96$ )

**Table 8** - Effect of energy levels with added glycerine or soybean oil on yield for carcass (CY), breast (BY), thigh (TY), upper-thigh (UTY), wing (WY), abdominal fat (AFY), liver (LY) and heart (HY) at the 34 to 42-day stage

Variable	Source	ME Level (kcal/kg)				Mean	CV (%)	P>F		
		3025	3100	3175	3250			Source	ME	SxME
CY (%)	Glycerine	69.64	68.89	68.78	69.23	69.13	1.28	0.116	0.662	0.276
	Soy oil	69.18	69.34	69.86	70.00	69.59				
	Mean	69.41	69.12	69.26	69.61					
BY (%)	Glycerine	34.71	34.46	35.18	35.18	34.87	2.70	0.319	0.396	0.784
	Soy oil	34.93	34.10	34.68	34.60	34.58				
	Mean	34.82	34.28	34.93	34.86					
TY (%)	Glycerine	15.26	15.19	14.98	15.23	15.17	3.21	0.711	0.927	0.809
	Soy oil	15.19	15.11	15.29	15.31	15.22				
	Mean	15.22	15.15	15.15	15.27					
UTY (%)	Glycerine	16.64	16.96	16.67	16.66	16.73	3.30	0.096	0.617	0.910
	Soy oil	16.31	16.55	16.59	16.31	16.44				
	Mean	16.47	16.76	16.63	16.48					



Continuation Table 8

	Glycerine	11.67	11.75	11.59	11.51	11.63				
WY (%)	Soy oil	11.80	11.71	11.63	11.62	11.69	3.48	0.619	0.716	0.964
	Mean	11.73	11.73	11.61	11.56					
	Glycerine	2.38	3.15	3.05	2.66	2.81				
AFY (%)	Soy oil	2.67	2.42	2.65	2.64	2.60	16.64	0.146	0.389	0.082
	Mean	2.52	2.79	2.85	2.65					
	Glycerine	2.74	2.95	2.77	2.48	2.72				
LY (%)	Soy oil	2.80	2.63	2.68	2.42	2.65	8.42	0.210	0.017	0.403
	Mean <sup>1</sup>	2.77	2.77	2.72	2.46					
	Glycerine	0.63	0.68	0.69	0.63	0.66				
HY (%)	Soy oil	0.67	0.66	0.63	0.63	0.65	8.32	0.597	0.312	0.210
	Mean	0.65	0.67	0.66	0.63					

Mean values with the same lowercase letter in a column do not differ statistically by the SNK test ( $P < 0.05$ ). <sup>1</sup>Linear effect ( $LY = 6.77 - 0.00013ME$ ,  $r^2 = 0.72$ )

## CONCLUSION

The metabolisable energy requirement of broilers, regardless of the source of increased energy (glycerine or soybean oil), was estimated at 3,075; 3,130; 3,200 and 3,250 kcal ME/kg feed for stages 1 to 7, 8 to 21, 22 to 33 and 34 to 42 days respectively. However, the use of glycerine as a source of increased energy resulted in better efficiency during the first two stages (1 to 7 and 8 to 21 days of age).

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