Optimizing cost, growth performance, and nutrient absorption with a bio-emulsifier based on lysophospholipids for broiler chickens

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ABSTRACT Two experiments **(Exp.)** were conducted to evaluate effects of a lysophospholipid-based bio-emulsifier **(LPL)** on growth performance, nutrient digestibility and energy utilization of broilers as well as the return on investment **(ROI)**. In Exp. 1, 392 chicks were housed in battery cages in a completely randomized design with 8 treatments and 7 replicates of 7 birds each from d 0 to 21 posthatch. In Exp. 2, 1,400 chicks were allocated in floor pens and fed the same 8 treatments, with 7 replicates and 25 birds each from d 0 to 43 posthatch. Treatments consisted of 6 degummed soybean oil-based diets: positive control **(PC1)**; PC1 formulated with 500 g/ton LPL **(PC1+LPL on top)**; PC1 formulated with 60 kcal LPL matrix **(PC1+LPL60)**; PC1 formulated with 100 kcal LPL matrix **(PC1+LPL100)**; and two negative controls NC-60 and NC-100 with reductions of 60 and 100 kcal/kg ME, respectively. Two other diets were formulated with acid soybean oil: positive control **(PC2)** and PC2 formulated with 60 kcal LPL matrix **(PC2+LPL60)**. In Exp. 1, performance was evaluated from d 0 to 21, ME and ileal digestibility of DM, CP and energy were determined on d 21. In Exp. 2, growth performance was evaluated from d 0 to 42, and on d 43 carcass and abdominal fat yields were calculated. There were no effects of soybean oil sources in any parameter. Inclusion of LPL increased ($P < 0.05$) BW gain and ileal digestibility of DM, fat and CP. Broilers fed the PC1+LPL on top diet had increased ($P < 0.05$) performance, ileal digestibility and energy utilization as well as decreased abdominal fat compared to NC-60 or NC-100. The use of LPL on top had a ROI of 8:1 vs. PC1, considering the gains in revenue of the slaughtered broilers in relation to the investment with LPL in feed. In conclusion, a lysophospholipid-based bio-emulsifier increased performance, digestibility and return on investment of broilers fed standard or reformulated diets.

Key words: biosurfactant, broiler, digestibility, lysophospholipid, performance

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INTRODUCTION

Lipids are commonly added to poultry diets as a concentrated energy source that provides fat-soluble vitamins and essential fatty acids (**FAs**) and enhances absorption of fat-soluble nutrients, playing important roles in biochemistry, physiology, and nutrition (Brindley, 1984; Hossain and Das, 2014). In the feed milling processes, fat addition can help to reduce dusting after mixing, decrease particle separation in mash diets, and improve quality of pellets (Abdollahi et al., 2013).

Fat has been obtained from animal or vegetable sources, and having differences in their FA content and chemical or physical characteristics can affect the digestibility of fat and oils (Wiseman and Salvador, 1991). Because of the rising cost of commercially available fats, such as degummed soybean oil (**DSO**), and following a trend of constant growth in the biodiesel production in recent years, there is an increased interest in maximizing the use of fat in diets (Ravindran et al., 2016). In this context, some by-products of the soy oil industry can be used to reduce energy costs in poultry diets, especially because these sources are obtained during the processing of soy oil or biodiesel production, as acid soybean oil (**ASO**) (Borsatti et al., 2018). The ASO is not efficiently used by poultry because it presents high

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contents of nonesterified FA that results in lower ME value for poultry than neutral fats, such as DSO (Sklan, 1979; Borsatti et al., 2018). Therefore, productivity and production profitability can be influenced by the choice of fat sources used in feed formulation combined with the purchase price, stability, and quality of the raw material.

The addition of fats has also been used to meet the requirements of ME for fast-growing birds when the dietary energy density increases. It has been reported that lipids contribute to increased growth rates and feed conversion efficiency (Pesti et al., 2002). Additional effects are expected in immature birds because the fat digestion is poor, due to a lack of lipase activity and bile salts production (Maisonnier et al., 2003), and then as the bird ages, lipase activity and bile salts are known to increase (Noy and Sklan, 1995). Limitation in lipases activity as well as presence of high number of substrates may cause an inability to form mixed micelles in the intestinal lumen, which further decreases fat digestion and absorption of nutrients (Leeson and Atteh, 1995). Consequently, the use of biosurfactants such as lysophospholipids (**LPL**), lecithins, and lysolecithins is of practical and commercial interest in broiler diets (Soares and Lopez-Bote, 2002).

Biosurfactants have been used generally formulated with different mixtures of emulsifiers, such as LPL. LPL are obtained from an enzymatic hydrolysis of phospholipids and constructed with a monoacylglycerol in either position *sn*-1, 1-LPL, or *sn*-2, 2-LPL, and a phosphate residue in position *sn*-3 (Belaunzaran et al., 2011). The mode of action has been reported as increase in release of monoglycerides and diglycerides by emulsion of dietary fat (Schwarzer and Adams, 1996; Van Nieuwenhuyzen and Tomás, 2008). Owing to the removal of one FA, LPL are high hydrophilic and have high oil-in-water emulsifying properties (Liu and Ma, 2011). Therefore, LPL have been able to improve the digestion of fats and oils, resulting in increased digestibility and energy retention, with increased villus length as well as growth performance (Zhang et al., 2011; Jansen et al., 2015; Brautigan et al., 2017).

In addition to LPL, it has been reported that the use of a suitable synthetic surfactant and a monoglyceride improves the lipid absorption process. The synthetic surfactant actuates in synergy with LPL to improve the emulsification step of the lipid absorption process; however, its concentration must be carefully studied as it can impair the hydrolysis step by steric hindering. The mixture introduces small quantities of synthetic emulsifier and monoglycerides to improve respectively emulsification and micelle formation to fully exploit the benefits of LPL. This product allows to investigate the synergistic effect of adding small amounts of glycerol polyethyleneglycol ricinoleate to the mixture to improve the emulsification but avoid negative effects on lipid hydrolysis (Jansen et al., 2015).

Diets formulated with differences in oil sources, ME levels, and biosurfactant can bring an interesting discussion about their contribution to improve metabolism and performance as well as their cost-benefit. Thus, the objective of the 2 experiments (**Exp.**) was to evaluate the effects of a LPL-based bio-emulsifier added to corn-soy diets on energy utilization, ileal digestibility, and growth performance of broiler chickens in Exp. 1 as well as on performance, carcass yield, and cost-benefit of broilers in Exp. 2. These effects were assessed both in a standard diet formulation or in reformulated diets having LPL matrix contribution, which was also compared to ME reductions and DSO or ASO as oil sources.

MATERIALS AND METHODS

All procedures used in the present study were approved by the Ethics and Research Committee of the Federal University of Santa Maria, Santa Maria, RS, Brazil (approval numbers 3770301018 and 4832250419).

Birds and Management

A total of 1,792 slow feathering male broiler chicks (Cobb 500) vaccinated for Marek's disease were acquired from a local hatchery (Vibra Group, Rio Grande do Sul, Brazil), obtained from the same broiler breeder parent flock, selected at placement, and distributed by body weight. The average BW on day 0 was 48 g in both experiments. The average temperature was 32°C at placement, being reduced by 1°C every 2 d until 23°C to provide comfort throughout the studies with the use of thermostatically controlled heaters, evaporative plates, and exhaust fans. In Exp. 1, lighting was continuous until day 21 posthatch. In Exp. 2, a continuous lighting schedule was used until day 7 posthatch, whereas a 16L:8D cycle with constant intensity was used thereafter. Birds had ad libitum access to water and mash diets (Tables 1–4).

In Exp. 1, 392 birds were reared in 56 wire battery cages (0.8×0.4 m^2) in a temperature-controlled room from day 0 to 21 posthatch. Each cage was equipped with one feeder and 2 nipple drinkers. In Exp. 2, 1,400 birds were allocated into 56 floor pens (1.65×1.75 m^2) in a climate-controlled poultry house with one feeder, 5 nipple drinkers, and new wood shavings as litter from day 0 to 43 posthatch. The experiments consisted of the same 8 treatments and 7 replicates, with 7 birds each in Exp. 1 or 25 birds per replicates in Exp. 2, both distributed in a completely randomized design.

Experimental Diets

Experimental diets were formulated using corn-soybean meal (**SBM**) with DSO (BSBIOS, Passo Fundo, RS, Brazil) or acid soy oil (Meridional Oleochemicals & Ingredients, Londrina, PR, Brazil) as the main energy-yielding ingredients (Tables 1–4). The tested additive, a synthetic emulsifier + monoglycerides + LPL, was added at 500 g/ton. The LPL consisted of a

Table 1. Ingredient and nutrient composition of the prestarter diet (as-is basis) fed to broilers from day 0 to 7 posthatch in Exp. 1 and 2.

Item	PC1[1]	PC1 + LPL on top[2]	NC-60[3]	PC1 + LPL60[4]	NC-100[5]	PC1 + LPL100[6]	PC2[7]	PC2 + LPL60[8]
Ingredients, %								
Corn	48.27	48.17	49.41	49.53	50.33	50.44	47.55	49.07
Soybean meal, 46% CP	44.92	44.94	44.90	44.72	44.75	44.58	45.03	44.80
Degummed soybean oil	3.01	3.04	1.90	1.90	1.13	1.13	-	-
Acid soybean oil	-	-	-	-	-	-	3.62	2.28
Dicalcium phosphate	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Limestone	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56
Salt	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
DL-Met, 99%	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
L-Lys HCl, 78%	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
L-Thr, 98.5%	0.04	0.04	0.03	0.04	0.03	0.04	0.04	0.04
Choline chloride, 60%	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Vit. and min. premix[9]	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Emulsifier[10]	-	0.05	-	0.05	-	0.05		0.05
Nutrient and energy composition, % or as shown[11]								
ME, kcal/kg	3,000	3,000	2,940	3,000	2,900	3,000	3,000	3,000
Crude protein	24.97 (24.9)	24.96 (24.8)	25.00 (25.0)	25.00 (24.9)	25.00 (25.0)	24.98 (24.9)	24.96 (24.8)	24.97 (24.9)
Ca	1.01 (1.0)	1.01 (1.0)	1.01 (0.99)	1.01 (0.99)	1.01 (1.0)	1.01 (1.1)	1.01 (1.0)	1.01 (0.99)
Av. P	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Total P	0.51 (0.52)	0.51 (0.51)	0.51 (0.52)	0.51 (0.50)	0.51 (0.51)	0.51 (0.50)	0.51 (0.52)	0.51 (0.51)
Na	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Choline, mg/kg	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600
Lys dig.[12]	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36
Met + Cys dig.	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02
Thr, dig.	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Trp dig.	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Arg dig.	1.58	1.58	1.58	1.57	1.58	1.57	1.58	1.57
Val dig.	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Ile dig.	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Leu dig.	1.87	1.87	1.88	1.88	1.88	1.88	1.87	1.87
Ether extract	5.72	5.74	4.65	4.64	3.92	3.91	6.29	5.01
Analyzed GE, kcal/kg	4,509	4,503	4,402	4,504	4,359	4,516	4,506	4,519

[1]PC1 = positive control formulated with soybean degummed oil (ME = 8,800 kcal/kg).
[2]PC1 + LPL on top = PC1 formulated with 500 g/ton of a synthetic emulsifier + monoglycerides + lysophospholipids (LPL) product.
[3]NC-60 = negative control with 60 kcal/kg ME reduction of the PC1.
[4]PC1 + LPL60 = PC1 reformulated with LPL matrix (60 kcal/kg).
[5]NC-100 = negative control with 100 kcal/kg ME reduction of the PC1.
[6]PC1 + LPL100 = PC1 reformulated with LPL matrix (100 kcal/kg).
[7]PC2 = positive control formulated with acid soy oil (ME = 7,900 kcal/kg).
[8]PC2 + LPL60 = PC2 reformulated with LPL matrix (60 kcal/kg).
[9]Composition per kilogram of feed: vitamin A, 8,000 UI; vitamin D_3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg. Phytase with 10,000 fungal phytase units/g, using available P and total Ca (g/kg) matrix values of 1,500 and 1,700, respectively.
[10]LYSOFORTE eXtend (Kemin do Brasil Ltda, Indaiatuba, SP, Brazil).
[11]Values in parenthesis were analyzed.
[12]Ratios of digestible amino acids to digestible Lys were maintained at TSAA 0.75; Thr 0.65; Val 0.77; Trp 0.17; Arg 1.08; Ile 0.67 (Rostagno et al., 2017).

Table 2. Ingredient and nutrient composition of the starter diet (as-is basis) fed to broilers from day 8 to 21 posthatch in Exp. 1 and 2.

Item	PC1[1]	PC1 + LPL on top[2]	NC-60[3]	PC1 + LPL60[4]	NC-100[5]	PC1 + LPL100[6]	PC2[7]	PC2 + LPL60[8]
Ingredients, %								
Corn	49.71	49.63	51.07	51.00	51.98	51.91	48.70	50.24
Soybean meal, 46% CP	42.72	42.73	42.50	42.51	42.36	42.37	42.88	42.63
Degummed soybean oil	4.17	4.19	3.03	3.04	2.26	2.27	-	-
Acid soybean oil	-	-	-	-	-	-	5.02	3.68
Dicalcium phosphate	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Limestone	1.41	1.41	1.41	1.41	1.41	1.41	1.41	1.41
Salt	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52
DL-Met, 99%	0.35	0.35	0.34	0.34	0.34	0.34	0.35	0.35
L-Lys HCl, 78%	0.14	0.14	0.15	0.15	0.15	0.15	0.14	0.14
L-Thr, 98.5%	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Choline chloride, 60%	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Vit. and min. premix[9]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Emulsifier[10]	-	0.05	-	0.05	-	0.05	-	0.05
Nutrient and energy composition, % or as shown[11]								
ME, kcal/kg	3,100	3,100	3,040	3,100	3,000	3,100	3,100	3,100
Crude protein	24.07 (24.2)	24.07 (24.1)	24.08 (24.4)	24.08 (24.1)	24.06 (24.0)	24.07 (24.2)	24.07 (24.4)	24.08 (24.5)
Ca	0.91 (0.93)	0.91 (0.91)	0.91 (0.92)	0.91 (0.92)	0.91 (0.93)	0.91 (0.90)	0.91 (0.92)	0.91 (0.91)
Av. P	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Total P	0.46 (0.48)	0.46 (0.47)	0.46 (0.46)	0.46 (0.45)	0.46 (0.48)	0.46 (0.46)	0.46 (0.47)	0.46 (0.47)
Na	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Choline, mg/kg	1,600	1,600	1,600	1,600	1,600	1,600	1,600	1,600
Lys, dig.[12]	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31
Met + Cys dig.	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Thr, dig.	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Trp dig.	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Arg dig.	1.51	1.51	1.51	1.51	1.51	1.51	1.51	1.51
Val dig.	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Ile dig.	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Leu dig.	1.81	1.81	1.82	1.82	1.82	1.82	1.81	1.82
Ether extract	6.88	6.90	5.79	5.80	5.06	5.06	7.68	6.40
Analyzed GE, kcal/kg	4,629	4,631	4,527	4,624	4,459	4,626	4,636	4,619

[1]PC1 = positive control formulated with soybean degummed oil (ME = 8,800 kcal/kg).

[2]PC1 + LPL on top = PC1 formulated with 500 g/ton of a synthetic emulsifier + monoglycerides + lysophospholipids (LPL) product.

[3]NC-60 = negative control with 60 kcal/kg ME reduction of the PC1.

[4]PC1 + LPL60 = PC1 reformulated with LPL matrix (60 kcal/kg).

[5]NC-100 = negative control with 100 kcal/kg ME reduction of the PC1.

[6]PC1 + LPL100 = PC1 reformulated with LPL matrix (100 kcal/kg).

[7]PC2 = positive control formulated with acid soy oil (ME = 7,900 kcal/kg).

[8]PC2 + LPL60 = PC2 reformulated with LPL matrix (60 kcal/kg).

[9]Composition per kilogram of feed: vitamin A, 8,000 UI; vitamin D3, 2,000 UI; vitamin E, 30 UI; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg. Phytase with 10,000 fungal phytase units/g, using available P and total Ca (g/kg) matrix values of 1,500 and 1,700, respectively.

[10]Lysofortetm eXtend (Kemin do Brasil Ltda, Indaiatuba, SP, Brazil).

[11]Values in parenthesis were analyzed.

[12]Ratios of digestible amino acids to digestible Lys were maintained at TSAA 0.75; Thr 0.65; Val 0.77; Trp 0.17; Arg 1.08; Ile 0.67 (Rostagno et al., 2017).

Table 3. Ingredient and nutrient composition of the grower diet (as-is basis) fed to broilers from day 22 to 35 posthatch in Exp. 2.

Item	PC1[1]	PC1 + LPL on top[2]	NC-60[3]	PC1 + LPL60[4]	NC-100[5]	PC1 + LPL100[6]	PC2[7]	PC2 + LPL60[8]
Ingredients, %								
Corn	52.29	52.21	53.63	53.55	54.54	54.46	51.04	52.58
Soybean meal, 46% CP	39.54	39.55	39.33	39.34	39.19	39.20	39.73	39.50
Degummed soybean oil	5.14	5.16	4.01	4.03	3.24	3.26	-	-
Acid soybean oil	-	-	-	-	-	-	6.20	4.86
Dicalcium phosphate	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Limestone	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-Met, 99%	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
L-Lys HCl, 78%	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Thr, 98.5%	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Choline chloride, 60%	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Vit. and min. premix[9]	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Emulsifier[10]	-	0.05	-	0.05	-	0.05	-	0.05
Nutrient and energy composition, % or as shown[11]								
ME, kcal/kg	3,200	3,200	3,140	3,200	3,100	3,200	3,200	3,200
Crude protein	22.82 (22.9)	22.82 (22.9)	22.83 (22.7)	22.83 (22.7)	22.84 (22.8)	22.84 (22.8)	22.81 (22.7)	22.82 (22.8)
Ca	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Av. P	0.34 (0.34)	0.34 (0.35)	0.34 (0.34)	0.34 (0.33)	0.34 (0.35)	0.34 (0.35)	0.34 (0.35)	0.34 (0.35)
Total P	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Na	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Choline, mg/kg	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500
Lys, dig.[12]	1.24	1.24	1.24	1.24	1.24	1.24	1.24	1.24
Met + Cys dig.	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Thr, dig.	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81
Trp dig.	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Arg dig.	1.42	1.42	1.42	1.42	1.42	1.42	1.42	1.42
Val dig.	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Ile dig.	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89
Leu dig.	1.74	1.74	1.74	1.74	1.75	1.75	1.74	1.74
Ether extract	7.89	7.91	6.80	6.82	6.07	6.08	8.88	7.60
Analyzed GE, kcal/kg	4,732	4,730	4,637	4,728	4,561	4,726	4,746	4,721

[1]PC1 = positive control formulated with soybean degummed oil (ME = 8,800 kcal/kg).
[2]PC1 + LPL on top = PC1 formulated with 500 g/ton of a synthetic emulsifier + monoglycerides + lysophospholipids (LPL) product.
[3]NC-60 = negative control with 60 kcal/kg ME reduction of the PC1.
[4]PC1 + LPL60 = PC1 reformulated with LPL matrix (60 kcal/kg).
[5]NC-100 = negative control with 100 kcal/kg ME reduction of the PC1.
[6]PC1 + LPL100 = PC1 reformulated with LPL matrix (100 kcal/kg).
[7]PC2 = positive control formulated with acid soy oil (ME = 7,900 kcal/kg).
[8]PC2 + LPL60 = PC2 reformulated with LPL matrix (60 kcal/kg).
[9]Composition per kilogram of feed: vitamin A, 8,000 UI; vitamin D_3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg. Phytase with 10,000 fungal phytase units/g, using available P and total Ca (g/kg) matrix values of 1,500 and 1,700, respectively.
[10]LYSOFORTE eXtend (Kemin do Brasil Ltda, Indaiatuba, SP, Brazil).
[11]Values in parenthesis were analyzed.
[12]Ratios of digestible amino acids to digestible Lys were maintained at TSAA 0.75; Thr 0.65; Val 0.77; Trp 0.17; Arg 1.08; Ile 0.67 (Rostagno et al., 2017).

biosurfactant mixture of soy lecithin, glycerol ricinoleate, monoglycerides and diglycerides, and tocopherol, commercially available as LYSOFORTE eXtend (Kemin do Brasil Ltda, Indaiatuba, SP, Brazil).

Treatments consisted of 6 corn-SBM basal diets formulated with DSO: a positive control diet (**PC1**); PC1 formulated with 500 g/ton LPL (**PC1 + LPL on top**); 2 negative controls (**NC**), **NC-60** and **NC-100**, with reductions of 60 and 100 kcal/kg ME, respectively, as well as 2 reformulated diets, PC1 formulated with 60 kcal/kg LPL matrix (**PC1 + LPL60**) and PC1 formulated with 100 kcal/kg LPL matrix (**PC1 + LPL100**). Two other diets were formulated with corn-SBM and ASO: a positive control formulated with ASO (**PC2**) and PC2 formulated with 60 kcal/kg LPL matrix (**PC2 + LPL60**). All positive control diets were formulated according to Rostagno et al. (2017), and nutrient concentrations were maintained similar through the dietary treatments, except for ME. The formulated ME in the PC1 and PC2 control feeds were 3,000; 3,100; 3,200; and 3,250 kcal/kg for prestarter, starter, grower, and finisher phases, respectively.

In Exp. 1, a two-phase feeding program was used, with prestarter (day 0–7) and starter (day 8–21) diets. Celite was included at 1% in the starter diet as an indigestible index marker to calculate diets' metabolizability and digestibility. In Exp. 2, a four-phase feeding program was used with the same prestarter (day 0–7) and starter (day 8–21) diets as well as grower (day 22–35) and finisher (day 36–42) diets.

Experimental Procedures

Experiment 1 A total of 392 birds were reared in wire battery cages from day 0 to 21 posthatch. Chicks were individually weighed into groups of 7 birds per cage before placement. Birds and feed were weighed weekly per cage to monitor the performance. Feed intake (**FI**), BW gain, and feed conversion ratio (**FCR**) corrected for the weight of dead broilers were determined. Mortality was recorded immediately after noticed, and dead broilers were weighed.

Excreta were collected twice daily on wax papers from day 19 to 20, being immediately mixed and pooled by cage and stored at −20°C until analysis. Before calorimetry, excreta were dried in a forced air oven at 55°C (Fisher Isotemp Oven, Waltham, MA) and ground to pass a 0.5-mm screen. On day 21, all birds were euthanized by cervical dislocation, and ileal digesta, contents from the two-third distal ileum, defined as the region between Meckel's diverticulum to approximately 2 cm cranial to the ileocecal junction, were collected by flushing with distilled water into plastic containers. Ileal digesta was subsequently pooled by cage and immediately stored at −20°C. Digesta samples were dried in a forced air oven at 55°C. Diet and dried samples of ileal digesta were ground to pass a 0.5-mm screen in a grinder (Tecnal, R-TE-648, Sao Paulo, SP, Brazil).

Dry matter analysis of diets, excreta, and ileal digesta was performed after oven drying (Nade, FA2104 N, Zhejiang, China) the samples at 105°C for 16 h (method 934.01; AOAC International, 2006). Diets, excreta, and ileal digesta were also analyzed for gross energy (**GE**) using an adiabatic bomb calorimeter (6400 Calorimeter; Parr Instrument Company, Moline, IL) using benzoic acid as a calibration standard. Nitrogen of diets and ileal digesta was determined using the combustion method (Thermo-Finnigan Flash EA 1112, Waltham, MA) with EDTA as a calibration standard. Acid insoluble ash concentrations in the diets, excreta, and ileal digesta samples were determined using the method described by Vogtmann et al. (1975) and Choct and Annison (1992). Ether extract (**EE**) in diets and ileal digesta samples was determined by extracting in petroleum ether using a Soxhlet apparatus for approximately 8 h (method 934.01; AOAC International, 2000).

Apparent ileal digestibility of CP, DM, EE, and energy as well as apparent total tract metabolizability of DM and energy were calculated using the following equations (Kong and Adeola, 2014; Stefanello et al., 2019, 2020):

$$\text{Digestibility}(\%) = [1-(\text{Mi}/\text{Mo}) \times (\text{Eo}/\text{Ei})] \times 100,$$

where Mi represents the concentration of acid insoluble ash in the diet in g/kg of DM; Mo represents the concentration of acid insoluble ash in the ileal digesta or excreta in g/kg of DM output; Ei represents the concentration of CP, DM, or EE in g/kg of DM or GE in kcal/kg of DM in the diet; and Eo represents the concentration of CP, DM, or EE in g/kg of DM or GE in kcal/kg of DM in the ileal digesta or excreta.

The AME and ileal digestible energy (**IDE**) (kcal/kg) of experimental diets were calculated using the analyzed content of acid insoluble ash and GE (Kong and Adeola, 2014), as follows:

$$\text{AME or IDE}(\text{kcal}/\text{kg}) = \text{GEi}-[\text{GEo} \times (\text{Mi}/\text{Mo})],$$

where GEi is the GE (kcal/kg DM) in the diet; GEo is the GE (kcal/kg) in the ileal content or excreta, on DM basis.

Experiment 2 A total of 1,400 birds were allocated in floor pens from day 0 to 43 posthatch. Chicks were weighed into groups of 25 birds per pen before placement. Birds and feed weights were recorded per pen on day 0, 7, 21, 35, and 42 posthatch. Growth performance (BW gain, FI, and FCR) was calculated for each period and from day 0 to 21 and day 0 to 42 posthatch. Mortality was recorded immediately after noticed when dead broilers were weighed.

On day 43, 4 birds were selected from each pen (n = 224) and processed for carcass and abdominal fat evaluation at a commercial poultry slaughterhouse. Before processing, broilers were fasted for 8 h and individually weighed. Birds were humanely rendered insensible using electrical stunning, then bled through a jugular vein cut for 3 min, scalded at 60°C for 45 s, and lastly defeathered. Evisceration was manually performed, and carcasses were chilled in ice for approximately 2 h. Eviscerated carcasses (without feet and neck) were hung for 3 min to remove excess water before weighing. Commercial cuts were performed by a crew of industry-trained personnel into bone-in drumsticks, thighs, wings, as well as deboned breast. Abdominal fat was weighed separately. Carcass yield was expressed relative to live weight, while commercial cuts and abdominal fat were expressed as percentage of the eviscerated carcass.

Return on investment (**ROI**) was calculated taking into consideration the feed cost for 42 d, cost of emulsifier product recovered through sale of broilers on day 42. As there were equal number of chicks in all groups, cost incurred on purchase of chicks and labors were not considered while calculating the ROI as these expenditures remained same for all groups (Selvam et al., 2015; Abdurofi et al., 2017; Wealleans et al., 2020a). ROI was calculated for LPL, considering also diet emulsifier and diet costs, FI, FCR, and final BW. Gross income was the product of market price of broiler kg and total weight of birds. Net income was calculated by subtracting diet cost from gross income. The ROI was calculated considering the net income difference between PC1 + LPL on top vs. PC1; PC1 + LPL60 vs. PC1; PC1 + LPL100 vs. PC1 or PC2 + LPL60 vs. PC2, divided by the investment with the emulsifier product for each treatment.

Statistical Analysis

Data were subjected to one-way analysis of variance using the GLM procedure of SAS Institute (SAS, 2009), and significance was accepted at $P < 0.05$. Means were compared by Tukey test. Orthogonal contrast analyses were also conducted comparing PC1 against PC1 + LPL on top, PC1 against PC2 as

Table 4. Ingredient and nutrient composition of the finisher diet (as-is basis) fed to broilers from day 36 to 42 posthatch in Exp. 2.

Item	PC1[1]	PC1 + LPL on top[2]	NC-60[3]	PC1 + LPL60[4]	NC-100[5]	PC1 + LPL100[6]	PC2[7]	PC2 + LPL60[8]
Ingredients, %								
Corn	62.15	62.07	63.52	63.45	64.43	64.35	61.19	62.71
Soybean meal, 46% CP	31.2631.27	31.04	31.04	30.90	30.91	31.40	31.17	
Degummed soybean oil	4.10	4.12	2.95	2.97	2.18	2.20	-	-
Acid soybean oil	-	-	-	-	-	-	4.93	3.58
Dicalcium phosphate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Limestone	1.07	1.07	1.07	1.07	1.07	1.07	1.06	1.07
Salt	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
DL-Met, 99%	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
L-Lys HCl, 78%	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
L-Thr, 98.5%	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride, 60%	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Vit. and min. premix[9]	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Emulsifier[10]	-	0.05	-	0.05	-	0.05	-	0.05
Nutrient and energy composition, % or as shown[11]								
ME, kcal/kg	3,250	3,250	3,190	3,250	3,150	3,250	3,250	3,250
Crude protein	19.83 (19.8)	19.83 (19.7)	19.84 (19.9)	19.84 (19.9)	19.85 (19.9)	19.85 (19.8)	19.82 (19.7)	19.83 (19.8)
Ca	0.66 (0.65)	0.66 (0.66)	0.66 (0.67)	0.66 (0.65)	0.66 (0.66)	0.66 (0.66)	0.66 (0.66)	0.66 (0.66)
Av. P	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Total P	0.33 (0.35)	0.33 (0.34)	0.34 (0.33)	0.34 (0.33)	0.34 (0.33)	0.34 (0.33)	0.33 (0.34)	0.33 (0.34)
Na	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline, mg/kg	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500
Lys, dig.[12]	1.07	1.07	1.07	1.07	1.07	1.07	1.07	1.07
Met + Cys dig.	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Thr, dig.	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Trp dig.	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Arg dig.	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Val dig.	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Ile dig.	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Leu dig.	1.57	1.57	1.57	1.57	1.58	1.58	1.57	1.57
Ether extract	7.06	7.08	5.96	5.98	5.23	5.24	7.84	6.56
Analyzed GE, kcal/kg	4,763	4,770	4,666	4,752	4,599	4,766	4,771	4,765

[1]PC1 = positive control formulated with degummed oil (ME = 8,800 kcal/kg).
[2]PC1 + LPL on top = PC1 formulated with 500 g/ton of a synthetic emulsifier + monoglycerides + lysophospholipids (LPL) product.
[3]NC-60 = negative control with 60 kcal/kg ME reduction of the PC1.
[4]PC1 + LPL60 = PC1 reformulated with LPL matrix (60 kcal/kg).
[5]NC-100 = negative control with 100 kcal/kg ME reduction of the PC1.
[6]PC1 + LPL100 = PC1 reformulated with LPL matrix (100 kcal/kg).
[7]PC2 = positive control formulated with acid soy oil (ME = 7,900 kcal/kg).
[8]PC2 + LPL60 = PC2 reformulated with LPL matrix (60 kcal/kg).
[9]Composition per kilogram of feed: vitamin A, 8,000 UI; vitamin D_3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg. Phytase with 10,000 fungal phytase units/g, using available P and total Ca (g/kg) matrix values of 1,500 and 1,700, respectively.
[10]LYSOFORTE eXtend (Kemin do Brasil Ltda, Indaiatuba, SP, Brazil).
[11]Values in parenthesis were analyzed.
[12]Ratios of digestible amino acids to digestible Lys were maintained at TSAA 0.75; Thr 0.65; Val 0.77; Trp 0.17; Arg 1.08; Ile 0.67 (Rostagno et al., 2017).

well as NC-60 and NC-100 against PC1 + LPL60 and PC1 + LPL100.

RESULTS

Experiment 1

Feeds were formulated to contain different oil sources and ME levels, and the analyzed GE, CP, Ca, and total P composition of the prestarter and starter diets were similar to the formulated values. GE values were 4,629; 4,631; 4,527; 4,624; 4,459; 4,626; 4,636; and 4,619 kcal/kg for PC1, PC1 + LPL on top, NC-60, PC1 + LPL60, NC-100, PC1 + LPL100, PC2, and PC2 + LPL60, respectively (Tables 1–4).

Growth performance, total tract metabolizability, and ileal digestibility of the experimental diets for broilers in Exp. 1 are presented in Table 5. There were no effects of treatments on FI and mortality (grand mean = 2.0%). No effects were observed between sources of oil on growth performance ($P > 0.05$); however, diets with ME reduction presented decreased performance. Cumulative BW gain from day 0 to 21 was not affected by LPL matrix ($P > 0.05$); however, broilers fed PC1 + LPL on top diet had higher ($P < 0.10$) BW gain than birds fed the NC-100 diet. Cumulative FCR from day 0 to 21 also was lower when broilers were fed the PC1 + LPL on top diet than when fed the NC-100 diet ($P < 0.10$). The PC1 formulated with DSO as energy source resulted in similar ($P > 0.05$) growth performance and digestibility of nutrients and energy compared with PC2, which was formulated with ASO and same ME.

Broilers fed diets formulated with LPL on top presented higher AME and total tract metabolizability of

Table 5. Growth performance, total tract metabolizability, and ileal digestibility of broilers fed diets containing different soybean oils, energy levels, and an emulsifier based on lysophospholipids (LPL), Exp. 1.

Item[1]	Performance (Day 0–21)			Total tract metabolizability			Apparent ileal digestibility				
	BW gain/bird, g	Feed intake, g	FCR	Dry matter, %	AME, kcal/kg	Energy, %	Dry matter, %	IDE, kcal/kg	Ether extract, %	Crude protein, %	Energy, %
PC1	1,013[a,b]	1,287	1.272[a,b]	69.7[a,b,c]	3,373[a,b,c]	73.6[a,b,c]	68.8[b,c]	3,208[a,b]	71.0[a,b]	82.0[a,b]	70.4[a,b]
PC1 + LPL on top	1,055[a]	1,317	1.249[b]	72.5[a]	3,434[a,b]	75.6[a]	72.8[a]	3,297[a]	78.5[a]	85.1[a]	73.6[a]
NC-60	982[a,b]	1,277	1.301[a,b]	68.2[b,c]	3,334[c]	72.9[c]	67.7[b,c]	3,186[a,b]	69.5[a,b]	79.1[b]	68.1[a,b]
PC1 + LPL60	1,011[a,b]	1,277	1.262[a,b]	70.8[a,b,c]	3,449[a]	75.2[a,b]	69.4[a,b]	3,318[a]	73.3[a,b]	83.2[a]	72.4[a,b]
NC-100	963[b]	1,297	1.352[a]	63.0[d]	3,321[c]	69.3[d]	65.6[c]	3,118[b]	66.9[b]	78.7[b]	66.8[b]
PC1 + LPL100	1,004[a,b]	1,285	1.281[a,b]	71.1[a,b]	3,429[a,b]	74.9[a,b,c]	70.0[a,b]	3,305[a]	72.3[a,b]	82.4[a,b]	71.8[a,b]
PC2	1,007[a,b]	1,269	1.263[a,b]	67.3[c]	3,369[b,c]	70.5[b,c]	67.2[b,c]	3,185[a,b]	69.4[a,b]	81.7[a,b]	69.8[a,b]
PC2 + LPL60	1,001[a,b]	1,259	1.258[a,b]	69.8[a,b,c]	3,382[a,b,c]	73.4[a,b,c]	68.1[b,c]	3,222[a,b]	71.1[a,b]	82.4[a,b]	70.5[a,b]
SEM	7.11	8.34	0.008	0.46	11.37	0.38	0.50	19.51	0.85	0.37	0.56
P value	0.077	0.796	0.063	0.001	0.010	0.001	0.012	0.048	0.036	0.001	0.042

[a-d]Means with different superscript letters differ ($P < 0.10$) based on Tukey's honest significant difference test.

Abbreviations: FCR, feed conversion ratio; IDE, ileal digestible energy.

[1]Dietary treatments: PC1 = positive control formulated with soybean degummed oil; PC1 + LPL on top = PC1 formulated with 500 g/ton of a synthetic emulsifier + monoglycerides + lysophospholipids (LPL) product; NC-60 = negative control with 60 kcal/kg ME reduction of the PC1; PC1 + LPL60 = PC1 reformulated with LPL matrix (60 kcal/kg); NC-100 = negative control with 100 kcal/kg ME reduction of the PC1; PC1 + LPL100 = PC1 reformulated with LPL matrix (100 kcal/kg); PC2 = positive control formulated with acid soy oil; PC2 + LPL60 = PC2 reformulated with LPL matrix (60 kcal/kg).

energy and DM as well as higher ileal digestibility of DM ($P < 0.05$) than broilers fed NC-60 or NC-100. Effects of ME reductions and LPL utilization were observed when broilers fed diets with 60 or 100 kcal/kg ME reductions presented lower ($P < 0.05$) IDE and energy retention than broilers fed PC1 + LPL60 or PC1 + LPL100. The reformulation protocol, with diets formulated with 60 or 100 kcal/kg LPL matrix (PC1 + LPL60 or PC1 + LPL100, respectively) presented similar performance and digestibility when compared with the PC1.

Experiment 2

Feeds were formulated to contain different oil sources and ME levels, and the analyzed GE, CP, Ca, and total P composition was similar to the formulated values. Growth performance of broilers in Exp. 2 is presented in Table 6 and demonstrated that LPL on top led to improved BW gain and FCR ($P < 0.05$). Broilers fed the PC1 + LPL on top diet had higher ($P < 0.05$) BW gain than birds fed the NC-60 or NC-100 diets from day 8 to 21, day 22 to 35, and day 36 to 42. Birds on PC1 treatment had similar BW gain and FCR to the PC2, which was similar to PC2 + LPL60. The FCR was lower ($P < 0.05$) when broilers were fed PC1 + LPL on top than that when fed NC-100 from day 8 to 21 and day 36 to 42. There were no effects of dietary treatments on FI and mortality (grand mean = 1.7%) evaluated per feeding phase or in the overall period.

From day 0 to 21 and day 0 to 42 in Exp. 2 (Table 7), birds on PC1 + LPL on top treatment had higher BW gain and lower FCR than NC-60 or NC-100 treatments ($P < 0.05$). For the overall period, birds on reformulated diets, PC1 + LPL60 and PC1 + LPL100 treatments, had lower FCR than those on NC-60 and NC-100 treatments, respectively ($P < 0.05$).

Carcass and yields of commercial cuts obtained in the present study are shown in Table 8. No statistical significance was found for carcass and commercial cuts weight as well as yields. Decreased abdominal fat ($P < 0.05$) occurred when broilers were fed PC1 + LPL on top diet or PC1 + LPL60 compared with the NC-100 diet. No differences were observed on carcass, commercial cuts, and abdominal fat yields when the PC1 formulated with DSO was compared with the PC2 formulated with ASO and same ME.

Contrasts between PC1 against PC2 and PC1 formulated with LPL are shown in Table 9. Contrasts between PC1 against PC1 + LPL on top confirmed improvements ($P < 0.05$) on BW gain, FCR, and ileal digestibility of DM, EE, and CP as well as a decrease on abdominal fat when the LPL was used. When comparing NC-60 and NC-100 with PC1 + LPL60 and PC1 + LPL100, it was observed that broilers fed LPL had increased ($P < 0.05$) IDE and ileal digestibility of DM, EE, and CP as well as improved BW gain and FCR.

ROI was evaluated only in Exp. 2 where broilers were raised until day 42 (Table 10). The cost-benefit calculation considered diets' cost and production parameters when broilers were fed diets with LPL (PC1 + LPL on

Table 6. Growth performance of broilers fed diets containing different soybean oils, energy levels, and an emulsifier based on lysophospholipids (LPL), Exp. 2.

Item[1]	BW gain/bird, g				Feed intake/bird, g				Feed conversion ratio			
	Day 0-7	Day 8–21	Day 22–35	Day 36–42	Day 0–7	Day 8–21	Day 22- 35	Day 36–42	Day 0–7	Day 8–21	Day 22–35	Day 36–42
PC1	160^{a,b,c}	968^{a,b}	1,638^{a,b}	704^{a,b}	214	1,184	2,246	1,289	1.266	1.223^{b,c}	1.372	1.829^{b}
PC1 + LPL on top	173^{a,b}	996^{a}	1,644^{a}	748^{a}	212	1,208	2,228	1,360	1.223	1.213^{c}	1.355	1.824^{b}
NC-60	165^{c}	946^{b}	1,615^{b}	671^{b}	213	1,198	2,250	1,291	1.288	1.267^{a}	1.393	1.925^{a,b}
PC1 + LPL60	172^{a,b,c}	968^{a,b}	1,638^{a,b}	700^{a,b}	212	1,209	2,218	1,321	1.234	1.249^{a,b}	1.354	1.887^{a,b}
NC-100	165^{b,c}	940^{b}	1,605^{b}	667^{b}	214	1,193	2,248	1,324	1.297	1.269^{a}	1.401	1.986^{a}
PC1 + LPL100	170^{a,b,c}	966^{a,b}	1,637^{a,b}	695^{a,b}	216	1,208	2,236	1,375	1.267	1.251^{a,b}	1.364	1.980^{a}
PC2	174^{a}	971^{a,b}	1,629^{a,b}	689^{a,b}	214	1,10	2,242	1,296	1.229	1.246^{a,b}	1.376	1.885^{a,b}
PC2 + LPL60	171^{a,b,c}	968^{a,b}	1,639^{a,b}	705^{a,b}	214	1,204	2,226	1,321	1.246	1.244^{a,b}	1.358	1.875^{a,b}
SEM	0.74	3.60	3.65	6.01	1.53	3.85	11.83	10.60	0.009	0.003	0.007	0.014
P value	0.005	0.003	0.055	0.023	0.999	0.664	0.997	0.359	0.328	0.001	0.645	0.015

$^{a\text{-}c}$Means with different superscript letters differ ($P < 0.05$) based on Tukey's honest significant difference test.

[1]Dietary treatments: PC1 = positive control formulated with soybean degummed oil; PC1 + LPL on top = PC1 formulated with 500 g/ton of a synthetic emulsifier + monoglycerides + lysophospholipids (LPL) product; NC-60 = negative control with 60 kcal/kg ME reduction of the PC1; PC1 + LPL60 = PC1 reformulated with LPL matrix (60 kcal/kg); NC-100 = negative control with 100 kcal/kg ME reduction of the PC1; PC1 + LPL100 = PC1 reformulated with LPL matrix (100 kcal/kg); PC2 = positive control formulated with acid soy oil; PC2 + LPL60 = PC2 reformulated with LPL matrix (60 kcal/kg).

top, PC1 + LPL60, and PC1 + LPL100 against PC1 or PC2 + LPL60 against PC2). Based on the obtained performance results, it was observed a profitability in reformulated diets having 60 kcal/kg from LPL, which reduced feed costs and increased ROI (3.0) compared with PC1. The highest growth performance resulted in the highest ROI (8.0) when LPL on top was used compared with PC1. The obtained ROI for LPL 100 kcal/kg matrix was 1.8. Using ASO as the oil source and 60 kcal/kg from LPL provided the lowest ROI (2.8) compared with PC2.

DISCUSSION

The present study had the objective of evaluating the effects that a synthetic emulsifier + monoglycerides + LPL product would have as an exogenous emulsifier or absorption enhancer on energy utilization, digestibility of nutrients, and broiler performance using 2 oil sources in the diet. In addition, control diets with practical energy and nutrient levels were compared with reformulated diets, which presented reduced soy oil inclusion. The results in this study supported the hypothesis that a synthetic emulsifier + monoglycerides + LPL supplementation would enhance performance of broilers by increasing energy values and digestibility of DM, protein, and fat.

The addition of selected quantities of synthetic emulsifier and monoglycerides to LPL and applying it as a mixture have been previously reported to improve in vitro lipid emulsification and hydrolysis (Jansen et al., 2015). These authors concluded that the mixture more than doubled the absorption of monoglycerides and increased the absorption of free FAs by more than 75%, demonstrating that diets could be reduced with

Table 7. Cumulative growth performance of broilers fed diets containing different soybean oils, energy levels, and an emulsifier based on lysophospholipids (LPL), Exp. 2.

Item[1]	BW gain/bird, g		Feed intake/bird, g		Feed conversion ratio	
	Day 0–21	Day 0–42	Day 0–21	Day 0–42	Day 0–21	Day 0–42
PC1	1,137^{a,b}	3,479^{a,b}	1,403	5,063	1.234^{b,c}	1.456^{b,c,d}
PC1 + LPL on top	1,169^{a}	3,562^{a}	1,426	5,042	1.220^{c}	1.415^{d}
NC-60	1,111^{b}	3,397^{b,c}	1,427	5,079	1.285^{a,b}	1.496^{a,b}
PC1 + LPL60	1,140^{a,b}	3,478^{a,b}	1,408	5,026	1.235^{b,c}	1.445^{c,d}
NC-100	1,106^{b}	3,377^{c}	1,437	5,179	1.300^{a}	1.533^{a}
PC1 + LPL100	1,136^{a,b}	3,470^{a,b,c}	1,616	5,125	1.246^{a,b,c}	1.477^{b,c}
PC2	1,146^{a,b}	3,463^{b,c}	1,429	5,057	1.247^{a,b}	1.460^{b,c,d}
PC2 + LPL60	1,139^{a,b}	3,484^{a,b}	1,418	5,073	1.245^{a,b,c}	1.456^{b,c,d}
SEM	3.86	10.09	5.87	15.06	0.006	0.006
P value	0.003	0.001	0.881	0.223	0.001	0.001

$^{a\text{-}d}$Means with different superscript letters differ ($P < 0.05$) based on Tukey's honest significant difference test.

[1]Dietary treatments: PC1 = positive control formulated with soybean degummed oil; PC1 + LPL on top = PC1 formulated with 500 g/ton of a synthetic emulsifier + monoglycerides + lysophospholipids (LPL) product; NC-60 = negative control with 60 kcal/kg ME reduction of the PC1; PC1 + LPL60 = PC1 reformulated with LPL matrix (60 kcal/kg); NC-100 = negative control with 100 kcal/kg ME reduction of the PC1; PC1 + LPL100 = PC1 reformulated with LPL matrix (100 kcal/kg); PC2 = positive control formulated with acid soy oil; PC2 + LPL60 = PC2 reformulated with LPL matrix (60 kcal/kg).

Table 8. Carcass, commercial cuts, and abdominal fat yields (%) and weights (g) of broilers fed diets containing different soybean oils, energy levels, and an emulsifier based on lysophospholipids (LPL), on day 43 in Exp. 2.

	Carcass[2]		Breast[3]		Thighs		Drumsticks		Wings		Abdominal fat	
Item[1]	g	%	g	%	g	%	g	%	g	%	g	%
PC1	2,763	78.4	905	32.7	342	12.5	503	18.3	339	12.4	27.4[a,b]	0.95[a,b]
PC1 + LPL on top	2,779	78.5	906	32.8	352	12.8	511	18.6	342	12.4	21.4[b]	0.83[b]
NC-60	2,732	78.3	890	32.1	350	12.8	505	18.5	334	12.1	25.1[a,b]	0.87[a,b]
PC1 + LPL60	2,742	78.4	892	32.5	341	12.6	505	18.6	332	12.2	22.3[b]	0.85[b]
NC-100	2,722	78.1	894	32.0	345	12.5	491	17.9	339	12.1	29.6[a]	0.98[a]
PC1 + LPL100	2,731	78.2	897	32.6	342	12.5	503	17.9	341	12.6	24.4[a,b]	0.86[a,b]
PC2	2,759	78.3	907	32.7	343	12.5	509	18.6	338	12.3	27.7[a,b]	0.98[a]
PC2 + LPL60	2,762	78.4	905	32.7	345	12.5	504	18.4	342	12.4	25.8[a,b]	0.93[a,b]
SEM	5.86	0.106	4.06	0.109	1.79	0.057	2.40	0.102	1.59	0.066	0.623	0.010
P value	0.187	0.993	0.929	0.451	0.705	0.652	0.630	0.417	0.653	0.618	0.008	0.050

[a,b]Means with different superscript letters differ ($P < 0.05$) based on Tukey's honest significant difference test.
[1]Dietary treatments: PC1 = positive control formulated with soybean degummed oil; PC1 + LPL on top = PC1 formulated with 500 g/ton of a synthetic emulsifier + monoglycerides + lysophospholipids (LPL) product; NC-60 = negative control with 60 kcal/kg ME reduction of the PC1; PC1 + LPL60 = PC1 reformulated with LPL matrix (60 kcal/kg); NC-100 = negative control with 100 kcal/kg ME reduction of the PC1; PC1 + LPL100 = PC1 reformulated with LPL matrix (100 kcal/kg); PC2 = positive control formulated with acid soy oil; PC2 + LPL60 = PC2 reformulated with LPL matrix (60 kcal/kg).
[2]Eviscerated carcass as a percentage of body weight at 43 d. There were 4 slaughtered birds per replicate pen and 7 replications per treatment with a total of 224 evaluated birds.
[3]Skinless boneless *Pectoralis major* as proportion of carcass at 43 d.

60 to 80 kcal/kg using this mixture, while maintaining broiler performance and carcass yield.

There are published data available on the effect of LPL on broiler performance, and most of the studies have evaluated the effect of "on top" supplementation, where improved performance is expected (Melegy et al., 2010; Zaefarian et al., 2015; Zampiga et al., 2016). In commercial diet formulation, it is common to apply energy reductions when supplementing diet with biosurfactants or bio-emulsifiers to maintain bird performance while reducing cost. Few publications have reported the LPL utilization on standard compared with reformulated poultry diets, where fats and oils have been reduced or substituted (Zhao and Kim, 2017; Papadopoulos et al., 2018; Chen et al., 2019). Reductions in soybean oil are more important in scenarios where the oil cost increases.

Although growth performance was evaluated in Exp. 1, it was designed mainly to evaluate ileal digestibility and energy utilization. Differences were observed between Exp. 1 and 2 in the average of BW gain and FCR, which could be explained by the lower number of birds per experimental unit in Exp. 1 as well as differences in environmental conditions and rearing system. However, it was also observed that effects of dietary treatments were similar in both experiments. Reformulated diets having 60 or 100 kcal/kg from LPL matrix (PC1 + LPL60 or PC1 + LPL100, respectively) presented similar performance and digestibility when compared with the control PC1, showing that the bio-emulsifier was able to enhance energy as expected.

In addition, broilers fed diets with LPL on top presented the highest digestibility and energy values. These results are consistent in part with findings by Wealleans et al. (2020a), who reported increased digestibility of DM, EE, and N as well as AME when broilers were fed diets formulated with 250 g/ton LPL on day 21. Zhang et al. (2011) observed increased AME; however, digestibility of DM and CP had no differences when broilers were fed diets with 500 g/ton LPL on day 17 ($P < 0.10$) and day 38 ($P < 0.01$).

Papadopoulos et al. (2018) observed increased intestinal mucosal height on day 14 when 500 g/ton LPL was included in broiler diets compared with positive control diets; however, this difference was not observed at 28 and 42 d of age. Villi structure is important because

Table 9. Contrasts of energy retention, nutrient digestibility, growth performance, and abdominal fat (%) of broiler chickens fed diets containing different soybean oils, energy levels, and supplemented or not with lysophospholipids (LPL).

	Ileal digestibility, % (Exp.1)				Day 0–21 (Exp.1)		Day 0–21 (Exp.2)		Day 0–42 (Exp.2)		Day 43 (Exp.2)
Contrasts (*P* value)[1]	IDE, kcal/kg	Dry matter	Ether extract	Crude protein	BW gain, g	FCR	BW gain, g	FCR	BW gain, g	FCR	Abdominal fat, %
PC1 vs. PC1 + LPL on top	0.243	0.028	0.022	0.012	0.081	0.469	0.014	0.449	0.009	0.007	0.009
PC1 vs. PC2	0.767	0.335	0.608	0.810	0.836	0.773	0.500	0.490	0.619	0.768	0.202
NC-60, NC-100 vs. PC1 + LPL60, PC1 + LPL100	0.002	0.018	0.048	0.001	0.056	0.022	0.002	0.001	0.001	0.001	0.014

Abbreviations: FCR, feed conversion ratio; IDE, ileal digestible energy.
[1]Dietary treatments: PC1 = positive control formulated with soybean degummed oil; PC1 + LPL on top = PC1 formulated with 500 g/ton of a synthetic emulsifier + monoglycerides + lysophospholipids (LPL) product; NC-60 = negative control with 60 kcal/kg ME reduction of the PC1; PC1 + LPL60 = PC1 reformulated with LPL matrix (60 kcal/kg); NC-100 = negative control with 100 kcal/kg ME reduction of the PC1; PC1 + LPL100 = PC1 reformulated with LPL matrix (100 kcal/kg); PC2 = positive control formulated with acid soy oil.

Table 10. Return on investment (ROI) of experimental diets containing different soybean oils, energy levels, and supplemented or not with lysophospholipids (LPL) for broilers[1].

	Degummed soybean oil				Acid soybean oil	
Parameters	PC1	PC + LPL on top	PC1 + LPL60	PC1 + LPL100	PC2	PC2 + LPL60
Total kg slaughtered broilers per treatment	617.05	631.57	617.05	615.65	614.42	617.92
Feed conversion ratio at 42 d	1.456	1.415	1.445	1.477	1.46	1.456
Total feed intake, kg	898.42	893.68	891.64	909.32	897.06	899.70
Cost of emulsifier per kg of feed ($)	0	0.002	0.002	0.002	0.000	0.002
Average cost (mixture) per kg feed ($)	0.291	0.294	0.286	0.282	0.284	0.281
Market value kg of broilers ($), march/2019	1.20	1.20	1.20	1.20	1.20	1.20
Total revenue of slaughter per kg of broilers	740.46	757.89	740.46	738.78	737.31	741.51
Total cost feed per treatment ($)	254.41	255.27	248.29	249.01	247.73	246.15
Total net income per treatment	486.05	502.62	492.17	489.77	489.58	495.36
Investment with the emulsifier per treatment ($)	0.00	2.06	2.06	2.10	0.00	2.08
ROI treatments *vs.* control diets	-	8.0	3.0	1.8	-	2.8

[1]Dietary treatments: PC1 = positive control formulated with soybean degummed oil; PC1 + LPL on top = PC1 formulated with 500 g/ton of a synthetic emulsifier + monoglycerides + lysophospholipids (LPL) product; PC1 + LPL60 = PC1 reformulated with LPL matrix (60 kcal/kg); PC1 + LPL100 = PC1 reformulated with LPL matrix (100 kcal/kg); PC2 = positive control formulated with acid soy oil; PC2 + LPL60 = PC2 reformulated with LPL matrix (60 kcal/kg).

LPL can increase fluidity and permeability of cell membranes, having direct or indirect effects on membrane protein formation (Lundbaek, 2006). It can influence the uptake of lipids across enterocytes in the small intestine. Lundbaek (2006) also concluded that by increasing LPL content in the lumen, smaller micelles were formed, and micelle transportation and lipid absorption can be improved.

Improvements on EE, DM, and CP digestibility as well as AME and IDE were followed by improvements on BW gain and FCR when LPL was used, mainly on top. Frequently, the increased broiler performance has been associated with improvements in energy utilization and availability of nutrients from feed (Olukosi et al., 2008; Vieira and Angel, 2012). Wealleans et al. (2020a) observed increases in AME and DM and fat digestibility when broilers were fed corn-SBM-wheat and palm oil basal diets supplemented with LPL from day 0 to 7 posthatch.

In the present study, broilers fed diets with LPL on top presented the highest growth performance, which was supported by increased energy and nutrient utilization. Melegy et al. (2010) reported improved BW gain and FCR when broilers were fed 250 or 500 g/ton LPL until day 42 compared with a control diet with reduced oil. Zhang et al. (2011) also reported increased BW gain and lower FCR in birds fed diets supplemented with LPL until day 21, but differences were not observed from day 0 to 42. Wealleans et al. (2020b) demonstrated that “on top” supplementation produced differences in FCR and calculated that supplementation at 125 and 250 g/ton could recover average dietary energy reductions of 57.88 and 73.11 kcal/kg in feed, respectively. This effect was noted in the present study, although to an extended degree, the synthetic emulsifier + monoglycerides + LPL product recovered performance when 60 kcal/kg or 100 kcal/kg LPL matrix was used in reformulated diets.

In the present study, carcass and commercial cuts yield were not affected by 500 g/ton LPL; however, broilers had reduced abdominal fat when fed PC1 + LPL on top compared with other treatments. Melegy et al. (2010) did not observe effects of 250 g/ton LPL on meat yield at 42 d; however, Jansen et al. (2015) found reduced abdominal fat on 42-day-old broilers fed diets with 500 g/ton LPL. This may indicate an increase in efficiency of energy usage for growth because birds that received reformulated diets, with LPL matrix and lower oil content, had lower abdominal fat and improved performance than those fed NC diets. The efficiency of LPL to improve energy can be also explained because in the absorption process, FA aggregate to form micelles to pass through the liquid phase of the small intestine to get absorbed as hydrophobic components. This process is naturally mediated by emulsifiers, such as bile salts, which can be limited in young birds, or if the amount of fat is high or using more saturated fat sources (Jansen et al., 2015). This limitation causes an inability to form mixed micelles in the intestinal lumen that further decreases fat digestion and absorption of nutrients (Leeson and Atteh, 1995). Therefore, exogenous emulsifiers can contribute in fat emulsification optimizing lipase activity and FA incorporation into micelles (Zhang et al., 2011).

Different fat sources have been used in LPL supplementation studies to evaluate the improvements in absorption produced by its addition (Zhang et al., 2011; Jansen et al., 2015; Wealleans et al., 2020a). In the present study, different oil sources were used; however, this comparison was not the main objective, and FA composition was not presented. The high free FA and low monoglycerides concentration in ASO was previously demonstrated (Sklan, 1979), and it interferes in emulsification in the intestine, resulting in lower absorption of fat and energy retention by broilers if compared to DSO. Vieira et al. (2002) reported poorer FCR in broilers fed ASO than when fed DSO from day 7 to 42; however, similar performance and carcass yield were observed by Vieira et al. (2006), which is consistent with results from the present study.

Limitation of ASO inclusion in poultry diets has been mainly due to the lack of consistency in FA composition, higher humidity, impurities, and standardization of FA, which have affected broiler performance (Vieira et al., 2002). Nevertheless, the industry of oil has changed, and more technology has been applied to obtain ASO. For this reason, ASO provides lower AME for broilers; however, it represents lower cost to feed formulations, increasing the potential of using ASO in animal nutrition because of its good quality, lower price, and similar FA compared with DSO (Borsatti et al., 2018).

The ROI for the treatment groups was calculated in comparison with the respective control group at 42 d of age. Among the treated groups, PC1 + LPL on top was supplemented at 0.5 g/kg of diet and showed an ROI of 8:1 over the control diet PC1. The PC1 + LPL60 had 60 kcal/kg AME reduction and was supplemented with 0.5 g/kg of diet, showing an ROI of 3:1 over the PC1. Using ASO, the PC2 + LPL60 with 60 kcal/kg AME reduction and supplemented with 0.5 g/kg of diet showed an ROI of 2.8:1 over the PC2. Using the same calculation, Selvam et al. (2015) reported an ROI of 24:1, when a multivitamin and amino acid supplement was administered for broilers. Lokapirnasari et al. (2017) provided probiotics at 0.005% in quail's diets for 4 wk resulting in an ROI of 15% over the control diet.

Diets formulated with differences in oil sources, ME levels, and a bio-emulsifier can become an interesting material for discussion and cost analysis. The cost-benefit considered diets' cost and production parameters when broilers were fed diets with LPL (on top or reformulation) against the respective positive control (standards with DSO or ASO). Based on production indexes, profitability was observed in reformulated diets having 60 kcal/kg or 100 kcal/kg from LPL, demonstrating reduced feed costs and increased ROI, 3.0 or 1.8, respectively. The highest growth performance resulted in the highest ROI (8.0) when LPL on top was used. It demonstrates that reducing feed cost through lower oil inclusion can provide similar or improved performance with higher cost-benefits if a biosurfactant is used.

Considering the ME levels, in the present study, broilers fed the control diet presented increased BW gain and decreased FCR compared with birds fed diets with 60 or 100 kcal/kg ME reductions. Dozier et al. (2006) observed decreased FCR when broilers were fed diets varying in ME from 3,175 to 3,310 kcal/kg from 30 to 59 d of age. Decreased growth performance between low and high energy levels was also observed by Stefanello et al. (2017). In this study, broilers were fed corn-SBM basal diets formulated with 3,050 and 3,170 kcal/kg from day 0 to 21 and day 22 to 40, respectively, having 50 or 100 kcal/kg ME reductions. In addition, no differences in abdominal fat or meat yield were observed when broilers were fed decreased ME, and these results are in agreement with those of Saleh et al. (2004) and Dozier et al. (2006) who evaluated broilers on day 63 and day 56, respectively.

No differences between dietary treatments were observed on FI in the present study, and this is in accordance with the study by Vieira et al. (2015) when birds were fed corn-SBM diets with 100 kcal/kg AME reduction, presenting similar FI until day 35. Stefanello et al. (2017) fed broilers with standard corn-SBM diets having 50 or 100 kcal/kg AME reduction and observed similar FI until day 40. On the other hand, dietary fat increases showed decreased FI of broilers (Cheng et al., 1997; Dozier et al., 2006, 2007). These results are still inconsistent in the literature as to whether the fast-growing broiler has the ability to adjust caloric intake when fed diets varying in AME (Leeson et al., 1996; Dozier et al., 2006) or to eat to a certain capacity regardless of dietary energy content (Hidalgo et al., 2004; Vieira et al., 2006).

In conclusion, no differences were observed on digestibility and performance using DSO or ASO in both experiments. The synthetic emulsifier + monoglycerides + LPL product improved digestibility of dry matter, protein, and fat as well as apparent metabolizable energy and IDE, which reflected in improved body weight gain and FCR. The LPL-based bio-emulsifier was able to enhance energy absorption when used in reformulated diets with reduced oil, as well as the LPL on top provided the highest digestibility and performance with reduced abdominal fat. It was economically beneficial to apply the LPL-based bio-emulsifier in broiler corn-soy diets. Therefore, the biosurfactant may be added to feed formulation to decrease the usage of costly added dietary fat or to maximize growth performance.

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DISCLOSURES

The authors declare no conflicts of interest.

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