



Lysolecithin, but not lecithin, improves nutrient digestibility and growth rates in young broilers

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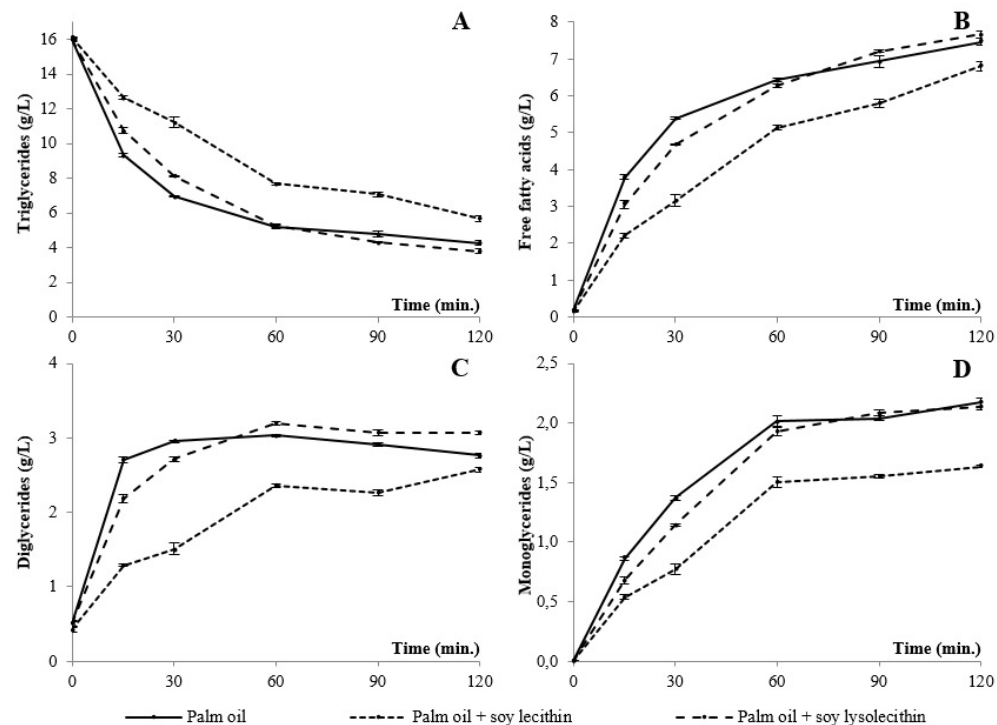


Figure 1. Hydrolysis of (A) triglycerides and accumulation of (B) free fatty acids, (C) diglycerides and (D) monoglycerides during the in vitro hydrolysis of palm oil (solid line), palm oil with soybean lecithin (dotted line) and palm oil with soybean lysolecithin (dashed line). The experimental treatments were carried out in triplicate. The mean concentrations of the lipids (mg/ml) are given over time (min), with error bars indicating the standard error values. (Experiment 1).

179x134mm (120 x 120 DPI)

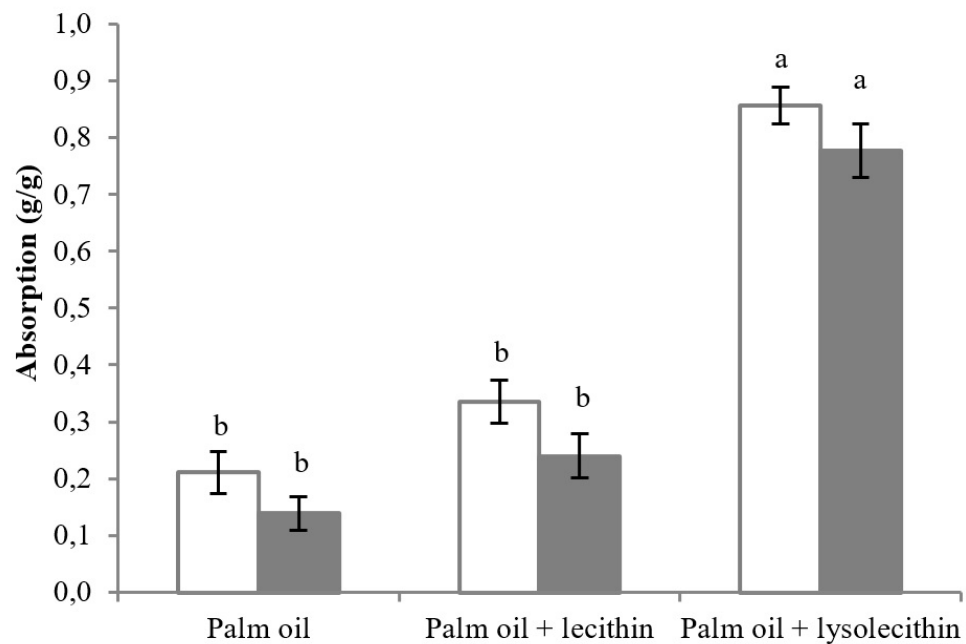


Figure 2. Absorption of monoglycerides (white bars) and free fatty acids (grey bars) generated during in vitro hydrolysis of palm oil, palm oil with soybean lecithin and palm oil with soybean lysolecithin by differentiated Caco-2 monolayers and expressed as percentage of applied monoglycerides and free fatty acids. Data are means of three or more observations per treatment, with error bars indicating the standard error values. (Experiment 1).

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Supplemental Information Table 1. Fatty acid composition (g/kg) in palm oil and the ether extract of the experimental starter diet (0-8 days of age) formulated with palm oil

Fatty acid ^a	Palm oil	Diet ^b
C14:0	10.1	4.7
C16:0	429.0	306.0
C18:0	45.7	37.2
C18:1 (ω -9)	401.6	339.3
C18:2 (ω -6)	102.1	289.4
C18:3 (ω -3)	3.8	11.3
Total unsaturated fatty acids	507.5	643.8
Total saturated fatty acids	492.4	356.2
Ratio unsaturated/saturated	1.03	1.81

^a Fatty acids present at levels below 5 g/kg in all samples are not listed.

^b Values represent the mean of the three experimental diets with standard error of the means of 0.1, 0.1, 0.4, 2.3, 1.6 and 0.2 g/kg for C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3, respectively.

Supplemental Information Table 2. Phospho- and lysophospholipid contents¹ (g/kg) of soybean lecithin and lysolecithin

(Lyso)phospholipid	Soybean lecithin	Soybean lysolecithin
Phosphatidylcholine	125.3	90.4
Lysophosphatidylcholine	7.8	39.2
Phosphatidylinositol	88.0	81.9
Lysophosphatidylinositol	ND	13.0
Phosphatidylethanolamine	97.2	33.6
Lysophosphatidylethanolamine	3.3	40.4
Phosphatidic acid	72.2	13.8
Lysophosphatidic acid	3.0	26.2
Total phospholipids	382.7	219.7
Total lysophospholipids	14.1	118.8

¹ Contents of non-phospholipid compounds such as triglycerides, glycolipids, water and minerals are not presented.

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3 **13 Supplemental Information Table 3.** Levels of moisture, impurities and unsaponifiables, and
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6 **14** free fatty acids, U:S ratio, and predicted AME values for broilers <21 days in commercial oil
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8 **15** samples used to make experimental diets (Experiment 3)
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Oil Type	MIU, %	FFA, %	U:S ratio	AME, MJ/kg, <21 days	AME, MJ/kg, >21 days
Lecithin	3.70	24.8	3.7	32.30	34.31
Lecithin	3.60	24.6	4.1	32.72	34.56
Lecithin	3.50	18.7	3.7	32.89	34.73
Soybean	0.70	1.9	4.8	36.57	37.40
Soybean	-- ¹	1.9	4.7	36.57	37.66

16 ¹Below limit of detection
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3 1 **Lysolecithin, but not lecithin, improves nutrient digestibility and growth rates in young**
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6 **broilers**
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26 ABSTRACT

27 Young broilers have an underdeveloped ability for lipid digestion. The potential of lecithin and
28 lysolecithin to improve lipid digestion and growth performance was investigated in 3
29 experiments: an *in vitro* model that mimics the intestinal conditions of the chick, a digestibility
30 trial with chicks (5 to 7 days of age), and a performance trial until 21 days of age. In Experiment
31 1, palm oil (PO), palm oil with lecithin (PO+L), and palm oil with lysolecithin (PO+LY) were
32 subjected to *in vitro* hydrolysis and applied to Caco-2 monolayers to assess lipid absorption.
33 The *in vitro* hydrolysis rate of triglycerides was higher in PO+LY ($k= 11.76 \times 10^{-3} \text{ min}^{-1}$) than
34 in either PO ($k= 9.73 \times 10^{-3} \text{ min}^{-1}$) or PO+L ($k= 8.41 \times 10^{-3} \text{ min}^{-1}$), and the absorption of
35 monoglycerides and free fatty acids was highest ($P<0.01$) for PO+LY. In Experiment 2, 90
36 broilers were assigned to three dietary treatments: a basal diet with 4% palm oil, and the basal
37 diet supplemented with either 250 ppm lecithin or lysolecithin. ATTD of crude fat was higher
38 in broilers supplemented with lysolecithin, but was lower in broilers supplemented with
39 lecithin. DM digestibility and AMEn in birds supplemented with lysolecithin were significantly
40 higher (3.03% and 0.47 MJ/kg, respectively). In Experiment 3, 480 broilers were randomly
41 allocated to four dietary treatments: basal diet with soybean oil (2%), basal diet with lecithin
42 (2%), soybean oil diet with 250 ppm lysolecithin, or lecithin oil diet with 250 ppm lysolecithin.
43 Lecithin diets significantly reduced weight at day 10 and 21 compared with soybean oil.
44 However, the addition of lysolecithin to lecithin-containing diets significantly improved bird
45 performance. The results of these studies show that, in contrast to lecithin, lysolecithin is able
46 to significantly improve the digestibility and energy values of feed in young broilers.

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48 **KEYWORDS:** broiler; digestibility; fat; lecithin; lysolecithin; performance

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51 INTRODUCTION

52 After hatch, lipid digestion in young birds is physiologically limited, and is a target for
53 improvement and support. Roy et al. (2010) have ascribed the inefficient digestion and
54 absorption of lipids by young chickens to a combination of a low duodenal secretion of lipase,
55 a low level of lipase activity and a low rate of bile salt synthesis. Kroghdahl (1985) and Maiorka
56 et al. (2004) showed, however, that lipase concentration and activity in young chickens (7 days
57 of age) are modulated according to the inclusion level of fats in the diet. Moreover, in a study
58 of Meng et al. (2004), lipase addition at 0.2 g/kg did not affect the apparent fat digestibility in
59 broilers of one to 14 days of age. Therefore, the inability to utilize fats has been attributed to
60 low bile salt concentrations in the intestines, rather than to deficiencies in lipase secretion or
61 activity (Maiorka et al., 2004; Maisonnier et al., 2003). Although dietary supplementation of
62 bile salt has been shown to improve lipid utilization in chickens (Kroghdahl, 1985; Polin et al
63 1980), supplementation is generally not applied on a commercial scale due to economic
64 drawbacks (Roy et al., 2010).

65 One strategy for improvement of fat digestion is the dietary application of molecules
66 with proven ability to improve lipid digestion, for example lecithin and its derivative
67 lysolecithin. Huang et al. (2007) showed that in diets supplemented with 2% of soybean oil,
68 replacement of 25% of the soybean oil with soybean lecithin (0.5% lecithin in the diet)
69 improved crude fat digestion and performance in broilers. On the other hand, complete
70 replacement of the soybean oil with lecithin (2% lecithin in the diet) resulted in adverse effects.
71 Moreover, in a study of Blanch et al. (1996) the AME of a basal diet with tallow as the main
72 fat source was not improved by the addition of 0.2% soybean lecithin.

73 Lysolecithins are produced by phospholipase to cleave one hydrophobic fatty acid from
74 phospholipids (Joshi et al., 2006). This changes the stereochemical structure of the
75 phospholipids in the lecithin into lysophospholipids. The resulting mixture, lysolecithin, has

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3 76 an increased hydrophilic-lipophilic balance (Van Nieuwenhuyzen and Tomás, 2008) and
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5 77 lower critical micelle concentration (0.02 to 0.2 mM/L). Both phospho- and lysophospholipids
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7 78 consist of a hydrophilic head group (phosphatidyl substituent) and a hydrophobic tail (fatty
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9 79 acid chains). But, due to the removal of one fatty acid, lysophospholipids are more hydrophilic
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11 80 and thus have better oil-in-water emulsifying properties than phospholipids (Joshi et al., 2006;
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13 81 Liu and Ma, 2011). In the animal, the pancreas secretes native phospholipase (EC 3.1.1.4) to
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15 82 convert the phospholipids secreted by the gall bladder into lysophospholipids (Karray et al.,
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17 83 2011). These lysophospholipids were shown to play an important role in mixed micelle
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19 84 formation (Lo and Tso, 2009). Therefore, it is hypothesized that lysolecithin possess a greater
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21 85 ability to improve the digestion of fats and oils than lecithin. Inclusion of 3.2% of rice bran
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23 86 lysolecithin in a broiler feed formulated with rice bran oil increased the crude fat digestibility
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25 87 (Raju et al., 2011). Additionally, Zhang et al. (2011) observed an increased fatty acid
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27 88 digestibility in broilers using 0.125% of soybean lysolecithin on soybean oil, tallow and poultry
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29 89 fat. These improvements in digestibility consistently lead to improved growth performance and
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31 90 efficiency in growing broilers (Wealleans et al., 2019), as well as in other species (Wang et al.,
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33 91 2019; Zhao et al., 2017; Papadopoulos et al., 2014).

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35 92 The limited comparative studies available on lecithin and lysolecithin application in
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37 93 broiler diets have, however, all been performed with birds of 14 days of age or older. Therefore,
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39 94 the potential of both lecithin and lysolecithin from soybean to improve lipid digestion was
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41 95 investigated in 3 experiments: an *in vitro* model, a digestibility trial with young broilers (5 to
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43 96 7 days of age), and a performance trial until 21 days of age.

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46 47 98 METHODS AND MATERIALS

48 49 99 *Experiment 1: In Vitro Fat Absorption*

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3 100 Crude palm oil (single batch) was sourced from a commercial feed mill in Belgium.
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5 101 The FA composition of the palm oil was analysed (ISO, 2002, 17764). Soybean lecithin and
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7 102 soybean lysolecithin were sourced from Kemin Europa NV (Herentals, Belgium). Lecithin and
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9 103 lysolecithin were analysed for their phospho- and lysophospholipid content by phosphorus-31
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11 104 nuclear magnetic resonance spectroscopy (³¹P-NMR, Spectral Service AG, Cologne,
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13 105 Germany).

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16 106 The lipid hydrolysis model previously employed by Jansen et al. (2015) was slightly
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18 107 modified. Briefly, 1.00 g of lecithin or lysolecithin were first dispersed into 49.00 g of palm
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20 108 oil. Fasted state simulated intestinal fluid (FaSSIF) was prepared by adding 2.24 g of FaSSIF
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22 109 powder (Biorelevant.com Ltd, Croydon, United Kingdom) to 1 L of phosphate buffer (35 mM,
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24 110 pH 6.5) containing 106 mM NaCl. According to the manufacturer, the FaSSIF contained 3 mM
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26 111 bile salt (sodium taurocholate). Aliquots of 0.25 g of each of the respective fat treatments and
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28 112 14.75 ml of FaSSIF were added into 50 ml centrifuge tubes. The content of each tube was
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30 113 mixed for 30 seconds with a high shear mixer (24000 rpm; IKA ultra-turrax T18, Staufen,
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32 114 Germany). Next, 24 mg of pancreatin (P7545, Sigma Aldrich) was added to each tube and they
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34 115 were incubated for two hours at 40 °C while shaking (250 rpm). The final contents in the digests
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36 116 were 106 mM NaCl, 1.6 g/L pancreatin, 1.6 g/L bile salts and 16.7 g/L palm oil. At 0, 15, 30,
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38 117 60, 90 and 120 minutes of incubation, a 0.5 ml sample of each digest was taken and diluted in
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40 118 9.5 ml tetrahydrofuran (HPLC grade, VWR International, Leuven, Belgium) to inactivate the
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42 119 enzymes and prepare the appropriate dilution for lipid analysis. Each digestion was performed
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44 120 in triplicate. Samples of the digests were submerged in liquid nitrogen and stored at -180 °C
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46 121 till absorption experiments started.

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49 122 Human colonic adenocarcinoma cells (Caco-2) were obtained from the European
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51 123 Collection of Cell Cultures (Public Health England, Porton Down, Salisbury, UK). Caco-2 cell
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53 124 work stock was used between passages 54 and 60. Cells were cultured in Dulbecco's modified

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3 125 eagle medium supplemented with 100 ml/L heat-inactivated fetal bovine serum (Hyclone,
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5 126 Thermo scientific, Leuven, Belgium), 10 ml/L non-essential amino acids, 100 U/ml of
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7 127 penicillin and 100 U/ml of streptomycin. The cells were maintained at 37 °C in a humidified
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9 128 atmosphere of 5% CO₂ and routinely passaged. Unless stated otherwise, the cell culture media
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11 129 and supplements were provided by Westburg (Leusden, The Netherlands).

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14 130 Caco-2 cells were seeded on collagen-coated Transwell-COL inserts (1.12 cm², pore
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16 131 size 0.4 µm, Corning Costar Corporation, Cambridge, MA) in 24-well plates at a density of 2
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18 132 x 10⁵ cells per insert and incubated for 21 days to allow the cells to differentiate. During
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20 133 incubation the medium (apical and basal) was changed three times a week and the trans-
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22 134 epithelial electrical resistance was monitored (Millicell-ERS, Millipore, Overijse, Belgium).
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24 135 Next, the different digests obtained with the lipid hydrolysis model were diluted 25-fold in
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26 136 FaSSIF and applied at the apical side of the monolayer. Simultaneously, Hank's balanced salt
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28 137 solution was applied at the basal side of the monolayer. The digest concentration and the
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30 138 differentiation protocol were optimized during the development of the model. Similar to Vors
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32 139 et al. (2012), a 25-fold dilution of the digests was selected to avoid toxicity while still
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34 140 presenting sufficient monoglycerides (MG) and free fatty acids (FFA) to the monolayers. At
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36 141 the start and after 60 minutes of incubation, a sample of the apical fluid was taken and diluted
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38 142 twofold in tetrahydrofuran. Each absorption experiment performed in at least three replicates.

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40 143 In each sample obtained during the *in vitro* lipid digestion, the degree of lipid
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42 144 hydrolysis was analyzed by HPLC. The lipids were separated into triglycerides (TG),
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44 145 diglycerides (DG), MG and FFA by a gel permeation column (PL 1110-6520, 5 µm 100A 300
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46 146 x 7.5 mm, Agilent Technologies, Diegem, Belgium) and detected by an Evaporative Light
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48 147 Scattering Detector (ELSD 85, VWR International). tetrahydrofuran was used as the mobile
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50 148 phase at a flow rate of 0.5 ml/min. Likewise, samples obtained with the lipid absorption model
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52 149 were analysed for their MG and FFA content.
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3 150 The hydrolysis of palm oil at each sample time was calculated and the apparent rate
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5 151 constant for TG hydrolysis and FFA, MG and DG release were determined as described by
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8 152 Jansen *et al.* (2015). The absorption of MG (g/g) in each well was calculated as follows:
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$$10 \text{ MG absorption} = \frac{MG_0 - MG_{60}}{MG_0}$$

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14 154 where MG_0 and MG_{60} are the respective MG contents (g/L) before and after 60 minutes of
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17 155 incubation. Correspondingly, FFA absorption (g/g) was calculated from the respective FFA
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19 156 contents.

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21 157 For statistical comparison of the in vitro hydrolysis, the apparent rate constants for TG
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24 158 hydrolysis and FFA, MG and DG release and the MG and FFA absorption were analysed as a
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26 159 one factorial arrangement. Analysis of variance (ANOVA) of the experimental treatments was
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29 160 done with STATGRAPHICS Centurion XVI software (Statpoint Technologies Inc.,
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31 161 Warrenton, VA), and means were separated by the least significant differences procedure. All
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33 162 statements of significance were based on a P-value equal to or less than 0.05.
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37 164 *Experiment 2: Digestibility Trial*

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40 165 A broiler digestibility trial was performed at the experimental research facility of the
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42 166 Laboratory of Livestock Physiology (Leuven, Belgium). The experiments were conducted in
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44 167 strict accordance with the European Communities Council Directive (2003/65/EC) and were
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47 168 approved by the Ethical Commission for Experimental Use of Animals of the KU Leuven
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49 169 (P213/2015). A total of 90 one-day-old male Ross 308 chickens were obtained from
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51 170 Belgabroed NV (Merksplas, Belgium) and assigned randomly at day zero, in groups of ten
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54 171 birds, to three dietary treatments: a basal diet without lecithin and lysolecithin, the basal diet
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56 172 supplemented with 250 ppm lecithin and the basal diet supplemented with 250 ppm
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3 173 lysolecithin. Lecithin and lysolecithin were applied to diets at the same rate in order to allow
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5 174 direct comparison of the effects of phospholipids and lysophospholipids.
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8 175 To prepare the experimental diet (Table 2), first all raw materials were milled together
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10 176 to obtain homogeneous batches. Next, the feed was divided into three batches and successively
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12 177 mixed in a small mixer with different premixes in order to produce the three experimental diets.
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14 178 Titanium dioxide (E171 titanium dioxide, IMCD Benelux N.V., Mechelen, Belgium) was
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16 179 added to all diets as an indigestible marker at an inclusion rate of 3 g/kg. All diets were fed as
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18 180 mash diets and also contained a commercial enzyme blend (KEMZYME Plus Concentrate Dry
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20 181 50 ppm, Kemin Europa NV) and phytase (RONOZYME P-(CT) 100 ppm, DSM Nutritional
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22 182 products, Deinze, Belgium).
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26 183 Birds were housed in nine digestibility cages (three replicates per treatment), consisting
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28 184 of a wire bottom with a plastic tray for excreta collection, two feed troughs and a drinking cup.
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30 185 The facility was kept under conventional EU conditions for lighting, heating and ventilation.
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32 186 The trial consisted of a pre-period of six days to minimize interference of egg yolk digestion
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34 187 (days 0 to 5) and a 48-hour collection period (days 5 to 7). Drinking water and feed were
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36 188 provided *ad libitum*. During the collection period, total excreta were collected, and pooled and
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38 189 homogeneous samples of the mixed wet excreta were freeze-dried and stored until analysis.
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42 190 The titanium dioxide was determined using the method of Short et al. (1996) with
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44 191 modifications according to Myers et al. (2004). Samples of the feed and freeze-dried excreta
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46 192 were analyzed in the accredited laboratory of the Institute for Agricultural and Fisheries
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48 193 Research (ILVO, Merelbeke, Belgium) for dry matter (DM), gross energy (GE), crude protein
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50 194 and crude fat according to EC (1971), ISO (1998, 9831), ISO (2005, 5983-2, N × 6.25) and
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52 195 ISO (1999, 6492), respectively. Additionally, the FA distribution of the ether extracts of the
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54 196 diet and excreta samples were determined (ISO, 2002, 17764).
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3 197 The coefficient of total tract apparent digestibility (CTTAD) of DM, crude protein and
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5 198 crude fat were determined by the use of the concentrations of titanium dioxide in the excreta
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8 199 and in the feed and calculated as described by Jansen et al. (2015). For the titanium dioxide in
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10 200 the feed a single value, averaged over the diets, was used for all calculations. Average diet
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12 201 titanium recovery was within acceptable limits for all diets. The coefficients of total tract
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14 202 apparent digestibility of individual fatty acids (CTTADF) were calculated as follows:

$$CTTADF = \frac{[FA_{diet} - (FA_{excreta} \times (1 - CTTAD \text{ of crude fat}))]}{[FA_{diet}]}$$

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21 204 where FA_{diet} and $FA_{excreta}$ are the respective FA contents (g/kg) analyzed in the ether extracts
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23 205 of the diet and excreta samples. The AME contents of the experimental diets were calculated
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25 206 from their respective titanium dioxide ratios and corresponding GE contents, as described by
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27 207 Jansen et al. (2015).

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31 208 The values for CTTAD of DM and crude fat, CTTADF, N-retention and AMEn were
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33 209 analysed as a one factorial arrangement and subjected to ANOVA with STATGRAPHICS
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35 210 Centurion XVI software (Statpoint Technologies Inc.). Repeated measures techniques were not
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37 211 used. Means were separated by the least significant differences procedure. All statements of
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39 212 significance were based on a P-value equal to or less than 0.05.

213 214 *Experiment 3: Performance Trial*

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216 All experimental procedures were conducted in strict accordance with the European
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218 Communities Council Directive (2003/65/EC) and were approved by the Ethics Committee of
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220 Nottingham Trent University.

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222 Birds were sourced from PD Hook Cote hatchery, Oxford. Birds were feather sexed on
day of hatch and collected by Nottingham Trent University (NTU) personnel to reduce travel
stress. A total of 480-day-old male Ross 308 chicks were individually weighed before random

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3 221 allocation to 80*80 cm mesh sided pens bedded on clean wood shavings. Unhealthy or
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5 222 unusually sized chicks were discarded from the trial upon arrival. Birds were individually
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7 223 weighed and only birds between 33 and 46 g were placed.
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10 224 Birds were allocated to four dietary treatments in a 2 x 2 factorial design. Treatments
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12 225 were: basal diet with soybean oil (2%), basal diet with lecithin oil (2%), basal diet with soybean
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14 226 oil with 250 ppm lysolecithin, or basal diet with lecithin oil with 250 ppm lysolecithin.
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16 227 Compared to Experiment 2, where dietary fat type and level were used to create challenging
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18 228 diets that could elucidate clearly the mode of action of lysolecithin, in Experiment 3 the basal
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20 229 diets were formulated to reflect relevant commercial compositions and to meet all nutrient
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22 230 requirements of the birds (Table 5). The lecithin-based oil used in this study is commercially
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24 231 available as Leciol (Adams and Green, East Yorkshire, UK). The lysolecithin was
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26 232 supplemented as LYSOFORTE® EXTEND (Kemin Animal Nutrition and Health, Belgium).
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28 233 Each treatment consisted of 12 pens, with 10 birds per pen. Diets were manufactured by Target
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30 234 Feeds (Whitchurch, UK) and supplied bagged as crumb for both starter and grower. Feed and
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32 235 water were available *ad libitum*, with care taken to ensure the birds ate and drank as soon as
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34 236 possible after placement.
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40 237 The lighting regimen was maintained in accordance with commercial practice with 15
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42 238 minutes of dusk at the start and end of each dark period. Temperature was set at 31 °C on day
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44 239 1 and reduced by approximately 1 °C per day until 21 °C was reached.
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47 240 Bird observations were used to monitor the environment and if the birds appeared
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49 241 uncomfortable, the temperature and/or ventilation was altered accordingly. Birds were
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51 242 observed twice daily during the trial and any observations related to health recorded in a trial
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53 243 diary. Any dead birds were weighed, and reasons recorded if culled. Birds were weighed by
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55 244 pen on arrival, on day 10 and at the end of the trial on day 21. Initially, individual weighed
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57 245 bags of feed were prepared containing precisely weighed feed quantities for each phase. Each
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pen of birds was fed from their designated bag for each phase. Extra feed was added to the bags as required and the quantity recorded. Total feed eaten was calculated as the difference between remaining feed in the bag and the amount weighed into the bag for each phase.

Energy conversion efficiency (ME MJ/kg gain) was calculated according to the following formula, as per Salah et al. (2004):

$$\text{Calorie conversion} = \frac{\text{Total Feed Consumption} \times \text{Energy content of feed}}{\text{Sum weight of all birds at trial end}}$$

Calorie conversion was calculated using the calculated ME contents on the feed, using both on the literature energy value of the two fat sources, as claimed by the manufacturers, and with the energy value as determined by the Wiseman equation (Wiseman et al., 1991).

Statistical analysis was carried out using SPSS v.24. After KS testing to confirm normality, data were analysed using one-way ANOVA to investigate the effect of dietary treatment on FCR, feed intake (per bird) and individual bodyweight gain for each weigh period of the study, and cumulatively. Where appropriate, Bonferroni post hoc testing was used to elucidate differences between diets/treatments.

RESULTS

Experiment 1: In Vitro Fat Absorption

In Experiment 1, the palm oil contained a high amount of saturated FA (492.4 g/kg), especially palmitic acid (429.0 g/kg), which was largely reflected in the FA in the ether extract of the diet (Supplemental Information Table 1). The total lysophospholipid fraction was confirmed to be much higher for soybean lysolecithin than for soybean lecithin (118.8 g/kg lysolecithin vs 14.1 lecithin; Supplemental Information Table 2). The majority of lysophospholipids in soybean lysolecithin were lysophosphatidylcholine (LPC) and lysophosphatidyl-ethanolamine.

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3 270 The hydrolysis of TG and the accumulation of FFA, DG and MG during the *in vitro*
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5 271 hydrolysis of palm oil, palm oil with lecithin and palm oil with lysolecithin are shown in Figure
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8 272 1. Over the whole incubation period of 120 min, the amounts of TG hydrolysed and FFA, DG
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10 273 and MG accumulated in palm oil with lecithin are markedly lower than those of palm oil and
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12 274 palm oil with lysolecithin. During the first 60 min of incubation, the amounts of TG hydrolysed
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14 275 and FFA and MG accumulated were slightly higher in palm oil without lysolecithin or lecithin
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16 276 than in palm oil with lysolecithin. After 120 min, however, the amounts of TG hydrolysed and
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18 277 FFA and DG accumulated was slightly higher in palm oil with lysolecithin than in palm oil
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20 278 without lysolecithin.
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24 279 A comparison of the apparent first-order rate constants for TG hydrolysis and the
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26 280 accumulation of FFA, DG and MG for each treatment is presented in Table 1. Addition of
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28 281 lecithin or lysolecithin to the palm oil had significant ($P < 0.01$) impact on the rates of TG
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30 282 hydrolysis and FFA, DG and MG release. TG were hydrolysed faster when lysolecithin was
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32 283 added to the palm oil ($k = 11.76 \times 10^{-3} \text{ min}^{-1}$) compared to palm oil without (lyso)lecithin ($k =$
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34 284 $9.73 \times 10^{-3} \text{ min}^{-1}$). In contrast, TG were hydrolysed slower when lecithin was added ($k =$
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36 285 $8.41 \times 10^{-3} \text{ min}^{-1}$). Similarly, the release of monoglycerides was the fastest in palm oil with
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38 286 lysolecithin ($k = 3.07 \times 10^{-3} \text{ min}^{-1}$) and the slowest in palm oil with lecithin ($k = 2.23 \times 10^{-3}$
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40 287 min^{-1}). In contrast, DG release was the slowest in palm oil without lecithin or lysolecithin.
41
42 288 There was no statistically significant difference observed in the rate of free fatty acid release
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44 289 between palm oil and palm oil with lecithin.
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49 290 The absorption of MG and FFA generated during *in vitro* hydrolysis of palm oil, palm
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51 291 oil with lecithin and palm oil with lysolecithin is presented in Figure 2. The absorption of
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53 292 monoglycerides was significantly higher ($P < 0.01$) for palm oil with lysolecithin (85.6%) than
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55 293 for palm oil (21.1%) and palm oil with lecithin (35.5%). The overall absorption of FFA was
56
57 294 slightly lower ($P > 0.1$) than that of MG. Nevertheless, the absorption of FFA was significantly
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295 higher ($P < 0.01$) for palm oil with lysolecithin (77.8%) than for palm oil (13.9%) and palm oil
296 with lecithin (24.0%).

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298 *Experiment 2: Digestibility Trial*

299 For Experiment 2, during the collection period, the observed feed intake values were
300 similar for all treatments with an average of 30.6 g per bird per day. Likewise, bodyweight gain
301 values were similar for all treatments with an average of 26.35 g per bird per day. Apparent
302 faecal digestibility values and AMEn are presented in Table 3.

303 There were significant differences ($P < 0.05$) between the treatments on all parameters
304 investigated. Compared to the basal diet, lysolecithin supplementation increased ($P < 0.01$) the
305 DM digestibility by 1.04%, CF digestibility by 2.25% and AMEn by 0.47 MJ/kg. On the other
306 hand, supplementation of lecithin had no effect on dry matter digestibility or AMEn, but
307 reduced ($P < 0.01$) the crude fat digestibility by 3.2%. When compared to the basal diet, N-
308 retention was not significantly affected by lecithin or lysolecithin supplementation. However,
309 the N-retention of the basal diet supplemented with lysolecithin (27.75 g/kg DM) was higher
310 ($P < 0.05$) than that of the basal diet supplemented with lecithin (25.26 g/kg DM). The fatty acid
311 distribution in the ether extracts of the faeces is presented in Table 4. There was no difference
312 ($P > 0.05$) between any of the dietary treatments in the fatty acid distribution in the faeces, nor
313 in the ratio of unsaturated over saturated fatty acids.

314

315 *Experiment 3: Performance Trial*

316 Table 6 shows the performance of the birds across the whole experimental period of
317 Experiment 3. During the starter phase, days 0-10, there were no significant differences in
318 average daily gain, feed intake or FCR between the treatment groups. But, birds fed the diet
319 with lecithin-based oil supplemented with lysolecithin were significantly heavier than those

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3 320 fed the diet with lecithin-based oil alone. During the grower phase there was a significant
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5 321 difference in bird weight. Birds fed the diet with lecithin-based oil alone were significantly
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7 322 lighter in weight when compared to the birds fed the diet with lecithin-based oil supplemented
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9 323 with lysolecithin ($p=0.047$). Similarly, when the whole trial phase was evaluated there were
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11 324 significant differences in average daily gain. Birds fed diets with lecithin-based oil alone gained
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13 325 less on average daily ($p=0.036$) than the birds fed the diet with lecithin-based oil supplemented
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15 326 with lysolecithin ($p=0.036$).

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19 327 The energetic values of each oil determined through analysis of chemical composition
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21 328 (Supplemental Information Table 3) demonstrated that, due to high levels of free fatty acids in
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23 329 the oils and an altered unsaturated: saturated ratio (Wiseman et al., 1991), the lecithin oil had
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25 330 a reduced predicted AME value for broilers <21 days compared to the soybean oil (36.57 MJ/kg
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27 331 soybean vs 32.64 MJ/kg lecithin). When calculating diet density based on the lipid analysis,
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29 332 therefore, rather than assumed energetic equivalence between the two oil sources, the effective
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31 333 energy conversion rate for soybean oil alone was 16.04 MJ/kg BWG, while the effective energy
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33 334 conversion rate for lecithin oil alone was worse, at 16.78 MJ/kg BWG. With the addition of
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35 335 lysolecithin, the energy conversion rate was improved across both fat sources (15.58 MJ/kg
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37 336 BWG soybean oil + lysolecithin; 16.23 MJ/kg BWG lecithin plus lysolecithin); the effective
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39 337 efficiency of use of the lecithin oil was brought closer to that of unsupplemented soya oil.
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46 47 339 DISCUSSION

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49 340 The structural differences in the composition of lecithin and lysolecithin leads to
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51 341 fundamentally diverse effects on both *in vitro* and *in vivo* lipid digestion. In Experiment 1,
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53 342 compared to palm oil without lecithin or lysolecithin, *in vitro* the apparent rate constants for
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55 343 triglyceride hydrolysis and monoglyceride release were significantly higher for palm oil
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57 344 supplemented with lysolecithin and significantly lower for palm oil supplemented with
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3 345 soybean lecithin (Figures 1 and 2). Likewise, in Experiment 2, compared to the basal diet, the
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5 346 crude fat digestibility was significantly higher in broilers supplemented with lysolecithin, while
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7 347 it was significantly lower in broilers supplemented with lecithin. Though the limitations of this
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9 348 initial study require further data to confirm these findings, Zhang et al. (2011) postulated that
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11 349 while both lecithin and lysolecithin may act as an emulsifier within the first stages of lipid
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13 350 digestion, for lipid hydrolysis to take place, the pancreatic colipase-lipase complex first must
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15 351 adsorb onto the emulsion droplets (Reis et al., 2010). The adsorption and activity of lipase at
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17 352 the oil-water interface, however, is affected by various surface-active compounds such as
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19 353 phospholipids and lysophospholipids (Reis et al., 2010, 2009).

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24 354 Possibly, the observed effect of lecithin on crude fat digestion in young broilers in
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26 355 Experiment 2 could be explained by a study of Chu et al. (2010) who showed that colipase and
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28 356 lipase adsorbed exclusively onto regions covered by phosphatidylcholine and bile salts and not
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30 357 to regions covered with phosphatidylcholine solely. This would suggest that, although
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32 358 phospholipids in lecithin may have aided the formation of smaller emulsion droplets, in the
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34 359 conditions of the young broiler insufficient bile salts are present – especially with diets
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36 360 containing high levels of palm oil where, due to the low amount of unsaturated fatty acids,
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38 361 digestibility is more challenging than that of other, more unsaturated, vegetable oils such as
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40 362 soybean oil (Tancharoenrat et al., 2013) – to facilitate the adsorption of the colipase-lipase
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42 363 complex to the surface of the droplet. The combination of the challenging basal fat type (palm
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44 364 oil) and level with the immature digestive system in young broilers led to a relatively low crude
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46 365 fat digestibility in the basal diet (69.52%) seen in Experiment 2; it is well known that relative
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48 366 digestibility coefficients of fat are limited when fats and oils are presented at a high level
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50 367 (Croom et al., 1999; Rampone, 1961), as the ability of the digestive system and liver for
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52 368 lipolysis and absorption become rate limiting. However, in contrast to the phospholipids
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54 369 contained in lecithin, it has been suggested that once in the small intestine, lysophospholipids
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3 370 tend to dissolve into mixed micelles and in this way leave the interface (~~Malaki-Nik~~ et al.,
4 371 20120). Moreover, through their participation in the formation of mixed micelles,
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6 372 lysophospholipids may play an additional role by displacing monoglycerides and FFA from
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8 373 the interface allowing the lipid hydrolysis process to continue (Lairon, 200913). Recent studies
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10 374 have also demonstrated the effect of lysolecithin on the intestinal mucosa – Papadopoulos et
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12 375 al. (2018) reported significantly thinner mucosa in birds supplemented with lysolecithin than
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14 376 in unsupplemented birds, while Chen et al. (2019) reported elevated claudin-3 levels following
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16 377 lysolecithin supplementation, indicating better sealing of tight junctions (Milatz et al., 2010).
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18 378 Brautigan et al. (~~2016~~2017) also showed increased collagen deposition and villus height
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20 379 following lysolecithin supplementation. Together, these results suggest that as lysolecithin
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22 380 becomes incorporated into the epithelial cell walls it also improves mucosal absorptive capacity
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24 381 in a way lecithin cannot.

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26 382 Although in this proof of concept study lecithin had a negative effect on lipid
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28 383 hydrolysis, no adverse effect was observed on the absorption of generated monoglycerides and
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30 384 FFA by the differentiated Caco-2 monolayer (Figure 2). This also supports the argument that
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32 385 phospholipids likely reside at the interface of emulsion droplets and in this way do not
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34 386 participate in the absorption process at the enterocytes. In contrast, the absorption of
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36 387 monoglycerides and FFA was higher with digests from the palm oil supplemented with
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38 388 lysolecithin than with digests from palm oil and palm oil supplemented with lecithin. Similarly,
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40 389 in a study by Sugawara et al. (2001) the uptake of lipid-soluble carotenoids was greatly
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42 390 improved in Caco-2 cells exposed to micelles containing lysophosphatidylcholine in
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44 391 comparison to cells incubated with micelles containing phosphatidylcholine.

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46 392 In addition to the improved CF digestibility, the DM digestibility and AMEn of basal
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48 393 diet supplemented with lysolecithin was also significantly higher than that of the basal diet and
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50 394 the basal diet supplemented with lecithin. This is in line with previous studies, which have
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3 395 shown improvements in CF and N digestibility following lysolecithin supplementation, though
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5 396 the effect of fat source on response is still uncertain: Zaefarian et al. (2015) reported improved
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7 397 AMEn values in supplemented soy oil diets, but little effect of lysolecithin on diets formulated
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9 398 with tallow, while conversely, Jansen et al. (2015) reported improvement of the AMEn of diets
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11 399 formulated with pig lard but not those formulated with soybean oil. Across 33 studies,
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13 400 Wealleans et al. (2019) found little evidence that the magnitude of response to lysolecithin
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15 401 supplementation was consistently altered by dietary fat type. In the present study, the N-
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17 402 retention in the basal diet supplemented with lysolecithin was also significantly higher than
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19 403 that in the basal diet supplemented with lecithin. Furthermore, the AMEn improvement of 0.47
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21 404 MJ/kg by lysolecithin supplementation can only be partially attributed to the 2.25%
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23 405 improvement in CF digestibility.

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28 406 Honda et al. (2009) found that fats incorporated in the feed matrix could cover other
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30 407 nutrients, lowering their digestion. As a consequence of the improved CF digestibility,
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32 408 lysolecithins could in this way enhance the digestion of other nutrients. Another possible
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34 409 explanation for the large improvement in AMEn may be found at the enterocyte level. As
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36 410 supported by the improved absorption of MG and FFA in the present study, lysolecithin may
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38 411 enhance the uptake of multiple nutrients across the enterocyte membrane. Lysophospholipids
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40 412 are known to alter membrane structure by inducing local curvatures of the bilayer (Lundbaek,
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42 413 2006; Wendel, 2000; Maingret et al., 2000). Moreover, they can affect proteins embedded in
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44 414 the membrane (Lundbaek, 2006; Maingret et al., 2000; Lundbaek and Andersen, 1994). In this
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46 415 way lysophospholipids could enhance the uptake of nutrients across the membrane of the
47
48 416 microvilli in the intestinal epithelium; this is supported by the work of Brautigan et al.
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50 417 (~~2016~~2017), who reported increased villus height and collagen cross-linkages, driven by
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52 418 upregulation of collagen-related genes, in the intestinal epithelium of broilers supplemented
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54 419 with lysolecithin.
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3 420 These differential effects on fat hydrolysis and overall digestibility between lecithin
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5 421 and lysolecithin are supported by the performance results of Experiment 3, although the basal
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7 422 diet formulations were substantially different – designed to be more commercially relevant –
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9 423 from those used in Experiment 2 Substituting soya oil for lecithin oil at the same concentration
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11 424 numerically reduced broiler growth and increased FCR until day 10 (1.45 soya oil vs 1.55
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13 425 lecithin oil, $P=0.161$), while weight at day 10 was 3% lower in birds fed on the lecithin oil diet
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15 426 than diets formulated with soya oil. Though differences between unsupplemented soya and
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17 427 lecithin oil treatments were not statistically significant, the large difference in FCR during the
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19 428 starter phase is commercially important to overall production profitability. Similarly, Huang et
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21 429 al. (2007) reported that complete replacement of the soybean oil with lecithin (2% lecithin in
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23 430 the diet) resulted in adverse effects. The adverse effect of lecithin on bird performance was
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25 431 greatest in young birds, and after day 10 the gap in feed conversion ratio between soya oil and
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27 432 lecithin oil diets narrowed (6.9% increase in FCR lecithin vs soya to day 10, 3.1% increase in
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29 433 FCR lecithin vs soya day 10-21), with birds on all treatments performing similarly. Until 14
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31 434 days of age, fat digestion is severely limited in chicks due to a lack of bile salt secretion
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33 435 (Krogdahl, 1985), after which the rates of synthesis increase fourfold. Therefore, it is likely
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35 436 that birds fed the lower available energy diets containing lecithin oil were able to achieve
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37 437 compensatory growth once bile salt synthesis reached sufficient levels and fat digestion
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39 438 improved (Krogdahl, 1985), thus closing the performance gap with those fed diets containing
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41 439 soya oil, despite energy conversion efficiency rates remaining lower throughout the trial.
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43 440 Future research on the comparative effects of lecithin and lysolecithin should continue to assess
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45 441 the effect on performance and digestibility until slaughter, when the bird is physiologically
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47 442 more mature. Meanwhile, the performance increases seen with supplemental lysolecithin are
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49 443 in line with previous research, which has demonstrated improved weight gain and feed
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51 444 conversion ratio (FCR) (Wealleans et al., 2019; Allahyari-Bake and Jahanian, 2017; Zaefarian
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3 445 et al., 2015; Zampiga et al., ~~2013~~2016), while others have reported increases in apparent
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5 446 metabolizable energy (AME) (Majdolhosseini et al., 2019; Melegy et al., 2010; Jansen et al.,
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7 447 2015).

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10 448 To conclude, this study presents evidence that lysolecithins – but likely not lecithin
11
12 449 itself – are able to significantly improve the digestibility and energy values of feed, especially
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14 450 in young broilers. These improvements may be due to a combined effect of lysophospholipids
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16 451 on lipid hydrolysis and nutrient absorption, though further research is required to confirm the
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18 452 multifactorial mode of action
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609 **Table 1.** Effect of soybean lecithin and soybean lysolecithin on the apparent first-order rate
 610 constant (k , $\times 10^3 \text{ min}^{-1}$) of triglyceride hydrolysis and diglyceride, monoglyceride and free
 611 fatty acid release during in vitro digestion of palm oil (Experiment 1)

	Triglyceride hydrolysis	Diglyceride release	Monoglyceride release	Free fatty acid release
Treatment ¹				
Palm oil	9.73 ^b	1.15 ^c	2.91 ^b	10.85 ^b
Palm oil + lecithin ²	8.41 ^c	1.58 ^b	2.23 ^c	9.49 ^b
Palm oil + lysolecithin ³	11.67 ^a	1.70 ^a	3.07 ^a	12.49 ^a
Pooled SEM	0.28	0.02	0.04	0.45
<i>P</i> -value	0.001	0.000	0.000	0.009

612 ^{a-c} Values within columns with different superscripts are significantly different ($P < 0.05$).

613 ¹ Data are means of three observations per treatment.

614 ^{2,3} Lecithin and lysolecithin were applied at 1g, dispersed into 49 g of oil

615

616 **Table 2.** Ingredients and nutrient composition of the basal¹ experimental diet (Experiment 2)

Item (g/kg, unless noted)	Starter diet
Ingredient	
Corn	452.9
Wheat	100.0
Soybean meal (45.3% CP)	363.2
Palm oil	40.0
Monocalcium phosphate	13.6
Vitamin and mineral premix ²	10.0
Methionine	3.0
Lysine HCl	2.3
Threonine	0.6
Limestone	6.8
NaCl	2.0
NaHCO ₃	2.5
TiO ₂	3.0
Calculated composition	
AMEn (MJ/kg)	11.41
Crude fiber	3.19
Lysine	1.15
Methionine + cysteine	0.86
Threonine	0.75
Calcium	0.85
Total phosphorus	0.69
Available phosphorus	0.40
Sodium	0.15
Analyzed composition ³	
Dry matter	90.43
Crude protein	23.05
Crude fat	7.25
TiO ₂	0.29
Gross energy (MJ/kg)	17.50

617 ¹ For experimental treatments, lysolecithin was added on top of the specified basal diet at 250
618 ppm

619 ² Supplied per kilogram of diet: manganese, 99 mg; zinc, 60 mg; iron, 49 mg; copper, 20 mg;
620 iodine, 1.2 mg; selenium, 0.4 g; vitamin A, 13,500 IU (retinyl acetate); vitamin E, 55 IU
621 (DL- α -tocopheryl acetate); cholecalciferol, 75 μ g; vitamin B₁, 2 mg; vitamin B₂, 5 mg;
622 vitamin B₃, 30 mg; vitamin B₅, 15 mg; vitamin B₆, 4 mg; vitamin B₁₂, 2 mg; vitamin K, 2.5
623 mg; folic acid, 1 mg; biotin, 0.2 mg; choline, 600 mg; etoxyquine, 33 mg; butylated
624 hydroxytoluene (BHT), 0.4 mg; propyl gallate, 0.12 mg; citric acid, 0.69 mg; sepiolite, 4 mg.

625 ³ Values represent the mean of the three experimental diets.

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627

628 **Table 3.** Effect of soybean lecithin and soybean lysolecithin on the apparent faecal dry matter
 629 and crude fat digestibility, nitrogen retention and AMEn of young broilers fed a palm oil rich
 630 diet (Experiment 2)

	Digestibility (%)		N-retention (g/kg DM)	AMEn (MJ/kg DM)
	DM	Crude fat		
Dietary treatment ¹				
Basal diet	69.85 ^b	69.52 ^b	27.03 ^{ab}	13.03 ^b
Basal diet + lecithin	69.47 ^b	66.32 ^c	25.26 ^b	13.03 ^b
Basal diet + lysolecithin	72.88 ^a	71.77 ^a	27.75 ^a	13.50 ^a
Pooled SEM	0.16	0.71	0.53	0.16
<i>P</i> -value	0.000	0.003	0.039	0.008

631 ^{a-c} Values within columns with different superscripts are significantly different ($P < 0.05$).

632 ¹ Data are means of three observations per treatment.

633

634 **Table 4.** Fatty acid distribution (%) in the ether extracts of the faeces of young broilers fed a
 635 basal palm oil diet, the basal diet supplemented with lecithin and the basal diet supplemented
 636 with lysolecithin (Experiment 2)

Fatty acid ¹²	Basal diet	Basal diet + lecithin	Basal Diet + lysolecithin	Pooled SEM	<i>P</i> -value
C16:0	40.59	40.47	41.23	0.69	NS
C18:0	6.29	6.89	6.91	0.15	NS
C18:1 (ω-9)	23.71	23.47	23.75	0.12	NS
C18:2 (ω-6)	26.01	26.46	25.55	0.75	NS
C18:3 (ω-3)	0.69	0.71	0.65	0.02	NS
Total unsaturated fatty acids	49.34	49.13	49.83	0.84	NS
Total saturated fatty acids	50.66	50.87	50.17	0.84	NS
Ratio unsaturated / saturated	1.03	1.04	1.01	0.03	NS

637 ¹Fatty acids that are not listed were present for less than 0.5% in all samples.

638 ² Data are means of three observations per treatment.

639 **Table 5.** Ingredients and nutrient composition of the basal experimental diets¹ (Experiment 3)

Item (g/kg, unless noted)	Soybean Oil	Lecithin Oil
Wheat	541.6	541.6
Pura ²	100.0	100.0
Soybean Meal (46.6% CP)	304.0	304.0
Soybean Oil	20.0	--
Lecithin Oil	--	20.0
Limestone	5.6	5.6
Salt	1.6	1.6
Sodium Bicarbonate	2.8	2.8
DCP	12.4	12.4
Lysine HCl	2.7	2.7
DL Methionine	3.4	3.4
Threonine	1.0	1.0
Ronozyme P5000	0.15	0.15
Ronzyme WX	0.15	0.15
Vit/Min Premix ³	4.63	4.63
Calculated composition, %		
ME, MJ/kg	12.609 MJ/kg	
Crude Protein	22.47	
Crude Fat	6.207	
Crude Fibre	3.38	
Dig Lys	1.247	
Dig Met	0.623	
Dig Met+Cys	0.938	
Ca	0.898	
Av P	0.449	
Na	0.150	

640 ¹ Lysolecithins (as LYSOFORTE[®] EXTEND) were added on top of the basal diet formulations
 641 at 500 ppm

642 ² Pura is a commercially available blend of whole rapeseed and pulses (field beans or peas)
 643 which has been ground, heat treated and pelleted. Typical analysis 21% oil, 21% protein.

644 ³Supplied per kilogram of diet: manganese, 99 mg; zinc, 60 mg; iron, 49 mg; copper, 20 mg;
 645 iodine, 1.2 mg; selenium, 0.4 g; vitamin A, 13,500 IU (retinyl acetate); vitamin E, 55 IU
 646 (DL- α -tocopheryl acetate); cholecalciferol, 75 μ g; vitamin B₁, 2 mg; vitamin B₂, 5 mg;
 647 vitamin B₃, 30 mg; vitamin B₅, 15 mg; vitamin B₆, 4 mg; vitamin B₁₂, 2 mg; vitamin K, 2.5
 648 mg; folic acid, 1 mg; biotin; 0.2 mg; choline, 600 mg; etoxyquine, 33 mg; butylated
 649 hydroxytoluene (BHT), 0.4 mg; propyl gallate, 0.12 mg; citric acid, 0.69 mg; sepiolite, 4 mg.

650 **Table 6.** Growth performance of young broilers from day 0-21 fed diets formulated with soya oil or lecithin oil, with or without supplemental
 651 lysolecithin. (Experiment 3).

	Soya Oil	Soya Oil + Lysolecithin	Lecithin Oil	Lecithin Oil + Lysolecithin	Pooled SEM	P-value
Day 0-10¹						
Weight at day 0, g	38.3	38.3	38.6	38.9	0.72	0.906
Average daily gain, g	21.2	21.5	20.8	22.0	0.41	0.198
Average daily feed intake, g	32.0	29.5	32.3	31.6	1.13	0.326
FCR	1.45	1.36	1.55	1.43	0.060	0.161
Day 10-21						
Weight at day 10, g	256.2 ^{ab}	258.9 ^{ab}	248.9 ^b	263.8 ^a	3.18	0.015
Average daily gain, g	61.3	61.4	59.4	63.4	0.11	0.105
Average daily feed intake, g	80.6	81.9	79.9	83.9	1.24	0.183
FCR	1.31	1.33	1.35	1.33	0.020	0.723
Day 0-21						
Weight at day 21, g	934.1 ^{ab}	941.6 ^{ab}	906.9 ^b	961.7 ^a	1.38	0.047
Average daily gain, g	42.5 ^{ab}	42.8 ^{ab}	41.3 ^b	43.9 ^a	0.50	0.036
Average daily feed intake, g	56.5	56.5	57.0	58.6	0.89	0.356
FCR	1.33	1.32	1.38	1.33	0.024	0.225
Energy conversion rate (MJ/kg BWG)	16.04	15.88	16.60	16.06	--	--

652 ¹ Data are means of twelve observations per treatment.

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3 653 **Figure 1.** Hydrolysis of (A) triglycerides and accumulation of (B) free fatty acids, (C)
4
5 654 diglycerides and (D) monoglycerides during the *in vitro* hydrolysis of palm oil (solid line),
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7 655 palm oil with soybean lecithin (dotted line) and palm oil with soybean lysolecithin (dashed
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9 656 line). The experimental treatments were carried out in triplicate. The mean concentrations of
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11 657 the lipids (mg/ml) are given over time (min), with error bars indicating the standard error
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13 658 values. Statistical analysis of the treatments is performed based on apparent rate constants (see
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15 659 Table 1). For enzyme kinetic comparison, apparent rate constants are used. (Experiment 1).
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22 661 **Figure 2.** Absorption of monoglycerides (white bars) and free fatty acids (grey bars) generated
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24 662 during *in vitro* hydrolysis of palm oil, palm oil with soybean lecithin and palm oil with soybean
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26 663 lysolecithin by differentiated Caco-2 monolayers and expressed as percentage of applied
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28 664 monoglycerides and free fatty acids. Data are means of three or more observations per
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30 665 treatment, with error bars indicating the standard error values. (Experiment 1).
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3 1 **Lysolecithin, but not lecithin, improves nutrient digestibility and growth rates in young**
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10 4 AL Wealleans^{†1}, J Buyse^{#‡}, D Scholey^{*}, L van Campenhout^{‡§}, E Burton^{*}, M Di Benedetto[†], S
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26 ABSTRACT

- 27 1. The potential of lecithin and lysolecithin to improve lipid digestion and growth
28 performance was investigated in three experiments: 1. an *in vitro* model that mimics
29 the intestinal conditions of the chick, 2. a digestibility trial with chicks (5-7 days of
30 age), and 3. a performance trial until 21 days of age.
- 31 2. In experiment 1, palm oil (PO), palm oil with lecithin (PO+L), and palm oil with
32 lysolecithin (PO+LY) were subjected to *in vitro* hydrolysis and applied to Caco-2
33 monolayers to assess lipid absorption.
- 34 3. The *in vitro* hydrolysis rate of triglycerides was higher in PO+LY ($k = 11.76 \times 10^3/\text{min}$)
35 than in either PO ($k = 9.73 \times 10^3/\text{min}$) or PO+L ($k = 8.41 \times 10^3/\text{min}$), and the absorption
36 of monoglycerides and free fatty acids was highest ($P < 0.01$) for PO+LY. In experiment
37 2, 90 broilers were assigned to three dietary treatments: a basal diet with 4% palm oil,
38 and the basal diet supplemented with either 250 ppm lecithin or lysolecithin.
- 39 4. ATTD of crude fat was higher in broilers supplemented with lysolecithin, but was lower
40 in broilers supplemented with lecithin. DM digestibility and AMEn in birds
41 supplemented with lysolecithin were significantly higher (3.03% and 0.47 MJ/kg,
42 respectively).
- 43 5. In experiment 3, 480 broilers were randomly allocated to four dietary treatments: basal
44 diet with soybean oil (2%), basal diet with lecithin (2%), soybean oil diet with 250 ppm
45 lysolecithin, or lecithin oil diet with 250 ppm lysolecithin.
- 46 6. Lecithin diets significantly reduced weight at day 10 and 21 compared with soybean
47 oil. However, the addition of lysolecithin to lecithin-containing diets significantly
48 improved bird performance.
- 49 7. The results of these studies showed that, in contrast to lecithin, lysolecithin was able to
50 significantly improve the digestibility and energy values of feed in young broilers.

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5 52 KEYWORDS: broiler; digestibility; fat; lecithin; lysolecithin; performance
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10 54 INTRODUCTION

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12 55 After hatch, lipid digestion in young birds is physiologically limited, and is a target for
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14 56 improvement and support. Roy *et al.* (2010) have ascribed the inefficient digestion and
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16 57 absorption of lipids by young chickens to a combination of a poor duodenal secretion of lipase,
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18 58 a low level of lipase activity and a reduced rate of bile salt synthesis. Kroghdahl (1985) and
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20 59 Maiorka *et al.* (2004) showed, however, that lipase concentration and activity in young
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22 60 chickens (at seven days of age) are modulated according to the inclusion level of fats in the
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24 61 diet. Moreover, in a study of Meng *et al.* (2004), lipase addition at 0.2 g/kg did not affect the
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26 62 apparent fat digestibility in broilers aged one to 14 days of age. Therefore, the inability to utilise
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28 63 fats has been attributed to low bile salt concentrations in the intestines, rather than to
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30 64 deficiencies in lipase secretion or activity (Maiorka *et al.*, 2004; Maisonnier *et al.*, 2003).
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32 65 Although dietary supplementation of bile salt has been shown to improve lipid utilisation in
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34 66 chickens (Kroghdahl, 1985; Polin *et al.* 1980), supplementation is generally not applied on a
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36 67 commercial scale due to economic considerations (Roy *et al.*, 2010).

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38 68 One strategy for improving fat digestion is the dietary application of molecules with proven
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40 69 ability to improve lipid digestion, for example lecithin and its derivative, lysolecithin. Huang
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42 70 *et al.* (2007) showed that, in diets supplemented with 2% of soybean oil, replacement of 25%
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44 71 of the soybean oil with soybean lecithin (0.5% lecithin in the diet) improved crude fat digestion
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46 72 and performance in broilers. On the other hand, complete replacement of the soybean oil with
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48 73 lecithin (2% lecithin in the diet) resulted in adverse effects. Moreover, in a study of Blanch *et*
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50 74 *al.* (1996) the AME of the basal diet containing tallow as the main fat source was not improved
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52 75 by the addition of 0.2% soybean lecithin.
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3 76 Lysolecithins are produced by phospholipase which cleaves one hydrophobic fatty acid from
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5 77 phospholipids (Joshi *et al.*, 2006). This changes the stereochemical structure of phospholipids
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7 78 in lecithin into lysophospholipids. The resulting lysolecithin mixture has an increased
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9 79 hydrophilic-lipophilic balance (Van Nieuwenhuyzen and Tomás, 2008) and lower critical
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11 80 micelle concentration (0.02 to 0.2 mM/l). Both phospholipids and lysophospholipids consist of
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13 81 a hydrophilic head group (phosphatidyl substituent) and a hydrophobic tail (fatty acid chains).
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15 82 But, due to the removal of one fatty acid, lysophospholipids are more hydrophilic and thus have
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17 83 better oil-in-water emulsifying properties than phospholipids (Joshi *et al.*, 2006; Liu and Ma,
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19 84 2011). In the animal, the pancreas secretes native phospholipase (EC 3.1.1.4) to convert the
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21 85 phospholipids secreted by the gall bladder into lysophospholipids (Karray *et al.*, 2011). These
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23 86 have been shown to play an important role in mixed micelle formation (Lo and Tso, 2009).
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25 87 Therefore, it can be hypothesised that lysolecithin possesses a greater ability to improve the
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27 88 digestion of fats and oils than lecithin. Inclusion of 3.2% of rice bran lysolecithin in a broiler
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29 89 feed formulated with rice bran oil increased the crude fat digestibility (Raju *et al.*, 2011).
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31 90 Additionally, Zhang *et al.* (2011) observed increased fatty acid digestibility in broilers using
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33 91 0.125% of soybean lysolecithin in partial replacement for soybean oil, tallow and poultry fat.
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35 92 These improvements in digestibility consistently lead to improved growth performance and
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37 93 efficiency in growing broilers (Wealleans *et al.*, 2019), as well as in other species (Wang *et al.*,
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39 94 2019; Zhao *et al.*, 2017; Papadopoulos *et al.*, 2014).
40
41 95 The limited comparative studies available on lecithin and lysolecithin application in broiler
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43 96 diets have all been performed with birds of 14 days of age or older. Therefore, the potential of
44
45 97 both lecithin and lysolecithin from soybean to improve lipid digestion was investigated in three
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47 98 experiments: an *in vitro* model, a digestibility trial with young broilers (5-7 days of age), and
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49 99 a performance trial until 21 days of age.
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101 METHODS AND MATERIALS

102 *Experiment 1: In vitro fat absorption*

103 Crude palm oil (single batch) was sourced from a commercial feed mill in Belgium. The FA
104 composition of the palm oil was analysed (ISO, 2002, 17764). Soybean lecithin and
105 lysolecithin were sourced from Kemin Europa NV (Herentals, Belgium). Lecithin and
106 lysolecithin were analysed for their phospho- and lysophospholipid content by phosphorus-31
107 nuclear magnetic resonance spectroscopy (³¹P-NMR, Spectral Service AG, Cologne,
108 Germany).

109 The lipid hydrolysis model, previously employed by Jansen *et al.* (2015), was slightly
110 modified. Briefly, 1 g of lecithin or lysolecithin were first dispersed into 49 g of palm oil.
111 Fasted state, simulated intestinal fluid (FaSSIF) was prepared by adding 2.24 g of FaSSIF
112 powder (Biorelevant.com Ltd, Croydon, United Kingdom) into 1 L of phosphate buffer (35
113 mM, pH 6.5) containing 106 mM NaCl. According to the manufacturer, the FaSSIF contained
114 3 mM bile salt (sodium taurocholate). Aliquots of 0.25 g of each of the respective fat treatments
115 and 14.75 ml of FaSSIF were added into 50 ml centrifuge tubes. The content of each tube was
116 mixed for 30 seconds with a high shear mixer (24000 rpm; IKA ultra-turrax T18, Staufen,
117 Germany). Next, 24 mg of pancreatin (P7545, Sigma Aldrich) was added to each tube and
118 incubated for two hours at 40°C while shaking (250 rpm). The final contents in the digests were
119 106 mM NaCl, 1.6 g/l pancreatin, 1.6 g/l bile salts and 16.7 g/l palm oil. At 0, 15, 30, 60, 90
120 and 120 minutes of incubation, a 0.5 ml aliquot of each digest was taken and diluted in 9.5 ml
121 tetrahydrofuran (HPLC grade, VWR International, Leuven, Belgium) to inactivate the enzymes
122 and prepare the appropriate dilution for lipid analysis. Each digestion was performed in
123 triplicate. Samples of the digests were submerged in liquid nitrogen and stored at -180°C until
124 the absorption experiments started.

1
2
3 125 Human colonic adenocarcinoma cells (Caco-2) were obtained from the European Collection of
4
5 126 Cell Cultures (Public Health England, Porton Down, Salisbury, UK). Caco-2 cell work stock
6
7
8 127 was used between passages 54 and 60. Cells were cultured in Dulbecco's modified eagle
9
10 128 medium supplemented with 100 ml/l heat-inactivated foetal bovine serum (Hyclone, Thermo
11
12 129 scientific, Leuven, Belgium), 10 ml/l non-essential amino acids, 100 U/ml of penicillin and
13
14 130 100 U/ml of streptomycin. The cells were maintained at 37°C in a humidified atmosphere of
15
16
17 131 5% CO₂ and routinely passaged. Unless stated otherwise, the cell culture media and
18
19 132 supplements were provided by Westburg (Leusden, The Netherlands).

20
21 133 Caco-2 cells were seeded on collagen-coated Transwell-COL inserts (1.12 cm², pore size 0.4
22
23 134 µm, Corning Costar Corporation, Cambridge, MA) in 24-well plates at a density of 2 x 10⁵
24
25 135 cells per insert and incubated for 21 days to allow the cells to differentiate. During incubation
26
27 136 the medium (apical and basal) was changed three times a week and the trans-epithelial electrical
28
29 137 resistance was monitored (Millicell-ERS, Millipore, Overijse, Belgium). Next, the different
30
31 138 digests obtained with the lipid hydrolysis model were diluted 25-fold in FaSSIF and applied at
32
33 139 the apical side of the monolayer. Simultaneously, Hank's balanced salt solution was applied at
34
35 140 the basal side of the monolayer. The digest concentration and the differentiation protocol were
36
37 141 optimised during the development of the model. Similar to Vors *et al.* (2012), a 25-fold dilution
38
39 142 of each digest was selected to avoid toxicity while still presenting sufficient monoglycerides
40
41 143 (MG) and free fatty acids (FFA) to the monolayers. At the start and after 60 minutes of
42
43 144 incubation, a sample of the apical fluid was taken and diluted twofold in tetrahydrofuran. Each
44
45 145 absorption experiment performed in at least three replicates.

46
47 146 In each sample obtained during the *in vitro* lipid digestion, the degree of lipid hydrolysis was
48
49 147 analysed by HPLC. The lipids were separated into triglycerides (TG), diglycerides (DG), MG
50
51 148 and FFA by a gel permeation column (PL 1110-6520, 5 µm 100A 300 x 7.5 mm, Agilent
52
53 149 Technologies, Diegem, Belgium) and detected by an Evaporative Light Scattering Detector
54
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2
3 150 (ELSD 85, VWR International). Tetrahydrofuran was used as the mobile phase at a flow rate
4
5 151 of 0.5 ml/min. Likewise, samples obtained with the lipid absorption model were analysed for
6
7
8 152 their MG and FFA content.

9
10 153 The hydrolysis of palm oil at each sample time was calculated and the apparent rate constant
11
12 154 for TG hydrolysis and FFA, MG and DG release were determined as described by Jansen *et al.*
13
14
15 155 (2015). The absorption of MG (g/g) in each well was calculated as follows:

$$\text{MG absorption} = \frac{MG_0 - MG_{60}}{MG_0}$$

16
17
18 156
19
20
21 157 where MG_0 and MG_{60} are the respective MG contents (g/l) before and after 60 minutes of
22
23
24 158 incubation. Correspondingly, FFA absorption (g/g) was calculated from the respective FFA
25
26 159 contents.

27
28 160 For statistical comparison of the *in vitro* hydrolysis, the apparent rate constants for TG
29
30
31 161 hydrolysis and FFA, MG and DG release and the MG and FFA absorption were analysed as a
32
33 162 factorial arrangement. Analysis of variance (ANOVA) of the experimental treatments was done
34
35 163 with STATGRAPHICS Centurion XVI software (Statpoint Technologies Inc., Warrenton,
36
37 164 VA), and means were separated by the least significant difference procedure. All statements of
38
39
40 165 significance were based on a P-value equal to or less than 0.05.

41 42 166 43 44 167 *Experiment 2: Digestibility*

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46 168 A broiler digestibility trial was performed at the experimental research facility of the
47
48
49 169 Laboratory of Livestock Physiology (Leuven, Belgium). The experiments were conducted in
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51 170 strict accordance with the European Communities Council Directive (2003/65/EC) and were
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53
54 171 approved by the Ethical Commission for Experimental Use of Animals of the KU Leuven
55
56 172 (P213/2015). A total of 90, one-day-old male Ross 308 chickens were obtained from
57
58 173 Belgabroed NV (Merksplas, Belgium) and assigned randomly at day zero, in groups of ten
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3 174 birds, to three dietary treatments; a basal diet without lecithin and lysolecithin, the basal diet
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5 175 supplemented with 250 ppm lecithin and the basal diet supplemented with 250 ppm
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7
8 176 lysolecithin. Lecithin and lysolecithin were applied to diets at the same rate in order to allow
9
10 177 direct comparison of the effects of phospholipids and lysophospholipids.

11
12 178 To prepare the experimental diet (Table 1), all raw materials were milled together to obtain
13
14 179 homogeneous batches. Next, the feed was divided into three batches and successively mixed
15
16
17 180 in a small mixer with different premixes in order to produce the experimental diets. Titanium
18
19 181 dioxide (E171, IMCD Benelux N.V., Mechelen, Belgium) was added to all diets as an
20
21 182 indigestible marker at an inclusion rate of 3 g/kg. All diets were fed in mash form and contained
22
23
24 183 a commercial enzyme blend (KEMZYME Plus Concentrate Dry 50 ppm, Kemin Europa NV)
25
26 184 and phytase (RONOZYME P-(CT) 100 ppm, DSM Nutritional products, Deinze, Belgium).

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29 185

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31 186 Table 1 here

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35 188 Birds were housed in nine digestibility cages (three replicates per treatment), constructed with
36
37 189 a wire bottom and a plastic tray for excreta collection, two feed troughs and a drinking cup.
38
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40 190 The facility was maintained under conventional EU conditions for lighting, heating and
41
42 191 ventilation. The trial consisted of a pre-period of six days to minimise interference of egg yolk
43
44 192 digestion (days 0 to 5) and a 48-hour collection period (days 5 to 7). Drinking water and feed
45
46 193 were provided *ad libitum*. During the collection period, total excreta were collected, and pooled
47
48 194 and homogeneous samples of the mixed wet excreta were freeze-dried and stored until analysis.
49
50
51 195 The titanium dioxide content was determined using the method of Short *et al.* (1996) with
52
53 196 modifications according to Myers *et al.* (2004). Samples of the feed and freeze-dried excreta
54
55 197 were analysed in the accredited laboratory of the Institute for Agricultural and Fisheries
56
57 198 Research (ILVO, Merelbeke, Belgium) for dry matter (DM), gross energy (GE), crude protein
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59
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199 and crude fat according to EC (1971), ISO (1998, 9831), ISO (2005, 5983-2, N × 6.25) and
200 ISO (1999, 6492), respectively. Additionally, the FA distribution of the ether extract of the
201 diets and excreta samples were determined (ISO, 2002, 17764).

202 The coefficient of total tract apparent digestibility (CTTAD) of DM, crude protein and crude
203 fat were determined by the use of the concentrations of titanium dioxide in the excreta and in
204 the feed, and calculated as described by Jansen *et al.* (2015). For the titanium dioxide in the
205 feed a single value, averaged over the diets, was used for all calculations. Average titanium
206 recovery was within acceptable limits for all diets. The coefficients of total tract apparent
207 digestibility of individual fatty acids (CTTADF) were calculated as follows:

$$CTTADF = \frac{[FA_{diet} - (FA_{excreta} \times (1 - CTTAD \text{ of crude fat}))]}{[FA_{diet}]}$$

209 where FA_{diet} and $FA_{excreta}$ are the respective FA contents (g/kg) analysed in the ether extract
210 of the diets and excreta samples. The AME contents of the experimental diets were calculated
211 from their respective titanium dioxide ratios and corresponding GE contents, as described by
212 Jansen *et al.* (2015).

213 The values for CTTAD of DM and crude fat, CTTADF, N-retention and AMEn were analysed
214 as a one factorial arrangement and subjected to ANOVA with STATGRAPHICS Centurion
215 XVI software (Statpoint Technologies Inc.). Repeated measures techniques were not used.
216 Means were separated by the least significant difference procedure. All statements of
217 significance were based on a P-value equal to or less than 0.05.

219 *Experiment 3: Performance*

220 All experimental procedures were conducted in strict accordance with the European
221 Communities Council Directive (2003/65/EC) and were approved by the Ethics Committee of
222 Nottingham Trent University.

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3 223 Birds were sourced from PD Hook Cote hatchery, Oxford, UK. Birds were feather sexed on
4
5 224 day of hatch and collected by Nottingham Trent University (NTU) personnel to reduce travel
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8 225 stress. A total of 480-day-old male Ross 308 chicks were individually weighed before random
9
10 226 allocation to 80 x 80 cm mesh sided pens bedded on clean wood shavings. Unhealthy or
11
12 227 unusually sized chicks were discarded from the trial upon arrival. Birds were individually
13
14 228 weighed and only birds between 33 and 46 g were placed in trial cages.
15
16
17 229 Birds were allocated to four dietary treatments in a 2 x 2 factorial design. Treatments were;
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19 230 basal diet with soybean oil (2%), basal diet with lecithin oil (2%), basal diet with soybean oil
20
21 231 with 250 ppm lysolecithin or basal diet with lecithin oil with 250 ppm lysolecithin. Compared
22
23 232 to experiment 2, where dietary fat type and level were used to create challenging diets that
24
25 233 could elucidate clearly the mode of action of lysolecithin, in experiment 3 the basal diets were
26
27 234 formulated to reflect relevant commercial compositions and to meet all nutrient requirements
28
29 235 of the birds (Table 2). The lecithin-based oil used in this study was commercially available as
30
31 236 Leciol (Adams and Green, East Yorkshire, UK). The lysolecithin was supplemented as
32
33 237 Lysoforte® Extend (Kemin Animal Nutrition and Health, Belgium). Each treatment consisted
34
35 238 of 12 pens, with 10 birds per pen. Diets were manufactured by Target Feeds (Whitchurch, UK)
36
37 239 and supplied bagged as mash for both starter and grower. Feed and water were available *ad*
38
39 240 *libitum*, with care taken to ensure the birds ate and drank as soon as possible after placement.
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41 241 The lighting regimen was maintained in accordance with commercial practice with 15 minutes
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43 242 of dusk at the start and end of each dark period. Temperature was set at 31°C on day 1 and
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45 243 reduced by approximately 1°C per day until 21°C was reached.
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3 247 Bird observations were used to monitor the environment and if the birds appeared
4
5 248 uncomfortable, the temperature and/or ventilation was altered accordingly. Birds were
6
7 249 observed twice daily during the trial and any observations related to health recorded in a trial
8
9 250 diary. Any dead birds were weighed, and reasons recorded if culled. Birds were weighed by
10
11 251 pen on arrival, on day 10 and at the end of the trial (day 21). Initially, individual weighed bags
12
13 252 of feed were prepared containing weighed feed quantities for each phase. Each pen of birds
14
15 253 was fed from their designated bag for each phase. Extra feed was added to the bags as required
16
17 254 and the quantity recorded. Total feed eaten was calculated as the difference between remaining
18
19 255 feed in the bag and the amount weighed into the bag for each phase.
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24 256 Energy conversion efficiency (ME MJ/kg gain) was calculated according to the following
25
26 257 formula, as per Salah *et al.* (2004):
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$$28 \quad \text{Calorie conversion} = \frac{\text{Total Feed Consumption} \times \text{Energy content of feed}}{\text{Sum weight of all birds at trial end}}$$

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32 259 Calorie conversion was calculated using the calculated ME contents on the feed, using both on
33
34 260 the literature energy value of the two fat sources, as claimed by the manufacturers, and with
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36 261 the energy value as determined by Wiseman *et al.* (1991).
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38
39 262 Statistical analysis was carried out using SPSS v.24. After testing to confirm normality, data
40
41 263 were analysed using one-way ANOVA to investigate the effect of dietary treatment on FCR,
42
43 264 feed intake (per bird) and individual bodyweight gain for each weigh period of the study, and
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45 265 cumulatively. Where appropriate, Bonferroni *post hoc* testing was used to elucidate differences
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47 266 between diets/treatments.
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51 52 53 268 RESULTS

54 55 269 *Experiment 1: In vitro fat absorption*

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57 270 In experiment 1, palm oil contained a high amount of saturated FA (492.4 g/kg), especially
58
59 271 palmitic acid (429.0 g/kg), which was largely reflected in the FA in the ether extract of the diet

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3 272 (Supplemental Table 1). The total lysophospholipid fraction was confirmed to be much higher
4
5 273 for soybean lysolecithin than for soybean lecithin (118.8 g/kg lysolecithin vs. 14.1 lecithin;
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7
8 274 Supplemental Table 2). The majority of lysophospholipids in soybean lysolecithin were
9
10 275 lysophosphatidylcholine (LPC) and lysophosphatidyl-ethanolamine.
11
12 276 The hydrolysis of TG and the accumulation of FFA, DG and MG during the *in vitro* hydrolysis
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14 277 of palm oil, palm oil with lecithin or with lysolecithin are shown in Figure 1. Over the whole
15
16 278 incubation period of 120 min, the amounts of TG hydrolysed and FFA, DG and MG
17
18 279 accumulated in palm oil with lecithin are markedly lower than those of palm oil and palm oil
19
20 280 with lysolecithin. During the first 60 min of incubation, the amounts of TG hydrolysed and
21
22 281 FFA and MG accumulated were slightly higher in palm oil without lysolecithin or lecithin,
23
24 282 than in palm oil with lysolecithin. After 120 min, however, the amounts of TG hydrolysed and
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26 283 FFA and DG accumulated was slightly higher in palm oil with lysolecithin than in palm oil
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28 284 without lysolecithin.
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35 286 Fig 1 here
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40 288 A comparison of the apparent first-order rate constants for TG hydrolysis and the accumulation
41
42 289 of FFA, DG and MG for each treatment is presented in Table 3 Addition of lecithin or
43
44 290 lysolecithin to the palm oil had significant ($P < 0.01$) impact on the rates of TG hydrolysis and
45
46 291 FFA, DG and MG release. TG were hydrolysed faster when lysolecithin was added to the palm
47
48 292 oil ($k = 11.76 \times 10^3/\text{min}$) compared to palm oil without (lyso)lecithin ($k = 9.73 \times 10^3/\text{min}$). In
49
50 293 contrast, TG were hydrolysed slower when lecithin was added ($k = 8.41 \times 10^3/\text{min}$). Similarly,
51
52 294 the release of MG was the fastest in palm oil with lysolecithin ($k = 3.07 \times 10^3/\text{min}$) and the
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54 295 slowest in palm oil with lecithin ($k = 2.23 \times 10^3/\text{min}$). In contrast, DG release was the slowest
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296 in palm oil without lecithin or lysolecithin. There was no statistically significant difference
297 observed in the rate of free fatty acid release between palm oil and palm oil with lecithin.

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299 Table 3 here

300

301 The absorption of MG and FFA generated during *in vitro* hydrolysis of palm oil, palm oil with
302 lecithin or with lysolecithin is presented in Figure 2. The absorption of MG was significantly
303 higher ($P<0.01$) for palm oil with lysolecithin (85.6%) than for palm oil (21.1%) or palm oil
304 with lecithin (35.5%). The overall absorption of FFA was slightly lower ($P>0.1$) than that of
305 MG. Nevertheless, the absorption of FFA was significantly higher ($P<0.01$) for palm oil with
306 lysolecithin (77.8%) than for palm oil alone (13.9%) or with lecithin (24.0%).

307

308 Fig 2 here

309

310 *Experiment 2: Digestibility*

311 In experiment 2, during the collection period the observed feed intake values were similar for
312 all treatments with an average of 30.6 g per bird per day. Likewise, bodyweight gain values
313 were similar for all treatments with an average of 26.35 g per bird per day. Apparent faecal
314 digestibility values and AMEn are presented in Table 4.

315

316 Table 4 here

317

318 There were significant differences ($P<0.05$) between the treatments for all parameters
319 investigated. Compared to the basal diet, lysolecithin supplementation increased ($P<0.01$) the
320 DM digestibility by 1.04%, CF digestibility by 2.25% and AMEn by 0.47 MJ/kg. On the other

1
2
3 321 hand, supplementation of lecithin had no effect on dry matter digestibility or AMEn, but
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5 322 reduced ($P<0.01$) crude fat digestibility by 3.2%. When compared to the basal diet, N-retention
6
7 323 was not significantly affected by lecithin or lysolecithin supplementation. However, N-
8
9 324 retention of the basal diet supplemented with lysolecithin (27.75 g/kg DM) was higher ($P<0.05$)
10
11 325 than that of the basal diet supplemented with lecithin (25.26 g/kg DM). Fatty acid distribution
12
13 326 in the ether extracts of the faeces is presented in Table 5. There was no difference ($P>0.05$)
14
15 327 between any of the dietary treatments in the fatty acid distribution in the faeces, nor in the ratio
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17 328 of unsaturated over saturated fatty acids.
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24 330 Table 5 here

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28 332 *Experiment 3: Performance Trial*

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30 333 Table 6 shows the performance of the birds across the whole experimental period of experiment
31
32 334 3. During the starter phase, days 0-10, there were no significant differences in average daily
33
34 335 gain, feed intake or FCR between the treatment groups. However, birds fed the diet containing
35
36 336 lecithin-based oil supplemented with lysolecithin were significantly heavier than those fed the
37
38 337 diet with lecithin-based oil alone. During the grower phase there was a significant difference
39
40 338 in bird weight. Birds fed the diet with lecithin-based oil alone were significantly lighter in
41
42 339 weight when compared to the birds fed the diet with lecithin-based oil supplemented with
43
44 340 lysolecithin ($P=0.047$). Similarly, when the whole trial phase was evaluated there were
45
46 341 significant differences in average daily gain. Birds fed diets with lecithin-based oil alone gained
47
48 342 less daily ($P=0.036$) than the birds fed the diet with lecithin-based oil supplemented with
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50 343 lysolecithin ($P=0.036$).
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58 345 Table 6 here
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6 347 The energetic values of each oil determined through analysis of chemical composition
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8 348 (Supplemental Information Table 3) demonstrated that, due to high levels of FFA in the oils
9
10 349 and an altered unsaturated: saturated ratio (Wiseman *et al.*, 1991), the lecithin oil had a reduced
11
12 350 predicted AME value for broilers <21 days compared to the soybean oil (36.57 MJ/kg soybean
13
14 351 vs. 32.64 MJ/kg lecithin). When calculating diet density based on lipid analysis, rather than
15
16 352 assuming energetic equivalence between the two oil sources, the effective energy conversion
17
18 353 rate for soybean oil alone was 16.04 MJ/kg BWG, while lecithin oil alone was worse, at 16.78
19
20 354 MJ/kg BWG. With the addition of lysolecithin, the energy conversion rate was improved across
21
22 355 both fat sources (15.58 MJ/kg BWG soybean oil + lysolecithin; 16.23 MJ/kg BWG lecithin
23
24 356 plus lysolecithin); the effective efficiency of use of the lecithin oil was brought closer to that
25
26 357 of unsupplemented soya oil.
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33 359 DISCUSSION

34
35 360 The structural differences in the composition of lecithin and lysolecithin leads to fundamentally
36
37 361 diverse effects on both *in vitro* and *in vivo* lipid digestion. In experiment 1, compared to palm
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39 362 oil without lecithin or lysolecithin, the apparent rate constants *in vitro* for triglyceride
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41 363 hydrolysis and monoglyceride release were significantly higher for palm oil supplemented with
42
43 364 lysolecithin and significantly lower for palm oil supplemented with soybean lecithin (Figures
44
45 365 1 and 2). Likewise, in experiment 2, compared to the basal diet, crude fat digestibility was
46
47 366 significantly higher in broilers supplemented with lysolecithin, but was significantly lower in
48
49 367 broilers supplemented with lecithin. Though the limitations of this initial study require further
50
51 368 data to confirm these findings, Zhang *et al.* (2011) postulated that, while both lecithin and
52
53 369 lysolecithin may act as an emulsifier within the first stages of lipid digestion, for hydrolysis to
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55 370 take place, the pancreatic colipase-lipase complex first must be adsorbed onto the emulsion
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3 371 droplets (Reis *et al.*, 2010). The adsorption and activity of lipase at the oil-water interface,
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5 372 however, is affected by various surface-active compounds such as phospholipids and
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7
8 373 lysophospholipids (Reis *et al.*, 2010; 2009).

9
10 374 Possibly the observed effect of lecithin on crude fat digestion in young broilers in experiment
11
12 375 2 could be explained by a study of Chu *et al.* (2010), who showed that colipase and lipase
13
14 376 adsorbed exclusively onto regions covered by phosphatidylcholine and bile salts and not to
15
16
17 377 regions covered with phosphatidylcholine solely. This suggested that, although phospholipids
18
19 378 in lecithin may have aided in the formation of smaller emulsion droplets, the young broiler
20
21 379 produces insufficient bile salts. This is especially the case in diets containing high levels of
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23
24 380 palm oil where, due to the low amount of unsaturated fatty acids, digestibility is more
25
26 381 challenging than with other, more unsaturated, vegetable oils, such as soybean oil
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28
29 382 (Tanchaorenrat *et al.*, 2013) to facilitate the adsorption of the colipase-lipase complex to the
30
31 383 surface of the droplet. The combination of the challenging basal fat type (palm oil) and level
32
33 384 with the immature digestive system in young broilers led to a relatively low crude fat
34
35 385 digestibility in the basal diet (69.52%) seen in experiment 2. It is well known that relative
36
37 386 digestibility coefficients of fat are limited when fats and oils are present at a high levels (Croom
38
39 387 *et al.*, 1999; Rampone, 1961), as the ability of the digestive system and liver for lipolysis and
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41
42 388 absorption become rate limiting. However, in contrast to the phospholipids contained in
43
44 389 lecithin, it has been suggested that, once in the small intestine, lysophospholipids tend to
45
46
47 390 dissolve into mixed micelles and, in this way, leave the interface (Nik *et al.*, 2010). Moreover,
48
49 391 through their participation in the formation of mixed micelles, lysophospholipids may play an
50
51 392 additional role by displacing MG and FFA from the interface, allowing lipid hydrolysis to
52
53 393 continue (Lairon, 2009). Recent studies have demonstrated the effect of lysolecithin on the
54
55 394 intestinal mucosa. Papadopoulos *et al.* (2018) reported significantly thinner mucosa in birds
56
57 395 supplemented with lysolecithin than in unsupplemented birds, while Chen *et al.* (2019)

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2
3 396 reported elevated claudin-3 levels following lysolecithin supplementation, indicating better
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5 397 sealing of tight junctions (Milatz *et al.*, 2010). Brautigam *et al.* (2017) showed increased
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7 398 collagen deposition and villus height following lysolecithin supplementation. Together, these
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9 399 results suggested that, as lysolecithin becomes incorporated into the epithelial cell walls, it
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11 400 improves mucosal absorptive capacity in a way lecithin cannot.

12
13
14 401 Although in this 'proof of concept' study lecithin had a negative effect on lipid hydrolysis, no
15
16 402 adverse effect was observed on the absorption of generated MG and FFA by the differentiated
17
18 403 Caco-2 monolayer (Figure 2). This supported the argument that phospholipids likely reside at
19
20 404 the interface of emulsion droplets and do not participate in the absorption process in
21
22 405 enterocytes. In contrast, the absorption of MG and FFA was higher with digests from the palm
23
24 406 oil supplemented with lysolecithin than with digests from palm oil and palm oil supplemented
25
26 407 with lecithin. Similarly, in a study by Sugawara *et al.* (2001) the uptake of lipid-soluble
27
28 408 carotenoids was greatly improved in Caco-2 cells exposed to micelles containing
29
30 409 lysophosphatidylcholine in comparison to cells incubated with micelles containing
31
32 410 phosphatidylcholine.

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34
35 411 In addition to the improved CF digestibility and DM digestibility, AMEn of the basal diet
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37 412 supplemented with lysolecithin was significantly higher than that of the basal diet or basal diet
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39 413 supplemented with lecithin. This is in line with previous studies, which have shown
40
41 414 improvements in CF and N digestibility following lysolecithin supplementation, although the
42
43 415 effect of fat source on response is still uncertain: Zaefarian *et al.* (2015) reported improved
44
45 416 AMEn values in supplemented soy oil diets, but little effect of lysolecithin on diets formulated
46
47 417 with tallow. Conversely, Jansen *et al.* (2015) reported improvements in AMEn of diets
48
49 418 formulated with pig lard but not those formulated with soybean oil. Across 33 studies,
50
51 419 Wealleans *et al.* (2019) found little evidence that the magnitude of response to lysolecithin
52
53 420 supplementation was consistently altered by dietary fat type. In the present study, N-retention
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3 421 for the basal diet supplemented with lysolecithin was significantly higher than in the basal diet
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5 422 supplemented with lecithin. Furthermore, the AMEn improvement of 0.47 MJ/kg by
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7 423 lysolecithin supplementation was only partially attributed to the 2.25% improvement in CF
8
9 424 digestibility.

10
11
12 425 Honda *et al.* (2009) found that fats incorporated in the feed matrix could encapsulate other
13
14 426 nutrients, lowering their digestion. As a consequence of improved CF digestibility,
15
16 427 lysolecithins could, in this way, enhance the digestion of other nutrients. Another possible
17
18 428 explanation for the large improvement in AMEn may be found at the enterocyte level. As
19
20 429 supported by the improved absorption of MG and FFA in the present study, lysolecithin may
21
22 430 enhance the uptake of multiple nutrients across the enterocyte membrane. Lysophospholipids
23
24 431 are known to alter membrane structure by inducing local curvatures in the bilayer (Lundbaek,
25
26 432 2006; Wendel, 2000; Maingret *et al.*, 2000). Moreover, they can affect proteins embedded in
27
28 433 the membrane (Lundbaek, 2006; Maingret *et al.*, 2000; Lundbaek and Andersen, 1994). In this
29
30 434 way lysophospholipids could enhance the uptake of nutrients across the membrane of the
31
32 435 microvilli in the intestinal epithelium, which is supported by the work of Brautigan *et al.*
33
34 436 (2017), who reported increased villus height and collagen cross-linkages, driven by
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36 437 upregulation of collagen-related genes in the intestinal epithelium of broilers supplemented
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38 438 with lysolecithin.

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41 439 These differential effects on fat hydrolysis and overall digestibility between lecithin and
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43 440 lysolecithin were supported by the performance results of experiment 3, although the basal diet
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45 441 formulations were substantially different, and designed to be more commercially relevant, from
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47 442 those used in experiment 2. Substituting soya oil for lecithin oil at the same concentration
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49 443 numerically reduced broiler growth and increased FCR until day 10 of age (1.45 soya oil vs.
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51 444 1.55 lecithin oil, $P=0.161$), while weight at day 10 was 3% lower in birds fed the lecithin oil
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53 445 diet compared to those formulated with soya oil. Although differences between
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3 446 unsupplemented soya and lecithin oil treatments were not statistically significant, the large
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5 447 difference in FCR during the starter phase was commercially important to overall production
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8 448 profitability. Similarly, Huang *et al.* (2007) reported that complete replacement of the soybean
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10 449 oil with lecithin (2% lecithin in the diet) resulted in adverse effects. This was greatest in young
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12 450 birds, and, after day 10, the gap in feed conversion ratio between soya oil and lecithin oil diets
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14 451 narrowed (6.9% increase in FCR lecithin vs. soya to day 10, 3.1% increase in FCR lecithin vs.
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16 452 soya day 10-21), with birds on all treatments performing similarly. Until 14 days of age, fat
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18 453 digestion was severely limited in chicks due to a lack of bile salt secretion (Krogdahl, 1985),
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20 454 after which the rates of synthesis increased fourfold. Therefore, it was likely that birds fed the
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22 455 lower available energy diets containing lecithin oil were able to achieve compensatory growth
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24 456 once bile salt synthesis reached sufficient levels and fat digestion improved (Krogdahl, 1985),
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26 457 thus closing the performance gap with those fed diets containing soya oil, despite energy
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28 458 conversion efficiency rates remaining lower throughout the trial.
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31 459 Future research on the comparative effects of lecithin and lysolecithin should continue to assess
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33 460 the effect on performance and digestibility until slaughter, when the bird is physiologically
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35 461 more mature. Meanwhile, the performance increases seen with supplemental lysolecithin were
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37 462 in line with previous research, which demonstrated improved weight gain and FCR (Wealleans
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39 463 *et al.*, 2019; Allahyari-Bake and Jahanian, 2017; Zaefarian *et al.*, 2015; Zampiga *et al.*, 2016),
40
41 464 while others have reported increases in AME (Majdolhosseini *et al.*, 2019; Melegy *et al.*, 2010;
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43 465 Jansen *et al.*, 2015).
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45
46 466 To conclude, this study presented evidence that lysolecithins, but likely not lecithin itself, are
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48 467 able to significantly improve the digestibility and energy values of feed, especially in young
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50 468 broilers. These improvements may be due to a combined effect of lysophospholipids on lipid
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52 469 hydrolysis and nutrient absorption, although further research is required to confirm any
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54 470 multifactorial mode of action
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625 **Table 3.** Effect of soybean lecithin and soybean lysolecithin on the apparent first-order rate
 626 constant ($k \times 10^3/\text{min}$) of triglyceride hydrolysis and diglyceride, monoglyceride and free fatty
 627 acid release during *in vitro* digestion of palm oil (Experiment 1)

	Triglyceride hydrolysis	Diglyceride release	Monoglyceride release	Free fatty acid release
Treatment ¹				
Palm oil	9.73 ^b	1.15 ^c	2.91 ^b	10.85 ^b
Palm oil + lecithin ²	8.41 ^c	1.58 ^b	2.23 ^c	9.49 ^b
Palm oil + lysolecithin ³	11.67 ^a	1.70 ^a	3.07 ^a	12.49 ^a
Pooled SEM	0.28	0.02	0.04	0.45
<i>P</i> -value	0.001	0.000	0.000	0.009

628 ^{a-c} Values within columns with different superscripts are significantly different ($P < 0.05$).

629 ¹ Data are means of three observations per treatment.

630 ^{2,3} Lecithin and lysolecithin were applied at 1g, dispersed into 49 g of oil

631

632 **Table 1.** Ingredients and nutrient composition of the basal¹ experimental diet (Experiment 2)

Item (g/kg, unless noted)	Starter diet
Ingredient	
Corn	452.9
Wheat	100.0
Soybean meal (45.3% CP)	363.2
Palm oil	40.0
Monocalcium phosphate	13.6
Vitamin and mineral premix ²	10.0
Methionine	3.0
Lysine HCl	2.3
Threonine	0.6
Limestone	6.8
NaCl	2.0
NaHCO ₃	2.5
TiO ₂	3.0
Calculated composition	
AMEn (MJ/kg)	11.41
Crude fibre	3.19
Lysine	1.15
Methionine + cysteine	0.86
Threonine	0.75
Calcium	0.85
Total phosphorus	0.69
Available phosphorus	0.40
Sodium	0.15
Analysed composition ³	
Dry matter	90.43
Crude protein	23.05
Crude fat	7.25
TiO ₂	0.29
Gross energy (MJ/kg)	17.50

633 ¹ For experimental treatments, lysolecithin was added on top of the specified basal diet at 250
634 ppm

635 ² Supplied per kilogram of diet: manganese, 99 mg; zinc, 60 mg; iron, 49 mg; copper, 20 mg;
636 iodine, 1.2 mg; selenium, 0.4 g; vitamin A, 13,500 IU (retinyl acetate); vitamin E, 55 IU
637 (DL- α -tocopheryl acetate); cholecalciferol, 75 μ g; vitamin B₁, 2 mg; vitamin B₂, 5 mg;
638 vitamin B₃, 30 mg; vitamin B₅, 15 mg; vitamin B₆, 4 mg; vitamin B₁₂, 2 mg; vitamin K, 2.5
639 mg; folic acid, 1 mg; biotin, 0.2 mg; choline, 600 mg; etoxyquine, 33 mg; butylated
640 hydroxytoluene (BHT), 0.4 mg; propyl gallate, 0.12 mg; citric acid, 0.69 mg; sepiolite, 4 mg.

641 ³ Values represent the mean of the three experimental diets.

642

643

644 **Table 4.** Effect of soybean lecithin and soybean lysolecithin on the apparent faecal dry matter
 645 and crude fat digestibility, nitrogen retention and AMEn of young broilers fed a palm oil rich
 646 diet (Experiment 2)

	Digestibility (%)		N-retention (g/kg DM)	AMEn (MJ/kg DM)
	DM	Crude fat		
Dietary treatment ¹				
Basal diet	69.85 ^b	69.52 ^b	27.03 ^{ab}	13.03 ^b
Basal diet + lecithin	69.47 ^b	66.32 ^c	25.26 ^b	13.03 ^b
Basal diet + lysolecithin	72.88 ^a	71.77 ^a	27.75 ^a	13.50 ^a
Pooled SEM	0.16	0.71	0.53	0.16
<i>P</i> -value	0.000	0.003	0.039	0.008

647 ^{a-c} Values within columns with different superscripts are significantly different ($P < 0.05$).

648 ¹ Data are means of three observations per treatment.

649

650 **Table 5.** Fatty acid distribution (%) in the ether extracts of the faeces of young broilers fed a
 651 basal palm oil diet, the basal diet supplemented with lecithin and the basal diet supplemented
 652 with lysolecithin (Experiment 2)

Fatty acid ¹²	Basal diet	Basal diet + lecithin	Basal Diet + lysolecithin	Pooled SEM	<i>P</i> -value
C16:0	40.59	40.47	41.23	0.69	NS
C18:0	6.29	6.89	6.91	0.15	NS
C18:1 (ω-9)	23.71	23.47	23.75	0.12	NS
C18:2 (ω-6)	26.01	26.46	25.55	0.75	NS
C18:3 (ω-3)	0.69	0.71	0.65	0.02	NS
Total unsaturated fatty acids	49.34	49.13	49.83	0.84	NS
Total saturated fatty acids	50.66	50.87	50.17	0.84	NS
Ratio unsaturated / saturated	1.03	1.04	1.01	0.03	NS

653 ¹Fatty acids that are not listed were present for less than 0.5% in all samples.

654 ² Data are means of three observations per treatment.

655 **Table 2.** Ingredients and nutrient composition of the basal experimental diets¹ (Experiment 3)

Item (g/kg, unless noted)	Soybean Oil	Lecithin Oil
Wheat	541.6	541.6
Pura ²	100.0	100.0
Soybean Meal (46.6% CP)	304.0	304.0
Soybean Oil	20.0	--
Lecithin Oil	--	20.0
Limestone	5.6	5.6
Salt	1.6	1.6
Sodium Bicarbonate	2.8	2.8
DCP	12.4	12.4
Lysine HCl	2.7	2.7
DL Methionine	3.4	3.4
Threonine	1.0	1.0
Ronozyme P5000	0.15	0.15
Ronzyme WX	0.15	0.15
Vit/Min Premix ³	4.63	4.63
Calculated composition, %		
ME, MJ/kg	12.609 MJ/kg	
Crude Protein	22.47	
Crude Fat	6.207	
Crude Fibre	3.38	
Dig Lys	1.247	
Dig Met	0.623	
Dig Met+Cys	0.938	
Ca	0.898	
Av P	0.449	
Na	0.150	

656 ¹ Lysolecithins (as LYSOFORTE[®] EXTEND) were added on top of the basal diet formulations
 657 at 500 ppm

658 ² Pura is a commercially available blend of whole rapeseed and pulses (field beans or peas)
 659 which has been ground, heat treated and pelleted. Typical analysis 21% oil, 21% protein.

660 ³Supplied per kilogram of diet: manganese, 99 mg; zinc, 60 mg; iron, 49 mg; copper, 20 mg;
 661 iodine, 1.2 mg; selenium, 0.4 g; vitamin A, 13,500 IU (retinyl acetate); vitamin E, 55 IU
 662 (DL- α -tocopheryl acetate); cholecalciferol, 75 μ g; vitamin B₁, 2 mg; vitamin B₂, 5 mg;
 663 vitamin B₃, 30 mg; vitamin B₅, 15 mg; vitamin B₆, 4 mg; vitamin B₁₂, 2 mg; vitamin K, 2.5
 664 mg; folic acid, 1 mg; biotin; 0.2 mg; choline, 600 mg; etoxyquine, 33 mg; butylated
 665 hydroxytoluene (BHT), 0.4 mg; propyl gallate, 0.12 mg; citric acid, 0.69 mg; sepiolite, 4 mg.

666 **Table 6.** Growth performance of young broilers from day 0-21 fed diets formulated with soya oil or lecithin oil, with or without supplemental
 667 lysolecithin. (Experiment 3).

	Soya Oil	Soya Oil + Lysolecithin	Lecithin Oil	Lecithin Oil + Lysolecithin	Pooled SEM	P-value
Day 0-10¹						
Weight at day 0, g	38.3	38.3	38.6	38.9	0.72	0.906
Average daily gain, g	21.2	21.5	20.8	22.0	0.41	0.198
Average daily feed intake, g	32.0	29.5	32.3	31.6	1.13	0.326
FCR	1.45	1.36	1.55	1.43	0.060	0.161
Day 10-21						
Weight at day 10, g	256.2 ^{ab}	258.9 ^{ab}	248.9 ^b	263.8 ^a	3.18	0.015
Average daily gain, g	61.3	61.4	59.4	63.4	0.11	0.105
Average daily feed intake, g	80.6	81.9	79.9	83.9	1.24	0.183
FCR	1.31	1.33	1.35	1.33	0.020	0.723
Day 0-21						
Weight at day 21, g	934.1 ^{ab}	941.6 ^{ab}	906.9 ^b	961.7 ^a	1.38	0.047
Average daily gain, g	42.5 ^{ab}	42.8 ^{ab}	41.3 ^b	43.9 ^a	0.50	0.036
Average daily feed intake, g	56.5	56.5	57.0	58.6	0.89	0.356
FCR	1.33	1.32	1.38	1.33	0.024	0.225
Energy conversion rate (MJ/kg BWG)	16.04	15.88	16.60	16.06	--	--

668 ¹ Data are means of twelve observations per treatment.

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3 669 **Figure 1.** Hydrolysis of (A) triglycerides and accumulation of (B) free fatty acids, (C)
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5 670 diglycerides and (D) monoglycerides during the *in vitro* hydrolysis of palm oil (solid line),
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7 671 palm oil with soybean lecithin (dotted line) and palm oil with soybean lysolecithin (dashed
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9 672 line). The experimental treatments were carried out in triplicate. The mean concentrations of
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11 673 the lipids (mg/ml) are given over time (min), with error bars indicating the standard error
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13 674 values. Statistical analysis of the treatments is performed based on apparent rate constants (see
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15 675 Table 1). For enzyme kinetic comparison, apparent rate constants are used. (Experiment 1).
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22 677 **Figure 2.** Absorption of monoglycerides (white bars) and free fatty acids (grey bars) generated
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24 678 during *in vitro* hydrolysis of palm oil, palm oil with soybean lecithin and palm oil with soybean
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26 679 lysolecithin by differentiated Caco-2 monolayers and expressed as percentage of applied
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28 680 monoglycerides and free fatty acids. Data are means of three or more observations per
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30 681 treatment, with error bars indicating the standard error values. (Experiment 1).
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