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NONRUMINANT NUTRITION

Refined crude glycerin in sow feed: A sustainable alternative

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ABSTRACT. The objective of this work was to evaluate the effect of semi-purified glycerin added to the feed of pregnant and lactating sows on performance including dorsal diameter, body condition, daily weight gain, feed intake, and feed conversion rate. Growth and feeding parameters were evaluated in born piglets: litter average, survival, daily weight gain, feed conversion rate, and daily milk consumption. Pregnant sows (n = 60; initial weight 180 ± 22 kg) were randomly assigned to the Control or Glycerin group (2%). Semi-purified glycerin was supplied from the beginning of gestation until farrowing. The inclusion of semi-purified glycerin did not affect any of the parameters evaluated in sows during gestation. The inclusion of semi-purified glycerin had a significant effect (p = 0.04) on the body condition of sows at weaning and appeared to have a negative effect on piglet survival. However, semi-purified glycerin caused no negative effects on milk production during lactation and did not affect piglet performance parameters. The addition of semi-purified glycerin as a caloric source could be an economically viable alternative to be implemented in feeding pregnant and lactating sows. However, additional tests are suggested.

Keywords: Sow performance; daily weight gain; feed intake; feed conversion rate.

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Introduction

Currently, there is a growing trend in clean energy research and the use of biodiesel is a viable clean energy option. The production of biodiesel from renewable biological sources such as vegetable oils and fats has been extensively reviewed (Aransiola et al., 2014). Biodiesel is a fuel whose origin can be vegetable or animal fats, whose qualities allow it to be used as fuel in some types of engines (Van Gerpen et al., 2004). During the biodiesel production process, several by-products are generated, among which are: methanol, fatty acids, catalysts, solid residues, and crude glycerin (Varanda et al., 2011). Crude glycerin (CG) is generated during the saponification process and biodiesel production and is widely used in the pharmaceutical and food industries (Ethier et al., 2011). However, only in biodiesel production is it referred to as crude glycerin (Dobroth et al., 2011; Ethier et al., 2011).

CG is a by-product of the transesterification reaction that takes place between fatty acids with methanol or ethanol to produce methyl esters and accounts for about 10% of biodiesel production and is considered a residual ethanol or methanol, fatty acid ethyl (or methyl) esters and residual fatty acids are mixed with it (Dobroth et al., 2011; Sousa et al., 2012). These impurities can be removed from CG to produce a feedstock for the pharmaceutical and food industries, but purification and refining are too expensive to be sustainable in the biodiesel industry (Ethier et al., 2011; Sabourin & Hallenbeck, 2009), thus resulting in a semi-purified glycerin (SG). The application of SG in the food sector, especially in animal feed, is due to its high energy value and rapid absorption in the intestine, which allows lipid formation or energy production through glycolysis and the citric acid cycle (Gallego et al., 2014). Therefore, several working groups have included CG cattle feed for several decades. In 1995, Kijora and his working group investigated the inclusion of crude glycerin as a component in the diet of fattening pigs, replacing barley with glycerol, and found that the glycerol diet did not alter carcass yield, meat quality, or important organs such as the kidney or liver (Kijora & Kupsch, 1995). Thinking of CG as an energy source in the diet of animals such as pigs is possible because the supply of glycerol contributes to metabolism during the energy state balance involving glycerolipids and fatty acids. In the body, the liver is the most important producer of glycerol (Clow et al., 2008; Quiniou et al., 2002). Much of the body's glycerol is obtained from glucose metabolism, fat diet, and glycerolgenesis (Brisson et al., 2001; Nye et al., 2008). Thus, triacylglycerol found in the bloodstream interacts with the lipase enzyme that catalyzes the generation of Free Fatty Acids (FFA) and Glycerol. Intracellular glycerol is converted to glycerol-3-phosphate by the action of the enzyme glycerol kinase. Glycerol-3-phosphate is an intermediate metabolite that acts in pathways such as the glycerolipid/free fatty acid pathway and in energy metabolism through transfer to the mitochondrion (Figure 1) (Possik et al., 2021). A condition characterized by strong energy demand is gestation, and fatty acids and glycerol coexist in physiology during gestation, where sows need to meet the demands of developing fetuses and achieve some weight gain. Maintenance accounts for 75-85% of total and environmental needs, which affects the temperature and activity of the animals (Noblet et al., 1990). During this metabolic process, the pregnant sow uses about 80% of the energy intake from the diet and approximately 5% is used for litter development (Aherne et al.,1999). During fetal development, glucose is the most important energy source that can cross the placenta (Hay, 1994; Lasuncion et al., 1987; Testar et al., 1985). In mammals, during the last third of gestation, the activity of the hormone-sensitive lipase enzyme increases because its mRNA expression is higher than normal (Elliott, 1975; Knopp et al., 1970; Sivan et al., 1999). Therefore, the present study focused on the evaluation of the effect of the inclusion of SG on performance parameters during gestation and lactation in sows, as well as growth and feeding parameters in piglets during the lactation period.

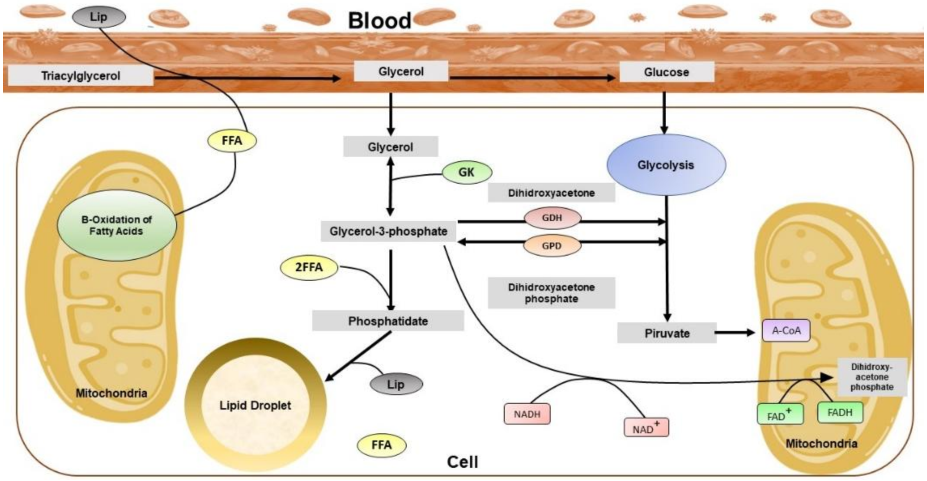

Figure 1. Metabolism of glycerol. Triacylglycerol circulating in the blood is influenced by lipase (Lip) generating Free Fatty Acids (FFA) and glycerol. Blood glycerol enters the cell and is catalyzed by glycerol kinase (GK) to produce glycerol-3-phosphate. Source: modified from Robergs and Griffin (1998).

Materials and methods

This experiment was carried out at Granja Santa Rosa (Puebla, Mexico) and the Biofuels Engineering Laboratory of the Benemérita Universidad Autónoma de Puebla for six months. The present work was approved by the ethics committee of the Benemérita Universidad Autónoma de Puebla in act number 230511.

Treatment of glycerin and its inclusion in the feed

CG was obtained from biodiesel production and subjected to acidification with sulfuric acid to pH 5.6. The solution obtained was subjected to heat to remove residual methanol. Subsequently, filtration was carried out with the aid of a metal filter to separate the inorganic salts and then poured into a separatory funnel. After 48 h, semi-purified glycerol (SG) was obtained and stored at 18°C until use. SG was added at a 2% concentration to the diet upon preparation. The substances obtained during each purification step are shown in Figure 2.

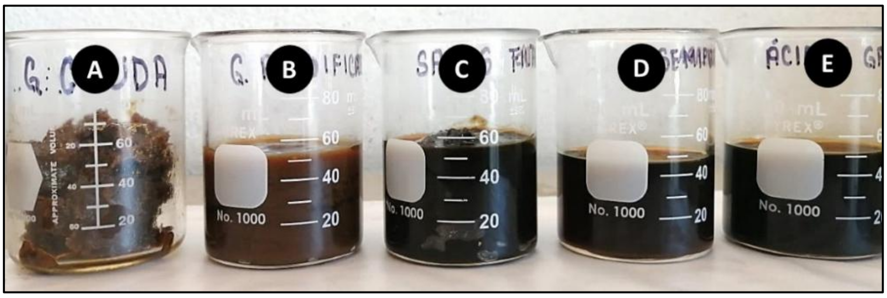

Figure 2. Glycerin and co-products at each stage of the semi-purification process. A) Crude glycerin; B) Crude glycerin with pH adjusted to 5.6; C) Inorganic salts obtained by filtration; D) Liquid and semi-purified glycerin obtained; E) Free fatty acids. Source: Prepared by the authors.

Animals, diet and experimental design

The research protocol in this work followed the Regulations for Animal Experimentation, having as legal basis:

1) Federal Law of Animal Health;

2) General Law of Ecological Equilibrium and Environmental Protection;

3) Regulation of the Federal Law of Animal Health;

4) Official Mexican Standard-024-ZOO-1995, on the specifications and animal health characteristics for the transport of animals, their products and by-products, pharmaceutical, biological, and food chemical products for use in animals or consumed by them;

5) Official Mexican Standard-033-SAG/Z00-2014, on the humane slaughter of domestic and wild animals

6) Official Mexican Standard-051-Z00-1995, on the humane treatment in the mobilization of animals

7) Official Mexican Standard-062-Z00-1999, on the Technical Specifications for the Production, Care and Use of Laboratory Animals.

A total of 120 Duroc x Landrace (pregnant) animals were used. The experimental area for each sow was $5m^2$ $animal^{-1}$ and the experimental unit was each pregnant sow and subsequently each pregnant sow and her piglets. Sows were randomly assigned to each treatment, obtaining the following groups: Control pregnant (n = 60); 2% Gly pregnant (n = 60); they were subsequently evaluated in the lactation stage. Sixty piglets from the sows in the control group and 60 piglets from the group of sows receiving 2% Gly were evaluated to quantify performance for 21 days corresponding to the lactation phase. The diets were formulated to be isoenergetic and isoaminoacidic as shown in Table 1. The composition of glycerin is listed in Table 2. Bromatological analyses were carried out for Crude Protein (CP) (%), Calcium (Ca) (%), Available Phosphorus (%), Crude Fiber (CF) (%), Ether Extract (EE) (%), Digestible Lysine (DL) (%), Ash (%). The analyses were performed in triplicate and determined according to Association of Official Analytical Chemists (Helrich, 1990).

Table 1. Diet composition of the control and glycerin group during gestation and lactation.

	Diet			
	Gestation		Lactation	
Ingredient	Control	Gly2%	Control	Gly2%
	%	%	%	%
Corn	59	59	65	65
Sorghum	10	10	10	10
Wheat bran	2	2	2	2
Rice Meal	4.9	4.9	4.9	4.9
Semipurifyed Glycerin	0	2	0	2
Soy oil	1.32	0	1.32	0
Soybean meal	18.01	18.01	12.01	12.01
salt	0.3	0.3	0.3	0.3
Sodium Bicarbonate	0.59	0.59	0.59	0.59
Calcium Carbonate	0.58	0.58	0.58	0.58
Monocalcium phosphate	1.99	1.31	1.99	1.31
DL-Methionine	0.07	0.07	0.07	0.07
L-Lisine H CL	0.34	0.34	0.34	0.34
L-Threonine	0.13	0.13	0.13	0.13
Coline	0.07	0.07	0.07	0.07
Vitamin and mineral premix	0.7	0.7	0.7	0.7
	100			
		Nutrient content		
Chemical composition				
DM (Dry Matter) (%)	88.94	88.94	89	89
NE (Net Energy) kcal kg^{-1}	2508	2508		
CP (Crude Prtotein) (%)	19.24	19.24	19.24	19.24
Ca (Calcium) (%)	0.72	0.72	0.72	0.72
AP (Available Phosphorus) (%)	0.4	0.4	0.4	0.4
CF (Crude Fiber) (%)	3.79	3.79	3.79	3.79
EE (Ether Extract) (%)	3.93	3.93	3.93	3.93
DL (digestible lysine) (%)	1.145	1.145	1.145	1.145

Table 2. Composition of crude glycerin included in the diet of lactating and pregnant sows.

Crude Glycerine composition	
Density	1.1-1.26 g mL^{-1}
Moisture	15-20%
Purity	> 93%
pH	5-7
Ether extract) % weight	2.3
Protein (%)	< 0.1
Ash (% peso)	4.6
Peroxide index (meq O2 kg^{-1} fat)	<0.02
Total Carbohidrates (%)	78.2
Total energy (kcal 100 g^{-1})	333.5; 3335 cal g^{-1}; 3.335 Mcal Kg^{-1}
Energetic content (KJ 100 g^{-1})	1414.5; 14.145 MJ Kg^{-1}

Results and discussion

Sow performance during gestation and lactation.

The variables evaluated in pregnant sows (Control and 2% Gly) were thoracic diameter, body weight, and body condition during early (35d) and late (85d) pregnancy. For lactating sows (Control and 2% Gly), all the above mentioned variables plus the mean number of piglets born, mean number of piglets dead, and daily weight gain were evaluated.

Piglet performance

Once the piglets were born, they were evaluated for 21 days for average daily gain (ADG), average daily feed intake (milk) (ADFI), and feed conversion ratio. Initial body weight and body weight at 21D were also obtained.

Similar postpartum weight recovery was observed between both groups (Table 3). An ANOVA test was applied to analyze the data obtained, followed by Tukey's post-hoc multiple comparisons test. Interestingly, Shieck et al. (2010) reported that sows fed CG have similar performance to sows fed a standard diet. The effect of glycerol on the feeding of lactating sows as well as their performance during gestation has been an understudied field. During farrowing, nutrient demand requiring high energy supply and some factors such as farrowing duration and farrowing intervals (Oliviero, 2010; Zaleski & Hacker, 1993). The inclusion of SG did not affect the parameters evaluated at 24h post-parturition. However, body condition at weaning was affected in the treated group (p = 0.04). This is probably because the litters of the glycerin-treated group presented a higher average, although the statistical analysis showed no significant difference.

Table 3. Performance of sows in the lactation period.

	Lactant Sows			
	Diet with Gly			
	0%	2%	SEM	*p*-value
24 H Post-partum				
Body Weight (kg)	207.3	195	8.076	0.13
Thoracic Diameter (Inch)	54.36	56.08	0.9107	0.06
Body Condition	3.16	3.38	0.111	0.05
Weaning 21D				
Body Weight (kg)	201	182.8	9.2	0.05
ADL (Average Dayli loss) kg	0.87	0.88	0.02	0.6
ADFI (Average Dayli Feed Intake) kg	2.79	2.74	0.12	0.68
Thoracic Diameter (Inch)	52.92	52.68	0.91	0.7
Body Condition	2.54	2.34	0.09	0.04
Average litter size	9.6	10.36	0.56	0.06
Average number of dead piglets	0.6	0.84	0.29	0.4

Many factors influence sow weight gain during gestation such as body weight at onset of pregnancy, gestational stage, and farrowing. During pregnancy, there is an increase in energy and nutritional requirements due to the exponential growth of the fetus and the effect on the sow weight. This increase is most clearly observed during late pregnancy (McPherson et al., 2004). During gestation (Table 4), the addition of SG did not affect any of the parameters evaluated at any of the stages. This fact contributes to showing that the use of SG can be a good alternative for the inclusion of a caloric supplement in the diets for pregnant sows due to its easy access and low cost, since during gestation, the amount of feed required by sows is greater. Therefore, the inclusion of this caloric byproduct could represent a positive impact on producers.

Table 4. Sow performance during early and late gestation. ADG (average daily weight gain), ADFI (average daily feed intake) and FC (feed conversion ratio).

	Pregnant Sows			
	Diet with Gly			
	0%	2%	SEM	*p*-value
	Early pregnant 35D			
Body Weight (kg)	199.32	200.04	6.08	0.9
Thoracic Diameter (Inch)	53.52	53.4	0.4	0.83
Body Condition	2.64	2.68	0.08	0.72
ADG (kg) (Average Dayli Gain)	0.27	0.253	0.026	0.54
ADFI (kg) (Average Dayli Feed Intake)	2.23	2.24	0.029	0.91
FC (Feed Convertion)	8.95	10.52	1.013	0.12
	Late pregnant 85D			
Body Weight (kg)	229.92	228.26	6.03	0.84
Thoracic Diameter (Inch)	56.48	56.39	0.58	0.91
Body Condition	3.76	2.91	0.64	0.29
ADG (kg) (Average Dayli Gain)	0.384	0.365	0.019	0.32
ADFI (kg) (Average Dayli Feed Intake)	2.68	2.7	0.06	0.7
FC (Feed Convertion)	7.2	7.6	0.42	0.3

*No significant differences were found between the groups evaluated. Source: own elaboration.

Piglet performance during lactation

During the lactation phase, the piglets showed no significant differences in the parameters evaluated. Piglets were not fed glycerin. The effects of 2% Gly diet on sows during gestation and lactation did not affect birth weight, body weight at weaning, milk production, ADG, and ADFI. Glycerol is a potent osmotic dehydrating agent and hydrophilic solute that can be transported by passive transport and can be released into the intestinal tract (Table 5). During fetal development, the concentration of metabolites that cross the placenta is dependent on their presence in the circulating blood. Glucose concentration is a function of the glycerol present in the body and glucose is the most important energy source that can cross the placenta, followed by amino acids (Hay, 1994; Herrera et al., 1985; Lasuncion et al., 1987).

Table 5. Piglet performance during the lactation period.

	Piglets performance		
	Diet		
	0%	2%	*p*-value
Initial Body Weight (kg)	1.68	1.52	0.83
Body Weight at Weaning (kg)	7.19	7.3	0.93
ADG (Average Dayli Gain) g	272.47	275.27	0.78
ADFI (Average Dayli Feed Intake) g	544.72	542.44	0.88
G:F (Growth/ Feeding) g g^{-1}	2.01	1.98	0.47

Previous studies have mentioned the fact that the amount of fatty acids and glycerides from glucose progressively increases until 20d in pregnant rat, and then important factors that influence piglets at the preweaning stage, such as birth weight or litter size, decrease (Quiniou et al., 2002). Even, changes in body condition during lactation or gestation can influence piglet birth weight and litter uniformity. The feed intake by the sow during gestation can affect the birth weight of piglets (Campos et al., 2012). However, glycerol absorption is saturable in the small intestine and glycerol is an important precursor of sn-glycerol 3-phosphate (g3p) during gluconeogenesis by transporting reducing equivalents involved in central metabolic pathways in the cytosol and mitochondria such as oxidative phosphorylation (Brisson et al., 2001; Hirschmann, 1960; Mattson & Volpenhein, 1964; Yuasa et al., 2003). Under normal conditions, the main sources of glycerol are 1) the adipose tissue by lipolysis, and 2) triglyceride hydrolysis in blood lipoproteins. On the other hand, the role of the microbiota in the digestive system is crucial for proper absorption because part of the microbiota can express phospholipases that hydrolyze cell membrane phospholipids to produce glycerol (Weir et al., 2013).

In addition, a comparative table (Table 6) is presented with feed cost per ton for the gestation and lactation stages. Diets that included SG had a lower cost per ton. It is important to mention that, if the cost is taken to an industrial scale, the difference is even more evident.

Table 6. Comparison between the cost of diets (per ton) with vegetable oil and semi-purified glycerin for pregnant and lactating sows.

		Gestación control		Gestación Gly 2%		Lactancia Control		Lactancia 2%	
Ingredients	Price per kg	Kg ton^{-1}	Total price	Kg ton^{-1}	Total price	Kg ton^{-1}	Total price	Kg ton^{-1}	Total price
Corn	$0.48	590	$283	590	$283	650	$312	650	$312
Sorghum	$0.75	100	$75	100	$75	100	$75	100	$75
Wheat bran	$0.38	20	$8	20	$8	20	$8	20	$8
Rice Meal	$3.00	49	$147	49	$147	49	$147	49	$147
Semipurifyed Glycerin	$1.16	0	$0	20	$23	0	$0	20	$23
Soy oil	$2.01	13.2	$27	0	$0	13.2	$27	0	$0
Soybean meal	$1.75	180.1	$315	180.1	$315	120.1	$210	120.1	$210
salt	$0.47	3	$1	3	$1	3	$1	3	$1
Sodium Bicarbonate	$1.16	5.9	$7	5.9	$7	5.9	$7	5.9	$7
Calcium Carbonate	$1.11	5.8	$6	5.8	$6	5.8	$6	5.8	$6
Phosphate monocalcic	$1.11	19.9	$22	13.1	$14	19.9	$22	13.1	$14
DL-Methionine	$4.95	0.7	$3	0.7	$3	0.7	$3	0.7	$3
L-Lisine H CL	$5.53	3.4	$19	3.4	$19	3.4	$19	3.4	$19
L-Threonine	$5.18	1.3	$7	1.3	$7	1.3	$7	1.3	$7
Coline	$4.08	0.7	$3	0.7	$3	0.7	$3	0.7	$3
Vitamin and mineral premix	$5.71	7	$40	7	$40	7	$40	7	$40
	38.826657	1000 kg	US$962.53	1000 kg	US $951.78	1000 kg	US $886.49	1000 kg	US $875.74

Conclusion

Glycerol has been included in animal feed with the objective of ensuring an adequate caloric intake but there is little or no regulation on its implementation. Semi-purification processes prove important to ensure the removal of residual substances. With the use of SG, a longer food preservation in optimal conditions of the feed is possible, as it has been recognized as a safe component in animal feed by the FDA. The inclusion of SG can result in a viable and economical alternative to provide a caloric source in the feed of sows.

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References

Aherne, F., Foxcroft, G., & Pettigrew, J. E. (1999). Nutrition of the sow. In B. E. Straw, S. D'Allaire, W. L. Mengeling, & D. J. Taylor (Eds.), *Diseases of swine* (8th ed.). Iowa State University Press.

Aransiola, E. F., Ojumu, T. V., Oyekola, O. O., Madzimbamuto, T. F., & Ikhu-Omoregbe, D. I. O. (2014). A review of current technology for biodiesel production: State of the art. *Biomass and Bioenergy, 61*, 276-297. https://doi:10.1016/j.biombioe.2013.11.014

Brisson, D., Vohl, M. C., St-Pierre, J., Hudson, T. J., & Gaudet, D. (2001). Glycerol: a neglected variable in metabolic processes?. *BioEssays*, 23(6), 534-542. http://dx.doi.org/10.1002/bies.1073

Campos, P. H. R. F., Silva, B. A. N., Donzele, J. L., Oliveira, R. F. M., & Knol, E. F. (2012). Effects of sow nutrition during gestation on within-litter birth weight variation: a review. *Animal*, *6*(5), 797-806. http://dx.doi.org/10.1017/S1751731111002242

Clow, K. A., Ewart, K. V., & Driedzic, W. R. (2008). Low temperature directly activates the initial glycerol antifreeze response in isolated rainbow smelt (Osmerus mordax) liver cells. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *295*(3), R961-R970. http://dx.doi.org/10.1152/ajpregu.90372.2008

Dobroth, Z. T., Hu, S., Coats, E. R., & McDonald, A. G. (2011). Polyhydroxybutyrate synthesis on biodiesel wastewater using mixed microbial consortia. *Bioresource Technology, 102*(3), 3352-3359. http://dx.doi.org/10.1016/j.biortech.2010.11.053

Elliott, J. A. (1975). The effect of pregnancy on the control of lipolysis in fat cells isolated from human adipose tissue. *European Journal of Clinical Investigation*, *5*, 159-163. http://dx.doi.org/10.1111/j.1365-2362.1975.tb00442.x

Ethier, S., Woisard, K., Vaughan, D., & Wen, Z. (2011). Continuous culture of the microalgae Schizochytrium limacinum on biodiesel-derived crude glycerol for producing docosahexaenoic acid. *Bioresource Technology*, *102*, 88-93. http://dx.doi.org/10.1016/j.biortech.2010.05.021

Gallego, A. G., Moreira, I., Pozza, P. C., Carvalho, P. L. de O., Peñuela-Sierra, L. M., & Costa Filho, C. de L. (2014). Neutral semi-purified glycerin in starting pigs feeding. *Semina: Ciências Agrárias*, *35*(5), 2831–2842. https://doi.org/10.5433/1679-0359.2014v35n5p2831

Hay, W. W., Jr. (1994). Placental transport of nutrients to the fetus. *Hormone Research*, *42*(4-5), 215-222. http://dx.doi.org/10.1159/000184196

Helrich, K. (1990). *Official methods of analysis* (15th ed.). The Association of Official Analytical Chemists Inc.

Herrera, E., Palacin, M., Martin, A., & Lasuncion, M. A. (1985). Relationship between maternal and fetal fuels and placental glucose transfer in rats with maternal diabetes of varying severity. *Diabetes, 34*(Supplement 2), 42-46. http://dx.doi.org/10.2337/diab.34.2.s42

Hirschmann, H. (1960). The nature of substrate asymmetry in stereoselective reactions. *Journal of Biological Chemistry, 235*(10), 2762-2767. https://doi.org/10.1016/S0021-9258(18)64537-5

Kijora, C., & Kupsch, S.-D. (1996). Evaluation of technical glycerols from "Biodiesel" production as a feed component in fattening pigs. *Fett/Lipid, 98*(7-8), 240–245. https://doi.org/10.1002/lipi.19960980703

Knopp, R. H., Herrera, E., & Freinkel, N. (1970). Carbohydrate metabolism in pregnancy: Metabolism of adipose tissue isolated from fed and fasted pregnant rats during late gestation. *The Journal of Clinical Investigation*, *49*(7), 1438-1446. http://dx.doi.org/10.1172/JCI106361

Lasuncion, M. A., Lorenzo, J., Palacin, M., & Herrera, E. (1987). Maternal factors modulating nutrient transfer to fetus. *Biology of the Neonate, 51*(2), 86–93. https://doi.org/10.1159/000242637

Mattson, F. H., & Volpenhein, R. A. (1964). The digestion and absorption of triglycerides. *Journal of Biological Chemistry*, *239*(9), 2772-2777. https://doi.org/10.1016/S0021-9258(18)93813-5

McPherson, R. L., Ji, F., Wu, G., Blanton Jr, J. R., & Kim, S. W. (2004). Growth and compositional changes of fetal tissues in pigs. *Journal of Animal Science*, *82*(9), 2534–2540. https://doi.org/10.2527/2004.8292534x

Noblet, J., Dounnad, J. Y., & Etienne, M. (1990). Energy utilization in pregnant and lactating sows: modeling of energy requirements. *Journal of Animal Science*, *68*(2), 562-572. https://doi.org/10.2527/1990.682562x

Nye, C., Kim, J., Kalhan, S. C., & Hanson, R. W. (2008). Reassessing triglyceride synthesis in adipose tissue. *Trends in Endocrinology & Metabolism*, *19*(10), 356–361. http://dx.doi.org/10.1016/j.tem.2008.08.003

Oliviero, C. (2010). Successful farrowing in sows. https://doi.org/10138/18971

Possik, E., Peyot, M., Ahmad, R., Madiraju, S. R., & Prentki, M. (2021). New mammalian glycerol-3-phosphate phosphatase: role in β-cell, liver and adipocyte metabolism. *Frontiers in Endocrinology*, *12*. https://doi.org/10.3389/fendo.2021.706607

Quiniou, N., Dagorn, J., & Gaudré, D. (2002). Variation of piglets, birth weight and consequences on subsequent performance. *Livestock Production Science*, *78*(1), 63-70. https://doi.org/10.1016/S0301-6226(02)00181-1

Robergs, R. A., & Griffin, S. E. (1998). Glycerol. Biochemistry, pharmacokinetics and clinical and practical applications. *Sports Medicine*, *26*(3), 145–167. https://doi.org/10.2165/00007256-199826030-00002

Sabourin, P. G., & Hallenbeck, P. C. (2009). High yield conversion of a crude glycerol fraction from biodiesel production to hydrogen by photofermentation. *Bioresource Technology*, *100*(14), 3513–3517. https://doi.org/10.1016/j.biortech.2009.03.027

Schieck, S. J., Kerr, B. J., Baidoo, S. K., Shurson, G. C., & Johnston, L. J. (2010). Use of crude glycerol, a biodiesel coproduct, in diets for lactating sows. *Journal of Animal Science*, *88*(8), 2648-2656. https://doi.org/10.2527/jas.2009-2609

Sivan, E., Homko, C. J., Chen, X., Reece, E. A., & Boden, G. (1999). Effect of insulin on fat metabolism during and after normal pregnancy. *Diabetes*, *48*(4), 834-838. https://doi.org/10.2337/diabetes.48.4.834

Sousa, M., Melo, V. M., Rodrigues, S., Sant'ana, H. B., & Gonçalves, L. R. (2012). Screening of biosurfactant-producing Bacillus strains using glycerol from the biodiesel synthesis as main carbon source. *Bioprocess and Biosystems Engineering*, *35*(6), 897-906. https://doi.org/10.1007/s00449-011-0674-0

Testar, X., Lasunción, M. A., & Chieri, R. (1985). Effects of exogenous insulin on placental transfer of maternal glucose to the rat fetus. *Diabetologia*, *28*(10), 743–748. https://doi.org/10.1007/BF00265022

Van Gerpen, J., Shanks, B., Pruszko, R., Clements, D., & Knothe, G. (2004). *Biodiesel Production Technology*. National Renewable Energy Laboratory. https://doi.org/10.2172/15008801

Varanda, M. G., Pinto, G., & Martins, F. (2011). Life cycle analysis of biodiesel production. *Fuel Processing Technology*, *92*(5), 1087–1094. https://doi.org/10.1016/j.fuproc.2011.01.003

Weir, T. L., Manter, D. K., Sheflin, A. M., Barnett, B. A., Heuberger, A. L., & Ryan, E. P. (2013). Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults. *PLoS One, 8*(8), e70803. https://doi.org/10.1371/journal.pone.0070803

Yuasa, H., Hamamoto, K., Dogu, S. Y., Marutani, T., Nakajima, A., Kato, T., Hayashi, Y., Inoue, K., & Watanabe, J. (2003). Saturable absorption of glycerol in the rat intestine. *Biological and Pharmaceutical Bulletin, 26*(11), 1633–1636. https://doi.org/10.1248/bpb.26.1633

Zaleski, H. M., & Hacker, R. R. (1993). Variables related to the progress of parturition and probability of stillbirth in swine. *The Canadian Veterinary Journal, 34*(2), 109-113.