Arch Med Vet **46, 111-120 (2014)**

ORIGINAL ARTICLE

Use of biodiesel co-products (Glycerol) as alternative sources of energy in animal nutrition: a systematic review

Uso de co-productos de biodiesel (Glicerol) como fuentes alternativas de energía en la alimentación animal: una revisión sistemática

VO Silva[a*], E Lopes[a], EF Andrade[a], RV Sousa[a], MG Zangeronimo[a], LJ Pereira[a]

[a]Department of Veterinary Medicine, Physiology and Pharmacology Area, Federal University of Lavras, Lavras, Minas Gerais, Brasil.

RESUMEN

El reciente aumento en el uso de biodiesel en Brasil y en el extranjero, junto con la disponibilidad de gran cantidad de su residuo, el glicerol, ha generado un gran interés en el uso de este co-producto de varias maneras, entre ellas su aplicación en la alimentación animal. El uso de glicerol en la formulación de dietas despierta interés inmediato para obtener un producto rico en energía y alta eficiencia de utilización por los animales. El objetivo de este estudio fue evaluar los efectos del uso de la glicerina resultante de la producción de biodiesel como suplemento energético en la producción de alimentos para animales, buscando establecer protocolos adecuados para cada especie a partir de estudios anteriores. En los trabajos analizados, hubo estudios con cerdos, vacas, toros, ovejas, gallinas ponedoras y pollos de engorde. Del análisis realizado se puede inferir que el glicerol es un ingrediente alimenticio de remplazo adecuado en dietas en animales de producción.

Palabras clave: alimentación, ingrediente alimentario, producción animal, desempeño.

SUMMARY

The recent surge in the use of biodiesel in Brazil and abroad, coupled with the availability of large amounts of glycerol, are generating interest in the use of this co-product in several ways, such as its use in animal feed. The use of glycerol in the formulation of diets caused immediate interest to obtain a highly efficient energy rich product to use in animal production. The aim of this study was to evaluate the effects of the use of glycerol resulting from biodiesel production as an energy supplement in animal feed, as well as establishing appropriate protocols for each species based on previous studies. Most of them using pigs, cows, bulls, sheep, laying hens and broilers. It was possible to infer from these studies that glycerol was a food ingredient suitable for replacement in diets of different animal species.

Key words: diet, food ingredients, animal production, performance.

INTRODUCTION

Brazil is a country with great diversity of oilseed crops for biodiesel production and it has advanced technology and industrial structure with a high potential for the development of biofuels. Resolution Nº. 6/2009 of the National Energy Political Council (ANP 2009) requires the mandatory addition of 5% of biofuel to diesel oil sold throughout the country since January 2010. According to the National Petroleum Agency (ANP 2011), in 2010 biodiesel production in Brazil was approximately 2.4 billion of liters and for every 50 gallons of biodiesel approximately 4 to 5 kg of its co-residual product, glycerol, are generated (He and Thompson 2006). Glycerol (propane-1 ,2,3-triol) is an organic compound belonging to the alcohol function, liquid at room temperature (25 °C), hygroscopic, odorless, viscous and presenting sweet taste (IUPAC 1993[1], cited Menten[2] *et al* 2009).

The recent surge in the use of biodiesel in Brazil and abroad, coupled with the availability of large amounts of glycerol, are generating interest in the use of this co-product in several ways, among them, its application in animal feed. The U.S. legislation assigned to glycerol GRAS status (Generally Recognized as Safe) for use as a food additive according to good manufacturing standards and food, including for humans (FDA 2006).

The use of glycerol in the formulation of diets stimulated immediate interest to obtain a rich in energy product

Accepted: 13.06.2013.

* vivian_osbio@yahoo.com.br

[1] IUPAC, International Union of Pure and Applied Chemistry. 1993. www.iupac.org. Visitado noviembre 2011.

[2] Menten JFM, VS Miyada, B Berenchtein. 2009. Glicerol na Alimentação Animal. www.agrolink.com.br/downloads/glicerol_2009-03-13.pdf. Visitado noviembre 2011.

(4320 kcal gross energy per kg of pure glycerol) and high efficiency of utilization by animals (Berenchtein *et al* 2010). Moreover, serving as an energy source, glycerol may also have a positive effect on nitrogen retention, improving the recovery of amino acids and reducing the emission of pollutants in the enviroment (Cerrate *et al* 2006, Berenchtein *et al* 2010). Several studies involving pigs (Della Casa *et al* 2009, Seneviratne *et al* 2011), cattle (Wang *et al* 2009[a], Wang *et al* 2009[b], Abo El-Nor *et al* 2010) and sheep (Terre *et al* 2011), have been developed to determine the effects of glycerol from different sources on performance, meat and milk quality and digestibility of nutrients.

Glycerol is a normal component of animal metabolism, it is produced by lipolysis of adipose tissue or blood lipoproteins. However, there is no agrement on the metabolic implications of exogenous supplementation of glycerol in the diet, particularly when supplementation becomes the major energy ingredient of the diet. Additionally, protocols for adequate supplementation for different species are unknown.

Thus, the objective of this study was to evaluate the effects of the use of glycerol resulting from biodiesel production as energy supplement in animal feed production, to establish appropriate protocols for each species from previous studies.

MATERIAL AND METHODS

RESEARCH STRATEGY

An electronic search of the database Science Direct[3] was carried out in October 2011 using the following keywords: glycerol and biodiesel, and food and nutrition. To confirm the results and obtain additional studies, a similar strategy was used in Pubmed[4], ISI Web of Science[5] and Scielo[6] databases, using the same keywords in Portuguese and Spanish, when applicable.

SELECTION OF STUDIES

Studies in which glycerol was used as nutrient for animal feed production were selected. There was no restriction for animal species, sample size, dose and period of administration of glycerol. Additionally, there was no restriction on date or language for the selected articles.

DATA COLLECTION

Two researchers conducted the searches separately, taking care that the work conforms to the criteria selected for inclusion. In case of any discrepancy between the documents, all criteria were reviewed and discussed. Table 1 shows the data related to the experimental design of the retrieved articles.

QUALITY CRITERIA

The criteria were adapted based on other systematic reviews (Noli *et al* 2005, Negre *et al* 2009, Pereira *et al* 2011). The parameters used were:

- Randomization: randomized trials received score 2, whereas non-randomized experiments or when this fact was not clearly described in the text received a score 1.
- Blind Assessment: experiments in which evaluation was performed by a blind examiner received score 2; when this fact was not clearly described in the text it received a score 1.
- Control group: studies that used control groups received score 2 and studies that control groups were not mentioned in the text received a score 1.
- sample size: the number of repetitions were determined according to the species: pig (less than 3 animals - a score 1, between 3 and 6 - score 2 and above 6 animals per group - score 3), birds (less than 10 - score 1, between 10 and 20 - score 2 and above 20 - score 3), cattle and sheep (less than 10 - score 1; between 10 and 20 - score 2 and above 20 - score 3).
- Quality parameters evaluated: studies performing molecular analyzes or ELISA / RIA received score 2, those which underwent other types of analyzes (colorimetric / enzymatic, substrate analysis, milk and eggs, performance, etc) received score 1;

  The maximum total score was 11 points.

Additional variables such as species, breed, experimental period, collected material, among others, were used for descriptive purposes only, without punctuation, in order to contribute to the discussion (table 1).

RESULTS

The search using ISI Web of Science database resulted in 132 articles, 38 were excluded for dealing with glycerol production, three were excluded because used *in vitro* essays, nine for not using glycerol in the diet (experiments with other products), 40 articles were literature reviews and 27 did not fit in the selection criteria (book chapter, book index, summary of congress, etc.). Thus, 15 out of 132 articles were selected for this study.

The search in PubMed database resulted in two additional articles. The research done in the ISI Web of Science Scielo databases did not result in additional articles. Therefore, we selected 17 articles for this review (table 2).

Retrieved studies had been carried out on pigs, cows, bulls, sheep, laying hens and broilers. Sample sizes varied widely among articles, a fact that may have occurred due to the variety of species and different statistical

[3] www.sciencedirect.com

[4] www.ncbi.nlm.nih.gov

[5] http://apps. isiknoledge.com

[6] www.scielo.org/php/index.php

Table 1. Summary of the selected studies.
Resumen de los estudios seleccionados.

Author and year of publication	Species	Randomization	Blind assessments	Control group	Number of animals per experimental group	Experimental period	Glycerol level	Collected material	Parameters evaluated
Abo El-Nor *et al* 2010	Holstein cow	UC	UC	Yes	4	4 períod of 10 days	0, 36,72, 108 g/kg/DM	Ruminal liquid	Digestibility; ammonia nitrogen; acetate; propionate; butyrate; isobutyrate; valerate; isovalerate; VFA; DNA concentration of ruminal bacteria.
Boyd *et al* 2011	Holstein cow	UC	UC	Yes	15	69 days	400 g/animal/day	Milk; blood; diet; feed; feces	Milk fat and protein percentage, SCC, NE and milk yield; blood glucose and urea nitrogen; feed DM, NDF, ADF, CP, EE, NDF, ADF; DMI; NE intake; NE Bdy; Nutrient digestibility of diets; feed intake; BW change; feces DM, NDF, ADF and CP.
Carvalho *et al* 2011	Holstein cow	Yes	UC	Yes	11 and 12	84 days	11.5 and 10.8% pre and postpartum diets	Diet; milk; blood; hepatic tissue; ruminal liquid	Feed intake, feed efficiency, BW and body condition; diet DM, CP, ADF, NDF, starch and minerals; milk production, protein, lactose, total solids, fat, SCC and MUN; blood glucose; glycerol; BHBA; NEFA; ruminal pH, VFA; hepatic tissue (lipids).
Chung *et al* 2007	Holstein cow	UC	UC	Yes	19 and 20	21days	162.5 g/day	Diet; blood; urine	BW; consumption; body condition; feed efficiency; diet DM, CP, ADF, NDF and minerals; blood glucose, BHBA, NEFA, urea and insulin; urine (acetoacetate); milk production, protein, fat, lactose, SCC and MUN.
DeFrain *et al* 2004	Holstein cow	UC	UC	Yes	10	91 days	430 g/d/ animal and 860 g/day	Milk; urine; blood; ruminal liquid; hepatic	DM intake, BW and body condition score; milk energy-corrected, production, protein, fat, lactose, MUN and SCC, non-fat solids; urine (ketones); blood glucose, BHBA, NEFA, insulin and glycerol; ruminal pH, VFA and NH_3N; hepatic tissue (lipids, glycogen).

Della Casa *et al* 2009	Swine Italian Duroc x Italian Large White	UC	UC	Yes	16	154 days	0, 5 and 10%	Carcass	Growth performance; hot carcass weights and wholesale cut weights; ham muscle quality characteristics; loin quality characteristics; fatty acid composition of ham subcutaneous fat; fatty acid composition of intramuscular fat from gluteus medius; mean score for the sensory attributes of loin.
Donkin *et al* 2009	Holstein cow	Yes	UC	Yes	15	56 days	5, 10 and 15% in diets	Milk; blood; feed; urine; feces	BW and condition scores; milk fat, protein, lactose, total solids, MUN and SCC; blood glucose concentrations; feed DM, CP, ADF, NDF and minerals; feces nitrogen, ADF, NDF; apparent digestibility coefficients , cumulative efficiency of feed utilization for milk, maintenance, and gain.
Kovács *et al* 2011	Swine Norwegian Landrace x Duroc	Yes	UC	Yes	12	UC	0, 5 and 10%	Urine; feces	Apparent digestible; metabolizable energy.
McLea *et al* 2011	Broiler	Yes	UC	Yes	63	21 days	33, 67, 100 g/kg	Feed; feces	Feed conversion; digestibility; metabolizable energy; crude energy.
Osborne *et al* 2009	Holstein cow	Yes	UC	Yes	30	14 days	20 g/L Water	Feed; milk; blood	DMI; BW; body condition score; water intake; energy balance; triacylglycerol; NEFA; BHBA; glucose; yield and milk composition.
Osman *et al* 2008	Holstein cow	UC	UC	Yes	6 and 4	14 days	400 mL/animal/day Water orally	Blood	Glucose, NEFA, BHBA, urea nitrogen, triacylglycerol, glucagom and insulin.
Seneviratne *et al* 2011	Swine Duroc x Large White/ Landrace	Yes	UC	Yes	48	28 days	Canola meal solvent-extracted or expeller-pressed - 0 and 50 g/kg Soybean meal(control)	Feed; feces	Apparent total tract digestibility and coefficients of crude protein and energy and digestible energy content of diets; average daily consumption of food; average daily gain; feed efficiency.

Swiatkiewicz and Koreleski 2009	Bovans Brown hen	Yes	UC	Yes	18	25 weeks	2, 4 and 6%	Eggs; excrecion	Feed intake; rates of egg quality; yolk color; thickness and density of the shell; egg weight; albumen height; resistance to rupture of the shell; nitrogen intake; calcium and phosphorus content of nitrogen; calcium and phosphorus in excreta; nitrogen retention; calcium and phosphorus; crude energy.
Terré *et al* 2011	Ripollesa Lamb	UC	UC	Yes	39 and 63	25 kg aprox. 4 weeks	0, 50 and 100 g/kg	Blood; ruminal mucosa; carcass	Performance; insulin; glucose; carcass yield; hot carcass weight; fatty acids in the *Longissimus dorsi*; measuring of rumen papillae.
Wang *et al* 2009[a]	Chinese Simmental Steer	UC	UC	Yes	8	21 days	0, 100, 200, 300 g/animal/ day	Feed; ruminal liquid; urine	pH ruminal; ammonia nitrogen; VFA; *in situ* ruminal digestion kinetics and effective degradability of corn stover and concentrate mix; urinary purine derivatives; digestibilidade.
Wang *et al* 2009b	Holstein cow	Yes	UC	Yes	9	59 days	0, 100, 200, 300 g/animal/day	Feed; milk; urine; blood	DM intake; milk production; fat content; protein and lactose in milk; BW; energy balance; glucose; NEFA; BHBA concentration of ketones in the urine.
Yalçin *et al* 2010	Lohmann Brown hen	Yes	UC	Yes	45	16 weeks	2,5; 5; 7,5%	Feed; eggs; excretion; blood	Fatty acids profile; initial weight; final weight; feed intake egg production; egg weight, feed efficiency; egg characteristics; percentage of shares of the egg; egg yolk cholesterol.

UC: unclear; VFA: volatile fatty acid; DNA: deoxyribonucleic acid; DM: dry matter; BW: body weight ; NEFA: non-esterified fatty acids; BHBA: beta-hydroxybutyrate; DMI: Dry Matter Intake; CP: Crude Protein; ADF: Acid Detergent Fiber; NDF: Neutral Detergent Fiber; NE: Net Energy; EE: Ether Extract; SCC: Somatic Cell Count; MUN: Milk Urea Nitrogen; NH_3N Ammonia Nitrogen.

Table 2. Scores for evaluation criteria of articles selected.
Puntuaciones de los criterios de evaluación de los artículos seleccionados.

Author	Randomization*	Blind assessments**	Control group***	Sample number+	Quality parameters evaluated++	Total
McLea *et al* 2011	2	1	2	3	1	9
Seneviratne *et al* 2011	2	1	2	3	1	9
Terré *et al* 2011	1	1	2	3	2	9
Yalçin *et al* 2010	2	1	2	3	1	9
Osborne *et al* 2009	2	1	2	3	1	9
Carvalho *et al* 2011	2	1	2	2	1	8
Kovács *et al* 2011	2	1	2	2	1	8
Abo El-Nor *et al* 2010	1	1	2	2	2	8
Donkin *et al* 2009	2	1	2	2	1	8
Wang *et al* 2009[b]	2	1	2	2	1	8
Osman *et al* 2008	1	1	2	2	2	8
Swiatkiewicz and Koreleski, 2009	2	1	2	2	1	8
Chung *et al* 2007	1	1	2	3	1	8
DeFrain *et al* 2004	1	1	2	2	2	8
Boyd *et al* 2011	1	1	2	2	1	7
Della Casa *et al* 2009	1	1	2	2	1	7
Wang 2009[a]	1	1	2	2	1	7

* Nonrandomized experiments or when randomization was not described clearly in the text (score 1) and randomized experiments (score 2).
** Experiments without blind assessments or those in which blind assessments were not clearly reported in the text (score 1) and experiments with blind assessments (score 2).
*** Studies without control groups or those which did not clearly mention a control group in the text (score 1) and studies with a control group (score 2).
\+ Sample size scores were determined according to the species: pig (less than 3 animals - score 1, between 3 and 6, score 2 and 6 above, score 3), birds (less than 10, score 1, between 10 and 20, scores 2 and above 20, score 3), cattle and sheep (less than 10, score 1; between 10 and 20, scores 2 and above 20, score 3).
\++ Research protocols that performed molecular or ELISA/RIA analyzes received score 2, those which underwent other types of analyzes (colorimetric/enzymatic, substrate analysis, milk and eggs, performance only, etc) received score 1.

designs used. Seventy percent of the studies conducted randomized trials. All articles included a control group, but none of them have declared blind evaluations.

Glycerol was fed to animals in 88% of the selected articles, while in 12% of them it had been administered in water. There was a large variation in the level of glycerol supplied, without a pattern even within the same species. The time of administration of glycerol also varied greatly (ten days to 25 weeks).

In experiments performed with bovines, the most frequently collected materials for laboratory analysis were: blood, food and milk. In pigs, the collection of faeces was the most commonly assessed parameter due to digestibility analyses. Excreta were also collected in three experiments with birds in order to evaluate the nitrogen, calcium and phosphorus content, food intake and digestibility. In one experiment conducted with lambs, ruminal mucosa, blood and carcass were collected.

Glycerol is classified according to its purity; in general, crude glycerol contains about 80% purity; technical glycerol has a purity exceeding 90% and pharmaceutical-grade glycerol has purity higher than 99.7% (Vincent *et al* 2010). Products with lower purity, as in the case of crude glycerol, may present contaminants originating from the extraction process, such as methanol, a toxic substance which is considered unsafe at levels above 150 ppm (Dasari 2007). However, pigs fed diets containing glycerol and 2,900 ppm (Hansen *et al* 2009) and 3,200 ppm (Lammers *et al* 2008) of methanol showed no signs of intoxication. Out of the 17 studies used in this review, seven (41%) used glycerol with purity between 65 and 90%; seven (41%) reported the use of glycerol between 90% and 99.7% purity and three (18%) used products with a purity greater than 99.7%. Regarding the levels of methanol, only three studies reported specific amounts of this substance - 1.2% (DeFrain et al 2004); 0.05% (Terré *et al* 2011, Kovacs *et al* 2011). Some other studies reported non-significant levels of methanol.

The replacement of 5% of the dietary energy source by glycerol did not modify the *in vivo* or at slaughtering performance in pigs (Della Casa *et al* 2009). Similar results were observed in lambs after about four weeks (Terre *et al* 2011). However, a substitution of 10% caused growth and feed efficiency deterioration (Della Casa *et al* 2009). In pigs, the addition of glycerol in the diet did not affect the average daily consumption, average daily weight gain or feed efficiency, in a period of 0-28 days, initiated seven days after weaning (Seneviratne *et al* 2011).

The performance of dairy cows was variable, particularly in relation to food intake. Some authors have reported that the use of glycerol reduced the dry matter intake. Defrain *et al* (2004), evaluating animals in the pre-birth, found that levels of glycerol from approximately 2.7 to 5.4% (430 g/day and 860 g/day) reduced dry matter intake by 17%. Similar results were obtained by Osborne *et al* (2009) in cows fed with diets containing up to about 13% glycerol (800 g/day to 1600 g/day) and soybean oil in water, during the pre-delivery and postpartum. Donkin *et al* (2009) also found a reduction in feed intake during the first seven days of use in diets containing 15% of glycerol in dry matter. On the other hand, diets with 10.8 and 11.5% glycerol during 28 days before calving until 56 days postpartum (Carvalho *et al* 2011), approximately 0.6 to 1.9% (100 g/day 200 g/day and 300 g/day) for 59 days postpartum (Wang *et al* 2009[b]), or raw glycerin containing approximately 0.7% glycerol (162.5 g/day) for 21 days post- delivery (Chung *et al* 2007), did not cause deleterious effects on feed intake and milk production (Chung *et al* 2007, Donkin *et al* 2009, Wang *et al* 2009[b], Carvalho *et al* 2011). Still, there was a tendency to increase milk production in cows supplemented with glycerol during the sixth week of lactation, probably due to changes in metabolism (DeFrain *et al* 2004). Regarding the composition of milk, no differences were found with the use of up to 13% glycerol (Donkin *et al* 2009, Osborne *et al* 2009, Carvalho *et al* 2011). There was only a subtle reduction in the levels of milk fat and protein within 63 days of early lactation, even when using 1.9% (300 g/d) glycerol in dry matter (Wang *et al* 2009[b]).

In chickens, the use of glycerol up to 7,5% did not affect body weight, and weight of egg production and feed efficiency (Yalcin *et al* 2010). The egg quality parameters were not changed (Swiatkiewicz and Koreleski 2008, Yalcin *et al* 2010). Moreover, the inclusion of glycerol obtained from soybean biodiesel production, at 5% and 7.5% increased cholesterol content of the egg yolk, increased the percentage of myristic, palmitic, palmitoleic and linolenic acids and reduced the percentage of oleic acid, compared to eggs of hens fed with control diet. The total monounsaturated fatty acids of egg yolk were lower only in animals fed with the diet containing 7.5% of glycerol compared to the control group (Yalcin *et al* 2010).

The addition of glycerin in the diet caused no significant effect when evaluating parameters such as carcass yield (Della Casa *et al* 2009, Terre *et al* 2011), lean content and weight and lean cuts (Della Casa *et al* 2009),. Additionally, no effect on fatty acid composition (Terre *et al* 2011) and intramuscular fatty acids (Della Casa *et al* 2009) were found. Higher doses of glycerol resulted in higher octadecenoic acid content and a trend towards lower levels of palmitic, stearic and linoleic acids (Della Casa *et al* 2009).

Glycerol has also influenced the pattern of rumen fermentation. Its use in about 3.3% (100g/day, 200 g/day and 300 g/day) caused a linear increase in the concentration of volatile fatty acids in the rumen of calves, especially propionate (Wang *et al* 2009[a]). These changes in fermentation profile were also found in lactating cows, while increasing proportions of propionate and butyrate (DeFrain *et al* 2004, Abo El-Nor *et al* 2010), valerate (Abo El-Nor *et al* 2010) and isovalerate with reduction in the proportion of acetate (DeFrain *et al* 2004, Abo El-Nor *et al* 2010).

Studies also reported the effects of glycerol in the digestion process. The use of glycerol in the levels of 1.11% to 3.33% (100 g/day, 200 g/day and 300 g/day) increased ruminal degradation of Neutral Detergent Fiber (NDF) of corn stover, digestibility and urinary excretion of purine derivatives (Wang *et al* 2009[a]). Supplementation at levels close to 1.5% (400 g/day) in dry matter during lactation also increased apparent digestibility of dry matter and Acid Detergent Fiber (ADF), during lactation (Boyd *et al* 2011). However, other studies have reported that the addition of up to 10.8% glycerol did not alter the measurements of rumen papillae (Terre *et al* 2011), fermenting bacteria in the rumen, the concentration of ammonia nitrogen and dry matter digestibility (Abo El-Nor *et al* 2010). There was a lower degradability of protein with use of 0.6% to 1.9% (100g/day 200 g/day and 300 g/day) glycerol (Wang *et al* 2009[a]) and reduction of NDF digestibility and concentration *Butyrivibrio fibrisolvens* and *Selenomonas ruminantium* at the inclusion levels of 7.2 and 10.8% (72 g/kg and 108 g/kg) glycerol in the diet from bovines (Abo El-Nor *et al* 2010).

With regard to digestibility of nutrients and energy, the use of glycerol up to 10% in the diet did not influence the digestible and metabolizable energy in pigs (Kovács *et al* 2011). Moreover, in canola meal diets *Solvent-extracted* and *Expeller-pressed* also for pigs, glycerol increased digestible energy content (Seneviratne *et al* 2011). In birds, ammonia, calcium and phosphorus levels in excreta were not affected by adding glycerin (Swiatkiewicz *et al* 2008).

With regard to metabolic effects, in cows there was an increase in blood glucose (Chung *et al* 2007, Osman *et al* 2008, Wang *et al* 2009[b]) and insulin (Osman *et al* 2008), reduction of serum β-hydroxybutyrate (Chung *et al* 2007, Osman *et al* 2008, Osborne *et al* 2009, Wang *et al* 2009[b]) and non-essential fatty acids in the blood (DeFrain *et al*

2004, Osman *et al* 2008, Wang *et al* 2009[b]), and reduction of ketones in urine (Chung *et al* 2007, Wang *et al* 2009[b]). These results were correlated with glycerol levels ranging from about 0.7 to 13% (162.5 g / day to 1600 g / day) in dry matter and for periods between 14-60 days. However, in other studies, supplementation up to 13% (or up to 1600 g / d) glycerol did not affect the concentration of glucose or insulin in cows and in lambs respectively (DeFrain *et al* 2004, Osborne *et al* 2009). Glycerol levels above 13% did not affect the concentrations of non-essential fatty acids in cows (Osborne *et al* 2009) and total protein, uric acid, triglycerides, cholesterol, alanine aminotransferase, aspartate amino transferase and alanima phosphatase in hens (Yalcin *et al* 2010). Additionally, the use of an average level of glycerol near to 5,4% dry matter (860 g/day) in cow diets, reduced glucose and β-hydroxybutyric acid increase in plasma for a period of seven to 21 days of lactation (DeFrain *et al* 2004).

Regarding metabolizable energy (ME), Kovács *et al* (2011) found that including glycerol (86.76% purity) in the diet of growing pigs, provided 3,218 kcal/kg of energy. For finishing pigs, the addition of 30% glycerol with a purity of 97.5%, the ME was 3,723 kcal/kg (Mendoza *et al* 2010). For chickens aged between 7 and 10 days, a diet containing from 3% to 6% glycerol showed ME values of 3,621 kcal/kg, turning to 3,331 kcal/kg and 3,349 kcal/kg, for chickens aged between 21-24 days and between 42-45 days of life respectively (Dozier *et al* 2007). Also in chickens, it was found that the ME values of feeds containing glycerol were 3,598, 4,911 and 3,777 kcal / kg for diets containing 70% glycerol from soybean oil, 9.92% from mixture of frying oil and lard and 79.32% (from semi-purified process) respectively (Lima *et al* 2012). Considering the high digestibility of glycerol in the diet of chickens, Cerrate *et al* (2006) assumed the value of 3,527 kcal/kg based on the gross value obtained from the calorimetric bomb (3,596 kcal/kg of ME for glycerol with purity between 2.5% and 10%), however the inclusion of 10% caused negative effects on the performance of these animals. Thus, a better understanding of the correlation between the purity of glycerol and its role as source of dietary energy will allow the formulation of balanced diets in order to improve animal production performance (Alvarenga *et al* 2012).

DISCUSSION

Systematic reviews can promote much insight into the scientific community in general, since they allow a more complete and clear view of the results from different studies in the same field and also suggest the best protocols to be used and / or searched. Study limitations should be highlighted as some publications may not have been retrieved due to the different title and keyword indexes, although the authors have endeavored to ensure that no article was excluded. It may also be mentioned that the assessment of methodological quality was based on previous research and experience of the authors.

In order to produce a quality evaluation, we used defined criteria such as presence or absence of randomization, blind assessment of measurements, presence of a control group, sample size, etc. These evaluations were of great importance in this study in order to contribute in characterisation of the study, classifying them according to their attributes and their statistical analyses. As an example, blind and randomized evaluations are of great importance to bring greater reliability to scientific work, since it prevents study participants to know which treatment is being applied. In the case of randomized trials, the distribution is made randomly (Pereira *et al* 2011).

The conduction of this study yielded results with large amplitude both with respect to the species addressed, such as the protocols used in the supplementation of glycerol. For a given species, there were considerable variations in the level of glycerol and the administration period.

Considering the performance of animals, starting with dairy cows, there was no consensus among authors mainly in relation to consumption. Some authors have suggested a reduction in food intake using varying levels of glycerol (intervals between approximately 2.7% and 15% dry matter) (DeFrain *et al* 2004, Donkin *et al* 2009, Osborne *et al* 2009), whereas others using similar levels found no such effects on consumption (ranges between 0.6% and 11.5% glycerol in dry matter) (Chung *et al* 2007, and Swiatkiewicz Koreleski 2009, Carvalho *et al* 2011). In the production and composition of milk there was greater consensus among authors, since most of them found no deleterious effects on these parameters (Chung *et al* 2007, Donkin *et al* 2009, Osborne *et al* 2009, Wang *et al* 2009[b], Carvalho *et al* 2011). In general, no changes in performance levels up to approximately 7.5% of glycerol were found in poultry and pigs and sheep (Della Casa *et al* 2009, Swiatkiewicz and Koreleski 2009, Yalcin *et al* 2010, Terre *et al* 2011). However, using about 10% in pigs, some detrimental effects on their growth were reported (Della Casa *et al* 2009) and at levels of 5% to 7.5% for birds the composition of eggs, especially in relation to the yolk were altered (Yalcin *et al* 2012).

The digestion process seems to be altered or at least influenced by the use of glycerol in different species. In cattle the pattern of ruminal fermentation was modified, however, there was no agreement regarding the obtained results. Glycerol can provide cattle with some benefits such as increased fiber degradation and dry matter digestibility in steers when used at levels ranging 1.11% to 3.33% (Wang *et al* 2009[a]), however, it can also negatively affect the degradation of crude protein in dairy cows (Wang *et al* 2009[b]), and also according to Abo El-Nor *et al* (2010) it reduces NDF digestibility, negatively affecting the process of digestion. In pigs and poultry, glycerol did not bring major changes in relation to digestibility (Swiatkiewicz and Koreleski 2009, Kovács *et al* 2011).

The metabolic effects of glycerol influenced biochemical parameters and hormone release in blood and urine. However, there was great variability in results. The supply of glycerol of about 0.7% to 13% in dry matter increased the availability of energy in some studies (Chung *et al* 2007, Osman *et al* 2008, Wang *et al* 2009[b]), but did not change it in others (glycerol in the diet about up to 13% dry matter) (DeFrain *et al* 2004, Osborne *et al* 2009, Terre *et al* 2011), while it decreased in additional studies (glycerol in the diet up to 5.4% of dry matter), increasing the susceptibility to ketosis during lactation in cows (DeFrain *et al* 2004). This diversity of results may be due to factors such as the wide variability in levels of glycerol supplied, periods of administration and interference of simultaneous treatment in animals. Besides, these differences among species in relation to age, weight and race were very important, generating mixed results and making comparison difficult.

Based on the analysis of results on animal performance and the quality of milk, eggs and carcasses, no harmful changes to animals were found. The range of scores among the retrieved articles was only two points, with nine being the highest score.

Since some studies may possibly have not been retrieved because of the search strategy used in this review, new systematic reviews are encouraged using other terms (such as glycerin) related to biodiesel components in order to produce more reliable data regarding its use in animal feeding.

Based on selected studies for laying hens, it can be concluded that the addition of up to 6% of glycerol in diet did not influence the egg quality parameters when administered up to 16 weeks. In pigs, the replacement of an energy source up to 5% of glycerol in the diet did not seem to alter the performance of animals. In cattle, especially lactating cows, adding up to 15% of glycerol in the diet did not affect negatively their metabolism, performance or production. Therefore, glycerol may be a food ingredient suitable for replacement in diets for different animal species.

ACKNOWLEDGEMENT

This work was financially supported by FAPEMIG (Research Sponsoring Agency of the State of Minas Gerais) and CNPq (National Counsel of Technological and Scientific Development).

REFERENCES

Abo El-Nor S, AA Abughazaleh, RB Potu, D Hastings, MSA Khattab. 2010. Effects of differing levels of glycerol on rumen fermentation and Bacteria. *Anim Feed Sci Technol* 162, 99-105.

Alvarenga RR, EMC Lima, MG Zangeronimo, PB Rodrigues, Bernardino VMP. 2012. Use of glycerine in poultry diets. *World Poultry Sci J*, 68, 637-644.

ANP, Agência Nacional do Petróleo, Gás Natural e Biocombustíveis. 2009. Resolução Conselho Nacional de Política Energética Nº 6, de 16.9.2009.

ANP, Agência Nacional do Petróleo, Gás Natural e Biocombustíveis. 2011. Biocombustíveis: Biodiesel.

Berenchtein B, LB Costa, DB Braz, VV Almeida, MLP Tse, VS Miyada. 2010. Utilização de glicerol na dieta de suínos em crescimento e terminação. *R Bras Zootec* 39, 1491-1496.

Boyd J, JW West, JK Bernard. 2011. Effects of the addition of direct-fed microbials and glycerol to the diet of lactating dairy cows on milk yield and apparent efficiency of yield. *J Dairy Sci* 94, 4616-4622.

Carvalho ER, NS Schmelz-Roberts, HM White, PH Doane, SS Donkin. 2011. Replacing corn with glycerol in diets for transition dairy cows. *J Dairy Sci* 94, 908-916.

Cerrate S, F Yan, Z Wang, C Coto, P Sacakli, PW Waldroup. 2006. Evaluation of glycerine from biodiesel production as a feed ingredient for broilers. *Int J Poult Sci* 5, 1001-1007.

Chung YH, DE Rico, CM Martinez, TW Cassidy, V Noirot, A Ames, GA Varga. 2007. Effects of feeding dry glycerin to early postpartum Holstein dairy cows on lactational performance and metabolic profiles. *J Dairy Sci* 90, 5682-5691.

Dasari M. 2007. Crude glycerol potential described. *Feedstufs* 79, 1-3.

DeFrain JM, AR Hippen, KF Kalscheur, PW Jardon. 2004. Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance *J Dairy Sci* 87, 4195-4206.

Della Casa G, D Bochicchio, V Faeti, G Marchetto, E Poletti, A Rossi, A Garavaldi, A Panciroli, N Brogna. 2009. Use of pure glycerol in fattening heavy pigs. *Meat Sci* 81, 238-244.

Donkin SS, SL Koser, HM White, PH Doane, MJ Cecava. 2009. Feeding value of glycerol as a replacement for corn grain in rations fed to lactating dairy cows. *J Dairy Sci* 92, 5111-5119.

Dozier WA, BJ Keer, A Corzo, MT Kidd, TE Weber, K Bregendahl. 2007. Apparent metabolizable energy of glycerin for broiler chickens. *Poult Sci*, 87, 317-322.

FDA, Food and Drug Administration. 2006. Substances generally recognized as safe: general purpose food additives-glycerin. Code of Federal Regulations.

Hansen CF, A Hernandez, BP Mullan, K Moore, M Trezona-Murray, RH King, JR Pluske. 2009. A chemical analysis of samples of crude glycerol from the production of biodiesel in Australia, and the effects of feeding crude glycerol to growing-finishing pigs on performance, plasma metabolites and meat quality at slaughter. *Anim Prod Sci*, 49, 154-161.

Kovács P, E Zsédely, A Kovács, G Virág, J Schmidt. 2011. Apparent digestible and metabolizable energy content of glycerol in feed of growing pigs. *Livest Sci* 142, 229-234.

Lammers PJ, BJ Kerr, TE Weber, K Bregendahl, SM Lonergan, KJ Prusa, DU Ahn, WC Stoffregen, WA Dozier, MS Honeyman. 2008. Growth performance, carcass characteristics, meat quality, and tissue histology of growing pigs fed crude glicerine-supplemented diets. *J Anim Sci,* 82, 62-70.

Lima EMC, PB Rodrigues, RR Alvarenga, VMP Bernardino, L Makiyama, RR Lima, VS Cantarelli, MG Zangerônimo.

2012. The energy value of biodiesel glycerine products fed to broilers at different ages. *J Anim Physiol Anim Nutr,* DOI: 10.1111/j.1439-0396.2012.01335.x

McLea L, MEE Ball, D Kilpatrick, C Elliott. 2011. The effect of glycerol inclusion on broiler performance and nutrient digestibility. *Br Poult Sci* 52, 368-375.

Mendonza OF, M Ellis, FK McKeith, AM Gaines. 2010. Metabolizable energy content of refined glycerin and its effects on growth performance and carcass and pork quality characteristics of finishing pigs. *J Anim Sci*, 88, 3887-3895.

Menten JFM, VS Miyada, B Berenchtein. 2009. Glicerol na Alimentação Animal. Disponível em: <http://www.agrolink.com.br/downloads/glicerol_2009-03-13.pdf>. Acesso em: 11/2011.

Negre A, E Bensignor, J Guillot. 2009. Evidence-based veterinary dermatology: a systematic review of interventions for Malassezia dermatitis in dogs. *Vet Dermatol* 20, 1–12.

Noli C, ST Auxilia. 2005. Treatment of canine Old World visceral leishmaniasis: a systematic review. *Vet Dermatol* 16, 213–232.

Osborne VR, NE Odongo, JP Cant, KC Swanson, BW McBride. 2009. Effects of supplementing glycerol and soybean oil in drinking water on feed and water intake, energy balance, and production performance of periparturient dairy cows. *J Dairy Sci* 92, 698–707.

Osman MA, PS Allen, NA Mehyar, G Bobe, JF Coetzee, KJ Koehler, DC Beitz. 2008. Acute metabolic responses of postpartal dairy cows to subcutaneous glucagon injections, oral glycerol, or both. *J Dairy Sci* 91, 3311-3322.

Pereira UP, DGS Oliveira, LR Mesquita, LJ Pereira. 2011. Efficacy of *Staphylococcus aureus* vaccines for bovine mastitis: A systematic review. *Vet Microbiol* 148, 117-124.

Seneviratne RW, E Beltranena, LA Goonewardene, RT Zijlstra. 2011. Effect of crude glycerol combined with solvent-extracted or expeller-pressed canola meal on growth performance and diet nutrient digestibility of weaned pigs. *Anim Feed Sci Tech* 170, 105-110.

Swiatkiewicz S, J Koreleski. 2009. Effect of crude glycerin level in the diet of laying hens on egg performance and nutrient utilization. *Poult Sci* 88, 615-619.

Terré M, A Nudda, P Casado, A Bach. 2011. The use of glycerine in rations for light lamb during the fattening period. *Anim Feed Sci Tech* 164, 262-267.

Thompson JC, BB He. 2006. Characterization of crude glycerol from biodiesel production from multiple feedstocks. *Appl Eng Agric* 22, 261-265.

Vicente G, JA Melero, G Morales, M Paniagua, E Martín. 2010. Acetalisation of bio-glycerol with acetone to produce solketal over sulfonic mesostructured silicas. *Green Chem* 12, 899-907.

Wang C, Q Liu, WJ Huo, WZ Yang, KH Dong, YX Huang, G Guo. 2009[a]. Effects of glycerol on rumen fermentation, urinary excretion of purine derivatives and feed digestibility in steers. *Livest Sci* 121, 15-20.

Wang C, Q Liu, WZ Yang, WJ Huo, KH Dong, YX Huang, XM Yang, DC He. 2009[b]. Effects of glycerol on lactation performance, energy balance and metabolites in early lactation Holstein dairy cows. *Anim Feed Sci Tech* 151, 12-20.

Yalçin S, H Erol, B Özsoy, İ Onbaşılar, S Yalçın, A Üner. 2010. Effects of glycerol on performance, egg traits, some blood parameters and antibody production to SRBC of laying hens. *Livest Sci* 129, 129-134.