Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

CRUDE GLYCEROL: BY-PRODUCT OF BIODIESEL INDUSTRIES AS AN ALTERNATIVE ENERGY SOURCE FOR LIVESTOCK FEEDING

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Received – October 15, 2017; Revision – November 30, 2017; Accepted – December 16, 2017

Available Online – December 27, 2017

DOI: [http://dx.doi.org/10.18006/2017.5\(6\).755.766](http://dx.doi.org/10.18006/2017.5(6).755.766)

KEYWORDS

Biodiesel

Crude glycerol

Glycogenic

Green house gases

Livestock feeding

ABSTRACT

Increased concern on greenhouse gas emission, concomitant global warming and decreasing fossil fuels have created the necessity to find an alternative source of energy for the transportation vehicles. Biodiesels is an alternative renewable fuel source, which releases less carbon dioxide, carbon monoxide, sulfur and nitrogen oxides upon combustion. These environmental friendly features of biodiesel make it a potential alternative to the conventional one. Biodiesel can be extracted from the edible or non-edible source of triglycerides. During this extraction process, crude glycerol (CG) is obtained at the rate of 10% as a byproduct of trans-esterification reaction. Chemically, CG so obtained is composed of catalysts, salts and fatty acids. Purification of CG to >95% involves the cost (0.28 USD/L production) and time consuming process. Hence, economical management of CG is the need of hour, one such alternative way is its entry in animal food chain as an energy source. Basically, glycerol is a glycogenic intermediate product of fat digestion converted to glucose by the action of key enzyme glycerol kinase. The CG undergo glycolysis and TCA cycle to liberate ATP. The digestible and metabolizable energy content of CG are comparable with the commonly used cereal grains in livestock feeding. Thus, CG acts as a bridging gap between the biodiesel industry and livestock production. Studies have showed that CG

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
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can be used as an energy source as a replacement of cereals in pig, poultry, goat, feedlot cattle and dairy cows. This review provides an overview of utilization of CG and its effect on production performance of different livestock species.

1 Introduction

Green house gases (GHG) emission is continued to be a major concern over many centuries due to increase in earth atmospheric temperature by 0.8°C since 1880 (NASA, 2015). Transportation sector contributes 14% to total global GHG emission (IPCC, 2014). To combat the negative impact of GHG emission from transportation fuels, developing countries shifting towards the alternative source of energy such as biodiesel production. Biodiesel is renewable, nontoxic, biodegradable, aromatic and free from sulfur gases. Additionally, its better lubricity improves the engine performance and lifespan (Al-Zuhair, 2007). These environmental friendly features made feasible to utilize biodiesel as an alternative to diesel in vehicles, thereby decreasing dependency on rapidly depleting fossil fuel resources. Biodiesel can be obtained from sources of triglycerides such as vegetable oil

or animal fat by a process called trans-esterification in the presence of catalyst methanol or ethanol (Hu et al., 2012). During the extraction process of biodiesel, glycerol is obtained at the rate of 10% as a byproduct of trans-esterification reaction (Posada et al., 2012). Glycerol so obtained is called crude glycerol (CG) since it is mixed with the impurities like catalysts, salts and fatty acids. Use of vegetable oils as a source of triglycerides for biodiesel production competes with the human food chain. Keeping this in mind many research studies took place to use non-edible oilseeds, waste oils, byproducts of the slaughter house, algae etc., as an alternative source of triglycerides. Various improvements in the method of extraction process by using different types of catalysts, enzymes, alcohols, different temperature and pressure combination has led to decrease in the cost involved and improving the efficiency of biodiesel extraction (Refaat, 2010) (Figure 1). Currently, the biodiesel production in

Figure 1 Steps involved in biodiesel extraction process (Shuit et al., 2010).

the world is around 29.7 billion liters which could create a huge amount of CG availability (REN21, 2015). Purified CG (>95%) are being used in the food, cosmetic and pharma industries. Purification of CG to 99% purity is a costlier affair, as it requires 0.28 US\$/L of production. The presence of impurities in CG affects the operational cost of the industrial process and also not feasible under small or medium scale biodiesel plants (Posada et al., 2012).

CG has a high Chemical Oxygen Demand (COD). Disposal as such in the open environment may pose environmental pollution. An alternative to purification and disposal, CG can also be used in the synthesis of many other commercial products by chemical (syn- gas, acrolein, 1, 2- propanediol) and bio-chemical (ethanol, 1, 3- propanediol, D- lactic acid, succinic acid, propionic acid and poly-3-hydroxybutyrate) processes. However, these are time consuming and involve use of chemicals, microorganisms and sophisticated facilities (Posada et al., 2012). Various researches are being carried out for an economical and environmentally friendly utilization of the by-product throughout the world. One such alternate utility will be an entry in the animal food chain as an energy source. The gross energy (GE, 3.3 - 6.7 Mcal/kg) and metabolizable energy (ME, 2.9 - 4.8 Mcal/kg) values of CG are

comparable with maize grains, the most commonly used ingredient in concentrate feed preparation (Jung & Batal, 2011; Benedet et al., 2015). Moreover, glycerol being the intermediate product of glycolysis serves as an energy source which can be utilized in ketosis. In this review, an attempt has been made to reveal the nutritional significance of CG, its composition, level of inclusion in different kind of animals, and effect on production are discussed in different headings.

2 Composition of CG

After biodiesel extraction, CG left over consists of impurities like methanol, soap, fatty acids, esters, and catalysts. The color of CG depends on the pigmentation of the source used for biodiesel extraction and varies from light brown to dark brown (Hansen et al., 2009). The density of CG ranged from 1.01 to 1.20 g/cm³ and pH ranges from 6.4 to 10 and that of pure glycerol is 1.31 g/cm³ and 6.4 respectively (Hu et al., 2012). Chemical composition of CG obtained from different sources is given in table 1. Source of triglyceride, the catalyst used and the process of extraction defines the composition of CG (Yang et al., 2012). CG derived from six different sources of triglycerides (mustard, rapeseed, canola, crambe, soybean and waste cooking oil) contained 0.06 to 0.44%,

Table 1 Composition of crude glycerol extracted from different sources

Source of triglyceride	Glycerol	Methanol	Moisture	Crude protein	Ash	Crude fat	Reference
Waste vegetable oil	63.42	4.38	13.93	0.03	7.41	9.57	Chanjula et al., (2016b)
Soybean oil	86.95	0.028	9.22	0.41	3.19	5.33	Lammers et al., (2008a)
Vegetable oil	87.42	0.05	7.98	----	5.87	----	Orengo et al., (2014)
Palm oil	88.91	0.52	5.6	0.01	3.51	----	Chanjula et al., (2016b)
Rapeseed oil	80.00	0.50	---	---	8.00	---	Barton et al., (2013)
Soybean oil	80.34	----	12.02	----	5.03	1.59	Lage et al., (2014a)
Soybean oil	80.30	0.40	12.40	---	----	---	Shin et al., (2012)
Pork fat	74.74	0.0	10.32	0.88	----	----	Silveira et al., (2015)
Castor, soybean, sunflower, cotton seed oil.	36.2	8.7	6.2	0.4	2.00	----	Lage et al., (2014b)
Soybean, sunflower	83	0.01	11	----	6.00	----	Carvalho et al., (2015)
Source not available*	82.6	0.4	7.1		9.30	0.6	Kass et al., (2013)
Soybean	80.34	0.003	12.02	1.15	5.72	1.81	San et al., (2015)
Source not available*	86.6	0.003	7.5	----	5.88	----	Egea et al., (2016)
Source not available*	86.6	0.028	9.22	0.41	3.19	0.12	Lammers et al., (2008b)
Source not available*	84.37	0.01	10.72	0.65	3.21	0.33	Kim et al., (2013)
From 11 different sources	72.4	4.12	5.5	----	5.20	----	Hansen et al., (2009)

* Obtained from biodiesel plant but source not available.

1 to 13% and 75 to 83% as protein, fat and carbohydrate, respectively. Whereas, the fat and carbohydrate in waste cooking oil was about 60 and 27%, respectively (Thompson & He, 2006). Almost 90% of ash in CG consists of sodium chloride derived from the catalyst used during extraction process (Orengo et al., 2014; Chanjula et al., 2016b). Other minerals such as Ca, P, K, Cu, Zn, Mg, and Mn are present in negligible amount have lesser importance in feeding (Jung & Batal, 2011; Shin et al., 2012).

Among the different constituents of the CG, methanol is more important due to its toxic effect on central nervous system and causes blindness by converting into toxic metabolites formaldehyde and formic acid (Dorman et al., 1993). However,

till today no such adverse effects had been reported in any species of animals fed with the CG. The lower level of lipid to water partition coefficient of methanol shows that microorganisms having a better tolerance to methanol than the ethanol (Patterson & Ricke, 2015) and it is advisable to use methanol instead of ethanol during biodiesel extraction process intended to use CG in animal feeding. Methanol concentration in the CG depends on the manufacturing process and stock feed. European Food Safety Authority (EFSA, 2010) limited amount of methanol in CG to 0.5% in the vegetable origin feedstock whereas, FDA (2010) allowed maximum up to 150 ppm of methanol in the final animal feed. However, Lage et al. (2014b) demonstrated no deleterious effects even at 8.7% of methanol in feed lot lambs. It has been seen that the residual methanol will evaporate during feed processing and storage due to its volatile nature. Silveira et al. (2015) in his pig experiment used CG derived from pork fat which was liquefied at 65.5°C found 0.0% of methanol indicating evaporation at that temperature. In addition to methanol, sodium is considered as next contaminant of concern. As maximum sodium content in CG should be around 1.07 to 1.2%, it may reach up to 1.4% (EFSA, 2010). Excess sodium is not a problem in ruminants until sufficient water is available for drinking; accordingly NRC (2001) has allowed maximum sodium 1.6% on the dry basis in the diet of lactating cattle without any negative impact on rumen fermentation and health status.

3 Scope for feeding of CG in animals

Limited availability of fossil fuels and increased global warming in recent years made biofuel a viable source of energy with low carbon emission, renewable and economical to use in transportation fuels. In 2014, global biofuel production reached around 123.7 billion liters of which 94 billion liters were ethanol and 29.7 billion liters were biodiesel (REN21, 2015). United States, Brazil, Germany, China and Argentina are the top countries in biofuel production. According to OECD/FAO (2015) prediction, expected world biodiesel production in 2024 will be

around 35 billion liters. India's share in crude oil production is about 1 percent of global crude oil production, whereas consumption amounts to 3.1 percent of global consumption (Energy Statistics Report, 2003). Currently, India has 20 large biodiesel plants producing around 135 million liters of biodiesel and 2,219 million liters of bioethanol per year (USDA, 2015). National Biofuel Policy of India (2015) decided to blend 20% of bioethanol and biodiesel with petrol and diesel respectively by the end of 12th five year plan i.e. 2017 to decrease GHG emission, climate change mitigation for environmentally sustainable development, apart from other advantages like creating new employment opportunities. In order to achieve the target of 20% blending national biofuel policy set a target of dedicating 11.2 to 13.4 million hectares (Mha) of land to *Jatropha* cultivation by the end of its 11th Five-Year Plan. However, it was a failure due to unawareness of farmers regarding agronomic practices of *Jatropha* cultivation, lack of field validation and standardization of oil yield and many other reasons which is behind the scope of this review (Singh et al., 2013; Edrisi et al., 2015). Several state governments along with the central government are providing incentives to farmers for planting *Jatropha* and other non-edible oilseeds. The expected increase in biodiesel production creates the excess availability of CG as a byproduct which can be utilized as an energy source for the livestock's which in turn reduces the dependence on the cereals and cereal by-products.

4 Nutritive value of CG

Chemically, glycerol is a sugar alcohol and highly soluble in water. Its sweetish taste increases the palatability of the diet (Groesbeck et al., 2008). Ghrelin a peptide hormone secreted from the stomach mucosa is considered as hunger hormone, increase in acyl ghrelin in pigs fed with CG in their diet indicated the tendency of increase in appetite (Orengo et al., 2014). Ingested glycerol absorbed by passive diffusion and acts as a readily available source of energy. Glycerol kinase is a key enzyme in the liver for initiation of glycerol utilization. It converts the glycerol to glycerol 3-phosphate, which in turn get converted to glucose by gluconeogenesis or oxidized to give energy by glycolysis or citric acid cycle (Figure 3). Viscous nature of CG reduces the dustiness of the feed in mash type of diet, but increasing it more than 8% of the diet in pig resulted in feeding difficulty due to the formation of an aggregate of feed to firm mass (Hansen et al., 2009) (Table 2). A similar finding was reported by Cerrate et al. (2006) in poultry birds increasing CG up to 10% affected the flow rate of feed in tube feeder and pellet quality. Whereas, the addition of CG prior to pelleting increased the pellet durability and feed mill production efficiency at 9% level in piglet diet preparation (Groesbeck et al., 2008). Lammer et al., (2008a) from his five experimental results, each consisting of different levels of CG in growing pig's diet concluded the average digestible energy (DE)

Table 2 Different levels of inclusion of CG and methanol in animal diet without any adverse effects

Source of Biodiesel	Purity of Glycerol %	Methanol (%)	Inclusion level on total DM basis	Species	Research findings	Reference
Vegetable oil (CGS) Mixed oil (animal fat and soybean oil, CGA)	55.95 (CGS) 55.45 (CGA)	10.96 (CGS) 5.05 (CGA)	4,8 and 12 %	Piglets	1. DE and ME values of CGS and CGA are 5,070 and 4,556, 5,143 and 4,488 kcal/kg respectively. 2. Source of CG had no significant effect on performance of the animals. 3. CG of both the types included up to 12% reduced the feed cost by 11%.	Carvalho et al., (2012b)
Anonymous	82.6	0.4	1, 2 and 3kg/head	Cows	1. Increased level of feed intake with increase in dose. 2. No effect on milk yield, milk protein increased significantly in all treatment group. 3. Lower blood NEFA and higher blood urea. 4. CG can be used to replace barley without any detrimental effect on microbial fermentation and lactation performance.	Kass et al., (2012)
	88.8	0.75	7.5 and 15 %	Weaned pigs	1. Caecal <i>Clostridium spp.</i> and <i>Lactobacillus spp.</i> were linearly reduced by CG inclusion level. 2. The glycerol kinase and relative mRNA expression in liver increased linearly with increasing CG. 3. CG up to 15% can be adopted easily by weaned piglets	Papadomichelakis et al., (2012)
Rapeseed oil	80	<0.5	5 and 10 %	Bulls	1. No significant effect on growth performance, carcass and meat quality traits. 2. No effect on blood and rumen metabolites. 3. CG can be used as a long-term substitution for barley meal up to 10% in finishing bulls.	Barton et al., (2013)
Anonymous	84.37	<0.01	5 %	Broiler chicken	1. Apparent total tract digestibility and gross energy was more than the basal diet. 2. No effects of CG on intestinal transit time of birds and nutrient utilization in diets.	Kim et al., (2013)
Vegetable oil	36.20	8.7	3, 6, 9 and 12 %	Lambs	1. Quadratic effect of level of CG was observed on final body weight and carcass weight. 2. Inclusion of CG tended to reduce the Zn content in meat. 3. No effect on performance and carcass traits of the animals, CG may be used in the diets of finishing lambs as a replacement to concentrate	Lage et al., (2014b)
Anonymous	87.5	0.05	2 and 4%	Young bulls	1. No effect on daily feed intake, weight gain, feed conversion efficiency and carcass characteristics. 2. CG can be used to replace barley up to 4% without any adverse effect on animal performance.	Egea et al., (2014)
Vegetable oil	87.42	0.05	10 %	Pigs	1. Higher average daily gain and feed intake. 2. Tendency of increase in acyl-ghrelin in CG fed group was observed. 3. CG can replace wheat up to 10% without any adverse effect.	Orengo et al., (2014)
Anonymous	85	<0.5	9 and 18 %	Weaning pigs	1. No effect on nutrient digestibility and blood metabolites. 2. At higher level of CG increased the excretion of glycerol in the urine indicating saturation of metabolism. 3. Cell structure and cytokine expression in the intestinal cell could be affected with higher glycerin addition in piglet diet.	Oliveira et al., (2014)
	73.5	1.52	3, 6, 9 and 12 %	Steer	1. Dry matter intake, nutrient digestibility and microbial protein synthesis were not affected by CG. 2. Intestinal and total digestibility of lipids improved by CG inclusion. 3. CG can be used as a good alternative to ground corn up to 12% of total DM.	Serrano et al., (2014)
Soybean oil	80.34	-----	10%	Bull	1. CG as a substitute for corn or soybean hull had no significant effect on carcass or meat quality traits however insignificant increase in monounsaturated fatty acids and CLA was noticed.	Lage et al., (2014a)
Soya bean oil	70	0.018	1.75, 3.5, 5.25 and 7%	Broiler birds	1. More weight gain, better feed conversion and highest breast yield was observed at 3.55% inclusion level.	Bernardino et al., (2015)

Source of Biodiesel	Purity of Glycerol %	Methanol (%)	Inclusion level on total DM basis	Species	Research findings	Reference
Soybean and sunflower	83	0.01	7.5, 15, 22.5 and 30 %	Lambs	<ol style="list-style-type: none"> 1. At 30% addition reduced carcass weight and yield. 2. Odd-chain fatty acids, oleic, palmitoleic, total unsaturated, and monounsaturated fatty acids were increased. 3. Tendency of increasing CLA in glycerin-fed lambs (P=0.06) was observed. 4. CG decreased stearic, palmitic trans-vaccenic, total saturated fatty acids, and atherogenicity index. 	Carvalho et al., (2015)
Soybean oil	87.98	0.003	7, 14, 21 and 28 %	Bulls	<ol style="list-style-type: none"> 1. No change in carcass characteristics and meat quality. 2. pH decreased linearly, and margaric acid (17:0) increased in the longissimus muscle with the addition of glycerin in the diet. 3. CG up to 28% can be included in the diet without any adverse effect. 	San et al., (2015)

and ME value of CG 3,344 $\pm$ 8 and 3,207 $\pm$ 10 kcal/kg, respectively. Average ME content of CG was reported to be 3,434 kcal/kg in broiler chickens (Dozier et al., 2008).

5 CG as in livestock feed

5.1 Non-ruminants

Use of glycerol as an energy supplement in animal feeding is not a new concept. Increased availability of CG as a byproduct from biodiesel industry paves the way for its use in formulating least-cost animal feed. Pure glycerol as a source of carbohydrate in transition animals to cure ketosis was reported by Fisher et al. (1971). However, the concept of glycerol utilization in pig feeding was started in 1990's (Kijora & Kupsch, 1996). Animals require time to adjust for the introduction of CG in the basic diet, this adjustment period is different for different kind of animals. In weaned pigs, supplementation in initial 35days decreased expression of glycerol kinase indicating decreased utilization followed by increased expression (Papadomichelakis et al., 2012).

Glycerol being the intermediate product of glycolysis acts as an energy source. Research studies have been conducted on replacement of energy sources such as cereal grains in the diet of the animals by isocaloric CG proved, CG as a better alternative (Orengo et al., 2014; Chanjula et al., 2016a). The inclusion of CG at various levels found safe with no adverse effects on feed utilization and body weight. Inclusion of CG (10%) in growing pig diet had no significant effect on feed intake, feed conversion ratio, carcass traits and average daily gain (Hansen et al., 2009; Carvalho et al., 2012b; Egea et al., 2016). Replacing isocaloric cereals with CG at 12% level in piglet diet reduced feed cost by 11% (Carvalho et al., 2012b). In contrary, inclusion level higher than 10% in porcine diet resulted in excretion of glycerol without metabolism in urine due to saturation of enzymes in the liver

(Lammers et al., 2008a). Nursery piglet diet with added glycerol did not affect the intake, digestibility, growth and development of GIT (Oliveira et al., 2014). No effect of CG on blood insulin or glucose concentration was observed in many studies (Lammers et al., 2008a; Hansen et al., 2009; Orengo et al., 2014).

Inclusion level of 2.5 and 5% was acceptable without any adverse effect on feed intake, feed conversion efficiency, growth rate and digestibility in broilers (Cerrate et al., 2006). Papadomichelakis et al. (2015) concluded that feeding CG at 7% level improved the feed efficiency in broilers but did not affect the growth rate. Further increase in CG to 10 to 21% affected the bird's performance, feed intake and increased the water intake which affected litter quality (Cerrate et al., 2006; Papadomichelakis et al., 2015). Loose litter quality may result in coccidiosis and mortality. Egg production, egg weight were not affected at 5, 10 and 15% inclusion level of CG in layers (Lammers et al., 2008b) (Table 2).

5.2 Effect of CG feeding on rumen fermentation and digestibility of CG

Glycerol utilization in ruminants is different from that of mono gastric animals. However, functionality is due to the glycogenic property only. Glycerol after ingestion 50 to 80% disappears from the rumen within 4 h of feeding (Donkin, 2008). Glycerol which escapes from the rumen fermentation gets converted to glucose in the liver. It enters the gluconeogenesis pathway at the triose phosphate level and is not dependent on the rate-limiting enzymes pyruvate carboxylase and phosphoenolpyruvate carboxykinase for its conversion (Leng, 1970). A portion of glycerol subjected to microbial fermentation increases the propionate concentration and decreases the acetate to propionate ratio (Benedeti et al., 2015). The proportion of increased propionate and decreased acetate concentration decides the total volatile fatty acids concentration

(TVFA). The increase in TVFA concentration was observed by Benedeti et al. (2015) and Lokesha et al., (2017), whereas, Chanjula et al. (2014) reported no change in the TVFA in spite of increased propionate production. Some of the researchers have also reported increased butyric acid and short chain fatty acids like valeric acid and isovaleric acid concentrations (Abo El-Nor et al., 2010; Shin et al., 2012) by CG fermentation (Table 2; Figure 2).

Megasphaera elsdenii and *Selenomonas ruminantium* are the two main glycerol utilizing bacteria found in the rumen (Krehbiel, 2008). Butyric acid is the main end product of *Megasphaera elsdenii*, which helps in maintaining the health of rumen epithelium, whereas, *Selenomonas ruminantium* ferments glycerol to propionate (Van et al., 2015). The addition of starch-rich readily available energy sources (grains, molasses) causes the decreased rumen pH due to lactic acid accumulation. Glycerol as a source of carbohydrate replaced with corn starch at 10 and 20% level had no significant effect on rumen pH and $\text{NH}_3\text{-N}$ level (Abo El-Nor et al., 2010; Long et al., 2015). The tendency to decrease pH and $\text{NH}_3\text{-N}$ was observed at 6% of CG feeding in goats and steers at 300g per day (Wang et al., 2009; Chanjula et al., 2016b). Decreased pH may be due to the accumulation of lactic acid in the rumen and decreased $\text{NH}_3\text{-N}$ indicates the decreased proteolytic activity or increased utilization of nitrogen by the rumen microbes. Paggi et al. (1999) reported 20% reduction in proteolytic activity with CG addition which could be explained with two mechanisms. First might be due to the structural property of glycerol having three

Figure 2 Effect of crude glycerol feeding in animals

Figure 3 Metabolism of glycerol (McDonald, 2010)

hydroxyl groups and lack of hydrophobic chain this would make the medium where glycerol is dissolved less suitable for the activity of the proteolytic enzymes. Another possible reason may be due to the presence of alcohol in the CG which will bind with protein and increases the resistance to proteolytic activity of rumen microbes (Patterson & Ricke, 2015).

Crossbred goats fed with CG at 6% of DM significantly reduced dry matter intake (DMI), organic matter, crude protein, ether extract and fiber digestibility; however, no such adverse effect was noticed when fed at 2 and 4% levels (Chanjula et al., 2016b). Similarly, Shin et al. (2012) in lactating cows replacing concentrate with 10% CG also observed 30% reduction in neutral detergent fiber (NDF) digestibility. The negative effect of CG on fiber digestibility may be due to a preferential increase in propionate production at the cost of acetate or due to decreased fibrolytic bacteria *Butyrivibrio fibrisolvens* and *Selenomonas ruminantium* associated with the feed particles prevented adhesion and utilization of fiber (Abo El-Nor et al., 2010; Van et al., 2015).

The recent experimental result with increased CG also showed a significant decrease in fiber utilizing bacteria *R. albus* and *R. flavefaciens* and protozoa *Entodinium* and starch utilizing *Isotricha*, whereas, gram-negative fiber utilizing bacteria *F. succinogenes* showed resistant (San et al., 2016). However, several experimental results are available with no effect on fiber digestibility (Long et al., 2015; Benedetti et al., 2015; Lokesha et al., 2017) to CG addition (Table 2).

Wang et al. (2009) reported the dose-dependent effect of glycerol and suggested 200 g/d/h as an effective in stimulating the rumen fermentation activity and microbial protein production. Long-term feeding of CG at 10% level replaced at isoenergetic levels with barley grains in finishing bull's for 266 days had no adverse effect on blood metabolites, growth and carcass characteristics (Barton et al., 2013). Many short-terms and long-term trails showed effective utilization of CG as an alternative to cereal grains in finishing bulls, goats, sheep and dairy cows without any effect on nutrient intake, digestibility and blood metabolites (Avila-Stagno et al., 2013; Kass et al., 2013; Lage et al., 2014b). The reason for the adverse effect of CG on fiber digestibility is not clear and may depend on the total dietary constituents and CG composition.

5.3 Effect of CG feeding on lactation

Glycerol as an energy supplement to dairy cows during early lactation (DeFraen et al., 2004; Wang et al., 2009; Carvalho et al., 2012a) or mid-lactation (Donkin, 2008) has been demonstrated. Dairy cows fed with food grade glycerol (purity 99.8%) showed positive energy balance by increased blood glucose and decreased blood NEFA, BHBA concentrations. However, the milk yield and its composition was not altered (Wang et al., 2009). In contrary,

tendency of increasing milk yield and milk protein content was reported with high purity glycerol (99.5%) than low purity glycerol (88.1%) (Omazic et al., 2013). Kass et al., (2013) observed that oral drenching of CG (500 ml/d) in early lactating cows increases milk yield and milk lactose content than the control group. Donkin (2008) in dairy cows diet replaced 5, 10 and 15% corn with pure glycerol for 56 days of the experimental period, observed no change in milk yield and composition but a linear increase in blood glucose indicated the positive energy balance. Carvalho et al. (2012a) did not observe any change in milk production and composition in transition dairy cows fed glycerol 28 days before expected date of calving to 56 days postpartum at 11.5% and 10.8% of DM respectively in pre and post-partum animals.

5.4 Effect of CG on Ruminal Methanogenesis

Increased propionate in conjugation with decreased acetate concentration, in turn, decreases the availability of H₂ for methane production (Van et al., 2015; Chanjula et al., 2016b). However, few studies, *in vitro* (Avila et al., 2011) and *in vivo* (Avila-Stagno et al., 2013) revealed an increase in propionate production and did not alter the methane production with CG feeding. In contrary, increased methane production with the linear increase in CG concentration in rumen simulation techniques was reported by Avila-Stagno et al. (2014). The author further predicted two possible explanation for increased methane production as, i. proportionate increase in butyrate resulted in release of reducing equivalents which increased methane production; ii. conversion of glycerol to methane precursors such as formate or ethanol. Patterson & Hespell (1979) reported that methane production may be due to *Methanosarcina*, methanogens which is capable of utilizing the methanol as a source for methane production. However, no experimental results are available to confirm the hypothesis of alteration in the methanogenic *archae* population in the rumen. Paschoaloto et al. (2016) carried out successive *in vitro* studies with different CG levels with varying concentrate: roughage diets. The authors concluded that production of methane does not depend on the CG inclusion level rather it depends on NDF content of the substrate.

5.5 Effect of feeding of CG on Carcass traits

Most of the research studies conducted on broilers (Min et al., 2008), pigs (Hansen et al., 2009), steers (Francozo et al., 2013; Eiras et al., 2014) and goats (Lage et al., 2014b; Chanjula et al., 2014) had no significant impact on the carcass traits. It may be due to the fact that CG is having similar metabolism as that of the replaced cereals. However, few researchers such as Cerrate et al. (2006) reported a significant increase in breast yield in the dressed carcass of broiler at 2.5 and 5% level of CG compared to control group. Steers fed with 2 to 12% of CG (Egea et al., 2014) had

higher meat arachidonic and linolenic acid content. The authors pointed out that decreased bio-hydrogenation in the rumen might be the reason behind it. Decreased fat digestion in the rumen fed with CG (Edwards et al., 2012) expected to decrease the bio-hydrogenation process. Addition of CG at 7.5, 15 and 22.5% in feed lot lambs also had positive impact on odd-chain fatty acids (oleic, palmitoleic), total unsaturated and monounsaturated fatty acids which in turn increased the quality of the meat. Further increase in CG to 30% reduced the carcass weight and yield (Carvalho et al., 2015). In ruminants, digestion of fat involves the lipolysis and bio-hydrogenation in the rumen. Added glycerol interrupting the bio-hydrogenation results in increased concentration of conjugated linolenic acid (CLA) in meat (Lage et al., 2014a; Carvalho et al., 2015). CLA having added beneficial effect in human health helps in protection against cancer, diabetes and cardiovascular diseases (Schmid et al., 2006). Avila-Stagno et al. (2013) fed lambs with increasing CG concentration (7, 14 and 21%) by replacing with barley and observed no effect on total saturated fatty acids or mono unsaturated fatty acids; however, poly unsaturated fatty acids were linearly decreased. Humectant property of glycerol makes it to absorb moisture from the surrounding; because of this property glycerol is more commonly used in food and beverage industry. Similarly, it is expected to increase the water holding capacity of meat which in turn increases the juiciness (Freund et al., 1995). Increase in muscle water content was reported by Parker et al. (2007) in steers, however, no such effect was reported in other studies (Eiras et al., 2014; Chanjula et al., 2014; Carvalho et al., 2015). Based on the several research studies EFSA (2010) has approved the use of CG in animal feeding without any deleterious effect up to 15% in ruminants and 10% in monogastric animals.

Conclusion and future prospective

Increased concern about environmental pollution and call for use of biodiesel is expected to increase the biodiesel production globally. Use of CG as an energy source by replacing grains in animal feeding can be a better alternative than disposal or purification. Nutrient composition of CG depends on the substrate and procedure involved in the biodiesel extraction. Methanol content of CG can be minimized up to the permeable limit by a simple process of evaporation. More than 10% of CG in mash type of diet decreases the feed quality in poultry and pig. Up to 15% or even more than that also showed no negative impact on feed utilization and production in ruminants. Feeding CG did not affect the feed intake and nutrient digestibility except fibre, on which their effect is still uncertain. Carcass dressing percentage, bodyweight gain is not altered by CG feeding. Further studies are needed to assess their effect on meat fatty acid profile, CLA content and methanogenesis in ruminants.

Conflict of interest

Authors declare that there is no conflict of interest.

Acknowledgments

All the authors acknowledge the support from their respective institutions and universities.

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