

Metabolic aspects related to the use of crude glycerin and ractopamine in pig feeds

Beta-adrenergic agonist, biodiesel, enzyme, glycerol and metabolism.

Luciana de Paula Naves^{1*}

Cesar Augusto Pospissil Garbosa²

Vinicius de Souza Cantarelli²

Raimundo Vicente de Sousa¹

¹ Departamento de Medicina Veterinária, Universidade Federal de Lavras, Lavras, MG, Brasil. *E-mail: luciana.naves@hotmail.com.

² Departamento de Zootecnia, Universidade Federal de Lavras, Lavras, MG, Brasil.

ABSTRACT

The use of crude glycerin as an energy source in animal feed has been considered for several years; however, there are few scientific studies about glycerol metabolism in pigs. Similarly, although ractopamine is recognized as an animal metabolism modifier additive, it is still controversial whether its main mechanism of action in pigs is through inhibition of lipogenesis and/or lipolysis stimulation. The understanding of the metabolism contributes for that nutritionists formulate diets more balanced and adequate nutritionally. For any living organism, including pigs, an important parameter that allows us to understand metabolic changes is by evaluation of changes in the expression and activity of key enzymes in certain metabolic pathways. Therefore the objective of this literature review is to discuss about the main metabolic aspects related to the use of crude glycerin and ractopamine in the pigs feeding. From this review it can be seen that both the inclusion of glycerin and the addition of ractopamine in the diet of pigs promote metabolic adjustments by changing the gene expression and activity of enzymes related to the metabolism of carbohydrates, lipids and proteins. However, further research is needed to better understand the integrated metabolism of pigs fed diets containing glycerin and/or ractopamine.

Keywords: beta-adrenergic agonist, biodiesel, enzyme, glycerol and metabolism.

ASPECTOS METABÓLICOS RELACIONADOS AO USO DE GLICERINA BRUTA E RACTOPAMINA EM RAÇÕES DE SUÍNOS

RESUMO

A utilização de glicerina bruta como ingrediente energético na ração animal tem sido considerada há alguns anos, porém, ainda há poucos estudos científicos sobre o metabolismo do glicerol em suínos. De maneira semelhante ainda é controverso se o seu mecanismo da ractopamina em suínos é por meio da inibição da lipogênese e/ou de estímulo da lipólise. Para qualquer organismo vivo, incluindo os suínos, um importante parâmetro que permite compreender mudanças metabólicas é avaliando-se alterações na expressão e atividade de enzimas-chaves de determinadas rotas metabólicas. Portanto, objetiva-se com esta revisão bibliográfica discutir sobre os principais aspectos metabólicos relacionados ao uso de glicerina bruta e ractopamina na alimentação de suínos. A partir da presente revisão verifica-se que tanto a inclusão de glicerina quanto a adição de ractopamina na dieta de suínos promovem adaptações metabólicas alterando a expressão gênica e a atividade de enzimas relacionadas ao metabolismo de carboidratos, lipídeos e proteínas. Todavia, novas pesquisas são necessárias para compreendermos melhor o metabolismo integrado de suínos alimentados com dietas contendo glicerina e/ou ractopamina.

Palavras-chave: agonista beta-adrenérgico, biodiesel, enzima, glicerol e metabolismo.

INTRODUCTION

Crude glycerin is a by-product derived from the production of biodiesel and as the production of this bio-fuel has been growing in recent years, the supply of crude glycerin in the market has also increased (ANP, 2015). Due to high glycerol content in the crude glycerin (typically between 80 to 95%) and the high energetic value of this compound (approximately 4,320 kcal of gross energy/kg), glycerin has been considered as an energetic alternative ingredient for animal nutrition, such as pigs (MOUROT et al., 1994; BERENCHTEIN et al., 2010; GOMIDE et al., 2012; CARVALHO et al., 2013).

Ractopamine has been added to the feed of pigs with the main objective of reducing the fat content in carcass, which makes the pork more competitive in the market because the consumers have prioritized the purchase of healthy foods and with better quality (FERREIRA et al., 2013; ARAÚJO et al., 2014). Moreover, ractopamine can also promote greater protein deposition in the muscle; however it is not fully understood if their predominant effect is the reduction of the rate of protein degradation or is the stimulation of the protein synthesis rate associated with hypertrophy of muscle fibers (DUNSHEA et al., 2005; MOORE et al., 2009).

According to FERREIRA et al. (2013), the understanding of metabolic events resulting from ractopamine supplementation in the diet can contribute to the formulation of diets with better balance of nutrients promoting greater zootechnical results in pigs, and thus, this is an interesting area for further scientific studies. Therefore, considering the potential of the glycerin as an energetic ingredient in feed for pigs and the scientific reports about the improvements from the supplementation of pig diets with ractopamine, the aim of this review is to discuss the main metabolic aspects related to use of these products in the swine feeds.

LITERATURE REVIEW

Biodiesel production and use of glycerin in swine feeds

Biofuels, such as biodiesel, are important alternatives of replacement to the petroleum-based fuels that are expensive fossil fuels derived from non-renewable natural resources. Moreover the biofuels cause less en-

vironmental impact (BOSO et al., 2013). Crude glycerin is a by-product from biodiesel production, obtained from transesterification reactions between fatty acids of a fat source and an alcohol in the presence of a catalyst which is typically a basic substance such as, the sodium hydroxide or potassium (THOMPSON & HE, 2006).

The raw materials for the biodiesel production can be vegetable oils (cotton, peanuts, babassu, canola, palm, sunflower, castor beans, soy, among other), animal fats (beef tallow, fish oils, swine lard) or even residual oil and fat derived from domestic, commercial or industrial processing (ANP, 2015). The type of raw materials and biodiesel production process influence the composition and quality of crude glycerin (HANSEN et al., 2009). Besides that, the crude glycerin can undergo to some degree of processing or purification, which can also affect its final quality and chemical composition, however, the partial or total purification of glycerin is a costly process (MENTEN et al., 2010). Thus, a lot of researchers focused on the use of crude glycerin in animal nutrition, since this co-product is usually cheaper than the purified glycerin.

Around 10% of the total volume of produced biodiesel corresponds to crude glycerin. Just in Brazil, the biodiesel production in 2014 was approximately 3.64 billion of liters, generating 364 million of liters of crude glycerin. The biodiesel production has been growing every year resulting in crude glycerin in a surplus amount to the capacity of utilization by chemical industry (ANP, 2015). Moreover, there is still no established laws to regulate the proper disposal of excess glycerin, which can cause environmental problems if all the glycerin produced is not utilized (MENTEN et al., 2010). Therefore, one of the great challenges of today is the development of technologies that enable new ways to use glycerin, avoiding its disposal in the environment and providing new methods to profit with this co-product.

In pig raising, feed represents about 70% of the production cost, being the energy one of the most expensive components of the formulations (BERTECHINI, 2012), thus justifying the interest of nutritionists in assessing alternative energetic sources that can efficiently replace conventional ingredients without

harming animal performance (GOMIDE et al., 2012). Thus, the glycerin has been studied as a possible energetic ingredient in feed for pigs (MOUROT et al., 1994; BERENCHTEIN et al., 2010; GOMIDE et al., 2012; CARVALHO et al., 2013).

In the USA and Europe, glycerin is utilized by food industry as a food additive and has "GRAS" (Generally Regraded as Safe) status (MENTEN et al., 2010). In Brazil, the glycerin use in food products was permitted by Resolution No. 386 of August 5th, 1999 (LOPES et al., 2012). However, to avoid cases of poisoning in animals and try to standardize the composition of the produced glycerin, the Ministry of Agriculture, Livestock and Supply (MAPA, Brazil) determined that the glycerin added as an ingredient in the animal diet must contain a amount maximum of 150 mg of methanol/kg and 13% of moisture and the other hand, a amount minimum of 80% of glycerol and low concentrations of sodium or other electrolyte (LOPES et al., 2012).

The energy content of the glycerin from the biodiesel is variable, being dependent of their contents of glycerol and fatty acids and also of the species and age of the animals studied, among other factors (CERRATE et al., 2006). However, in general, the glycerin is a good energy source in the feed of pigs in the finishing phase, being related in the literature the values of metabolizable energy (ME) of 3,267 Kcal of ME/kg of crude glycerin derived from rape oil (KOVÁCS et al., 2011) and 3,475 Kcal of ME/kg of crude glycerin derived from soybean oil (MELO, 2012).

The inclusion of up to 9% of glycerin derived from beef tallow in pig diets in growing and finishing phases was considered safe by BERENCHTEIN et al. (2010), based on the parameters of performance (daily feed intake, daily weight gain and feed conversion), carcass traits (hot carcass yield, carcass length, backfat thickness, loin eye area, and relation fat/meat) and pork quality (pH, color, and water loss by dripping of the *Longissimus dorsi* muscle).

Similarly, CARVALHO et al. (2013) did not find loss in performance, backfat thickness and loin depth when pigs in the growing and finishing phases were fed diets containing up to 12% of crude glycerin derived from soybean oil or mixed glycerin derived from ani-

mal fat and soybean oil. In addition, was related that the glycerin use can be economically viable with reduction of the cost with animal feed in approximately 11%.

GOMIDE et al. (2012) also evaluated the effect of partial substitution of corn by crude glycerin in diets for barrows in finishing phase. They concluded that the use of up to 16% glycerin did not affect performance and carcass characteristics (carcass length, weight of warm and chilled carcass, estimated carcass yield, estimated weight loss in cooling, loin eye area, backfat thickness at P2 point, muscle depth, and estimated yield of meat in cold housing), and increase the water holding capacity and tenderness of the meat, which are desirable characteristics by the consumer and by the food industry. The lower loss of water in pork due to supplementation of the feed with glycerol has been reported since the 90's (MOUROT et al., 1994). This improvement may probably be related to the hypothesis that part of ingested glycerol may be stored in muscle tissue and, considering that glycerol can exert high osmotic pressure in the cell, the glycerol may have induced a greater water holding capacity in the pork. However, both MOUROT et al. (1994) as GOMIDE et al. (2012) did not quantified the glycerol content in the muscle tissue of pigs which would prove or disprove the hypothesis established to justify the data observed.

On the other hand, when growing and finishing pigs were fed diets containing 30% of crude glycerin of vegetable origin, the animals showed lower weight gain and poor feed conversion, without any effect on feed intake (KIJORA et al., 1995). MENDOZA et al. (2010) also reported negative effect due to the addition of 30% of purified glycerin in the feed of pigs, because the complete oxidation of the urine of these animals yielded 540% more gross energy than determined in the urine of the pigs fed a diet without glycerin (control diet) representing a large energy loss. Therefore, these results suggest that there is a metabolic limitation when high levels of glycerin are added in the pig diets.

Absorption and metabolism of glycerol

HANSEN et al. (2009) evaluated increasing levels of inclusion of mixed crude glycerin from vegetable oil and tallow in feed for pigs in growing and finishing

phases and verified that plasma concentrations of glucose and free fatty acids were not influenced by the glycerin levels. However, the increase of 0 to 16% of glycerin in the feed resulted in gradual increase of the plasma glycerol. Similar results for plasma glycerol content were observed in two experiments with broilers fed with diets containing of 0 to 7% of glycerin, during periods of 22 to 35 and 33 to 42 days of age (BERNARDINO et al., 2014b). These results confirm the report of that glycerol present in the glycerin can be absorbed by the digestive tract of monogastric animals and is transported by the blood to the liver (TAO et al., 1983) and also indicate that the amount of glycerol absorbed by the animal is dependent of their inclusion level in the diet.

Although it is known that the glycerol present in the glycerin can be absorbed by the pigs, it is important that the inclusion level of crude glycerin in the feed is not high enough to saturate the metabolic capacity of the organism. Because if it happens, the glycerol excess absorbed is excreted via urine resulting in lower energetic use of the glycerin, which may even harm the animal performance (BARTLET & SCHNEIDER, 2002).

In this regard, MELO (2012) evaluated the effect of increasing levels of crude glycerin (0, 5, 10, 15, and 20%) on performance, carcass and pork quality, and economic viability of the use of this co-product for barrows in the finishing phase. The evaluated parameters were harmed when the feed contained 15% or 20% of glycerin, probably because there was the saturation of the activity of enzymes that participate in the glycerol metabolism, being that a great amount of this compound was not metabolized and was excreted in the urine.

The enzyme glycerol kinase (GK, EC 2.7.1.30; ATP: glycerol 3-phosphotransferase) converts glycerol to glycerol-3-phosphate and this is a key enzyme in the metabolism of glycerin. In the absence of phosphorylation, the glycerol is excreted by the animal resulting in double nutritional loss, because besides the lower generation of energy that could be produced, the animal must expend energy to promote the glycerol excretion (CHAMPE et al., 2009). The expression of GK enzyme gene was determined by RT-PCR in the liver

of male piglets (Large White x Pietrain) weaned and fed during the period of 30 to 72 days of age with diets not containing or containing glycerin at inclusion level of 7.5 or 15% (PAPADOMICHELAKIS et al., 2012). The amount of mRNA on the GK increased linearly with increasing inclusion of glycerin in the feed. This signifies that there was a positive regulation at the transcriptional level of the GK gene indicating that the piglets increased their ability to metabolize glycerol to glycerol 3-phosphate. However, although this result of gene expression represents an adaptation metabolic, an important parameter to measure the efficiency of an enzyme is determining its catalytic activity.

Until now, was not found any scientific study reporting the effect of dietary crude glycerin on the activity of hepatic GK in finishing barrows. However, BERNARDINO et al. (2014b) conducted two experiments with broilers fed diets containing from 0 to 7% of glycerin, during the rearing periods of 22-35 and 33-42 days of age. For both experiments they found that the increase of the dietary glycerin concentration increased the GK activity showing a self-regulation of the body to allow a better use of the dietetic glycerol.

In the sequence of the metabolic pathway, glycerol-3-phosphate can be converted to dihydroxyacetone phosphate by enzyme glycerol-3-phosphate dehydrogenase (EC 1.1.1.8 and EC 1.1.5.3 for the fractions cytosolic and mitochondrial). Then, the dihydroxyacetone phosphate can be converted to glyceraldehyde 3-phosphate by triosephosphate isomerase and depending on the energy status of the animal, this intermediate can be used for glucose synthesis (gluconeogenesis), lipids synthesis (lipogenesis) or to be completely oxidized for the energy production via glycolysis and Krebs cycle (CHAMPE et al., 2009).

Therefore, the glycerol metabolism is of great interest, since this compound participates of the intermediate energetic metabolism. HAGOPIAN et al. (2008) reported that the activity of liver enzymes GK and glycerol-3-phosphate dehydrogenase increased when the diet of rats was formulated with caloric restriction, demonstrating that there was a metabolic adaptation for that the glycerol derived from the breakdown of triglycerides could be used in a greater ratio as an energy source.

There is still the hypothesis that besides serving as energy source, the glycerol can also have positive effect over the retaining of amino acids or nitrogen. The explanation for this hypothesis is based in the possibility of that glycerol could inhibit the activity of enzymes, such as phosphoenolpyruvate carboxykinase and glutamate dehydrogenase, resulting in economy of gluconeogenic amino acids favoring, consequently, the body protein deposition (STEELE et al., 1971; CERRATE et al., 2006). The glutamate dehydrogenase (EC 1.4.1.2) converts of reversible manner the glutamate to α -ketoglutarate, representing an important link between metabolism of carbon and nitrogen (NELSON & COX, 2011). Recently, BERNARDINO et al. (2014a) reported that the increase of mixed glycerin inclusion in the broiler diet from 1.75 to 7% inhibited by 47% the activity of the gluconeogenic enzyme glutamate dehydrogenase and increased in 13% the amount of crude protein in the broiler breast. However, the real effect of dietary glycerin on the protein metabolism in pigs remains controversial of manner that more researches in this area are needed.

Ractopamine as animal metabolism modifier

Ractopamine is a synthetic beta-adrenergic agonist with an analogous structure to the catecholamines which are hormones amino-derivate from the catechol. The ractopamine is a additive considered an animal metabolism modifier because it can change the protein and lipid metabolism (SCHINCKEL et al., 2003). According to FERREIRA et al. (2013), the ractopamine dose commonly used in pig diets ranges between 0 and 20 mg/kg of feed.

The sarcoplasmic proteome comprises soluble proteins and enzymes, constitutes approximately one-third of the total proteins in skeletal muscles, and governs the biochemical processes influencing muscle metabolism. In a recent study, COSTA-LIMA et al. (2015) evaluated the sarcoplasmic proteome profile of the muscle *Longissimus thoracis* of pigs fed diets with or without ractopamine and they observed evidence of change of the muscle energy metabolism, because in the pork of pigs fed diet containing ractopamine there was greater relative abundance of L-lactate dehydrogenase and fructose-bisphosphate aldolase, that are enzymes involved in glycolytic metabolism. In addition, there are reports that ractopamine can stimulate

the increase in diameter of muscle fibers, improving performance and pig carcass characteristics (AGOSTINI et al., 2011; LI et al., 2015).

Several experiments confirmed that the ractopamine supplemented in the swine diet can reduce the fat content of the carcass (CARR et al., 2009; FERREIRA et al., 2011; ANDRETTA et al., 2012), which is desirable due to the greater acceptance of this product by the consumer. In general, the results indicate that the action of ractopamine on reducing the deposition of lipids in the carcass of pigs is mainly explained by lipogenesis inhibition and no by lipolysis stimulation (FERREIRA et al., 2013). The lipogenesis is regulated by a variety of factors including dietary nutrients, hormones, nuclear transcription factors, and lipogenic enzymes. For example, the catalytic activity of the malic enzyme (EC 1.1.1.40) generates NADPH which is as agent reducing in the fatty acid biosynthesis in hepatocytes and adipocytes (NELSON & COX, 2011). MILLS et al. (1990) reported lower activity of the malic enzyme in pigs fed diet containing 20 mg of ractopamine/kg, when compared with the enzyme activity determined in pigs fed diet without inclusion of ractopamine.

The fatty acids biosynthesis occurs in the cell cytoplasm and is initiated by the transfer of acetyl-CoA from the mitochondria to the cytosol, a process which occurs when there is high supply of ATP in the mitochondria, with consequent increase of the concentration of acetyl-CoA in the interior this organelle. Subsequently, there is the formation of malonyl-CoA by carboxylation of the acetyl-CoA catalyzed by acetyl-CoA-carboxylase (EC 6.4.1.2), which is a limiting step of the lipogenic pathway. Malonyl-CoA units are then used for the fatty acids synthesis through a sequence of reactions that are repeated several times, catalyzed by an enzyme system called the fatty acid synthase (EC 2.3.1.85). After the action of fatty acid synthase occurs the formation of palmitic fatty acid [$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$], which can be further lengthened and/or added of unsaturation according with the requirements and enzymatic capacity of the organism (CHAMPE et al., 2009; NELSON & COX, 2011).

The determination of the acetyl-CoA carboxylase activity in hepatocytes and adipocytes can probably con-

tribute to a more detailed understanding of the lipid metabolism of pigs fed diets containing ractopamine. However, to this moment, these information were not find in the literature probably because the most of the specific kinetic assays known uses radioactive substrates (KROEGER et al., 2011), which are expensive and require specialized laboratories.

Finally, studies on gene expression are also important tools for the understanding of energy metabolism. In this regard, REITER et al. (2007) reported reduction in the mRNA concentration of the fatty acid synthase and GLUT-4 transporter in the adipocytes of pigs fed diet containing 20 mg of ractopamine/kg, when compared to that determined in pigs fed diet without ractopamine. As the GLUT-4 is responsible for glucose transport into adipocytes, the reducing of the gene expression of this transporter means that there was lower uptake of glucose by the cell contributing to reduce fat deposition in the adipose tissue.

CONCLUSION

The glycerol contained in the crude glycerin added in monogastric diets can be absorbed by the digestive tract, however, there is a metabolic limitation in their use (especially at the level of enzyme saturation) when the swine diets contains high inclusion levels of glycerin (usually above 15% for barrows in finishing phase). Lipogenesis inhibition (and not the lipolysis stimulation) seems to be the major metabolic effect to justify the lower fat content in the carcass of swine fed diets supplemented with ractopamine. However, new studies evaluating the activity of lipogenic enzymes need to be conducted for a better understanding of energy metabolism of pigs fed diets containing glycerin and/or ractopamine.

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