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# Student Report No. 27

## The use of glycerol in diets for broilers


by

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## Abbreviations

| | | | |
|---|---|---|---|
| AME | Apparent metabolisable energy | LWG | Live weight gain |
| FCR | Feed conversion ratio | GC-FID | Gas chromatography-flame ionizing detector |
| DM | Dry matter | NIRS | Near infra-red spectroscopy |
| DMI | Dry matter intake | ATP | Adenosine triphosphate |

# 1. ABSTRACT

For every litre of biodiesel produced, thirty percent of crude glycerol is also produced as a by-product. Thompson and He (2006) analysed the nutrient content of crude glycerol and reported that the main component was carbohydrate. Previous research is lacking into the optimum inclusion level of glycerol in broiler diets, the energy potential of glycerol, nutrient digestibility within broilers and the subsequent effect on broiler meat quality. As a result, this project aimed to investigate these parameters.

During this study it was reported that glycerol inclusion had positive effects on feed efficiency. The AME content of glycerol was determined as 16.8MJ/kg and the optimum inclusion level of glycerol was 6.7% when incorporated into broiler diets. Glycerol had significant positive effects on total starch and amino acid digestibility, specifically threonine digestibility, which was significantly increased with glycerol inclusion. In regards to meat quality, glycerol inclusion had no effect of the moisture content and tenderness of broiler breast meat.

Glycerol was also integrated into broiler diets as a partial/complete replacement for the soya oil content. It was reported that birds offered the diets containing a partial replacement of soya oil with glycerol showed increased feed efficiency compared to birds offered only glycerol. It was also observed that glycerol may provide some of the same beneficial attributes of soya oil.

Glycerol contains methanol, which is toxic and so it is important to find a rapid means to measure the methanol. Therefore, crude glycerol samples containing known quantities of methanol were scanned using NIR (near infra-red) and Raman spectroscopy. It was found that both methods have the potential to measure the methanol content in crude glycerol, and Raman spectroscopy gave rise to more accurate predictive findings than NIRS.

In conclusion, glycerol had positive effects on performance, nutrient digestibility and the AME (apparent metabolisable energy) content of diets, with no subsequent decline in meat quality. Depending on the market price of wheat and glycerol, glycerol could be successfully incorporated into broiler diets as a partial replacement of wheat.

## 2. INTRODUCTION

In November 2005, the United Kingdom announced the Renewable Transport Fuels Obligation (RTFO). It requires that 5% of the volume of all fuel sold on UK forecourts should originate from a renewable source by 2010. The European Union Directive (2009/28/EC) on the promotion of the use of energy from renewable sources also states targets of 5.75% market share of biofuels by 2010 and 10% by 2020. To achieve this amount of biodiesel, around 3Mt of oilseed rape would be required, resulting in 1.4Mt of the by-product glycerol. The glycerol liberated during transesterification has substantial commercial value if refined to yield various commercial grades, such as United States Pharmacopeia (USP) grade glycerol. This is of high quality and economic value, but the refining process is expensive and not well suited to small-scale biodiesel plants. As a result of this, glycerol is commonly only partially purified and of low economic value (Haas *et al.,* 2005). If a means can be found to increase the market value of crude glycerol, this in turn would obviously help offset some of the production cost of the biodiesel and, thus, biodiesel production could become economically favourable, with benefit to all (Haas *et al*., 2005).

Thompson and He (2006) analysed the nutrient content of glycerol produced from various oil sources and reported that the main component was carbohydrate (Table 1). Therefore, glycerol could be used as an energy source in animal diets.

**Table 1.** Nutrient analysis of crude glycerol from different sources (Thompson and He, 2006).

| Feed stock | Ida gold mustard | Pacific gold mustard | Rapeseed Canola | | Soybean | Crambe | Waste vegetable oil |
|---|---|---|---|---|---|---|---|
| *Crude Glycerol* | | | | | | | |
| Fat (%) | 2 | 1 | 9 | 13 | 8 | 1 | 6 |
| Carbohydrate (%) | 83 | 84 | 76 | 75 | 76 | 79 | 27 |
| Protein (%) | 0.10 | 0.20 | 0.07 | 0.06 | 0.05 | 0.40 | 0.20 |
| Calories (MJ/kg) | 14.60 | 14.50 | 16.30 | 17.50 | 15.80 | 16.30 | 27.20 |
| Ash (%) | 2.80 | 1.90 | 0.70 | 0.60 | 2.70 | 0.20 | 5.50 |

Previous work has demonstrated that intestinal absorption of glycerol can range from 70–90% in rats (Lin, 1977) and to more than 97% in pigs and laying hens (Bartelt and Schneider, 2002). Due to the small molecular weight of glycerol, it can be absorbed passively, and at a rapid rate by diffusion across the stomach wall. Once glycerol enters the liver, it can be converted to glucose (Emmanuel *et al.,* 1983) via gluconeogenesis or oxidized for energy production via the glycolytic and Krebs cycles (Rosebrough *et al.,* 1998). As an energy source, one mole of glycerol can be oxidized, yielding twenty-two moles of ATP (Min *et al*., 2010). Therefore, glycerol has the potential to be incorporated into animal diets as an energy source.

However, there is a lack of information in the literature regarding the integration of glycerol into poultry diets. There is no information on the optimum inclusion level of glycerol within a wheat-based broiler diet in terms of broiler performance. There is also a lack of information regarding the effect of glycerol inclusion on total starch, dry matter (DM), glycerol and amino acid digestibility. The actual energy potential of glycerol is also unknown. Therefore, research needs to be carried out to determine the actual energy available to birds when incorporated into diets. From this information, feed producers and feed formulators would be able to place a monetary value upon glycerol when formulating poultry diets, thus facilitating accurate, least-cost formulations. If glycerol can be of benefit in broiler production, there is also a need to investigate the effect on the quality of meat produced.

Crude glycerol may also be contaminated with small traces of methanol when it leaves the biodiesel plant. Methanol is toxic and the only accurate method to measure it in glycerol is using the gas chromatography-flame ionizing detector (GC-FID) technique, which is expensive and time-consuming. Therefore, if glycerol has the potential to be used in poultry diets with no compromise in the quality of the meat produced, a rapid method to measure the methanol content would be of benefit to feed manufacturers.

## 2.1. Aims

Investigations were carried out to (1) determine the optimum inclusion level of glycerol as a partial replacement for the wheat content in broiler diets through measuring growth performance and nutrient digestibility; (2) to measure the energy potential of glycerol; (3) to investigate the effect of glycerol on broiler breast meat quality; (4) to determine if glycerol had the potential to be successfully integrated into a broiler diets as a partial or complete replacement for the soya oil content and finally, (5) to investigate a more rapid and cheaper solution to measure the methanol in glycerol.

# 3. MATERIALS AND METHODS

**Five trials were carried out:**

**Trial 1**: Diets were formulated to contain 0, 3.3, 6.7 and 10% glycerol from two sources (Table 2) as a partial replacement for the wheat content resulting in seven diets in total. The sample size was 8 birds per experimental diet resulting in 63 birds in total. This was to find the optimum inclusion level of glycerol through examining the effect on broiler performance and nutrient digestibility.

**Table 2.** Characterisation of the combined glycerol sources (%) used in trial 1.

| Glycerol | Source A | Source B | Method |
|---|---|---|---|
| | 52 | 81 | BS 5711-3 |
| Glycerol | 49 | 85 | Megazyme assay kit (Weiland, 1988) |
| Methanol | 1.6 | 0.3 | (Gas Chromatography Flame Ionization detector |
| Water | 5.2 | 12.8 | BS 5711-8 |
| Ash | 3.7 | 5.8 | BS 5711-6 |
| MONG* | 38.8 | 4.0 | BS 5711-9 |
| Free fatty acids | 0.74 | 0.62 | BS EN ISO 660 |
| Gross energy (MJ/kg) | 20.70 | 14.41 | 1271 Isoperibol bomb colorimeter |

*Matter Organic Non Glycerol: Poly-glycerol, sugar components, free fatty acids and partial glycerides in the crude glycerol, (Yong, 2001)

**Trial 2**: Diets were formulated to contain 0, 7, 8, 9, and 10% glycerol as a partial replacement for the wheat content. The sample size was 10 birds per experimental diet resulting in 50 birds in total. This was to define further the optimum inclusion level of glycerol and to investigate amino acid digestibility.

**Trial 3**: Diets were formulated to contain 0 and 7.5% wheat pollard (fine bran) and 0, 0, 6, 0 and 8% glycerol as a partial replacement for the wheat content. The sample size was 10 birds per experimental diet resulting in 50 birds in total. This was to determine if glycerol and wheat by-products could be possible partial alternatives to wheat-based broiler diets. This trial also examined the quality of the meat produced.

**Trial 4**: Diets were formulated to contain glycerol as a complete and partial replacement for the soya oil content in the diet to observe the effects on broiler performance (Table 3). The sample size was 8 birds per experimental diet resulting in 64 birds in total.

**Table 3.** Trial 4 diet formulations containing glycerol and soya oil.

| Diet | Glycerol (%) | Soya oil (%) |
|---|---|---|
| 1 | 0 | 6 |
| 2 | 6 | 0 |
| 3 | 0 | 8 |
| 4 | 8 | 0 |
| 5 | 3 | 3 |
| 6 | 4 | 4 |
| 7 | 4 | 2 |
| 8 | 5 | 3 |

**Trials 1–4**: At 7d, birds were housed in individual cages and offered experimental diet and water *ad libitum* up to 28d of age. At 14, 21 and 28d the birds were weighed to determine growth performance and the feed that remained at the end of each week was weighed to determine dry matter intake and feed efficiency. At 14–21d a complete excreta collection was made from under each cage in order to measure AME of the diets.

At 28d of age, a kill order was formulated and each bird was humanely killed by dislocation of the spinal cord. Various samples were collected from the digestive tract of the birds and analysed in the laboratory in order to measure total starch, DM and glycerol digestibility.

Additional analysis was also carried out during **Trial 2**, in which samples of the diet and excreta were measured for their amino acid content in the laboratory in order to determine amino acid digestibility.

Additional analysis was also carried out at the end of **Trial 3**, in which samples of the breast tissue were removed. This was to determine various meat quality attributes.

**Experiment 5**: Nine crude glycerol samples from different batches were obtained containing residual methanol were obtained from four biodiesel plants. Concurrently, laboratory grade glycerol samples were placed into individual containers and methanol was added to them in various quantities. All of the samples were then measured for their methanol content using gas chromatography. The same samples were then scanned and correlated with their methanol content using NIR and Raman spectroscopy to make a comparison with the results observed from the gas chromatography.

# 4. RESULTS

## 4.1. Trial 1

Feed conversion efficiency (FCE), AME and live weight gain (LWG) were found to be positively affected by the addition of glycerol (Table 3). Birds at 7–14d were most efficient at converting their feed intake into increased weight gain when offered the diet containing 10% glycerol inclusion (Figure 1). However, as the birds grew older, feed efficiency declined and the greatest feed utilization was with birds offered the diet containing 6.7% (Figure 2). These birds were significantly heavier than birds offered the diet containing no glycerol. This was also in parallel with an increase in energy available to the birds, in contrast to birds offered the diet containing no glycerol. The energy available to the birds from the diets containing glycerol increased linearly with increasing glycerol (Figure 3).

Glycerol inclusion had no negative effects on dry matter and total starch digestibility. However, glycerol digestibility was observed to increase with increasing glycerol (Table 3).

**Table 4.** The combined effect of both sources of glycerol inclusions on growth performance and nutrient digestibility

| | Glycerol inclusion (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0.0 | 3.3 | 6.7 | 10.0 | S.E.M. | P= Value |
| 7-28d Feed conversion Efficiency | 1.30[c] | 1.20[b] | 1.16[a] | 1.20[b] | 0.01 | <0.01 |
| Apparent metabolisable energy (MJ/kg) | 14.2[a] | 14.6[b] | 15.0[c] | 15.1[c] | 0.17 | <0.001 |
| 7–28d Live weight gain (g) | 1332 | 1395 | 1423 | 1390 | 31.88 | <0.05 |
| 7–28d dry matter intake (g) | 1723 | 1668 | 1642 | 1658 | 38.81 | NS |
| Dry matter digestibility (%) | 72 | 71 | 67 | 68 | 0.02 | NS |
| Total starch digestibility (%) | 95 | 96 | 94 | 95 | 0.01 | NS |
| Glycerol digestibility (%) | 17[a] | 69[b] | 88[c] | 87[c] | 0.03 | <0.001 |

[abc] Superscript indicates significant difference (*P<0.05*)

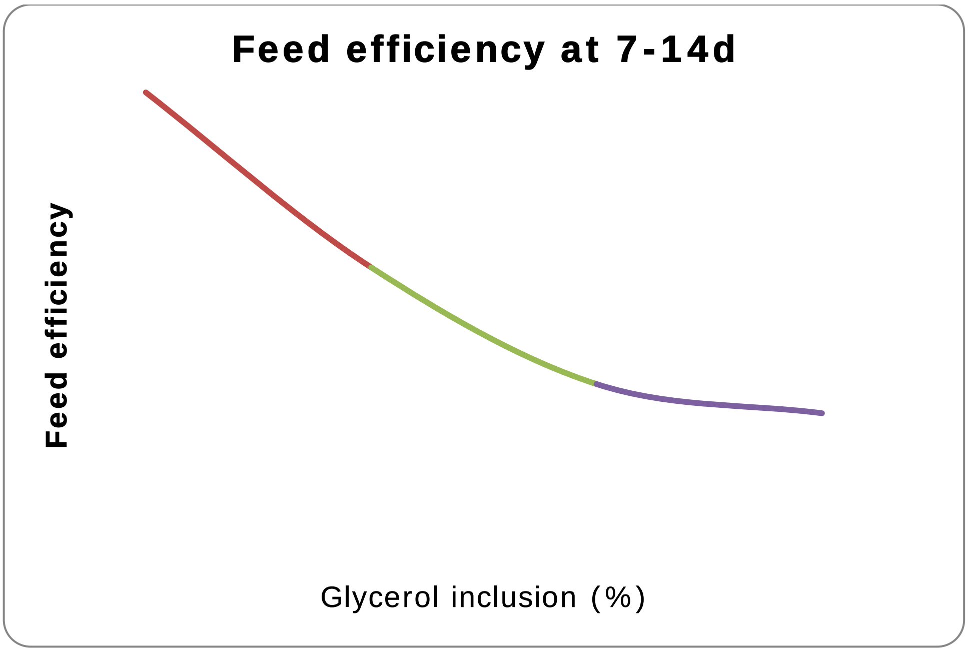


**Figure 1.** The effect of glycerol inclusion on 7–14d feed efficiency.

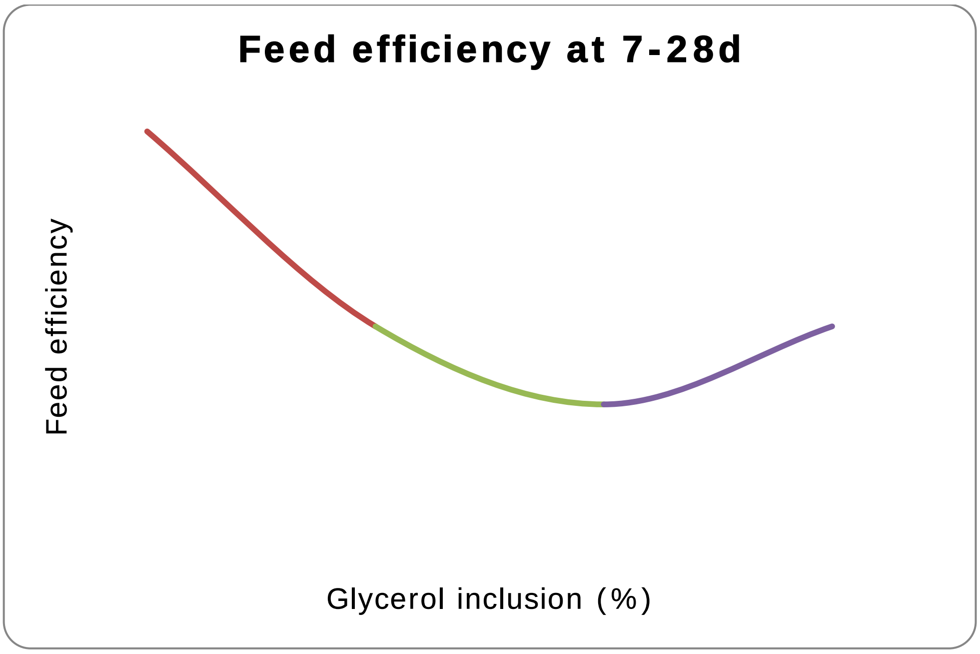


**Figure 2.** The effect of glycerol inclusion on feed conversion efficiency at 7–28d.

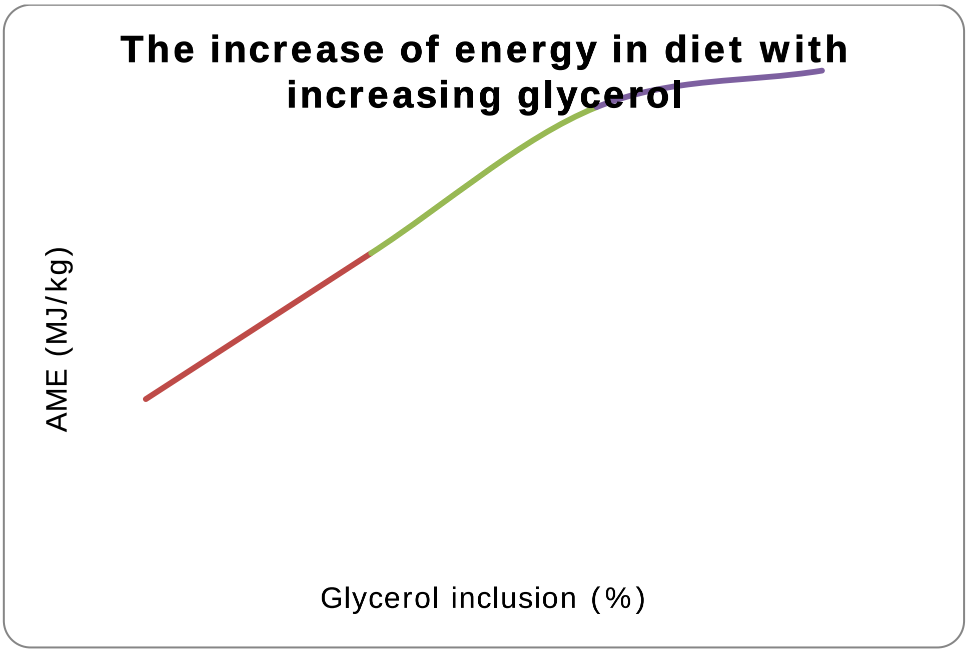


**Figure 3.** The effect of glycerol inclusion on the AME content of the diets.

## 4.2. Trial 2

It was observed that birds offered the diets containing glycerol at any level were more efficient at feed per unit gain and heavier than birds offered the diet containing no glycerol over the total 7-28d experimental period**.** However, this was not reflected in an increase in AME (Table 4), in contrast to Trial 2 findings.

Birds offered the diet containing no glycerol were least efficient at digesting the total starch content of the diet, and total starch digestibility was significantly greatest in birds offered the diet containing

9% glycerol inclusion. However, above this point, digestibility declined, although even at the 10% glycerol inclusion level the birds were still able to digest the total starch content of the diet better than the birds offered the diet containing no glycerol (Table 4).

Birds offered the diets containing glycerol between 7–10% inclusions were able to digest the glycerol most efficiently, in contrast to the birds offered the diet containing no glycerol. This is likely a result of no free glycerol being present (Table 4).

**Table 5.** The effect of glycerol inclusion on growth performance and nutrient digestibility.

| | Glycerol inclusion (%) | | | | |
|---|---|---|---|---|---|
| | 0 | 7 | 8 | 9 | 10 |
| **Parameters** | | | | | |
| Total LWG (g) | 1212 | 1341 | 1397 | 1317 | 1349 |
| Total DM intake (g) | 1625 | 1632 | 1700 | 1585 | 1652 |
| Total FCR | 1.35 | 1.24 | 1.24 | 1.24 | 1.27 |
| AME (MJ/kg) | 14.89 | 14.77 | 14.54 | 15.17 | 14.72 |
| DM digestibility (%) | 0.73 | 0.73 | 0.77 | 0.73 | 0.75 |
| Starch digestibility (%) | 91 | 94 | 97 | 98 | 95 |
| Glycerol digestibility (%) | 6 | 96 | 97 | 96 | 97 |

Amino acid digestibility was also investigated and it was found that the birds offered the diet containing 8% glycerol had significantly greater digestibility for aspartic acid, threonine, valine and histidine in contrast to birds offered the diet containing no glycerol (Table 5 and Figure 4).

**Table 6.** The effect of glycerol inclusion on amino acid digestibility

| Glycerol inclusion level (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 7 8 | | | 9 10 | | s.e.m. | P value | P Linear |
| Aspartic acid | 0.90 | 0.91 | 0.92 | 0.91 | 0.91 | 0.006 | | |
| Threonine | $0.89^{a}$ | $0.92^{b}$ | $0.94^{c}$ | $0.93^{b,c}$ | $0.92^{b}$ | 0.005 | <0.001 | <0.001 |
| Serine | 0.91 | 0.92 | 0.93 | 0.91 | 0.92 | 0.006 | NS | NS |
| Glutamic acid | 0.93 | 0.93 | 0.94 | 0.93 | 0.92 | 0.007 | NS | NS |
| Proline | 0.92 | 0.93 | 0.94 | 0.92 | 0.92 | 0.007 | NS | NS |
| Glycine | 0.89 | 0.90 | 0.91 | 0.90 | 0.89 | 0.007 | NS | NS |
| Alanine | 0.87 | 0.91 | 0.92 | 0.91 | 0.90 | 0.007 | NS | NS |
| Valine | 0.86 | 0.88 | 0.90 | 0.89 | 0.87 | 0.009 | NS | <0.05 |
| Isoleucine | 0.91 | 0.90 | 0.92 | 0.91 | 0.91 | 0.018 | NS | NS |
| Leucine | 0.91 | 0.92 | 0.93 | 0.91 | 0.92 | 0.008 | NS | NS |
| Tryosine | 0.93 | 0.94 | 0.94 | 0.93 | 0.93 | 0.005 | NS | NS |
| Phenlalanine | 0.92 | 0.92 | 0.93 | 0.92 | 0.92 | 0.009 | NS | NS |
| Lysine | 0.94 | 0.94 | 0.94 | 0.93 | 0.93 | 0.009 | NS | NS |
| Histidine | $0.89^{a}$ | $0.92^{a,b}$ | $0.94^{b}$ | $0.92^{a,b}$ | $0.91^{a}$ | 0.011 | <0.05 | <0.05 |
| Arginine | 0.92 | 0.94 | 0.94 | 0.93 | 0.93 | 0.006 | NS | NS |

[abc] Superscript denotes significant difference (*P<0.05*)

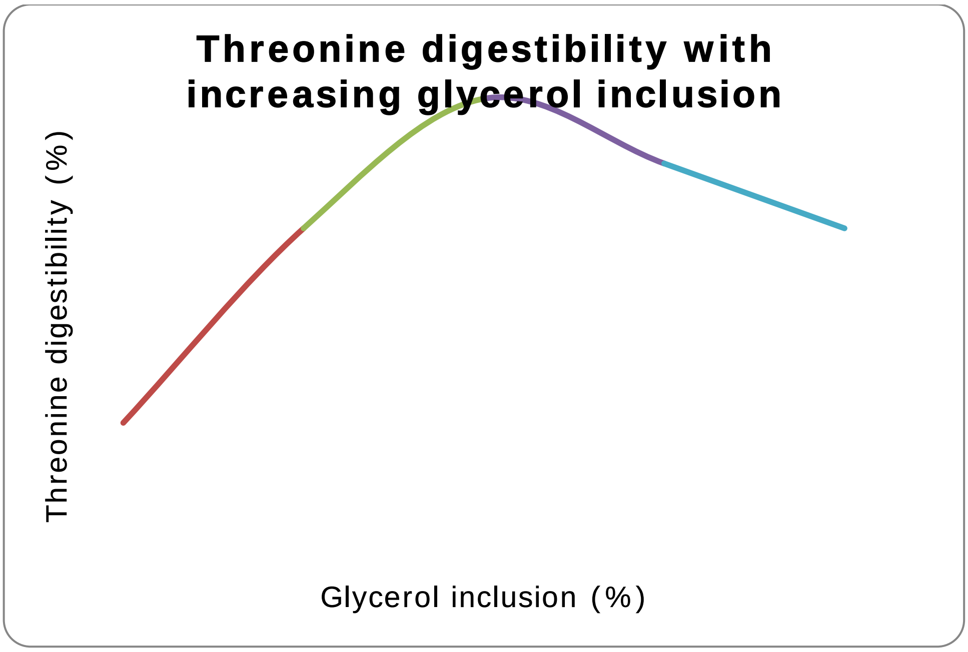


**Figure 4.** Threonine digestibility with glycerol inclusion.

## 4.3. Trial 3

During this trial, the AME of glycerol was investigated, as was the effect of glycerol inclusion on the moisture content of broiler breast meat and its subsequent tenderness. Diets were formulated to contain varying AME contents based on an assumed AME content for glycerol and it was found that glycerol had a much higher AME value than that assigned to it. Based upon calculations of diets 3 and 5, containing 6 and 8% glycerol, respectively, the AME content of glycerol was 16.8 MJ/kg; much higher than was anticipated.

The moisture loss of broiler breast meat was measured pre- and post-cooking and it was found that glycerol had no negative effects on moisture loss from the meat. It was also observed that, broiler breast meat was just as tender as meat containing no glycerol (Table 7).

**Table 7.** The effect of glycerol on AME available from the diets and broiler breast meat.

| Diet | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Glycerol (%) | 0 | 0 | 6 | 0 | 8 |
| Wheat pollard (%) | 0.0 | 7.5 | 7.5 | 7.5 | 7.5 |
| ***Parameters*** | | | | | |
| Drip loss (%) | 1.48 | 1.41 | 1.33 | 1.46 | 1.43 |
| Cooking loss (%) | 23 | 22 | 21 | 22 | 20 |
| Tenderness | 1.56 | 1.50 | 1.62 | 1.54 | 1.43 |

## 4.4. Trial 4

When comparing performance parameters for birds offered the diets containing only soya oil at 6 and 8% soya oil vs. 6 and 8% glycerol, there was a reduction in LWG and feed efficiency observed for birds offered 6 and 8% glycerol.

However, it was observed that glycerol did increase DM digestibility when birds were offered 6% glycerol in contrast to the birds offered 6% soya oil. Although, there was no significant difference in DM digestibility for diets containing 8% soya oil or 8% glycerol.

Observations were also made on the performance of birds offered the diets containing 3: 3, 4: 4, 4: 2 and 5: 3% glycerol: soya oil, with the birds offered the diets containing only soya oil at 6 and 8% inclusion level, and no significant differences were found in the growth performance of the birds offered each diet.

The diets containing partial replacement of soya oil with glycerol were then compared with the two diets containing complete replacement of soya oil with glycerol at 6 and 8% inclusion level. There was a tendency for DMI to decline with birds offered the diets containing the high inclusion levels of either glycerol or the glycerol: soya oil ratio. Birds offered the diets containing a partial replacement of soya oil with glycerol were more efficient at converting feed to gain in contrast to the birds offered the diets containing the complete replacement of soya oil with glycerol.

Although significant differences were found for DMI, LWG and FCR of birds offered diets containing complete or partial replacement of soya oil with glycerol, this did not affect the birds' ability to digest the DM or the total starch content in the diets (Table 8).

**Table 8.** The effect of glycerol as a complete and partial replacement for the soya oil content of the diet.

| Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | S.E.M | P-Value |
|---|---|---|---|---|---|---|---|---|---|---|
| Glycerol inclusion (%) | 0 | 6 | 0 | 8 | 3 | 4 | 4 | 5 | | |
| Soya oil inclusion (%) | 6 | 0 | 8 | 0 | 3 | 4 | 2 | 3 | | |
| ***Parameters*** | | | | | | | | | | |
| 7–28d DMI (g) | 1520 | 1408 | 1418 | 1240 | 1472 | 1286 | 1509 | 1320 | 52.8 | <0.01 |
| 7–28d LWG (g) | 1197 | 1011 | 1072 | 858 | 1163 | 989 | 1168 | 1064 | 39.0 | <0.001 |
| 7–28d FCR | 1.27 | 1.40 | 1.32 | 1.46 | 1.27 | 1.30 | 1.29 | 1.24 | 0.03 | <0.001 |
| DM Digestibility (%) | 67 | 74 | 71 | 73 | 70 | 73 | 72 | 70 | 0.01 | <0.05 |
| Total starch digestibility (%) | 93 | 95 | 90 | 94 | 93 | 94 | 93 | 90 | 0.02 | NS |

## 4.5. Experiment 5

***NIR spectroscopy***

A similar trend between the actual and NIRS predicted values was obtained from the crude glycerol samples and the laboratory prepared glycerol samples containing methanol. This was reflected by a high $R^2$ value of 0.871, suggesting that the predicted content of methanol was very close to that obtained from the more accurate method using gas chromatography. Robust cross validation statistics were also achieved for the predicted methanol content in the crude and laboratory prepared samples with $R^2_{cv}$ 0.75. Ideally, the percentage error should be ≤ 2% for laboratory analysis; however, during this investigation the value was very high at 63.53%.

***Raman spectroscopy***

Similar to the samples scanned using NIRS, the methanol in the crude and laboratory prepared glycerol samples were well predicted with a high $R^2$ value of 0.86. The abundance of methanol found in the samples using the gas chromatography method and Raman spectroscopy were then plotted to show the accuracy of the Raman spectroscopy results (Figure 5). Robust cross validation statistics were also reported with $R^2_{cv}$ 0.87. Despite this, the percentage error was again high at 50.85%.

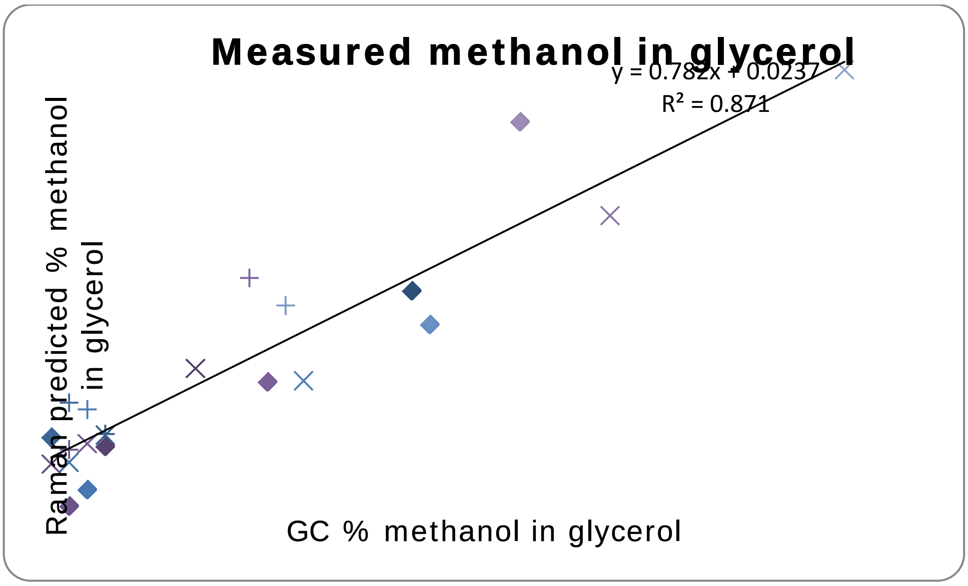


**Figure 5.** GC actual and Raman predicted quantity of methanol in glycerol.

## 5. GENERAL DISCUSSION

The poultry meat industry has been the most successful of any of the animal industries (Leeson, 2008). By 2015, it is predicted that world consumption of poultry meat will be around 64 million tonnes, equating to a yearly production of 40 billion birds and requiring approximately 16 million tonnes of feed. According to Elson *et al.* (2010), feed constitutes about 70% of the cost of broiler and egg production with the cost of energy being a major consideration, given that birds eat to their energy requirements (NRC, 1994; Huges, 2003). To produce poultry meat, growing birds must eat enough feed to provide additional energy over maintenance requirements for the synthesis of body tissue (Latshaw and Moritz, 2009). After energy, amino acids are the most critical dietary factors influencing feed costs and performance in the broiler industry (Wijlyein *et al*., 2004). The protein: energy ratio within the diet must also be correct in order to prevent or minimise energy wastage through fat deposition, and to drive lean growth. Excessive fat deposition is of great concern to producers, the processing industries and consumers alike (Buyse *et al.,* 1998).

The UK chicken meat industry is highly dependent on the supply of energy and protein from wheat and soya, for which current (2014) market prices are: wheat £166.50 per tonne, soya bean meal £310.99 per tonne, full fat soyabean meal £403.00 per tonne and soya oil £528.23 per tonne. Wheat is the main ingredient of UK broiler diets and can contribute up to 70% of the ration. However, wheat varies in energy and, thus, quality content (Owens *et al*., 2007). This variability, when combined with the fluctuating cost, makes other ingredients, which may be added as a partial substitute for wheat, attractive to feed formulators and broiler producers.

Crude glycerol is the primary by-product from biodiesel production and has the potential to be used as an energy source (Thompson and He, 2006). While the price of glycerol is dictated by the production of biodiesel, crude glycerol could be an economical partial substitute for wheat in broiler diets.

The current project explored the effects of glycerol inclusion in broiler diets. The primary aim was to determine the optimum inclusion level and energy potential of glycerol. Additional aims where to investigate the subsequent effect on nutrient digestibility and on meat quality characteristics, and to identify a means to rapidly determine the methanol content of crude glycerol.

## 5.1. The optimum inclusion rate of glycerol

Previous studies on the use of glycerol in broiler diets have given somewhat conflicting results/findings. Cerrate *et al.* (2006) carried out a broiler feeding trial to investigate the effect of glycerol inclusion level (0–10%) as a partial replacement for maize in the diets. They reported that the performance of birds offered the diet containing 5% glycerol inclusion was similar to that of birds offered the diet containing no glycerol, with no negative effects. However, birds offered diets containing 10% glycerol inclusion showed a significant decline in weight and feed efficiency when compared to birds offered the diets containing 0–5% glycerol inclusion. These findings conflict with those of Simon *et al.* (1996); Barteczko and Kaminski (1999); Swiatkiewicz and Koreleski (1999) and Yalcin *et al.* (2010), who each reported no negative effects on broiler performance when glycerol was incorporated into diets at 10% inclusion level. These observations are consistent with those found in the first trial carried out by Mclea *et al.* (2011a) where birds between 7–14d of age, glycerol inclusion linearly improved feed efficiency. However, at 21–28 and 7–28d a decline in utilisation of the diets was observed with the greatest efficiency observed at the 6.7% glycerol inclusion level. It was determined that the optimum inclusion level of glycerol into broiler diets was 6.7%, based on feed to gain values obtained. This is the first time that an optimum inclusion rate for glycerol has been established and this will be valuable information for the poultry industry.

McLea *et al.* (2011a) observed a reduction in feed efficiency at the 10% glycerol inclusion level as birds grew older. It has been suggested that the reduction in feed efficiency may be a result of saturation of the glycerol kinase enzyme at inclusion levels of glycerol above 5% (Doppenberg and Van der Aar, 2007). Doppenberg and Van der Aar (2007) reported that glycerol included above 5% may not be converted to glycerol-3-phosphate for subsequent use as an energy source due to the saturation of glycerol kinase. As a result, Doppenberg and Van der Aar (2007) speculated that excess glycerol is excreted. This is in line with the observations by Bartlet and Schneider (2002), who incorporated glycerol into broiler diets and found elevated levels of glycerol within the excreta when glycerol was included at levels above 5%. However, this conflicts with Simon *et al.* (1996), Barteczko and Kaminski (1999), McLea *et al.* (2011a) and McLea *et al*. (2011b) who all found no negative effects on growth performance when birds were offered diets containing glycerol at 10% inclusion. Although McLea *et al.* (2011a) found a significant reduction in feed efficiency between

6.7 and 10% glycerol inclusion, birds offered the diet containing 10% glycerol were still more efficient at utilising glycerol, in contrast to birds offered the control diet containing no glycerol.

The findings of the current studies suggest that the optimum inclusion rate of glycerol in broiler diets is 6.7%. In contrast to the findings of Doppenberg and Van der Aar (2007), saturation of the glycerol kinase enzyme may occur at glycerol inclusion levels above the 6.7% level. However, because there are also conflicting results in relation to how the glycerol is utilised at high inclusion levels, further investigations of effects on glycerol kinase are needed to provide a clearer understanding of glycerol utilisation.

## 5.2. The effect of glycerol on nutrient digestibility

There is a lack of information in the literature regarding the effect of glycerol inclusion on dry matter, total starch, glycerol and amino acid digestibility. McLea *et al*. (2011b) did observe a significant effect on total starch digestibility which was greatest at the 9% glycerol inclusion level with 98% of the total starch content of the diet being digested.

Glycerol effects on amino acid digestibility were also investigated by McLea *et al.* (2011b) and a significant positive effect reported. Threonine digestibility increased linearly in birds with up to 9% dietary glycerol inclusion. Threonine is the second most limiting amino acid after lysine for broilers and is important for optimum growth of broilers (Kidd *et al*., 1999), and has an important role as a precursor of glycine and serine (Ojano-Diranin and Waldroup, 2001) and is also involved in immune responses (Lemme, 2001). The effect on threonine digestibility observed in the current study, may have important economic implications.

## 5.3. The energy value of glycerol

No research has been carried out into the energy potential of glycerol or on the effect on growth performance when glycerol and cereal by-products partially replace the wheat content within broiler diets. Hence (McLea *et al*., 2011c), formulated diets to contain a specific AME content with the inclusion of glycerol at 6 and 8% and the inclusion of wheat pollard at 7.5%. The aim of this trial was to investigate if less expensive biodiesel by-product-based diets could supply the same energy as wheat-based diets through the inclusion of glycerol.

McLea *et al.* (2011c) found that the actual AME content of the diets containing glycerol was higher than the formulated AME content of the diets. Thus, indicating that glycerol can be used to increase the energy value of a less expensive diet containing wheat by-product. The mean AME value of glycerol was determined by the following equation; (Total AME of the diet - total AME of main energy ingredients within the diet)*(100/glycerol inclusion level) according to National

Research Council (NRC), 1994). It was found that the mean AME value of glycerol was 16.8 MJ/kg. It has been speculated that the AME value of crude glycerol is very much dependent upon the presence or absence of free fatty acids, unreacted triglycerides, residual methanol and the amount of glycerol within the sample (Dozier *et al*., 2008). Unfortunately for the feed industry, there is currently no rapid analysis that can quickly determine the AME of glycerol. Dozier *et al.* (2008) did find that the AME of glycerol was 95% of its gross energy, but the current work appears to suggest that this underestimates the AME value.

## 5.4. Glycerol as a replacement for soya oil

All of the research that has incorporated glycerol into broiler diets has done so to partially replace the main carbohydrate source within the diets. However, although soya oil has 2.25 times the energy potential of wheat, it is very expensive. This study investigated the effect of including glycerol in broiler diets as a partial or complete replacement for soya oil (McLea *et al*., 2011d). Glycerol did not have a positive effect on broiler performance when it completely replaced the soya oil content of the diet and this was reflected in a decline in growth performance. This may have been due to reduced palatability, consistent with the findings of McCann *et al.* (2009) who concluded that glycerol may have reduced the palatability of pig diets. However, during this current work, when glycerol partially replaced the soya oil content, positive effects were observed on nutrient digestibility. Therefore, glycerol may provide some of the beneficial attributes reported in the use of soya oil in broiler diets.

## 5.5. Pellet stability

It was interesting to note that pellets produced for the glycerol diets in the current studies, were less dusty than the diets containing no glycerol and appeared to be of better physical quality. This is potentially beneficial for the feed industry because there are many advantages to feeding a good quality pelleted diet. Grosbeck *et al.* (2008) reported that glycerol inclusion up to 9% improved the pellet durability index by 2–6%, in contrast to a diet containing no glycerol. Pelleting alters the density of the diet, as well as the size and hardness of the feed particles and reduces dust (Nir *et al*., 1994). It has also been found that when feed is supplied as a pellet, the amount of dietary energy lost through heat loss is considerably reduced (Leroy, 1961). The benefits of pelleting include the enhanced handling characteristics of the feeds, as well as improved animal performance. It increases bulk density and flowability, and decreases spillages and improves feed efficiency, compared with mash (Choi *et al*., 1986). However, the quality of the pellet must be taken into account. Research has shown that feeding poor quality pellets diminishes the benefits of pelleting (Scheideter, 1991). The current studies identified that glycerol has the potential to be successfully incorporated into broiler diets with positive effects on performance and nutrient digestibility. However, pelleting of glycerol is a logistical issue that needs to be addressed if glycerol is be successfully used in commercial broiler diets. The diets used in the current work were cold pelleted, so that a direct comparison with commercially produced diets is not possible as, under commercial conditions, the pelleting process involves steam conditioning at high temperatures (≥80$^{o}$C). Temperature was not recorded during the pelleting process applied in the current studies, but it is unlikely to have exceeded 60$^{o}$C. A recommendation from this work is to establish the effect on pellet quality of commercial pelleting of diets containing glycerol.

## 5.6. The effect of glycerol on meat quality

Moisture loss of any sort can have a significant effect on the texture, tenderness and end weight of the meat. Therefore, the current works investigated the effect of glycerol and wheat pollard inclusion on the moisture content and shear force tenderness of the broiler breast meat. Previous work by Mourot *et al*. (1994) reported a significant decline in drip and cooking loss when pigs were offered glycerol at 5% inclusion level. They (Mourot *et al.,* 1994) concluded that this may have resulted because glycerol is stored within the muscle, promoting increased osmotic pressure within the muscle.

However, in contradiction to Mourot *et al.* (1994), when we analysed broiler breast meat from birds offered diets containing levels of glycerol up to 8%, there were no significant differences in moisture loss and the tenderness of meat between those birds and birds offered diets containing different levels of glycerol (McLea *et al.,* 2011c). This is consistent with the observations of Casa *et al.* (2009) who incorporated glycerol into pig diets at 5 and 10% inclusion level. It can be concluded that glycerol has no effect on the moisture content of meat before and after the cooking stage. Glycerol did not affect the tenderness of the meat.

## 5.7. Determining the methanol content

The crude glycerol obtained from biodiesel production typically contains unreacted triglycerides, residual methyl esters, residual potassium or sodium salts and methanol (Thompson and He, 2006). Methanol is toxic and, upon ingestion, can pose a health risk to the animal (Medinsky and Dorman, 1995). The only method at present that can accurately measure the methanol content within crude glycerol is GC-FID, which is time-consuming, expensive and impractical if glycerol were to be used by animal feed manufacturers (Dambergs *et al*., 2002). The current work investigated two rapid means of measuring the methanol content within crude glycerol samples. These were NIR and Raman spectroscopy. A strong correlation for the prediction of all the samples with both the NIR and Raman spectra was observed with an $R^2_{cv}$ value of 0.75 for NIR and 0.87 for Raman spectroscopy. However, the SECV% was substantially higher than the threshold of 2–3% error to be accepted for repeatability, being at 63% and 50% for NIR and Raman spectra, respectively. As a consequence, it can be concluded that methanol may be estimated using the scanning techniques of NIR and Raman spectroscopy, but with a compromised accuracy. However, it must be stressed that the data set was small and needs to be increased before firm conclusions can be drawn.

## 5.8. The use of glycerol in commercial diets

Crude glycerol also contains residual sodium or potassium (depending on the identity of the catalyst used during the transesterification process). Excess sodium or potassium can result in production problems and wet litter (Cerrate *et al.*, 2006). When formulating broiler diets using glycerol, the amount of sodium or potassium present in the crude glycerol sample needs to be determined and then accounted for within the diet. Cerrate *et al.* (2006) noted that diets containing glycerol increased litter moisture content and it would be important to investigate the contributing factors in future studies. It has been found that NIR (Cozzolino *et al*., 2008) and Raman spectroscopy (Walrafen and Douglas, 2006) have the potential to measure sodium and potassium in wine and aqueous solutions, therefore they may be useful tools in predicting sodium or potassium levels in glycerol.

## 5.9. Housing

During this study, birds were housed in metabolism cages under scientific research conditions rather than under commercial housing conditions. Validation of the results under commercial housing conditions are required before recommendations can be made to the poultry industry, as it has been shown that the performance of birds differs between housing conditions (Fouad *et al.,* 2008).

# 6. CONCLUSIONS

- This work has shown that the optimum inclusion rate of glycerol in broiler diets is 6.7%. However, validation studies are required under commercial conditions before firm recommendations are given to the poultry industry.
- The AME content of glycerol was determined to be 16.8 MJ/kg. However, the AME value varies with the composition of the crude glycerol source and more work is required to examine the factors contributing to this variability.
- Glycerol added into broiler diets at an inclusion level between 3.3 and 6.7% can be used to increase the AME of less expensive cereal by-product diets.
- Glycerol at the inclusion level of 8-9% appears to have positive effects on starch and amino acid digestibility.

- Glycerol has the potential to be incorporated into broiler diets as a partial replacement for soya oil, thus reducing reliance on expensive soya oil. It also appears to improve pellet stability.
- Glycerol had no negative effects on the moisture content and tenderness of broiler breast meat. However, further sensory analysis is required to ensure that glycerol has no detrimental effect on the sensory quality of poultry meat.
- NIR and Raman spectroscopy have the potential to be accurate predictors of the methanol content in crude glycerol.

## 7. RECOMMENDATIONS FOR FURTHER STUDY

- It would be helpful to investigate the role of glycerol kinase enzyme in influencing feed efficiency as birds grow older.
- The effect of glycerol on pellet quality should be investigated for diets produced under commercial conditions.
- The effect of glycerol quality and quantity on broiler performance also needs to be examined in commercial housing conditions.
- It would be advantageous to find a rapid means to determine the sodium or potassium content of crude glycerol, in order to avoid production problems and wet litter when birds are housed in commercial settings.

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