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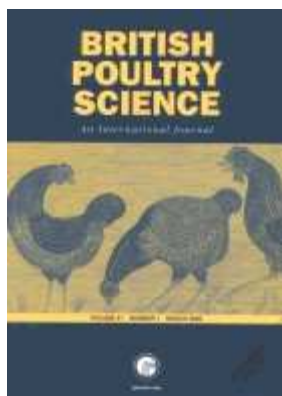
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The effect of glycerol inclusion on broiler performance and nutrient digestibility

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RUNNING TITLE: DIETARY GLYCEROL FOR BROILERS

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Abstract 1. Crude glycerol from biodiesel production was offered *ad libitum* to broiler chickens in a 21-d feeding and digestibility trial. The study was designed as a 3*2+1 factorial design with 3 concentrations (33, 67, 100 g/kg) of glycerol from 2 sources, A and B (PRS Environmental Ltd and John Thompson and Sons Ltd) and a control diet. The diets were formulated to contain apparent metabolisable energy (AME) of 12.95 MJ/kg (assuming 14.6 MJ/kg for glycerol).

2. No significant interactions occurred, so only the main effects were discussed. At 7-14 d, feed conversion ratio (FCR) showed a significant linear response with increased glycerol inclusion. However quadratic responses on FCR were observed for the 21-28 d period and 7-28 d.

3. Glycerol digestibility was significantly greater with birds offered the 67 g/kg and 100 g/kg glycerol-based diets in contrast to the digestibility of 33 g/kg glycerol-based diets.

4. Glycerol inclusion level also had an effect on AME, which increased linearly with increasing glycerol inclusion. Birds offered the diets containing glycerol also required less energy per unit gain in contrast to birds offered the control diet.

5. When examining the effect of source of glycerol, source A glycerol resulted in the highest AME (15.20 vs. 14.72 MJ/kg). There was no significant effect of glycerol source on the other performance parameters.

6. Glycerol digestibility was significantly greater with glycerol from source B (John Thompson and Sons, Ltd) with a mean value of 0.848 in contrast to source A (PRS Environmental), which had a somewhat lower mean glycerol digestibility of 0.757.

7. In conclusion, glycerol source did not affect performance and increasing level of glycerol improved FCR, with 67 g/kg inclusion resulting in the most efficient conversion of feed to gain without any negative effects upon nutrient digestibility.

INTRODUCTION

Glycerol is a by-product of biodiesel production and a need to find a viable use for crude glycerol is required. One suggestion has been to use it as an energy source within animal diets (Thompson and He, 2006). This theory does have its merits, because, as with glucose, glycerol can be converted by the glycerol kinase enzyme into an energy yielding molecule for cellular metabolism. It is the intermediate in both the glycolytic and gluconeogenic pathway depending on physiological conditions (Alberts *et al.*, 2002; Berg *et al.*, 2002; Rosebrough *et al.*, 1980).

However, there is little research on the actual energy value that can be placed upon glycerol as a nutrient within non-ruminant diets. Dozier *et al.* (2008) carried out one of the first studies to determine the apparent metabolisable energy corrected for nitrogen loss (AME_N) value on broiler chickens. According to Adeola, (2001), Dozier *et al.* (2008), determined the AME_N of glycerol by regressing the AME_N intake against feed intake (FI) and glycerol was added into the basal diets at varying inclusion levels. They noted that when glycerol was integrated into broiler diets at different ages that the AME_N value obtained across experiments of 0 g/kg and 60 g/kg glycerol inclusion and 0 g/kg, 30 g/kg, 60 g/kg and 90 g/kg glycerol inclusion resulted in a mean AME_N value of 14.37 MJ/kg for glycerol. This value was 95% of the gross energy (GE) content of the glycerol which was 15.05 MJ/kg. These findings have provided initial evidence to suggest that glycerol can be used as an energy source within broiler diets up to a point, after which a decline in AME occurs posing an energy loss when the birds may not be able to utilise the glycerol further. It needs to be evaluated if glycerol is utilised efficiently by broilers in terms of feed conversion ratio (FCR) by examining the optimum inclusion of glycerol when integrated into broiler rations. Cerrate *et al.* (2006) investigated the effect of

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3 including glycerol with an assumed AME value of 14.76 MJ/kg in the diets of
4 broilers as a partial replacement for maize. For birds aged 0-14 d, starter diets were
5 formulated to contain 12.91 MJ/kg AME with glycerol inclusions of 0, 50 and 100
6 g/kg glycerol inclusion. Performance results indicated that at inclusion
7 concentrations of 50 and 100 g/kg glycerol, birds at 0-14 d had increased feed intake
8 in contrast to the other concentrations. There was no significant difference in regard
9 to weight gain and FCR. However the opposite resulted when 35-42 d birds were
10 offered the 100 g/kg glycerol inclusion, resulting in feed conversion becoming less
11 efficient. Cerrate *et al.* (2006) reported that this effect may have been attributable to
12 pelleting instability and feeding equipment faults rather than the effect of glycerol
13 *per se*. Doppenberg and Van der Aar (2007), base their conclusions on performance
14 data and suggested another reason for the reduction in FCR at an inclusion
15 concentration of 100 g/kg glycerol, that the glycerol kinase enzyme may become
16 saturated above 50 g/kg inclusion (Doppenberg and Van der Aar, 2007). Thus,
17 further glycerol may not be utilised by the animal and must be excreted via the urine
18 which represents an energy cost. During a pig feeding trial, Bartelt and Schneider,
19 (2002) found that ileal absorption of glycerol to be >97% even at the inclusion
20 concentration of 150 g/kg and they also found very low urinary excretion of glycerol
21 at a low inclusion of 50 g/kg; however, this increased at higher inclusion
22 concentrations.
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50 While there is evidence to suggest that crude glycerol can be utilised as an
51 acceptable energy source for broilers, there is no information on the optimum
52 inclusion within a wheat-based broiler diet upon broiler performance. There is also a
53 lack of information regarding and the effect of glycerol inclusion on nutrient
54 digestibility such as total starch digestibility, dry matter digestibility and glycerol
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3 digestibility. Therefore the aims of this study were to define the optimum inclusion
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5 of glycerol in a wheat-based broiler diet and to establish the effect of glycerol
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7 inclusion on broiler performance and nutrient digestibility.
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10 MATERIALS AND METHODS

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12 This experiment compared 7 diets comprising 3 concentrations (33, 67, 100 g/kg) of
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14 glycerol from each of 2 sources (PRS Environmental Ltd, Craigavan, Northern
15
16 Ireland (NI) and John Thompson and Sons Ltd, Belfast, NI), and a control diet.
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18 Glycerol compositions from PRS Environmental and John Thompson and Sons Ltd
19
20 were analysed by ASG Analytik-Service Gesellschaft mbH, Augsburg, Germany
21
22 after the end of the experiment. Glycerol content was also determined using a
23
24 commercial enzyme assay kit from Megazyme International Ireland Ltd according to
25
26 Wieland (1988) Gross energy was also determined for both of the glycerol sources
27
28 using an isoperibol automated bomb calorimeter (PARR, Model 1271).
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34 **Experimental diets**

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36 Diets were formulated by Devenish Nutrition Ltd, Belfast, NI and produced at Agri-
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38 Food and Biosciences Institute (AFBI), Newforge. The diets were formulated to
39
40 contain 12.95 MJ/kg AME and 13.9 g/kg lysine. During the transesterification
41
42 process where biodiesel is produced, sodium hydroxide is added as the catalyst and
43
44 trace amounts are found in the crude glycerol. The sodium content of the glycerol
45
46 was assigned the assumed value of 20g/kg during the diet formulation and the
47
48 sodium bicarbonate (g/kg) content was reduced accordingly. However the sodium
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50 chloride (g/kg) content formulated into the diets remained unchanged in order to
51
52 maintain the balance of minerals (Table 1). Crude glycerol does not contain any
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54 vitamins and trace minerals. Crude glycerol contains the end products of the
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56 reactants that were entered into the process which include methanol and sodium
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3 hydroxide (Table 2). As such the vitamin and mineral (g/kg) contents within the diet
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5 formulation was included at the expected levels for starter, grower diets for broilers.
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7 In order to maintain a balance of minerals and nutrients for birds at 7-28 d the
8
9 concentration of calcium was a compromised concentration within the formulation
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11 matrix for starter/grower diets. The AME content of the crude glycerol was assumed
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13 to be 14.6 MJ/kg and the dietary ingredients are presented in (Table 1).
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Tables 1 and 2
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The wheat was firstly milled using a 5 mm sieve through a hammer mill. All of the dietary ingredients were then weighed and mixed in a Bental mixer and pelleted to 3 mm through a Lister cold pelleter. A small proportion of each diet was sampled, milled to 0.75 mm using a Glen Cross beater mill and various analyses were carried out including, dry matter (DM) content by oven drying at $100^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24 h and ash was determined in a muffle furnace at 450°C for 16 h (AOAC, 1990).

Total starch and glycerol content were determined using a commercial enzyme assay kit from Megazyme International Ireland Ltd (McCleary *et al.*, 1997; Wieland, 1988). Titanium dioxide (TiO_2) was used as an indigestible marker and determined according to Peddie *et al.* (1982). Gross energy of the diets was measured using an isoperibol automated bomb calorimeter (PARR, Model 1271) and the results are presented in Table 3.

Table 3 near here

Animal subjects

The study was conducted under the confines of the Animal Scientific Procedures Act 1986. A total of 100 Ross broiler birds were obtained from Moy Park Northern Ireland on day of hatch and housed in a brooder at 33°C and fed *ad libitum* crumbled diet and water until 7 d of age. At 7 d the birds were weighed and the 18 heaviest and 19 lightest birds were discarded from further analysis. The remaining 63 birds

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3 were arranged into 9 weight blocks and randomly allocated over 7 treatments and
4 placed into individual metabolism cages. The birds were kept under the appropriate
5 temperature, humidity and lighting regime. The birds were then offered experimental
6 feed and water *ad libitum* up to 28 d of age. At 14, 21 and 28 d the birds were
7 weighed to determine growth performance and feed remaining at the end of each
8 week was weighed to determine DM intake and FCR.
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10 11 12 13 14 15 16 17 18 **Excreta**

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20 Between 14-21 d of age a total excreta collection was made from under each cage
21 and placed into foil trays and stored daily at 4°C. At the end of the collection period,
22 it was oven dried for 48 h at 80°C, milled to 0.75 mm using a Glen Cross beater mill
23 and analysed for DM to determine GE content. Apparent metabolisable energy
24 (AME) not corrected for nitrogen loss and AME: gain was then calculated.
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32 At 28 d of age, the birds were humanely killed by dislocation of the spinal
33 cord. The contents of the ileum were removed and placed into ice and subsequently
34 stored at -80°C. The duodenum contents were removed for measurement of *in vivo*
35 supernatant viscosity of the diet through the digestive tract according to Bedford and
36 Classen (1993). The ileum contents were freeze dried for 3 d and milled to 0.80 mm
37 using a Polymix hammer mill (Kinematic Inc, Bohemia, New York, USA). The
38 ileum contents were then analysed for DM, total starch content and glycerol content
39 according to (McCleary *et al.*, 1997; Wieland, 1988) and titanium dioxide (as for
40 diets). Total starch digestibility, glycerol digestibility and DM digestibility were
41 determined.
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54 55 56 **Statistical analysis**

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58 Data for this study were analysed by analysis of variance (ANOVA) using Genstat
59 version 9.0 at AFBI, Newforge. The 7 diets comprised a 3 inclusion concentration
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(33, 67, 100 g/kg) * 2 sources (PRS, Thompsons) factorial plus a control. Hence contrasts were calculated to test for (a) linear and quadratic effects of glycerol inclusion averaged over the 2 sources; (b) differences between the two sources averaged over the inclusion concentrations; (c) interaction between inclusion concentration and source; and (e) differences between the control diet and the other 6 diets.

One bird had to be humanely killed on d 21 because it had become lame. Two more birds died on d 25 and d 27, another bird developed ascities; all of these birds were excluded from analysis. With regard to the factorial design, there were no interactions between the glycerol concentration and source and therefore only the main effects are discussed below.

RESULTS

Glycerol sources

Diet 1 (control) containing no added experimental glycerol was analysed for glycerol content as a baseline and contained 10.8 g/kg glycerol DM. Analytical results and characterisation of both sources of glycerol A and B are presented in Table 2.

The effect of glycerol inclusion concentration

FCR, AME, AME:gain and liveweight gain (LWG) were found to be significantly affected by the addition of glycerol. At 7-14 d FCR was seen to show a significant linear response, becoming more efficient as the inclusion level of glycerol increased. At 21-28 d and for the entire experimental period a quadratic response existed for FCR (Table 4).

Dietary AME content increased linearly with glycerol inclusion ($P<0.05$), (Table 4). No significant differences between the birds offered the glycerol diets and those offered the control diet were found for metabolisable energy intake (MEI)

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3 and ME:GE (Table 4). However it was observed that birds offered the diets
4 containing glycerol required less energy per unit gain in contrast to the birds offered
5 the control diet, which was reflected in a decline in AME: gain (Table 4).
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10 Although no linear or quadratic responses were observed, birds offered diets
11 containing glycerol gained significantly more weight which was reflected in 14-21 d
12 liveweight gain and total liveweight gain values ($P<0.05$) (Table 4).
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15 Glycerol inclusion level had no significant effects on either DM digestibility
16 or total starch digestibility at the ileal level. However glycerol digestibility was
17 significantly greater for birds offered the 67 g/kg and 100 g/kg glycerol-based diets
18 ($P<0.001$) in contrast to the glycerol digestibility of the diets containing 33 g/kg
19 glycerol-based diets (Table 4).
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Table 4 near here

29 **The effect of source of glycerol**

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31 The source of glycerol inclusion did not significantly affect FCR, liveweight gain or
32 DM intake over the entire experimental period. There was a significant difference
33 between the two sources in regards to dietary AME content with PRS glycerol
34 resulting in the highest ($P<0.05$) AME (Table 5).
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Table 5 near here

41 There was also a significant effect of glycerol source on DM digestibility,
42 which was higher when birds were offered glycerol diets from source B (John
43 Thompson and Sons Ltd) ($P<0.05$). Total starch digestibility and glycerol
44 digestibility coefficients were also significantly greatest for birds offered glycerol
45 diets from source B from (John Thompson and Sons Ltd) ($P<0.05$; Table 5).
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53 **DISCUSSION**

54 **The effect of glycerol inclusion level**

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56 The effect on FCR over the entire experimental period suggested that 67 g/kg
57 glycerol resulted in the best utilisation of the diets in this study. This result conflicts
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3 with previous findings of Cerrate *et al.* (2006) who found that birds at 0-14 d showed
4 no significant difference in FCR when offered diets containing 0-100 g/kg glycerol.
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6 It also conflicts with birds at 35-42 d of age offered the 100 g/kg glycerol diets
7 showing significantly higher FCR reflecting a reduction in converting feed to
8 increased growth in contrast to the birds offered the control and 50 g/kg diets. This
9 study showed that birds at 7-14 d were able to utilise the glycerol containing diets
10 most efficiently at the 100 g/kg inclusion level. These findings are also in contrast to
11 the suggestions of Doppenberg and Van der Aar (2007), from various performance
12 data, that glycerol kinase enzyme may become saturated above 50 g/kg inclusion in a
13 diet. In this study it is likely that saturation of glycerol kinase may have occurred
14 between 67 and 100 g/kg glycerol inclusion, given the quadratic effect on FCR.
15 Nevertheless, even at 100 g/kg inclusion of glycerol, feed conversion was more
16 efficient for birds offered glycerol than those offered the control diet and this is
17 reflected in LWG.
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36 The linear response of FCR at 7-14 d indicated that the birds being offered
37 100 g/kg could efficiently utilise the glycerol. However, as the birds grew older, a
38 significant decline in FCR was reported at glycerol concentrations above 67 g/kg.
39 This decrease in efficiency was specifically detected at 21-28 d, which was
40 consistent with findings of Simon, (1996) and Cerrate *et al.* (2006) who found that
41 older birds were less efficient at utilising greater levels of glycerol than younger
42 birds. However in regards to this current trial, more investigation would be required
43 in order to determine if there was an age effect on glycerol utilisation. The rationale
44 behind this was that, even at the 100 g/kg glycerol inclusion level, FCR values
45 indicated that birds offered this concentration could still convert feed into increased
46 growth more efficiently than birds offered diets containing no glycerol at 21-28 d of
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3 age and total FCR, thus suggesting no age effect. Glycerol digestibility remained
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5 high at high glycerol inclusion levels, which indicated that it was absorbed from the
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7 gastro-intestinal tract but not necessarily utilised.
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10 No feed mechanical problems arose when feeding the birds the 100 g/kg
11 glycerol which may have occurred in the study by Cerrate *et al.* (2006). In fact
12 glycerol inclusion at the level of 100 g/kg seemed to promote pellet stability, which
13 is in line with findings of Swiatkiewicz and Koreleski (2009) who found that
14 glycerol inclusion presented a much more stable pellet than the control diet pellets.
15 Glycerol inclusion also had no effect on viscosity of the diet indicating that, even
16 though glycerol is a viscous liquid, it did not affect normal transit time through the
17 digestive tract of the bird. AME increased linearly as glycerol inclusion increased
18 although at 67 g/kg and 100 g/kg glycerol inclusion the AME values were not
19 significantly different from each other. This was not reflected in the declining FCR
20 values with birds at 21-28 d of age with the 100 g/kg glycerol inclusion level. This
21 was neither reflected in an increase in DM digestibility. However there was also no
22 increase in the AME: GE ratio, therefore this may have been a result of a slightly
23 increased lipid content from the full fat soya content within the diets containing
24 glycerol. Birds required less energy per unit gain when offered the diets containing
25 glycerol in contrast to the birds offered the control diet, suggesting that glycerol can
26 be efficiently utilised as an energy source for growth. This study also noted that
27 although there was a decline at the 100 g/kg glycerol inclusion level, birds offered
28 this concentration were still able to convert feed into increased growth more
29 efficiently than birds offered the control diet containing no glycerol and the 33 g/kg
30 glycerol inclusion. However, it is in agreement with Dozier *et al.* (2008) who
31 reported an estimated AME_N value of 14.01 MJ/kg with glycerol inclusion level of 0
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3 and 30, 60 and 90 g/kg glycerol inclusion for 42-45 d-old broilers. Birds offered
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5 diets containing 90 g/kg glycerol inclusion had a higher AME_N value than the
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7 control however the AME_N value was no different from the AME_N values estimated
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9 for the 30 and 60 g/kg glycerol inclusion. This again suggests that an age effect may
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11 have arisen in which birds at a certain age cannot utilise glycerol above a certain
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13 inclusion, in this instance the 90 g/kg glycerol concentration. However this was not
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15 investigated during this study and requires further research.
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19 20 **The effect of glycerol inclusion level on nutrient digestibility**

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22 As glycerol inclusion level increased, so did glycerol digestibility; however it did not
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24 seem to improve FCR at 100 g/kg inclusion level, thus the glycerol may have been
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26 absorbed but not put to use as a nutrient and energy source within the bird. This may
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28 be because once all of the substrate (glycerol) has saturated the glycerol kinase
29
30 enzyme binding sites, further addition of glycerol will not be utilised and as such
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32 excess glycerol must be excreted out from the bird. However the fact that there was
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34 no difference in the AME: GE ratio at any level of glycerol inclusion is difficult to
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36 explain and warrants further investigation. At the 33 g/kg glycerol inclusion,
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38 glycerol digestibility values were significantly lower than the values obtained from
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40 the 67 and 100 g/kg glycerol concentrations but with no change in AME:GE values
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42 for all levels. This may have resulted because there was so little of the glycerol
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44 added to the diet on a g/kg basis and thus the most of the energy was generated from
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46 the wheat within the diet. This result is also in line with FCR values with birds at 7-
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48 21 d of age showing decreased efficiency to utilise the diets at the 33 g/kg inclusion
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50 level in contrast to the other levels.
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57 58 **The effect of source of glycerol**

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3 Although the two sources of glycerol differed greatly in their respective glycerol
4 content, there was no effect on FCR or growth performance. Evidence from this
5 study showed that the cruder of the two glycerol sources, source A (PRS
6 Environmental Ltd) resulted in the highest AME value. Source A PRS
7 Environmental Ltd glycerol contained many un-reacted tri, di and mono glycerides
8 in the form of Matter Organic Non-Glycerol (MONG) and as a result this could have
9 increased the AME. The fact that glycerol digestibility was lower in diets containing
10 source A (PRS Environmental Ltd) reflects the lower glycerol in the product.
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22 According to this study, it was evident that total starch is digested more
23 efficiently than glycerol and total starch digestibility was highest when glycerol was
24 included in the diets. Total starch digestibility was highest within birds offered
25 glycerol-based diets from source B (John Thompson and Sons Ltd). Therefore this
26 may provide evidence that glycerol may actually promote total starch digestibility
27 within a wheat-based broiler diet, however this requires further investigation. This
28 may involve integrating glycerol into diets with a constant wheat g/kg and
29 determining total starch and glycerol digestibility values. Glycerol was being
30 efficiently digested at 67 and 100 g/kg and AME values were highest at these
31 inclusion levels. However according to FCR results this energy may not have been
32 available to the birds. A possible reason for this, as suggested by Doppenberg Van
33 der Aar (2007), would be that the glycerol kinase enzyme does become saturated
34 above levels of 67 g/kg glycerol inclusion; thus further utilisation is inhibited. This
35 theory seems consistent with FCR results regarding source B (John Thompson and
36 Sons Ltd) product which contained 850 g/kg glycerol. However it seems conflicting
37 with FCR results from source A glycerol sourced from (PRS Environmental Ltd)
38 which had such a low content of glycerol (490 g/kg) where one would have expected
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3 to observe no decline in FCR at 100 g/kg. It was observed in this study that both
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5 sources of glycerol showed a decline in FCR at glycerol inclusion levels above 67
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7 g/kg and as such this warrants further investigation to determine if the glycerol
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9 kinase enzyme is the limiting factor preventing further utilisation.
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12 **Conclusions**

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14 Glycerol can be integrated into a broiler diet at least up to a concentration of 67 g/kg
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16 without any negative effects on growth performance, FCR, AME, AME:gain, DM
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18 digestibility, glycerol digestibility or total starch digestibility. As a result of this
19
20 research, it can be determined that glycerol can be included in broiler chicken diets
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22 as a partial energy replacement for wheat. Glycerol utilisation declined as the birds
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24 grew older but even at the highest glycerol inclusion (100 g/kg) FCR was improved
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26 in comparison to the control diet.
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32
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36
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38
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Table 1. *Composition of experimental diets*

Ingredients	Glycerol inclusion (g/kg)			
	0	33	67	100
Wheat	575	537	496	449
Soyabean meal	275	275	275	275
Full fat soya	72	84	96	112
Soya oil	40	36	32	29
Glycerol*	0	33	67	100
Roxazyme G [†]	0.1	0.1	0.1	0.1
Finase ^{®‡}	0.1	0.1	0.1	0.1
Sodium bicarbonate	3.1	0.6	0.0	0.0
Limestone	8.3	8.2	8.1	8.0
Sodium chloride	1.2	1.3	1.2	1.3
Mono dicalcium phosphate	9.2	9.3	9.5	9.7
L-lysine mono HCl	0.5	0.3	0.2	0.0
DL-methionine	2.9	2.9	3.0	3.0
DL-threonine	0.47	0.40	0.40	0.30
Premix [§]	5	5	5	5
Maxiban premix [#]	2.5	2.5	2.5	2.5
Titanium dioxide	3	3	3	3
<i>Formulated analysis (g/kg)</i>				
AME (MJ/kg)	12.95	12.95	12.95	12.95
Crude protein	220	220	220	220
Lysine	13.9	13.9	13.9	13.9
Threonine	8.7	8.7	8.7	8.7
Calcium	8.5	8.5	8.5	8.5
Phosphorus	5.8	5.8	5.8	5.8
Methionine + cystine	9.9	9.9	9.9	9.9

* Glycerol from two sources was included into the diet formulation matrix with an assumed AME value of 14.60MJ/kg and glycerol was added at the above inclusion levels resulting in seven experimental diets.

[†] Roxazyme G: Recommended usage level: 100g/tonne: Endo-1,4- β -xylanase EC/IUB No. 3.2.1.8-minimum activity 26,000 units/g and endo-1,3 (4)- β -D-Glucanase (EC/IUB No. 3.2.1.6- minimum activity 18,000 units/g and endo-1,4- β -glucanase (EC/IUB No. 3.2.1.4-minimum activity 8,000 units/g.

[‡] Finase[®]: Is an enzyme preparation of 3- phytase (E.C.3.1.3.8) minimum phytase activity of 5,000 PPU/g.

[§] Premix: This supplement was a standard proprietary product and the major nutrients are as follows 5kg/t. Declared vitamins/trace elements: Finished feed levels, Vit A 0.6 g/kg as acetate, Vit D₃ 0.25/kg, Vit E 0.05 g/kg as alpha-tocopherol acetate, 0.0175 g/kg sodium selenite, copper sulphate pentahydrate 1 g/kg.

[#] Maxiban premix: narasin 50mg/kg and nicarbazin 50mg/kg, excipients 822g/kg, anti-dusting oil 20g/kg, micro-tracerF-Red 10-20g/kg.

Table 2. *Characterisation of the crude glycerol source A and B*

	Source A (PRS Environmental) (g/kg)	Source B (John Thompson and Sons Ltd) (g/kg)	Method
Glycerol	523	810	BS 5711-3
Glycerol	490	854	Megazyme assay kit (Wieland, 1988)
Methanol	16	0.3	Gas chromatography –flame ionisation detection (GC-FID) DIN EN 14110
Water	52	128	BS 5711-8
Ash	37	58	BS 5711-6
Non glycerol organic matter*	388	4.0	BS 5711-9
Free fatty acids	7.4	6.2	BS EN ISO 660
Gross Energy (MJ/kg)	20.70	14.41	1271 Isoperibol bomb calorimeter

* Non-glycerol organic matter: polyglycerol, sugar components, free fatty acids and partial glycerides in the crude glycerine (Yong *et al.*, 2001).

Table 3. *The gross energy, total starch and glycerol content of the combined glycerol diets*

Glycerol inclusion level from both sources combined (g/kg DM)				
	0	33	67	100
Gross energy (GE) (MJ/kg)	19.31	19.59	19.90	20.28
Total starch (g/kg)	343	323	293	273
Glycerol content (g/kg)	10.8	31	57	75

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Table 4. The effect of glycerol inclusion level on broiler performance and nutrient digestibility parameters

N	Glycerol inclusion level from both sources combined (g/kg)				SEM	P Value	P=L	P=Q
	9	18	16	17				
	0	33	67	100				
7-14 d Liveweight gain (g)	249	250	242	261	13.26	NS	NS	NS
14-21 d Liveweight gain (g)	460 ^a	513 ^b	504 ^b	497 ^b	17.01	<0.05	NS	NS
21-28 d Liveweight gain (g)	622.1	646.8	670.4	627.8	20.01	NS	NS	NS
Total liveweight gain (g)	1332 ^a	1395 ^b	1423 ^b	1390 ^b	31.88	<0.05	NS	NS
7-14 d DM intake (g)	296	285	263	283	12.85	NS	NS	NS
14-21 d DM intake (g)	564	552	555	560	15.60	NS	NS	NS
21-28 d DM intake (g)	862	832	828	819	21.45	NS	NS	NS
Total DM intake (g)	1723	1668	1642	1658	38.81	NS	NS	NS
7-14 d FCR [*]	1.20 ^c	1.14 ^b	1.10 ^a	1.09 ^a	0.024	<0.01	<0.05	NS
14-21 d FCR	1.23 ^b	1.14 ^a	1.08 ^a	1.11 ^a	0.026	<0.01	NS	NS
21-28 d FCR	1.39 ^c	1.30 ^b	1.24 ^a	1.31 ^b	0.031	<0.01	NS	<0.05
Total FCR	1.30 ^c	1.20 ^b	1.16 ^a	1.20 ^b	0.016	<0.01	NS	<0.05
Viscosity (cpu)	2.73	3.26	2.59	2.67	0.298	NS	NS	NS
AMEI [†]	8.0	8.1	8.3	8.5	0.24	NS	NS	NS
AME:GE [‡]	0.73	0.75	0.75	0.74	0.009	NS	NS	NS
AME [§]	14.2 ^a	14.6 ^b	15.0 ^c	15.1 ^c	0.171	<0.001	<0.05	NS
AME:gain ^ζ	17.44 ^b	16.56 ^a	16.18 ^a	16.87 ^a	0.410	<0.05	NS	NS
Total DM digestibility	0.72	0.71	0.67	0.68	0.016	NS	NS	NS
Total starch digestibility	0.95	0.96	0.94	0.95	0.0132	NS	NS	NS
Glycerol digestibility	0.17 ^a	0.69 ^b	0.88 ^c	0.87 ^c	0.0263	<0.001	<0.001	<0.001

^{a,b,c} Superscripts indicate significance difference ($P < 0.05$).

^{*} FCR: Feed conversion ratio.

[†] AMEI: Apparent metabolisable energy intake (MJ/kg).

[‡] AME:GE: Apparent metabolisable energy: gross energy.

[§] AME: Apparent metabolisable energy (MJ/kg DM).

^ζ AME:gain: Apparent metabolisable energy.

Table 5. *The effect of the source of glycerol on broiler performance and nutrient digestibility parameters*

	Glycerol source		SEM	P value
	Source A (PRS Environmental)	Source B (John Thompson and Sons Ltd)		
	26	25		
7-14 d Liveweight gain (g)	254	249	13.26	NS
14-21 d Liveweight gain (g)	499	508	17.01	NS
21-28 d Liveweight gain (g)	633	664	20.01	NS
Total liveweight gain (g)	1388	1418	31.88	NS
7-14 d DM intake (g)	277	278	12.85	NS
14-21 d DM intake (g)	548	563	15.60	NS
21-28 d DM intake (g)	804	849	21.45	NS
Total DM intake (g)	1625	1686	38.81	NS
7-14 d FCR*	1.09	1.12	0.024	NS
14-21 d FCR	1.10	1.12	0.026	NS
21-28 d FCR	1.28	1.29	0.031	NS
Total FCR	1.18	1.19	0.016	NS
Viscosity (cpu)	2.86	2.82	0.298	NS
AMEI [†]	8.33	8.29	0.24	NS
AME:GE [‡]	0.75	0.75	0.009	NS
AME [§]	15.20 ^b	14.72 ^a	0.171	<0.01
Total DM digestibility	0.67 ^a	0.71 ^b	0.016	<0.05
Total starch digestibility	0.94	0.96	0.0132	NS
Glycerol digestibility	0.77 ^a	0.85 ^b	0.0263	<0.01

^{a,b,c} Superscripts indicate significance difference ($P < 0.05$).

* FCR = Feed conversion ratio.

[†] AMEI = Apparent metabolisable energy intake (MJ/kg).

[‡] AME:GE = Apparent metabolisable energy intake: gross energy (MJ/kg).

[§] AME = Apparent metabolisable energy (MJ/kg DM).