

**Chardon LL Hearing**

**An analysis of “the Chicken Study”  
The effect of Glufosinate Resistant Corn on Growth of Male  
Broiler Chickens.**

**Author: Department of Animal and Poultry Sciences,  
University of Guelph.  
Report No. A56379; July 12, 1996.**

**Joint Proof of Evidence**

**of**

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**on behalf of Friends of the Earth**

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This evidence was submitted at the National Seed List Hearings which ran from October to November 2000 in Manchester and London, England. In April 2000 the UK Government proposed to add the genetically modified maize seed, Chardon LL, to the UK National Seed List. Chardon LL is a variety of T25 maize developed by Aventis. The hearings considered public objections to this government proposal. On 15 November 2000 the National Seed List Hearings were indefinitely suspended by the UK Government.

This is part of a series of evidence submitted. For the rest of the evidence and for Friends of the Earth's case against Chardon LL maize see:

[www.foe.co.uk/campaigns/food\\_and\\_biotechnology/information/gm\\_food/](http://www.foe.co.uk/campaigns/food_and_biotechnology/information/gm_food/)

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## **1. Background and expertise**

- 1.1 We have been asked by Friends of the Earth to analyse the Methods, Results and Conclusions of the following paper (AV/2):

*The effect of Glufosinate Resistant Corn on Growth of Male Broiler Chickens.*

*Author: Department of Animal and Poultry Sciences, University of Guelph.*

*Report No. A56379*

*Date July 12, 1996.*

- 1.2 We set out the results of our analysis in this Joint Proof of Evidence.
- 1.3 I, Steve Kestin, am currently employed as a Research Fellow at the Department of Clinical Veterinary Science at the University of Bristol. I have a B.Sc (Agriculture) from the University of Reading and a Ph.D. from the University of Bristol entitled 'Lameness in Broiler Chickens – Welfare Implications and Control Strategies'. My research work and interests in animal welfare have evolved since 1990 when I obtained my first research grant to work on leg weakness or lameness in broiler chicken. I am currently employed full time on four projects relating to broiler chicken (and two relating to fish) welfare. The broiler chicken projects are:
- (1) a MAFF-funded project on the development of an objective method for assessing lameness in broiler chicken;
  - (2) a MAFF-funded project on understanding the epidemiology of the infectious causes of lameness in broiler chicken;
  - (3) a MAFF-funded project on evaluating extensive broiler production systems (as a sub-contractor); and
  - (4) an RSPCA-funded project on developing welfare assessment systems for broiler chicken.

- 1.4 Since 1983, I have authored or co-authored 76 published academic articles on animal welfare, 23 of which relate specifically to chickens. My work involves the design and execution of experiments with broilers.
  
- 1.5 I, Toby Knowles, am also employed as a Research Fellow at the Department of Clinical Veterinary Science at the University of Bristol. I have a B.Sc (Agriculture) from Seale-Hayne College, Devon and an M.Sc. in Applied Statistics from Sheffield Hallam University. In 1991 I was awarded a Ph.D. from the University of Cambridge for a thesis entitled 'The Welfare of Spent Hens in Transit and Related Effects of Housing System'. I am a Fellow and professional member of The Royal Statistical Society and a Fellow of the Cambridge Philosophical Society. I am a member of the Society for Veterinary Epidemiology and Preventative Medicine, the Association for the Study of Animal Behaviour, the International Society for Animal Ethology and the Sheep Veterinary Society.
  
- 1.6 I am currently employed as consultant statistician to the School of Veterinary Science and I also run four projects related to animal welfare and to agriculture. Since 1988, I have authored or co-authored 70 published academic articles, 22 of which relate specifically to chickens. I have considerable experience, over 10 years, of designing and executing agricultural experiments.
  
- 1.7 We have examined this report as if we were peer-reviewing, for publication in a scientific journal, a report of a new diet ingredient. We both regularly peer review scientific papers for a number of journals.

## **2. Introduction**

- 2.1 The report documents a small trial where two groups of broiler chicken were fed different diets, one based on normal maize, and the other based on maize genetically modified to be resistant to the herbicide glufosinate ammonium. The growth and performance of the broilers to 42 days was measured. The objective of the study seems to be set out in the Introduction, where it is stated that *“This study was conducted to compare the performance of broiler chickens fed glufosinate resistant corn with a standard commercially available corn hybrid”*.
- 2.2 Whilst this is not stated as such, considering the study as a whole it appears that the objective was to detect whether there were any differences in nutrient quality between the diets, using the broiler chicken as a model.
- 2.3 We note that no statistically significant effects were found in the study but:
- 2.3.1 that there was a trend for the variance in live weight of the GM fed birds, and the variation in most other variables, to be much higher than for the birds fed the non-GM maize (Table 1); and
  - 2.3.2 that there was a trend for higher mortality in the GM fed birds (twice as many broilers fed the GM T25 maize died (10) compared with those fed the non-GM maize (5) (Table 2)).
- 2.4 We also note that no comment is made by the authors on the much higher variations for the GM-fed birds. As to the higher number of deaths, the report states that *“Mortality was normal for this fast-growing strain of bird, where at our research facilities we normally see values of 5 to 8% in male broilers”* .
- 2.2 We set out our analysis of the report in five parts, under the following

numbered sections of this Proof of Evidence:

Section 3: Comments on the Overall Objective

Section 4: Comments on the Method

Section 5: Comments on the Statistical Analysis

Section 6: Comments on the Results

Section 7: Comments on the Conclusions

### **3. Comments on the Overall Objective**

- 3.1 We assume that this study is one of a number looking at all aspects of the maize and its likely effects on target and non-target organisms. Differences in nutrient quality are only one aspect that should be investigated. In particular, appropriate toxicological studies should be undertaken.
  
- 3.2 Similarly, growth of broiler chickens provides only one model. Before it could be concluded that the nutrient value of the GM corn was the same as commercially available corn, it would also be necessary to examine growth in both monogastric and ruminant mammals, where these are also target species. This is because they have very different digestive systems from birds, which react to feed ingredients in different ways.

### **4. Comments on the Method**

- 4.1 In this section we consider genotypes used, pen and treatment allocation, number of birds used, control, brooding and stocking density, lighting, diet formulation and transition and weight measurement.

- 4.2 The report states (first sentence of section entitled ‘Materials and Methods’) that “*Two hundred and eighty commercial strain Ross x Ross male broiler chickens were obtained at day of age*” and used in the study. Ross, a broiler chicken production company, market several genotypes of broiler, including the Ross 208, the Ross 308 and the Ross 508, but the report does not state which genotype was used. It is also possible to create custom genotypes by using different male and female lines. All these genotypes have different growth characteristics. The exact genotype of broiler used should be specified so that comparisons can be made with published information on growth performance and fat distributions. This would allow an objective identification to be made whether the birds on this trial were growing normally or not.
- 4.3 The allocation of birds to pens and the allocation of pens to treatments appears satisfactory as it was carried out completely at random.
- 4.4 In this study, 8 pens of birds were divided across 2 treatments, giving 4 pens per treatment. This is far too few. Regardless of the fact that the pens contained 35 birds each, these birds cannot be considered to provide independent measurements of treatment effects. For statistical analysis of the study, the number of independent measurements from each treatment population was only 4 (n=4). This means that the power of the study is very limited, i.e. it will only be possible to identify very large differences between populations.
- 4.5 It may be worth noting, in passing, that if one were seeking to show no effect, one of the best methods to do this is would be to use insufficient replication, a small n. In a study of this nature we would have carried out sample size calculations to determine the difference in weights that we would have liked to be able to detect (see below).

- 4.6 A further major flaw in the design of the study is that there is no positive control. A third treatment should have been included, where birds were fed a diet which was known to be nutritionally unbalanced, leading to, say, a 3 percent growth reduction. In an experiment like the one conducted, where there is no positive control, if no effects are found, it cannot be determined whether this is because there is no difference between the diets, or, put simply, because the experiment has not worked because of some unforeseen other factor. This could be because the birds were infected with a virus, or a range of other reasons. If the positive control is included, this allows us to identify whether the experimental set up is working and is sensitive enough to detect the expected difference. If we demonstrated that this third treatment reduced growth, but that the GM diet did not, then we could tentatively conclude that the diet had produced no deleterious effect, at least of a magnitude which the design of the experiment allows detection. Without this control, we cannot do this.
- 4.7 The brooding, stocking density etc. of the birds appears satisfactory.
- 4.8 The lighting schedule used, i.e., constant lighting, is not accepted UK/European practice. Usually, at least 1 hour dark is provided, and increasingly, longer periods of dark are used. However, the lighting pattern used in this study is not likely to have materially affected the findings. No indication of the lighting level is given. In the UK it should be at least 10 lux.
- 4.9 The formulation of the diets, and the change – over from starter to grower and then to finisher, appears satisfactory.
- 4.10 The measurement of live weight appears satisfactory but even though all birds

were weighed individually, for the purposes of analysis, pen means should have been used, leading to a sample size of only four.

4.11 Normally, in a feed trial where feed intakes are being measured, more details of how food spillage was avoided are given. This is because feed spillage can be a particular problem in *ad libitum* fed poultry, and can compromise the feed intake results. This particularly applies in studies where there is inadequate or barely adequate replication.

4.12 Measurement of eight birds from each replicate (32? from each treatment) for yield and fat pad weight is rather few.

## **5. Comments on the Statistical Analysis**

5.1 The reporting of the statistical analysis is inadequate. It is not clear how the data were dealt with. No degrees of freedom or t values are shown for the tests that have been carried out. It is not clear whether the errors associated with the means are standard deviations (SD) or standard errors of the means (SE). The data should more appropriately have been reported as confidence intervals as we are interested in the differences between means and the reliability of the estimate of this difference.

## **6. Comments on the Results**

6.1 Tables 1 and 2 do not tell us if they show the mean plus or minus SD or SE. Examining the data in detail and comparing it with some of our own, makes us think it is SD that is presented in the tables, but as this information is

fundamental to interpreting the data we should be told.

- 6.2 The trend for the variance in live weight of the GM fed birds and the variation in most other variables is much higher than that in the commercial corn hybrid fed birds. This is not a statistically significant effect, but is a trend which is suspicious and could be because of flaws in the design or execution of the study, or, alternatively, a real difference in the effect of the diets though the exact cause is impossible to say with any degree of certainty.
- 6.3 The lack of replication has been commented on above. Sample size calculations show that, given the variation in body weight at 42 days, and the 4 replicates used, the minimum difference in body weight at 42 days that could have been detected in the study between treatments would have been 137g or 5.48% of the 42 day live weight. In our experience in similar studies, this would have been a huge treatment effect. For example, in a study comparing the performance of different genotypes (Kestin et al. (2000) (FOE/46, an additional document submitted since start of the hearing; at the bottom of page 1088 of the original, Table 4)) we found a difference of 78 g between two Ross genotypes examined (Cross 1 and Cross 2)) at 35 days Body Weight. This was statistically significant. A sample size of between 22 and 26 was used in this study (page 1086, Table 1).
- 6.4 The trend for higher mortality in the GM fed birds - 10 birds, as against 5 birds - is also suspicious. Again, as for weight and other variables, it suggests either a fault in the study or a real effect of diet. At this point, however, it is impossible to say which with any degree of certainty.
- 6.5 The report states that "*Mortality was normal for this fast-growing strain of bird, where at our research facilities we normally see values of 5 to 8% in*

*male broilers*” . UK industry average mortality is approximately 4% to 42 days.

## **7. Comments on the Conclusions**

- 7.1 It will have been gathered from the above that there are serious flaws in the study. Summarised, these are that there was insufficient replication, which means that only very large effects on live weight could have been detected. Second, the study may not have been correctly statistically analysed and is not properly reported, and thirdly, that without a positive control, it is not possible to say whether a nil finding would have been real, or a function of poor experimental control. These flaws mean that it is not possible to draw any conclusions from the study as it is reported.
- 7.2 The correct way to undertake a study of this type is to first postulate that the test compound may produce, for example, a 3% reduction in growth (75g at 42 days). (This would be a very meaningful difference to the poultry industry, where even 1% matters.) Then a study is designed which is capable of identifying an effect of this size, assuming normal variability (identified from previous studies or from the literature). Modeling the study as presented using standard predictive packages, indicated that at least 14 replicates of each treatment are required to detect a 3% difference at the 5% level.
- 7.3 Having identified the scale of difference of interest, a third diet is designed which will achieve this scale of effect. For example, a diet which is slightly unbalanced in amino acid profile, such that one essential amino acid becomes limiting before full growth potential is reached, is made. The study is then

carried out with the three diets (test, normal and positive control) being fed to 14 replicates each. Then, provided the expected difference between the positive control diet and the normal diet is found, and no difference between the test diet and the normal diet is found, it would be possible to conclude that any differences in the nutritive value of the test and normal diets produces a less than 3% detrimental effect on growth.

- 7.4 Put simply, this study as reported is inadequate in terms of providing any evidence or conclusions. It is not of a standard that would be acceptable for publication in a scientific journal.
- 7.5 It follows that neither do we consider the study as reported to be adequate for being taken into account as evidence of safety in connection with decisions to approve the use of the relevant GM maize. If anything, the results as reported arouse suspicions of real differences between the treatments. This should act as a spur to further investigation (along the lines, in respect of safety for chicken feed, outlined in this section 7).

### **References.**

Kestin, S.C., Su, G. and Sorensen, P. 1999. Different commercial broiler crosses have different susceptibilities to leg weakness. *Poultry Science*. 78, 1085-1090 (FOE/45).