

# Case study 17: The effect of spent yeast (*Saccharomyces cerevisiae*) strain SAB5 on the performance of village chickens

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## Summary

This study describes an experiment carried out in Swaziland to determine the effect of supplementing the diet of young chickens with different levels of spent yeast on weight gain, feed conversion efficiency and mortality.

The case study highlights an error sometimes committed in the design of poultry or animal feeding experiments where researchers neglect the effect of competition that occurs among chickens or animals when fed as a group, and assume that measurements from them can be analysed individually. Suggested remedies in experimental design are made for dealing with this problem.

Summaries of the results from this experiment indicate that there were no differences in weight gain or feed efficiency for chickens fed the different diets, but it was not possible to evaluate the statistical significance of the results. There was an indication of a slight effect on mortality which might have been significant had a larger number of chickens been used.

## Background

Rural area poultry accounts for more than 60% of the total national poultry population in most African countries (Sonaiya et al. 1990). Village or indigenous chickens are more widely distributed in rural Africa than other livestock species (Sonaiya et al. 1990). In Swaziland, about 92.5% of the rural households keep poultry and 70% of these households keep indigenous chickens. The number of indigenous chickens in Swaziland is about 1.8 million which accounts for about 14% of the total poultry population. These village chickens, other than being a cheap source of protein to the rural resource poor, also serve as a source of income. However, they tend to have low growth rate and to suffer high mortality from disease (Ministry of Agriculture and Cooperatives (MOAC) 2006)



Source: Thulile Sgwane

The high prevalence of diseases in poultry has led to the introduction of a wide range of antibiotics. However, sub-therapeutic use of antibiotics in poultry feeds has become undesirable due to possible residuals effects in animal products leading to antibiotic resistance in humans. An alternative to the use of antibiotics is the use of probiotics which have been shown to improve performance in terms of weight gain, feed conversion efficiency and reduced mortality (Lange 2007). Spent yeast (*Saccharomyces cerevisiae*) is one of the probiotics being mostly researched. Probiotics are live microbial feed supplements that beneficially affect the host animal by improving its intestinal balance, leading to resistance to disease and lower mortality rates, especially during the first week of life (Gibson and Fuller 2000).

## Objectives

The objective of the study described here was to determine if spent yeast improves weight gain and feed conversion efficiency and reduces mortality of village chickens raised under the rural setting of Swaziland.



Source: Thulile Sgwane

## Questions to be addressed

The specific questions the case study aims to address are:

- Does spent yeast increase weight gain and feed efficiency and, if so, is there a relationship between increased performance and level of spent yeast?
- Does spent yeast reduce mortality and, if so, is there a relationship between decreased mortality and level of spent yeast?

As will be discovered, there are problems in the way that the study has been designed in order to enable the above questions to be addressed satisfactorily. We shall discuss:

- why the design is unsuitable for statistical analysis;
- how we can redesign the experiment to allow statistical analysis to be undertaken;
- how we can take into account particular features of the housing that might allow reduction of residual random variation.

## Study design

Two hundred day-old village chicks were randomly allocated using random tables into four 'brooding surrounds' which had been prepared for the experiment in the poultry house. Each surround was randomly assigned one of four diets.



Source: Thulile Sgwane

Surrounds were made of mesonite board. Dry hay straw was used as litter material for the first two weeks and sawdust thereafter. Litter was spread to a depth of 5 cm.

Clean drinkers and feed trays were evenly distributed as shown in each of the surrounds.



The initial brooding temperature in the house was set to 32°C, then reduced to 29°C in week 1, to 26°C in week 2 and then kept at 25°C from week 3 until the end of the experiment in week 8. After brooding, which lasted four weeks, chicks were spread out in four 2.24 m<sup>2</sup> compartments in the poultry house so that there were 10 birds per m<sup>2</sup>.

Chicks were fed starter crumbles for the first three weeks, then changed to mixed fowl feed in weeks 4 and 5. From week 6 to week 8 chickens were let out to forage on Kikuyu grass (*Penisetum clandestinum*) while supplemented with mixed fowl feed. All chickens were fed *ad libitum* using feeding troughs throughout the eight weeks of the experiment. Feeders were topped up to three quarters full at 0600 hours daily.



Source: Thulile Sgwane

The experimental diets included a control (Diet 1) in which no spent yeast was added. The other experimental diets constituted different rates of spent yeast. These were 0.05 g/kg (Diet 2), 0.10 g/kg (Diet 3) and 0.15 g/kg (Diet 4).

Diets fed to chickens during the eight week experiment			
Diets	Weeks 1-3	Weeks 4-5	Weeks 6-8
1	Starter crumbles	Mixed fowl feed	Mixed fowl feed + Kikuyu forage
2	Starter crumbles + 0.05 g/kg spent yeast	Mixed fowl feed + 0.05 g/kg spent yeast	Mixed fowl feed + 0.05 g/kg spent yeast + Kikuyu forage
3	Starter crumbles + 0.10 g/kg spent yeast	Mixed fowl feed + 0.10 g/kg spent yeast	Mixed fowl + 0.10 g/kg spent yeast + Kikuyu forage
4	Starter crumbles + 0.15 g/kg spent yeast	Mixed fowl feed + 0.15 g/kg spent yeast	Mixed fowl feed + 0.15 g/kg spent yeast + Kikuyu forage

Day old chicks were weighed in batches of 10 for the first three weeks and the mean value assigned individually to each chick in a batch. After the third week it was possible to weigh chickens individually. Chicks were individually tagged on a leg at the start of the experiment. Each day the feed left over from the day before was measured and the daily amounts added together to allow weekly feed intake to be calculated. Deaths were recorded daily.

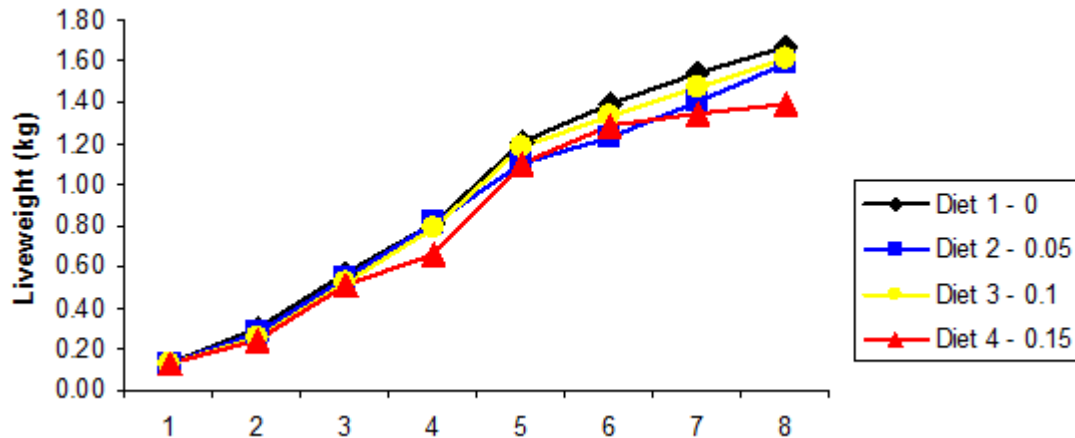
## Exploration and description

Mean weekly weights and feed conversion efficiencies (calculated as weight gained over the previous week divided by the feed consumed) were plotted for each diet against age of chicken in weeks.

## Weight gain

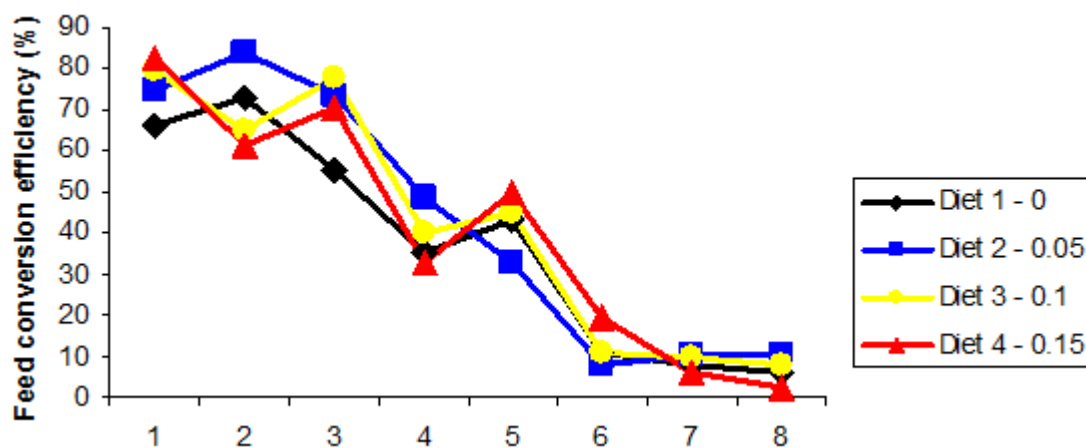
The line graph depicts the performance of the chickens with respect to weight gain. It can be seen that the observed trends were similar suggesting that inclusion of different rates of the *Saccharomyces cerevisiae* did not affect weight gain. Indeed between weeks 6 and 8 those

chickens receiving the highest level of spent yeast grew on average the least.



## Feed conversion efficiency

A line graph for feed efficiency against age similarly indicated that inclusion of *Saccharomyces cerevisiae* had little effect. Feed conversion efficiency is expected to decline with age as depicted by the trend, although in weeks 6 to 8 it needs to be remembered that the chickens were also foraging. Feed conversion efficiency estimates between weeks 6 and 8 ignore the consumed amounts of Kikuyu grass that is poorly digested by non-ruminants due to its high fibre content. The decline with age tends to be due to the fact that although chickens eat more as they get older their growth rate remains relatively constant. Nutrient requirements for muscle growth decrease with age, thus reducing the proportion of the feed that is converted into weight gain.



We have now realised that we do not have sufficient diet replication to allow us to undertake further statistical analysis. We had presumed that it would be possible to use the individual measurements recorded per chicken in an analysis of variance, but we had neglected the fact that there is competition among chickens for feed when they are fed together. The

consequence is that we cannot assume individual observations to be independent – one of the assumptions in an analysis of variance.

Thus, only one single mean value for birds on a group-fed diet can be used for comparison purposes, and we have no means for calculating standard errors. In other words the experimental or observational unit in this study is the surround or compartment.

## Mortality

Four of the 50 chickens in the control group died by eight weeks of age. In contrast, no chicken died in the group fed 0.5 g/kg of spent yeast, and just one and two, respectively, in the other two groups. Bird mortality is different from variables such as weight or feed efficiency in that deaths, especially when few, are generally likely to be individual occurrences and unrelated to the group to which the bird belongs. Thus, assuming that mortalities can be treated as independent observations we can combine the three spent yeast treated groups and compare 3/150 deaths against 4/50 deaths for the control group by a Chi<sup>2</sup> test. Using **Stats** → **Statistical Tests** → **Contingency Tables** in GenStat and creating a 2-way table with values 3, 147, 4 and 46 we obtain Chi<sup>2</sup> = 4.00 (P = 0.046), seemingly just significant. However, note the warning message in the GenStat output.

```
CHISQUARE [PRINT=test, prob; METHOD=pearson] Mortality
***** Warning from CHISQUARE:
some cells in the table of expected values have less than 5.

Pearson chi-square value is 4.00 with 1 df.
Probability level (under null hypothesis) p = 0.046
```

We need to heed this message. When some values are small we need to adjust the formula for a Chi<sup>2</sup> test by incorporating the value 0.5 as follows:

$$\text{Chi}^2 = \text{Sum} [ (|\text{Observed} - \text{Expected}| - 0.5)^2 / \text{Expected} ]$$

Calculating by hand we find that the Chi<sup>2</sup> value reduces to 2.42; this is well below the significance level.

Thus, although there was a trend to suggest that *Saccharomyces cerevisiae* helped to reduce mortality, the levels of mortality observed in this study were too low to detect statistical significance.



Source: Thulile Sgwane

## Findings, implications and lessons learned

Various lessons on study design can be learned from this case study.

- Firstly, as has already been mentioned, group feeding means that individuals in a

group cannot be assumed to be independent in relation to such measurements as weight gain or feed conversion efficiency. It is possible to be a little more relaxed in the case of mortality and assume that deaths occur independently, provided there is no evidence of transmissible infection within the group. For any variable that is to be measured it is important beforehand to understand what will determine the experimental or observational unit for statistical analysis.

- This study was part of the requirements for a Bachelor of Science in Animal Science degree. It has demonstrated how advisable it is, even in the smallest of degree projects, for a student to seek statistical advice before embarking on his or her study. Other case studies in this Teaching Resource focus on different aspects of study design and a student should look at the various case studies to see the examples that may be relevant to his/her project.
- For this case study a more appropriate experimental design would have been to randomly assign the chicks to a number of smaller surrounds for each feed. Since three levels of spent yeast were to be compared with the control diet and 200 chicks were available, 16 chicks could have been randomly assigned, say to each of 12 surrounds, with four surrounds per diet. This would have used 192 of the chicks and resulted in this skeleton analysis of variance for a completely randomised design.

Source	d.f.
Diet	3
Residual	8
Total	11

- This design could be improved further by taking into account the positions of the different surrounds within the poultry house and grouping them according to different environmental conditions apparent within the house, e.g. proximity to an extractor fan or to an exposed area. Let us suppose that three blocks can be defined to describe three areas within the poultry house, each containing four surrounds, each with one of the diets. This would lead to this randomised block analysis of variance:

Should blocking have a positive effect this would reduce the size of the residual variance:

Source	df
Block	2
Diet	3
Residual	6
Total	11

- Finally there was a reduction in mortality from 8% in the control group to an average of 2% in the spent yeast groups. In view of the comparatively small numbers of chickens this was not statistically significant. Study question 5 illustrates how one can evaluate ahead of time the size of sample that might be needed to achieve statistical significance.



## Study questions

1. Imagine a particular shape of a poultry house (maybe the one depicted in this case study) to be used to repeat this experiment. Assuming that 16 smaller surrounds have been made, each to contain 12 chicks. Describe how the experiment is to be laid out within the poultry house and how surrounds are to be allocated to diets. Sketch the analysis of variance.
2. Suppose that you find that the poultry house has room for only eight of the surrounds described in question 1. It is decided that the experiment should be run twice with different batches of chicks on each occasion. Describe how you will design the experiment and sketch the analysis of variance. Why do you think it is considered necessary to replicate this experiment when only eight surrounds are available?
3. The chickens in this study were of mixed sex. Describe how you would take sex into account within the experimental design and sketch the analysis of variance.
4. In poultry experiments it might sometimes be appropriate to take into account initial weight. Give an example from the literature where this has been done and describe how the researcher made use of initial weight, either in the design or in the analysis.
5. Assume that 5% of chickens fed the control diet died and that spent yeast reduced mortality, on average, by half. Assuming equal numbers of chickens per diet determine how many chickens will be needed in the experiment to demonstrate that the average effect of spent yeast on mortality is statistically significant ( $P < 0.05$ ).
6. Describe how you could include contrast terms in the analysis of variance (see Case Study 15) to investigate any pattern in the effect of level of spent yeast on weight gain or feed conversion efficiency.
7. Describe how logistic regression (see Case Study 14) might be used to analyse the effect of spent yeast on mortality in this experiment assuming that chickens are allocated to, say, 20 surrounds.
8. When individual chickens die during an experiment discuss what effect, if any, this might have on the performance of the surviving group. When analysing weight gain over particular sub-periods of an experiment discuss whether one should eliminate or retain all measurements for chickens that die during the experiment up to the point that they die.
9. Assessment of the amount of feed consumed can be affected by the design of the feeders, as there can be feed spill when the chickens feed. Describe how you might modify the feeder shown in this study to minimise feed wastage.

## Further reading

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