Evaluation of Ensiled Brewer's Grain in the Diet of Piglets by One Way Multiple Analysis of Variance, MANOVA

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Summary

The basic purpose of feeding trials is to find the optimum level of feed ingredients which give the highest economical returns to the farmers. This can be achieved through estimation and comparison of means of different rations. The example we have is a study of incorporation of different levels of ensiled brewers grains in the diet of 24 hybrids weaned piglets from Landrace x Duroc x Berkshire x Large White. They were randomly divided into four groups with three replicates of two piglets per pen. They were fed 0, 10, 20, 30% incorporation of ensiled brewer's grains on dry matter basis during post-weaning period followed by 0, 30, 40 and 50% during growing period and 0, 50, 60 and 70% during finishing period. We have one explanatory variable: initial weight, and four post treatment outcome variables recorded per piglets: final weight, dry matter consumption, weight gain and index of consumption. Comparing of several multivariate treatment means model design analysis is adapted. We obtain the MANOVA (Multiple Analyse of Variance) table of each phase, where the treatment differences exist by using Wilk's lambda distribution, and we find the treatment effect by using a confidence interval method of MANOVA. This model has the advantage of computing the responses of all variables in the matrix of sum of squares and more precisely in separation of the different means percentage of Ensiled Brewer's grain.

Résumé

Evaluation de la drêche ensilée des brasseries dans le régime des porcelets par une voie d'analyse de Variance Multiple, MANOVA

L'objectif global est de trouver un taux d'incorporation optimum de la drêche qui sera économique pour les éleveurs. Ceci sera atteint à travers une estimation et une comparaison des moyennes des différentes rations alimentaires. L'exemple que nous avons pour cette étude est l'incorporation du taux optimum en utilisant la drêche ensilée des brasseries dans 24 hybrides de porcelets croisés: Landrace x Duroc x Bershine x Large white. Ils ont été répartis au hasard en quatre lots de trois répétitions de deux porcelets par cage. Ils ont consommé 0, 10, 20, 30% d'incorporation de la drêche ensilée en période de post-sevrage, puis 0, 30, 40, 50% en période de croissance et 0, 50, 60, 70% pendant la période de finition. Nous avons une variable exploratrice: le poids initial et quatre variables après traitement pris pour un porc: poids final, consommation alimentaire en matière sèche, gain de poids et indice de consommation. La comparaison de variables multiple à plusieurs traitements de moyennes du modèle expérimental d'analyse est adaptée. Nous obtenons le tableau de MANOVA de chaque phase, où les différences des traitements existent en utilisant la distribution de Wilk's lambda et les effets traitements sont trouvés en utilisant la méthode de l'intervalle de confiance de MANOVA. Ce modèle a l'avantage de combiner les cinq réponses en matrice des sommes des carrés et en plus d'augmenter la précision dans la séparation des différentes moyennes de pourcentage de la drêche ensilée des brasseries des porcs.

Introduction

Optimisation of feed intake and composition is a continuing problem in animal production. Cameroon's livestock production is very important economic activity. There are about 5 million bovines, 1.1 million pigs, 6.5 million sheep and goats and 15.2 million poultry (2).

According to Ranjhan (13) there is a need to enrich animals diets using such by-products as blood flour, fish flour, palm oil cake, cotton cake, soya bean cake and ensiled brewer's grain. Little attention has been accorded by researchers at this moment to determine the optimal levels of incorporating these by-products in animals' diet.

As such developing an efficient system to determine optimum levels of dietary ingredients calls for concerted efforts to develop useful models. Statistical work concerned with calculating optimal amount has concentrated on characterising the response of an individual plot especially in agriculture, where Wallach (19) used hierarchical linear model approach to estimate parameters and obtain optimal fertilizer strategy which depends on the site and year characteristics. Similarly, Anderson *et al.* (1) and Heady *et al.* (9) statistically attempted to obtain optimal fertilizer amounts by characterizing the response of an individual plot to fertilizer applied.

D-optimality criteria have been applied in other ways for example by Hatzis *et al.* (8) to construct locally optimal designs in non-linear multi-response estimation using Poisson model for filter feeding. The calculated optimal design greatly reduces variances of model parameter estimates compared to variances from previously used empirical designs.

In animal data with multiple responses, the wider statistical problem is that in many cases, univariate analysis is generally used to see the effects of different levels of treatment by using each response separately. Meffeja *et al.* (11, 12) and Pond *et al.* (16) used univariate method to demonstrate that when you increase the level of wet ensiled brewer's grain you decrease feed intake and average daily weight gain, and increase feed conversion ratio. They looked also at the effect of dietary level of ensiled brewer's grains on growing and finishing pig performances. The same model has since then been widely used for evaluating poultry feeds. Scott *et al.* (17) noted that fish flour is a good source of animal proteins whereas Dafwang *et al.* (5) showed that these proteins gave a good performance of broiler chicks. More

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recently Dongmo *et al.* (6) used this univariate approach to demonstrate the importance of blood meal and showed that blood meal alone is not a good protein source for broilers. However, combination of different protein sources gave attractive results.

Bryan (4) compared multivariate versus univariate tests and noted that one important aspect of the use of a multivariate test as distinct from a series of univariate tests concerns the control of type I error rates. He noted that using the 5% level of significance a multivariate test gives a 0.05 probability of a type I error irrespective of the number of variates involved. It also has the added advantage of taking proper account of the correlation between variables.

The overall aim of this work is to tend to adapt a one way multivariate analysis of variance (MANOVA), using Richard and Dean (14) techniques to compare several treatment responses and to propose the model to animal scientist or biometricians. Basically, the objectives are:

- Obtain the multivariate treatment means of four levels of a pig's ration at various stages of pigs growth,
- Tests for the significance of the differences in the treatment means using the one way MANOVA model,
- Compute the confidence interval of the derived parameters of the model, and
- Make recommendations based on the finding of the analysis.

Methodology of data

The model is adapted to data on a piglet feeding trial using ensiled brewer's (EB) grain in the rations. There were used 24 hybrids piglets which were cross breeds of Landrace, Berkshire, Duroc and Large White, all 8 weeks old at the start of the trial. The piglets were initially assigned to the treatments completely at random in 12 pens which 2 piglets per pen, one male and one female. There were 4 treatments of EB in each of the three phases of piglets growth. In phase I; 0%, 10%, 20% and 30% of EB was included in the ration during post weaning period; then 0%, 30%, 40% and 50% of EB rations during the growing period and finally 0%, 50%, 60% and 70% during the finishing stage.

There were 3 replicates per treatment. The animals were fed once a day and water was available without restrictions. In the beginning of the trial, initial weights were recorded and then every 2 weeks until the pigs were 127 day old. The objectives of the study were, to determine the optimum level of ensiled brewer's incorporation in pig's diet at different life stages, and to evaluate the value of using EB by pig's farmers. The five outcome response variables in order are initial weight (kilogram), final weight (kilogram), daily dry matter consumption (gram), weight gain (gram) and the index of consumption. After logarithmic (ln(x)) transformation to normalize the original data, the multivariate analysis of variance methods are applied to achieve these objectives.

Dietary ingredient and nutrient composition of the ensiled brewers grain and basal diet

Ensiled brewer's grain derived to Anonyms societies of brewer of Cameroon (SABC), table 1 give their chemical composition and table 2 the basal diet base with maize and cotton meal.

Table 1
Chemical composition of Cameroon dry brewer's grains

Characteristics	Dry ensiled brewer's grain
Dry matter (%)	91.7
Digestible energy (kcal/kg)	2030
Crude proteins (%)	28.6
Ether extract (%)	7.6
Crude fibre (%)	15.7
Ash (%)	3.5
Calcium (%)	0.28
Phosphor (%)	0.60

Source: (10)

Table 2
Percentage composition of basal diet and calculated nutrients

Ingrédients	Composition in %
Maize	54.0
Cotton meal	15.0
Blood flour	4.0
Fish flour	3.0
Palm kernel meal	10.0
Bone flour	3.4
Paddy wheat	10.0
Salt	0.5
Concentration	0.1
Calculated nutrients	
Digestible energy (kcal/kg)	3164
Crude proteins (%)	19.80
Crude fibre (%)	5.30
Lysine (%)	1.00
Methionine and cystine (%)	0.70

Source: (12)

The model

Multivariate Analysis of Variance (One way MANOVA)

Let the data be indexed with a double subscript l_j, where l indicate the treatment (l=1, 2,..,g) and j is the number of animal for each treatment. We let g be the number of the group that received one level of a treatment. The total number of plots at treatment l is n_l, and the overall number of plots is n. The response of plot lj is X_{l_j}. MANOVA model describes responses as a function of diets rations for a fixed treatment. We assume that each component of the observation vector X_{l_j} satisfies the univariate model, and the errors for the components of X_{l_j} are correlated, but the covariance matrix $\sum$ is the same for all populations.

The model response X_{l_j} is: $X_{l_j} = \mu + t_l + e_{l_j}$ and a vector of observations may be decomposed as suggested by the model. Thus:

$$X_{l_j} = \overline{X} + (\overline{X}_l - \overline{X}) + (X_{l_j} - \overline{X}_l)$$

Where:

μ is overall means;

$X_{l_{jj}}$ are the observations;

$\overline{X}$ is the overall sample mean;

$\overline{X}_l - \overline{X}$ is the treatment effects;

$X_{l_j} - \overline{X}_l$ are the residual effects.

The decomposition leads to the multivariate of the univariate sum of squares by summing the cross product over l and j responses.

$$\sum_{l=1}^{g} \sum_{j=1}^{nl} (X_{\ell j} - \overline{X})(X_{\ell j} - \overline{X})'$$

$$= \sum_{l=1}^{g} n_\ell (\overline{X}_\ell - \overline{X})(\overline{X}_\ell - \overline{X})' + \sum_{l=1}^{g} \sum_{j=1}^{nl} (X_{\ell j} - \overline{X}_\ell)(X_{\ell j} - \overline{X}_\ell)'$$

Or
Total sum of squares and cross products = Treatment (between) sum of squares and cross products + Residual (within) sum of squares and cross products

The within sum of squares and cross products matrix can be expressed as:

$$W = \sum_{l=1}^{g} \sum_{j=1}^{nl} (X_{\ell j} - \overline{X}_{\ell})(X_{\ell j} - \overline{X}_{\ell})'$$

$$= (n_1 - 1)S_1 + (n_2 - 1)S_2 + \dots\dots\dots + (n_g - 1)S_g$$

Where S_l is sample covariance matrix for the l th sample treatment.

The hypothesis of no treatment effects, H_0: $T_1 = T_2 = \dots\dots = T_g = 0$ is tested by considering the relative sizes of the treatment and residual sum of squares and cross products. Formally we summarize the calculations leading to the test statistic in a MANOVA table.

Table 3
MANOVA table for comparing treatments means vectors

Source of Variation	Matrix of Sum of Squares and Cross Products (SSP)	Degrees of freedom (d .f)
Treatment	$B = \sum_{l=1}^{g} n_{\ell}(\overline{X}_{\ell} - \overline{X})(\overline{X}_{\ell} - \overline{X})'$	$g - 1$
Residual (error)	$W = \sum_{l=1}^{g} \sum_{j=1}^{nl} (X_{\ell j} - \overline{X}_{\ell})(X_{\ell j} - \overline{X}_{\ell})'$	$\sum_{l=1}^{g} n_{\ell} - g$
Total (corrected for the mean)	$B+W = \sum_{l=1}^{g} \sum_{j=1}^{nl} (X_{\ell j} - \overline{X})(X_{\ell j} - \overline{X})'$	$\sum_{l=1}^{g} n_{\ell} - 1$

One test of H_0: $T_1 = T_2 = \dots = T_g = 0$ involves generalized variances. We reject H_0 if the ratio of generalized variances:

$$\lambda^* = \frac{|W|}{|B+W|}$$

is too small, where λ* is a distribution of Wilk's lambda (20).
In this diet trial $n_{l_j} = n$ is large, we use the modification of λ* due to Bartlett (3), given by:

$$-\left(n-1-\frac{(p+g)}{2}\right) \ln\left(\frac{|W|}{|B+W|}\right) > \chi^2_{p(g-1)}(\alpha)$$

Where, p is the number of response variables, and $\chi^2_{p(g-1)}(\alpha)$ is the upper (100α), the percentile of a chi-square distribution with p(g-1) degrees of freedom.

Confidence intervals for treatments effects

When the hypothesis of equal treatment effect is rejected, those effects that led to the rejection of the hypothesis are of interest. Let T_{ki} be the ith component of T_k. $T_{l\,i}$ is estimated by

$$\hat{T}_{\ell i} = \overline{X}_{\ell i} - \overline{X}, \quad \hat{T}_{ki} = \overline{X}_{ki} - \overline{X} \quad \text{and} \quad \hat{T}_{ki} - \hat{T}_{\ell i} = \overline{X}_{ki} - \overline{X}_{\ell i}$$

is the difference between two independent sample means and the two sample t- based confidence interval is valid with an appropriately modified (α). Notice that:

$$\mathrm{Var}(\hat{T}_{ki} - \hat{T}_{\ell i}) = \mathrm{Var}(\overline{X}_{ki} - \overline{X}_{\ell i}) = \left(\frac{1}{n_k} + \frac{1}{n_\ell}\right)\sigma_{ii}$$

Where σ_{ii} is the i^{th} diagonal element of Σ. We estimate $(\overline{X}_{ki} - \overline{X}_{\ell i})$ by: $\mathrm{Var}(\overline{X}_{ki} - \overline{X}_{\ell i}) = \left(\frac{1}{n_k} + \frac{1}{n_\ell}\right) \frac{W_{ii}}{n-g}$

where W_{ii} is the i^{th} diagonal element of W and $n = n_1 + \dots\dots + n_g$. There are p variables and g(g-1)/2 pair wise differences, so each two sample t interval will employ the critical value t_{n-g} (/ 2m) , where m= pg (g-1)/2 is the number of simultaneous confidence statements, and for multivariate model, the confidence of at least
(1-) is $T_{ki} - T_{l\,i}$ belong to:

$$\hat{T}_{ki} - \hat{T}_{\ell i} \pm t_{n-g}\left(\frac{\alpha}{pg(g-1)}\right)\left(\frac{Wii}{n-g}\left(\frac{1}{n_k} + \frac{1}{n_\ell}\right)\right)^{\frac{}{2}}$$

Model application

Here, the number of variable (p) is 4, the number of the groups (g) and treatments are 4.
Sample mean vectors $\overline{X}_l$ for l th treatments in the reel data, Phase I, II, III.

Phase I

$$\overline{X}_1 = \begin{bmatrix} 9.75^a \\ 24.85^b \\ 812.4^c \\ 272.3^d \\ 2.97^e \end{bmatrix} \quad \overline{X}_2 = \begin{bmatrix} 9.46 \\ 25.18 \\ 806.7 \\ 281.5 \\ 2.86 \end{bmatrix} \quad \overline{X}_3 = \begin{bmatrix} 10.00 \\ 24.90 \\ 799.5 \\ 282.6 \\ 2.79 \end{bmatrix} \quad \overline{X}_4 = \begin{bmatrix} 8.64 \\ 26.76 \\ 805.1 \\ 321.5 \\ 2.50 \end{bmatrix} \quad \overline{X} = \begin{bmatrix} 9.45 \\ 25.41 \\ 805.9 \\ 288.9 \\ 2.79 \end{bmatrix}$$

[a] initial weight (kg), [b] final weight (kg), [c] dry matter consumption (g), [d] weight gain (g), [e] index consumption

Phase II

$$\overline{X}_1 = \begin{bmatrix} 24.77 \\ 52.46 \\ 1525.4 \\ 483 \\ 3.13 \end{bmatrix} \quad \overline{X}_2 = \begin{bmatrix} 25.03 \\ 48.91 \\ 1312.9 \\ 399.4 \\ 3.25 \end{bmatrix} \quad \overline{X}_3 = \begin{bmatrix} 25.03 \\ 48.91 \\ 1339.4 \\ 407.5 \\ 2.97 \end{bmatrix} \quad \overline{X}_4 = \begin{bmatrix} 26.58 \\ 47.94 \\ 1261.4 \\ 365.0 \\ 3.46 \end{bmatrix} \quad \overline{X} = \begin{bmatrix} 25.28 \\ 49.90 \\ 1352.9 \\ 411.6 \\ 3.29 \end{bmatrix}$$

Phase III

$$\overline{X}_1 = \begin{bmatrix} 53.52 \\ 60.95 \\ 1790.1 \\ 528.5 \\ 3.35 \end{bmatrix} \quad \overline{X}_2 = \begin{bmatrix} 48.91 \\ 53.52 \\ 1326.1 \\ 347.2 \\ 3.82 \end{bmatrix} \quad \overline{X}_3 = \begin{bmatrix} 48.91 \\ 51.93 \\ 1286.91 \\ 212.72 \\ 6.30 \end{bmatrix} \quad \overline{X}_4 = \begin{bmatrix} 47.94 \\ 49.90 \\ 1130 \\ 142.6 \\ 7.92 \end{bmatrix} \quad \overline{X} = \begin{bmatrix} 49.90 \\ 54.05 \\ 1366.5 \\ 262.43 \\ 5.05 \end{bmatrix}$$

Interpretation of results

From MANOVA table 1, we note that by equation of modification of the distribution$\alpha$of Wilk's lambda, λ*= 78.16, 30.36 and 93.44 for phases 1, 2, and 3 respectively reflecting significant differences in treatment means. After rejecting the null hypothesis at = 5% level in all the 3 phases, we conclude that treatments differences exist. We then constructed simultaneous interval estimates for all the pair-wise comparison of differences in treatment means (tables 4, 5 and 6). According to Pacock (15) when at 95% confidence limits, the lower and upper limits have same signs (positive or negative), the test is significant, other wise we note the absence of treatments differences. Thus:
Post-weaning (Phase I): All the pair-wise comparisons in dry matter consumption and index of consumption are different.

Table 4
Degree of significant of confidence interval of pair wise comparison for post weaning phase

Variables	Lower and Upper 95% Confidence Interval of Pair wise Comparison					
	T1 – T2	T1 – T3	T1 – T4	T2 – T3	T2 – T4	T3 – T4
Initial weight	[-0.32 0.38]	[-0.37 0.32]	[-0.29 0.47]	[-0.40 0.29]	[-0.26 0.44]	[-0.20 0.49]
Final weight	[-0.22 0.19]	[-0.21 0.21]	[-0.28 0.13]	[-0.20 0.21]	[-0.27 0.15]	[-0.28 0.14]
Dry matter consumption	[0.006 0.008]*	[0.015 0.017]*	[0.008 0.010]*	[0.007 0.010]*	[0.001 0.003]*	[-0.008 -0.006]*
Weight gain	[-0.26 0.19]	[-0.26 0.19]	[-0.39 0.06]	[-0.25 0.20]	[-0.36 0.09]	[-0.35 0.09]
Index consumption	[0.03 0.05]*	[0.05 0.06]*	[0.17 0.18]*	[0.005 0.02]*	[0.13 0.14]*	[0.11 0.13]*

*= significant

Table 5
Degree of significant of confidence interval of pair wise comparison for growing phase

Variables	Lower and Upper 95% Confidence Interval of Pair wise Comparison					
	T1 – T2	T1 – T3	T1 – T4	T2 – T3	T2 – T4	T3 – T4
Initial weight	[-0.24 0.22]	[-0.24 0.22]	[-0.30 0.16]	[-0.23 0.23]	[-0.29 0.17]	[-0.29 0.17]
Final weight	[-0.76 -0.30]*	[-0.76 -0.30]*	[-0.73 -0.28]*	[-0.23 0.23]	[-0.21 0.25]	[-0.21 0.25]
Dry matter consumption	[0.06 0.06]*	[0.04 0.04]*	[0.10 0.10]*	[-0.02 -0.02]*	[0.04 0.04]*	[0.06 -0.06]*
Weight gain	[-0.03 0.41]	[-0.05 0.39]	[0.05 0.50]*	[-0.24 0.20]	[-0.13 0.31]	[-0.11 0.33]
Index consumption	[-0.23 0.15]	[-0.24 0.14]	[-0.19 0.19]	[-0.20 0.18]	[-0.25 0.13]	[-0.24 0.14]

*= significant

Table 6
Degree of significant of confidence interval of pair wise comparison for finishing phase

Variables	Lower and Upper 95% Confidence Interval of Pair wise Comparison					
	T1 – T2	T1 – T3	T1 – T4	T2 – T3	T2 – T4	T3 – T4
Initial weight	[-0.13 0.31]	[-0.13 0.31]	[-0.11 0.33]	[-0.22 0.22]	[-0.20 0.24]	[-0.20 0.24]
Final weight	[-0.14 0.40]	[-0.11 0.43]	[-0.07 0.47]	[-0.24 0.30]	[-0.20 0.34]	[-0.23 0.31]
Dry matter consumption	[-0.03 0.63]	[0.001 0.66]*	[0.13 0.79]*	[-0.30 0.36]	[-0.17 0.49]	[-0.20 0.46]
Weight gain	[0.12 1.83]	[0.05 1.76]	[0.45 2.16]	[-0.92 0.78]	[-0.52 1.18]	[-0.45 1.25]
Index consumption	[-0.24 -0.02]*	[-0.74 -0.52]*	[-0.97 -0.75]*	[-0.61 -0.39]*	[-0.84 -0.62]*	[-0.78 -0.56]*

* = significant

Table 7
Cost ($ U.S) of feed per kg live weight gain per ration and phase

Phase I		Phase II		Phase III	
Treatment % of EB	Cost 1 kg	Treatment % of EB	Cost 1 kg	Treatment % of EB	Cost 1 kg
0	0.74	0	0.78	0	0.84
10	0.69	30	0.74	50	0.80
20	0.66	40	0.71	60	1.26
30	0.56	50	0.72	70	1.52

This table tells us that during post-weaning period 30% of E.B. have the lowest cost of production; in phase II, 40% and 50% of E.B. give lower costs while in phase III, 50% of E.B. minimises the costs.

Treatment four (30% of ensiled brewers grain) gives a better response in gain weight with lower index of consumption.

Growing (Phase II): For the variable final weight, treatment 1 (0% EB) is significantly different from the other treatments. There exist significant differences for all pair-wise comparisons for the variable dry matter consumption. For the variable weight gain, only treatment 1 and treatment 4 (50% EB) were significantly different.

Finishing (Phase III): For dry matter consumption, only treatment 1 versus treatment 3 and treatment 1 versus treatment 4 show significant differences, while for weight gain treatment 1 versus treatment 2 are significantly different. For the index of consumption all pair-wise comparisons show significant differences.

Economic analysis, conclusion and recommendation

Economic analysis

It is of interest to know which treatment is economically recommended at various stages of piglet's growth given the cost of feeding. Table 7 shows the cost incurred by farmers to obtain 1 kg of live weight gain per ration.

Conclusion

The purpose of this study was to develop a model to be applied in piglet feeding trials to separate treatment means effect. One way MANOVA was used to fit the data of different percentages of ensiled brewers grain in piglet ration. From the resulting analysis, all the three phases, post-weaning, growing and finishing show that treatment differences exist after obtaining different MANOVA tables and applying Wilk's lambda distribution modified by Bartlett and comparing to Chi-square distribution with p(g–1) degrees of freedom.

On the other hand, we found a confidence interval for treatments effects in each variable at different phases by a pair wise comparison for multivariate model. Using the theory that, if the test is significant at the 5% level then the 95% confidence limits will be in the same direction (15) we were able to separate pair-wise treatments which were significant and that were not significant.

Recommendations

From the foregoing explanation, one can make the following recommendation related to the use of different levels ensiled brewers grain in pig rations at various life stages of piglets.

i. during post-weaning period, 30% of ensiled brewers grain gives a good performance.
ii. during growing period, 40-50% of ensiled brewers grain is recommended, and
iii. during finishing phase of growing, 50% of EB gives better responses.

In consideration of comparing several multivariate treatment means, this model takes care of all the variables at the same time but does not give optimal solution in the choice of the level of ensiled brewer's grains. This can be extended in other related studies.

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