ISSN 1682-8356 ansinet.org/ijps



# POULTRY SCIENCE

ANSImet

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan Mob: +92 300 3008585, Fax: +92 41 8815544 E-mail: editorijps@gmail.com International Journal of Poultry Science 13 (2): 88-96, 2014 ISSN 1682-8356 

Asian Network for Scientific Information, 2014

## Experimental Design and Analysis with Emphasis on Communicating What Has Been Done: (II) Calculating Interaction Contrasts with SAS

L. Billard<sup>1</sup>, M.Y. Shim<sup>2</sup> and G.M. Pesti<sup>2</sup>

<sup>1</sup>Department of Statistics, University of Georgia, Athens, GA-30602, USA

<sup>2</sup>Department of Poultry Science, University of Georgia, Athens, GA-30602, USA

**Abstract:** Appropriate statistical analyses are of primary importance for understanding experimental results. Shim *et al.* (2014) detailed the influence of different statistical approaches on results of a two factor nutrition experiment with broiler chickens. However, frequently designs with more than two factors are needed because of the complexity of modern broiler and egg production. Statistical analyses need to be clearly communicated so that readers can properly interpret the results of experiments with poultry. Designs with two or more factors are frequent players in the world of experimental design. The computational burden of the attendant analysis of variance is somewhat eased by the presence of statistical packages. Contrary to expectation, it is not clear from texts or the Manual (s) how the package(s) can be used to find components of the interaction effects, whether the factors are qualitative or quantitative factors. We show how SAS can be persuaded to calculate these components (A x Linear B, etc., when A is a qualitative and B is a quantitative factor and Linear A x Linear B, etc., when both A and B are quantitative factors). The procedure can be adapted to fit other packages which have provision for contrast calculations. The results presented here extend and clarify the analyses of Shim *et al.* (2014) on the advantages and disadvantages of various techniques for analyzing results from experiments on poultry with more than two factors.

**Key words:** Qualitative and quantitative factors, A × linear B, linear A × linear B contrasts

#### INTRODUCTION

Shim et al. (2014) pointed out clear ambiguities in the way statistical procedures are presented in poultry science journals. Statements appearing like "Data were analyzed by using the GLM procedure of SAS (SAS Institute, 2006)" make it impossible for readers to understand and especially repeat, the methods that were applied. Shim et al. (2014) demonstrated the influence of several different statistical analytical approaches to a data set from an experiment with broiler chickens. They showed how different models may lead to different conclusions from the same experimental They listed possible advantages and disadvantages that may be applied to a relatively simple two factor treatment design. In this paper, we extend the results of Shim et al. (2014) and show how experiments with two or more factors, some are quantitative and others are qualitative, may be analyzed as they apply to experiments with poultry.

The availability of statistical packages has eased considerably the computational burden of many statistical analyses. Those who use them extensively are grateful. However, those same users are also painfully aware of the limitations of any particular package, limits that beguile the glossy "covers" (so-to-speak) seemingly promising so much more apparently than can be delivered and limits exposed when trying to

reconcile inconsistent answers generated supposedly clear but in fact oftentimes obscure Manual instructions. This paper focuses attention on the use of the SAS package and in particular on an aspect of the GLM procedure as used in the analysis of experimental design data. More specifically, we consider a standard factorial design with two (or possibly more) factors. The factors of interest are A and B. Suppose factor B is a quantitative factor. Then, among the usual quantities of interest, we can also find appropriate statistics relating to the components of B, such as Linear B, Quadratic B, The GLM procedure does this and the documentation is clear on how to carry out this task. The difficulties come when we try to find components of the interaction term A x B. If A is a quantitative factor, interest centers on components Linear A x Linear B, Quadratic A x Linear B, Linear A x Quadratic B, etc. The SAS Manual provides no evidence that its GLM procedure will calculate these components. If A is a qualitative factor, we may wish to consider components A x Linear B, A x Quadratic B, etc. Here too we are left to believe these components cannot be calculated by a SAS procedure, though there is evidence suggesting that components Linear B at a (specific) level of A, etc. can be found. Unfortunately, Manual instructions to do this are very oblique and are from a practical point of view nonexistent. Not surprisingly there is a wide spread

belief that SAS cannot calculate these components. This is unfortunate since the need for these components arises frequently, especially in agricultural and biological applications and in social science applications and too often such applied researchers therefore do not take their analyses these extra steps because they think they "cannot" and "need not".

However, in fact, SAS can be persuaded to yield calculations on these interaction components. Our purpose here is to indicate how this can be done. Thus, we consider the case that both factors are quantitative and we look at interaction components when one factor is qualitative and one is quantitative. We also draw attention to a related issue. First, we will assume there are only two factors, A and B, with replications. Then, generalization to more than two factors, with or without replications, follows readily, as shown. While the vehicle to develop these results is that for the SAS package, the principles described herein can be adapted to fit other software packages which allow the calculation of basic contrast components.

Our approach will be developed by way of an illustrative example using the data of Table 1. These data were extracted from the results of an experiment reported in Chamruspollert *et al.* (2002).

#### **MATERIALS AND METHODS**

**Both factors quantitative:** The vehicle for illustrating the methodology is a factorial design investigating the influence of two quantitative factors A (Arg) and B (Met) on the response variable (Average BW gain in 14 d) of chickens.

Any analysis starts by entering the data appropriately, typically by using an INFILE statement or a DATA LINE (or CARDS) statement followed by the actual data. Figure 1(I) shows one version. We note that in this example factor A has four equally spaced levels (1.52, 2.02, 2.52, 3.02%), factor B has three equally spaced levels (0.35, 0.45, 0.55%) and there are three replications. This Fig. 1(I) also shows SAS statements asking the procedure GLM to execute the standard analysis which produces the usual statistics associated with A, B and the interaction A x B. Also, it is reasonably straight-forward to calculate a linear component of A using SAS (SAS Institute, 2006). Thus, we include a CONTRAST statement contrast 'Linear A' A -3 -1 1 3, after the MODEL statement. Likewise, the statement contrast 'Linear B' B -1 0 I, will calculate the linear component of B. These determinations are easily made by following appropriate guidelines such as those in the SAS Manual. The numbers (-3, -1, 1, 3) used in the Linear A contrast are those weights needed to construct a linear function across the levels of A. We recall that, in general, a contrast across "treatments" T1,..., Tk is defined as, for equal replications per treatment:

$$z = \sum_{i=1}^{k} \omega_i T_i \text{ with } \sum_{i=1}^{k} \omega_i = 0$$
 (1)

In this context, the different levels of A constitute the treatments. Let us represent the weights as the vector:

$$W = (\omega_1, ..., \omega_k)$$
 (2)

Thus, the vector of weights associated with the Linear A and Linear B contrasts in our example are written, respectively, as:

$$A_i = (-3, -1, 1, 3)$$
 and  $B_i = (-1, 0, 1)$ 

Suppose now we wish to calculate the Linear A x Linear B component of the interaction A x B. This is achieved by inserting, with the CLASS statement "class A B", the CONTRAST statement:

between the MODEL and RUN statements. Similarly, to calculate the Linear A x Quadratic B, Quadratic A x Linear B and Quadratic A x Quadratic B components, we use the statements:

contrast 'Linear A x Quadratic B' A \* B -3 6 -3 -1 2 -1 1 -2 1 3 -6 3;

contrast 'Quadratic A x Linear B' A \* B -1 0 1 1 0 -1 1 0 -1 -1 0 1;

contrast 'Quadratic A x Quadratic B' A \* B 1 -2 1 -1 2 -1 -1 2 -1 1 -2 1;

respectively; likewise, for other components of the A  $\times$  B interaction.

These interaction component contrasts for Linear A x Linear B are but examples of the basic contrast definition in equation (1), where now the treatments correspond to the twelve (= 4 x 3) linear-linear levels of A x B. Formally, the weights are given by the vector:

$$C = vec(A' #B')$$
 (4)

where, if the column vector A' (of dimension a) has elements  $a_i$  and the row vector B (of dimension b) has elements  $b_{ij}$ , then the matrix D = (A'#B) is of dimension ab and has elements  $d_{ij} = a_ib_j$  and where vec(D') is the vector obtained by listing out the row elements of D' in order.

For example, Table 2 gives the matrix elements found from evaluating (-3, -1, 1, 3)'#(-1, 0, 1). Hence, the weights for the Linear A x Linear B contrast and in the order they are to be used become readily apparent. Table 2 also provides the weights and their ordering for the Linear A x Quadratic B, Quadratic A x Linear B and

Table 1: Illustrative data

	B1				<b>B</b> 2			Вз		
<b>A</b> 1	327.63	308.13	320.63	386.13	372.50	372.00	345.00	389.00	381.00	
<b>A</b> 2	278.75	264.38	211.36	363.63	359.88	345.75	331.93	349.38	352.00	
<b>A</b> 3	254.25	191.50	206.00	314.75	355.25	338.13	313.63	355.75	418.75	
<b>A</b> 4	181.50	144.50	157.50	176.63	240.50	290.50	369.00	336.50	385.86	

Table 2: Both A and B quantitative factors

	(	a) Linear B		(b	) Quadratic	В
Linear A	-1	0	1	1	-2	1
-3	3	0	-3	-3	6	-3
-1	1	0	-1	-1	2	-1
1	-1	0	1	1	-2	1
3	-3	0	3	3	-6	3
		A´ı#Bı			A´ı#Bq	
	(1	c) Linear B		(0	d) Quadratic	В
Quadratic A	-1	0	1	1	-2	1
1	-1	0	1	1	-2	1
-1	1	0	-1	-1	2	-1
-1	1	0	-1	-1	2	-1
1	-1	0	1	1	-2	1
		A´q#Bı			A´q#Bq	

Table 3: A qualitative and B quantitative factors weight matrix. Linear B at  $A_2$ 

		Linear B				
		-1	0	1		
<b>A</b> 1	0	0	0	0		
<b>A</b> 2	1	-1	0	1		
<b>A</b> 2 <b>A</b> 3	0	0	0	0		
<b>A</b> 4	0	0	0	0		

Quadratic A x Quadratic B contrast statements. The complete set of PROC GLM statements for these linear and quadratic contrasts is displayed in Fig. 1 (ii) and its output is given in Fig. 1 (iii).

Qualitative and quantitative factors: Let us now consider the case when one factor (A) is qualitative and one factor (B) is quantitative. First, the contrasts over B are evaluated at each level of A, separately. Thence, the final interaction contrast is subsequently calculated. Let us denote the levels of A by  $A_1, \ldots, A_4$ .

Therefore, to calculate the A x Linear B contrast, we first calculate the contrasts Linear B at the level  $A_i$ , i = 1,..., 4. For example, the Linear B at  $A_1$  contrast is evaluated by inserting the statement:

contrast 'Linear B at A1' B -1 0 1 A \* B -1 0 1 0 0 0 0 0 0 0 0; (5)

between the MODEL and RUN statements. Since weights not specified at the end of a weight vector are automatically set at zero, we can write this CONTRAST statement more simply as:

contrast 'Linear B at A<sub>1</sub>' B -1 0 1 A \* B -1 0 1;

In contrast to the case when both factors are quantitative (where only weights for A\*B were required, see, e.g., (3), note there are two parts to this statement, one with weights appropriate to the Linear B component, viz.,  $B_{\rm I} = (-1, 0, 1)$  and one appropriate to the A×B component. The weights associated with A \* B are as given by the

general formula of equation (4), but now the weights for A(•) equate to the vector of 0's except that a weight 1 appears in the ith place when dealing with the i<sup>th</sup> level of A. See Table 3 for the (A:#B) matrix for use in calculating the Linear B at the second level of component A, for example.

Therefore, to determine Linear B at A<sub>2</sub>, we include the statements:

contrast 'Linear B at A2' B -1 0 1 A \* B 0 0 0 -1 0 1;

similarly for levels  $A_3$  and  $A_4$ . We also need to calculate separately the Linear B component of the main effect of B. The complete set of SAS statements for the PROC GLM part of the program is displayed in Fig. 2(i) and the output is shown in Fig. 2(ii). While for the present purposes our ultimate goal is to calculate the overall A\*Linear B contrast, we note in passing that SAS outputs the F- and P-values for the contrast Linear B at a specific level of A; so, e.g., we can test whether or not there is a significant linear trend across the B levels at  $A_1$  considered alone.

The completed sum of squares (SS) value is then readily found from:

(A × Linear B)SS = 
$$\sum_{i=1}^{4}$$
 (Linear B at A<sub>j</sub>)SS - (Linear B)SS (6)

"by hand" if need be, but see below. Thence, for the data of our example:

Hence, the F- and P- statistics, etc., can be evaluated. Suppose further we wish to instruct SAS to carry out the calculations of Eq. (6). This is achieved by changing the PROC GLM, statement to the statement:

proc glm data = <datafilename> outstat = <filename>

where, in our case the datafile name is "one" and the outstat file name is "junk", and by adding the set of statements as provided in Fig. 3. Before elaborating on this, it may be instructive to look more closely at what SAS is doing internally.

The OUTSTAT option allows us to keep (for subsequent use) internal SAS (SAS Institute, 2006) output not automatically printed in the standard output. To see the contents of this OUTSTAT data set, we can print them in the usual way. Thus, Fig. 4(i) gives the SAS statements needed to affect this, with the printed output shown in Fig. 4(ii). Critically, the structure of this OUTSTAT data

set instructs us on how to write our program for the calculation of the sum of squares of the A x Linear B contrast of (6). More specifically, we want to add the SS terms for "observations" OBS = 6,..., 9 and to subtract that for OBS = 5.

Most importantly at this stage is the realization that there is a hidden DO loop, with the consequence that the intuitive step of doing a natural DO loop on the OBS variable does not work. This is circumvented by the IF/ELSE statements on the automatic variable N (SAS Language Manual; SAS Institute, 2006). Thus, to calculate the (A x Linear B) SS, we use the SAS (SAS Institute, 2006) statements:

```
retain sum 0;
if _N_ < 5 then SS = 0;
else if _N_ = 5 then SS = - SS;
sum = sum + SS;
```

as in Fig. 3. Running totals are still automatically retained; the required answer is the last value calculated, in this example, (A x Linear B)SS = 884.1875. Suppression of all but this last summation can be incorporated into the program [by asking for output only at the end, e.g., "if last then output;"].

In like manner, with appropriate use of the automatic variable \_N\_, the Error MS and hence the F- and P-statistics can be calculated. These have been done in the SAS statements of Fig. 3. The corresponding output is shown in Fig. 5.

An alternative compact code for obtaining the A x Linear B contrast statistics (suggested by a reviewer) as well as those for the A x Quadratic B contrast, is provided in Appendix A and the corresponding output is in Appendix B

Not to confuse the Issue ... But: For qualitative and quantitative factors, we developed program statements that would instruct the SAS package to calculate the Ax Linear B, etc., contrast statistics. In particular, appropriate weights to insert into a CONTRAST statement, such as those in Fig. 2(i), were determined. It is critical to note that the order in which the factors A and B are inserted into the CLASS statement

```
Fig. 1(i): Basic SAS Program
/* Quantitative × Quantitative Model. Interaction Components */
options Is=72 nodate pageno=1 formdlim=' '; /* List desired options */
title 'Quantitative/Quantitative Example';
data one:
do A = 1 to 4:
      do B = 1 to 3:
              do rep = 1 to 3;
                   input y@@;
                    output;
              end:
end;
data lines; /* Or, 'cards,' */
327.63 308.13 320.63 386.13 372.50 372.00 345.00 389.00 381.00
278.75 264.38 211.36 363.63 359.88 345.75 331.93 349.38 352.00
254.25 191.50 206.00 314.75 355.25 338.13 313.63 355.75 418.75
181.50 144.50 157.50 176.63 240.50 290.50 369.00 336.50 385.86
proc glm;
class A B:
model y = A|B /ss3;
Fig. 1(ii): Contrast Statements
/*Contrast statements*/
proc glm;
class A B:
model y = A|B /ss3;
contrast 'Linear A × Linear B' A * B 3 0 -3 1 0 -1 -1 0 1 -3 0 3;
contrast 'Linear A × Quadratic B' A * B -3 6 -3 -1 2 -1 1 -2 1 3 -6 3;
contrast 'Quadratic A × Linear B' A * B -1 0 1 1 0 -1 1 0 -1 -1 0 1;
contrast 'Quadratic A × Quadratic B' A * B 1 - 2 1 - 1 2 - 1 - 1 2 - 1 1 - 2 1;
```

			Quantitative factors of procedure		
Dependent variable:	Υ	THE GL	n procedure		
Source	DF	Sum of squares	Mean squares	F value	Pr > F
Model	11	173003.6059	15727.6005	17.62	<0.0001
rror	24	21419.8748	892.4948		
Corrected total	35	194423.48.07			
	R-Square	Coeff. Var.	Root MSE	Y mean	
	0.889829	9.698180	29.87465	3.8.0439	
Source	DF	Type III SS	Mean square	F value	Pr > F
	3	48038.59334	16012.86445	17.94	<0.0001
	2	97474.62894	48737.31447	54.61	<0.0001
A*B	6	27490.38357	4581.73060	5.13	0.0016
Contrast	DF	Contrast SS	Mean square	F value	Pr > F
in Ax Lin B	1	18882.98497	18882.98497	21.16	0.0001
in A x Quad B	1	3501.57656	3501.57656	3.92	0.0592
in Ax Lin B	1	109.52554	109.52554	0.12	0.7292
Lin A x Quad B	1	4947.30623	4947.30623	5.54	0.0271

is also important. Reversing the order from (A B) to (B A) necessitates changing the order of the coefficients in the CONTRAST statements.

To illustrate, let us suppose that now factor A is quantitative and factor B is qualitative and suppose we wish to calculate the (B x Linear A)SS. A set of CLASS and CONTRAST statements to be used is given in Fig. 6. Thus, we use:

#### class B A;

contrast 'Linear A at  $B_1$ ' A -3 -1 1 3 A\*B -3 -1 1 3; contrast 'Linear A at  $B_2$ ' A -3 -1 1 3 A\*B 0 0 0 0 -3 -1 1 3;

and so on. Or, we can use:

#### class A B:

contrast 'Linear A at B1' A-3-1 1 3 A\*B-3 0 0-1 0 0 1 0 0 3 0 0; (7) contrast 'Linear A at B2' A -3 -1 1 3  $\,$  A\* B 0 -3 0 0 -1 0 0 1 0 0 3 0;

and so on. However, the following will not work:

#### class A B;

contrast 'Linear A at B<sub>1</sub>' A -3 -1 1 3 A\*B -3 -1 1 3;

since the order of (A B) in the class statement is incorrect for this format of the CONTRAST weights. To see this, we refer to the matrix of weights appropriate to the Linear B at A<sub>1</sub> contrast in Table 4, when the "class A B," statement is used. It is immediately clear that the row vector C of weights (from equation (4)) produces the CONTRAST statement (7) above.

How a reversal of the factors in the CLASS statement affects the program when both factors are quantitative, if at all, is left as an exercise for the reader.

**Three or more factors:** The same principles used in the previous sections apply when there are three or more

factors, with each factor either qualitative or quantitative. We illustrate this briefly for the case where all factors are quantitative and where one factor is qualitative and two factors are quantitative factors. Suppose all factors have three levels.

When all three factors are quantitative, the methods of both factors quantitative apply. Suppose we want to find the contrast Linear A x Linear B x Linear C. Thus, we need to calculate A'#B#Cı. The weights for Linear A x Linear B are first calculated, as shown in both factors quantitative, i.e., A'#Bı = (1, 0,-1, 0, 0, 0,-1, 0, 1). These in turn are multiplied by the linear C weights Cı = (-1,0,1) again as shown in Eq. (4); see Table 5. Therefore, the CLASS and CONTRAST statements are:

#### class A B C;

contrast 'Linear A x Linear B x Linear C' A\*B\*C
-1 0 1 0 0 0 1 0 -1 0 0 0 0 0 0 0 0 1 0 -1 0 0 0 -1 0 1;

Consider now the case where factor A is qualitative and each of B and C is a quantitative factor. Suppose in particular we want to calculate the A x Linear B x Linear C contrast. Then, the appropriate linear weights are

#### Fig. 2(i): Contrast Statements

/\* Qualitative x Quantitative Model. Interaction Components \*/
/\* A is QUALitative, B is QUANTitative\*/
[Data input, etc., statements]
proc glm;
class A B;
model y = A|B /ss3;
contrast 'Linear B' B - 1 0 1;
contrast 'Linear B at A1' B - 1 0 1 A \* B - 1 0 1;
contrast 'Linear B at A2' B - 1 0 1 A \* B 0 0 0 - 1 0 1;
contrast 'Linear B at A3' B - 1 0 1 A \* B 0 0 0 0 0 0 - 1 0 1;
contrast 'Linear B at A3' B - 1 0 1 A \* B 0 0 0 0 0 0 0 0 0 - 1 0 1;

Fia.	2(ii):	SAS	out	put

Fig. 2(ii): SAS out pu	ıt				
		Qualitative x 0	Quantitative factors		
		The GLI	M procedure		
Dependent variable:	Υ				
Source	DF	Sum of squares	Mean squares	F value	Pr > F
Model	11	173003.6059	15727.6005	17.62	<0.0001
Errot	24	21419.8748	892.4948		
Corrected total	35	194423.48.07			
	R-Square	CoeffVar	Root MSE	Y mean	
	0.889829	9.698180	29.87465	3.8.0439	
Source	DF	Type III SS	Mean square	F value	Pr > F
Α	3	48038.59334	16012.86445	17.94	<0.0001
В	2	97474.62894	48737.31447	54.61	<0.0001
A*B	6	27490.38357	4581.73060	5.13	0.0016
Contrast	DF	Contrast SS	Mean square	F value	Pr > F
Linear B	1	91472.74954	91472.74954	102.49	<0.0001
Linear B @ A1	1	4192.85535	4192.85535	4.70	0.0403
Linear B @ A2	1	12956.76540	12956.76540	14.52	0.0009
Linear B @ A3	1	31737.91740	31737.91740	35.56	<0.0001
Linear B @ A4	1	61582.29660	61582.29660	69.00	<0.0001

#### Fig. 3: Computing the A x Linear B Contrast Statistics

```
/* Qualitative X Quantitative Model. Interaction Components */
/* A is QUALitative, B is QUANTitative*/
[Data input, etc., statements, see Table 2(I)]
proc glm data=one outstat=junk;
[Other statements for Ax Linear B contrast components, see Table 5(I)]
/* To compute (A x Linear B)MS */
data three:
set junk end=last;
title 'Ax Linear B Contrast':
retain div dfE dfA sum 0;
                                                                                                        /* Calculates Error MS */
if N = 1 then div = SS/DF:
else div = div +0;
if N = 1 then dfE = DF;
else dfE =dfE + 0;
                                                                                                        /* Keeps Error DF as dfE */
if N = 2 then dfA = DF;
else dfA =dfA + 0;
                                                                                                        /* Keeps A DF as dfA */
if N < 5 then SS = 0:
else if N = 5 then SS = -SS;
sum = sum + SS;
                                                                                                        /* Calculates (A × Linear B)SS */
/* To retain the contrast needed and to find the F- and P - statistics */
                                                                                                        /* Or, 'if N = 9 then do,' */
if last then do:
        output;
        MS = sum/dfA;
                                                                                                     /* Calculates (A × Linear B)MS */
        F = MS/div;
                                                                                                      /* Calculates (A × Linear B) F-value */
        p = 1 - probf(F, dfA, dfE);
                                                                                                        /* Calculates (A × Linear B) P-value */
        file print;
        put '(A × Linear B)SS = 'sum 10.4;
                                                                                                     /*Keep 4 decimal places */
        put '(A × Linear B)MS = 'MS 10.4;
        put 'F-value = 'F 8.4;
        put ' P-value = ' p 6.5;
end:
run;
```

 $B_i = C_i =$  (-1, 0, 1). Hence, first, from (4), the weights for Linear B × Linear C become B'#C<sub>i</sub> = (1, 0,-1, 0, 0, 0,-1, 0, 1) in the analogous manner to that described in both factors quantitative and Table 2(i). Since A is a qualitative factor, then these weights are applied at each level of A analogously to that described earlier for one each qualitative and quantitative factors.

The CLASS and CONTRAST statements become: class A B C;

contrast 'Linear B x Linear C' B\*C 1 0 -1 0 0 0 -1 0 1; contrast 'Linear B x Linear C@A<sub>1</sub>' B\*C 1 0 -1 0 0 0 -1 0 1 A\*B\*C 1 0 -1 0 0 0 -1 0 1;

contrast 'Linear B x Linear C@A<sub>2</sub>' B\*C 1 0 -1 0 0 0 -1 0 1 A\*B\*C 0 0 0 0 0 0 0 0 1 0 -1 0 0 0 -1 0 1;

contrast 'Linear B×Linear C @ A<sub>3</sub>' B\*C 1 0 -1 0 0 0 -1 0 1 A\*B\*C 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 -1 0 1;

Then, the different interaction component contrasts are used to find the overall (A  $\times$  Linear B  $\times$  Linear C)SS and hence the relevant F- and P- values as shown for the qualitative and quantitative factors. We leave as an exercise how the alternative code of Appendix A might be written for the case that all factors have three levels.

As a final comment, we remind ourselves that the weights in the various contrasts herein (e.g., Equation

(1) are those for equal replications per treatment. When there are unequal replications, Equation (1) becomes:

Table 4: Factor a quantitative and factor b qualitative weight matrix. Linear A at B<sub>1</sub>

	<b>B</b> 1	<b>B</b> 2	<b>B</b> 3
Linear A	1	0	0
-3	-3	0	0
-1	-1	0	0
1	1	0	0
3	3	0	0
		Δ΄:#R:	

Table 5: Factors A, B, C quantitative weight matrix. Linear A × Linear B × Linear C

Line	ear C			L	inear A	× Line	ear B-			
		1	0	-1	0	0	0	-1	0	1
<b>C</b> 1	-1	-1	0	1	0	0	0	1	0	-1
<b>C</b> 2	0	0	0	0	0	0	0	0	0	0
Сз	1	1	0	-1	0	0	0	-1	0	1
		A´ı#Bı#Cı								

Fig. 4(i): OUTSTAT data program

/\* Qualitative × Quantitative Model. Interaction Components \*/

 $/^*$  A is QUALitative, B is QUANTitative $^*$ /

[Data input and PROC GLM statements]

/\* To print outstat data \*/

proc print data=junk,

title 'Outstat data from PROC GLM',

run,

Fig. 4(ii) (continued): SAS OUTSTAT Output

Outstat data from PROC GLM - [N\_NAME SOURCE TYPE]

OBS	NAME	SOURCE	TYPE	DF	SS	F	PROB
1	Υ	ERROR	ERROR	24	21419.87		
2	Υ	Α	SS3	3	48038.59	17.942	0.000003
3	Υ	В	SS3	2	97474.63	54.608	0.000000
4	Υ	Α×Β	SS3	6	27490.38	5.134	0.001612
5	Υ	Linear B	CONTRAST	1	91472.75	102.491	0.000000
6	Υ	Linear B at A1	CONTRAST	1	4192.86	4.698	0.040343
7	Υ	Linear B at A2	CONTRAST	1	12956.77	14.517	0.000850
8	Υ	Linear B at A3	CONTRAST	1	31737.92	35.561	0.000004
9	Υ	Linear B at A4	CONTRAST	1	61582.30	69.000	0.000000

Fig. 5: SAS Output for A × Linear B Contrast Statistics

(A x Linear B)SS = 18997.085 (A x Linear B)MS = 6332.362 F = 7.0951 P = 0.00141

Fig. 6: Reversing the CLASS Statements

/\* Qualitative × Quantitative Model. Interaction Components \*/

/\* A is QUALitative and B is QUANTitative \*/

[Data input, etc. statements]

/\* To compute the B × Linear B \*/

proc glm data=one outstat=junkl;

class A B;

model y = A|B /ss3;

contrast 'Linear A' A -3 -1 13;

contrast 'Linear A at Bl' A - 3 - 1 1 3 A  $^{\star}$  B - 3 0 0 - 1 0 0 1 0 0 3 0 0;

contrast 'Linear A at B2' A -3 -1 1 3 A \* B 0 -3 0 0 -1 0 0 1 0 0 3 0; contrast 'Linear A at B3' A -3 -1 1 3 A \* B 0 0 -3 0 0 -1 0 0 1 0 0 3;

run;

/\*OR, Alternatively: \*/

proc glm data=one outstat=junkl;

class BA;

model y = A|B /ss3;

contrast 'Linear A' A -3 -1 1 3;

contrast 'Linear A at Bl' A - 3 - 1 1 3 A \* B - 3 - 1 1 3;

contrast 'Linear A at B2' A -3 -1 1 3 A \* B 0 0 0 0 -3 -1 1 3;

contrast 'Linear A at B3' A -3 -1 1 3 A  $^{\ast}$  B 0 0 0 0 0 0 0 -3 -1 1 3;

run;

$$z = \sum_{i=1}^k r_i \omega_i T_i \ \ with \ \sum_{i=1}^k r_i \omega_i = 0$$

where,  $r_1$  is the number of replications for treatment  $T_1$ . For example, if k = 3 with  $r_1 = 3$ ,  $r_2 = 3$ ,  $r_3 = 2$ , then the  $\omega_1$  weights of Eq. (2), for  $B_1$ , become  $W = (-1/3, 0, \frac{1}{2})$ . Likewise, the other contrast statements can be suitably adjusted.

#### **RESULTS AND DISCUSSION**

The interpretation of the various interaction components can be facilitated by reference to Fig. 7 which shows a surface plot of the means at each combination of the levels of A (Arg) and B (Met). For example, the analysis of the contrast interaction A x Linear B revealed this to be a significant component (P = 0.001, Fig. 5. This tells us that there is a significant linear trend in B (Met) across the different levels of A (Arg) and that this trend is different for different levels of A (Arg). These differences

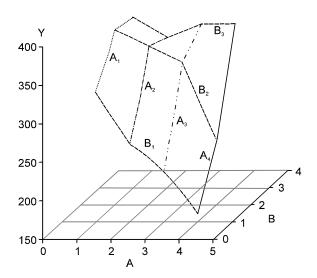


Fig. 7: Response Surface for Factors A and B

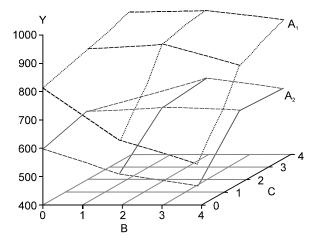


Fig. 8: Response Surfaces for B and C at Ai, I = 1, 2

for different A values are clearly evident in Fig. 7. Note that depending on the data, it can be that there is no significant linear trend in B, but there are significant A  $\times$  Linear B components (Shim *et al.*, 2014). Likewise, were we to consider the factor A as a quantitative factor and B as a qualitative factor (see discussion on "Not to

#### APPENDIX A

#### Alternative code for A × Linear B Contrasts:

#### Alternative code for A × Quadratic B Contrasts:

#### Alternative code for A × Linear B × Linear C Contrasts:

class A B C; model Y = A|B|C/ss3;

contrast 'A\*Linear B\*Linear C' A\*B\*C 1 0-1 0 0 0-1 0 1-1 0 1 0 0 0 1 0-1,

A\*B\*C10-1000-10100000000-10100010-1;

### Appendix B SAS Output: A x Linear B and A x Quadratic B Contrasts

Qualitative x Quantitative Example The GLM Procedure Dependent variable: Y Source DF Sum of squares Mean squares F value Pr > F Model 173003.6059 15727.6005 17.62 <0.0001 11 Errot 24 21419.8748 892.4948 Corrected total 35 194423.48.07 R-Square Coeff Var Root MSE Y mean 0.889829 9.698180 29.87465 3.8.0439 Source DF Type III SS Mean square F value Pr > F 3 48038.59334 16012.86445 17.94 < 0.0001 В 97474.62894 48737.31447 <0.0001 2 54.61 27490.38357 4581 73060 0.0016 A\*B 6 5 13 DF Contrast SS F value Pr > F Contrast Mean square Linear B 91472.74954 91472.74954 102.49 <0.0001 4192.85535 0.0403 Linear B @ A1 4192 85535 4 70 1 Linear B @ A2 12956.76540 12956.76540 14.52 0.0009 Linear B @ A3 31737.91740 31737.91740 35.56 < 0.0001 1 Linear B @ A4 61582.29660 61582.29660 <0.0001 69 00 (A\*Linear B Contrast)SS = 18997.0852 (A\*Linear B Contrast)MS = 6332.3617 F = 7.0951246503 p-value = 0.0014129691 A x Quadratic B Contrasts Contrast DF Contrast SS Pr > FMean square F value Linear B 6001.879401 6001.879401 6.72 0.0159 Linear B @ A1 2002 812050 0.1472 2002.812050 2 24 Linear B @ A2 6833.584356 6833.584356 0.0107 7.66 4243.661356 Linear B @ A3 4243.661356 0.0393 4 75 1 Linear B @ A4 1415.120000 1415.120000 1.59 0.2201 (A\*Linear B Contrast)SS = 8493.2984 (A\*Linear B Contrast)MS = 2831.0995 F = 3.1721187688 p-value = 0.0425295631

Confuse the Issue ... But"), then the dotted lines corresponding to the three levels of B suggest that there is a different trend line across the levels of A. Indeed, in this case, the B x Linear A component has a significant value (P<0.001) and also there is a significantly different quadratic trend across A for the differing levels of B (P = 0.043). That is, the linear trend of Arg across the levels of Met is significant and the quadratic trend across Arg for the differing levels of Met is significant at P<0.05 and different for differing levels of Met.

When there are one qualitative and two quantitative variables, the surfaces will be as in the example of Fig. 8. The data for this design were extracted from Chamruspollert et al. (2004) and consists of two levels (25, 35°C) of a qualitative factor A (Temperature) and three levels (1.52, 2.52, 3.52% and 0.35, 0.55, 0.75%) for each of quantitative factors B (Arg) and C (Met). Note that although A is actually a quantitative factor, when there are only two levels, it is better handled as a qualitative factor (as herein), unless there is prior evidence to support only a linear component across the two levels of A. For the purposes of this illustration, it is assumed the design here is a standard factorial design. For these data, the visual suggestion that the Linear B x Linear C interaction differs for the two levels of A is corroborated by the statistical analysis for which P<0.001. In these kinds of designs, the surfaces correspond to the different level of A (Ai, i = 1 ,..., r). There are linear surfaces across the levels of Arg and Met combinations, but this surface is different for different values of temperature. In this case, if there is a significant difference (P<0.05) in the A x Linear B x Linear C interaction component, then these surfaces will assume different 'shapes'.

As illustrated by Shim *et al.* (2014), there are multiple ways of using SAS and other statistical software packages to analyze experimental data. The approaches

illustrated here are capable of extracting more information and lead to more insightful interpretations, than are usually presented by researchers. Complex experiments with multiple input factors are becoming increasingly important as poultry producers seek to balance multiple factors to maximize performance and profits while trying to minimize environmental impacts. Going the extra steps illustrated here should aid researchers and producers in properly interpreting trials where multiple factors influence productivity.

Finally, this paper has illustrated one way showing how the SAS (SAS Institute, 2006) package can be adapted to obtain these interaction components; other ways (such as use of the difficult macro and/or proc iml methods) can be developed, using the same principles. Adaptation to other packages including later versions of SAS can also be based on these principles.

#### REFERENCES

- Chamruspollert, M., G.M. Pesti and R.I. Bakalli, 2002. Dietary interrelationships among arginine, methionine and lysine in young broiler chicks. Br. J. Nutr., 88: 655-660.
- Chamruspollert, M., G.M. Pesti and R.I. Bakalli, 2004. Chick responses to dietary arginine and methionine levels at different environmental temperatures. Br. Poult. Sci., 45: 93-100.
- SAS Institute, 2006. SAS User's Guide: Statistics. Version 9.1.3 ed. SAS Inst. Inc., Cary, NC.
- Shim, M.Y., L. Billard and G.M. Pesti, 2014. Experimental design and analysis with emphasis on communicating what has been done: I) A comparison of statistical models using general linear model with SAS. Int. J. Poult. Sci., 13: 76-87.