NUTRITIONAL TRIALS AND DATA ANALYSIS

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STATISTICS

- Research tool
- Deals with the collection, organization, analysis, and interpretation of data.
- Useful in drawing meaningful conclusions from a set of data
- You can make the data look the way you like, if you know how to do it (misuse)
- Should know why you are using?
- Should have focused questions to answer rather than reporting all possible relationships among all possible treatments

ANOVA

Analysis of Variance / Partitioning of variance

Understanding Variance:

- All individuals in a population are not similar
- They differ from each other.
- Population forms a bell shape curve
- We want to know whether this dissimilarity (variation) is a chance variation or otherwise
- Inherent variation of due to other factors
- Proportion of variation due to known variables is analysed

NUTRITIONAL TRIALS

- **Growth Trials**
- **Production Trials**
- **Testing different treatments on any other aspect(s)**

EXPERIMENTAL DESIGNS

- **Completely Randomized Designs (CRD)**
- **Randomized Complete Block Designs (RCBD)**
- **Latin Square Designs (LSD)**
- **Factorial Experiments**

LIMITATIONS OF EACH DESIGN

- **CRD**

  When experimental units are homogenous

  Have less variation

  Randomization carried out using Random Number Tables

- **RCBD**

  when experimental units can meaningfully grouped

  Such groups are called blocks

- **LSD**

  Double grouping

  Where two major sources of variation are present

ANOVA for CRD

When we have 4 treatments and 4 replicates

Source of variation	Degree of freedom	Degree of freedom
Treatment	(t-1)	3
Error	t(r-1)	12
Total	(n-1)	15

ANOVA for RCBD

When we have 4 treatments and 4 replicates

Source of variation	Degree of freedom	Degree of freedom
Treatment	(t-1)	3
Blocks	(b-1)	3
Error	(t-1)(b-1)	9
Total	(n-1)	15

ANOVA for 2 x 2 factorial arrangement

When we have 4 treatments and 4 replicates

Source of variation	Degree of freedom	Degree of freedom
Treatment	(t-1)	3
Factor A	(a-1)	1
Factor B	(b-1)	1
A x B	(a-1)(b-1)	1
Error	ab(r-1)	12
Total	(n-1)	15

ANOVA for factorial experiment

Two factor factorial 2 x 2 with 12 replicates each

Source of variation	Degree of freedom	Degree of freedom
Factor A	(a-1)	1
Factor B	(b-1)	1
Interaction AB	(a-1)(b-1)	1
Error	ab(r-1)	44
Total	(n-1)	47

ANOVA for Latin Square Design

When we have 4 treatments and 4 replicates

Source of variation	Degree of freedom	Degree of freedom
Treatments	(r-1)	3
Blocks (animals)	(r-1)	3
Periods	(r-1)	3
Error	(r-1)(r-2)	6
Total	(n-1)	15

ANOVA for Latin Square Design

Four treatments and 4 replicates with 2 x 2 factorial arrangement

Source of variation	Degree of freedom	Degree of freedom
Treatments	(r-1)	3
Factor A Factor B A x B	(a-1) (b-1) (a-1)(b-1)	1 1 1
Blocks (animals)	(r-1)	3
Periods	(r-1)	3
Error	(r-1)(r-2)	6
Total	(n-1)	15

Example I

Effect of bST and Enzose on DMI and production performance of buffaloes

Abu Bakar Sufyan’s MSc Data

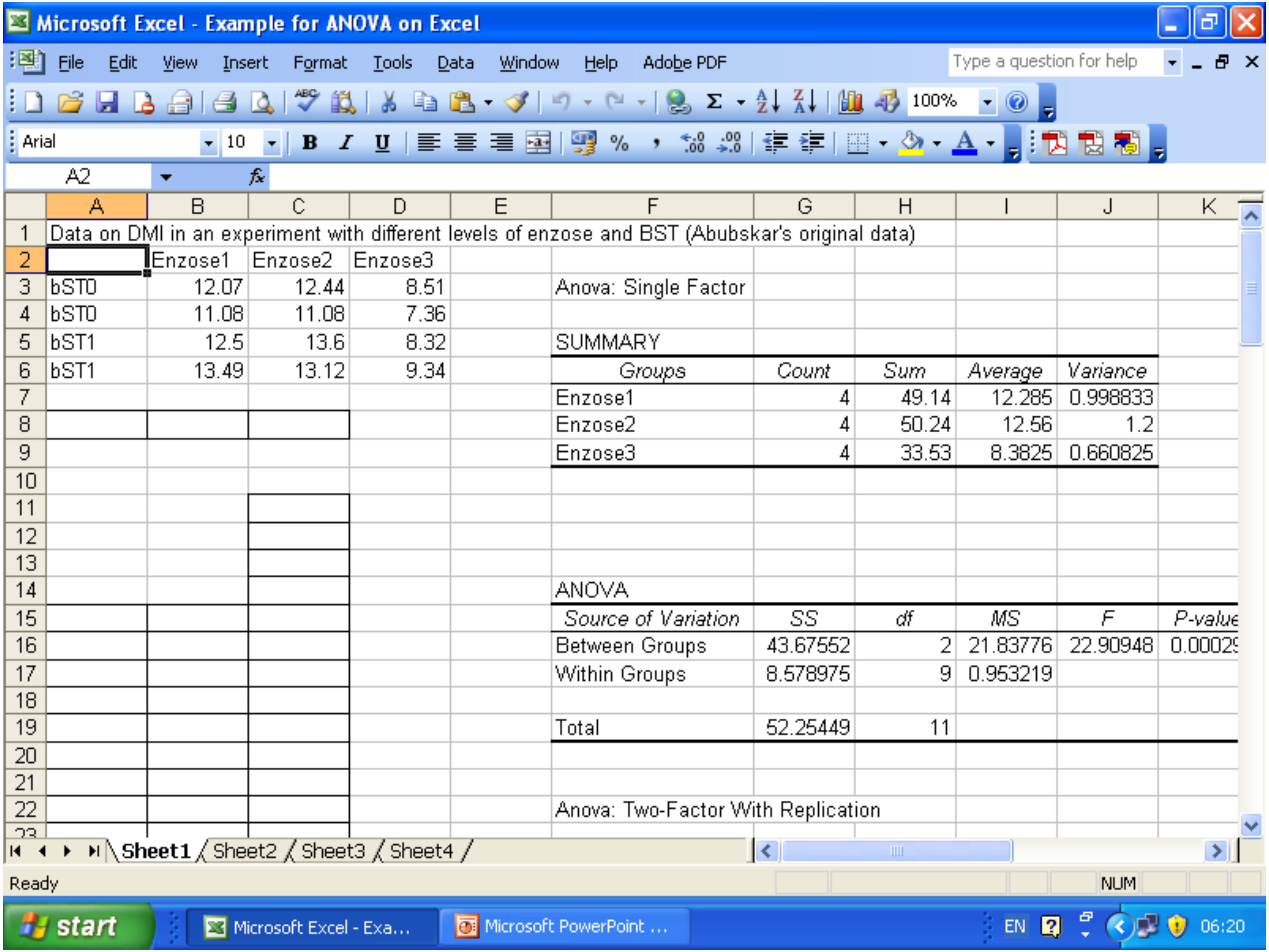
Two levels of bST: bST0 and bST1 (250 mg)

Three levels of Enzose: ENZ1, ENZ2 and ENZ3 (0,20,40)

Two replicates per treatment

Data analysis

2 x 2 factorial arrangements



Data on DMI in an experiment with different levels of enzose and BST (Abubskar's original data)

	Enzose1	Enzose2	Enzose3
bST0	12.07	12.44	8.51
bST0	11.08	11.08	7.36
bST1	12.5	13.6	8.32
bST1	13.49	13.12	9.34

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Enzose1	4	49.14	12.285	0.998833
Enzose2	4	50.24	12.56	1.2
Enzose3	4	33.53	8.3825	0.660825

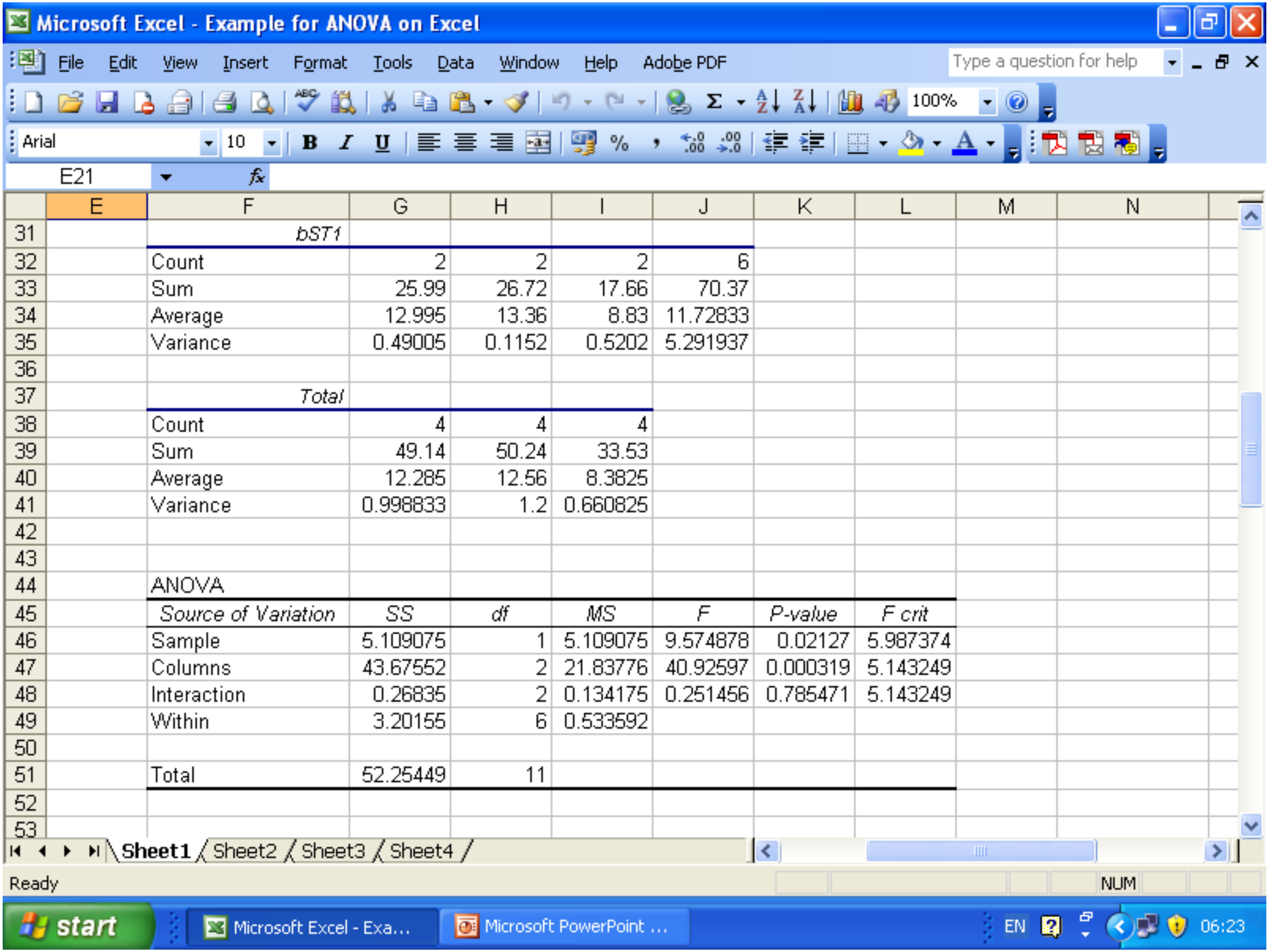
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	43.67552	2	21.83776	22.90948	0.00029
Within Groups	8.578975	9	0.953219		
Total	52.25449	11			

Anova: Two-Factor With Replication

Anova: Two-Factor With Replication

SUMMARY	Enzose1	Enzose2	Enzose3	Total
bST0				
Count	2	2	2	6
Sum	23.15	23.52	15.87	62.54
Average	11.575	11.76	7.935	10.42333
Variance	0.49005	0.9248	0.66125	4.137147
bST1				
Count	2	2	2	6
Sum	25.99	26.72	17.66	70.37
Average	12.995	13.36	8.83	11.72833
Variance	0.49005	0.1152	0.5202	5.291937
Total				
Count	4	4	4	
Sum	49.14	50.24	33.53	
Average	12.285	12.56	8.3825	
Variance	0.998833	1.2	0.660825	



Microsoft Excel - Example for ANOVA on Excel

	bST1			
Count	2	2	2	6
Sum	25.99	26.72	17.66	70.37
Average	12.995	13.36	8.83	11.72833
Variance	0.49005	0.1152	0.5202	5.291937

Total			
Count	4	4	4
Sum	49.14	50.24	33.53
Average	12.285	12.56	8.3825
Variance	0.998833	1.2	0.660825

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	5.109075	1	5.109075	9.574878	0.02127	5.987374
Columns	43.67552	2	21.83776	40.92597	0.000319	5.143249
Interaction	0.26835	2	0.134175	0.251456	0.785471	5.143249
Within	3.20155	6	0.533592			
Total	52.25449	11				

SAS - [PROGRAM EDITOR - Abubakar Data PROC GLM running]

```
Data one;
Input ENLEVEL$ BSTLEVEL$ DMI;
cards;
Enzose1      bST0      12.07
Enzose1      bST0      11.08
Enzose1      bST1      12.5
Enzose1      bST1      13.49
Enzose2      bST0      12.44
Enzose2      bST0      11.08
Enzose2      bST1      13.6
Enzose2      bST1      13.12
Enzose3      bST0      8.51
Enzose3      bST0      7.36
Enzose3      bST1      8.32
Enzose3      bST1      9.34
;
Proc GLM;
Class ENLEVEL BSTLEVEL;
Model DMI = ENLEVEL BSTLEVEL ENLEVEL*BSTLEVEL;
means ENLEVEL BSTLEVEL ENLEVEL*BSTLEVEL /duncan;
lsmeans ENLEVEL BSTLEVEL ENLEVEL*BSTLEVE/stderr;
Title 'Stat Analysis using factorial arrangements';
run;
```

NOTE: 23 Line(s) recalled.

SAS - [OUTPUT - (Untitled) PROC GLM running]

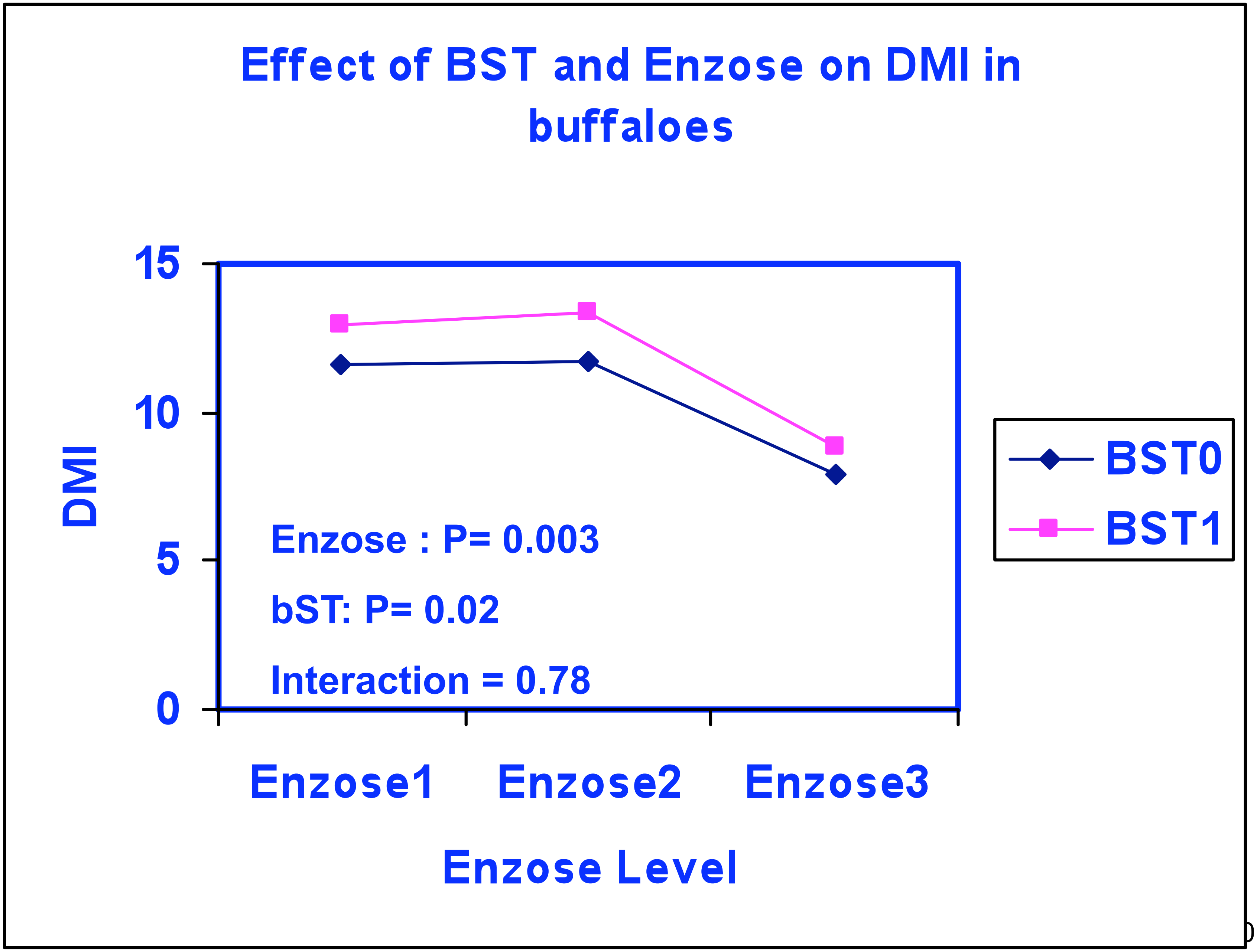
Dependent Variable: DMI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	49.05294167	9.81058833	18.39	0.0014
Error	6	3.20155000	0.53359167		
Corrected Total	11	52.25449167			

R-Square	C.V.	Root MSE	DMI Mean
0.938732	6.595202	0.7304735	11.07583333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ENLEVEL	2	43.67551667	21.83775833	40.93	0.0003
BSTLEVEL	1	5.10907500	5.10907500	9.57	0.0213
ENLEVEL*BSTLEVEL	2	0.26835000	0.13417500	0.25	0.7855

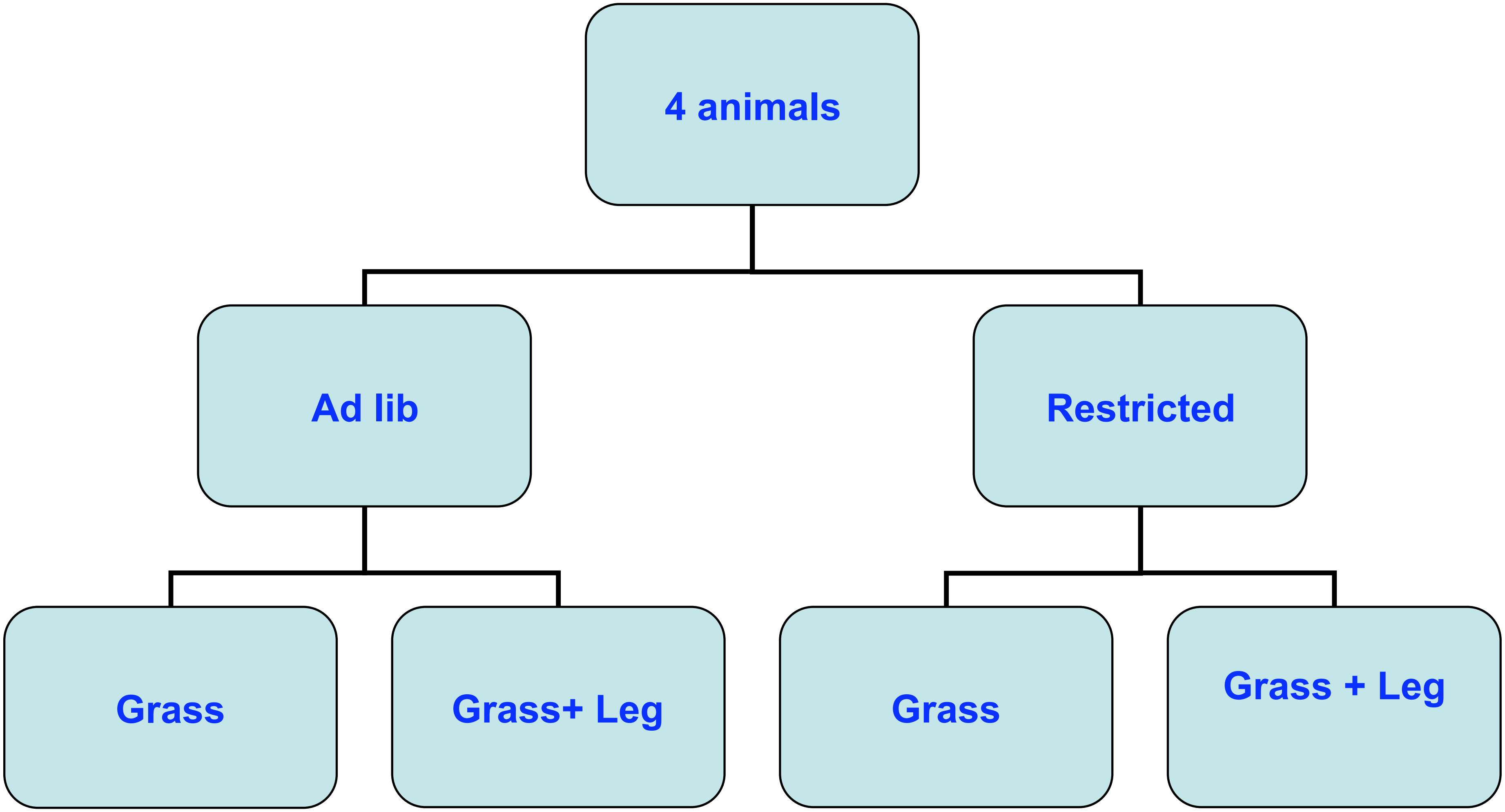
Source	DF	Type III SS	Mean Square	F Value	Pr > F
ENLEVEL	2	43.67551667	21.83775833	40.93	0.0003
BSTLEVEL	1	5.10907500	5.10907500	9.57	0.0213
ENLEVEL*BSTLEVEL	2	0.26835000	0.13417500	0.25	0.7855



Effect of BST and Enzose on DMI in buffaloes
15
10
5
0
DMI
Enzose : P= 0.003
bST: P= 0.02
Interaction = 0.78
BST0
BST1
Enzose1
Enzose2
Enzose3
Enzose Level

Example II

Effect of Intake level and forage source on kinetics of fibre digestion



4 x 4 Latin Square design

2 x 2 factorial Arrangement

Factor I: Forage Source

Factor II: Intake level

Model Statement

```
proc glm;
class anim per trmt;
model dm = anim per trmt;
contrast 'grass vs leg+grass' trmt +1 -1 +1 -1;
contrast 'restrict vs ad lib' trmt +1 +1 -1 -1;
contrast 'interaction' trmt +1 -1 -1 +1;
lsmeans trmt/stderr;
means trmt/duncan;
run;
```

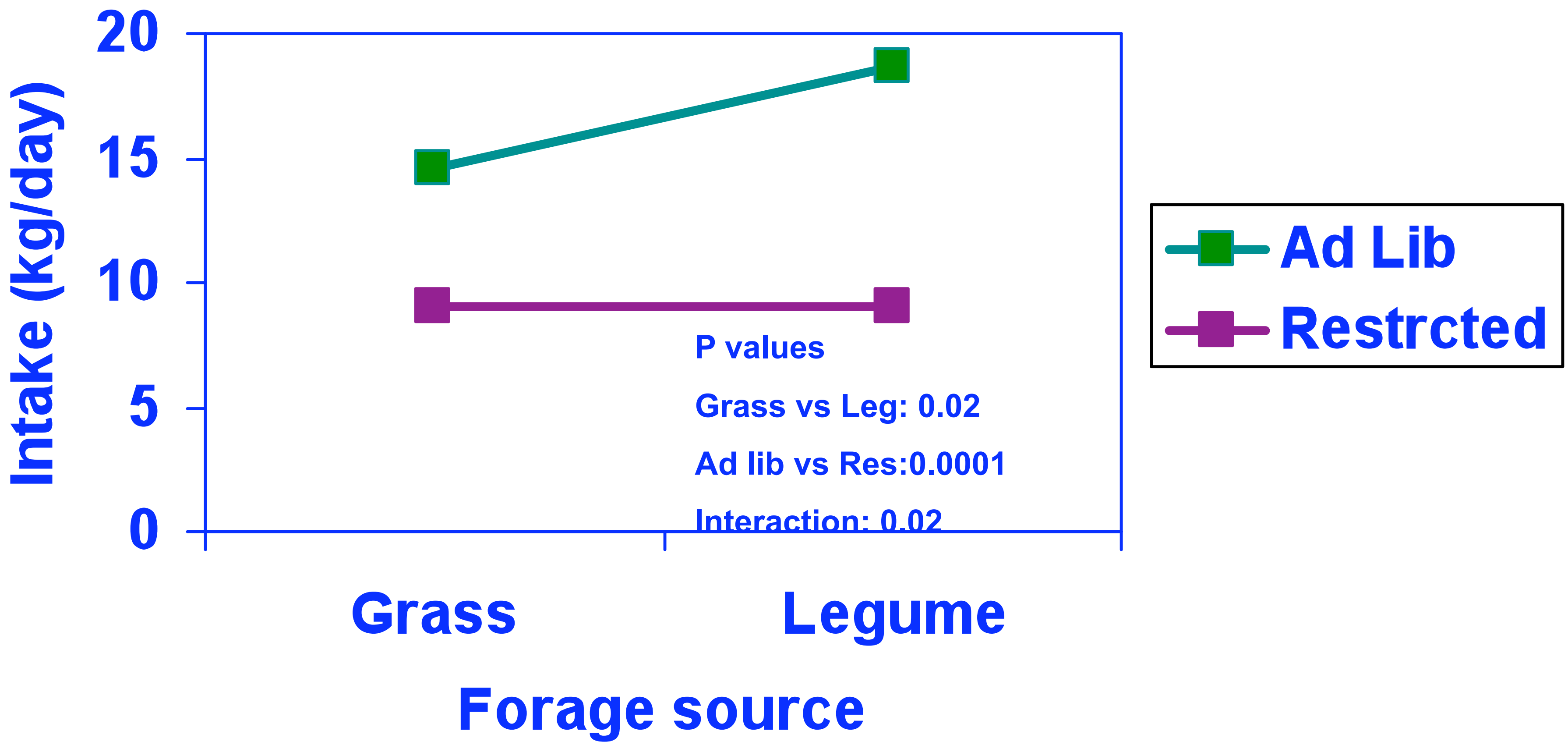
File: Latin Square Factorial Intake

SAS - [PROGRAM EDITOR - Latin square Factorial Intake]

```
8 4 AG 13.6625 12.34536 11.16585 8.853950 5.608422 0.833356 4.775066 3.245529
8 1 AGA 17.625 16.04820 14.92092 10.13611 6.606400 1.206767 5.399634 3.529713
7 2 AGA 22.55 20.54347 19.08752 12.78322 8.538755 1.507238 7.031517 4.244469
6 3 AGA 20 18.15679 16.83420 11.15296 7.353434 1.305841 6.047593 3.799528
5 4 AGA 22.3 20.00082 17.95293 12.82811 8.357711 1.405049 6.952662 4.470405
6 1 RG 10 9.127073 8.590611 6.192421 3.992915 0.689663 3.303252 2.199505
5 2 RG 10 9.079198 8.565062 6.240396 4.079563 0.660586 3.418977 2.160832
8 3 RG 10 9.087565 8.521612 6.123438 3.937581 0.637084 3.300497 2.185855
7 4 RG 10 8.916870 8.050991 6.413508 4.066170 0.617652 3.448518 2.347338
5 1 RGA 10 9.093575 8.455232 5.821648 3.785081 0.678893 3.106188 2.036566
8 2 RGA 10 9.062034 8.447763 5.820507 3.839257 0.659415 3.179841 1.981248
7 3 RGA 10 9.049997 8.405873 5.719421 3.742254 0.651482 3.090771 1.977165
6 4 RGA 10 8.921861 8.041454 5.883235 3.810460 0.618828 3.191632 2.072775
;
proc glm;
class anim per trmt;
model dm = anim per trmt;
contrast 'grass vs leg+grass' trmt +1 -1 +1 -1;
contrast 'restrict vs ad lib' trmt +1 +1 -1 -1;
contrast 'interaction' trmt +1 -1 -1 +1;
lsmeans trmt/stderr;
means trmt/duncan;
run;
```

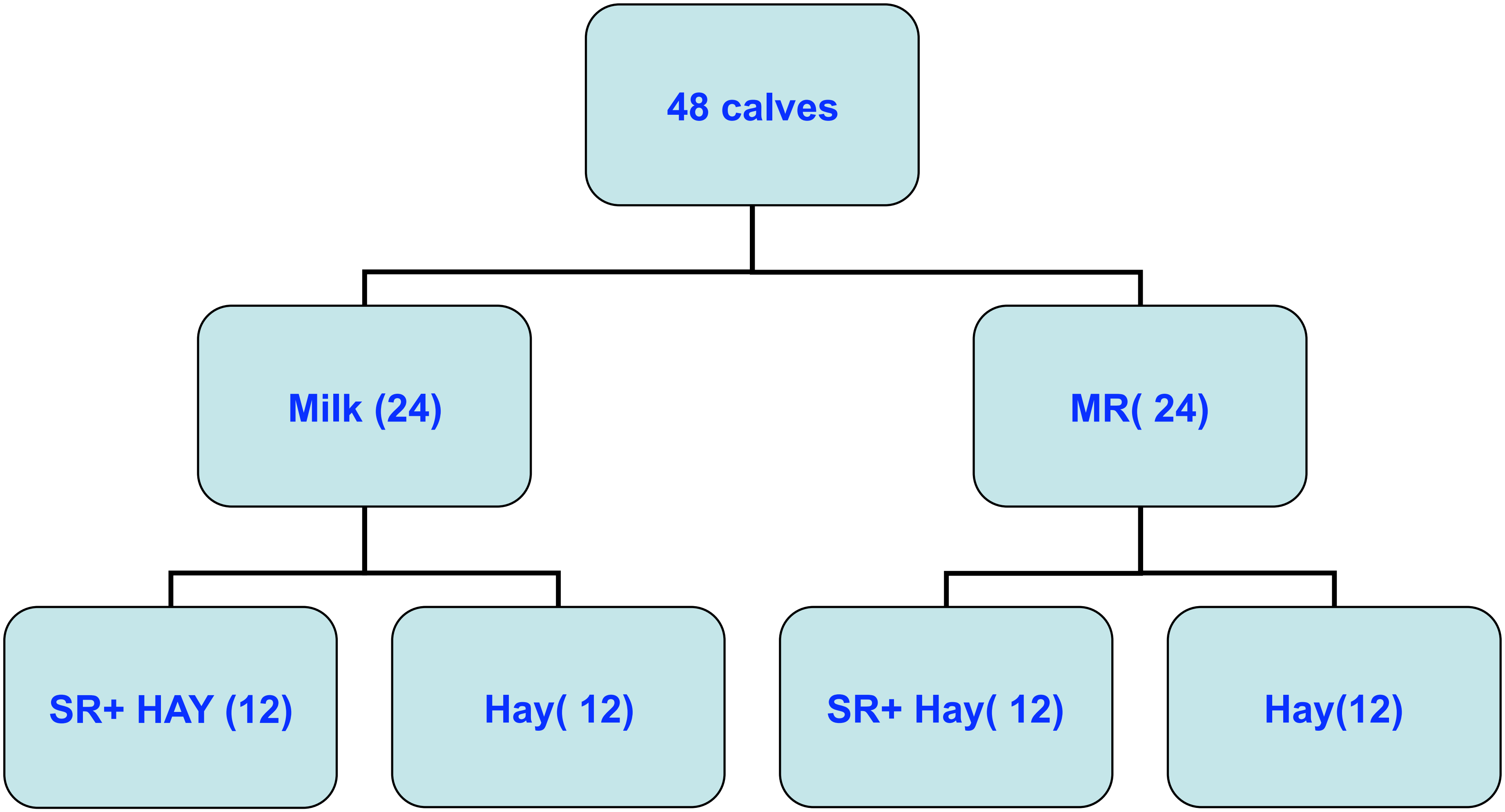
C:\SAS

Effect of feed intake level and forage source on Kinetics of fibre digestion.. in Beef cattle



Example III

Effect of different feeding regimens on the growth performance of Sahiwal Calves



STAT ANALYSIS

DIFFERENT OPTIONS:

CRD

Trtmt I = Milk and SR

Trtmt II = Milk and Hay

Trtmt III = MR and SR

Trtmt IV = MR and Hay

Birth weight as Covariance????

RCBD

Milk and Milk Replacer

Sex as Blocks

CRD

2 x 2 Factorial Arrangement

Factor I: Liquid Diet, Milk vs milk replacer

Factor II: Starter ration+ Hay vs Hay only

MODEL STATEMENTS IN SAS

CRD

Effect of different feeding regimens: milk and MR with or without SR

```
Proc GLM;
Class trt sex;
Model wwt TWGain DWGain TMilk FCR = trt sex bwt;
contrast 'Milk vs CMR' TRT  -1 -1 +1 +1;
contrast 'Fodder Vs Concen' TRT +1 -1 +1 -1;
means  trt sex /duncan;
lsmeans trt sex /stderr;
Title 'stat analysis using CRD';
run;
```

Model Finally used

$$Y_{ijkl} = \mu + F1_i + F2_j + (F1 \times F2)_{ij} + BWT_k + calf_l + e_{ijkl}$$

Model Statement in SAS

```
proc mixed;
class fone ftwo id;
model DWGain = fone|ftwo bwt;
random id(fone*ftwo);
lsmeans fone|ftwo / bylevel om pdiff;
run;
```

File: SWL PI mixed models weight etc.sas

Output STAT mixed models.excel

Performance of Sahiwal calves given different dietary treatments

Parameters	Milk vs MR		SR vs Hay		Milk		MR		F1	F2	F1*F2
	Milk	MR	SR	Hay	SR	Hay	SR	Hay			
Weaning weights (kg)	52±.8	35±.8	49±.8	38±.8	56±1	47±1	40±1	30±1	0.0001	0.0001	0.66
Total weight gain (kg)	30.0±.8	14±.8	26±.8	18±.8	34±1	26±1	18±1	10±1	0.0001	0.0001	0.66
Daily growth rate (g/d)	357±9	162±9	311±9	208±9	401±13	310±13	214±13	115±13	0.0001	0.0001	0.67

GROWTH TRIALS

- Repeated measure analysis
- What does it mean?

Model Statement

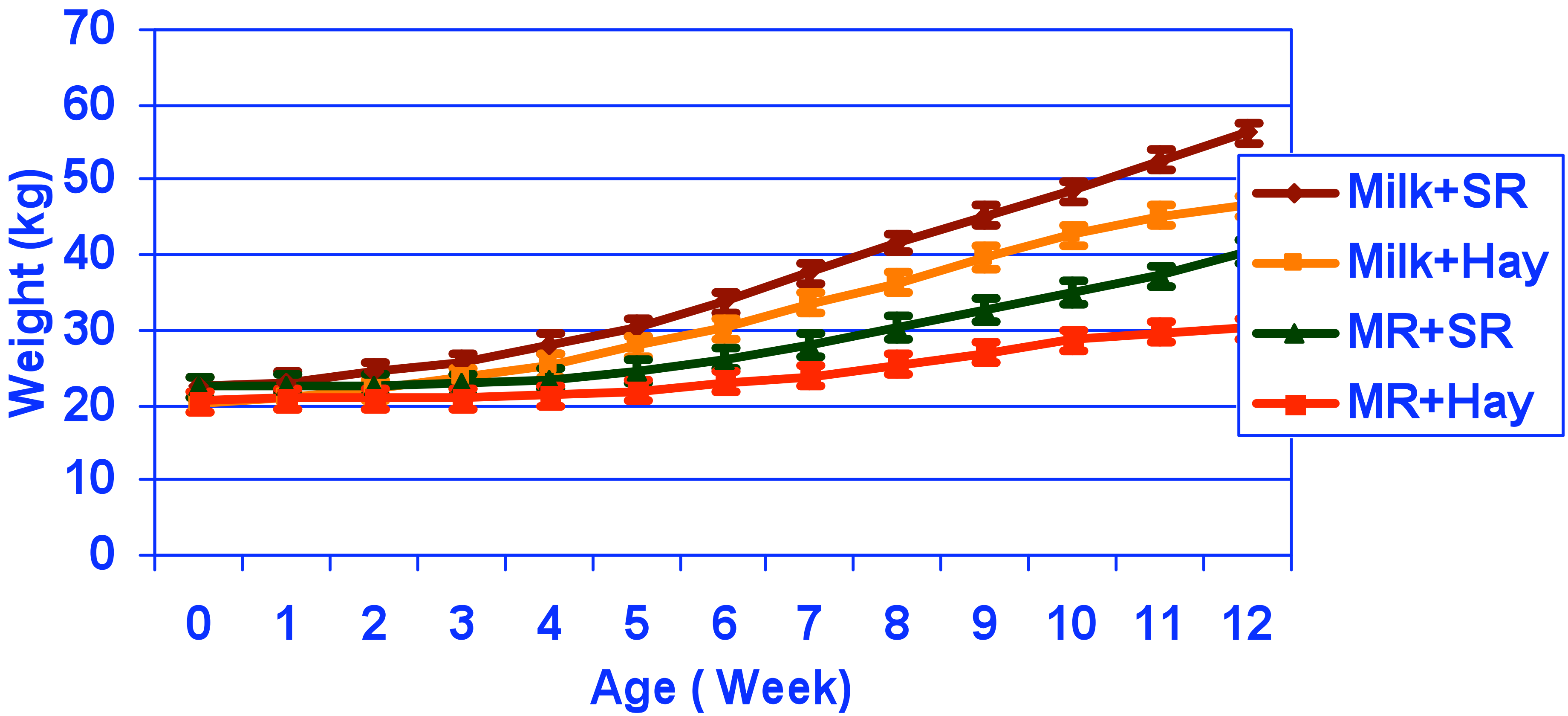
$$Y_{ijklm} = \mu + Sex_i + F1_j + F2_k + W_l + (SEX \times F1 \times F2 \times W)_{ijkl} + Calf_m + e_{ijklm}$$

Model Statement in SAS

```
proc mixed;
class sex fone ftwo id wk;
model wt = sex|fone|ftwo|wk;
random id(fone*ftwo);
repeated wk / sub=id(fone*ftwo) type = ar(1);
lsmeans sex|fone|ftwo|wk / bylevel om pdiff;
run;
```

File: SWL PI mixed model growth curve

Growth Curve of Sahiwal Calves on different pre-weaning dietary regimens



Example IV

Economic feasibility of raising Lohi sheep and Beetal goats for meat production under high input system

Effect of different protein levels on the performance of Lohi Sheep with or without ionophores and Probiotics

Treatments

Fodder

Concentrate

LP MP HP

With or without Ionophores

With or without Probiotics

Treatment plan

Fodder				Ionophores			Probiotics		
	LP	MP	HP	LP	MP	HP	LP	MP	HP

How to analyze this data?

- **Analyze separately: delete Fodder and analyze the rest using 2 x 3 factorial design**
- **Imbalance design?**
- **CRD?**
- **Nested design?**
- **Fodder Vs Concentrate**
- **Ionophores vs probiotics**
- **Concentrate vS Ionophores or Probiotics**
- **Linear Response?**
- **Quadratic Response?**

Model Statement in SAS

- proc glm;
- class trmt;
- model TDMI DMI DMIBW CPI NDFI ADFI TGAIN DGAIN FCR FEEDC ECONO = trmt;
- contrast 'Fodder vs concentrates' trmt +1 +1 +1 +1 +1 +1 +1 +1 +1 -9;
- contrast 'Conc vs I+P' trmt -2 -2 -2 +1 +1 +1 +1 +1 +1 0;
- contrast 'I vs P' trmt 0 0 0 -1 -1 -1 +1 +1 +1 0;
- contrast 'Linear conc' trmt -1 0 +1 0 0 0 0 0 0 0;
- contrast 'Quadratic conc' trmt +1 -2 +1 0 0 0 0 0 0 0;
- contrast 'Linear I' trmt 0 0 0 -1 0 +1 0 0 0 0;
- contrast 'Quadratic I' trmt 0 0 0 +1 -2 +1 0 0 0 0;
- contrast 'Linear P' trmt 0 0 0 0 0 0 -1 0 +1 0;
- contrast 'Quadratic P' trmt 0 0 0 0 0 0 +1 -2 +1 0;
- means trmt/duncan;
- lsmeans trmt/stderr;

- File: Linear Quadratic response

Linear, quadratic and cubic curves

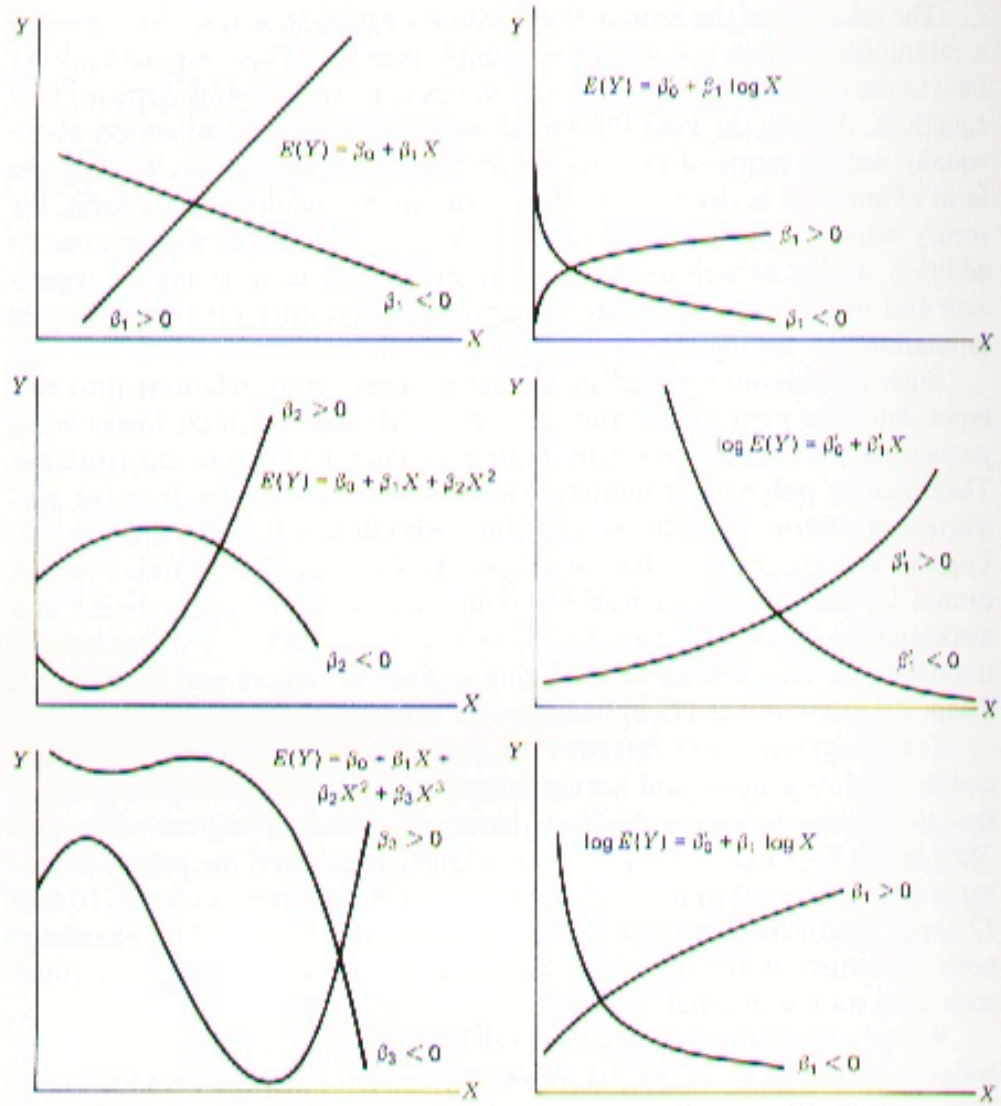


Figure 19.1 General types of curves.

Calculation of digestion rate of fibre or protein

Fractional digestion rate?

Example of a Tank filled with water

Model fitting

$C_t = C_0 . e^{(-kt)}$

Where

Ct = amount of potentially digestible fibre remaining at any time.

C0 = amount of substrate remaining at time zero

e = exponential

k = fractional digestion rate

t = time

To solve the above equation,

take natural logarithm (ln) of both the sides.

The above equation then becomes like the following:

$\ln C_t = \ln C_0 - kt$

Lag time= (ln 100-intercept)/rate of digestion.

Example: digestion rate calculation. excel

Non linear Model in SAS:

Example: nonlinear model for digestion rate.sas

As a Nutritionist you should know

- What you want to do?
- You can draw the desired conclusions by changing a design
- Precision and accuracy
- Coefficient of variation
- Probability level
- Type I and Type II Error
- Standard Deviation vs Standard Error
- Sample size
- Treatments well apart to detect the difference

- **Stat significance vs practical significance**
- **Interpretation of data: regression and correlation example**
- **Drawing conclusions**

HOPE YOU UNDERSTOOD IT

Additional slides

Type I Error:

Rejecting the null hypothesis when it is true

Type II

Accepting the null hypothesis when it is false

Precision and accuracy

Precision

the magnitude of difference between two treatments that an experiment is capable of detecting at a given level of significance

Accuracy

The degree of closeness with which a measurement can be made

The measurement can be accurate but not precise

Examples: Watch, Balance, Any equipment that change its results with calibration

Standard Deviation and Standard Error of mean

Standard Deviation:

Average Squared Deviation: Variance

$$s^2 = \frac{\Sigma(Y_1 - \bar{Y})^2}{(n-1)}$$

Root mean square Deviation:

Represented by small *s for a sample and σ for* a population

Deviation from mean of a Sample/ population

Standard Deviation of Mean or Standard Error

$$s_Y = \frac{s}{\sqrt{n}}$$

Standard Deviation applies to observation and Standard Error applies to means

Co efficient of variation:

A quantity used for evaluating results from different experiments

$$CV = \frac{100s}{\bar{Y}} \, percent$$

Interpretation of Results

Describing results

Explaining results

Regression

The magnitude of change in a dependant variable as a result of per unit change in an independent variable

Or

Increase of decrease in a dependant variable as a result of per unit increase or decrease in an independent variable

Example: FCR

Correlation:

Measurement of relationship between two variables

Relationship could be positive or negative

Relationship between the number of storks flown over Tokyo city and number of births