



# Effects of Dietary Chitosan for Mitigating Enteric Methane Emissions from Ruminants: A Meta-Analysis of *In Vitro* Experiments

Rakhmad P. Harahap, Duta Setiawan, Sri Suharti, Nahrowi, Anuraga Jayanegara

AFENUE (Animal Feed and Nutrition Modelling Research Group)

# Penentuan Topik

- Meta-analysis is one method of **research synthesis**.
- **Research synthesis** may be defined as review of **primary research** on a given topic with purpose of **integrating the findings** (e.g., for creating generalizations or resolving conflicts)
- Penentuan topik meta-analisis:
  - Update publikasi terkini (browsing internet)
  - Publikasi yang sedang **trend**
  - Penentuan X (independent var.) dan Y (dependent var.)
  - X : Kitosan; Y : Metanogenesis dan profil fermentasi rumen

# Pengumpulan Data

- Kriteria artikel yang dimasukkan dalam data base:
  - Artikel tersebut diterbitkan dalam Bahasa Inggris
  - Dosis kitosan dalam ransum dan emisi metan ( $\text{CH}_4$ )
  - Percobaan dilakukan dengan menggunakan sistem *in vitro batch* dengan sapi atau domba sebagai donor cairan rumen
- Terdapat 41 studi dari 12 artikel

# Input Data Base

<b>Insoluble chitosan</b>							
pH	6.43	6.44	6.43	6.46	0.013	NS	L*, Q†
NH <sub>3</sub> -N (mg L <sup>-1</sup> )	18.1	10.8	11.7	18.7	3.87	NS	Q†
Total VFAs (mmol L <sup>-1</sup> )	78.7	79.9	80.0	81.2	1.63	NS	NS
VFAs (mmol mol <sup>-1</sup> )							
Acetate	693	695	691	693	3.83	NS	NS
Propionate	109	146	146	147	2.66	NS	NS
Butyrate	125	122	126	123	2.81	NS	NS
BCVFA	21.9	21.4	21.8	21.7	0.58	NS	NS
Asymptotic GP (mL)	139	132	136	135	3.73	NS	NS
GP rate (μL h <sup>-1</sup> )	46.9	45.5	45.0	45.0	1.85	NS	NS
FOM (mg)	347	351	353	357	7.17	NS	NS
Methane (mL L <sup>-1</sup> )	145	146	145	144	3.00	NS	NS
Methane (mL d <sup>-1</sup> )	13.6	12.8	13	12.6	0.52	NS	NS
Methane (mL g FOM <sup>-1</sup> )	39.2	36.4	36.7	35.4	1.53	NS	L*, Q†
<b>Soluble chitosan</b>							
pH	6.43	6.45	6.46	6.44	0.016	NS	NS
NH <sub>3</sub> -N (mg L <sup>-1</sup> )	18.1	12.1	18.6	14.7	4.56	NS	NS
Total VFAs (mmol L <sup>-1</sup> )	78.7	77.6	76.6	75.2	1.77	NS	L*
VFAs (mmol mol <sup>-1</sup> )							
Acetate	693	692	691	687	4.40	NS	NS
Propionate	145 <sup>b</sup>	151 <sup>b</sup>	154 <sup>b</sup>	186 <sup>a</sup>	4.73	***	L***, Q***
Butyrate	125 <sup>a</sup>	120 <sup>a</sup>	118 <sup>a</sup>	94.7 <sup>b</sup>	3.58	***	L***, Q***
BCVFA	21.9	21.3	21.4	19.9	0.78	NS	L*, Q†
Asymptotic GP (mL)	139	132	129	125	6.39	NS	L*, Q†
GP rate (μL h <sup>-1</sup> )	46.9	45.0	43.2	45.7	1.85	NS	NS
FOM (mg)	347 <sup>a</sup>	340 <sup>a</sup>	336 <sup>ab</sup>	324 <sup>b</sup>	7.28	*	L**, Q*
Methane (mL L <sup>-1</sup> )	145 <sup>a</sup>	139 <sup>a</sup>	134 <sup>ab</sup>	122 <sup>b</sup>	6.00	*	L***, Q**
Methane (mL d <sup>-1</sup> )	13.6 <sup>a</sup>	11.9 <sup>ab</sup>	10.8 <sup>bc</sup>	9.67 <sup>c</sup>	0.95	**	L***, Q**
Methane (mL g FOM <sup>-1</sup> )	39.2 <sup>a</sup>	35.1 <sup>ab</sup>	32.3 <sup>b</sup>	30.0 <sup>b</sup>	3.07	*	L**, Q*

Belanche et al. 2016

# Input Data Base

$$\text{Mol} = \frac{\text{massa (gr)}}{Mr} = \frac{\text{volume}}{22,4 \text{ L (STP)}} = \frac{\text{jumlah molekul}}{6,02 \times 10^{23}}$$

$$\frac{\text{Mol}_1}{\text{Volume}_1} = \frac{\text{Mol}_2}{\text{Volume}_2}$$

					day		mg	ml	g/kg dm	mmol/l	mmol/ d	mmol/g dom
no	paper	study	year	repl	period	system	sampel incubation (DM)	rumen+buffer	chi	ch4v	ch4d	ch4dom/ dmd
1	belanche	1	2016	4	1	batch	500	50	0,00	6,473	0,607	1,750
2	belanche	1	2016	4	1	batch	500	50	0,50	6,518	0,571	1,625
3	belanche	1	2016	4	1	batch	500	50	1,00	6,473	0,580	1,638
4	belanche	1	2016	4	1	batch	500	50	2,00	6,429	0,563	1,580
5	belanche	2	2016	4	1	batch	500	50	0,00	6,473	0,607	1,750
6	belanche	2	2016	4	1	batch	500	50	0,50	6,205	0,531	1,567
7	belanche	2	2016	4	1	batch	500	50	1,00	5,982	0,482	1,442
8	belanche	2	2016	4	1	batch	500	50	2,00	5,446	0,432	1,339

# Model Meta-analisis (PROC. MIXED)

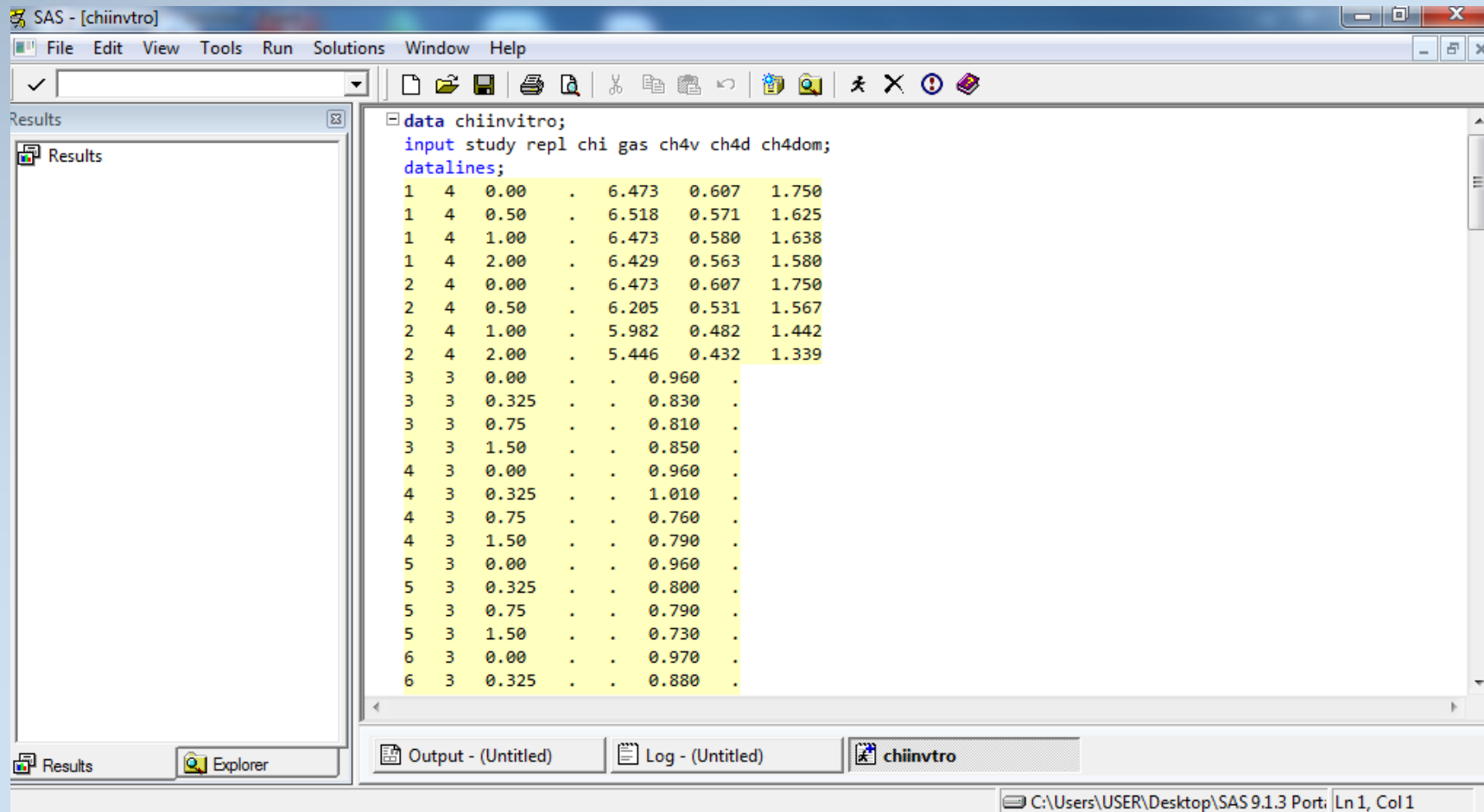
- Menurut St-Pierre (2001):

$$Y_{ij} = B_0 + B_1 X_{ij} + s_i + e_{ij}$$

- Keterangan:
  - $Y_{ij}$  = variabel dependen
  - $B_0$  = keseluruhan *intercept* di semua eksperimen (efek tetap)
  - $B_1$  = koefisien regresi linier Y pada X (efek tetap)
  - $X_{ij}$  = nilai variabel prediktor kontinu (level penambahan kitosan)
  - $s_i$  = efek acak percobaan i
  - $e_{ij}$  = kesalahan residual yang tidak dapat dijelaskan
- Disajikan dengan p-value dan *root mean square error* (RMSE).
- Analisis statistik dilakukan dengan perangkat lunak SAS versi 9.1 (SAS Institute Inc., Cary, NC, USA)



# Coding SAS 9.1



SAS - [chiinvtro]

File Edit View Tools Run Solutions Window Help

Results

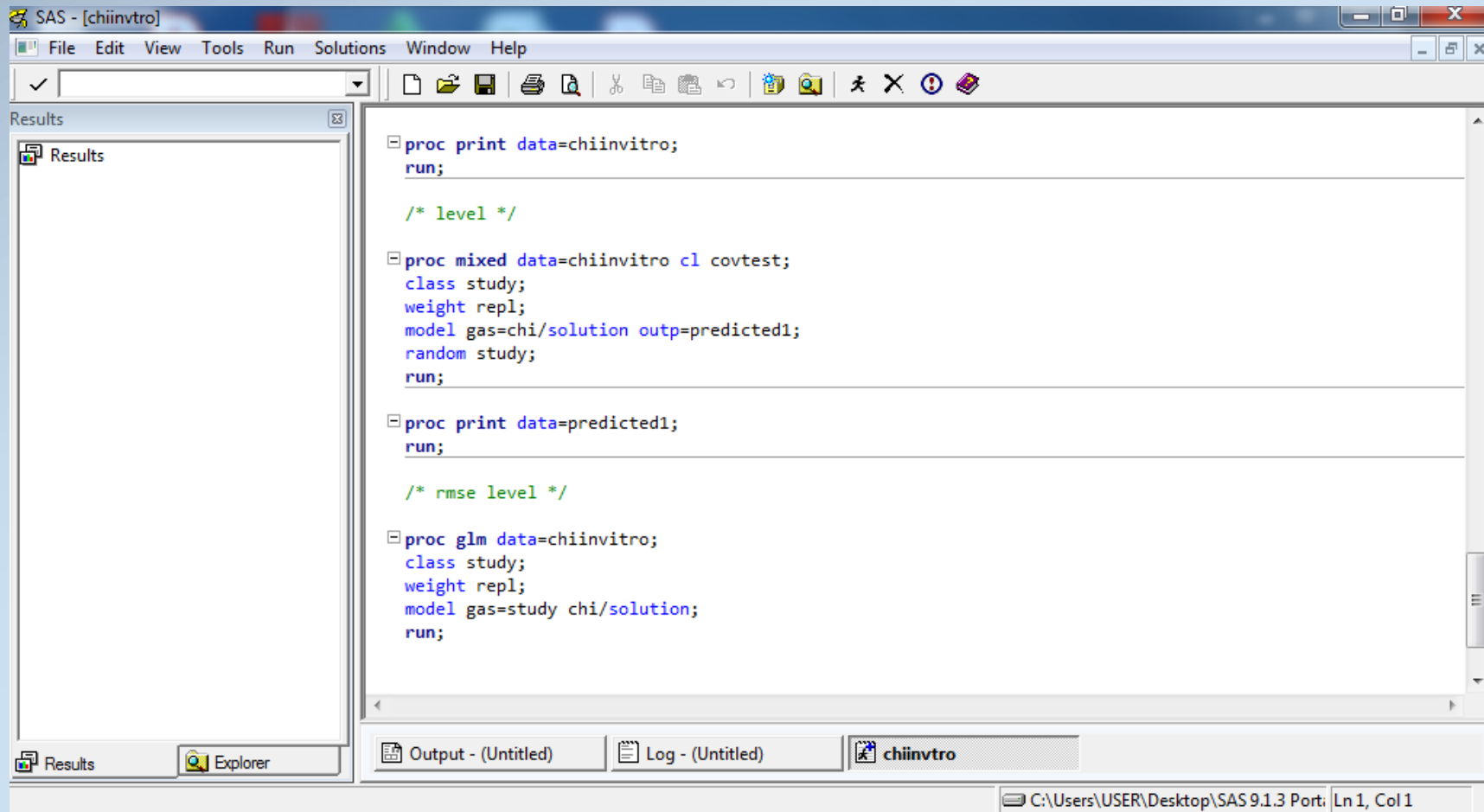
Results

```
data chiinvtro;
input study repl chi gas ch4v ch4d ch4dom;
datalines;
1 4 0.00 . 6.473 0.607 1.750
1 4 0.50 . 6.518 0.571 1.625
1 4 1.00 . 6.473 0.580 1.638
1 4 2.00 . 6.429 0.563 1.580
2 4 0.00 . 6.473 0.607 1.750
2 4 0.50 . 6.205 0.531 1.567
2 4 1.00 . 5.982 0.482 1.442
2 4 2.00 . 5.446 0.432 1.339
3 3 0.00 . . 0.960 .
3 3 0.325 . . 0.830 .
3 3 0.75 . . 0.810 .
3 3 1.50 . . 0.850 .
4 3 0.00 . . 0.960 .
4 3 0.325 . . 1.010 .
4 3 0.75 . . 0.760 .
4 3 1.50 . . 0.790 .
5 3 0.00 . . 0.960 .
5 3 0.325 . . 0.800 .
5 3 0.75 . . 0.790 .
5 3 1.50 . . 0.730 .
6 3 0.00 . . 0.970 .
6 3 0.325 . . 0.880 .
```

Output - (Untitled) Log - (Untitled) chiinvtro

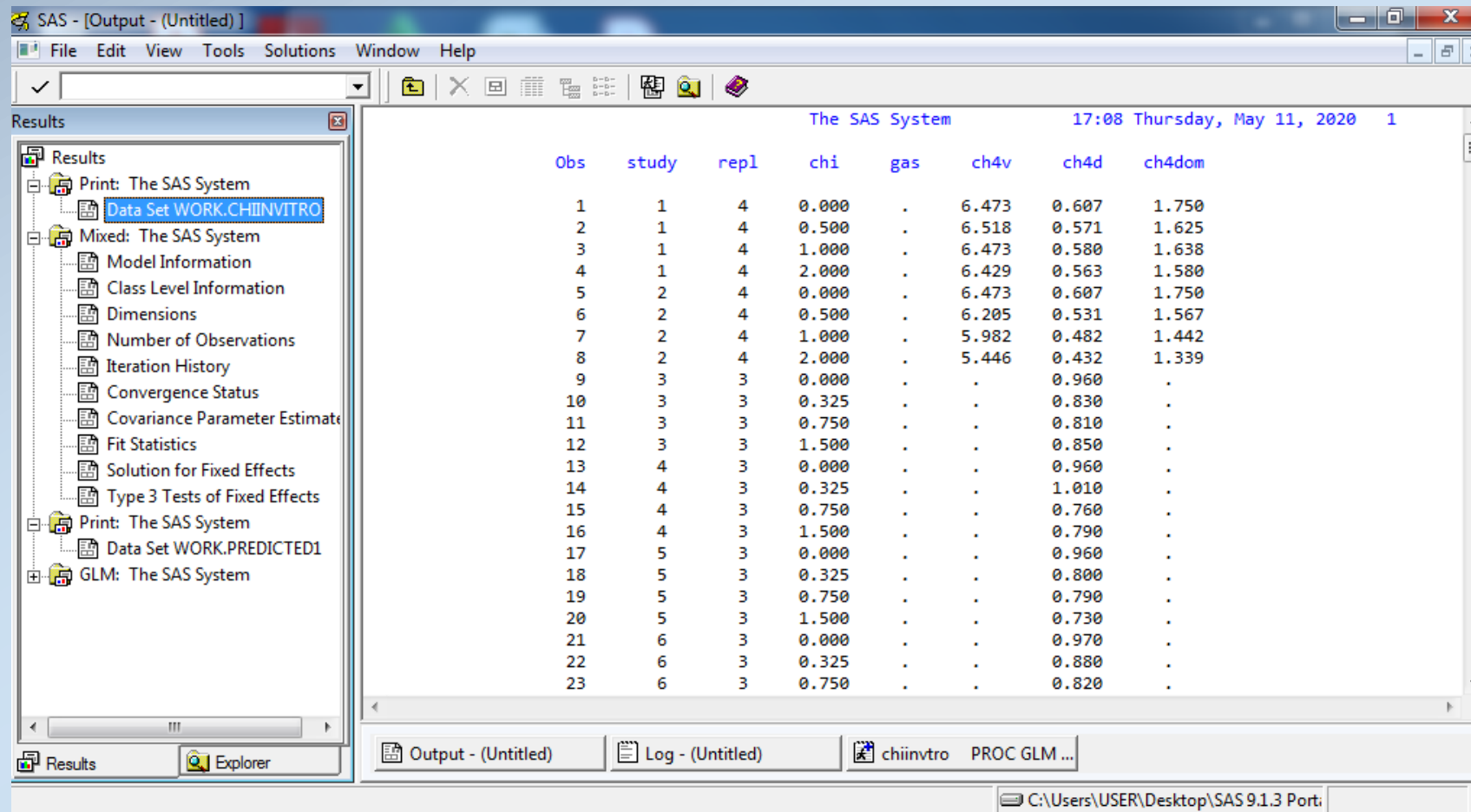
C:\Users\USER\Desktop\SAS 9.1.3 Port Ln 1, Col 1

# Coding SAS 9.1





# Coding SAS 9.1



The screenshot shows the SAS 9.1 Output window. The left pane displays the Results tree with 'Data Set WORK.CHINVTR' selected. The right pane shows the output for 'The SAS System' dated 17:08 Thursday, May 11, 2020. The output is a table with 9 columns: Obs, study, repl, chi, gas, ch4v, ch4d, and ch4dom. The data consists of 23 observations across 6 studies and 3 replicates. The bottom status bar indicates the current file is 'chiinvtr PROC GLM ...' and the path is 'C:\Users\USER\Desktop\SAS 9.1.3 Porti...'.

Obs	study	repl	chi	gas	ch4v	ch4d	ch4dom
1	1	4	0.000	.	6.473	0.607	1.750
2	1	4	0.500	.	6.518	0.571	1.625
3	1	4	1.000	.	6.473	0.580	1.638
4	1	4	2.000	.	6.429	0.563	1.580
5	2	4	0.000	.	6.473	0.607	1.750
6	2	4	0.500	.	6.205	0.531	1.567
7	2	4	1.000	.	5.982	0.482	1.442
8	2	4	2.000	.	5.446	0.432	1.339
9	3	3	0.000	.	.	0.960	.
10	3	3	0.325	.	.	0.830	.
11	3	3	0.750	.	.	0.810	.
12	3	3	1.500	.	.	0.850	.
13	4	3	0.000	.	.	0.960	.
14	4	3	0.325	.	.	1.010	.
15	4	3	0.750	.	.	0.760	.
16	4	3	1.500	.	.	0.790	.
17	5	3	0.000	.	.	0.960	.
18	5	3	0.325	.	.	0.800	.
19	5	3	0.750	.	.	0.790	.
20	5	3	1.500	.	.	0.730	.
21	6	3	0.000	.	.	0.970	.
22	6	3	0.325	.	.	0.880	.
23	6	3	0.750	.	.	0.820	.

# Coding SAS 9.1

The screenshot displays the SAS 9.1 Output window for a PROC GLM analysis. The left pane shows a tree view of results, with 'Fit Statistics' selected. The main pane displays the following statistics:

**Fit Statistics**

-2 Res Log Likelihood	387.7
AIC (smaller is better)	391.7
AICC (smaller is better)	392.0
BIC (smaller is better)	395.1

**Solution for Fixed Effects**

Effect	Estimate	Standard Error	DF	t Value	Pr >  t
Intercept	199.10	15.2440	15	13.06	<.0001
chi	-0.6569	0.6951	25	-0.95	0.3537

**Type 3 Tests of Fixed Effects**

Effect	Num DF	Den DF	F Value	Pr > F
chi	1	25	0.89	0.3537

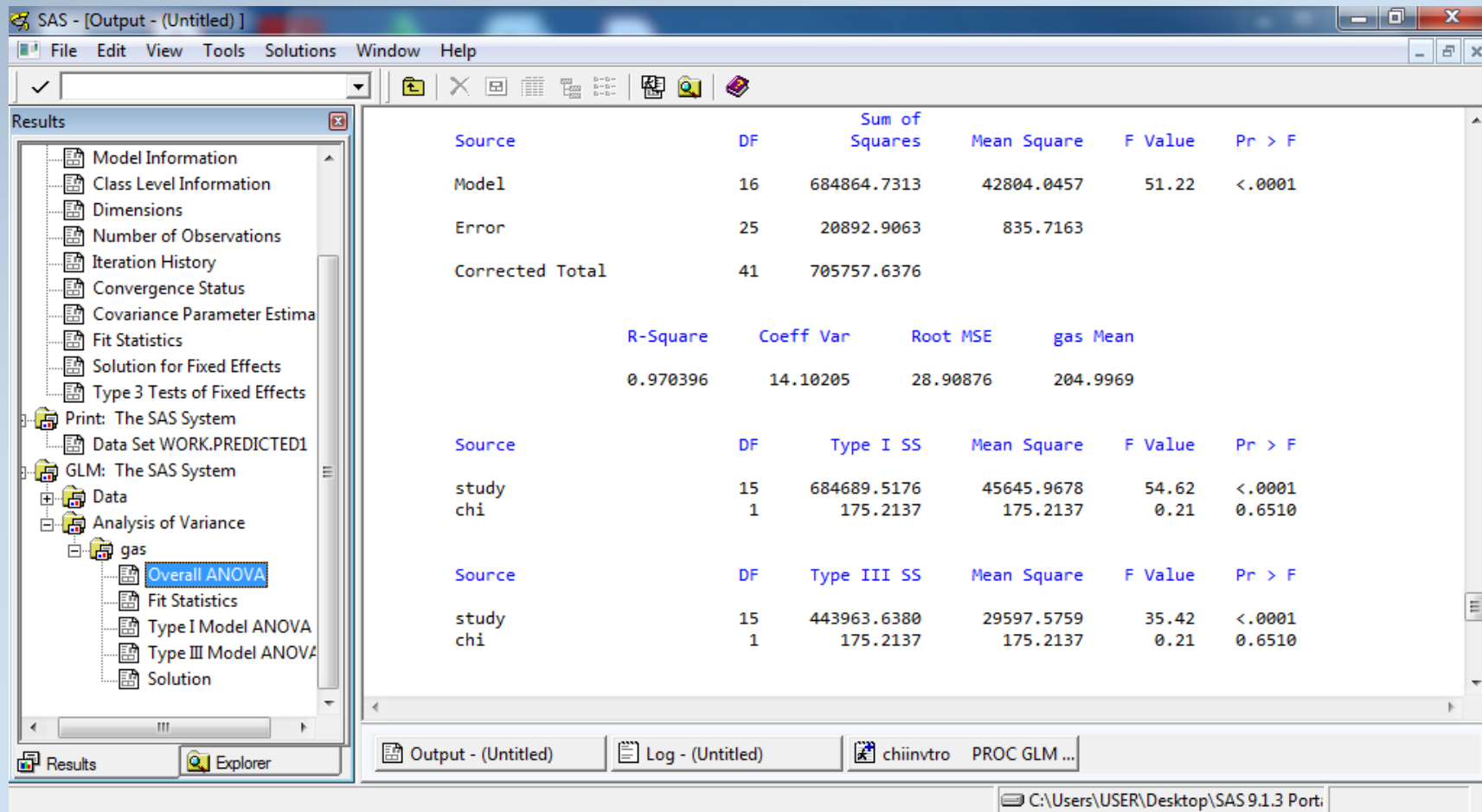
The bottom of the window shows the taskbar with 'Output - (Untitled)', 'Log - (Untitled)', and 'chiinvtr PROC GLM ...' open. The status bar indicates the path 'C:\Users\USER\Desktop\SAS 9.1.3 Porti'.

# Coding SAS 9.1

The screenshot displays the SAS 9.1 interface. The main window shows the output of a PROC GLM analysis. The left pane contains a tree view of results, with 'Data Set WORK.PREDICTED1' selected. The main window displays a table of results for the chiinvtr model. The table includes columns for observation number, study, replication, and various model parameters. The bottom status bar indicates the current directory is C:\Users\USER\Desktop\SAS 9.1.3 Port.

Obs	study	repl	chi	gas	ch4v	ch4d	ch4dom	Pred	StdErr	DF	Alpha	Lower	Upper	Resid
1	1	4	0.000	.	6.473	0.607	1.750	199.097	62.1650	25	0.05	71.0660	327.129	.
2	1	4	0.500	.	6.518	0.571	1.625	198.769	62.1583	25	0.05	70.7514	326.786	.
3	1	4	1.000	.	6.473	0.580	1.638	198.440	62.1535	25	0.05	70.4329	326.448	.
4	1	4	2.000	.	6.429	0.563	1.580	197.784	62.1498	25	0.05	69.7837	325.783	.
5	2	4	0.000	.	6.473	0.607	1.750	199.097	62.1650	25	0.05	71.0660	327.129	.
6	2	4	0.500	.	6.205	0.531	1.567	198.769	62.1583	25	0.05	70.7514	326.786	.
7	2	4	1.000	.	5.982	0.482	1.442	198.440	62.1535	25	0.05	70.4329	326.448	.
8	2	4	2.000	.	5.446	0.432	1.339	197.784	62.1498	25	0.05	69.7837	325.783	.
9	3	3	0.000	.	.	0.960	.	199.097	62.1650	25	0.05	71.0660	327.129	.
10	3	3	0.325	.	.	0.830	.	198.884	62.1604	25	0.05	70.8620	326.906	.
11	3	3	0.750	.	.	0.810	.	198.605	62.1557	25	0.05	70.5927	326.617	.
12	3	3	1.500	.	.	0.850	.	198.112	62.1507	25	0.05	70.1103	326.114	.
13	4	3	0.000	.	.	0.960	.	199.097	62.1650	25	0.05	71.0660	327.129	.
14	4	3	0.325	.	.	1.010	.	198.884	62.1604	25	0.05	70.8620	326.906	.
15	4	3	0.750	.	.	0.760	.	198.605	62.1557	25	0.05	70.5927	326.617	.
16	4	3	1.500	.	.	0.790	.	198.112	62.1507	25	0.05	70.1103	326.114	.
17	5	3	0.000	.	.	0.960	.	199.097	62.1650	25	0.05	71.0660	327.129	.
18	5	3	0.325	.	.	0.800	.	198.884	62.1604	25	0.05	70.8620	326.906	.
19	5	3	0.750	.	.	0.790	.	198.605	62.1557	25	0.05	70.5927	326.617	.
20	5	3	1.500	.	.	0.730	.	198.112	62.1507	25	0.05	70.1103	326.114	.
21	6	3	0.000	.	.	0.970	.	199.097	62.1650	25	0.05	71.0660	327.129	.
22	6	3	0.325	.	.	0.880	.	198.884	62.1604	25	0.05	70.8620	326.906	.

# Coding SAS 9.1



The screenshot displays the SAS 9.1 Output window for a PROC GLM analysis. The left pane shows a tree view of the output, with 'Overall ANOVA' selected under the 'gas' data set. The main pane displays the ANOVA table and fit statistics.

**ANOVA Table (Sum of Squares)**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	16	684864.7313	42804.0457	51.22	<.0001
Error	25	20892.9063	835.7163		
Corrected Total	41	705757.6376			

**Fit Statistics**

R-Square	Coeff Var	Root MSE	gas Mean
0.970396	14.10205	28.90876	204.9969

**ANOVA Table (Type I SS)**

Source	DF	Type I SS	Mean Square	F Value	Pr > F
study	15	684689.5176	45645.9678	54.62	<.0001
chi	1	175.2137	175.2137	0.21	0.6510

**ANOVA Table (Type III SS)**

Source	DF	Type III SS	Mean Square	F Value	Pr > F
study	15	443963.6380	29597.5759	35.42	<.0001
chi	1	175.2137	175.2137	0.21	0.6510

The bottom of the window shows the taskbar with 'Output - (Untitled)', 'Log - (Untitled)', and 'chiinvtr PROC GLM ...' open. The status bar indicates the path 'C:\Users\USER\Desktop\SAS 9.1.3 Porti'.

# Penyajian Data

Table 1. Effects of chitosan addition (in g/kg DM) on gas production and enteric methane (CH<sub>4</sub>) emission of *in vitro* batch culture study

Response parameter	Unit	n	Parameter estimates				Model statistics	
			Intercept	SE intercept	Slope	SE slope	p-Value	RMSE
<b>Gas production</b>	ml/g DM	42	199.1	15.2	-0.657	0.695	<.001	28.9
<b>CH<sub>4</sub></b>	mmol/d	65	1.03	0.13	-0.072	0.017	<.001	0.17
<b>CH<sub>4</sub></b>	mmol/g DOM	47	3.58	0.41	-0.050	0.055	<.001	1.76
<b>H<sub>2</sub>S</b>	μmol/d/g DM	6	5.55	3.72	0.121	0.046	0.376	0.79

Note: DM=dry matter, DOM=digested organic matter; n=number of treatment, RMSE=root mean square error, SE=standard error.

# Penyajian Data

Table 2. Effects of chitosan addition (in g/kg DM) on rumen fermentation, microbial population, and enzyme CMCase activity in the *in vitro* batch culture study

Response parameter	Unit	n	Parameter estimates				Model statistics	
			Intercept	SE intercept	Slope	SE slope	p-Value	RMSE
pH		82	6.40	0.05	0.002	0.004	<.001	0.12
NH <sub>3</sub>	mg/dL	82	25.2	1.43	-0.044	0.243	<.001	8.24
Total VFA	mmol/l	89	67.8	3.19	-0.228	0.366	<.001	7.49
C <sub>2</sub>	%	92	62.7	0.98	-0.024	0.071	<.001	2.29
C <sub>3</sub>	%	92	20.4	0.68	0.304	0.155	<.001	5.42
C <sub>4</sub>	%	94	11.5	0.47	-0.199	0.086	<.001	2.97
iso-C <sub>4</sub>	%	25	0.95	0.16	0.001	0.004	<.001	0.07
C <sub>5</sub>	%	27	2.13	0.17	0.016	0.009	<.001	0.17
iso-C <sub>5</sub>	%	25	1.86	0.33	0.003	0.011	<.001	0.20
C <sub>6</sub>	%	6	0.20	0.22	0.004	0.002	0.527	0.04
C <sub>2</sub> :C <sub>3</sub>		92	3.23	0.14	-0.041	0.024	<.001	0.81
BCVFA		25	3.39	0.44	-0.314	0.082	<.001	0.87
TVFA:TDS	mmol/g DM	36	8.75	0.53	1.80	0.227	<.001	1.33
Bacteria	×10 <sup>10</sup> /g	12	0.98	0.06	0.018	0.050	0.004	0.13
Methanogen	×10 <sup>3</sup> /g	12	3.86	1.12	-2.76	1.475	0.075	3.81
Fibrobacter succinogenes	×10 <sup>3</sup> /g	12	2.26	1.50	6.20	1.849	0.272	4.77
Anaerobic fungi	×10 <sup>3</sup> /g	12	1.86	0.84	0.014	0.303	0.158	0.78
Protozoa	×10 <sup>2</sup> /g	12	4.96	0.87	-0.636	0.821	0.030	2.12
CMCase	U/mL	12	0.56	0.10	-0.107	0.033	0.030	0.09

Note: C<sub>2</sub>=acetate, C<sub>3</sub>=propionate, C<sub>4</sub>=butyrate, C<sub>5</sub>=valerate, C<sub>6</sub>=caproate, NH<sub>3</sub>=ammonia, DM=dry matter, n=number of treatment, BCVFA=branch-chain volatile fatty acids, TVAS:TDS= total VFA-to-truly degraded substrate ratio, RMSE=root mean square error, SE=standard error.



# Penyajian Data

Table 3. Effects of chitosan addition (in g/kg DM) on *in vitro* batch culture digestibility study

			Parameter estimates				Model statistics	
Response parameter	Unit	n	Intercept	SE intercept	Slope	SE slope	p-Value	RMSE
DMD	g/kg	51	651	24.2	0.966	0.971	<.001	71.6
CPD	g/kg	10	503	95.2	3.60	2.999	0.006	98.7
NDFD	g/kg	15	601	15.0	1.98	0.955	<.001	73.9

Note: DMD=dry matter digestibility, OMD= organic matter digestibility, CPD=crude protein digestibility, NDFD=neutral detergent fiber digestibility, n= number of treatment, SE= standard error, RMSE= root mean square error.

# Penyajian Data

Table 4. Effects of chitosan addition (in g/kg DM) on rumen fatty acid profile in the *in vitro* batch culture study

Response parameter	Unit	n	Parameter estimates				Model statistics	
			Intercept	SE intercept	Slope	SE slope	p-Value	RMSE
C <sub>14:0</sub>	%	18	2.26	0.34	-2.01	1.68	<.001	1.13
C <sub>15:0</sub>	%	18	2.49	0.32	-2.70	1.38	<.001	0.93
C <sub>16:0</sub>	%	18	22.7	2.51	-18.1	8.38	<.001	5.62
C <sub>17:0</sub>	%	18	1.45	0.27	-0.858	0.702	<.001	0.47
C <sub>18:0</sub>	%	18	36.4	1.67	-39.9	21.1	<.001	14.2
C <sub>18:2n6</sub>	%	18	3.34	1.43	3.15	3.74	0.048	2.51
CLA	%	18	0.74	0.22	0.471	1.03	0.009	0.69
C <sub>18:3n3</sub>	%	18	0.74	0.31	2.39	1.78	0.043	1.19
SFA	%	18	75.4	5.33	-28.1	27.3	<.001	18.3
MUFA	%	18	19.2	4.38	23.1	24.3	0.002	16.3
PUFA	%	18	5.53	1.57	4.11	6.38	0.008	4.28

Note: CLA=*cis*9, *trans*11 C<sub>18:2</sub>, SFA=saturated fatty acid, MUFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid, n=number of treatment, RMSE=root mean square error, SE=standard error.

# Simpulan

- Chitosan seems to be suitable for use as a feed additive in ruminant diets.
- Chitosan addition is able to mitigate enteric methane emission, alters rumen fermentation profiles toward a favourable direction, and improves nutrient digestibility.
- Further, chitosan plays a role in inhibiting biohydrogenation of fatty acids in the rumen as indicated by the increase of PUFA and the decrease of SFA.

# Terimakasih

AFENUE (Animal Feed and Nutrition Modelling Research Group)