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 (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

Insoluble chitosan							
pH	6.43	6.44	6.43	6.46	0.013	NS	L*, Q†
NH_3-N (mg L^{-1})	18.1	10.8	11.7	18.7	3.87	NS	Q†
Total VFAs (mmol L^{-1})	78.7	79.9	80.0	81.2	1.63	NS	NS
VFAs (mmol mol^{-1})							
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^{-1})	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^{-1})	145	146	145	144	3.00	NS	NS
Methane (mL d^{-1})	13.6	12.8	13	12.6	0.52	NS	NS
Methane (mL g FOM^{-1})	39.2	36.4	36.7	35.4	1.53	NS	L*,Q†
Soluble chitosan							
pH	6.43	6.45	6.46	6.44	0.016	NS	NS
NH_3-N (mg L^{-1})	18.1	12.1	18.6	14.7	4.56	NS	NS
Total VFAs (mmol L^{-1})	78.7	77.6	76.6	75.2	1.77	NS	L*
VFAs (mmol mol^{-1})							
Acetate	693	692	691	687	4.40	NS	NS
Propionate	145^b	151^b	154^b	186^a	4.73	***	L***,Q***
Butyrate	125^a	120^a	118^a	94.7^b	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^{-1})	46.9	45.0	43.2	45.7	1.85	NS	NS
FOM (mg)	347^a	340^a	336^{ab}	324^b	7.28	*	L**, Q*
Methane (mL L^{-1})	145^a	139^a	134^{ab}	122^b	6.00	*	L***, Q**
Methane (mL d^{-1})	13.6^a	11.9^{ab}	10.8^{bc}	9.67^c	0.95	**	L***, Q**
Methane (mL g FOM^{-1})	39.2^a	35.1^{ab}	32.3^b	30.0^b	3.07	*	L**, Q*

Belanche et al. 2016

Input Data Base

$$\text{Mol} = \frac{\text{massa (gr)}}{Mr} = \frac{\text{volume}}{22{,}4\ \text{L(STP)}} = \frac{\text{jumlah molekul}}{6{,}02\times10^{23}}$$

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

					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

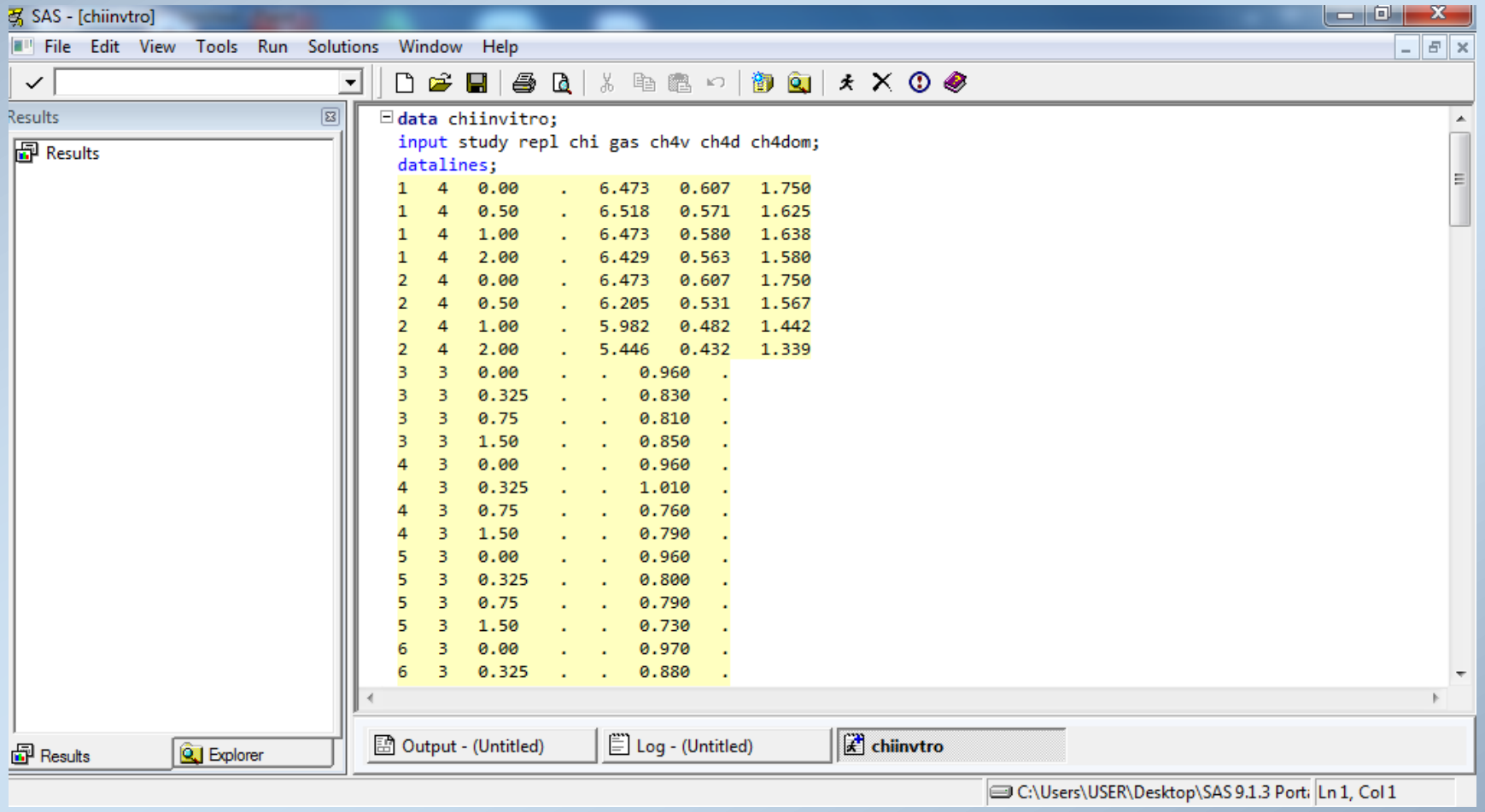
Model Meta-analisis (PROC. MIXED)

- Menurut St-Pierre (2001):

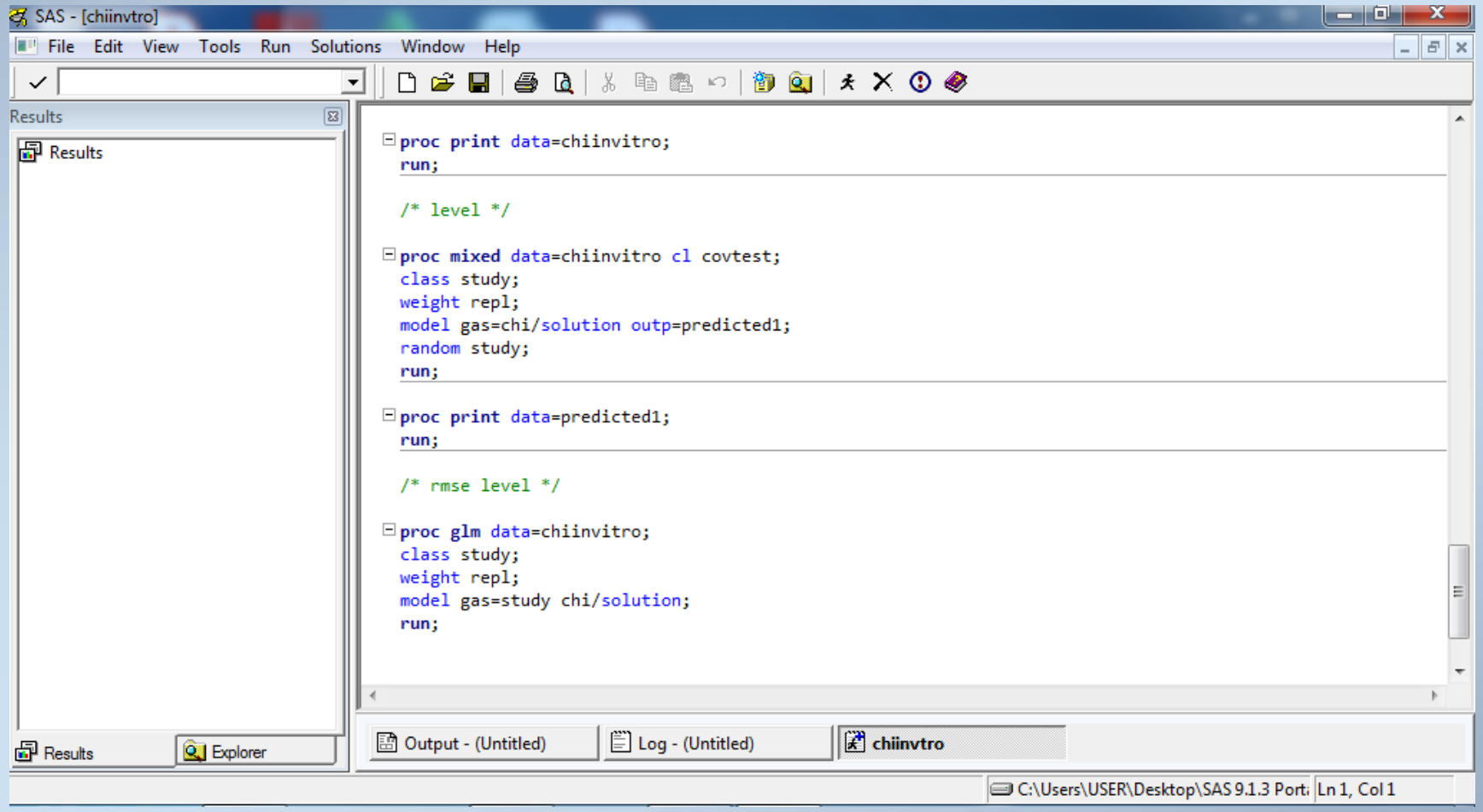
$$Y_{ij} = B_0 + B_1X_{ij} + s_i + e_{ij}$$

- 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



Coding SAS 9.1



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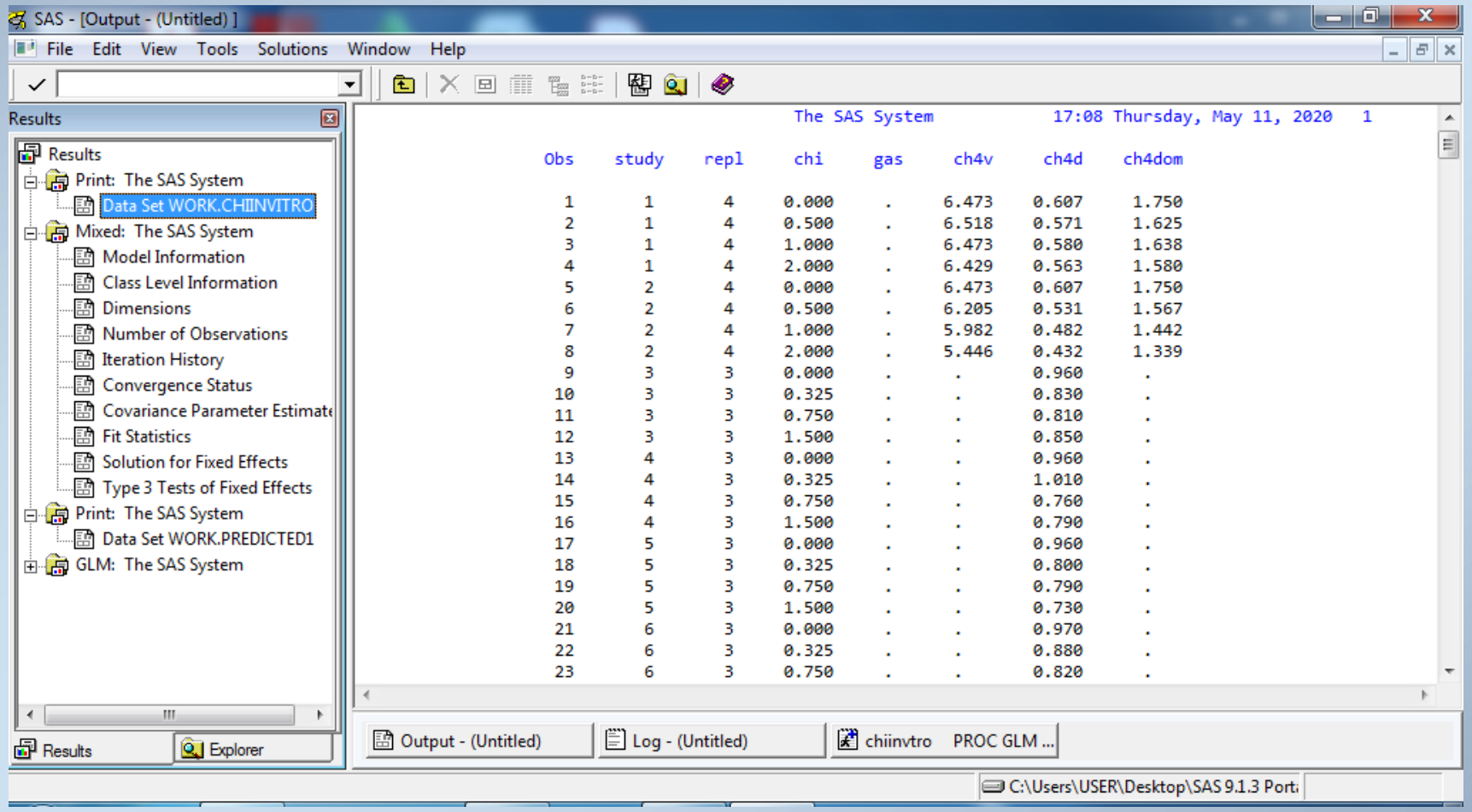

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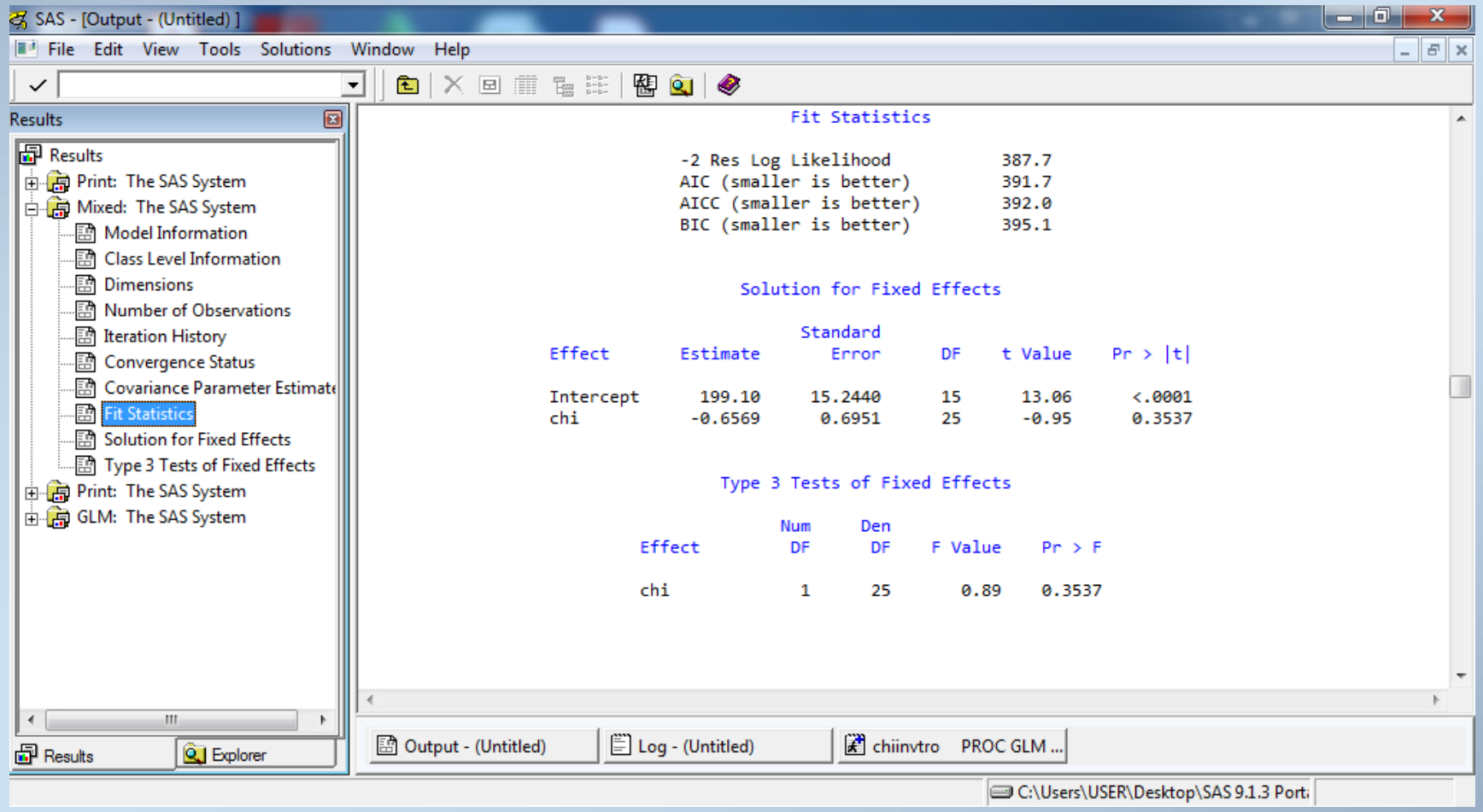

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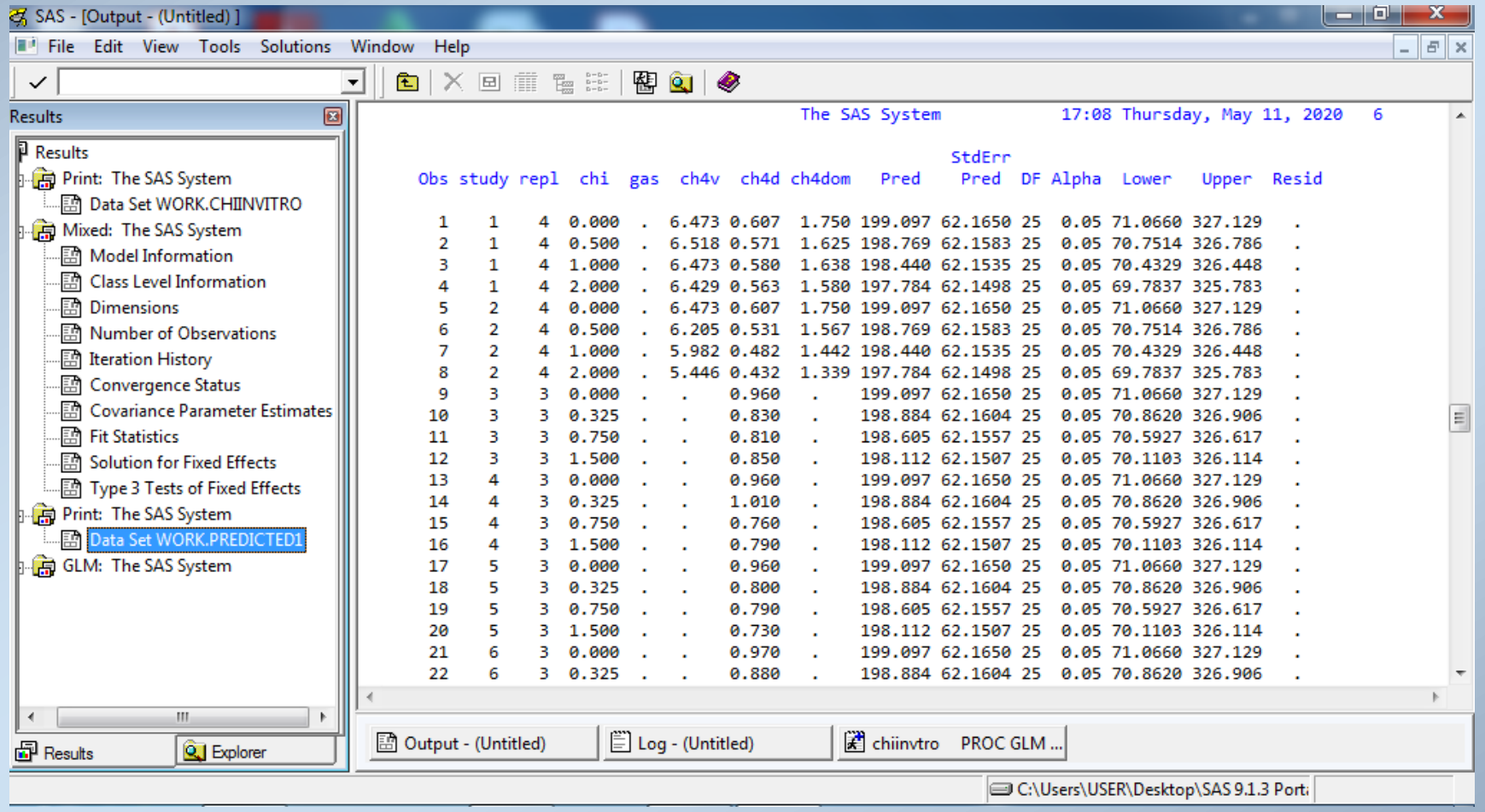
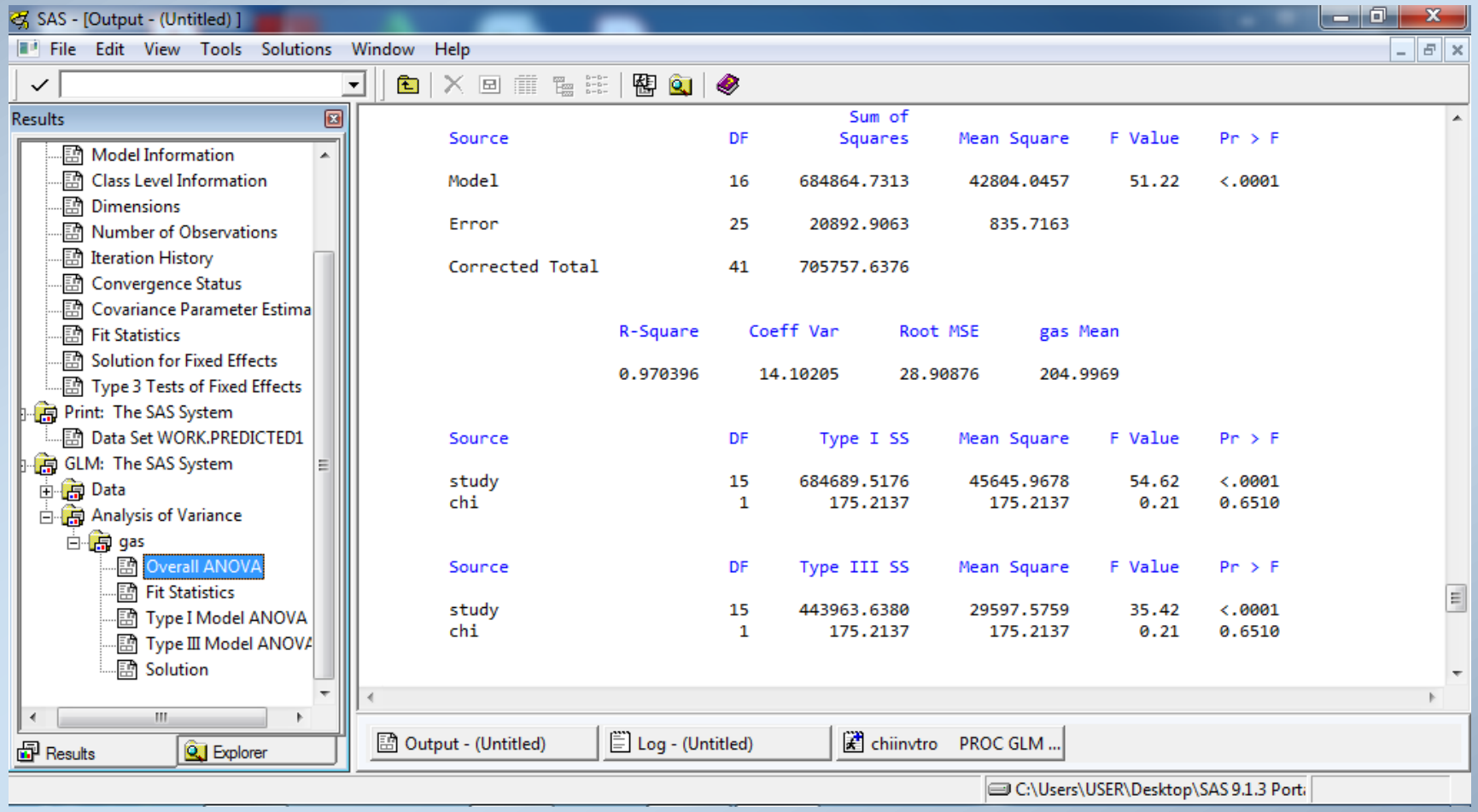


Obs	study	repl	chi	gas	ch4v	ch4d	ch4dom	Pred	StdErr Pred	DF	Alpha	Lower	Upper	Resid
1	1	4	0.000	.	6.473	0.607	1.750	199.097	62.1650	25	0.05	71.0660	327.129	.
2	1	4	0.500	.	6.518	0.571	1.625	198.769	62.1583	25	0.05	70.7514	326.786	.
3	1	4	1.000	.	6.473	0.580	1.638	198.440	62.1535	25	0.05	70.4329	326.448	.
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



Penyajian Data

Table 1. Effects of chitosan addition (in g/kg DM) on gas production and enteric methane (CH_4) emission of *in vitro* batch culture study

			Parameter estimates				**Model statistics**	
Response parameter	Unit	n	Intercept	SE intercept	Slope	SE slope	p-Value	RMSE
Gas production	ml/g DM	42	199.1	15.2	-0.657	0.695	<.001	28.9
CH_4	mmol/d	65	1.03	0.13	-0.072	0.017	<.001	0.17
CH_4	mmol/g DOM	47	3.58	0.41	-0.050	0.055	<.001	1.76
H_2S	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

			Parameter estimates				**Model statistics**	
Response parameter	Unit	n	Intercept	SE intercept	Slope	SE slope	p-Value	RMSE
pH		82	6.40	0.05	0.002	0.004	<.001	0.12
NH_3	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_2	%	92	62.7	0.98	-0.024	0.071	<.001	2.29
C_3	%	92	20.4	0.68	0.304	0.155	<.001	5.42
C_4	%	94	11.5	0.47	-0.199	0.086	<.001	2.97
iso-C_4	%	25	0.95	0.16	0.001	0.004	<.001	0.07
C_5	%	27	2.13	0.17	0.016	0.009	<.001	0.17
iso-C_5	%	25	1.86	0.33	0.003	0.011	<.001	0.20
C_6	%	6	0.20	0.22	0.004	0.002	0.527	0.04
C_2:C_3		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	$\times 10^{10}$/g	12	0.98	0.06	0.018	0.050	0.004	0.13
Methanogen	$\times 10^{3}$/g	12	3.86	1.12	-2.76	1.475	0.075	3.81
Fibrobacter succinogenes	$\times 10^{3}$/g	12	2.26	1.50	6.20	1.849	0.272	4.77
Anaerobic fungi	$\times 10^{3}$/g	12	1.86	0.84	0.014	0.303	0.158	0.78
Protozoa	$\times 10^{2}$/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_2=acetate, C_3=propionate, C_4=butyrate, C_5=valerate, C_6=caproate, NH_3=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

			Parameter estimates				**Model statistics**	
Response parameter	Unit	n	Intercept	SE intercept	Slope	SE slope	p-Value	RMSE
$C_{14:0}$	%	18	2.26	0.34	-2.01	1.68	<.001	1.13
$C_{15:0}$	%	18	2.49	0.32	-2.70	1.38	<.001	0.93
$C_{16:0}$	%	18	22.7	2.51	-18.1	8.38	<.001	5.62
$C_{17:0}$	%	18	1.45	0.27	-0.858	0.702	<.001	0.47
$C_{18:0}$	%	18	36.4	1.67	-39.9	21.1	<.001	14.2
$C_{18:2n6}$	%	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
$C_{18:3n3}$	%	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 $C_{18:2}$, 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

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