NEURAL NETWORK MODELS FOR GROWTH OF *SALMONELLA* SEROTYPES IN GROUND CHICKEN THIGH MEAT SUBJECTED TO TEMPERATURE ABUSE DURING REFRIGERATED STORAGE

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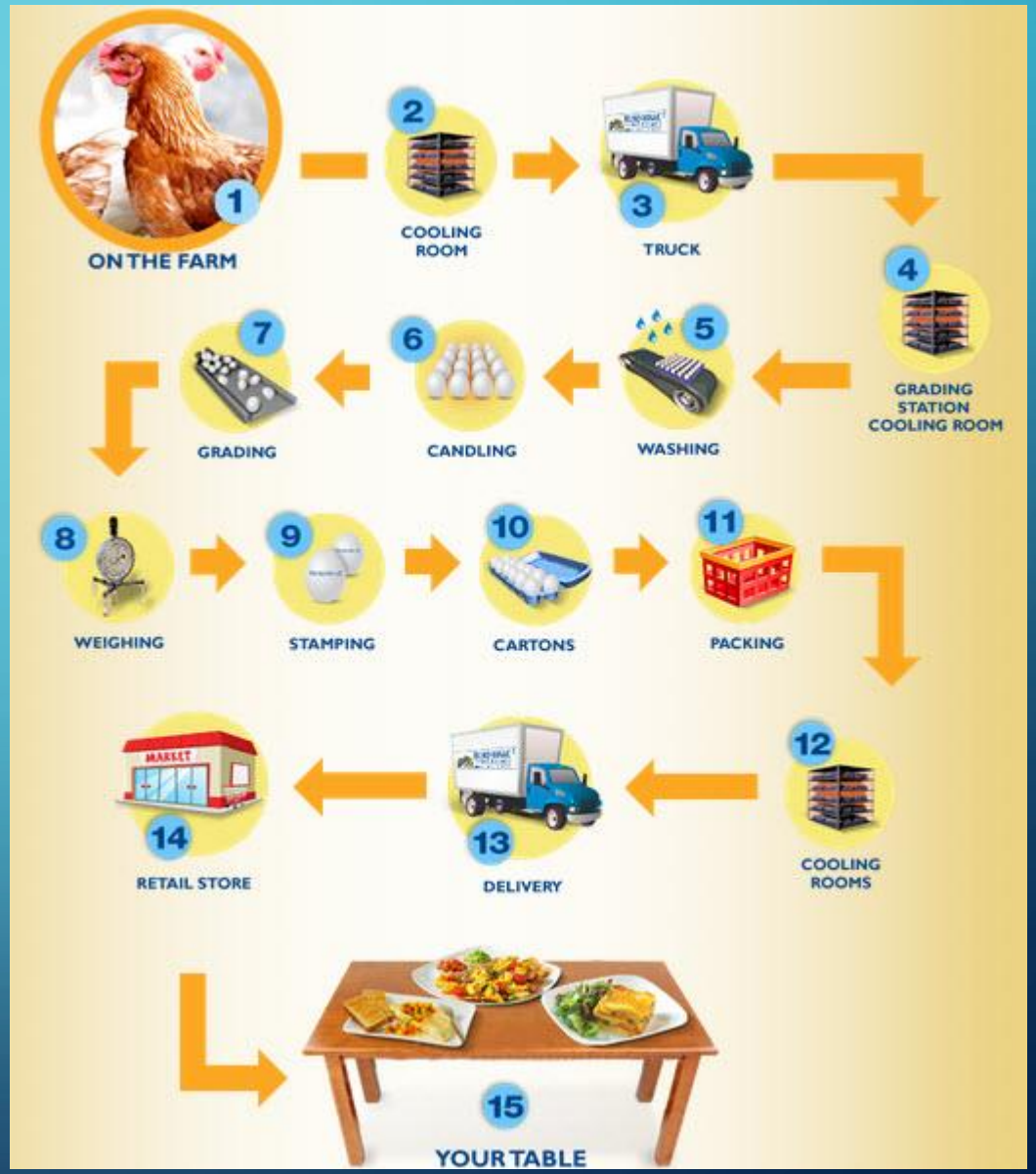
PRINCESS ANNE, MD

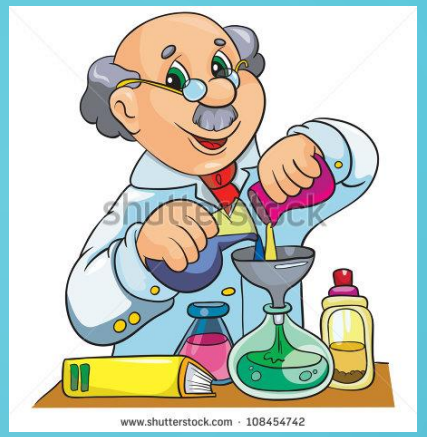
INTRODUCTION
PREDICTIVE MICROBIOLOGY

- Models that predict pathogen growth are valuable tools for food safety.
  - HACCP
  - QMRA

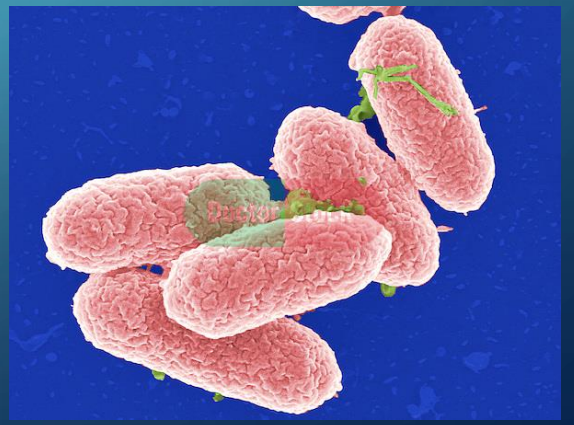
Salmonella



INTRODUCTION

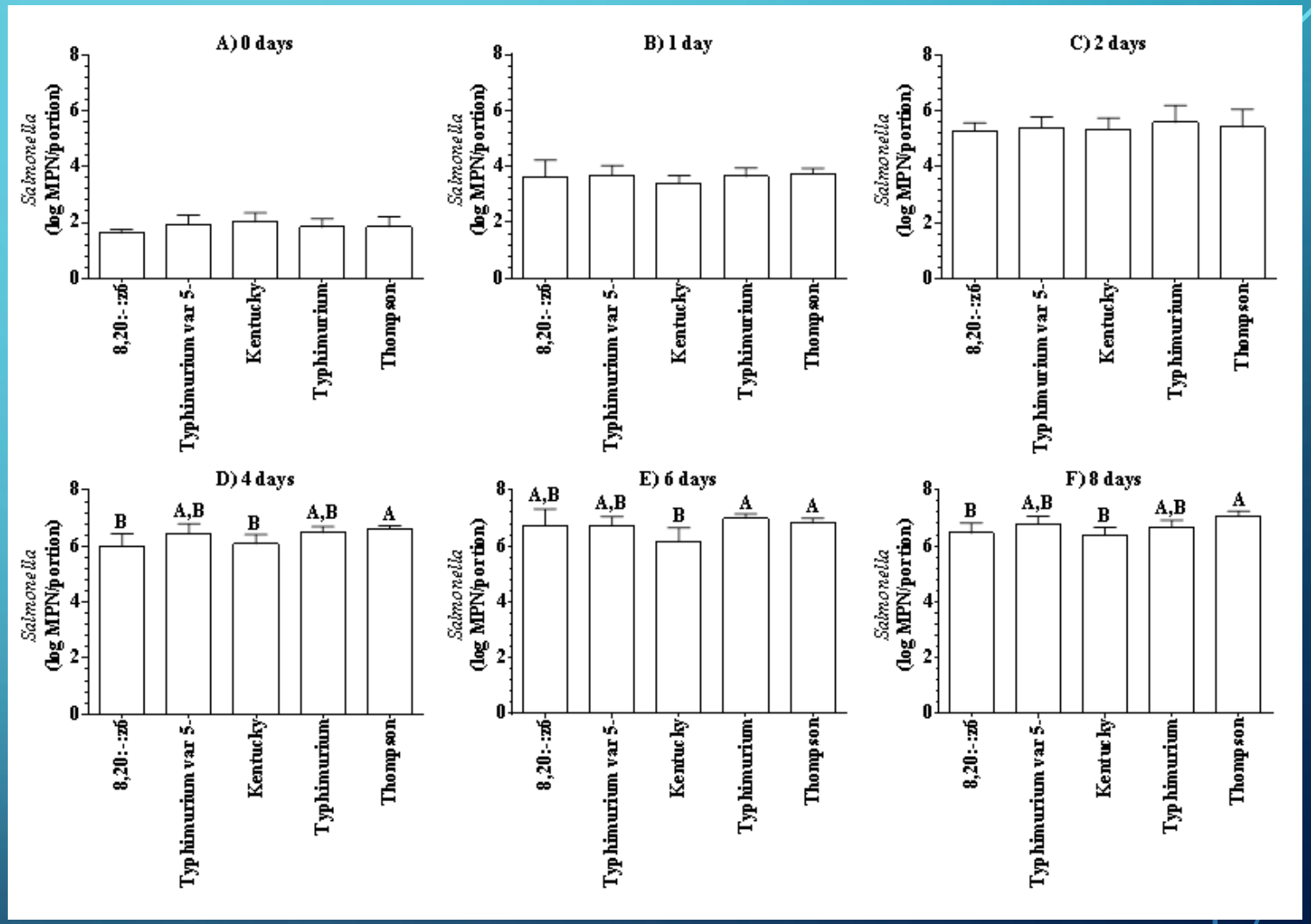


- Growth of pathogens in food is affected by time and:
  - Food factors (temperature, pH, Aw, native microflora…)
  - Pathogen factors (physiological state, strain variation…)



INTRODUCTION
GROUND CHICKEN

Serotypes 8,20:-:z_6 and Kentucky grew less than Thompson at 4 to 8 days at 16°C



Oscar, 2015. J. Food Prot. 78:1819-1827.

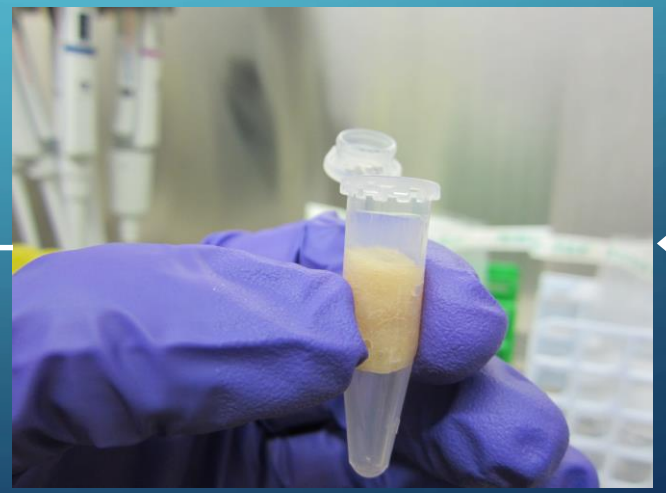
OBJECTIVE
SALMONELLA AND CHICKEN

- To investigate variation of growth among serotypes.
- To develop multiple versions of a model for use in HACCP and QMRA.

STORAGE TRIALS
EXPERIMENTAL DESIGN

- 6 x 8 full factorial at 16°C
  - 0, 1, 2, 4, 6, 8 days
  - 8,20:-:z_6, Kentucky, Typhimurium, Typhimurium var 5-, Thompson, Enteritidis, 4,5,12:Nonmotile, or 4,12:Nonmotile

Ground chicken thigh meat (0.75 cm^3)

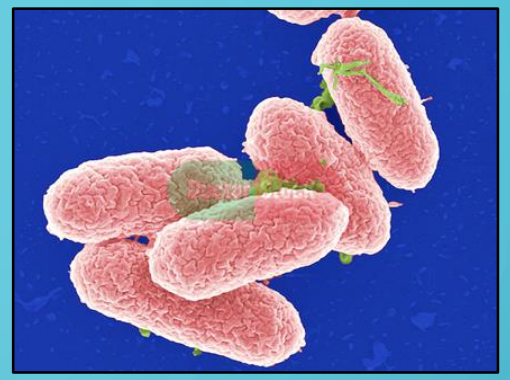


1.7 log

72 h
22°C
0 rpm

Previous history

EXPERIMENTAL DESIGN
JUSTIFICATION



SEROTYPE SELECTION

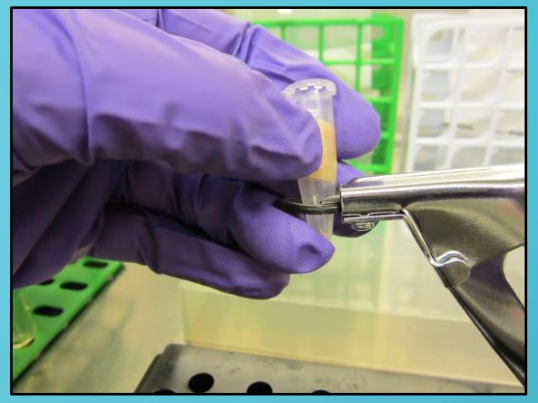
- Chicken isolate
  - All
- Top human clinical isolate
  - Typhimurium var 5-, Typhimurium, Thompson

SEROTYPE SELECTION

- Top chicken isolate
  - Typhimurium var 5-, Typhimurium, Kentucky, Enteritidis
- Grow slower on chicken
  - Kentucky, Enteritidis, 8,20:-:z_6
- Unknown growth on chicken
  - 4,5,12:Nonmotile, 4,12:Nonmotile

STORAGE TRIAL
SAMPLE PROCESSING

- One sample per time
  - 0.75 cm^3 portion
  - 9 ml buffered peptone water
  - 15 seconds in Pulsifier

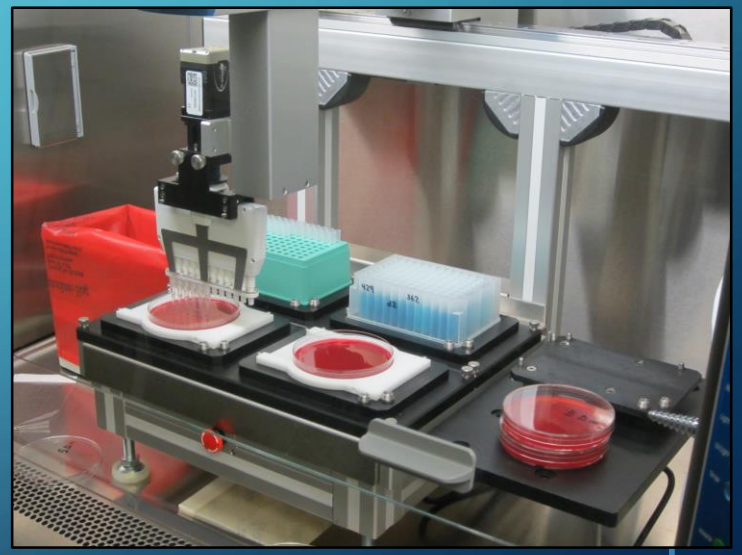


SALMONELLA ENUMERATION

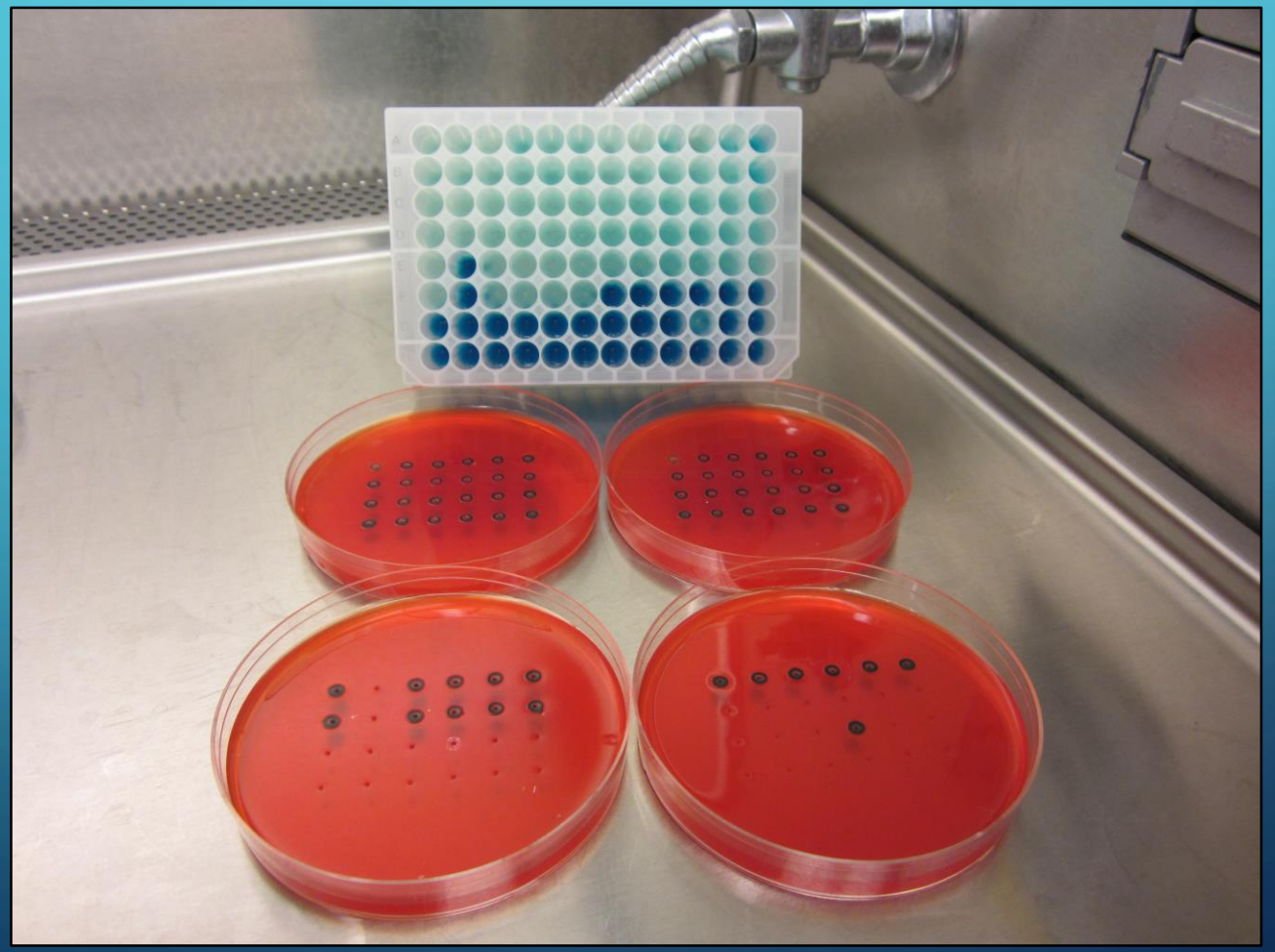
AUTOMATED MINI-MOST PROBABLE NUMBER METHOD

3 REPLICATE X 8 DILUTION

- Pre-enrichment in buffered peptone water.
  - Serial dilution (1:10) → 24 h at 40°C
- Selective enrichment in Rappaport Vassiliadis broth.
  - Transfer (10 µl) → 48 h at 42°C
- Selective plating on xylose lysine tergitol 4 agar.
  - Drop plating (2 µl) → 24 h at 40°C



DROP PLATING
(OPTIONAL)



Rapid confirmation

Later Flow Assay for *Salmonella*

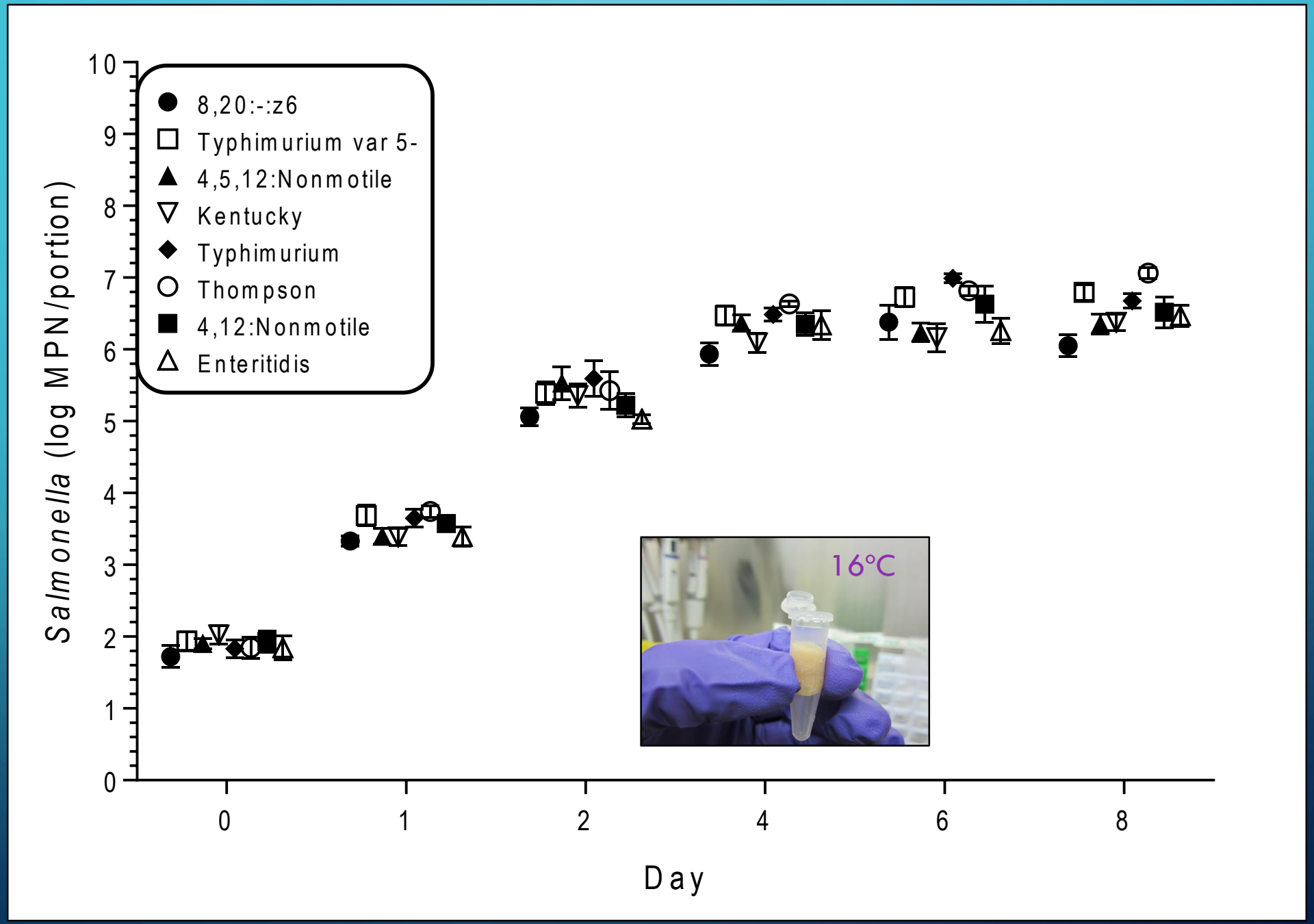

8,20:-:z6
Typhimurium var 5-
4,5,12:Nonmotile
Kentucky
Typhimurium
Thompson
4,12:Nonmotile
Enteritidis
Salmonella (log MPN/portion)
Day
0
1
2
4
6
8
0
1
2
3
4
5
6
7
8
9
10
16°C

Table 1. Results of the two-way analysis of variance for growth of *Salmonella* serotypes in ground chicken thigh meat subjected to temperature for 0 to 8 days at 16°C

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	0.585	0.2167	ns	No
Time	94.68	< 0.0001	****	Yes
Serotype	0.8763	< 0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	5.224	35	0.1493	F (35, 228) = 1.199	P = 0.2167
Time	845.4	5	169.1	F (5, 228) = 1358	P < 0.0001
Serotype	7.825	7	1.118	F (7, 228) = 8.980	P < 0.0001
Residual	28.38	228	0.1245		

Table 2. Effect of time on growth of *Salmonella* serotypes in ground chicken thigh meat subjected to temperature abuse at 16°C: Tukey's multiple comparison test ($P < 0.05$)

	8,20:-:z6		Typhimurium var 5-		4,5,12:Nonmotile		Kentucky	
Comparison	Mean Diff.	Significant?	Mean Diff.	Significant?	Mean Diff.	Significant?	Mean Diff.	Significant?
0 vs. 1	-1.61	Yes	-1.75	Yes	-1.49	Yes	-1.37	Yes
0 vs. 2	-3.34	Yes	-3.45	Yes	-3.63	Yes	-3.33	Yes
0 vs. 4	-4.21	Yes	-4.53	Yes	-4.46	Yes	-4.07	Yes
0 vs. 6	-4.66	Yes	-4.79	Yes	-4.33	Yes	-4.14	Yes
0 vs. 8	-4.33	Yes	-4.86	Yes	-4.45	Yes	-4.35	Yes
1 vs. 2	-1.73	Yes	-1.71	Yes	-2.14	Yes	-1.97	Yes
1 vs. 4	-2.60	Yes	-2.79	Yes	-2.97	Yes	-2.70	Yes
1 vs. 6	-3.05	Yes	-3.05	Yes	-2.84	Yes	-2.77	Yes
1 vs. 8	-2.72	Yes	-3.11	Yes	-2.95	Yes	-2.99	Yes
2 vs. 4	-0.87	Yes	-1.08	Yes	-0.84	Yes	-0.73	Yes
2 vs. 6	-1.32	Yes	-1.34	Yes	-0.70	Yes	-0.81	Yes
2 vs. 8	-0.99	Yes	-1.40	Yes	-0.82	Yes	-1.02	Yes
4 vs. 6	-0.45	No	-0.26	No	0.13	No	-0.07	No
4 vs. 8	-0.12	No	-0.32	No	0.02	No	-0.29	No
6 vs. 8	0.33	No	-0.07	No	-0.12	No	-0.22	No

Table 3. Effect of serotype on growth of *Salmonella* in ground chicken thigh meat subjected to temperature abuse at 16°C: Tukey's multiple comparison test ($P < 0.05$)

Day	Comparison	Mean Diff.	Significant?
4	8,20:-:z6 vs. Thompson	-0.70	Yes
6	4,5,12:Nonmotile vs. Typhimurium	-0.76	Yes
6	Kentucky vs. Typhimurium	-0.83	Yes
6	Kentucky vs. Thompson	-0.66	Yes
6	Typhimurium vs. Enteritidis	0.73	Yes
8	8,20:-:z6 vs. Typhimurium var 5-	-0.74	Yes
8	8,20:-:z6 vs. Typhimurium	-0.63	Yes
8	8,20:-:z6 vs. Thompson	-1.01	Yes
8	4,5,12:Nonmotile vs. Thompson	-0.71	Yes
8	Kentucky vs. Thompson	-0.69	Yes

MODEL DEVELOPMENT
NEURAL NETWORK

- Software
  - Excel
  - NeuralTools
- Data
  - Training (n = 192)
  - Testing (n = 84)

	A	B	C	D
1				
2	Tag	Serotype	Time	Log
3	Train	8,20:-:z6	0	2.20
4	Train	8,20:-:z6	0	2.01
5	Train	8,20:-:z6	0	1.82
6	Train	8,20:-:z6	0	1.69
7	Test	8,20:-:z6	0	1.39
8	Test	8,20:-:z6	0	1.22
9	Train	8,20:-:z6	1	3.56
10	Train	8,20:-:z6	1	3.44
11	Train	8,20:-:z6	1	3.37
12	Train	8,20:-:z6	1	3.29
13	Test	8,20:-:z6	1	3.22
14	Test	8,20:-:z6	1	3.10

STEP 1: DEFINE DATASET RANGE VARIABLE TYPES

NeuralTools - Data Set Manager [Book1]

New

Delete

Data Set #1

Data Set

Name: Data Set #1

Excel Range: A1:D277 Multiple...

Apply Cell Formatting

Variables

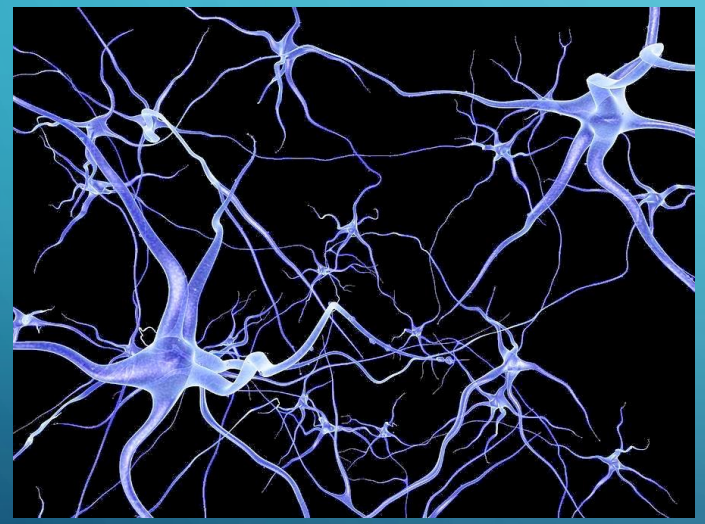
Excel Data Range	Variable Name	Variable Type
A2:A277	Tag	Tag
B2:B277	Serotype	Independent Category
C2:C277	Time	Independent Numeric
D2:D277	Log	Dependent Numeric

4 Variables, 276 Data Cells Per Variable

Import...

OK Cancel

STEP 2: SELECT
TYPE OF NEURAL NETWORK



NeuralTools - Training

Train | Net Configuration | Runtime

Type of Net: MLF Net

Options:

Perform Linear Regression (Numeric Prediction Only)

Number of Nodes in 1st Layer: 2

Number of Nodes in 2nd Layer: 0

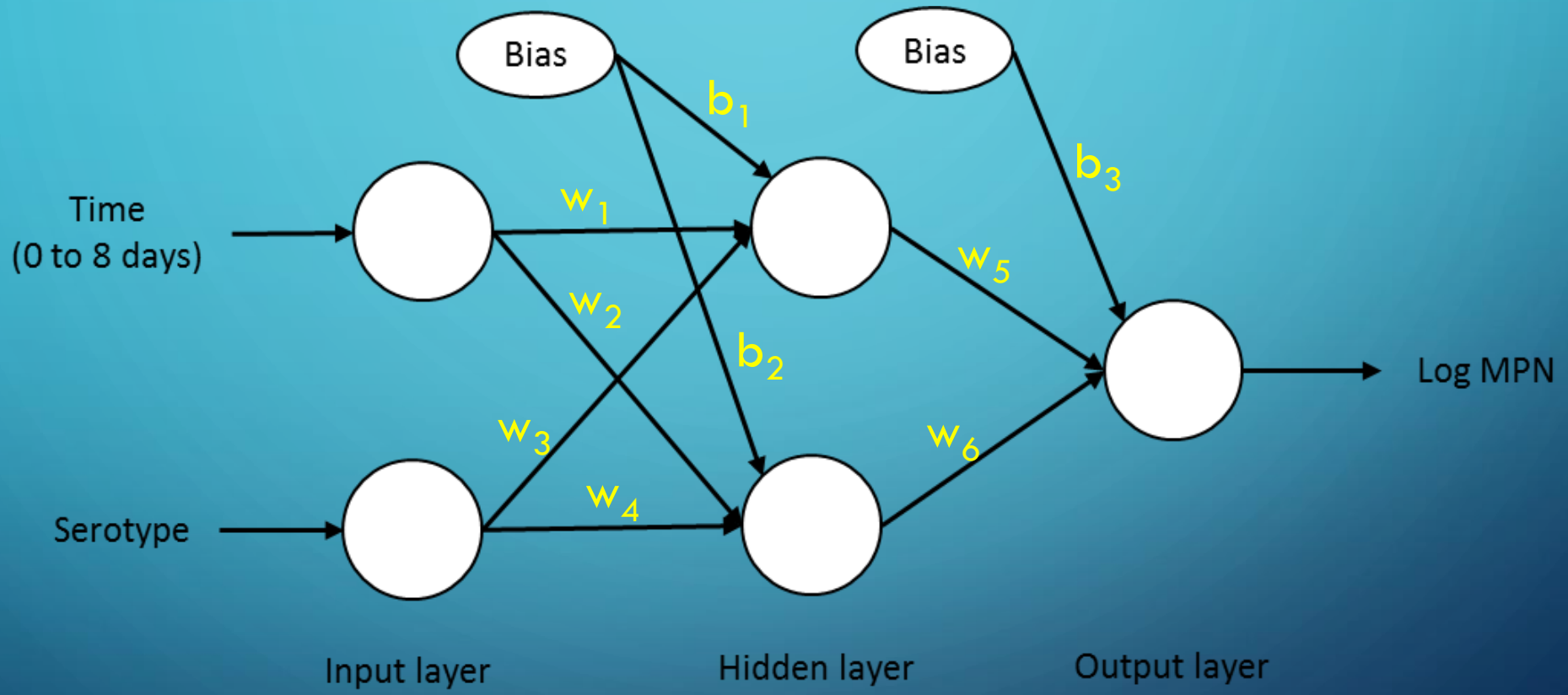
Description:

A Multi-Layer Feedforward Network consists of an input layer of nodes, one or two hidden layers, and an output layer. A net is configured by specifying the numbers of nodes in both hidden layers. By selecting zero nodes, the second layer is eliminated; it is seldom needed for better prediction accuracy. NeuralTools can auto-configure the net based on training data. If possible, use the more time-consuming Best Net Search to find the optimal configuration.

Next >> Cancel

MULTI-LAYER FEEDFORWARD NEURAL NETWORK

SINGLE HIDDEN LAYER WITH TWO NODES



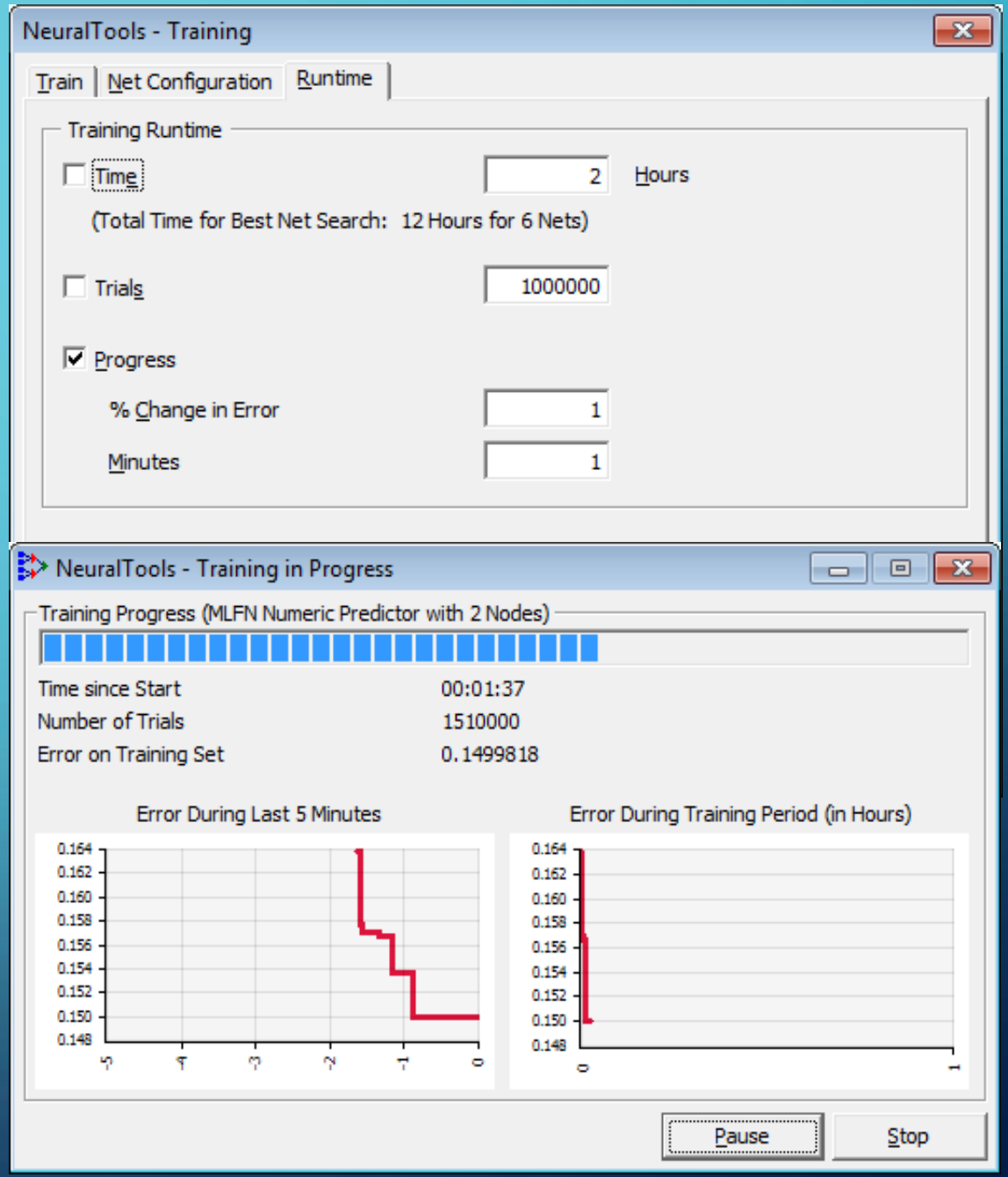
w = weight

b = bias

Hyperbolic Tangent Function

Identity Function

STEP 3: TRAIN
THE NEURAL NETWORK



STEP 4: EVALUATE

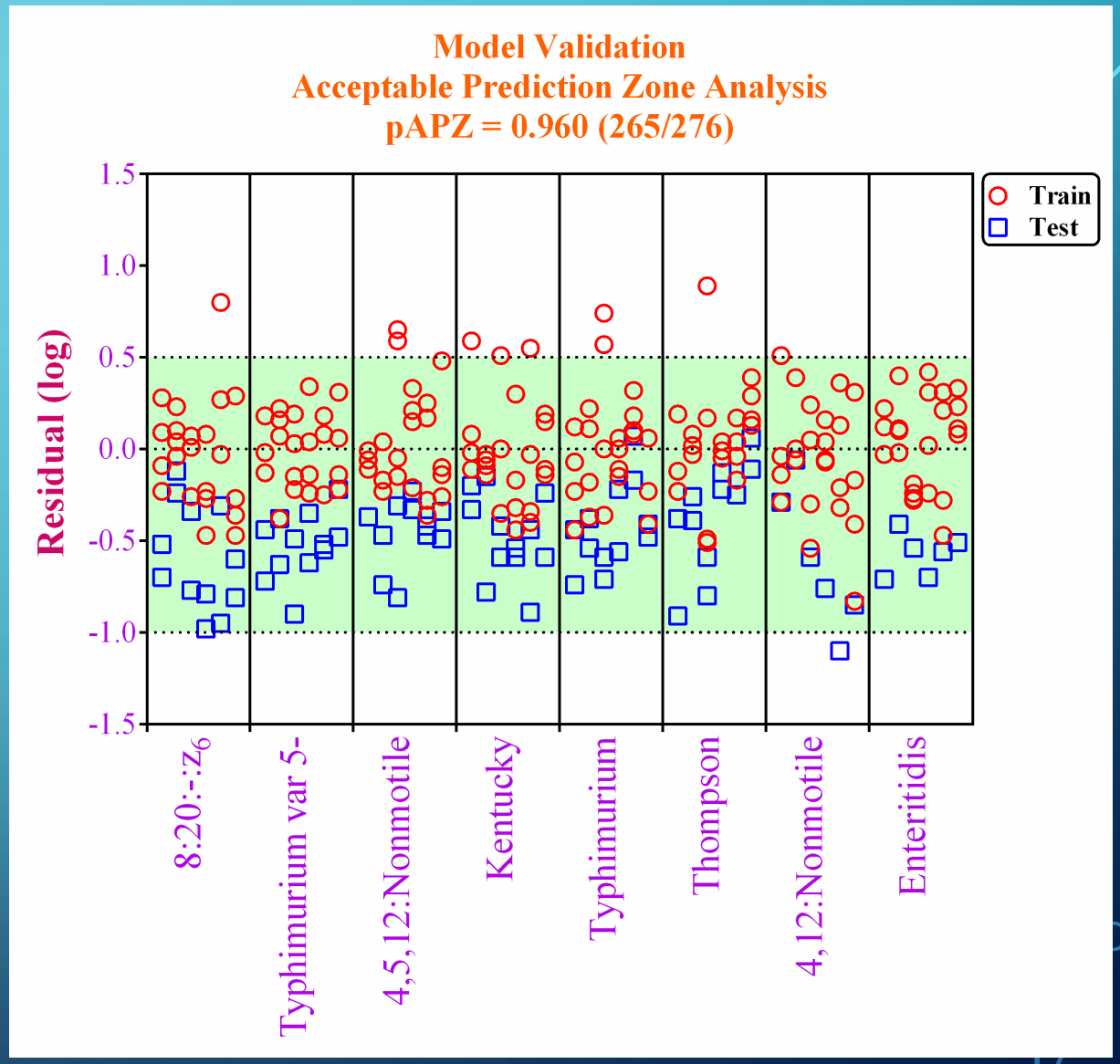
ACCEPTABLE PREDICTION ZONE METHOD

Acceptable Accuracy and Bias

pAPZ ≥ 0.7

-1 log (fail-safe) to 0.5 log (fail-dangerous)

Residual (log) = observed - predicted



Oscar, 2005, J. Food Prot. 68:2606-2613.

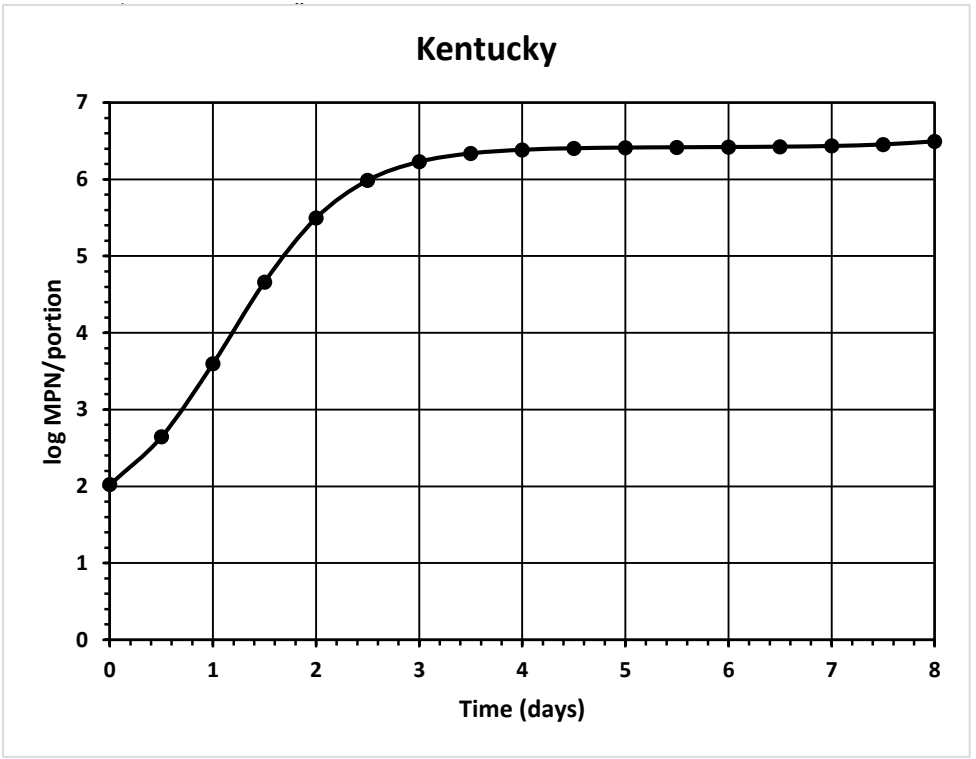
TABLE 4. Performance of the neural network model for growth of *Salmonella* serotypes in ground chicken thigh meat stored at 16°C for 0 to 8 days

	Train			Test			Train + Test		
Serotype	In	Total	pAPZ[a]	In	Total	pAPZ	In	Total	pAPZ
8,20:-:z6	23	24	0.958	12	12	1.000	35	36	0.972
Typhimurium var 5-	24	24	1.000	12	12	1.000	36	36	1.000
4,5,12:Nonmotile	22	24	0.917	12	12	1.000	34	36	0.944
Kentucky	21	24	0.875	12	12	1.000	33	36	0.917
Typhimurium	22	24	0.917	12	12	1.000	34	36	0.944
Thompson	23	24	0.958	12	12	1.000	35	36	0.972
4,12:Nonmotile	23	24	0.958	5	6	0.833	28	30	0.933
Enteritidis	24	24	1.000	6	6	1.000	30	30	1.000
	182	192	0.948	83	84	0.988	265	276	0.960

[a] Proportion of residuals (observed - predicted) in an acceptable prediction zone from -1 log (fail-safe) to 0.5 log (fail-dangerous).

STEP 5: DEVELOP
THE PREDICTIVE MODEL

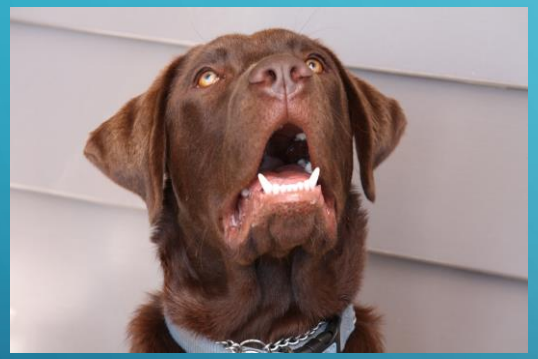
Serotype	Time	Log
Kentucky	0.0	2.02
Kentucky	0.5	2.64
Kentucky	1.0	3.60
Kentucky	1.5	4.66
Kentucky	2.0	5.49
Kentucky	2.5	5.99
Kentucky	3.0	6.23
Kentucky	3.5	6.34
Kentucky	4.0	6.38
Kentucky	4.5	6.40
Kentucky	5.0	6.41
Kentucky	5.5	6.42
Kentucky	6.0	6.42
Kentucky	6.5	6.42
Kentucky	7.0	6.43
Kentucky	7.5	6.45
Kentucky	8.0	6.49



8,20:-:z6
Typhimurium var 5-
4,5,12:Nonmotile
Kentucky
Typhimurium
Thompson
4,12:Nonmotile
Enteritidis

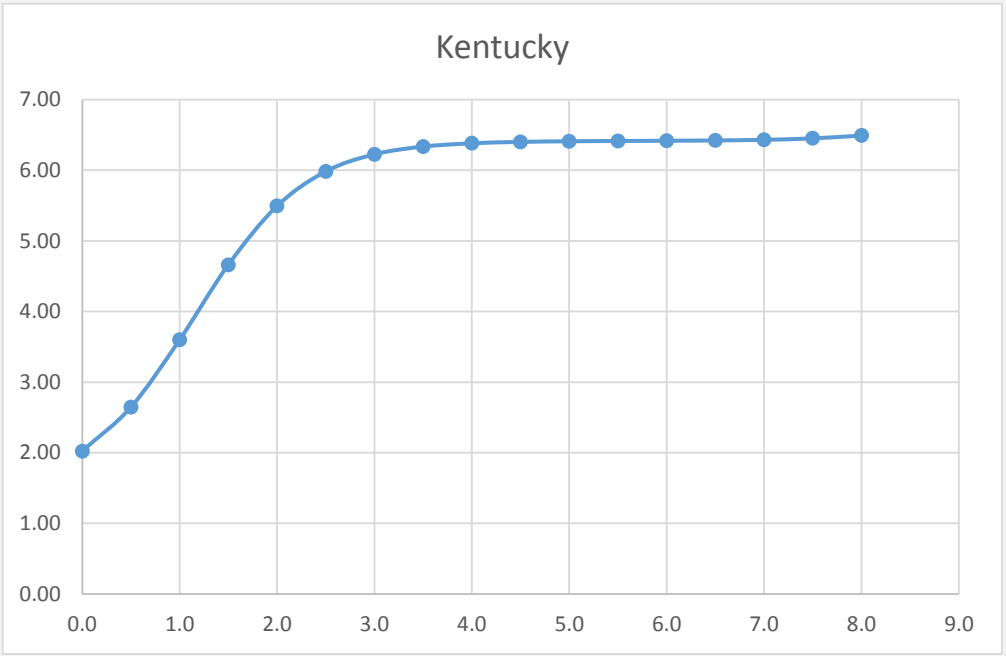
Excel

NeuralTools
Sophisticated Neural Networks for Excel



VLOOKUP

	A	B	C
1	Serotype	Time	Log
2	Kentucky	0.0	2.02
3	Kentucky	0.5	2.64
4	Kentucky	1.0	3.60
5	Kentucky	1.5	4.66
6	Kentucky	2.0	5.49
7	Kentucky	2.5	5.99
8	Kentucky	3.0	6.23
9	Kentucky	3.5	6.34
10	Kentucky	4.0	6.38
11	Kentucky	4.5	6.40
12	Kentucky	5.0	6.41
13	Kentucky	5.5	6.42
14	Kentucky	6.0	6.42
15	Kentucky	6.5	6.42
16	Kentucky	7.0	6.43
17	Kentucky	7.5	6.45
18	Kentucky	8.0	6.49
19			
20			
21			
22			
23	8,20:-:z6	0	1.92
24	Typhimurium var 5-	0	2.13
25	4,5,12:Nonmotile	0	2.06
26	Kentucky	0	2.02
27	Typhimurium	0	2.13
28	Thompson	0	2.12
29	4,12:Nonmotile	0	1.99
30	Enteritidis	0	1.93
31	8,20:-:z6	0.5	2.45
32	Typhimurium var 5-	0.5	2.83
33	4,5,12:Nonmotile	0.5	2.71
34	Kentucky	0.5	2.64
35	Typhimurium	0.5	2.83
36	Thompson	0.5	2.82



STANDALONE VERSION

HACCP

Excel

Iteration #1

	A	B	C	D	E	F	G
1							
2	**Serotype**	**Time**	**Log**		**Prevalence**	**Code**	**Serotype**
3	Enteritidis	0.0	1.93		2	1	Thompson
4	Enteritidis	1.3	3.90		24	2	Typhimurium var 5-
5	Output	Δ	1.97		8	3	Typhimurium
6					2	4	4,5,12:Nonmotile
7					2	5	4,12:Nonmotile
8					5	6	8,20:-:z6
9					4	7	Kentucky
10					2	8	Enteritidis
11							

- A3=VLOOKUP(RiskDiscrete(F3:F10,E3:E10),F3:G10,2,FALSE)
- B4=RiskLognorm(1,1,RiskTruncate(0,8))

Iteration #10,000

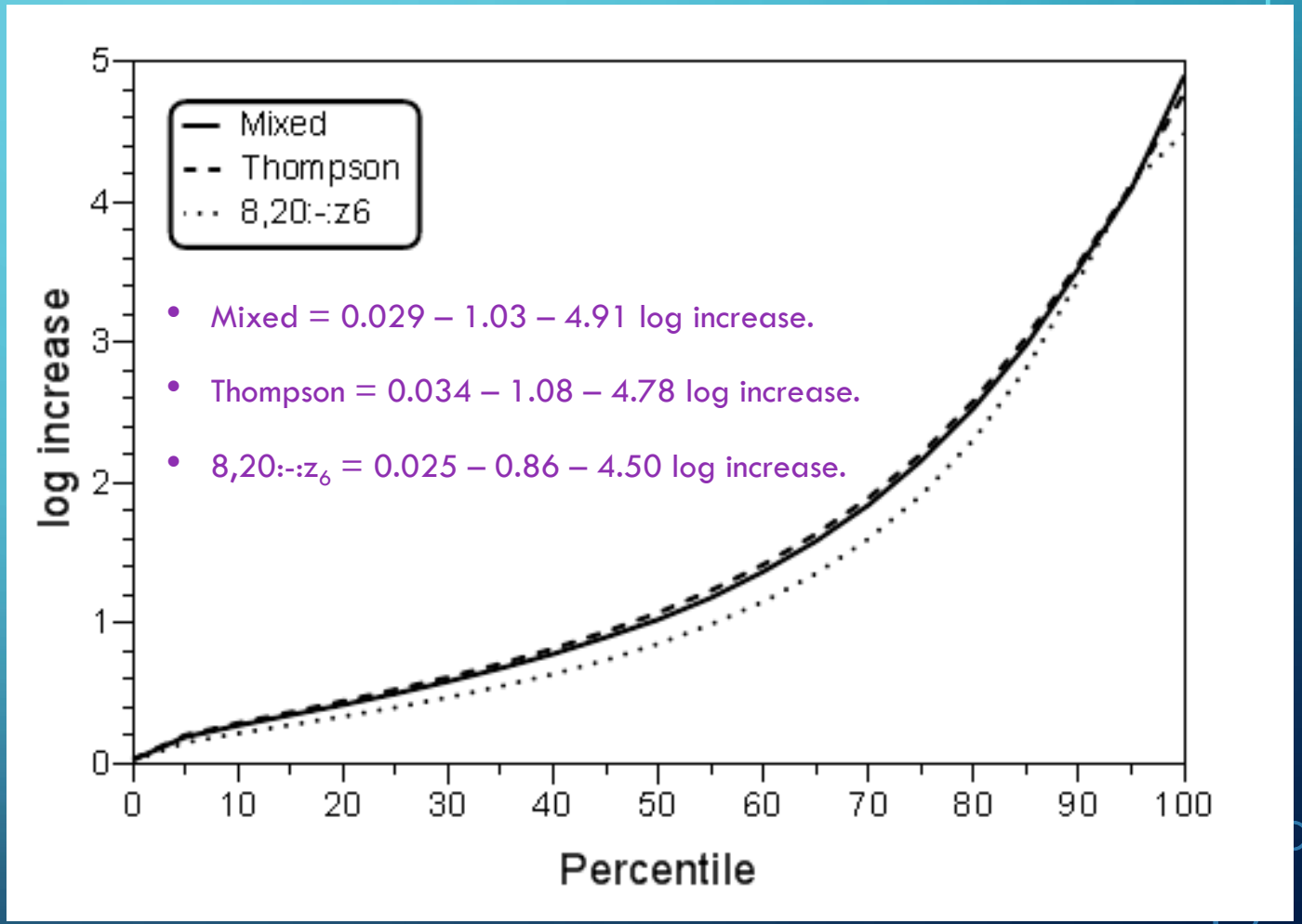
	A	B	C	D	E	F	G
1							
2	**Serotype**	**Time**	**Log**		**Prevalence**	**Code**	**Serotype**
3	4,12:Nonmotile	0.0	1.99		2	1	Thompson
4	4,12:Nonmotile	0.4	2.39		24	2	Typhimurium var 5-
5	Output	Δ	0.40		8	3	Typhimurium
6					2	4	4,5,12:Nonmotile
7					2	5	4,12:Nonmotile
8					5	6	8,20:-:z6
9					4	7	Kentucky
10					2	8	Enteritidis
11							

STOCHASTIC VERSION
RISK ASSESSMENT

MODEL SIMULATION RESULTS
RISK ASSESSMENT

Output

Cumulative distribution for log increase

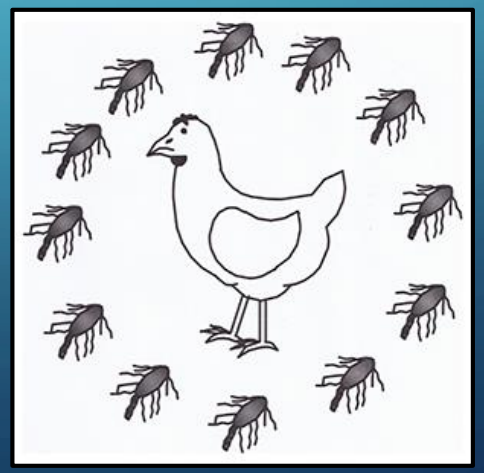


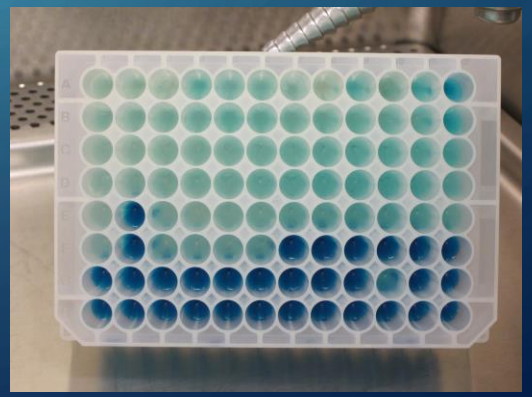
- Mixed = 0.029 – 1.03 – 4.91 log increase.
- Thompson = 0.034 – 1.08 – 4.78 log increase.
- 8,20:-:z_6 = 0.025 – 0.86 – 4.50 log increase.

SUMMARY

- Confirmed that serotype is an important independent variable for modeling growth of *Salmonella* in chicken.
- Used an automated mMPN method to acquire data for model development.
- Developed different versions of a neural network model for use in HACCP and QMRA.

www.ars.usda.gov/naa/errc/PoultryFARM





ACKNOWLEDGEMENT

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