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## Appendices

### Appendix A: The composition of Custom Mix mouse feed

Protein	21.50%
Fibre	4.00%
Sodium	0.24% minimum
Calcium	1.75% minimum
Phosphorus	1.20%

### Appendix B: The composition of Chick Starter Feed and Broiler Finisher Feed

#### 1. Chick Starter Feed :

Crude Protein, minimum	20.0%
Crude Fibre, maximum	4.50%
Crude Fat	3.70%
Salt	0.50%
Fluorine, maximum	0.02%
Urea, maximum	Nil

This feed was compounded from: Wheat, Sorghum, Pollard, Bran, Lucerne Meal, Rice Pollard, Meat and Bone Meal, Blood Meal, Soyabean Meal, Cottonseed Meal, Sunflower Seed Meal, Salt, Limestone, Methionine, Vitamins A, D3, E, K, B12, Riboflavin, Niacin, Pantothenic Acid, Folicin, Biotin, Choline, Oxides of Zinc and Manganese, Sulphates of Iron and Copper, Potassium Iodide, Ethoxyquin and/either 3-5 dinitro-o-Toluamide 125 ppm or Elancoban 90 ppm or Coxistac 60 ppm as a coccidiostat.

#### 2. Broiler Finisher Feed :

Crude Protein, minimum	19.0%
Crude Fibre, maximum	3.50%
Crude Fat	3.50%
Salt	0.50%
Fluorine, maximum	0.02%
Urea, maximum	Nil

This feed was compounded from: Wheat, Sorghum, Meat and Bone Meal, Blood Meal, Fish Meal, Soyabean Meal, Cottonseed Meal, Sunflower Seed Meal, Salt, Lime,



Vitamins A, D3, E, K and B12, Niacin, Calcium Pantothenate, Folic Acid, Pyridoxine, Choline Chloride, Riboflavin, Oxides of Zinc and Manganese, Sulphates of Iron and Copper, Potassium Iodide, Ethoxyquin, Methionine and either Coxistac 60 ppm or Elancoban 90 ppm or Avotec 90 ppm as a coccidiostat.

### Appendix C: Buffered 10% Formalin (pH 7)

Formaldehyde	100 mL
Distilled H <sub>2</sub> O	900 mL
Sodium dihydrogen phosphate (NaH <sub>2</sub> PO <sub>4</sub> .H <sub>2</sub> O) or [NaH <sub>2</sub> PO <sub>4</sub> .2H <sub>2</sub> O = 4.25 g]	4 g
Disodium sodium phosphate (Na <sub>2</sub> HPO <sub>4</sub> )	6.5 g

Dissolve sodium phosphate in water, add formaldehyde and mix.

### Appendix D: Mobile Phase for HPLC

#### Preparing buffer solution

1. 6.8045 g KH<sub>2</sub>PO<sub>4</sub> was weighed
2. Dissolve KH<sub>2</sub>PO<sub>4</sub> into 2 L of deionised water to make 0.025 M KH<sub>2</sub>PO<sub>4</sub>
3. Add 2 ml of Triethylamine (TEA)
4. Adjust pH to 7.15

#### Mobile Phase

To make mobile phase, the buffer solution was mixed with Acetonitrile (ACN) :

2:1 for hordenine analysis

6:4 for gramine analysis

This mixture was then filtered under vacuum through 0.5 µm filter paper (Waters Associates) before using.

### Appendix E: The Histokinetic Process

The series of solution used consecutively in the histokinetic process:

- 30% alcohol                      2 hours
- 50% alcohol                      2 hours
- 70% alcohol                      2 hours
- 80% alcohol                      2 hours
- 95% alcohol                      1 hour
- absolute alcohol I              1 hour

- absolute alcohol II	1 hour
- 50:50 alcohol/xylol	1 hour
- xylol I	1 hour
- xylol II	2 hours
- paraplast I	2 hours
- paraplast II	2 hours

## Appendix F: The Staining Process (Ehrlich's Haemotoxylin and Eosin)

### 1. The series of solution used consecutively in the staining process:

- xylol I	5 mins
- xylol II	5 mins
- absolute alcohol I	3 mins
- absolute alcohol II	3 mins
- 80% alcohol	2 mins
- 50% alcohol	2 mins
- distilled water	1 min
- Ehrlich's haemotoxylin	40 mins
- rinse in H <sub>2</sub> O	
- acid/alcohol rinse	3-5 mins
- running tap cold water	30 mins
- 70% alcohol	2 mins
- 90% alcohol	2 mins
- absolute alcohol III	2 mins
- alcoholic eosin	2-3 mins
- absolute alcohol IV	2 mins
- absolute alcohol V	2 mins
- xylol III	2 mins
- xylol IV	3 mins
- mount in DPX	

### 2. Alcoholic Eosin:

Eosin Y	10 g
Distilled H <sub>2</sub> O	200 mL
95% alcohol	800 mL

Dissolve eosin in distilled water and add alcohol

### 3. Acid Alcohol:

70% alcohol	990 mL
concentrated hydrochloric acid (HCl)	10 mL

**4. Ehrlich's Haemotoxylin:**

Haemotoxylin	5 g
Absolute alcohol	250 mL
Glycerol	250 mL
Distilled H <sub>2</sub> O	250 mL
Glacial Acetic Acid	50 mL
Potash Alum (Aluminium Potassium Sulphate)	37.5 g

Dissolve the haemotoxylin in the alcohol, dissolve the alum in the water, mix together and add other components.

**Appendix G: Body weights of mice (g) receiving different levels of alkaloids gramine and hordenine in feed during the feeding choice experiment**

Treatment Group	Week after Treatment								
	0	1	2	3	4	5	6	7	8
H 50	33.74 ± 2.36	35.34 ± 2.70	34.40 ± 2.64	35.40 ± 2.80	35.48 ± 3.08	36.66 ± 2.90	36.26 ± 3.35	36.06 ± 3.07	37.82 ± 3.35
H 500	32.44 ± 1.18	33.26 ± 1.63	33.92 ± 1.69	34.94 ± 1.86	34.34 ± 1.99	35.14 ± 1.84	35.60 ± 1.80	35.14 ± 1.62	37.90 ± 1.33
G 50	35.12 ± 1.78	34.68 ± 1.03	36.66 ± 1.92	36.94 ± 2.05	36.68 ± 2.20	37.60 ± 2.04	38.80 ± 2.14	36.86 ± 2.33	39.00 ± 2.38
G 500	33.44 ± 1.60	29.62 ± 2.55	34.52 ± 1.92	34.86 ± 2.17	34.42 ± 2.17	34.80 ± 2.41	36.80 ± 2.21	34.48 ± 2.52	37.28 ± 2.25

(Values are Mean ± SEM; H 50 = standard feed + feed containing 50 ppm hordenine; H 500 = standard feed + feed containing 500 ppm hordenine; G 50 = standard feed + feed containing 50 ppm gramine; G 500 = standard feed + feed containing 500 ppm gramine)

**Appendix H: Total feed intakes of mice (g) receiving different levels of alkaloids gramine and hordenine in feed during the feeding choice experiment**

Treatment Group	Week after Treatment							
	1	2	3	4	5	6	7	8
H 50	53.95	71.93	74.33	62.80	61.93	59.27	64.73	65.80
	± 10.1	± 16.9	± 15.0	± 13.5	± 16.0	± 14.4	± 14.5	± 13.6
H 500	77.20	80.20	92.00	86.67	93.00	89.73	85.67	82.13
	± 12.6	± 16.6	± 15.3	± 14.6	± 15.4	± 15.5	± 15.6	± 17.6
G 50	85.50	87.13	93.07	85.27	89.13	86.67	79.53	84.67
	± 17.1	± 16.7	± 16.7	± 13.6	± 14.7	± 14.7	± 12.1	± 15.3
G 500	70.70	89.93	105.80	97.07	99.73	101.93	103.60	90.80
	± 16.8	± 16.6	± 12.7	± 15.2	± 14.7	± 15.6	± 14.9	± 13.9

(Values are Mean ± SEM; H 50 = standard feed + feed containing 50 ppm hordenine; H 500 = standard feed + feed containing 500 ppm hordenine; G 50 = standard feed + feed containing 50 ppm gramine; G 500 = standard feed + feed containing 500 ppm gramine)

**Appendix I: Liver weights of mice (g) receiving different levels of alkaloids gramine and hordenine in feed during the feeding choice experiment**

Treatment Group			
H 50	H 500	G 50	G 500
2.2328	2.430	2.3564	2.1038
± 0.198	± 0.096	± 0.083	± 0.223

(Values are Mean ± SEM; H 50 = standard feed + feed containing 50 ppm hordenine; H 500 = standard feed + feed containing 500 ppm hordenine; G 50 = standard feed + feed containing 50 ppm gramine; G 500 = standard feed + feed containing 500 ppm gramine)

**Appendix J: Liver weights of mice (g) receiving different levels of alkaloids gramine and hordenine**

Treatment Group				
H 50	H 500	G 50	G 500	Control
1.9840	1.7928	2.2634	2.0880	2.0594
± 0.12	± 0.25	± 0.14	± 0.12	± 0.15

(Values are Mean ± SEM; H 50 and H 500 = feed containing 50 and 500 ppm hordenine respectively; G 50 and G 500 = feed containing 50 and 500 ppm gramine respectively; control = no gramine and hordenine)

**Appendix K: Liver weights of chickens (g) receiving different levels of alkaloids gramine and hordenine**

Treatment Group				
H 50	H 500	G 50	G 500	Control
61.29	43.69	58.63	58.62	56.74
± 8.39	± 1.27	± 6.81	± 6.30	± 5.08

(Values are Mean ± SEM; H 50 and H 500 = feed containing 50 and 500 ppm hordenine respectively; G 50 and G 500 = feed containing 50 and 500 ppm gramine respectively; control = no gramine and hordenine)

**Appendix L: Body weights of mice (g) receiving different levels of alkaloids gramine and hordenine in feed**

Treatment Group	Week after Treatment											
	0	1	2	3	4	5	6	7	8	9	10	11
H 50	25.06 ± 0.69	26.26 ± 1.19	27.79 ± 1.39	29.20 ± 1.43	30.99 ± 1.41	32.29 ± 1.58	33.51 ± 1.31	34.57 ± 1.43	35.48 ± 1.55	35.37 ± 1.31	36.94 ± 1.56	37.12 ± 1.66
H 500	22.23 +	23.55 +	25.11 +	26.89 +	28.62 +	28.83 +	29.30 +	29.80 +	30.98 +	31.27 +	31.86 +	31.95 +
G 50	1.74 ± 25.34	2.16 ± 27.40	2.21 ± 29.85	2.31 ± 31.58	2.63 ± 33.21	3.39 ± 33.94	3.68 ± 34.75	4.02 ± 35.13	3.99 ± 36.20	4.03 ± 35.96	3.98 ± 36.89	3.93 ± 36.75
G 500	1.26 ± 26.55	1.82 ± 27.85	2.15 ± 29.92	2.13 ± 32.29	2.06 ± 34.03	2.20 ± 34.54	2.18 ± 35.32	3.09 ± 37.73	3.03 ± 38.46	2.78 ± 38.44	2.77 ± 38.75	3.21 ± 39.79
Control	25.31 ± 1.06	27.11 ± 1.49	28.79 ± 1.72	31.12 ± 1.82	32.91 ± 1.78	33.04 ± 1.62	33.33 ± 1.54	35.21 ± 1.51	36.33 ± 1.75	35.89 ± 1.80	36.12 ± 1.53	36.21 ± 1.55

(Values are Mean ± SEM. Values within a column with different superscripts are significantly different from one another. H 50 and H 500 = feed containing 50 ppm and 500 ppm hordenine respectively, G 50 and G 500 = feed containing 50 ppm and 500 ppm gramine respectively, control = no hordenine and gramine)

**Appendix M: Feed intakes of chickens (g) receiving different levels of alkaloids  
gramine and hordenine in feed**

Treatment Group	Week after Treatment					
	1	2	3	4	5	6
H 50	291.1 ± 10.8	502.3 ± 26.8	380.9 ± 11.4	1088.0 ± 18.9	1046.9 ± 15.7	1202.9 ± 22.5
H 500	299.7 ± 15.9	511.8 ± 17.1	378.3 ± 19.8	1114.3 ± 19.7	1071.0 ± 11.0	1193.1 ± 33.3
G 50	300.6 ± 12.9	499.0 ± 12.5	402.5 ± 16.0	1079.4 ± 18.0	1045.7 ± 24.8	1190.9 ± 68.4
G 500	268.1 ± 15.0	472.6 ± 16.0	384.9 ± 18.6	1021.9 ± 57.1	1047.4 ± 56.3	1126.6 ± 63.6
Control	296.6 ± 10.4	510.0 ± 13.6	373.2 ± 27.7	1071.4 ± 26.0	1044.2 ± 36.7	1224.1 ± 63.8

(Values are Mean ± SEM; H 50 and H 500 = feed containing 50 and 500 ppm hordenine respectively; G 50 and G 500 = feed containing 50 and 500 ppm gramine respectively; control = no gramine and hordenine)

**Appendix N: Feed conversion ratios of chickens receiving different levels of  
alkaloids gramine and hordenine in feed**

Treatment Group				
H 50	H 500	G 50	G 500	Control
1.9974	1.9838	2.0255	1.9392	2.0459
± 0.02	± 0.04	± 0.05	± 0.02	± 0.05

(Values are Mean ± SEM; H 50 and H 500 = feed containing 50 and 500 ppm hordenine respectively; G 50 and G 500 = feed containing 50 and 500 ppm gramine respectively; control = no gramine and hordenine)



**Appendix O: Body weights of chickens (g) receiving different combination of alkaloids gramine and hordenine in feed**

Treatment Group	Week after Treatment						
	0	1	2	3	4	5	6
Control	107.3 ± 2.74	309.5 ± 6.36	609.9 ± 12.1	863.8 ± 19.7	1409.0 ± 35.7	1936.4 ± 44.2	2378.5 ± 56.4
G 500	107.3 ± 2.75	288.6 ± 9.72	568.7 ± 12.9	810.2 ± 20.0	1319.8 ± 26.0	1815.5 ± 31.5	2192.7 ± 27.6
H 500	107.5 ± 2.68	314.3 ± 13.3	591.5 ± 11.2	858.4 ± 18.5	1392.1 ± 31.2	1930.6 ± 48.5	2337.0 ± 67.3
G50H500	107.3 ± 2.71	314.3 ± 10.9	610.9 ± 13.0	806.7 ± 39.5	1421.7 ± 30.6	1849.4 ± 78.8	2250.5 ± 97.9
H50G500	107.4 ± 2.65	308.1 ± 8.73	607.3 ± 12.4	860.5 ± 29.5	1389.8 ± 43.4	1892.2 ± 40.4	2224.7 ± 35.2

(Values are Mean ± SEM, G 500 and H 500 = feed containing 500 ppm gramine and hordenine respectively, G50H500 = feed containing combination of 50 ppm gramine and 500 ppm hordenine, H50G500 = feed containing combination of 50 ppm hordenine and 500 ppm gramine, control = no gramine and hordenine)

**Appendix P: Feed intakes of chickens (g) receiving different combination of alkaloids gramine and hordenine in feed**

Treatment Group	Week after Treatment					
	1	2	3	4	5	6
Control	286.7 ± 6.56	527.3 ± 9.68	598.3 ± 39.1	1027.4 ± 25.2	1182.5 ± 25.5	1103.9 ± 26.0
G 500	257.2 ± 8.86	508.5 ± 12.9	581.9 ± 18.4	983.4 ± 29.8	1114.0 ± 44.6	1057.3 ± 43.6
H 500	292.5 ± 6.12	538.1 ± 6.90	614.2 ± 13.7	998.4 ± 47.0	1172.6 ± 21.5	1097.8 ± 23.4
G50H500	295.9 ± 8.46	538.8 ± 6.8	594.9 ± 17.8	988.0 ± 19.2	1126.5 ± 36.5	1081.3 ± 28.1
H50G500	260.3 ± 5.83	527.2 ± 3.30	616.4 ± 9.18	985.4 ± 38.9	1121.7 ± 23.6	1057.3 ± 43.6

(Values are Mean ± SEM, G 500 and H 500 = feed containing 500 ppm gramine and hordenine respectively, G50H500 = feed containing combination of 50 ppm gramine and 500 ppm hordenine, H50G500 = feed containing combination of 50 ppm hordenine and 500 ppm gramine, control = no gramine and hordenine)

**Appendix Q: Feed conversion ratios of chickens receiving different combination of alkaloids gramine and hordenine in feed**

Treatment Group				
Control	G 500	H 500	G50H500	H50G500
1.9918	2.0312	2.0035	2.0157	2.0166
± 0.06	± 0.03	± 0.04	± 0.06	± 0.05

(Values are Mean ± SEM, G 500 and H 500 = feed containing 500 ppm gramine and hordenine respectively, G50H500 = feed containing combination of 50 ppm gramine and 500 ppm hordenine, H50G500 = feed containing combination of 50 ppm hordenine and 500 ppm gramine, control = no gramine and hordenine)