POULTRY CRC LTD

FINAL REPORT

Sub-Project No: 2.1.1

PROJECT LEADER: Dr David Cadogan

Influence of betaine on embryo survival, hatchability and chick quality

DATE OF COMPLETION: 10 July 2014

ISBN 1 921010 63 0

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Published in August 2014

Executive Summary

Two experiments were conducted to first investigate the uptake of dietary betaine into the egg, and secondly to commercially evaluate the supplementation of natural anhydrous betaine on broiler breeder performance, using hatchability and chick quality as measures.

In experiment 1, Hy-Line Brown laying hens were offered a control diet and a treated diet with 1000ppm of natural anhydrous betaine over a 6 week period. After 1 week pre-treatment, eggs were collected, contents emptied into containers and pooled in weekly periods. The betaine supplementation increased betaine content from week 2, and levels stayed similar until week 5. Overall, the betaine supplementation significantly increased the betaine content of the eggs ($P<0.001$) from 0.46 mg/100g (control) to 1.37 mg/100g (treated), a 3 fold increase. Betaine had no significant effect on the production parameters of the laying hens, although there was a numerical 2.4% increase in egg weight by the treated diet.

The commercial evaluation of betaine (experiment 2) was tested in 3 sheds of 7000 Ross 308 broiler breeders, run in two blocks, with a partial crossover (one shed had both a control and a treated diet at different times) of treatments (control and betaine treated diets with 2000ppm). The broiler breeders were 32 weeks of age, and were offered the control and treated diets for 24 weeks. Betaine significantly improved hatchability from 84.75% to 86.89% ($P=0.004$), but had no effect on hatch weight or number of chicks culled.

The improvement in hatchability supports the work conducted in pig breeders, and strongly suggests betaine improves embryo survival in the egg during the incubation process. Further work is required to assess the effects of betaine on the broiler breeder progeny, to find whether the increased betaine content in the egg improves progeny growth performance and carcass yield.

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Introduction

Betaine is one of the most powerful osmolytes in nature. The presence of betaine enables microbes, plants and animals alike to be more resistant to osmotic, heat, cold, disease or environmental stresses. Betaine is also a very good source of methyl groups, which are used to spare the amino acid methionine, increase carnitine levels and methylate DNA to maximise its integrity and gene expression. In monogastrics, betaine has been shown to spare energy by reducing the demand of ATP in sodium and potassium pump, and thus improve growth and carcass yield. More recently it has been demonstrated that betaine significantly improves embryo survival in gestating sows by reducing blood homocysteine, a blood toxin and reducing (temperature) stress on the sow (Van Wettere et al., 2012). There is on-going research examining the benefits of betaine for pregnant women, post 10 weeks of pregnancy, reducing the toxin homocysteine in the blood and reducing the risk of embryo loss and abnormalities (Ekland et al., 2005).

In the commercial broiler and layer industry, the large scale incubation of fertile eggs is difficult to manage, particularly keeping an even, ideal temperature for each individual egg. Temperature variation and fluctuation does occur in commercial incubators, and can cause major problems with embryo development and survival. Impaired embryo development produces a considerable loss in broiler and layer performance and health. The supplementation of betaine should allow the incubated embryo to be more resistant to temperature fluctuations and reduce the potentially toxic homocysteine levels in the embryo. Increased levels of betaine in the egg also maximises the methylation of DNA, increasing its stability, in turn having a positive influence on epigenesis and allowing the bird to perform closer to its genetic potential.

Volume of birds, and hence eggs produced, has a large influence on the sustainability and returns to the broiler producer. If there are problems with producing enough viable chicks, then the proportion of the overhead costs increases significantly. Large incubators in hatcheries are fully automated, and the temperature and other environmental conditions can vary in different locations of the units. Variations of over 2°C can cause problems with chick development, and may cause increased embryo mortality.

If foetal programming and embryo development are maximised, this will allow the birds to reach their genetic potential and improve poultry industry efficiency, sustainability and carbon footprint. Improvements in chick quality and subsequent growth through betaine supplementation have the potential to increase feed efficiency by at least 2%, which is approximately $55 million in savings to the industry producing 550 million broilers per year.

The present Poultry CRC project 2.1.1 incorporated two experiments. The first experiment was designed to investigate whether a significant amount of dietary betaine can accumulate in the egg, using commercial laying hens. Experiment 2 assessed the benefits of natural betaine supplementation of Ross 308 broiler breeders, measuring the hatchability, percentage of chick culls and hatched weight under commercial conditions.

Experiment 1

The usefulness of betaine as a feed additive for laying hens

Methodology

A total of 60 laying hens of approximately 50 weeks of age were divided into 2 equal groups of 30 birds and allocated to one of two dietary treatments. The control diet was sorghum/soy-based (Table 1) formulated to meet the birds' nutrient requirements. One treatment was the control diet supplemented with 1kg/tonne natural anhydrous betaine (Betafin®) and the other unsupplemented diet served as a control. The diets were initially planned to contain 2kg/tonne (2000 ppm) of betaine, however, an error in communication meant half the dose rate was used. These diets were fed to the laying hens for a period of 42 days during which time weekly egg weights, feed intake and egg quality information was recorded. Prior to the initiation of the 42 day data capture period, the laying hens received the experimental diets for 10 days. Eggs were homogenised each week, freeze dried and sent to Danisco, Finland, for betaine analysis.

Diets

Diets were formulated by Dr David Creswell and mixed on site at the Poultry Research Foundation, University of Sydney. The dietary treatments were 0 and 1000ppm betaine.

Table 1. Diet composition and calculated analysis.

Ingredients	Amount, kg/t
Sorghum 9	702.4
Millrun	13
Soybean meal 48	143
MBM 50	45
Limestone	85
MDCP 21	1.7
Salt	2.7
Sodium bicarbonate	1.5
L Lysine HCL	1.3
DL Methionine	2.3
L Threonine	0.5
Choline chloride 60	0.6
Vitamins/trace minerals	1.0
Total, kg	1000
Nutrient minimums	
ME, kcal/kg (MJ/kg)	2800 (11.72)
Protein, % (actual)	(15.8)
SID	
Lysine, %	0.70
Methionine, %	0.382
MC, %	0.614
Tryptophan, %	0.154
Threonine, %	0.509
Arginine, %	0.749
Isoleucine, %	0.555
Valine, %	0.65
Calcium, %	3.6
Available P, %	0.32
Sodium, %	0.18
Choline, ppm	1250

Design

Laying hens were housed individually in battery cages and had ad libitum access to feed and water. For experimental purposes one replicate was considered to be 3 adjacent cages of 1 bird i.e. each of the two treatments were replicated 10 times (a total of 30 birds on each diet).

Egg weight and numbers were recorded daily and notes made on any apparent egg shell abnormality (frosting, cracked, soft-shelled). Feed consumption was monitored weekly.

All eggs laid over one week, within treatment, were homogenised, a sub-sample taken and freeze dried. The betaine content of the eggs was analysed through acetonitrile and methanol extraction, and betaine was determined as bromophenacyl ester derivatives with UV detection by HPLC.

Results

The effect of betaine addition to a sorghum-based diet on the performance of laying hens is presented in Table 2. There was no significant effect of treatment on any performance parameter, although there was a numerical 2.4% increase in egg weight (P=0.23).

Table 2. Effect of betaine addition to sorghum-based diets on laying hen performance over a six-week feeding period.

Treatment	**Egg Wt (g)**	**Egg Wt (g/wk/rep)**	**Egg No. (per wk/bird)**	**Feed Intake (g/rep/wk)**	**FCR (g:g)**	**Lay %**
Control	61.2	1129.2	5.88	2196.2	1.99	83.97
Betaine	62.7	1132.8	5.87	2219.3	2.00	83.81
P =	*0.23*	*0.96*	*0.95*	*0.75*	*0.92*	*0.92*
SEM	*0.829*	*49.55*	*0.11*	*51.28*	*0.094*	*1.02*

NB. One replicate is 3 adjacent cages, each of 1 bird. Thus, the feed intake per bird, per day, was approximately 105g. Lay percentage and egg numbers per week do not include seconds (cracked, frosted, soft-shelled). Observationally, egg quality was unaffected by treatment. When seconds are included in the analysis lay % increases to 88.3% for both treatment groups.

The addition of 1 kg/tonne or 1000ppm of betaine to the diet increased the content of betaine by 0.91 mg/100g in the egg (P<0.001).

Table 3. The dietary manipulation of the betaine content in eggs

Treatment	Week 2	Week 3	Week 4	Week 5	Week 6	Average
Control (mg/100g)	0.38	0.50	0.56	0.46	0.40	0.46
Betaine (mg/100g)	1.30	1.36	1.20	1.64	1.34	1.37

Conclusion

The beneficial effect of betaine on laying hen performance was not confirmed in the current study, although there was a numerical improvement of 2.4% in egg weight. It should be noted that these birds were already toward the end of the laying period and had also been fed on a wheat-based diet prior to the start of this experiment. It is possible that stress associated with the change from a wheat- to sorghum-based diet was responsible for obscuring treatment effects.

The study did, however, demonstrate that the betaine content of the egg can be manipulated by the diet. The addition of 1000ppm of Betafin S1 increased the betaine content by 0.91mg/100g. The significant increase in the betaine level of homgenised egg contents suggests there is a high potential to improve embyro survival, hatchability and general bird performance post hatch.

Experiment 2

Assessment of the effect of Betafin® S1 natural betaine in broiler breeders

Introduction

This study was undertaken to evaluate the effects of the use of Betafin natural betaine when used in the diets of broiler breeders. Unfortunately progeny growth performance could not be accurately evaluated during this study.

Design

Two treatments were used in a commercial breeder site.

The treatments were;

A. Control: broiler breeder diets with no betaine included
B. Treatment: broiler breeder diets with betaine included at 2 kg /t.

The hypotheses were;

1. H_0; Actual % chick hatched Group A = Actual % chick hatched Group B
   H_1; Actual % chick hatched Group A ≠ Actual % chick hatched Group B

And

2. $1H_0$; Chick weight Group A = Chick weight Group B
   $1H_1$; Chick weight Group A ≠ Chick weight Group B

And

3. H_0; % culled at hatch Group A = % culled at hatch Group B
   H_1; % culled at hatch Group A ≠ % culled at hatch Group B

Methodology

The study was conducted on one broiler breeder farm (Farm 3, Turi Foods, Victoria) with one Group A (control) and one Group B (treated) shed per farm. This was conducted as a commercial study and as such it was not possible to utilise more sheds at one time to reduce the pen effect, so the experiment was run in two experimental blocks, crossing over one shed from control to treatment in the second block of the study. A complete cross over with both the initial sheds did not occur due to the progress of fitting new nest systems on the farm.

The product (Betafin® S1 natural betaine, minimum 96% by HPLC feed grade anhydrous betaine) was supplied in “bag a batch premixes” to the feed mill. The sheds did not share silos and each ration was delivered to the appropriate shed. The diet formulations were varied as is standard depending on the age, intake, bodyweight and production parameters. Standard flock performance figures were obtained from the flocks which were then analysed for the actual chicks hatched percentage, chick weights and the percentage of chicks culled in the hatchery.

In the first block, Bannockburn Farm, Sheds 32 and 33 contained approximately 7,000 Ross 308 hens plus around 8% males. In block 1, run between 10/07/2012 to 10/12/2012, the sheds were 100% deep litter with manual nest boxes. In block 2, the trial started at 22/3/2013 and ended 26/9/2013, and this time the sheds had been fitted with Janssen nest systems. Betaine treatment with Betafin betaine commenced in Shed 33 when the birds were 32 weeks of age. In the second block (same numbers), the control diets were offered to birds in Shed 31 and the betaine treated diets went to shed 32. This meant there was a partial cross-over of shed 32, from treatment to control.

Results were analysed statistically using a single factor ANOVA utilising Microsoft® Office Excel®2007.The alternative hypotheses were accepted when $P \leq 0.01$. The effect of age on the observed variation was balanced between treated and control flocks as both groups aged at the same rate and the pen effect is partially discounted due to the cross over in one shed. The analysis is recorded in Appendix 2.

Results

A summary of the results can be seen in Table 1 below. Further details of the results can be found in Appendix 1.

Table 1 Broiler breeder performance with or without Betafin® S1 natural betaine in the diets

Farm/Parameter	Control	Treated	P values
Farm 3, Block 1, Average chick weight (g)	42.18	42.15	0.95191
Farm 3, Block 1, Actual percentage hatch	81.91	83.19	0.16075
Farm 3, Block 1, Actual percentage culls	0.62	0.61	0.87101
Farm 3, Block 2, Average chick weight (g)	39.62	39.28	0.48743
Farm 3, Block 2, Actual percentage hatch	84.75	86.89	0.00042
Farm 3, Block 2, Actual percentage culls	0.44	0.41	0.39734
Combined Average chick weight (g)	40.80	40.64	0.66011
Combined Actual percentage hatch	83.42	85.14	**0.00291**
Combined Actual percentage culls	0.52	0.50	0.53826

Hypothesis testing

The data supported acceptance of alternative hypothesis 1 that is the actual percentage hatched was higher for the treated group. This was the case for Block 2 and the overall combined data. For hypotheses 2 and 3 (chick weight and percentage culled at hatch) the alternate hypotheses were rejected.

Discussion

The present results suggest that betaine improves the number of hatched chicks in broiler breeders, which supports the observations in pigs that betaine improves the number of born alive in pig breeders (van Wettere et al., 2012). The suggested mode of action is that betaine improves embryo survival through its osmoregulation properties as well as limiting the amount of toxin, i.e., homocysteine, present. The lack of influence on chick weight and hatchery cull rates is not inconsistent with the improvement in the proportion hatched.

While there is no recorded research of anhydrous betaine in broiler breeders, Lu and Zou (2006) observed supplementation to ISA layer diets to increase egg production, while Park et al. (2006) recorded increased egg size, also in ISA brown layers. Harms and Russell (2002) found no effect on egg production, egg weight or weight gain of laying hens, similar to that observed in experiment 1.

There was a significant block effect on percentage hatched and chick hatched weight ($P<0.01$). Both blocks were conducted during the cooler months of autumn and spring, so the main difference was the change from manual nesting boxes to the Janssen automated nest system. The increase in hatchability (3.8%) in block 2 (compared to block 1) seemed to coincide with a reduction in hatched chick weight of 6.4% which suggests the lower weight chicks had a greater rate of survival. However, the 2.1% increase in percentage hatched by betaine supplementation (combined blocks) did not negatively influence chick weight. This suggests that dietary betaine influences hatchability by a different mode of action to the change to modern nesting systems, and therefore the response was additive.

Whilst we are not able to surmise that any effect of Betafin on in-ovo gut function is likely to lead to better hatchability, the results of this study adds to the data suggesting a beneficial effect from the inclusion of natural betaine in the diet of production animals.

Recommendations for further research

Unfortunately no accurate measurements could be taken on the progeny of the control and betaine supplemented broiler breeders. The potential in-ovo effects of betaine in chick development and subsequent growth and carcass characteristics could be significant and this strongly warrants further investigation.

The benefits of betaine on an increased percentage hatched, without affecting chick weight, need to be assessed under more controlled conditions to verify the response at the same dose (2000ppm) and at a lower more cost effective dose at 1000ppm.

Benefits to the Poultry Industry

If this is verified, a 2.0% improvement in hatchability is worth about 1.2 cents per egg (Peter Scott, personal communication), and if 750,000,000 eggs are produced p.a. to produce 600,000,000 broilers, then this is worth approximately $9.04 million to the broiler industry. It is likely that natural betaine may also improve hatchability in layer, duck and turkey breeders. The cost of natural betaine is between $5 and $6/kg, however betaine can spare and replace choline and some of the methionine. At 2kg/tonne dose rate, betaine would cost between $10 and

$12/tonne in breeder diets, so further research to assess lower, more cost effective doses is highly recommended.

Conclusion

The supplementation of natural anhydrous betaine in diets offered to Hy-Line laying hens at 1000ppm significantly increased the betaine content of the egg, which demonstrates the osmolyte and methyl group donor can be used to promote embryo health, development and survival. The significant increase in hatchability supports the benefits of betaine in broiler breeders, and could potentially produce a gross $11.3 million to the industry. Progeny growth performance and carcass yield may be also improved through a better developed embryo. Increasing betaine to layer diets could also promote a health benefit to egg consumers.

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Appendix 1 Production data

Setter No.	Fl No	grade	Fl Age	Dolly No.	Trays	Eggs set	Chicks exp.	Est %	Actual chick	Actual %	Culls	% Culls	Egg age	Ch Wgt
Data includes eggs set 10/7/2012 up to but not including 10/12/12														
2	BB3-33	1	41	116-185	90	12960	11146	86	10959	84.56	114	1.0	3	41
3	BB3-33	1	41	141-25	90	12960	11146	86	11123	85.83	91	0.8	1	41
7	BB3-33	1	41	204	45	6480	5573	86	5500	84.88	22	0.4	1	41.6
2	BB3-33	1	40	326-313	90	12960	11146	86	10869	83.87	108	1.0	3	42
1	BB3-33	1	40	270-234	90	12960	11146	86	11293	87.14	52	0.5	4	42.2
3	BB3-33	1	40	75-19	90	12960	11146	86	11290	87.11	55	0.5	4	41.8
2	BB3-33	1	39	22-256	90	12960	11146	86	10995	84.84	22	0.2	5	42.6
3	BB3-33	1	39	219-83	90	12960	11146	86	11304	87.22	70	0.6	3	39.8
2	BB3-33	1	38	43-37	90	12960	11146	86	11001	84.88	63	0.6	5	40
3	BB3-33	1	38	204-309	90	12960	11016	85	11297	87.17	63	0.6	3	42.4
6	BB3-33	1	38	60-50	90	12960	11016	85	10958	84.55	40	0.4	3	42.8
2	BB3-33	1	37	145-278	90	12960	11016	85	11210	86.50	47	0.4	4	38.2
5	BB3-33	1	37	124-226	90	12960	11016	85	11224	86.60	74	0.7	2	41
3	BB3-33	1	37	120-75	90	12960	11016	85	11532	88.98	36	0.3	3	41.4
2	BB3-33	1	36	57-234	90	12960	11016	85	11257	86.86	29	0.3	4	42
4	BB3-33	1	35	269-150	90	12960	11016	85	11302	87.21	44	0.4	6	40.2
7	BB3-33	1	36	73-289	90	12960	11016	85	11119	85.79	64	0.6	3	39.6
9	BB3-33	1	36	67-20	90	12960	11016	85	11450	88.35	63	0.5	3	40.8
13	BB3-33	1	34	170-8	90	12960	11146	86	11237	86.71	41	0.4	6	39.6
14	BB3-33	1	34	309	45	6480	5573	86	5731	88.44	22	0.4	5	40.2
3	BB3-33	1	34	151-230	90	12960	11146	86	11338	87.48	51	0.4	5	40.6
3	BB3-33	1	34	189-98	90	12960	11016	85	11456	88.40	47	0.4	3	39.8
2	BB3-33	1	33	342-23	90	12960	11016	85	11448	88.33	32	0.3	5	38.6
14	BB3-33	1	32	83-56	90	12960	11016	85	11614	89.61	46	0.4	4	38.2
7	BB3-33	1	32	22-340	90	12960	11016	85	11208	86.48	37	0.3	7	37.4
8	BB3-33	1	32	148-71	90	12960	11016	85	10607	81.84	47	0.4	6	37
9	BB3-33	1	32	7-104	90	12960	11016	85	10833	83.59	70	0.6	5	36.8
5	BB3-33	1	57	30-330	90	12960	9202	71	9761	75.32	50	0.5	4	44.2
12	BB3-33	1	57	162-253	90	12960	9202	71	9862	76.10	114	1.1	3	45.2
3	BB3-33	1	56	508-65	90	12960	9202	71	9770	75.39	102	1.0		45.2
16	BB3-33	1	55	16-305	90	12960	9202	71	9611	74.16	58	0.6		44.6
19	BB3-33	1	55	350-107	90	12960	9202	71	9915	76.50	100	1.0		43.6
19	BB3-33	1	54	255-8	90	12960	9202	71	9884	76.27	101	1.0	7	45
19	BB3-33	1	54	339-238	90	12960	9202	71	9663	74.56	90	0.9	4	45.8
19	BB3-33	1	52	98-7	90	12960	9850	76	10149	78.31	70	0.7	6	42.8
20	BB3-33	1	52	318-179	90	12960	9850	76	9930	76.62	43	0.4	5	45.2
6	BB3-33	1	51	80-257	90	12960	9850	76	10418	80.39	51	0.5	7	44.6
9	BB3-33	1	51	304-200	90	12960	9850	76	10263	79.19	44	0.4	5	44.6
10	BB03-33	1G	50	118-323	90	12960	9979	77	10300	79.48	53	0.5	7	44.6
20	BB3-33	1	49	194-55	90	12960	9979	77	10267	79.22	45	0.4		43.8
17	BB3-33	1	49	70-280	90	12960	10238	79	10284	79.35	100	1.0		43.2
12	BB3-33	1	48	93-229	90	12960	10368	80	10361	79.95	58	0.6		42.2
5	BB3-33	1	47	319	45	6480	5314	82	5301	81.81	18	0.3	5	45.4
9	BB3-33	1	47	257-272	90	12960	10627	82	10606	81.84	86	0.8	1	45.2
8	BB3-33	1	47	64-7	90	12960	10627	82	10591	81.72	39	0.4	2	43.4
2	BB3-33	1	46	254-19	90	12960	10627	82	10337	79.76	114	1.1	7	44.6
3	BB3-33	1	46	53-326	90	12960	10627	82	10843	83.67	81	0.7	6	44.4
13	BB3-33	1	45	56-315	90	12960	10757	83	11011	84.96	98	0.9	6	42.4
23	BB3-33	1	45	20-800	90	12960	10757	83	10752	82.96	85	0.8	2	42.4
3	BB3-33	1	44	123-185	90	12960	10886	84	11123	85.83	114	1.0	3	42.6
10	BB3-33	1	44	235-286	90	12960	10886	84	10814	83.44	67	0.6	2	43.6
12	BB3-33	1	44	223-330	90	12960	10886	84	10958	84.55	95	0.9	1	43.6
3	BB3-33	1	43	199-191	90	12960	11146	86	11012	84.97	95	0.9	3	42.8
2	BB3-33	1	42	55-27	90	12960	11146	86	10788	83.24	63	0.6	4	42.6

Data includes eggs set 10/7/2012 up to but not including 10/12/12

Setter No.	Fl No	grade	Fl Age	Dolly No.	Trays	Eggs set	Chicks exp.	Est. %	Actual chick	Actual %	Culls	% Culls	Egg age	Ch Wgt
3	BB3-32	1	41	272-300	90	12960	11146	86	11150	86.03	123	1.1	3	40.8
2	BB3-32	1	41	201-274	90	12960	11146	86	10951	84.50	112	1.0	2	41.4
1	BB3-32	1	40	120-226	90	12960	11146	86	10860	83.80	77	0.7	4	42.6
3	BB3-32	1	40	290-130	90	12960	11146	86	11022	85.05	37	0.3	5	42.8
2	BB3-32	1	40	237-57	90	12960	11146	86	10871	83.88	82	0.7	4	42.6
3	BB3-32	1	39	150-6	90	12960	11146	86	11008	84.94	47	0.4	4	42.8
5	BB3-32	1	39	129-182	90	12960	11146	86	11116	85.77	71	0.6	3	42.4
2	BB3-32	1	39	100-202	90	12960	11146	86	10935	84.38	71	0.6	3	39
3	BB3-32	1	38	117-185	90	12960	11146	86	11071	85.42	36	0.3	4	41.8
2	BB3-32	1	37	170-5	90	12960	11016	85	11133	85.90	41	0.4	5	42
3	BB3-32	1	37	130-344	90	12960	11016	85	11304	87.22	53	0.5	3	38.6
2	BB3-32	1	36	16-187	90	12960	11016	85	11129	85.87	67	0.6	5	42
4	BB3-32	1	37	98-256	90	12960	11016	85	11466	88.47	42	0.4	3	41
3	BB3-32	1	36	102-322	90	12960	11016	85	11305	87.23	71	0.6	4	42.4
8	BB3-32	1	36	315-227	90	12960	11016	85	10666	82.30	33	0.3	3	40.2
8	BB3-32	1	36	315-227	90	12960	11016	85	10666	82.30	33	0.3	3	40.2
8	BB3-32	1	36	315-227	90	12960	11016	85	10666	82.30	33	0.3	3	40.2
18	BB3-32	1	34	95-4	90	12960	11146	86	11299	87.18	71	0.6	4	40.7
2	BB3-32	1	34	332-35	90	12960	11146	86	11183	86.29	72	0.6	6	39.8
1	BB3-32	1	33	96-325	90	12960	11016	85	11459	88.42	61	0.5	5	40.4
3	BB3-32	1	33	262-349	90	12960	11016	85	11359	87.65	33	0.3	4	39.2
13	BB3-32	1	32	265-110	90	12960	11016	85	11233	86.67	39	0.3	5	37.8
10	BB3-32	1	32	219-18	90	12960	11016	85	11445	88.31	41	0.4	5	37.6
11	BB3-32	1	32	329-305	90	12960	11016	85	10769	83.09	24	0.2	4	38.6
12	BB3-32	1	32	179-139	90	12960	11016	85	10471	80.79	52	0.5	4	37.4
4	BB3-32	1	57	57-321	90	12960	9202	71	8928	68.89	81	0.9	5	
11	BB3-32	1	56	212-191	90	12960	9202	71	9007	69.50	74	0.8	5	45
2	BB3-32	1	56	156-28	90	12960	9202	71	9993	77.11	55	0.5		45
15	BB3-32	1	54	41-147	90	12960	9202	71	9013	69.54	58	0.6	8	45
16	BB3-32	1	54	45-3	90	12960	9202	71	9177	70.81	56	0.6	6	44.8
16	BB3-32	1	53	245-57	90	12960	9202	71	9263	71.47	54	0.6	9	44.2
20	BB3-32	1	53	35-198	90	12960	9850	76	9857	76.06	48	0.5	6	43.2
14	BB3-32	1	51	81-2	90	12960	9850	76	9840	75.93	125	1.3	4	44
15	BB3-32	1	51	117-201	90	12960	9850	76	10202	78.72	91	0.9	3	44.2
12	BB03-32	1G	50	258-135	90	12960	9979	77	9857	76.06	84	0.8	5	43.6
23	BB3-32	1	49	150-53	90	12960	9979	77	10096	77.90	71	0.7		44
16	BB3-32	1	49	100-110	90	12960	10238	79	10151	78.33	107	1.0		44.6
10	BB3-32	1	48	159-178	90	12960	10368	80	10422	80.42	72	0.7		43.6
4	BB3-32	1	47	250-101	90	12960	10627	82	10640	82.10	87	0.8	5	45.6
6	BB3-32	1	47	101-13	90	12960	10627	82	10403	80.27	39	0.4	3	45.4
3	BB3-32	1	46	237-308	90	12960	10627	82	10552	81.42	71	0.7	6	43.8
6	BB3-32	1	47	206-208	90	12960	10627	82	10422	80.42	41	0.4	3	43.8
5	BB3-32	1	46	16-120	90	12960	10627	82	10369	80.01	71	0.7	5	44.8
14	BB3-32	1	45	328-68	90	12960	10757	83	10701	82.57	57	0.5	6	42.2
19	BB3-32	1	45	283-132	90	12960	10757	83	10734	82.82	78	0.7	5	42.4
5	BB3-32	1	44	292-101	90	12960	10886	84	10832	83.58	75	0.7	3	43
4	BB3-32	1	43	58-92	90	12960	10886	84	10679	82.40	81	0.8	5	43.8
11	BB3-32	1	44	313-162	90	12960	10886	84	10585	81.67	64	0.6	1	43.2
2	BB3-32	1	43	74-8	90	12960	11146	86	10806	83.38	74	0.7	3	42.6
3	BB3-32	1	42	243-182	90	12960	11146	86	10808	83.40	80	0.7	3	42.2
2	BB3-32	1	42	32-318	90	12960	11146	86	10988	84.78	73	0.7	2	42
3	BB3-32	1	41	272-300	90	12960	11146	86	11150	86.03	123	1.1	3	40.8

Setter No.	Fl No	grade	Fl Age	Dolly No.	Trays	Eggs set	Chicks exp.	Est. %	Actual chick	Actual %	Culls	% Culls	Ch Wgt
10	BB3-31	1	25	341-5	90	12960	8424	65	9545	73.65	91	0.9	33.2
18	BB3-31	1	26	189	45	6480	4536	70	5212	80.43	33	0.6	33.8
9	BB3-31	1	27	318-226	90	12960	9720	75	10562	81.50	87	0.8	33.8
12	BB3-31	1	27	192-276	90	12960	9720	75	9910	**76.47**	110	1.1	34.4
11	BB3-31	1	27	30-102	90	12960	9720	75	11078	85.48	37	0.3	35.2
10	BB3-31	1	27	118-35	90	12960	10498	81	10963	84.59	37	0.3	36
23	BB3-31	1	28	323-175	90	12960	10498	81	11067	85.39	27	0.2	36.8
6	BB3-31	1	28	312-273	90	12960	10886	84	11133	85.90	31	0.3	35.8
22	BB3-31	1	29	300-132	90	12960	10886	84	11126	85.85	48	0.4	36.6
16	BB3-32	1	29	226	45	6480	5443	84	5733	88.47	56	1.0	36.8
7	BB3-31	1	29	127-328	90	12960	10886	84	10877	83.93	41	0.4	36.8
12	BB3-31	1	30	170-230	90	12960	10886	84	11305	87.23	82	0.7	36.6
10	BB3-31	1	30	111-247	90	12960	11016	85	10899	84.10	56	0.5	38.8
12	BB3-31	1	30	253-237	90	12960	11016	85	11155	86.07	41	0.4	38.6
20	BB3-31	1G	32	223-293	90	12960	11016	85	11279	87.03	24	0.2	38
13	BB3-31	1	31	338-324	90	12960	11016	85	11251	86.81	26	0.2	38
8	BB3-31	1	31	208-32	90	12960	11016	85	11243	86.75	33	0.3	38.6
20	BB3-31	1G	32	223-293	90	12960	11016	85	11279	87.03	24	0.2	38
1	BB3-31	1G	32	226	45	6480	5508	85	5727	88.38	16	0.3	38.4
3	BB3-31	1G	32	172-153	90	12960	11016	85	11281	87.04	32	0.3	38.8
5	BB3-31	1G	32	188-8	90	12960	11016	85	11308	87.25	38	0.3	40.8
4	BB3-31	1	33	191-136	90	12960	11016	85	11307	87.25	31	0.3	40
7	BB3-31	1	34	16-195	90	12960	11146	86	11143	85.98	22	0.2	38.2
8	BB3-31	1	34	41-74	90	12960	11146	86	11013	84.98	34	0.3	38
6	BB3-31	1G	34	213-93	90	12960	11146	86	11210	86.50	37	0.3	39.2
7	BB3-31	1	34	174-450	90	12960	11146	86	11214	86.53	50	0.4	38.8
3	BB3-31	1	35	68-19	90	12960	11146	86	11347	87.55	44	0.4	39.8
4	BB3-31	1	35	19-.12	90	12960	11146	86	11179	86.26	49	0.4	40.4
14	BB3-31	1G	36	12-198	90	12960	11146	86	11447	88.33	38	0.3	39.6
1	BB3-31	1G	36	241	45	6480	5573	86	5731	88.44	20	0.3	39.8
4	BB3-31	1G	36	318-17	90	12960	11146	86	11154	86.06	56	0.5	39.8
14	BB3-31	1G	37	201-35	90	12960	11146	86	11346	87.55	52	0.5	38.2
20	BB3-31	1G	37	147-195	90	12960	11146	86	11286	87.08	41	0.4	39.6
15	BB3-31	1	38	64	45	6480	5573	86	5619	86.71	34	0.6	40.8
18	BB3-31	1	38	341-238	90	12960	11146	86	11210	86.50	48	0.4	39.6
21	BB3-31	1G	38	273-156	90	12960	11146	86	11215	86.54	46	0.4	39.8
20	BB3-31	1G	39	326-186	90	12960	11146	86	11095	85.61	24	0.2	38.6
23	BB3-31	1G	39	71-302	90	12960	11146	86	11214	86.53	32	0.3	39.6
1	BB3-31	1	40	219-335	90	12960	11146	86	10908	84.17	55	0.5	41.2
22	BB3-31	1G	40	226-154	90	12960	11275	87	10896	84.07	31	0.3	39.8
18	BB3-31	1	41	180-258	90	12960	11146	86	10942	84.43	103	0.9	39.6
22	BB3-31	1	41	53-48	90	12960	11146	86	10859	83.79	60	0.5	39.2
13	BB3-31	1	41	101-346	90	12960	11146	86	11127	85.86	45	0.4	44.4
5	BB3-31	1	42	340-123	90	12960	11275	87	10998	84.86	22	0.2	42.6
12	BB3-31	1	43	25-334	90	12960	11275	87	11146	86.00	33	0.3	42
1	BB3-31	1	43	206-283	90	12960	11275	87	11059	85.33	37	0.3	42.2
5	BB3-31	1	43	55-185	90	12960	11275	87	10991	84.81	47	0.4	42.2
18	BB3-31	1G	44	126-194	90	12960	11146	86	10848	83.70	78	0.7	42.8
5	BB3-31	1	44	253-130	90	12960	11146	86	10732	82.81	42	0.4	43.2
19	BB3-31	1G	45	237	45	6480	5573	86	5609	86.56	9	0.2	39.6
1	BB3-31	1	45	244-300	90	12960	11016	85	10873	83.90	49	0.4	42.6
8	BB3-31	1	46	303-269	90	12960	11016	85	10654	82.21	31	0.3	43.2
9	BB3-31	1	46	332-270	90	12960	11016	85	10718	82.70	38	0.4	43.4
	BB3-31	1G	48	24-327	90	12960	11016	85	10425	80.44	64	0.6	43.4
11	BB3-31	1G	49	69	45	6480	5508	85	5252	81.05	22	0.4	44
22	BB3-31	1	50	262	45	6480	5443	84	5323	82.15	28	0.5	41
3	BB3-31	1	51	316	45	6480	5314	82	5426	83.73	23	0.4	42.8
4	BB3-31	1	51	258	45	6480	5314	82	4969	76.68	77	1.5	43
7	BB3-31	1	51	232	45	6480	5314	82	5049	77.92	20	0.4	43
18	BB3-31	1G	52	150-101	90	12960	10368	80	9822	75.79	73	0.7	43.2

Setter No.	Fl No	grade	Fl Age	Dolly No.	Trays	Eggs set	Chicks exp.	Est. %	Actual chick	Actual %	Culls	%Culls	Ch Wgt
Data from eggs set between 22/3/13 and 26/9/13													
11	BB3-32	1	25	324	45	6480	4212	65	4717	72.79	64	1.3	33.4
20	BB3-32	1	26	124-71	90	12960	9072	70	10038	77.45	87	0.9	33.4
10	BB3-32	1	27	183-23	90	12960	9720	75	10720	82.72	60	0.6	34.2
12	BB3-32	1	27	289-55	90	12960	9720	75	11299	87.18	47	0.4	34.6
9	BB3-32	1	27	297-203	90	12960	10498	81	11251	86.81	50	0.4	35.6
22	BB3-32	1	28	402-308	90	12960	10498	81	11160	86.11	39	0.3	36.2
8	BB3-32	1	29	27-.5.	90	12960	10886	84	11247	86.78	41	0.4	36.2
23	BB3-32	1	29	159	45	6480	5443	84	5729	88.41	22	0.4	36.2
5	BB3-32	1	29	316-255	90	12960	10886	84	11429	88.19	45	0.4	36.4
8	BB3-32	1	30	241-292	90	12960	10886	84	11426	88.16	30	0.3	37.2
7	BB3-32	1	30	184-26	90	12960	11016	85	11503	88.76	40	0.3	38.8
11	BB3-32	1	30	18-93	90	12960	11016	85	11205	86.46	58	0.5	39
19	BB3-32	1G	32	273-31	90	12960	11016	85	11626	89.71	33	0.3	37.4
14	BB3-32	1	31	130	45	6480	5508	85	5729	88.41	17	0.3	39
23	BB3-32	1	31	251-269	90	12960	11016	85	11365	87.69	31	0.3	38.8
9	BB3-32	1	31	307-236	90	12960	11016	85	11347	87.55	49	0.4	37.8
19	BB3-32	1G	32	273-31	90	12960	11016	85	11626	89.71	33	0.3	37.4
1	BB3-32	1G	32	.62.	45	6480	5508	85	5774	89.10	18	0.3	39.8
4	BB3-32	1G	32	162-227	90	12960	11016	85	11397	87.94	49	0.4	39.8
6	BB3-32	1	33	290-157	90	12960	11016	85	11452	88.36	54	0.5	40.2
7	BB3-32	1	33	311-201	90	12960	11016	85	11310	87.27	30	0.3	39.2
8	BB3-32	1	33	170-318	90	12960	11016	85	11516	88.86	29	0.3	39.8
5	BB3-32	1	33	101-30	90	12960	11146	86	11605	89.54	33	0.3	38.6
8	BB3-32	1G	34	253-13	90	12960	11146	86	11482	88.60	23	0.2	38.4
6	BB3-32	1	34	28-65	90	12960	11146	86	11401	87.97	36	0.3	38.4
10	BB3-32	1	35	130-67	90	12960	11146	86	11451	88.36	47	0.4	38
1	BB3-32	1	35	197-145	90	12960	11146	86	11400	87.96	52	0.5	39
2	BB3-32	1	35	60-141	90	12960	11146	86	11726	90.48	34	0.3	34
23	BB3-32	1	36	123-11	90	12960	11146	86	11494	88.69	38	0.3	38.6
21	BB3-32	1G	36	294-172	90	12960	11146	86	11508	88.80	37	0.3	39
2	BB3-32	1G	36	342	45	6480	5573	86	5704	88.02	22	0.4	38.6
3	BB3-32	1G	36	134-326	90	12960	11146	86	11748	90.65	46	0.4	39
19	BB3-32	1G	37	177-143	90	12960	11146	86	11588	89.41	44	0.4	39.4
16	BB3-32	1	38	110-2	90	12960	11146	86	11459	88.42	57	0.5	39.8
13	BB3-32	1G	38	104-308	90	12960	11146	86	11579	89.34	38	0.3	38.4
14	BB3-32	1G	38	292-301	90	12960	11146	86	11729	90.50	34	0.3	37.8
18	BB3-32	1G	39	157	45	6480	5573	86	5849	90.26	33	0.6	38.8
21	BB3-32	1G	39	269-284	90	12960	11146	86	11313	87.29	37	0.3	39
3	BB3-32	1	40	61-129	90	12960	11146	86	11498	88.72	48	0.4	39.8
14	BB3-32	1	40	143-331	90	12960	11146	86	11541	89.05	38	0.3	42
19	BB3-32	1	41	126-56	90	12960	11146	86	11556	89.17	37	0.3	39.4
14	BB3-32	1	41	7-27	90	12960	11146	86	11562	89.21	31	0.3	41.8
19	BB3-32	1	42	245	15.75	2268	1950	86	1936	85.36	13	0.7	42.4
22	BB3-32	1	42	28-249	90	12960	11146	86	11267	86.94	43	0.4	42
9	BB3-32	1	43	350	45	6480	5638	87	5586	86.20	32	0.6	41.6
4	BB3-32	1	43	159-110	90	12960	11275	87	11199	86.41	41	0.4	42.6
7	BB3-32	1	43	17-335	90	12960	11275	87	11291	87.12	26	0.2	42
17	BB3-32	1G	44	74-3	90	12960	11146	86	11240	86.73	43	0.4	39.8
6	BB3-32	1	44	288	45	6480	5573	86	5650	87.19	17	0.3	40.2
7	BB3-32	1	45	230-18	90	12960	11146	86	10962	84.58	26	0.2	41.4
20	BB3-32	1G	45	51	45	6480	5573	86	5720	88.27	11	0.2	39.2
2	BB3-32	1	45	214-56	90	12960	11016	85	11162	86.13	43	0.4	42
2	BB3-32	1	46	110	45	6480	5508	85	5569	85.94	22	0.4	44
7	BB3-32	1	46	98-294	90	12960	11016	85	11162	86.13	33	0.3	42.8
5	BB3-32	1G	48	77-229	90	12960	11016	85	10982	84.74	47	0.4	44.4
7	BB3-32	1G	48	89-46	90	12960	11016	85	11044	85.22	58	0.5	43.2
18	BB3-32	1	50	55	45	6480	5443	84	5301	81.81	31	0.6	42.2
22	BB3-32	1	50	335	45	6480	5443	84	5184	80.00	26	0.5	41.8
7	BB3-32	1	51	18	45	6480	5314	82	5025	77.55	52	1.0	43.6
15	BB3-32	1G	52	284-71	90	12960	10368	80	10686	82.45	45	0.4	43.4

Appendix 2 Production data

Statistics Block 1

Anova: Single Factor

SUMMARY Actual percentage hatch

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (32) Actual% hatch	52	4259.352	81.911	25.958
Treated (33) Actual % hatch	54	4492.755	83.199	18.272

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	43.9837	1	43.984	1.9955	0.160752	3.9324
Within Groups	2292.31	104	22.041			
Total	2336.29	105				

SUMMARY Percentage culled at hatchery

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (32) % Culls	52	32.13498	0.618	0.0545
Treated (33) % Culls	54	32.95563	0.6103	0.0636

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00157	1	0.0016	0.0265	0.87101	3.9324
Within Groups	6.15038	104	0.0591			
Total	6.15194	105				

SUMMARY Chick weights

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (32) Ch Weight	51	2151.1	42.178	4.6573
Treated (33) Ch Weight	54	2276.2	42.152	5.458

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.01853	1	0.0185	0.0037	0.951907	3.9333
Within Groups	522.141	103	5.0693			
Total	522.16	104				

Statistics Block 2

Note removed abnormal figure from hatch shed BB3-31 setter 12 dolly No 192-276 set on the 2/4/13 which was correlated with a hatchery problem

SUMMARY Actual hatch percentage

Groups	*Count*	*Sum*	*Average*	*Variance*
Actual % BB3-31 Control	59	5000.224	84.74956	9.627831
Actual % BB3-32 Betaine	60	5213.649	86.89414	11.09113

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	136.8182	1	136.8182	13.19909	**0.000417**	3.922172
Within Groups	1212.791	117	10.36574			
Total	1349.609	118				

SUMMARY Percentage culled at hatch

Groups	*Count*	*Sum*	*Average*	*Variance*
%Culls BB3-31	59	26.01311	0.4409	0.05587
%Culls BB3-32	60	24.45547	0.407591	0.035775

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.033005	1	0.033005	0.721631	0.397344	3.922172
Within Groups	5.351216	117	0.045737			
Total	5.384221	118				

SUMMARY Chick weight

Groups	*Count*	*Sum*	*Average*	*Variance*
Ch Weight BB3-31	59	2337.6	39.62034	7.185786
Ch Weight BB3-32	60	2356.8	39.28	7.01722

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.445718	1	3.445718	0.485259	0.487432	3.922172
Within Groups	830.7916	117	7.100783			
Total	834.2373	118				

Statistics Combined Blocks

Note removed abnormal figure from hatch shed BB3-31 setter 12 dolly No 192-276 set on the 2/4/13 which was correlated with a hatchery problem

Anova: Single Factor

SUMMARY Actual percentage hatch

Groups	*Count*	*Sum*	*Average*	*Variance*
Actual % Cont	111	9259.576	83.4196	19.13684
Actual % Treated	114	9706.403	85.14389	17.79504

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	167.211	1	167.211	9.059531	**0.002914**	3.883497
Within Groups	4115.892	223	18.45691			
Total	4283.103	224				

SUMMARY Percentage culls at hatchery

Groups	*Count*	*Sum*	*Average*	*Variance*
%Culls Controls	111	58.14809	0.523857	0.062592
%Culls Treated	114	57.4111	0.503606	0.058858

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.023063	1	0.023063	0.379957	0.538255	3.883497
Within Groups	13.53602	223	0.0607			
Total	13.55909	224				

SUMMARY Chick weight at hatch

Groups	*Count*	*Sum*	*Average*	*Variance*
Ave chick weight Control	110	4488.7	40.80636	7.602253
Ave chick weight Treated	114	4633	40.64035	8.29818

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.542881	1	1.542881	0.193915	0.660107	3.883687
Within Groups	1766.34	222	7.956486			
Total	1767.883	223				