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The Use of Ethanolamine for the Treatment of Eggs; Its Influence on the Postembryonic Biochemistry of Broiler Chicks and Indicators of Meat Quality

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Authors' contributions

Authors SYZ and MSN designed the study, author TOA performed the statistical analysis. Authors ISY and YNI managed the analyses of the study. Authors MAR and LJA managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To study the effect of ethanolamine on the development of broiler chickens, blood chemistry, and safety of meat of broiler chickens using ethanolamine in the incubation of eggs.
Place of Study: The experiments were carried out at the Michailovsky Broiler closed joint-stock company.
Methodology: Several experimental groups, as well as a control one, were formed of about 250 eggs each. These groups were analogous in regard to laying time and storage period, weight and quantity of the eggs. The incubating eggs were taken from Habbard F15 crossbreed hens (whose age was 295 days). Ethanolamine was sprayed on the eggs before incubation and again when the eggs were transferred to the hatchers. Biochemical and zootechnical parameters were measured according to standard procedures.
Results: The hatching of the experimental group was 88% against 8% for the control group level. The following biochemical parameters of the poults' blood were obtained: peroxidase and superoxide dismutase increased by 17.0% and 10.5%, respectively, but the Schiff bases and malondialdehyde decreased by 1.6% and 0.85%, respectively.

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The safety and quality indicators of the broiler meat for the experimental group were better than those for the control group; moreover, such indicators meet basic health standards.
The use of ethanolamine achieves an optimum level of the metabolic processes, protection of the cell structure and the cell's energetic homeostasis, i.e., it ensures a high-viability level for the chicks at the postembryonic stage and it guarantees high-quality and safe products.

Keywords: Embryogenesis; chicks; eggs; ethanolamine; antioxidants; lipid peroxidation.

ABBREVIATIONS

BAS - biologically active substance; AODS - antioxidant defense system; LP - lipid peroxidation; MDA - malondialdehyde; SB - Schiff bases.

1. INTRODUCTION

Researchers have studied the effect of biologically-active substances on animals; they have also determined the content of phospholipids and ethanolamine in the organs and blood of animals and have studied the metabolism of ethanolamine [1-4]. Ethanolamine is a component of phospholipids, in particular of certain plasmalogens and cephalins that can also be converted into both choline and lecithin. Moreover, on being converted into choline, ethanolamine has the ability through the cofactor flavine adenine dinucleotide to support the mitochondrial respiratory chain function, inhibiting its energy losses. In certain preliminary experiments we already proved the effectiveness of a one-off use of ethanolamine for the organisms of heavy-breed and egg-breed chicks [5]. We also established the possibility of its usage in combination with natural metabolites such as succinic acid and glycine. As a result of our research, the antioxidant, immunity- and metabolism-stimulating capacity of this BAS was demonstrated [5]. However, in order to improve on the aforementioned effect it has proven to be of particular interest that the incubating eggs be processed twice (firstly, before incubation and secondly before transferring them to the hatchers. The choice of such periods of time was determined by the following: in these periods the organism of the embryo is depressed due to two main stress factors: first, the change in the temperature level (when the eggs are being set for incubation); and second, the switch from allantoic to lung respiration, when there is also a temperature imbalance (when the eggs are transferred to the hatchers). Disturbances in the metabolic processes and a certain decrease in vitality in these periods, as was demonstrated earlier, correlate directly with disturbances in the biological oxygenation process and as a result with the activation of LP. In previous research conducted by many different groups [6-8] the following tendencies were clearly detected. The effect produced by the ethanolamine gradually decreased towards the end of the embryogenesis stage, which resulted in only a very slight rise in embryo vitality at the end of the period under analysis. On the other hand, at the start of early development, a notable effect of the medication was evident. This presupposed the need for an additional application of ethanolamine at a later stage of embryogenesis.

The main goal of our work was to study the positive effect of ethanolamine on the organism of broiler chicks when a twostage treatment of the eggs was applied. A second objective was to assess broiler meat quality when using ethanolamine. The significance of this research consists in the development of an effective way to stimulate the vitality of chickens during the

preparation of high quality and environmentally friendly products. Ethanolamine is a phospholipid carcass component of every cell membrane, including the muscular tissue, which contributes to muscle growth in broilers. The benefit is an increase in the number of viable specimens, and hence an increase in profitability.

2. MATERIAL AND METHODS

For the above purpose, several experimental groups, as well as a control one, were formed of about 250 eggs each. These groups were analogous in regard to laying time and storage period, weight and quantity of the eggs. Experimental lots of eggs were treated with ethanolamine. For the first day the 1st and the 2nd test groups were treated with 0.1% ethanolamine. For the 18th day, the 1st test group was treated with 1.5% ethanolamine, and the 2nd test group was treated with 2.5% ethanolamine, while the control group was treated with nothing. The dispersion source is a sprinkler. The eggs were stored at a temperature of 12°C and humidity of 75% for 5 days prior to treatment with ethanolamine. During the incubation the turning of eggs is performed automatically by means of inclination to 45º to both sides from the vertical position at intervals of one hour. The Petersime-like incubator was used for incubation of eggs. The incubator is produced by "Petersime" in the Russian Federation in the Moscow region. The incubation regime is as follows:

For the first 1-2 days: temperature 38°C, humidity 55%.
Days 3-6: temperature 37.9°C, humidity 55%.
Days 7-12: temperature 37.8C - humidity 55%:
Days 13-15: temperature 37.7°C, humidity 55%.
Days 16-18: 37.6°C, humidity 55%.

Then the eggs were placed in the hatchers, days 19-21: temperature 37.2°C, humidity 65%.

The experiments were carried out at the Michailovsky Broiler closed joint-stock company, and the incubating eggs were taken from Habbard F15 crossbreed hens (whose age was 295 days). BAS was sprayed on the eggs before incubation and again when the eggs were transferred to the hatchers. Biochemical and zootechnical parameters were measured according to standard procedures [9]. The protein detection method is based on the fact that proteins react with copper sulfate in an alkaline environment and violet compounds are formed during this process, whose intensity is measured using a photometer. The albumin detection principle is based on the electrophoretic division of molecules during their movement with different speeds in a constant electric field. The uric acid deoxygenates the phospho-tungstic reagent forming a light-blue compound. The color intensity is proportional to the concentration of uric acid. The activity of α-amylase is determined by the decrease in the color intensity of starch concentration. α-amylase hydrolyses the amylolysis forming final products which are non-responsive to color reaction with iodine. Glucose is detected by heating ortho-toluidine, thus forming a colored compound whose color density is directly proportional to the glucose content. The general lipid detecting method is based on the color reaction of breakdown products of unsaturated lipids in the sulfuric acid with a reagent consisting of vanillin and ortho-phosphoric acid. The calcium detection method is based on the fact that calcium with murexide in an alkaline environment forms a colored compound whose stability is increased by means of adding glycerin to the solution. Phosphorus in the protein-free filtrate becomes yellow-lemon during the reaction with a vanadyl-molybdenic agent. The degree of coloration is measured on a photoelectric colorimeter. In its reaction to alkaline sodium phosphatase, β-glycerphosphate is hydrolyzed with the release of inorganic phosphorus. The activity of the ferment is determined by its amount. The lysozyme detection

method is based on the measurement with a photoelectric colorimeter of the changes in optical density of the standard live suspension of micrococcus lysodeikticus. The peroxidase detection method is based on determining the reaction rate of benzidine oxidation to hydrogen peroxide which involves ferment, forming a colored reaction product, whose maximum absorption occurs at 532 nm. Detection of superoxide dismutase is based on the inhibiting action of superoxide dismutase applied to the reduction of colorless tetrazolium salts by superoxide anion radicals, during which they are transformed into colored compounds (formazans). Detecting of Schiff's bases is based on the fact that by interacting with N-terminal amino acid residues (phospholipid amino groups) they form conjugating fluorescent compounds like Schiff's bases – lipoprotein complexes being a part of the intercellular formation - lipofuscin. The malondialdehyde detection method is based on the fact that at a high temperature in an acidic environment malondialdehyde reacts with 2-theobarbituric acid forming a colored trimethine complex whose maximum absorption occurs at 532 nm.

Broilers were grown in L-112 cage batteries. Broilers were grown till 42 days in cages, 40 broilers in each; their growth was monitored by the personnel; the composition of the ready-mixed feed is demonstrated in the appendix; the feed was given out by an automatic chain feeder, the movement rate of the feeder was 6.2 meters per minute; nipple bird baths were used for drinking. The temperature and humidity over the whole period of growth varied from 18°C to 33°C and from 55 to 65%, respectively.

Feeding broilers with a premixed combined-feed. (we mean that ready-mixed feed was used for feeding the broilers).

We determined the mass of live chickens and weighed their organs using a laboratory balance. The laboratory equipment and chemicals are produced by "Laboratory equipment", Moscow, Russian Federation. We used a morphologic method of anatomic dissection for evaluating the organoleptic indicators of broiler carcasses. The evaluation of the quality of broth and sanitary indicators of broiler carcasses was conducted in a laboratory in accordance with the industry-specific standard of the Russian Federation. The statistical analysis was performed in accordance with the manual for counting of statistical data [10]. The statistic analysis of the data was performed using the Student's coefficient, with the help of the program Microsoft Office Excel 2007.

During performance of the experiments ethical requirements were complied with.

3. RESULTS AND DISCUSSION

The development of the chicks in the experimental groups exceeded that of the control group in a complex of histological indicators, as well as in many biochemical, zootechnical and morphological indicators. As a result, the chicks acquired a higher immunological status and a natural resistance.

As may be concluded from the Table 1 data, the natural metabolite ethanolamine has an optimizing influence on many metabolic processes. For example, the total lipid level in the first experimental group was 14.9% higher than in the control group, as a result of the stimulating influence of ethanolamine on the phospholipids synthesis. Ethanolamine also had a considerable effect on protein metabolism: in both experimental groups the total protein level was 12.2% – 13.9% higher and the level of lysozyme activity was 13.8% – 15.3% higher, compared with the control group.

Table 1. Biochemical, Blood and Serum Indicators of the Day-Old Chicks and LP and AODS Indicators (n=5)

Group / Marker	Control	Exp. Group 1	Exp. Group 2
Total protein, g/l	29.30±1.07	33.40±0.62*	32.87±0.39*
Albumine, g/l	11.10±0.35	12.50±0.4	12.66±0.43
Uric acid, umol/l	0.50±0.04	0.53±0.03	0.48±0.03
α-amylase, u/l	700.0±13.6	727.0±10.76	735.0±18.34
Glucose, Mmol/l	7.1±0.26	7.7±0.10	7.6±0.15
Total lipids, g/l	1.47±0.05	1.69±0.03*	1.61±0.05
Ca, mmol/l	4.9±0.23	5.3±0.16	4.7±0.10
P, mmol/l	2.51±0.08	2.66±0.05	2.75±0.02*
Alkaline phosphatase, Bod u.	1.5±0.05	1.6±0.05	1.6±0.1
Lysozyme, mkg/ml	27.5±0.90	31.7±0.63*	31.29±0.61*
Peroxidase, transmission density units/l*sec	41±3.55	48±2.32	42±1.64
Superoxide dismutase, act./mg of gemoglobine	3.8±0.68	4.2±0.46	4.0±0.34
SB, relative units/ml	0.5±0.07	0.3±0.04	0.2±0.06*
MDA, mkmol/l	2.0±0.16	1.7±0.04	1.8±0.07

** p<0.05, ** p<0.01, *** p<0.001*

Ethanolamine stimulated the antioxidant defense level by activating the following enzymes: superoxide dismutase increased by 5.6% – 10.5% and peroxidase by 2.4% – 17%, and also by protecting the biological oxygenation chain. It also reduced the activity of LP processes. In contrast, in the control group a high intensity of the free-radical oxygenation process and a low activity level of antioxidants were detected.

A 10% – 15% decrease in the SB level and MDA values that are 1.67 – 2.5 times lower than in the control group were found. Such levels were reflected in the membrane structure stabilization and considerably improved the functioning of all cellular systems.

The stress-based disturbances in the mitochondrial respiratory chain stimulate LP processes and reduce antioxidant defense levels, both of which lead to free radical formation [6,11]. Taking into consideration that the latter causes destruction of nucleic acids and nucleotide coenzymes, as well as disturbances in the functioning of enzymes and covalent modification of various biomolecules, etc., it may be concluded that excessive production of free radicals can result in sundry pathological alterations to the characteristics of blood vessels as well as tissues and organs. In light of the possibility of correcting the aforementioned stress situations with the help of the BAS, it may be possible to avoid the negative effect of stress to a great extent and thus to considerably increase the quality and viability of the chicks, which proves to by one of the main goals of any poultry farm.

The increase of the chicks' viability during their fetal life is shown in Table 2.

Table 2. Markers of Incubation Biocontrol, % (n=300)

group	neo fetus	blood rings	still fetus	addled eggs	fragile	hatchability	±Δ	hatching	±Δ
Control	8.67± 1.62	2.33± 0.87	6.33± 1.41	5.0± 1.26	2.67± 0.93	82.12± 2.21	__	75.00± 2.50	__
Exp.1	7.0± 1.47	1.33± 0.66	3.67± 1.09	3.0± 0.98	1.67± 0.74	89.61± 1.67*	+7.49	88.33± 2.15*	+8.33
Exp.2	7.33± 1.51	1.33± 0.66	5.00± 1.26	3.67± 1.09	1.67± 0.74	90.60± 1.68*	+5.29	80.71± 2.28*	+6.00

** p<0.05, ** p<0.01, *** p<0.001*

As opposed to previous research [5], in the experimental groups all categories of incubation waste were much lower than the control group. The hatchability and hatching in these groups exceeded the control group by 5.29% – 7.49% and by 6.00% – 8.33%, respectively. Hence, by twofold application of ethanolamine on the incubating eggs, we managed to stabilize and achieve the sought-after effect. The results were quite similar for a series of repeated experiments.

These results were confirmed by the autopsies of the day-old chicks (Table 3).

Table 3. The Interior Measurements of Day-old Chicks, g (n=10)

Measurement	Control	Exp. Group 1	Exp. Group 2
Chick weight	42.231±0.45	44.027±0.65	43.950±0.059
Yolk sack with the residual yolk	7.018±0.43	5.487±0.28	5.581±0.35
Heart	0.307±0.02	0.317±0.02	0.289±0.02
Liver	1.199±0.08	1.237±0.04	1.234±0.04
Spleen	0.015±0.004	0.017±0.004	0016±0.005
Masticaroty stomach	2.578±0.09	2.657±0.08	2.613±0.06
Forestomach	0.396±0.02	0.372±0.01	0.412±0.01
Bursa of Fabricius	0.067±0.01	0.077±0.02	0.075±0.02

** p<0.05, ** p<0.01, *** p<0.001*

The use of kolamin had a stimulating effect on the organogenesis, apart from which, the chicks from the experimental groups had better markers of body weight, which were 4.1% – 4.3% higher than those of the control group.

In the experimental groups, the mass of the residual yolk (a temporary organ of nutrition and respiration) was 20.4% – 21.8% lower than that of the control group. This is direct evidence of more intensive metabolic processes and facilitation of hatching.

The mass of the "bursa Fabricius" (the central immunopoesis organ of poultry) increased by 10.7% - 13 % in the experimental group.

The anatomic research that we conducted enabled us to determine the following features. In the experimental group and in the control group of day-old chicks the organs are located anatomically correctly, with no deviations from the norm being visually evident. During the autopsy the following was found in the experimental group (in contrast to the control): the thymus is well developed; the yolk sack is small; the "bursa Fabricius" is not fully visible due to its location under the rectum, which is more anatomically correct.

These features indicate a certain improvement in the energy metabolism of the organism under analysis. It becomes clear that the chicks of the experimental groups have an additional reserve of energy due to the application of kolamin. It is important to note that the fatty deposits do not exceed the admissible level. All these data prove that ethanolamine is really capable of influencing the lipid, energetic and certain other types of metabolism in a positive way.

The anatomical investigation was supported by a histological investigation of the immune system.

The histologic specimens of internal organs of the day-old chicks had no pathological alterations. All the organ systems were well developed. In the experimental groups the immune defense organs (Liver, Spleen, Bursa of Fabricius) were more developed than in the control group. This proves the positive influence of ethanolamine on the immune system of the chicks and also supports the above-mentioned zootechnic and biochemical data.

The increase of the latter resulted in a higher status of natural resistance and viability of the experimental chicks in the embryonic and postembryonic periods (Table 4).

Table 4. Livability of Chickens, %, (n=100)

Groups of chicks			Control	Exp. Group 1	Exp. Group 2
					1
		mortality	3	1	
	1 - 10	livability	97	99	99
		mortality	1	__	1
	10 - 20	livability	99	100	99
		mortality	2	1	2
Age (days)	20 - 30	livability	98	99	98
		mortality	2	2	1
	30 - 42	livability	98	98	99
Total number (within 42		mortality	8	4	5
days)		livability	92	96	95

During the breeding period, the mortality rate of the experimental groups was on average 3.0% – 4.0% lower than the control group. This shows that the natural metabolite ethanolamine has a "long-lasting" residual effect. Organoleptic and sanitary indicators of broiler meat and broth have also been studied, with the results shown in Table 5:

Table 5. Organoleptic characteristics of the meat and broth of broilers

Group	Appearance	Flavor	Taste	Broth Fat	Consistence	Average
Control Group	6.10	6.80	6.70	6.70	6.20	6.50
Exp. Group 1	**6.70**	**7.30**	**6.90**	**7.00**	**6.90**	**6.96**
Exp. Group 2	6.60	7.20	6.80	6.90	6.70	6.84

From Table 5 it may be concluded that ethanolamine has a positive effect on the organoleptic characteristics of broiler meat and broth, with the experimental groups receiving higher scores. In the study sample of carcasses obtained from the experimental and control groups, a whitish-yellow color of their surface and subcutaneous adipose tissue was

observed, which lends the surface a characteristic smell, peculiar to fresh poultry meat. The serosa-phrenic cavities were moist and shiny, with no sign of slime or mold. The cut muscle was pale pink and slightly damp, not leaving wet spots on the filter paper and free of any foreign smell. Tight muscles with elastic consistency were found, as determined by pressing a finger deep into the muscle and quickly removing it.

Our study of the quality and safety of the broiler meat of the control and experimental groups showed that all samples met the standards (Table 6).

Table 6. Indicators of quality and safety of broiler meat

Parameter Name	Real Results			Maximum Permissible Concentration	
	control	Exp. Group 1	Exp. Group 2	Control Group	Exp. Groups 1,2
Salmonella	Not found			Not present in 25 g	
L. Monocytogenes	Not found			Not present in 25 g	
Number of mesophilic aerobic and facultative anaerobic microorganisms, colony forming units per gram	2.5x 103	Less than $1.5x10^3$	Less than $2.0x10^3$	$1.0x10^4$	
Volatile fatty acids (mg KOH per 100 g)	2.18± 0.16	2.22 ± 0.18	2.19±0.20	Max. 8.0	
Fatty acid number (mg KOH / g)	0.454± 0.021	0.442± 0.023	0.450±0.020	Max. 3.0	
The reaction of ammonia with Nessler reagent	Negative			-	

** p<0.05, ** p<0.01, *** p<0.001*

The table shows that the sample of the experimental groups is not inferior to the microbiological control group and contains less mesophyllic aerobic and facultative anaerobic microorganisms compared with the control group. In addition, no significant changes in the chemical characteristics of the meat and fat of the broilers were established. None of the above results reported in Table 6 exceeds the allowable values.

4. CONCLUSION

Ethanolamine produces a protective effect on the biological oxygenation chain. Ethanolamine protects the organism against energy loss due to the reduction in the formation of free radicals and to the activation of the enzymatic antioxidant defense system that provides additional protection against active oxygen forms.

By suppressing the free radical formation process, the medication produces an optimizing effect on metabolic processes, stimulating the synthesis of phospholipids, which in turn stabilizes the structures of the cell membranes.

The optimization of the level of metabolic processes and the absence of excess free radicals provide for a higher immunological status, natural resistance and viability of the chicks and

therefore a more balanced development of the embryo. As a result, ethanolamine has a positive impact on the indicators of broiler meat.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Certain Poultry-yard Microclimate Indicators for Broiler Chickens

Light Regime

Age in days	Days, hours	Nights, hours
1-5	24	0
6-42	23	1

Humidity and temperature growth regime

Days	Mass, g	Temperature,ºC	Relative humidity, %
1	2	3	4
0	40	33	55
1	52	32.4	55
5	110	30	60
1	2	3	4
10	220	27	65
15	430	25	65
20	740	23	60
25	1,050	21	65
30	1,370	20	65
35	1,700	19.5	65
40	2,100	19	65
42	2,350	18	65

Structure of premixes for Hubbard F-15 crossing

VITAMINOUS							
No.	Composition	Unit of measure	Broilers			Replacements 0-22 weeks (0.15%)	Parent stock (0.15%)
			0-10 days (0.15%)	11-33 days (0.15%)	34-40 days (0.15%)		
1	Vitamin A	Ths. IU	10,000	8,333	6,667	8,000	10,000
2	Vitamin D3	Ths. IU	2,667	2,333	2,000	1,000	1,000
3	Rovimix Hy-D	Ths. IU	0	0	0	1,000	1,000
4	Vitamin E	mg	66,667	66,667	66,667	53,333	66,667
5	Vitamin K3	mg	2,000	1,333	1,333	1,333	3,333
6	Vitamin B1	mg	2,000	1,333	1,333	1,333	2,000
7	Vitamin B2	mg	5,333	4,000	4,000	5,333	6,667
8	Vitamin B3	mg	10,000	6,667	6,667	6,667	10,000
9	Vitamin B5	mg	40,000	26,667	26,667	40,000	40,000
10	Vitamin B6	mg	2,667	2,000	2,000	2,000	3,333
11	Vitamin Bc	mg	1,000	667	667	667	1,333
12	Vitamin B12	mg	13.3	13.3	13.3	13.3	20
13	Vitamin H	mg	240	257	240	240	333
14	Cobalt	mg	333	333	333	333	333
15	Iodine	mg	667	667	667	1,333	1,333
16	Selenium	mg	133	133	133	267	267
17	Antioxidant – Endox	mg	66,667	66,667	66,667	66,667	66,667

18	Rovabio	mg	33,333	33,333	33,333	33,333	33,333
19	Natufos 10000	mg	0	33,333	33,333	26,667	26,667
20	Bachilichmum	mg	166,667	166,667	166,667	0	0
MINERAL							
No.	Composition	Unit of measure	Broilers (0.15%)			Replacements 0-22 weeks (0.15%)	Parent stock (0.15%)
			0-10 days	11-33 days	34-40 days		
1	Iron	mg	40,000			40,000	
2	Copper	mg	16,667			6,667	
3	Zinc	mg	53,333			66,667	
4	Manganese	mg	53,333			66,667	
5	Luctarom Fruity	mg	133,333			0	
6	Bachilichmum	mg	0			166,667	

Structure of combined feed for Hubbard F-15 crossing

No	**List of feed components**	**Unit of measure**	**Diet structure**				
			0 - 5 days	**6 - 10 days**	**11 -21 days**	**22-33 days**	**34 -42 days**
1	2	3	4	5	6	7	8
1	Corn gluten meal	%	8.970	8.970	9.960	4.000	4.000
2	Corn	%	29.505	29.505	35.200	45.110	45.110
3	Wheat	%	0.000	0.000	3.531	6.570	6.570
3	Crushed wheat	%	20.025	20.025	8.239	0.000	0.000
4	Soy bean meal 44%	%	0.000	0.000	0.000	0.000	0.000
5	Bean cake 36%	%	9.180	9.180	5.000	3.000	3.000
6	Full-fat soy bean	%	25.000	25.000	26.000	28.500	28.500
7	Sunflower cake 36%	%	1.770	1.770	5.640	2.800	2.800
8	Sunflower meal 36%	%	0.000	0.000	0.000	0.000	0.000
9	Nonfat dry milk	%	0.000	0.000	0.000	0.000	0.000
10	Fish flour	%	0.000	0.000	0.000	0.000	0.000
11	Meat and feather flour	%	0.000	0.000	0.000	4.000	4.000
12	Soy bean oil	%	0.000	0.000	0.910	1.420	1.420
13	Wheat chop	%	0.335	0.235			
14	Premix 0-10 days vit.	%	0.150	0.150			
15	Premix 0-10 days min.	%	0.150	0.150			
16	Normaflor	%	0.000	0.150			
17	Apex	%	0.000	0.000			
18	Cygro	%	0.050	0.050			
19	Selenium	%	0.015	0.015			
20	Allzyme Vegpro	%	0.150	0.150			
21	Fungistat	%	0.200	0.200			
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
22	Laevomycetin	%	0.000	0.000			
23	Sodium sulfate	%	0.100	0.100			
24	Lacture	%	0.050	0.000			
13	Wheat chop	%			0.230	0.230	
25	Premix 11-33 days vit.	%			0.150	0.150	

26	Premix 11-33 days min.	%			0.150	0.150	
16	Normaflor	%			0.000	0.000	
17	Apex	%			0.000	0.000	
18	Cygro	%			0.050	0.050	
19	Selenium	%			0.020	0.020	
20	Allzyme Vegpro	%			0.150	0.100	
21	Fungistat	%			0.200	0.250	
22	Laevomycetin	%			0.000	0.000	
23	Sodium sulfate	%			0.200	0.200	
24	Lacture	%			0.050	0.050	
13	Wheat chop	%					0.280
27	Premix 34-42 days vit.	%					0.150
28	Premix 34-42 days min.	%					0.150
16	Normaflor	%					0.000
17	Apex	%					0.000
19	Selenium	%					0.020
20	Allzyme Vegpro	%					0.100
21	Fungistat	%					0.250
23	Sodium sulfate	%					0.200
24	Lacture	%					0.050
29	Limestone	%	1.500	1.500	1.480	1.470	1.470
30	Monocalcium phosphate	%	1.360	1.360	1.390	0.830	0.830
31	Sodium chloride	%	0.100	0.100	0.100	0.110	0.110
32	Sodium bicarbonate	%	0.150	0.150	0.150	0.060	0.060
33	Methionine	%	0.290	0.290	0.240	0.330	0.330
34	Lysine	%	0.450	0.450	0.460	0.260	0.260
35	Threonine	%	0.070	0.070	0.050	0.040	0.040
36	Euroguard	%	0.200	0.200	0.200	0.000	0.000
37	Choline chloride B4	%	0.230	0.230	0.250	0.300	0.300
	Total		100.0	100.0	100.0	100.0	100.0

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=359&id=22&aid=2692