Appendix 22

Performance of Chickens fed with Feed containing Soybean Meal derived from Genetically Modified Imidazolinone-tolerant CV127 Soybean as compared to Conventional Soybean Meals

STUDY REPORT

Project Number:

10.06.21.033-01

Study Title:

"Performance of chickens fed with feed containing soybean meal derived from genetically modified imidazolinone-tolerant CV127 soybean as compared to conventional soybean meals"

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Sponsor:

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1. Objective

The objective of this study was to evaluate the performance of chickens fed with feed containing soybean meal derived from genetically modified imidazolinone herbicide tolerant CV127 soybean as compared to conventional soybean meals.

In order to attain the objective, it was necessary to first determine the values of apparent metabolisable energy corrected for nitrogen (Preliminary experiment 1) and the true digestibility values of the amino acids (Preliminary experiment 2) contained in genetically modified soybean meal, conventional soybean meal from a closely related non-genetically modified soybean variety (CONQUISTA) and in two commercial soybean meals (COODETEC 217 and MONSOY 8001).

The Study was conducted following the guidelines provided by the Study Plan (SP) (*Schedule 1).*

2. Information Referring to the Test Substance and to the Reference Substances

The soybean grain from each of the varieties used to produce the soybean meals tested in this study was produced in the 2006/2007 growing season at Santo Antonio de Posse (SP). The soybeans were cultivated according to standard agricultural practices as described in the report entitled “Soybean Genetically Modified with the AHAS Gene for Tolerance to Imidazolinone Herbicides: Agricultural and Ecological Experiments 2006/07” that was prepared by Carlos Arias of Empresa Brasileira de Pesquisa Agropecuaria, Brasil (Embrapa). The CV127 soybean plants were from the imidazolinone-tolerant line CV603 and they were treated with 70 g imazapyr/ha, while the conventional soybean varieties Conquista, Monsoy 8001, and Coodetec 217 were treated with the conventional soybean herbicide Volt. After harvest of the soybean grain, the grain was analyzed by GeneScan, Brazil using an event specific PCR for the detection of the inserted gene that encodes the imidazolinone-tolerant AHAS enzyme in order to verify the identity of each grain lot. Of the four grain samples, only the CV127 grain tested positive by PCR, thereby verifying the identity of this grain and that there was no contamination of the conventional soybean grain with grain from CV127 soybean. After the production of the soybean meals, all samples were subjected to similar PCR testing that verified the identity of the meal samples and confirmed that there was no contamination of the conventional soybean meals with meal from CV127 soybean. In addition, the grain and the soybean meal that was produced from it were tested for the presence of important mycotoxins, including aflatoxins B1, B2, G1, and G2, zearalenone, and ochratoxin A by the Instituto de Technologia de Alimentos (ITAL) in Campinas, Brazil. None of these mycotoxins were detected in any of the soybean grain or meal samples. The grain samples were also tested by Bioensaios (Viamão, Brazil) and GENCS (Guaratinguetá, Brazil) for the presence of residues of pesticides that were used in the cultivation of the soybeans. For all pesticides tested, residues were either not detectable or were well below the minimum residue levels established for each of the pesticides.

All soybean meals were produced from whole soybean grain by the Instituto de Technologia de Alimentos (ITAL) in Campinas, Brazil**.** First, the soybeans were cleaned of impurities such as dust, stones, branches and weed seeds using manual separation and sieves. The soybeans were flaked in an expeller type press with a 40 kg/h processing capacity. Approximately 5 % of the oil was removed during this preparation. The flaked soybean material was extracted in a batch type extractor, using indirect steam to heat the n-hexane solvent to between 45 – 50 ºC. In the production of the defatted and toasted soybean meal, the extractors were heated with indirect steam during 20 minutes in order to evaporate the solvent in the meal. Subsequently, direct steam (30 psi) was applied for 30 minutes through an orifice plate with a 2.5 mm aperture. The residual solvent was removed by treating with direct steam for 30 minutes under vacuum

(250 mm Hg). To finish the process, the meal was subjected to a pressure of 0.2 kg/cm^2 for 10 minutes. The meal moisture was reduced to less than 12 % (w/w) in a flash dryer with a temperature of 200-250 ºC for 54 seconds.

NOTE: The actions, as well as the information presented in the paragraphs above, in item 2, were not generated by Embrapa Suínos e Aves and were not part of the Study Plan. The data and information presented here were issued by the laboratories indicated above and preceded the initiation of the Study Plan.

2.1. Identification of the Test Substance

Name: Soybean meal obtained from genetically modified CV127 soybean, tolerant to imidazolinone herbicides.

2.2. Identification of the Reference Substances

Control Meal: Soybean meal obtained from the non transgenic parental soybean CONQUISTA variety (BRSMG046) from which the CV127 soybean was developed by transformation.

Standard Meal 1: Soybean meal obtained from the conventional, non transgenic soybean COODETEC 217 variety, cultivated at the same time and at the same venues as the CV127 and CONQUISTA soybeans.

Standard Meal 2: Soybean meal obtained from the conventional, non transgenic soybean MONSOY 8001 variety, cultivated at the same time and at the same venue as the CV127 and CONQUISTA soybeans.

2.3. Receipt and control of the Test Substance and of Referential Substances

Soybean meals were received from the Pilot Feed Plant of Embrapa Suínos e Aves on October 25, 2007; they were packaged in forty-four (44) duly identified double raffia bags. Eleven (11) bags were received of each of the four (4) meals. After these bags were weighed, it was determined that the following quantities had been received: 375.7 kg of CV127 soybean meal, 378.5 kg of the CONQUISTA soybean meal, 365.4 kg of the COODETEC 217 (Standard 1) soybean meal and 379 kg of the MONSOY 8001 (Standard 2) soybean meal. Upon their receipt, an inspection was carried out, and the products that were declared as suitable were released for stocking purposes.

The Test Substance (CV127 soybean meal) was stored on 20 cm high pallets at a distance of 40 cm from the walls, inside an appropriate room, duly identified and provided with a compartment made of a metallic, 2 mm screen to prevent rodents from entering. Both the room and the compartment were always kept closed, and only authorized personnel had access to them.

The identified Reference Substances were stored on 20 cm high pallets at a distance of 40 cm from the walls, in the Ingredients Storage Room.

The Test and Reference Substances were registered at the time they were used in the respective Custody Chains.

The remaining ingredients that were necessary to the elaboration of the experimental feed were also stored in this venue, on 20 cm high pallets at a distance of 40 cm from the walls. Corn was packed in raffia bags, while the other ingredients were stored in plastic buckets closed with a cover and identified with the name of the product, lot No., manufacture date, expiration date, active principle, purity and project number.

At the end of the study, the unused Test and Reference substances, the bags and other residues were incinerated.

3. Information Referring to the Test System

Species: Chicken *(Gallus gallus domesticus)*

Type: Broiler Chicken

Lineages: Ag Ross508 Chicken

Date Received: February 1st, 2008

Origin: Sadia S.A.

4. Records and Observations

Throughout the study, information, data, measurements and calculations were recorded on specific forms at the time they were obtained.

All the records were analyzed from a critical point of view and include the identification of the individual responsible for carrying out the activity and of the individual responsible for checking the results.

The bulk original data is kept at Embrapa Suínos e Aves under the responsibility of the Study Director (SD).

5. Preliminary Experiments

5.1. Preliminary Experiment 1: Determination of apparent metabolisable energy corrected for nitrogen (AMEn) for the four soybean meals.

The experiment was conducted at Embrapa Suínos e Aves, in its Energy Metabolisation Room, at the Metabolism Building, during the* period *from November 07 to 29, 2007.

The instructions specified in the current version of POP-CP.001 were complied with (*Schedule 2*).

All the stages of the experiment were performed by properly trained personnel, always under the supervision of the Lead Researcher (LR) and/or SD (*Schedule 3*).

The measurement equipment and/or tests that could have a direct impact on the final result of the experiment were properly calibrated by qualified laboratories.

5.1.1. Material and Methods

5.1.1.1 Birds and Housing

The experiment used six hundred (600) broiler chickens of the AgRoss508 lineage, separated by sex and lodged in heated metallic pens.

During the pre-experimental period (chickens ranging from 1 to 14 days of age), four (4) boxes of 2.43 m^2 were used, two (2) for males and two (2) for females.

During the experimental period (15 to 22 days of age), the chickens were transferred to six (6) metallic pens measuring 2.7 x 0.9m and containing twelve (12) boxes each. This stage was subdivided into two (2) periods: the adaptation period (15 to 18 days of age) and the total excreta collection period (from 19 to 22 days of age).

A design was defined for the distribution of the treatments during the pre-experimental and experimental stages.

5.1.1.2 Experimental Design

The experimental design was accomplished in blocks, by initial weight, in random order, with five (5) treatments and twelve (12) repetitions of ten (10) chickens each (5 males and 5 females).

5.1.1.3 Experimental Diets

The Experimental Feeds were produced at the Pilot Feed Plant of Embrapa Suínos e Aves.

The feeds consisted of 60 % of the basal diet and 40 % of each tested soybean meal (Test Substance and Reference Substances). The basal diet consisted of all feed ingredients except soybean meal, including corn, vitamin and mineral premixes, soybean oil, and other nutrients shown in *Table 1a*. The amount of protein in corn and soybean meal, calcium, dicalcium phosphate, phosphorus, and calcitic lime calcium were determined at the Physical-Chemical Analyses Laboratory (LAFQ) of Embrapa Suínos e Aves prior to the production of the feeds. The values of the apparent metabolisable energy corrected for nitrogen (AMEn) on a wet weight basis were recorded, as well as the remaining nutrients found in the ingredients referenced by Rostagno et al. (2005).

The experimental treatments were:

Witness Treatment:

- T1: Reference Feed: based on corn and commercial soybean meal and formulated to contain 22 % gross protein and 3000 kcal of AMEn/kg, as specified in Tables 1a and 1b.

Test Treatments:

- T2: Experimental Feed 2: 60 % Basal Diet and 40 % Event CV127 soybean meal;
- T3: Experimental Feed 3: 60 % Basal Diet and 40 % Conventional CONQUISTA soybean meal;
- T4: Experimental Feed 4: 60 % of Basal Diet and 40 % Standard 2 (MONSOY 8001) soybean meal;
- T5: Experimental Feed 5: 60 % of Basal Diet and 40 % Standard 1 (COODETEC 217) soybean meal.

All feed and water were supplied *ad libitum* during the pre-experimental and experimental stages.

The quantities of the experimental feed that were prepared are listed in *Table 2.*

Following production, the feed bags were identified with the number of the project, the name of the SD (study director), treatment, individual bag identification (bag number /total bags), destination, date of manufacture and expiration date.

The feed was stored in the Metabolism Room stacked on 20 cm high pallets, properly cleaned and kept at a distance of 40 cm from the walls. Besides the standard identification, the bags with feed containing the CV127 soybean meal were also identified with the biological risk symbol.

Table 1a: Ingredients composition of the reference feed used during the pre-experimental and experimental periods.

Ingredients	**(%)**
Corn	49.426
Commercial soybean meal	41.164
Dicalcium phosphate	1.706
Calcitic limestone	1.400
Soybean oil	4.841
DL-Methionine	0.222
Salt	0.558
Antioxidant	0.015
Mycotoxin Adsorbent	0.200
Growth Promoter	0.013
Coccidiocide	0.025
Choline chloride	0.280
Vitamin Premix	0.100
Mineral Premix	0.050
Total	100.000

Table 1b: Nutritional composition of the reference feed used during the pre-experimental and experimental periods.

Nutritional Composition	**Reference Feed**
Crude Protein (%)	22.000
AME_n (kcal/kg)	3000
Calcium (%)	0.980
Total Phosphorus (%)	0.693
Available Phosphorus (%)	0.470
Digestible Lysine (%)	1.154
Digestible Methionine +Cystine (%)	0.840
Digestible Methionine (%)	0.420
Digestible Arginine (%)	1.495
Digestible Valine (%)	0.967
Digestible Threonine (%)	0.780
Digestible Tryptophan (%)	0.260
Digestible Isoleucine (%)	0.919

Table 2: Quantities of experimental feeds prepared

Feed	Quantities prepared (kg)
T1: Reference Feed	170
T2: Experimental Feed 2	50
T3: Experimental Feed 3	50
T4: Experimental Feed 4	50
T5: Experimental Feed 5	50

5.1.1.4 Methodology Used

At the beginning of the study, a sample of fifty (50) male chicks and fifty (50) female chicks were weighed in order to determine the average weight and variability and establish twelve (12) weight ranges that were used to populate the twelve (12) blocks in each treatment. Once the weight ranges were defined, all chicks were individually weighed and placed in plastic boxes according to their corresponding weight range. After the chickens were weighed and distributed into boxes by weight, the number of chickens that should form each block for each type of treatment was defined. Then chickens were distributed among the boxes that were previously identified in the pens by treatment, repetition and project number. As a result, each weight range (blocks) was represented in all treatments.

The quantity of chickens for all the treatments of the same block was the same (5 males and 5 females), and all chickens were within the same weight ranges, according to their sex.

In order to determine the AMEn of the soybean meals, the total excreta collection method was used (Hill & Anderson, 1958). The AMEn values were obtained using Matterson *et al.*'s (1965) formula, being adjusted based on nitrogen retention.

Stainless steel trays were placed under each bin compartment, so as to individualize the collection of the excreta by experimental unit and to avoid losses. The collection of excreta was performed twice a day at approximately 08:30 and 14:30 hrs for four (4) days for chickens ranging between 19 to 22 days of age. Before the collection trays with excreta samples were removed, feathers and feed residues that had fallen into the trays were removed.

The excreta was packed in plastic bags, identified by treatment, repetition, collection number and project number, being then weighed and stored inside a freezer. At the end of the collection period, the excreta was thawed, gathered by repetition within each treatment, homogenized and samples of approximately 500 g were collected.

These samples were placed inside aluminium trays and submitted to pre-drying in a dry oven under forced ventilation and a temperature ranging between 55 ± 5 °C for approximately 48 hours. After drying, the samples were ground, placed in plastic bags, identified (treatment, repetition and Study number), and sent to LAFQ for analysis of nitrogen content, dry matter and gross energy.

The slaughter of the chickens was accomplished by cervical displacement in the Metabolism Room. Both the dead chickens and the feed and excreta leftovers were packed in raffia bags, identified and sealed, being later taken to the Suruvi Experimental Field for incineration.

5.1.1.5 Measurements

In order to determine the AMEn of the four (4) soybean meals, the following variables were evaluated: feed consumption, the amount of excreta and analytic variables.

Feed consumption was determined through the difference between the total feed supplied and the feed leftovers for each repetition.

The amount of excreta obtained was the sum of the quantities of excreta that had been collected through repetition throughout the experimental period.

The analytic variables determined for the feed and the excreta were as follows: dry matter, gross energy and nitrogen. The dry matter was determined for each type of soybean meal.

In addition, the room temperature inside the Metabolism Room and the temperature for the storage of the samples inside the freezer were continually monitored using appropriate equipment.

Mortality was determined by the occurrence of death during the experimental period.

5.1.2. Results

The AMEn results for the four (4) soybean meal types studied are presented in *Table 3*. These values were used to formulate the feed for the study “Performance of chickens fed with feed containing soybean meal derived from genetically modified imidazolinone-tolerant CV127 soybean as compared to conventional soybean meals.”

Analyzing the remaining variables, it was determined that the average temperature (27.1 $^{\circ}$C) of the Metabolism Room was within a range considered adequate for chickens (25 $\pm$ 4 $^{\circ}$C). It was also considered that some temperature deviations from the specified temperature range did not affect the results of the experiment.

As regards the monitoring of the storage temperature of the excreta inside the freezer, it was determined that the average temperature had been –16.9 °C. This was a little above the temperature specified in the method used for the procedure (–18 °C), but due to the fact that the samples remained frozen throughout the time of storage, it was considered that the variation did not interfere in the results of the experiment.

No deaths were recorded throughout the experimental period.

Table 3: Results (average) of apparent metabolisable energy corrected for nitrogen (AMEn) for the types of soybean meal studied

Soybean Meal	AMEn (kcal/kg)
CV127	2198 ± 16
CONQUISTA	2182 ± 17
COODETEC 217.	2156 ± 16
MONSOY 8001	2136 ± 16

5.2. Preliminary Experiment 2: Determination of digestible amino acids in the soybean meal.

The experiment was carried out at Embrapa Suínos e Aves in the Amino Acids Metabolism Room at the Metabolism Building during the period from November 3 to 7, 2007.

The instructions specified in POP-CP.002 (current release) were complied with (*Schedule 2*).

All the stages of the experiment were carried out by trained personnel, always under the supervision of LR and/or of SD (*Schedule 3*).

The measurement equipment and/or tests used to determine the results that would have a direct impact on the final results of the experiment were calibrated by accredited laboratories.

5.2.1. Material and Methods

5.2.1.1 Birds and Housing

Thirty (30) cecectomised roosters of Embrapa's CSG lineage with an average weight of 3.3 kg that were forty-one (41) weeks of age were used.

Before getting settled, the roosters were weighed individually and distributed among the experimental units in order to keep the treatments as homogenous as possible.

The roosters were lodged individually in metal cages provided with stainless steel trays used to collect their excreta.

A design was defined to distribute the treatments during the experimental stage.

The roosters were submitted to the regime of approximately fourteen (14) hours of being exposed to light, interspersed with ten (10) hours of darkness throughout the experimental period.

5.2.1.2 Experimental Design

The experimental design was made completely at random, with five (5) treatments and six (6) repetitions of one (1) rooster per experimental unit.

5.2.1.3 Experimental Diets

Six (6) samples of 30 g each of soybean meal were weighed at the LAFQ. The samples were packed in plastic bags, identified with the number of the project, the treatment and the repetition, and sent to the Amino Acids Metabolism Room.

The experimental treatments were:

Witness Treatment:

- T1: No meal (Fasting rooster)

Test Treatments:

- T2: CV127 Soybean Meal
- T3: Conventional CONQUISTA Soybean Meal
- T4: Standard 2 (MONSOY 8001) Soybean Meal
- T5: Standard 1 (COODETEC 217) Soybean Meal

Throughout the test period water was supplied *ad libitum* from a nipple type drinking fountain.

5.2.1.4 Methodology Used

The procedures used to determine the digestible amino acids were those described by Sibbald (1979) with modification according to Parsons (1985).

At the start of the experiment, the roosters were kept under fasting conditions for a period of 36 hours to empty out their digestive tracts.

After the fasting period, the roosters were forced to ingest 30g of each type of soybean meal, according to each treatment, which was introduced into the ingluvium (craw) through a funnel inserted via esophagus.

In parallel, six (6) roosters were kept fasting for the collection of excreta and for the determination of endogenous and metabolic losses.

After the forced feeding, the excreta were collected from individual roosters for a period of forty-eight (48) hours, two (2) times a day (at approximately 0830 and 1530 hrs). Any visible feathers were removed from each excreta collection to prevent possible interactions of the nitrogen contained in the excreta with the nitrogen contained in the feathers. The excreta from each rooster were pooled to form a single sample per bird. After each collection, the excreta were placed in plastic pots of known weight, having been previously identified with the number of the study and the sequential number. The pots were packed individually in plastic bags, which were identified and stored in a freezer.

It was necessary to repeat the collection of the excreta for two (2) treatments because the roosters lodged in cages G28, G42, G62 (Treatment 3) and G40 and G46 (Treatment 4) regurgitated. The repetition of the experiment was necessary because the reduced number of rooster repetitions from Treatments T3 and T4 could affect the determination of the amino acid digestibility coefficient. For safety purposes, eight (8) roosters were used in this repetition per treatment instead of six (6). Nevertheless, the excreta of only six (6) roosters per treatment were sent for analysis, as determined in the SP (Study Plan).

At the end of the experiment, the frozen excreta samples were forwarded to LAFQ for lyophilisation. After being lyophilized, and after being properly packed and identified, the

samples were sent to the Foods Technology Institute (ITAL) for total amino acids determination.

Also at the end of the experiment, the roosters were slaughtered by cervical dislocation at the Metabolism Room. Both the dead roosters and the leftover feed and excreta were packed in raffia bags, identified and sealed, being then taken to the Suruvi Experimental Field for incineration.

5.2.1.5 Measurements

In order to determine the percentage of digestible amino acids in the four (4) types of soybean meals, the following variables were evaluated: the quantity of test feed, the quantity of excreta, the analytical variables and the digestibility coefficients of the amino acids.

The analytical variables determined in the soybean meals were the following: dry matter, nitrogen and total amino acids. As to the excreta, the dry matter, the nitrogen and total amino acids were determined.

The quantity of excreta obtained was the sum of the quantity of excreta collected per repetition throughout the experimental stage.

The digestibility coefficient of the amino acids was computed as the difference between the amino acids ingested and those excreted, considering the endogenous secretion of amino acids.

The percentage of digestible amino acids was determined based on the total quantity of amino acids in the meals and the amino acid digestibility coefficients.

In addition, the room temperature inside the Metabolism Room and the storage temperature of the samples inside the freezer were continually monitored by appropriate equipment.

Mortality was determined by the incidence of death during the experimental stage.

5.2.2. Results

The estimated percentages of the digestible amino acids for the four (4) types of soybean meals studied are listed in *Table 4*. The estimated digestible amino acid contents were used for the formulation of the experimental feed of the experiment called “Performance of chickens fed with feed containing soybean meal derived from genetically modified imidazolinone-tolerant CV127 soybean as compared to conventional soybean meals”.

As mentioned in item 5.2.1.5, the determination of total amino acids in the soybean meals was one of the analytical variables measured. These analyses were carried out by ITAL and the total amino acid values found in the soybean meals were used for the calculation of the percentage of digestible amino acids in the soybean meal, with the exception of those values determined for the sulfur amino acids methionine and cysteine. The levels of the sulfur amino acids methionine and cysteine determined by ITAL were discarded since they were below the referenced literature values for these amino acids that are typical for soybean meal. These values were low for the reason that ITAL did not follow the specific protocol for determination of methionine and cysteine by acid hydrolysis (Oxidation with performic acid followed by acid hydrolysis), according to AOAC (1995). So, the methionine and cysteine values were underestimated due to losses that occurred when the acid hydrolysis was carried out without the pre oxidation with performic acid.

Therefore, the methionine and methionine plus cysteine values, from the 4 (four) types of soybean meals utilized in the feed formulations were based in Rostagno et al. (2005) (*Table 4*). Subsequently, soybean meals samples were shipped to Eurofins Scientific Analytics Laboratory in Hamburg, Germany, for determination of total sulfur amino acids levels. The results of these analysis demonstrated that the total amino acid levels for methionine and cysteine found in soybean meal samples were within the typical parameters of these amino acids in conventional soybean meal and were similar with the values referenced by Rostagno et al. (2005). The results from Eurofins Scientific Analytics indicated that the different soybean meal samples had respective methionine and methionine plus cysteine values as follows: CV127 soybean meal (48,7 % PB), 0,67 % and 1,33 %; CONQUISTA (47,8 % PB) soybean meal, 0,68 % and 1,35 %; MONSOY 8001 (45,1 % PB) soybean meal, 0,65 % and 1,30 %; and COODETEC 217 (45,9 % PB), 0,62 % and 1,24 %. The data referenced by Rostagno et al. (2005) for total amino acids (methionine and methionine plus cysteine) present in soybean meal is 0,64 % and 1,27 % for 45 % PB, respectively; and for soybean meals with 48 % PB, 0,66 % and 1,37 %, respectively.

Upon analysis of the remaining variables, it was determined that the average temperature in the Metabolism Room during the first period of collection was 21.8 °C. When treatments T3 and T4 were repeated, the average temperature was 23.4 °C. These temperatures were within the range considered to be adequate for the chickens (25 ± 4 °C). It was considered that the occurrences of temperatures outside this range did not affect the results of the experiment.

As regards the monitoring of the storage temperature of the excreta in the freezer, it was determined that the average temperature was –17.7 °C, and that it had been a little above the temperature that had been specified in the procedure for the method (–18 °C), although due to the fact that the samples had been kept frozen throughout the storage time, it was considered that the variation did no interfere in the results of the experiment.

No deaths were recorded during the experimental period.

Table 4: Percent of digestible amino acids present in the soybean meal adjusted to a standard moisture level.

Amino acids[1] (%)	Treatments			
	T1	T2	T3	T4
	CV127 (%)	CONQUISTA (%)	MONSOY 8001 (%)	COODETEC 217 (%)
Aspartic acid	4.94	4.48	4.57	4.82
Threonine	1.59	1.45	1.49	1.49
Serine	2.35	2.00	2.08	2.29
Glutamic acid	8.29	7.50	7.55	8.01
Proline	1.42	1.82	1.82	1.37
Glycine	1.64	1.54	1.57	1.64
Alanine	1.94	1.68	1.73	1.83
Cysteine	0.39	0.54	0.50	0.42
Valine	1.74	1.91	1.91	1.55
Methionine	0.43	0.45	0.45	0.26
Isoleucine	1.68	1.66	1.67	1.51
Leucine	3.27	2.90	2.94	3.13
Tyrosine	1.64	1.28	1.33	1.65
Phenylalanine	2.31	1.97	2.02	2.30
Lysine	2.76	2.32	2.36	2.68
Histidine	1.41	1.43	1.38	1.27
Arginine	3.28	2.99	2.95	3.10
Tryptophan	0.56	0.63	0.64	0.62

6. Study: Performance of chickens fed with feed containing soybean meal derived from genetically modified imidazolinone-tolerant CV127 soybean as compared to conventional soybean meals.

The study comprised the development of a performance experiment carried out in Test Shed No. 4A at the Suruvi Experimental Field at Embrapa Suínos e Aves during the period from February 01 to March 14, 2008.

The instructions specified in POP-CP.003 (current release) were complied with (*Schedule 2).*

The measurement equipment and/or tests that could have a direct impact on the final result of the experiment were properly calibrated by qualified laboratories.

6.1. Material and Methods

6.1.1. Birds and Housing

Five hundred and seventy-six (576) broiler chickens were used, of which 50 % were males and 50 % were females, all of them of the AgRoss508 lineage.

The chickens were separated by sex in the period ranging from one to forty-two (42) days of age, lodged in forty-eight (48) boxes that provided 1.5 m^2 of space per bird, thereby abiding by the dimensions of 800 to 950 cm^2 (approximately 30 cm by 30 cm) per chicken, as recommended in ILSI (2003). Besides these boxes, eight (8) boxes were used at the extremity of each queue as edges, each containing twelve (12) chickens.

A design was defined for the distribution of the treatments during the experimental period.

Throughout the testing period, the chickens were subjected to the natural light regime.

6.1.2. Experimental Design

The experimental outline was accomplished randomly by blocks, according to corporal weight, with four (4) treatments and twelve (12) repetitions, of which six (6) repetitions were made with males and six (6) with females. Each repetition contained twelve (12) chickens, for a total of one hundred and forty-four (144) chickens per treatment.

6.1.3. Experimental Diets

The experimental feed was produced at the Feed Plant of Embrapa Suínos e Aves.

The nutritional requirements complied with the recommendations for mixed lots, which are contained in the AgRoss 508 (AGROCERES, 2004) Lineage Manual, even though the males and females had been raised separately.

The feed was produced in bran form, and together with water they were supplied *ad libitum* to the chickens.

Values for apparent metabolisable energy corrected for nitrogen (AMEn) and for digestible amino acids for the soybean meals, obtained during Preliminary Experiment 1 and Preliminary Experiment 2, respectively, were used in the formulation of the experimental feeds. However, for the values of methionine and methionine plus cysteine for the four (4) soybean meals were based on Rostagno et al. (2005) because the total amino acids determined by ITAL were below the levels mentioned in the literature and the results for the determinations by Eurofins Scientific Analytics were not available at

the time that the feed was prepared. As regards the remaining nutrients of the soybean meals, the values determined by ITAL were used.

As regards corn protein and calcitic lime calcium, the values determined by LAFQ were used. Two (2) dicalcium phosphate lots were used, the first one, the phosphorus and the calcium being determined in the LAFQ, while for the second lot these levels were determined at Sadia S/A's Laboratory. For the remaining nutrients, and for the AMEn of the corn and of the refined soybean oil, the data referenced by Rostagno et al. (2005) was used.

The feed was formulated to be isoenergetic and isoproteic.

Synthetic amino acids were used as needed to supply the minimum quantity required for the lineage.

According to ILSI (2003), it is recommended that the coccidiocide and the growth promoter be withdrawn before slaughter. According to the Brazilian legislation, these two additives must be removed seven (7) days before the chickens are slaughtered. As a result of the above, the final phase of feeding was subdivided into two (2) subphases. Therefore, the experiment consisted of four (4) experimental stages: initial (1 to 10 days of age), growth (11 to 28 days of age), final 1 (29 to 35 days of age), and final 2 (36 to 42 days of age). The coccidiostat was removed from the feed one week prior to slaughter of the animals.

The experimental treatments were:

Test Treatment:

- T1: Feed made of corn and CV127 soybean meal.

Witness Treatments:

- T2: Feed made of corn and conventional CONQUISTA soybean meal.
- T3: Feed made of corn and Standard 1 (MONSOY 8001) soybean meal.
- T4: Feed made of corn and Standard 2 (COODETEC 217) soybean meal.

The composition of the ingredients of the experimental feed is presented in *Tables 5a, 6a, 7a and 8a,* while the nutritional composition is presented in *Tables 5b, 6b, 7b and 8b,* according to the stage of the experiment.

Following production, the feed bags were identified with the number of the project, the SD name (study director), treatment, individual identification of the bags (bag number/total bags), destination, manufacture date, and expiration date. At the Test Shed, the feed was stored over properly cleaned 20 cm high pallets, and placed at a distance of 40 cm from the walls, inside a room protected with a 2 mm metal screen offering restricted access. Specifically as regards bags containing CV127 soybean meal, besides the standard identification, the symbol representing the biological risk was printed on the label.

The quantities of the experimental feed prepared are shown in *Table 9.*

Table 5a: Ingredient composition of the experimental feed used during the Initial stage when chickens ranged from 1 to 10 days in age.

Ingredients (%)	Treatments			
	T1 CV 127	T2 CONQUISTA	T3 MONSOY 8001	T4 COODETEC 217
Corn	49.047	47.999	43.207	44.749
Soybean Meal CV127	41.628	-	-	-
Soybean Meal CONQUISTA	-	42.323	-	-
Soybean Meal MONSOY8001	-	-	45.885	-
Soybean Meal COODETEC	-	-	-	44.842
Dicalcium phosphate	1.900	1.899	1.980	2.056
Calcitic limestone	1.173	1.147	1.159	1.009
Soybean oil	4.851	5.099	6.345	5.903
L-Lysine	-	0.119	0.008	-
DL-Methionine	0.207	0.202	0.220	0.227
L-Threonine	-	0.017	-	-
L-Valine	-	-	-	0.019
Salt	0.584	0.584	0.585	0.585
Antioxidant	0.015	0.015	0.015	0.015
Mycotoxin Adsorbent	0.100	0.100	0.100	0.100
Growth Promoter	0.025	0.025	0.025	0.025
Coccidiocide	0.025	0.025	0.025	0.025
Choline chloride	0.296	0.296	0.296	0.296
Vitamin Premix	0.100	0.100	0.100	0.100
Mineral Premix	0.050	0.050	0.050	0.050
Total	**100.000**	**100.000**	**100.000**	**100.000**

Table 5b: Nutritional composition of the experimental feed used during the Initial stage when chickens ranged from 1 to 10 days in age

Nutritional Composition	Treatments			
	T1 CV 127	T2 CONQUISTA	T3 MONSOY 8001	T4 COODETEC 217
Crude Protein (%)	24.063	24.063	24.063	24.063
AME_n (kcal/kg)	3010	3010	3010	3010
Calcium (%)	1.000	1.000	1.000	1.000
Total Phosphorus (%)	0.767	0.768	0.735	0.701
Available Phosphorus (%)	0.480	0.480	0.480	0.480
Digestible Lysine (%)	1.235	1.180	1.180	1.296
Digestible Methionine +Cystine (%)	0.870	0.870	0.870	0.870
Digestible Methionine (%)	0.533	0.530	0.553	0.556
Digestible Arginine (%)	1.550	1.426	1.509	1.551
Digestible Valine (%)	0.879	0.981	1.028	0.870
Digestible Threonine (%)	0.799	0.760	0.796	0.785
Digestible Tryptophan (%)	0.263	0.295	0.320	0.305
Digestible Isoleucine (%)	0.827	0.832	0.879	0.780

Table 6a: Ingredients composition of experimental feeds used during the growth stage when chickens ranged from 11 to 28 days in age.

Ingredients (%)	Treatments			
	T1	**T2**	**T3**	**T4**
	CV 127	**CONQUISTA**	**MONSOY 8001**	**COODETEC 217**
Corn	52.692	51.795	47.565	48.892
Soybean Meal CV 127	36.861	-	-	-
Soybean Meal CONQUISTA	-	37.442	-	-
Soybean Meal MONSOY 8001	-	-	40.578	-
Soybean Meal COODETEC	-	-	-	39.706
Dicalcium phosphate	1.777	1.776	1.848	1.915
Calcitic limestone	1.153	1.130	1.141	1.008
Soybean oil	6.247	6.457	7.556	7.177
L-Lysine	-	0.127	0.029	-
DL-Methionine	0.182	0.178	0.195	0.200
L-Threonine	-	0.007	-	-
L-Valine	-	-	-	0.013
Salt	0.509	0.509	0.510	0.510
Antioxidant	0.015	0.015	0.015	0.015
Mycotoxin Adsorbent	0.100	0.100	0.100	0.100
Growth Promoter	0.025	0.025	0.025	0.025
Coccidiocide	0.025	0.025	0.025	0.025
Choline chloride	0.263	0.263	0.263	0.263
Vitamin Premix	0.100	0.100	0.100	0.100
Mineral Premix	0.050	0.050	0.050	0.050
Total	**100.000**	**100.000**	**100.000**	**100.000**

Table 6b: Nutritional composition of the experimental feed used during the growth stage when chickens ranged from 11 to 28 days in age.

Nutritional Composition	Treatments			
	T1	**T2**	**T3**	**T4**
	CV 127	**CONQUISTA**	**MONSOY 8001**	**COODETEC 217**
Crude Protein (%)	22.000	22.000	22.000	22.000
AME_n (kcal/kg)	3150	3150	3150	3150
Calcium (%)	0.950	0.950	0.950	0.950
Total Phosphorus (%)	0.719	0.719	0.690	0.660
Available Phosphorus (%)	0.450	0.450	0.450	0.450
Digestible Lysine (%)	1.113	1.080	1.080	1.167
Digestible Methionine Cystine (%)	0.800	0.800	0.800	0.800
Digestible Methionine (%)	0.486	0.484	0.504	0.506
Digestible Arginine (%)	1.406	1.295	1.368	1.407
Digestible Valine (%)	0.811	0.900	0.942	0.800
Digestible Threonine (%)	0.732	0.690	0.729	0.720
Digestible Tryptophan (%)	0.238	0.267	0.288	0.276
Digestible Isoleucine (%)	0.756	0.760	0.801	0.715

Table 7a: Ingredients composition of experimental feeds used during the final stage 1 when chickens ranged from 29 to 35 days in age

Ingredients (%)	Treatments			
	T1	**T2**	**T3**	**T4**
	CV 127	**CONQUISTA**	**MONSOY 8001**	**COODETEC 217**
Corn	62.231	61.575	58.303	59.304
Soybean Meal CV 127	28.594	-	-	-
Soybean Meal CONQUISTA	-	28.995	-	-
Soybean Meal MONSOY 8001	-	-	31.419	
Soybean Meal COODETEC	-	-	-	30.798
Dicalcium phosphate	1.670	1.670	1.725	1.777
Calcitic limestone	1.149	1.132	1.140	1.037
Soybean oil	5.238	5.388	6.238	5.952
L-Lysine	-	0.122	0.046	-
DL-Methionine	0.140	0.137	0.150	0.154
L-Threonine	-	0.003	-	-
Salt	0.433	0.433	0.433	0.433
Antioxidant	0.015	0.015	0.015	0.015
Mycotoxin Adsorbent	0.100	0.100	0.100	0.100
Growth Promoter	0.025	0.025	0.025	0.025
Coccidiocide	0.025	0.025	0.025	0.025
Choline Chloride	0.230	0.230	0.230	0.230
Vitamin Premix	0.100	0.100	0.100	0.100
Mineral Premix	0.050	0.050	0.050	0.050
Total	**100.000**	**100.000**	**100.000**	**100.000**

Table 7b: Nutritional composition of experimental feeds used during the final stage 1 when chickens ranged from 29 to 35 days in age

Nutritional Composition	Treatments			
	T1	**T2**	**T3**	**T4**
	CV 127	**CONQUISTA**	**MONSOY 8001**	**COODETEC 217**
Crude Protein (%)	18.663	18.663	18.663	18.663
AME_n (kcal/kg)	3200	3200	3200	3200
Calcium (%)	0.900	0.900	0.900	0.900
Total Phosphorus (%)	0.663	0.663	0.640	0.617
Available Phosphorus (%)	0.420	0.420	0.420	0.420
Digestible Lysine (%)	0.908	0.900	0.900	0.950
Digestible Methionine +Cystine (%)	0.690	0.690	0.690	0.690
Digestible Methionine (%)	0.410	0.409	0.424	0.426
Digestible Arginine (%)	1.168	1.080	1.137	1.168
Digestible Valine (%)	0.704	0.772	0.804	0.685
Digestible Threonine (%)	0.626	0.590	0.622	0.616
Digestible Tryptophan (%)	0.198	0.220	0.236	0.227
Digestible Isoleucine (%)	0.642	0.644	0.676	0.610

Table 8a: Ingredients composition of experimental feeds used during the final stage 2 when chickens ranged from 36 to 42 days in age

Ingredients (%)	Treatments			
	T1	T2	T3	T4
	CV 127	CONQUISTA	MONSOY 8001	COODETEC 217
Corn	62.323	61.668	58.397	58.935
Soybean Meal CV 127	28.585	-	-	-
Soybean Meal CONQUISTA	-	28.986	-	-
Soybean Meal MONSOY 8001	-	-	31.409	-
Soybean Meal COODETEC	-	-	-	30.863
Dicalcium phosphate	1.670	1.669	1.725	1.778
Calcitic limestone	1.149	1.132	1.140	1.036
Soybean oil	5.205	5.355	6.204	6.305
L-Lysine	-	0.122	0.046	-
DL-Methionine	0.140	0.137	0.150	0.155
L-Threonine	-	0.003	-	-
Salt	0.433	0.433	0.433	0.433
Antioxidant	0.015	0.015	0.015	0.015
Miycotoxin Adsorbent	0.100	0.100	0.100	0.100
Choline chloride	0.230	0.230	0.230	0.230
Vitamin Premix	0.100	0.100	0.100	0.100
Mineral Premix	0.050	0.050	0.050	0.050
Total	**100.000**	**100.000**	**100.000**	**100.000**

Table 8b: Nutritional composition of experimental feeds used during the final stage 2 when chickens ranged from 36 to 42 days in age

Nutritional Composition	Treatments			
	T1	T2	T3	T4
	CV 127	CONQUISTA	MONSOY 8001	COODETEC 217
Crude Protein (%)	18.666	18.666	18.666	18.666
AME_n (kcal/kg)	3200	3200	3200	3220
Calcium (%)	0.900	0.900	0.900	0.900
Total Phosphorus (%)	0.663	0.663	0.640	0.617
Available Phosphorus (%)	0.420	0.420	0.420	0.420
Digestible Lysine (%)	0.908	0.900	0.900	0.951
Digestible Methionine +Cystine (%)	0.690	0.690	0.690	0.690
Digestible Methionine (%)	0.410	0.408	0.424	0.426
Digestible Arginine (%)	1.168	1.080	1.137	1.169
Digestible Valine (%)	0.704	0.772	0.804	0.685
Digestible Threonine (%)	0.626	0.590	0.623	0.616
Digestible Tryptophan (%)	0.198	0.220	0.236	0.227
Digestible Isoleucine (%)	0.642	0.644	0.676	0.610

Table 9. Quantities of experimental feed prepared per stage.

Experimental Stage (days of age)	Quantity of Feed Prepared (kg)			
	Treatments			
	T1	T2	T3	T4
	CV 127	CONQUISTA	MONSOY 8001	COODETEC 217
Initial (01 to 10)	45	45	45	45
Growth (11 to 28)	270	270	270	270
Final 1 (29 to 35)	190	190	190	190
Final 2 (36 to 42)	250	250	250	250

6.1.4. Methodology Used

The methodology was designed to evaluate the effect of the addition of a test substance to the feed of broiler chickens on their performance compared to other reference substances.

Protection circuits were assembled over a wood shavings bed (at an approximate height of 10 cm), and bell-shaped heating lamps were installed inside each box to heat it.

Four appropriate measurement equipment items were also installed to continuously measure the temperature of the surroundings.

One day before the experiment was started the initial stage experimental feed was packed at the venue.

The experimental feeds were weighed together with the tube-like feedbins already identified with the box number pertaining to the treatment and to the repetition. Cup type drinking fountains were supplied and placed inside the protection circle.

In order to distribute the chickens in the boxes, a sample of 100 (one hundred) male chickens and 100 (one hundred) female chickens which had been removed from several boxes were initially weighed to determine the average of the lot, and thus estimate the distribution of the chickens by weight ranges.

After the weight of the chickens was determined for each weight range, these chickens were weighed individually, and placed inside plastic boxes according to their corresponding weight.

After all the chickens had been weighed and distributed among the boxes, the number of chickens that should form each block inside each treatment was defined according to their weight, being then distributed into the boxes previously identified by treatment, repetition and project number.

The number of chickens for all treatments and belonging to the same block was the same (12 males or 12 females) and all the chickens were within the same weight range according to their sex.

Before each chicken was weighed, the feeding bin inside the box was removed and weighed to determine the amount of unconsumed feed.

Chickens that were incorrectly sexed (male/female) and the refuse were segregated, weighed separately and lodged in the bordering boxes.

The birds that died during the experiment were first weighed and then frozen for later incineration.

At the end of each experimental period, the chickens and the feed were weighed and their weights were recorded on a specific form. After weighing, the unconsumed feed was removed from the feeding pens and the feed for the next experimental phase was then weighed in the respective feeding pens.

At the end of the experiment, the chickens were slaughtered by cervical dislocation inside the Test Shed. The dead chickens, unconsumed feed, and beds were packed in raffia bags, identified and sealed, being then incinerated at the Surivi Experimental Field.

6.1.5. Measurements

During the study of broiler chickens, the following variables were evaluated: initial weight, corporal weight, weight gains, feed intake and the feed conversion at the end of each phase (at 10, 28, 35 and 42 days of age for both males and females).

In addition, the air temperature in the housing area was measured and recorded. Chickens that died during the experimental phase were evaluated for physical defects, smaller than average size, or other causes or mortality. Birds that were the subject of errors in sex determination were removed from the experiment. All evaluations were replicated during the experimental period.

To determine the initial weight, the chickens were weighed together at each repetition before being put into the boxes.

The corporal weight of each repetition was determined by weighing all the chickens of 11, 28, 35 and 42 days of age.

Weight gains were computed through the difference between the corporal weight at each weighing age and the initial weight by repetition, generating weight gains for each period, from 1 to 10, 11 to 28, 29 to 35 and 42 days of age.

Feed intake was determined through the difference existing between the total feed supplied and the unconsumed feed for each repetition, when feed was changed (weighting dates), generating the consumption for period (1 to 10, 11 to 28, 29 to 35 and 42 days of age).

The feed conversion was computed by dividing feed intake by weight gains during the period.

The air temperature inside the Test Shed was continuously monitored using appropriate measurement equipment.

6.1.6. Statistical Analyses

The data was statistically analyzed using the SAS (2003) software, and including in the variance analysis model the block effect, the sex, the treatment and the interaction between these two factors.

The analysis was accomplished with the Dunnett Test (Zimmermann, 2004) that was used to compare treatment (T1), containing soybean meal from CV127 soybean with each of the remaining treatments studied.

As regards the detailing of the statistical analysis, the SP (study plan) provided for the use of Tukey's Test to compare the treatments. However, since the objective of the experiment was to evaluate the performance of broiler chickens fed with soybean meal from CV127 soybean as compared to the reference soybean meals, the Dunnett's Test

was selected, given that it is more appropriate for this type of comparison. This test is used to compare the main treatment to be tested with each one of the remaining treatments that has been studied.

6.2. Results

The performance results are presented in *Table 10.* Since no interaction was detected between the sex of the chickens and the treatment, the statistical analysis was performed for twelve (12) repetitions.

It was determined that the treatment using soybean meal from CV127 soybean did not differ significantly (P>0.05) from treatments that contained the CONQUISTA and MONSOY 8001 soybean meals as regards the feed intake, corporal weight, weight gains or feed conversion during any of the periods studied. These results show that when corn diets and soybean meals were used, both the CV127 soybean meal and the CONQUISTA and MONSOY 8001 soybean meals in isoproteic and isoenergetic diets resulted in a similar performance for broiler chickens ranging from 1 to 42 days of age.

The treatment using CV127 soybean meal was significantly different (P<0.05) for some of the parameters studied when compared to the treatment containing the COODETEC 217 soybean meal for the four (4) periods evaluated. For the period from 1 to 10 days of age, for corporal weight, weight gain and feed intake, the treatment using soybean meal from CV127 soybean gave significantly higher values (P<0.5) compared to the treatment using COODETEC 217 soybean meal (Table 10). However, no statistical differences were seen for feed conversion between these treatments. Lower corporal weight and weight gain for chickens that received the treatment using COODETEC 217 soybean meal, as compared to those that were fed with treatments containing CV127 soybean meal were observed. These results may be explained by the lower consumption of feed containing soybean meal from COODETEC 217 soybean compared to that from soybean meal derived from CV127 soybean (P<0.05).

For periods ranging from 1 to 28 days, 1 to 35 days and 1 to 42 days, however, significant differences (P<0.5) were only detected between treatments using CV127 soybean meals and COODETEC soybean meal as regards the following variables: corporal weight, and weight gain. During these periods, the weight and weight gains determined to exist in chickens that were fed feeds with CV127 soybean meals were also higher than those of the chickens that were fed feeds with COODETEC 217 soybean meal. However, the chickens that received the two soybean meals presented similar feed intake and feed conversions (P>0.5). Even though during these periods no differences were detected as to feed intakes, it was determined that numerically the feed consumption of chickens fed with feed containing COODETEC 217 soybean meal was lower than that of those fed with feed containing CV127 soybean meal. This fact was probably the determinant for the lower corporal weight and the weight gain for chickens fed with COODETEC 217 soybean meal.

Table 11 shows the number of chickens that were discarded per treatment and per repetition, as well as the reasons for their discarding. It may be seen that only five chickens died during the entire experiment. On the other hand, five chickens were discarded due to physical defects, and six chickens were discarded due to an error in determining their sexes. These figures may also be considered as being normal, in view of the number of chickens lodged.

No unusual circumstances occurred during the study that would have interfered with the experiment or had an effect on the results. The average temperature determined inside the Testing Shed was 23.6 °C, which was considered as being satisfactory since the study did not require a controlled environment.

Table 10. The effect of using four types of soybean meal on corporal weight (CW), weight gain (WG), feed intake (FI), and feed conversion (FC) with their respective average standard errors for broiler chickens during the periods studied.

Performance	Treatments			
	T1	T2	T3	T4
	CV127	CONQUISTA	MONSOY 8001	COODETEC 217
Initial weight (g)	44.44 ± 0.05	44.40 ± 0.05	44.40 ± 0.05	44.43 ± 0.05
Period from 1 to 10 days of age				
CW (g)	289 ± 4	295 ± 4	292 ± 4	273 ± 4 *
WG (g)	244 ± 4	251 ± 4	247 ± 4	228 ± 4 *
FI (g)	272 ± 3	273 ± 3	271 ± 3	258 ± 3*
FC	1.11 ± 0.01	1.09 ± 0.01	1.10 ± 0.01	1.13 ± 0.01
Period from 1 to 28 days of age				
CW (g)	1480 ± 10	1503 ± 10	1507 ± 10	1443 ± 10 *
WG (g)	1436 ± 10	1459 ± 10	1463 ± 10	1398 ± 10 *
FI (g)	1955 ± 15	1983 ± 15	1970 ± 15	1915 ± 15
FC	1.36 ± 0.01	1.36 ± 0.01	1.35 ± 0.01	1.37 ± 0.01
Period from 1 to 35 days of age				
CW (g)	2068 ± 18	2106 ± 18	2101 ± 18	2004 ± 18 *
WG (g)	2024 ± 18	2062 ± 18	2057 ± 18	1959 ± 18 *
FI (g)	3023 ± 18	3055 ± 18	3037 ± 18	2975 ± 18
FC	1.50 ± 0.01	1.49 ± 0.01	1.48 ± 0.01	1.52 ± 0.01
Period from 1 to 42 days of age				
CW (g)	2620 ± 15	2644 ± 15	2666 ± 15	2567 ± 15 *
WG (g)	2576 ± 15	2600 ± 15	2621 ± 15	2522 ± 15 *
FI (g)	4183 ± 22	4187 ± 22	4210 ± 22	4125 ± 22
FC	1.63 ± 0.01	1.62 ± 0.01	1.61 ± 0.01	1.64 ± 0.01

Note: *The averages followed by an asterisk (*) on the lines represent significant differences ($P<0.05$) as compared with the CV127 soybean meal treatment when the Dunnett's Test was used.*

Table 11: Number of chickens discarded during the experiment per treatment, per repetition and the reasons for the discardings.

Treatment	Number of Repetitions	Reason for discarding*				
		D	PD	W	S	O
Stage Initial						
T2	1	1	-	-	-	-
T3	1	1	-	-	-	-
T4	5	-	1	-	-	-
Growth stage						
T1	1; 2; 4 and 6	-	2	-	3	-
T2	4	1	-	-	-	-
T3	1; 4 and 6	1	-	-	2	-
T4	1 and 6	1	-	-	1	-
Final Stage 1						
T3	4 and 6	-	2	-	-	-

** Reason for discarding: Death (D); physical defect (PD); below average weight (W); error in determining the sex (S); and other causes (O).*

6.3. Conclusions

The study, therefore, supports the conclusion that the CV127 soy meal is at least equivalent to the tested conventional soy meals, meaning that CV127 can be used in feed without affecting the performance of the chickens during the period ranging from 1 to 42 days of age. However, the use of the COODETEC 217 soy meal resulted in a lower corporal weight and weight gain, when compared to the CV127 soy meal.

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