**Title**

**EFFECT OF FEEDING SOLID STATE FERMENTED COTTON SEED CAKE ON GROWTH PERFORMANCE OF BROILERS.**

**KAKDE D. N.1, MHASE P. P. 1, MAJEE S. B.1, MAGESHWARAN V.2, LONKAR V.D.3,BANNALIKAR A. S.1, PAWAR P.D.\*4, PAWADE M. M.5**

1 Department of Microbiology, Bombay Veterinary College, Parel, Mumbai -400012, India.2 Chemical and Biochemical Processing Division, Central Institute of Research on Cotton Technology, Matunga, Mumbai – 400016, India

3Department of Poultry Science, KNP College of Veterinary Science, Shirwal, Dist. Satara.

4Department of Veterinary Parasitology, KNP College of Veterinary Science, Shirwal, Dist. Satara.

5Department of Microbiology, KNP College of Veterinary Science, Shirwal, Dist. Satara.

**Abstract**

Present study dealt with microbial detoxification of free gossypol from cotton seed cake and its utility as cheaper protein substitute against soya meal of broiler diet. Upon evaluation for growing ability in minimum essential medium containing only gossypol as carbon source, the two culture combinations were included as inoculum I (Pleurotus / Lintinus sajor-caju + Saccharomyces cerevisiae MTCC 6933) and inoculum II (Saccharomyces cerevisiae + Candida tropicalis) respectively, in this study. The freshly grown broth cultures of inoculums I and II were employed for solid state fermentation by spraying them on a raw autoclaved cotton seed cake which were incubated at 280+2 C for 48 Hrs. The inoculum I and II fermented substrates yielded reduction of free gossypol by 72 % and 53.3 %, respectively. The shelf life studies of fermented substrate for a period of nine months did not reveal any major alteration in their nutritive values except negligible depletion of lysine content (0.93% to 0.88%). Studies of broiler performance after feeding fermented substrate at 20% and 40% w/v level up to 5 weeks period was carried out in 350 birds that were divided into six different groups. Results of performance parameters observed did not reveal any statistical differences between control and treatment groups pertaining to traits studied. No major difference was detected in their average live weights (ranged between 1463-1659 g), feed consumption (ranged between 2792.49-3067.15 g), gain in weight (ranged between 376.46-577.57 g), FCR (ranged between 1.46-1.62 g/g) and mortality (ranged between 4 - 10%) except in 40% control (12%). Thus, it was concluded that the microbial method of solid state fermentation of cotton seed cake for reduction of free gossypol yielded promising results and it can be included in place of soybean meal upto 40% level of substitution in broiler diet. However, further studies on optimization of substrate treatment and its economic assessment are necessary.

**Key words: Broiler, feed, fermentation, gossypol, performance.**

**INTRODUCTION**

The Indian poultry sector has reported around 8-10 percent growth annually over the last decade as reported by Investment information and credit rating agency (2014) and still higher rate is focused in order to cater the need of growing local and overseas demand of protein. Major share of the nutrients produced in India are now consumed locally and there is growing demand from commercially flourishing animal husbandry and poultry industry. Poultry industry is also facing intense competition for maize and soybean from human, livestock and aquaculture industry. It is the need of the day to find alternative sources to cope up with these demands so as to meet a cherished goal of ‘food security’ in India. Only the feed itself constitutes approximately 60–75% of the total cost of commercial poultry production (Ogundipe, 1987) and a marginal imbalance in feed cost results in major setback to industry. In such circumstances it is necessary to evaluate feasibility of some other conventional substitute to meet if not total then at least partial demand of costly feed ingredients. Inclusion of any nutrient in poultry diet is governed by its availability, price and nutritive value and nutrients that are less competed by other vertebrate species. Agro-by-products like cottonseed meal, sunflower meal, rapeseed meal, groundnut cake, sesame meal, etc. were included earlier in poultry feed by many workers successfully to mitigate this demand. Cottonseed being the second largest oilseed protein source of animal feed (Smith, 1973) and approximately two million tons of it is primarily used as ruminant feed was considered for this study. However, its large scale inclusion in poultry diet comes with certain limitations because of its high fibre content and anti-nutritional factor gossypol as well as it contains less crude protein, less dietary energy, and low available lysine content than the soybean meal used in conventional diet (Lordelo et al., 2004). Feeding trials conducted with CSM replacement in poultry diets have always presented the data stressing the need of reduction of gossypol to fairly low levels in order to avoid its unfavorable physiological outcome (National Animal Production Research Institute Report, 1984).

Gossypol is found in bound and unbound free state, the latter being toxic researchers studied it for many years to device a suitable technique for removing it from cotton seed and proposed a number of methods, such as, solvent extraction (Rahma and Rao, 1984), liquid cyclone and / or acetone (Gardner et al., 1976); chemical treatment with iron sulfate (Tabatabai et al., 2002) or calcium hydroxide treatment (Nagalakshmi et al., 2002), microbial fermentation (Shi et al., 1998) and so on. Unfortunately, these methods also adversely affected its nutritive quality and were not adopted by stakeholder (Zhang et al., 2006). Even glandless cottonseed was developed to eliminate gossypol (Ryan et al., 1986) which was not promoted as it required genetic interventions. Microbial method of detoxification by Solid State Fermentation (SSF) happens to be promising technique with added benefit of complete degradation of FG (Zhang et al., 2006). Few microorganisms like Candida tropicalis, Torulopsis candida, Saccharomyces cerevisae, Aspergillus flavus, Aspergillus niger,, Aspergillus oryzae and A. terreus are found capable of degrading FG in CSM (Weng and Sun, 2006; Khalaf and Meleigy, 2008). Organically fermented CSC usually contain few exo-enzyme (secreted by microorganisms) such as cellulolytic enzymes, amylases, proteases and lipolytic enzymes, variety of vitamins and some unknown active substance (Brock et al., 1994). But there is very scanty literature citing SSF and its FG reduction efficiency (Mageshwaran et al. 2011) had reported promising results of in-vitro biodegradation of gossypol and a sharp decrease in gossypol concentrations as well as marginal improvement in the lysine level. Hence, present research was undertaken with an objective to assess the utility of CSC after SSF in commercial broiler diet.

**MATERIALS AND METHODS**

**Microorganisms for SSF**

The culture of Pleurotus sajor-caju, Saccharomyces cerevisiae MTCC 6933 and Candida tropicalis were procured and maintained by Chemical and Biochemical Processing Division, Central Institute of Research on Cotton Technology, Matunga, Mumbai and Department of Micrbiology, Bombay Veterinary College, Mumbai. Cultures were maintained respectively on Sabrauds Dextrose Agar, Potato Dextrose Agar and Yeast peptone Dextrose medium (HiMedia, Ltd. India), incubated at 280C for 7 days and fungal spores were preserved at 40C in refrigerator until further use (Chang et al., 1981; Bacha et al., 2011; Dhanasekaran et al., 2011). Gossypol utilization studies of organisms were performed by growing them in minimum essential medium composed of Sodium nitrate (0.5g), Di potassium hydrogen orthophosphate (0.65g), Potassium hydrogen orthophosphate (0.2g), magnesium sulphate (0.1g) supplemented with gossypol acetic acid (10 ppm) as only carbon source and all these ingredients were dissolved in 1 litre distilled water (Mageshwaran et al,. 2011).

**Acute Toxicity assessment in Rats**

The necessary ethical permission for carrying out present research was taken in the meeting held in August 2013 of Institutional Animal Ethics Committee (IAEC, CPCSEA, India- 230/CPCSEA; Dated: 01/08/2000). Prior to inclusion of SSF CSC in poultry diet, for its safety assessment oral acute toxicity trials on the filtrates of culture biomass was conducted. It was carried out in twenty four adult healthy wistar rats of either sex according to OECD (Organization for Economic Cooperation and Development, 2001) guideline no. 420. For toxicity trial Rats were supplied by Central Lab Animal House facility, Bombay Veterinary College, Mumbai, India. The broth extract of culture was centrifuged and supernatant was filtered (2 micron) and filtrate thus obtained was used for assessment of its acute toxicity by oral gavage at the calculated limit dose level of 2000 mg/kg body weight.

**Solid State Fermentation**

The SSF of CSC was carried out at Central Institute for Research on Cotton Technology (CIRCOT), Matunga (E), Mumbai, with pretreatment to CSC by autoclaving it at 1210 C for 15 minutes to achieve 80% moisture content in it and then adding inoculums to it. Two different inoculums combinations were selected for this study after assessment of their gossypol utilization. Inoculum-1 comprised of cultures in equal quantity of Pleurotus sajor-caju and Saccharomyces cerevisiae MTCC 6933 and Inoculum-2 comprised of equal quantity of Saccharomyces cerevisiae plus Candida tropicalis, respectively. Freshly grown Inoculums were sprayed in 20% and 40% concentration on separate CSC substrates and were allowed to ferment substrates at 28 ± 2°C for 48 hours. Biochemical characterization of biodegraded substrates was carried out by calorimetric methods which indicated around 72 percent reduction in FG when fermented with inoculums I and around 53.3 percent when treated with inoculums II (Mageshwaran et al., 2013).The shelf life studies of fermented substrates were performed by keeping feed samples in triplicates in airtight plastic containers and periodically testing it to ascertain the qualitative changes during a period of total nine months duration (A.O.A.C., 1999).

**Table 1:** Broiler Feed Formulation

|  |  |  |  |
| --- | --- | --- | --- |
| **Ingredients** | **Negative Control** | **20 % Substitution of** **SSF CSC** | **40 % Substitution of** **SSF CSC** |
| Pre-starter | Starter | Finisher | Pre-starter | Starter | Finisher | Pre-starter | Starter | Finisher |
| Maize | 53.47 | 54.62 | 59 | 39 | 41.14 | 27.75 | 24.9 | 47.4 | 36.22 |
| Oil | 1.95 | 3.33 | 4.3 | 7 | 7.98 | 12.61 | 11.82 | 8.3 | 12.17 |
| Soybean doc | 40.45 | 38 | 32.7 | 35.25 | 33.11 | 28.18 | 30 | 28.5 | 24.37 |
| Cotton seed meal | 0 | 0 | 0 | 14.56 | 13.68 | 27.36 | 29.12 | 11.77 | 23.2 |
| Trace Mineral Mixture  | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Dicalcium Phosphate | 1.8 | 1.85 | 1.87 | 1.82 | 1.85 | 1.86 | 1.84 | 1.88 | 1.9 |
| Limestone Powder | 1.4 | 1.35 | 2.76 | 1.4 | 1.35 | 1.31 | 1.32 | 2.72 | 2.64 |
| Salt | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Vitamin Premix | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Choline 60 % | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Anticoccidial | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| DL-Methionine | 0.21 | 0.17 | 0.14 | 0.21 | 0.17 | 0.17 | 0.21 | 0.15 | 0.15 |
| Lysine | 0.11 | 0.07 | 0 | 0.15 | 0.11 | 0.15 | 0.18 | 0.03 | 0.06 |
| Toxin binder | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **Total** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |

**Experimental Protocol of Feeding Trials of SSF CSC in Broilers:**

The grinded SSF CSC blended successfully with the other ingredients and the gross texture, consistency and appearance of the feed meal was found same as the conventional poultry feed. The proximate analysis of fermented substrate substituted for soya-meal was performed for computation of broiler feed. Performance of broiler birds was evaluated by feeding them on diets containing SSF CSC replacing 20% and 40% of soya-meal, respectively, as discussed by Adeyemo et al., (2007). The trial was conducted on 350 broiler (Cobb 400) birds for a period of five weeks. The commercial day-old broiler chicks were randomly divided into 7 equal groups of 50 birds. All the birds were reared in deep litter system of housing under ideal and identical managemental and environmental conditions. The diets fed were isocaloric and isonitrogenous. The performance parameters studied during experimental period were weekly average live weight, weekly average gain in weight, average feed consumption per bird, feed conversion ratio (FCR) and percent mortality.

**Table 2:** The experimental design of feeding trial of SSF CSC.

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Groups** | **Treatment** |
| 1. | Group A | Control diet as per the requirement of commercial broiler birds |
| 2. | Group B | Diet substituting 20% Soyameal with Inoculum -1 SSF CSC  |
| 3. | Group C | Diet substituting 20% Soyameal with Inoculum -II SSF CSC |
| 4. | Group D | Control Diet substituting 20% Soyameal with CSC  |
| 5. | Group E | Diet substituting 40% Soyameal with Inoculum -1 SSF CSC |
| 6. | Group F | Diet substituting 40% Soyameal with Inoculum -II SSF CSC |
| 7. | Group G | Control Diet substituting 40% Soyameal with CSC |

**Statistical Analysis**

All rough data was statistically analyzed by computing for their statistical Means, Standard Deviation and Standard Error in MS Excel and subjected to Completely Randomized Block Design using free online software of Indian Council of Agriculture Research, Goa, India (http://icargoa.res.in/wasp2.0/).

**RESULTS AND DISCUSSION**

Microbial SSF is promising method of value addition of agro by-products and it has recently evolved as potential tool, because of utilization of otherwise a non digestible matter in it, through secretion of exo-enzymes such as cellulolytic enzyme, amylase, protease and lipolytic enzyme, some variety of vitamins, and some unknown active substances (Brock et al., 1994; Prabhakar et al., 2005). The cultures when used in combinations in present research were found promising for SSF which was in agreement with similar earlier reports (Zhang et al., 2011).

In acute toxicity studies of SSF CSC extract mortality was not observed in any rat throughout the experimental period of 14 days and the rats remained healthy. Their feed intake and water intake remained normal. There were no signs of intoxication on the body weights during the observation period as no significant difference was noticed in their average bodyweights as compared to untreated control. All rats were sacrificed and on necropsy, gross pathological changes were not observed in any of the experimental rats. The microscopic histopathological examination of the vital body organs of rats (liver, Spleen & kidneys) did not reveal any observable alterations.

The observations of shelf life studies of SSF CSC (Table 3) indicated non-significant alteration in the lysine content of feed preserved up to 3 months duration in all samples, but when the samples were preserved for nine months duration detectable reduction (P<0.05) in Lysine percent was observed. The results of periodic estimation of change in FG indicated non-significant but gradual fall in control sample and increase in SSF treated samples. Observations of total gossypol content of feed revealed slight and gradual non-significant decrease in the control after keeping it for nine months while the values of inoculums 1 and inoculums 2 treated CSC remained significantly unaltered. Results of crude fibre content also remained almost unchanged (P>0.05) during entire period of nine months in all samples. On estimation of crude protein of control sample results indicated that the values remained constant throughout the period on the contrary slight but non-significant improvement in total protein content was observed in treated samples that showed improving trend with increase in keeping time. Total fat content of all samples remained almost unchanged while there was slight but non-significant depletion in the total moisture content during entire study period of nine months. SSF CSC revealed good keeping quality without any depletion of nutrients upto 9 months period except lysine. The slight but insignificant increase in total protein content may be because of growth of biomass on CSC.

Performance of broiler birds fed on SSF CSC at the rate of 20% and 40% substitution of soyameal are depicted in table 4. The average weekly weights amongst the age groups differed non-significantly (P>0.05) when compared with negative control and the results of CSC fermented by both the Inoculums I and II fed at 20% and 40 % level of substitution for Soyameal did not differ from the non fermented control group D significantly. The results of average weight gain in all age groups taken weekly did not reveal any significant differences from each other and figures were almost comparable. Hedarinia and Malakian, (2011) and Kanyinji and Sichanwa (2014) had presented similar results which were proved in our work that feeding SSF CSC did not have any significant impact over weight gain of broilers. The effect of substitution of SSF CSC on average feed consumption amongst treatment group did not differ significantly (P>0.05) as compared to negative control at both level of substitution however slight increased intake in treatment groups may be attributed to its high fibre content. Marginally increased feed consumption recorded in treatment groups could be attributed to the fact that substitution of soyameal with CSC might lower the actual energy content tending towards high feed intake for satisfying their energy requirements as discussed earlier by Nzekwe and Olomu (1984)and Ojewola, (1993).The average FCR calculated from the available data did not differ significantly amongst any age groups and appeared in the range of 1.46 to 1.62, but revealed insignificant but slight increasing trend with inclusion of higher level of CSC. Our findings were in close accordance with Kinyinji et al. (2014)revealing insignificant influence upon inclusion of SSF CSC upto 15 - 20% substitution levels or even when raised upto 30% level as observed by Watkins et al., (1993) and Baber et al., (1995). The mean mortality percent in all groups, control as well as treatment remained well below the recommended average between the range of 4 – 10 percent except in group G (12 %) fed on 40 % CSC without treatment. Reasons of mortality were variable and post mortem examination did not reveal any pathological changes and microscopic examination was not indicative of toxicity and/ or mycotic infections. The results showing impact on performance of broiler birds pertaining to average weekly live weights remained unaffected in accordance with findings of Bamgbose (1988); Fernandez et al. (1994; 1995); Henry et al. (2001).

**CONCLUSIONS**

CSC can be successfully included upto 20 - 40 % level in the diet of commercial broilers upon its SSF with organism Pleurotus sajor-caju plus Saccharomyces cerevisiae as well as Saccharomyces cerevisiae plus Candida tropicalis when used in combination. However, further trials on large scale are suggested for the wide acceptance in the field level.

**CONFLICT OF INTEREST DISCLOSURE**

There are no conflicts of interest pertaining to financial matters concerned with research and “ the authors declare no competing financial interest.”

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**TABLES AND FIGURES**

**Table 1:** Broiler Feed Formulation

|  |  |  |  |
| --- | --- | --- | --- |
| **Ingredients** | **Negative Control** | **20 % Substitution of** **SSF CSC** | **40 % Substitution of** **SSF CSC** |
| Pre-starter | Starter | Finisher | Pre-starter | Starter | Finisher | Pre-starter | Starter | Finisher |
| Maize | 53.47 | 54.62 | 59 | 39 | 41.14 | 27.75 | 24.9 | 47.4 | 36.22 |
| Oil | 1.95 | 3.33 | 4.3 | 7 | 7.98 | 12.61 | 11.82 | 8.3 | 12.17 |
| Soybean doc | 40.45 | 38 | 32.7 | 35.25 | 33.11 | 28.18 | 30 | 28.5 | 24.37 |
| Cotton seed meal | 0 | 0 | 0 | 14.56 | 13.68 | 27.36 | 29.12 | 11.77 | 23.2 |
| Trace Mineral Mixture  | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Dicalcium Phosphate | 1.8 | 1.85 | 1.87 | 1.82 | 1.85 | 1.86 | 1.84 | 1.88 | 1.9 |
| Limestone Powder | 1.4 | 1.35 | 2.76 | 1.4 | 1.35 | 1.31 | 1.32 | 2.72 | 2.64 |
| Salt | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Vitamin Premix | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Choline 60 % | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Anticoccidial | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| DL-Methionine | 0.21 | 0.17 | 0.14 | 0.21 | 0.17 | 0.17 | 0.21 | 0.15 | 0.15 |
| Lysine | 0.11 | 0.07 | 0 | 0.15 | 0.11 | 0.15 | 0.18 | 0.03 | 0.06 |
| Toxin binder | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **Total** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |

**Table 2:** The experimental design of feeding trial of SSF CSC.

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Groups** | **Treatment** |
| 1. | Group A | Control diet as per the requirement of commercial broiler birds |
| 2. | Group B | Diet substituting 20% Soyameal with Inoculum -1 SSF CSC  |
| 3. | Group C | Diet substituting 20% Soyameal with Inoculum -II SSF CSC |
| 4. | Group D | Control Diet substituting 20% Soyameal with CSC  |
| 5. | Group E | Diet substituting 40% Soyameal with Inoculum -1 SSF CSC |
| 6. | Group F | Diet substituting 40% Soyameal with Inoculum -II SSF CSC |
| 7. | Group G | Control Diet substituting 40% Soyameal with CSC |

**Table 3.** Result of Shelf life estimation of SSF CSC

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatments** | **Day 0** | **After 3 months** | **9 months** |
| **Lysin content** |
| Control CSC | 0.94 % a | 0.93 % a | 0.88% b |
| Inoculum I treated CSC | 1.09 % a | 1.1 % a | 0.68% b |
| Inoculum II treated CSC | 1.18 % a | 1.17 % a | 0.66% b |
| **FG content** |
| Control CSC | 0.22 % | 0.22 % | 0.16 % |
| Inoculum I treated CSC | 0.049 % | 0.056 % | 0.143 % |
| Inoculum II treated CSC | 0.048 % | 0.053 % | 0.194 % |
| **Total Gossypol** |
| Control CSC | 2.3 % | 2.33 % | 1.3 % |
| Inoculum I treated CSC | 0.89 % | 0.9 % | 0.87 % |
| Inoculum II treated CSC | 0.88 % | 0.92 % | 0.97 % |
| **Crude fibre** |
| Control CSC | 37.2 % | 37.2 % | 37.27 % |
| Inoculum I treated CSC | 26.4 % | 28.5 % | 28.65 % |
| Inoculum II treated CSC | 28.3 % | 27.3 % | 28.35 % |
| **Crude protein** |
| Control CSC | 20.6 % | 20.4 % | 20.09 % |
| Inoculum I treated CSC | 27.6 % | 28.63 % | 29.67 % |
| Inoculum II treated CSC | 27.31 % | 29.25 % | 29.8 % |
| **Ether Extract** |
| Control CSC | 5.4 % | 5.44 % | 5.44 % |
| Inoculum I treated CSC | 4.37 % | 4.4 % | 4.38 % |
| Inoculum II treated CSC | 4.43 % | 4.43 % | 4.44 % |
| **Moisture content** |
| Control CSC | 9.8 % | 8.10 % | 7.3 % |
| Inoculum I treated CSC | 11.7 % | 11.27 % | 10.7 % |
| Inoculum II treated CSC | 11.2 % | 9.3 % | 6.3 % |

**\*a b** Means with different superscripts are significantly different (*P* ˂ 0.05)

**Table 4.** Performance of birds fed on SSF CSC fed at 20% and 40% substitution levels of Soya-meal (Result analysis for within row variation)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Age in** **Week** | **Negative Control** | **Inoculum I****@ 20%** | **Inoculum II****@ 20%** | **Control** **20%** | **Inoculum I****@ 40%** | **Inoculum II****@ 40%** | **Control** **40%** |
| **Group A** | **Group B** | **Group C** | **Group D** | **Group E** | **Group F** | **Group G** |
| **Average Weight (g)** |
| **I**  | 135.67 | 134.12 | 133.88 | 122.5 | 129.51 | 133.58 | 124.29 |
| **II** | 347.09 | 335.53 | 336.48 | 318.7 | 340.09 | 319.96 | 291.50 |
| **III** | 628.02 | 657.23 | 651.19 | 640.23 | 631.96 | 622.44 | 573.06 |
| **IV** | 1111.69 | 1078.97 | 1118.97 | 1082.21 | 1095.6 | 993.90 | 1037.59 |
| **V** | 1560.40 | 1566.7 | 1632.64 | 1659.78 | 1472.0 | 1463.61 | 1507.8 |
|  | **Average Weight Gain (g)** |
| **I** | 90.78 | 89.72 | 88.24 | 77.96 | 84.15 | 88.11 | 78.80 |
| **II** | 211.42 | 201.41 | 202.60 | 196.20 | 210.58 | 186.38 | 167.21 |
| **III** | 280.93 | 321.70 | 314.71 | 321.53 | 291.87 | 302.48 | 281.56 |
| **IV** | 483.67 | 421.74 | 467.78 | 441.98 | 463.60 | 371.46 | 464.53 |
| **V** | 448.71 | 487.69 | 513.67 | 577.57 | 376.46 | 469.71 | 470.21 |
|  | **Average Feed Consumption / week (g)** |
| **I** | 124.85 | 125.69 | 137.17 | 131.62 | 123.42 | 137.17 | 141.86 |
| **II** | 362.57 | 385.03 | 363.28 | 344.65 | 375.37 | 371.15 | 311.07 |
| **III** | 490.35 | 516.28 | 569.77 | 497.67 | 525.72 | 501.89 | 473.65 |
| **IV** | 857.45 | 953.63 | 923.38 | 864.15 | 855.23 | 809.09 | 994.58 |
| **V** | 957.27 | 1086.52 | 1046.1 | 1106.97 | 974.79 | 1000.81 | 997.00 |
|  | **Total Average Feed Consumption (g) up to Week** |
| **I** | 124.85 | 125.69 | 137.17 | 131.62 | 123.42 | 137.17 | 141.86 |
| **II** | 487.42 | 510.72 | 500.45 | 476.27 | 498.79 | 508.32 | 452.93 |
| **III** | 977.77 | 1152.69 | 1070.22 | 973.94 | 1024.51 | 1010.21 | 926.58 |
| **IV** | 1835.17 | 2106.32 | 1993.6 | 1838.09 | 1879.74 | 1819.3 | 1921.16 |
| **V** | 2792.49 | 3067.15 | 3039.7 | 2945.06 | 2854.53 | 2820.11 | 2918.16 |
|  | **Mean Feed Conversion Ratio (FCR) (g/g)** |
| **I** | 0.92 | 0.94 | 1.02 | 1.07 | 0.95 | 1.03 | 1.14 |
| **II** | 1.40 | 1.52 | 1.49 | 1.49 | 1.47 | 1.59 | 1.55 |
| **III** | 1.56 | 1.56 | 1.64 | 1.52 | 1.62 | 1.62 | 1.62 |
| **IV** | 1.65 | 1.83 | 1.78 | 1.70 | 1.72 | 1.83 | 1.85 |
| **V** | 1.79 | 1.98 | 1.86 | 1.77 | 1.94 | 1.93 | 1.94 |
| **Average** | **1.46** | **1.57** | **1.56** | **1.51**  | **1.54** | **1.60** | **1.62** |
|  | **Mortality (%)** |
| **I** | 00 | 2.00 | 2.00 | 00 | 00 | 2.00 | 4.00 |
| **II** | 00 | 00 | 00 | 00 | 00 | 00 | 4.00 |
| **III** | 2.00 | 4.00 | 2.00 | 2.00 | 00 | 4.00 | 2.00 |
| **IV** | 2.00 | 4.00 | 00 | 2.00 | 4.00 | 00 | 2.00 |
| **V** | 00 | 00 | 00 | 2.00 | 00 | 00 | 00 |
| **Percent** | **4.00 %** | **10.00 %** | **4.00 %** | **6.00 %** | **4.00 %** | **6.00 %** | **12.00 %** |

\*All the values remained unaffected (*P* ˃ 0.05).