

The objective of this study was to analyze the environmental performance of aquaculture in Colorado of the West City, Rondônia State, Brazil. Fifteen fish farmers were interviewed. For data collection, structured interviews were carried out, using a questionnaire based on information supplied by the United Nations Food and Agriculture Organization (FAO). High temperatures also reduce the weight of organs in broilers, in order to reduce the metabolic rate of animals with consequent reduction of heat production. The protein level of broiler rations created under high temperatures may be reduced by supplementing with synthetic amino-acids without modifying their performance. Therefore, considering the different responses associated with the effect of high temperatures, it is evident that the broiler requirements vary not only due to the lineage but also in function of the thermal environment to which such animals are submitted in the different periods. The current industry demand for dairy products with extended shelf life has resulted in new challenges for milk quality maintenance. The processing of milk with high bacterial counts compromises the quality and performance.



Borges is a Doctor and Post-Doctor in Forest Engineering. **Marco** is a high school student at Paulo de Assis Ribeiro in Colorado of the West City, in Rondonia State Brazil. **Moraes** is a psychologist, and Master's student in the Professional Master in Intellectual Property and Technologies for Innovation in Rondonia State Brazil.

Aurélio Ferreira Borges
Marco Túlio Silva Borges
Raquel Nogueira de Moraes (Organizers)

Environmental performance of aquaculture, chickens and milk in Brazil

Study cases



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Marco Túlio Silva Borges
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I. Chapter 1

Total bacterial count and somatic cell count in refrigerated raw milk stored in communal tanks

Edmar da Costa Alves¹

Aurélio Ferreira Borges²

Alice Maria Dahmer³

Abstract

The current industry demand for dairy products with extended shelf life has resulted in new challenges for milk quality maintenance. The processing of milk with high bacterial counts compromises the quality and performance of industrial products. The study aimed to evaluate the total bacteria counts (TBC) and somatic cell count (SCC) in 768 samples of refrigerated raw milk, from 32 communal tanks. Samples were collected in the first quarter of 2010, 2011, 2012 and 2013 and analyzed by the Laboratory of Milk Quality – LQL. Results showed that 62.5%, 37.5%, 15.6% and 27.1% of the means for TBC in 2010, 2011, 2012 and 2013, respectively, were above the values established by legislation. However, we observed a significant reduction in the levels of total bacterial count (TBC) in the studied periods. For somatic cell count, 100% of the means indicated values below 600.000 cells/mL, complying with the actual

¹ I'm a Master's in Food Engineering.

² I'm a Doctor and Post-Doctor in Forest Engineering.

³ I'm a Doctor in Food Engineering.

Brazilian legislation. The values found for the somatic cell count suggests the adoption of effective measures for the sanitary control of the herd. However, the results must be considered with caution as it highlights the need for quality improvements of the raw material until it achieves reliable results effectively.

Key words: Normative instruction; Legislation; Nonconformity; Microbiological quality.

A atual demanda da indústria por produtos lácteos com *shelf life* prolongado tem resultado em novos desafios para a manutenção da qualidade microbiológica do leite, pois o processamento da matéria-prima com altas contagens microbianas compromete a qualidade e o rendimento industrial de seus derivados. O estudo objetivou avaliar a contagem bacteriana total (CBT) e a contagem de células somáticas (CSS) de 768 amostras de leite cru refrigerado provenientes de 32 tanques comunitários. As amostras foram coletadas no primeiro trimestre dos anos de 2010, 2011, 2012 e 2013 e analisadas pelo Laboratório de Qualidade do Leite. Os resultados apontaram que 62,5%, 37,5%, 15,6% e 28,1% das médias para a CBT em 2010, 2011, 2012 e 2013, respectivamente, estavam acima dos valores preconizados pela legislação. Entretanto observou-se uma redução significativa nos níveis de contagem bacteriana total (CBT) nos períodos analisados. Para a contagem de células somáticas, 100% das médias apontaram valores abaixo de 600 mil células/mL, estando essa contagem de acordo com as legislações vigentes no Brasil. Portanto, os valores encontrados para a contagem de células somáticas sugerem a adoção de medidas efetivas de controle sanitário do rebanho. Porém, os resultados obtidos para a contagem bacteriana total devem ser considerados com cautela, uma vez que eles indicam a necessidade de

melhoria da qualidade da matéria-prima, até que se atinjam resultados efetivamente confiáveis.

Palavras-chave: Instrução normativa; Legislação; Não conformidade; Qualidade microbiológica.

Introduction

Through the technical regulation of identity and quality of refrigerated raw milk, the Brazilian legislation provides quality microbiological standards that indicate the hygienic conditions of processed milk. In establishments under federal inspection, the entire volume of milk began to be analyzed by the Brazilian Network for Milk Quality (BNMQ) laboratories, credentialed by the Ministry of Agriculture (BRASIL, 2011).

An important resource for monitoring the microbiological quality of refrigerated raw milk is the somatic cell count (SCC), whose function is to combat bacteria which cause mastitis and possible economic losses arising there from (DONG et al., 2012). All cells in milk and those originating from both apoptotic desquamation of alveoli, as those removed during the flow of the bloodstream, are considered somatic cells (GAIATO et al., 2012).

Another measure that allows knowing the concentration of microorganisms in milk is the total bacterial count (TBC) that assists the evaluation of milking and storage procedures in the rural property and, at the same time, allows us to infer the likely adverse effects on industrial productivity and safety of the milk (GARGOURI et al., 2013; SILVA, 2011).

The objective of this study was to verify the characteristics related to the microbiological quality through somatic cell count and total bacterial count of

refrigerated raw milk stored in communal tanks for industrial processing.

Material and methods

Refrigerated raw milk was collected in 32 communal tanks, from rural properties at Colorado do Oeste City, Rondonia State Brazil. The collection procedure consisted of milk homogenization, through activation of the tank agitator for 5 minutes, followed by the collection and transference of the samples to appropriate containers, which were sent to the Milk Quality Laboratory, at the Federal University in Goiás State (CPA, 2010).

Data collection refers to samples analyzed during the months of January, February and March, in 2010, 2011, 2012 and 2013, period classified as rainy (October to April) (RONDONIA, 2007). Two samples of milk were collected every month, once for CBT and one for CCS. Therefore, in the quarter, six samples per tank were analyzed, allowing the calculation of the geometric mean, according to the methodology suggested by the Normative Instruction nº 51 (NI-51) and the Normative Instruction nº 62 (NI-62) (BRASIL, 2002; 2011).

Table 1 shows the amount of communal tanks of the milk suppliers and the geometric mean size used to infer the results.

Results of the microbiological quality presented in this study were extracted from reports issued by LQL for a dairy industry inserted in the Federal Inspection System (FIS), at Colorado do Oeste City, in Rondonia State Brazil.

According to information provided by the laboratory LQL (CPA, 2010), TBC analyses were performed using the equipment BactoScan FC[□], with a capacity of 150 samples/hour. For SCC analysis, the Fossomatic 5000 Basic equipment was used, with capacity of 300 samples/hour. Both

devices are based on the analytical principle of flow cytometry.

We start from the following premise in assessing the microbiological quality of refrigerated raw milk:

- The collected sample is suitable for industrial processing if the results of the geometric mean for SCC and TBC are up to 1.000.000 cells/mL for samples of 2010; 750.000 cells/mL for samples of 2011 and 2012, and 600.000 cells/mL for samples of 2013.
- If the samples are above the specified value, it will be considered unsuitable for processing and/or for marketing purposes (BRASIL, 2002; 2011).

The research project was approved by the Ethics Committee in Research of the Federal Institute of Education, Science and Technology in Rondonia State Brazil.

Results and discussion

The results indicated that, in the studied period, milk TBC showed geometric mean in disagreement with the legislation (Table 2).

The microbiological content for TBC of refrigerated raw milk was above the maximum permissible limit

Table 1. Sample data and geometric mean of milk suppliers in Colorado do Oeste in the first quarter of 2010 - 2013.

Amount of tanks	Geometric mean of the quarter	Total of analyzed samples
32	128	768

Table 2. Percentage results of geometric means (n=128) of refrigerated raw milk for TBC in the first quarter of 2010 - 2013 (Colorado do Oeste City).

(Jan. Feb.)	First quarter		TBC (cells/mL)			
	≤ 600 k		> 750 k	> 1000 k	> 10.000 k	
2010	25%	(8)	12.5%	(4)	50% (16)	12.5% (4)
2011	62.5%	(20)	6.25%	(2)	31.2% (10)	-
2012	84.3%	(27)	3.1%	(1)	12.5% (4)	-
2013	69.6%	(23)	18.1%	(6)	9.0% (3)	-

(Table 2), that is 1.0×10^6 cells/mL for 2010; 7.5×10^5 cells/mL for 2011 and 2012 and 6.0×10^6 cells/mL for 2013 (BRASIL, 2002; 2011).

Considering the values of geometric means for total bacterial counts, 20 (62.5%) samples had counts exceeding 1.000.000 cells/mL in 2010; 12 (37.5%) and 5 (15.6%) samples were higher than 750.000 cells/mL in 2011 and 2012, respectively; and 9 (27.1%) samples were above 600.000 cells/mL in 2013 (Table 2), considering the maximum limits established by legislation (BRASIL, 2002; 2011).

In several studies on the incidence of TBC in milk, it was observed that high microbial counts are sourced from disability in washing and sanitizing of the equipments, milking utensils and inadequate cooling system (TRONCO, 2008). In Lacerda et al. (2010), high bacterial counts were due to failure in handling, especially with regard to hygiene and health care in milking. Another explanation for high values of TBC is the season, which influences the feeding

of the herd and milk production, interfering with the bacterial content (HOOGERHEIDE and MATTIODA, 2012; PAIVA et al., 2012). This aspect can be seen in a study about the quality of refrigerated raw milk in Ouro Preto do Oeste City, Rondonia State Brazil, showing that the mean TBC in March, a period considered rainy (October-March), of 1.891.000 cells/mL, was higher than the average of July, a period considered dry, of 760.000 cells/mL (ALMEIDA, 2010).

According to the results, there were significant reductions in the levels of TBC of refrigerated raw milk; however, values in disagreement with the legislation were found (Figure 1). The values in disagreement to TBC found in 2010 (62.5%, Figure 1) is a serious problem for the industry regarding to quality, especially in the results above 10.000 cells/mL. Buying milk with high bacterial counts, the company may be putting at risk the safety of dairy products produced with this type of raw material. TBC values above the reference parameters of the legislation are among the major deficiencies in microbiological quality of milk as stated by Ponsano et al. (2011), based on studies on training farmers to improve the quality of raw milk produced in Araçatuba City, Sao Paulo State Brazil. Then, it becomes evident the need to adopt control measures to improve the microbiological quality of milk before submitting it to industrial processing.

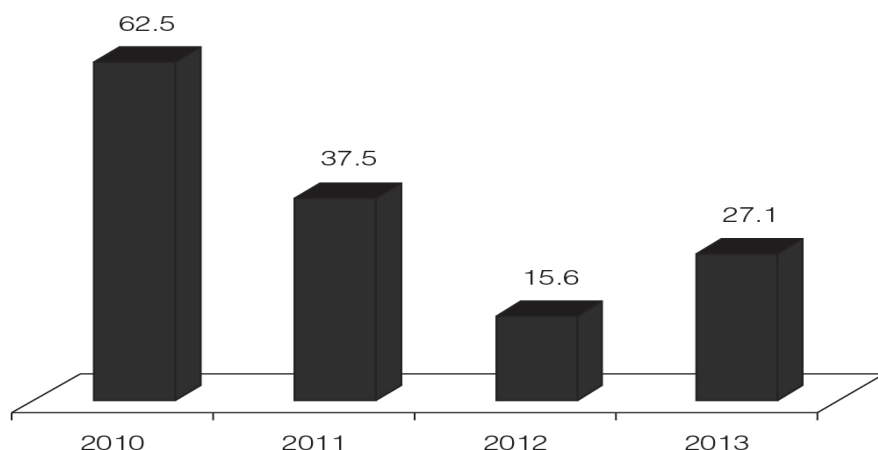


Figure 1. Percentage of quarterly geometric means (from 2010 to 2013; n=128) in disagreement with the legislation, for total bacterial count of refrigerated raw milk in Colorado do Oeste City.

It is observed that over the study period (Figure 1) there were reductions of TBC in compliance with the legislation in 2011 and 2012, with a slight increase in 2013. This result is alarming, since in the second half of 2014 the limit for the TBC must present 300.000 cells/mL. Increased TBC in the first quarter of 2013 can be explained by the gradual reduction of the maximum allowable values for the microbiological requirements of milk (BRASIL, 2011).

Although the percentage of TBC samples in disagreement with the legislation is high, it is noted (Figure 1) that, over the analyzed period, there were reductions of TBC, in compliance with the Brazilian legislation (BRASIL, 2002; 2011) with a slight increase in 2013. Studies by Paiva et al.

(2012) showed an annual quality evolution of refrigerated raw milk with a reduction rate of TBC due to technical guidance to farmers. However, Ponsano et al. (2011) warns that it is necessary a greater time working together with farmers so that they may assimilate information and put them into practice, until improvements in milk quality are met.

Therefore, in this research, one of the factors that may have contributed to improvements in the levels of TBC are the intensification of technical orientation performed by the milk collection companies in line with the requirements of Brazilian legislation, which gradually is being implemented throughout the national territory (BRASIL, 2002; 2011).

For somatic cell count, no statistical differences between the studied periods (Figure 2) occurred. In Figure 2, the SCC had amplitude ranging from 53.000 to 483.000 cells/mL, results compliant to the legislation (BRASIL, 2002; 2011).

Regarding the description of SCC variable, on average, samples showed 346.000 cells/mL, with a standard deviation of 93.33. The SCC variable had a coefficient of variation of 27%, showing an average variability (FONSECA and MARTINS, 1996).

Similarly, to the results of this survey, Silva (2011) found mean values below 600.000 cells/mL in the first six months of 2007, 2008 and 2009 in refrigerated raw milk stored in communal tanks of northeastern Brazil, concluding that the elevation of SCC in milk (up to 200.000 cells/mL), which indicates the occurrence of mastitis, affects the composition of the milk and the shelf- life of products. Hortet et al. (1999) found losses of 0.30 kg of milk, which occurred for 100.000 cells/mL, and 0.61 kg, for 200.000 cells/mL. In addition, with the increase in SCC, milk composition, enzymatic activity, clotting time, productivity and quality of dairy products, are negatively

influenced (KITCHEN, 1981).

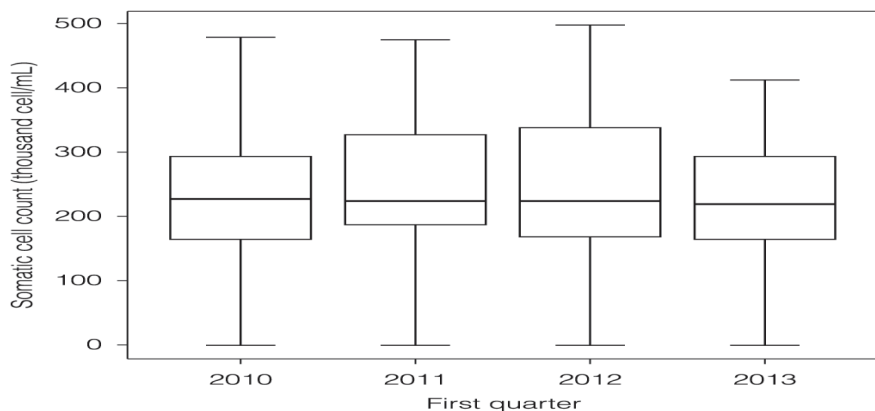


Figure 2. Box plot of the geometric mean for somatic cell count of refrigerated raw milk in the first quarter of 2010 – 2013 (Colorado do Oeste).

Considering the results in accordance to the SCC, it was expected a better result for TBC, since the practices that prevent mastitis are largely the same that prevent bacterial contamination of the milk. However, it must be considered that crossbreed and low production animals are less susceptible to mastitis, which most often affects purebreds and high production animals (BELOTI et al., 2011).

Further, according to the results shown in Figure 2, it is observed that the values for somatic cell showed a similar behavior. These results also indicate that milk somatic cells varied slightly depending on the period studied. Corroborating this behavior Mesquita et al. (2008), researching the quality of milk in the central west and northern Brazil in January 2007 to July 2008 found that the geometric mean for SCC (300.000 CFU/mL) had no significant change

over the period. With the results obtained in this research (Figure 2), milk suppliers would not have difficulties to meet the parameters set by legislation (BRASIL, 2002; 2011).

Conclusions

The high percentages for total bacterial count in milk indicate failures in milking hygiene procedures and/or storage that affect the quality of the raw material. However, there were reductions of TBC over the studied period probably due to the training of farmers in accordance with the requirements of the legislation.

Although all parameters of somatic cell counts were within the microbiological standards established by legislation, this result must be considered with caution as it highlights the need for improvements in the quality of the raw material until it achieves reliable results effectively.

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II. Chapter 2

Baselines of the lysine amino-acid in rations for broilers

Aurélio Ferreira Borges

Marco Túlio Silva Borges⁴

Raquel Nogueira de Moraes⁵

Abstract

Synthetic amino acid supplementation has provided facilities in the adjustment of feed formula, making it possible to obtain the required levels of essential amino acids. Lysine is pronounced the second greatest restrictive amino acid in broiler nutrition. The synthetic amino acid lysine is used as the orientation amino acid in poultry for the reason that it is mainly consumed for protein synthesis. In order to characterize the requirements of lysine, 240 male broilers of the lineage Avian Farms were kept in an environment with average temperature of 25.6°C. The 22 to 42 days old broilers presented initial average weight of 541 ± 3.6 g. The basal ration contained 19.57% of crude protein (CP), 3,100 kcal of metabolizable energy (ME)/kg and 0.88% of total lysine, which was supplemented with 0.000, 0.076, 0.153, 0.230 and 0.306% de L-lysine hydrochloric acid (HCL), resulting in rations with 0.88, 0.94, 1.00, 1.06 and 1.12% of total lysine. The experimental design was completely randomized with five treatments of total lysine, six repetitions and eight broilers per

⁴ I'm a high school student at Paulo de Assis Ribeiro in Colorado of the West City, in Rondonia State Brazil.

⁵ I'm a psychologist. I'm a Master's student in the Professional Master in Intellectual Property and Technologies for Innovation in Rondonia State Brazil.

repetition. During experiments, the temperature was kept at $25.6 \pm 0.24^{\circ}\text{C}$, relative moisture at $68.4 \pm 6.30\%$, black globe temperature at $25.7 \pm 0.25^{\circ}\text{C}$ and the wet-bulb globe temperature (WBGT) at $74 \pm 0.6^{\circ}\text{C}$. Treatments influenced the absolute weight of carcass, breast with bone, leg, thigh, abdominal fat and relative weight of breast with bone. Lysine levels had a quadratic effect over the food conversion (FC), which increased up to the level 1.03% of total lysine. Lysine levels had a quadratic influence over the weight gain (WG), which increased up to the level 1.05% of total lysine.

Key words: Chickens for meat production, growth phase, lysine, thermal environment.

Introduction

Birds, as well as mammals, are homeothermic animals, which indicated that means that even there are fluctuations at the environmental temperature, they can keep the body temperature constant (Borges et al., 2002). Any environmental change out of the thermal comfort of these animals requires behavioral, physical or physiological adjustments as attempts to adapt to the new condition. Oba et al. (2007) reported that the range of thermal neutrality for broilers is between 24 and 28°C . Considering these adjustments, the ration intake stands out, which are reduced as temperature increases, thus causing the decrease of growth rate and worsening of food conversion (Baziz et al., 1996). However, the effect of temperature over metabolism is more complex than how it is frequently reported. According to Mendes et al. (1997), the combination of high temperature and environment with high levels of protein reduces the growth rate and production of breast meat from broilers of the fast

growth commercial lineage.

High temperatures also reduce the weight of organs in broilers, in order to reduce the metabolic rate of animals with consequent reduction of heat production. Studies conducted by Zaboli et al. (2016) showed that the protein level of broiler rations created under high temperatures may be reduced by supplementing with synthetic amino-acids without modifying their performance. Therefore, considering the different responses associated with the effect of high temperatures, it is evident that the broiler requirements vary not only due to the lineage but also in function of the thermal environment to which such animals are submitted in the different periods.

In some tropical areas of Brazil State, such as the North Region, a temperature range of 35 to 45°C from August to May is very common, and the reduction of growth performance in poultry is the most important issue. The year-on-year amplification in growth rate of modern poultry due to constant genetic improvement, global heating, and the expanding of the poultry industry in hot climates requires correct ways to lighten the consequences of heat stress. Therefore, diverse methods of growth performance in poultry have been corroborated in the world, such as climate-controlled housing, low providing density, nutritional management, lowering marketing weight, among others. Though, in greatest situations many of these practices are overpriced with low efficiency (Zaboli et al. 2016).

Protein remains the most expensive dietary nutrient, though the use of crystalline amino acids offers multiple advantages in that they provide reductions in both dietary crude protein concentrations and the excretion of dietary nitrogen into the environment (Franco et al., 2017). Belloir et al., (2017) showed that the protein level of broiler rations created under high temperatures may be reduced by supplementing with synthetic amino-acids without

modifying their performance.

Lysine is considered the second most limiting amino acid in broiler diets. It is used as the reference amino acid in poultry and swine nutrition because it is mainly utilized for protein synthesis and accounts for 7.5% of carcass protein (Viola et al., 2009). The present work was conducted in order to characterize the lysine requirements for male broilers from 22 to 42 days old kept under average temperature of 25.6°C.

Materials and methods

Animals

The experiment was conducted in the climatic chambers of the Laboratory of Animal Bioclimatology of the Zootechny Department, Federal University of Viçosa (UFV), Brazil. Overall, 240 male broilers of the lineage Avian farms with initial average weight of 541 ± 3.6 g, vaccinated against the diseases Marek and Avian pox. Broilers remained in the experiment from the 22nd to 42th day at high temperature (25.6°C). The experimental design was completely randomized with five treatments (levels of lysine), six repetitions and eight broilers per repetition.

Experimental diets (Table 1), isoproteic and isoenergetic, based on maize, soybean meal and corn gluten were formulated to attend the nutritional requirements of broilers regarding protein, energy, calcium, phosphorous and amino acids, except for lysine. Rations were supplemented with 0.000, 0.076, 0.153, 0.230 and 0.306% of L-lysine HCl 78.4%, resulting in rations with 0.88, 0.94, 1.00, 1.06 and 1.12% of total lysine.

Experimental design

During the initial period (1 to 21 days old), broilers were created in a conventional shed under traditional management and feeding. When 22 days were completed, broilers were weighed and transferred to climatic chambers and the experimental period started, where they remained until 42 days old. Broilers were placed in metal batteries composed by 12 compartments with area of 0.72 m² per compartment, all of them trough-type, with each compartment representing an experimental unit.

The temperature and moisture monitoring of each room was made by thermometers of maximum and minimum, dry bulb, wet bulb and black globe placed at an intermediate height in relation to the battery central compartment. Temperatures were daily recorded at two moments (8 and 18 h) during all the experiment.

The thermal environment was expressed in terms of the Wet- bulb Globe Temperature (WBGT) proposed by Buffington et al. (1981) and calculated by the following equation: $WBGT = Bgt + 0.36 Dpt - 330.08$ in which Bgt is the black globe temperature in °K and Dpt the dew point temperature in °K. Rations and water were provided at will and water was changed twice a day to avoid heating.

A continuous light program was adopted along all the experiment 24 h of artificial light by means of two fluorescent lamps of 25 Watts per room. The studied variables were: ration intake, weight gain, food conversion, total lysine intake, carcass yield, protein deposition and absolute and relative weights of prime cuts (breast, thigh and drumstick).

The calculation of ration intake during the experimental period was obtained by the difference between the amount of ration provided and lost

and wastes of rations, which were weighed in the beginning and end of experiment. The weight gain of broilers was obtained by the difference between the weight in the end and beginning of experiment. The food conversion was calculated for the period from 22 to 42 days old based on data of ration intake and weight gain.

Analyses of crude protein and body composition

In the end of experiment, broilers were weighed after 12 h of fasting and posteriorly four of each repetition were chosen to be slaughtered considering the average weight of the experimental unit ($\pm 5\%$). After broilers were bled and plucked, eviscerated carcasses were weighed. Posteriorly the abdominal fat was removed and weighed. The two entire carcasses (including feet and head) of each repetition were ground during 15 min, one by one, in commercial cutter of 30 Horse Power (HP) and 1,775 rpm, and after homogenization one sample was collected. Due to the high fat content of carcasses, samples were pre-dried in forced ventilation stove at $\pm 60^{\circ}\text{C}$ for 72 h and pre-degreased by hot method in extractor *Soxhlet* for 4 h. After this step, samples were ground and placed in glasses for posterior evaluations. Analyses of crude protein were made in the Laboratory of Animal Nutrition of the Zootechny Department of Federal University of Viçosa.

Table 1. Calculated composition of the experimental diets (%).

Ingredients	Total lysine level (%)				
	0.88	0.94	1.00	1.06	1.12
Corn (7.98% PB)	65.500	65.500	65.500	65.500	65.500
Soybean meal (45.61% PB)	21.870	21.870	21.870	21.870	21.870
Corn gluten meal (60.38% PB)	7.122	7.122	7.122	7.122	7.122

Dicalcium phosphate	1.524	1.524	1.524	1.524	1.524
Limestone	1.228	1.228	1.228	1.228	1.228
Soybean oil	1.200	1.200	1.200	1.200	1.200
Salt	0.415	0.415	0.415	0.415	0.415
Mineral mix ¹	0.050	0.050	0.050	0.050	0.050
Vitamin mix ²	0.100	0.100	0.100	0.100	0.100
Butylated Toluene Hydroxide	0.010	0.010	0.010	0.010	0.010
Cocxistac [*]	0.050	0.050	0.050	0.050	0.050
Choline chloride	0.125	0.125	0.125	0.125	0.125
Virginiamicin	0.055	0.055	0.055	0.055	0.055
Caulin	0.596	0.520	0.443	0.366	0.290
L-Lysine HCL (78.4%)	0.000	0.076	0.153	0.230	0.306
DL-Methionine (99%)	0.153	0.153	0.153	0.153	0.153
Tryptophan (99%)	0.005	0.005	0.005	0.005	0.005
Calculated composition					
Crude protein (%)	19.570	19.570	19.570	19.570	19.570
Metabolizable energy (kcal/kg)	3.100	3.100	3.100	3.100	3.100
Calcium (%)	0.918	0.918	0.918	0.918	0.918
Available phosphorus (%)	0.389	0.389	0.389	0.389	0.389
Sodium (%)	0.200	0.200	0.200	0.200	0.200
Total lysine	0.880	0.940	1.000	1.060	1.120
Digestible lysine (%) ³	0.780	0.840	0.900	0.960	1.020
Digestible tryptophan (%) ³	0.150	0.150	0.150	0.150	0.150
Digestible valine (%) ³	0.814	0.814	0.814	0.814	0.814
Digestible threonine (%) ³	0.632	0.632	0.632	0.632	0.632
Digestible meth + cys	0.515	0.515	0.515	0.515	0.515

¹Content/kg – Mn, 60 g; Fe, 80 g; Zn, 50 g; Cu, 10 g; Co, 2 g; I, 1 g vehicle q.s.p. 500 g. 2 Content/kg - vit. A - 15,000,000 UI, vit. D3 - 1,500,000 UI, vit. E - 15,000 UI, vit. B1 - 2.0 g, vit. B2 - 4.0 g, vit. B6 - 3.0 g, vit. B12 - 0.015 g, nicotinic acid - 25 g, pantothenic acid - 10 g, vit. K3 - 3.0 g, pholic acid - 1.0 g, zinc bacitracin - 10 g, selenium - 250 mg and vehicle q.s.p. - 1,000. g. 3 Digestible amino acids calculated based on the coefficients of digestibility from Rhodimet-Rhône-Poulenc (1993) tables). ^{*}Active principle – salinomicine.

An additional group of 21 days old broilers was slaughtered to determine the body composition in the beginning of experiment. The protein deposition in the carcass was calculated by the difference between the values of carcass composition between 22 and 42 days old.

The two remaining carcasses of each repetition (total of 12 carcasses per treatment) were used to obtain the prime cuts in which the absolute weight (grams) and yield (%) of entire carcasses (with feet and head), legs, thigh, drumstick, breast, feathers and abdominal fat. The carcass yield was obtained by the ratio between the weight of clean and eviscerated carcass (with feet and head) and the live weight after fasting, while the yield of prime cuts was determined considering the weight of the eviscerated carcass without feathers.

Statistical analysis

Statistical analyses were carried out through the SAEG software (1997). Estimates of total lysine requirements were established by means of linear regression and/or quadratic models and by Linear Response Plateau (LRP) according to the better adjustment.

Results and discussion

Table 2 presents the average values of environmental conditions within the climatic chambers obtained during the experimental period. The Avian Farms manual recommends the temperature around 22.5°C and moisture around 70% for the category between 22 and 42 days. The temperature limit of thermal-neutrality is about 25°C, and then it is possible to infer that the experimental conditions of the present work represent a moderately hot environment. A comfortable environment for such category of animals those with Black Globe Humidity Index (BGHI) around 72 and as stressing those with BGHI of 84 (Borges et al., 2002).

Table 2. Average environmental conditions observed during the experimental period in the climatic chambers with broilers from 22 to 42 days old.

Variable	Values
Average air temperature (°C)	25.6 ± 0.24
Average relative humidity (%)	68.4 ± 6.30
Average black globe temperature (°C)	25.7 ± 0.25
Black globe humidity index (BGHI)	73.7 ± 0.60

Table 3. Performance, total lysine intake and protein deposition rate of male broilers from 22 to 42 days of age fed with rations containing different levels of lysine, under high temperature environment.

Parameter	Lysine levels (%)					CV (%)
	0.88	0.94	1.00	1.06	1.12	
Weight gain (g) ¹	1,219	1,265	1,293	1,315	1,283	4.53
Feed intake (g)	2,371	2,350	2,369	2,383	2,390	4.62
Feed:gain ratio ²	1.94	1.86	1.83	1.81	1.86	2.76
Total lysine intake (g) ³	21	22	2	25	27	4.81
			4			
Protein deposition rate (g)	164	166	177	180	173	3.46

^{1,2,4}Quadratic effect (P<0.09), (P<0.01) and (P<0.03), respectively. ³Linear effect (P<0.01).
CV: Coefficient of variation.

days. The temperature limit of thermal-neutrality is about 25°C, and then it is possible to infer that the experimental conditions of the present work represent a moderately hot environment. A comfortable environment for such category of animals those with Black Globe Humidity Index (BGHI) around 72 and as stressing those with BGHI of 84 (Borges et al., 2002).

Table 3 presents results of performance (weight gain, ration intake and food conversion), total lysine intake and rates of fat and protein deposition in broiler carcasses from 22 to 42 days old receiving rations with different levels

of lysine and kept at high temperature (25.6°C). A quadratic effect ($P<0.09$) was observed in the lysine levels of the ration over the weight gain (WG) of broilers up to the level 1.05%, which was associated to an intake of 25 g of total lysine. Such result was higher than 0.92 and 0.98% of total lysine obtained by Barboza (1998) for Hubbard and Ross male broilers from 22 to 40 days old, as well as to 0.85 and 1.00% of total lysine obtained by Barboza (1998) also for the weight gain of male broilers from 21 to 42 and 22 to 40 days old, respectively. On the other hand, Conhalato (1998) obtained a better result of weight gain for broilers from 22 to 42 days old created in hot periods of the year (average temperature superior to 26°C) with levels of total lysine (1.20%) higher than those found in the present work. Contrarily, Mendes et al. (1997) did not observe any influence of the lysine level over the weight gain in 22 to 42 days old broilers submitted to high environmental temperature (25.5 to 33°C).

The differences of results among the above-mentioned studies may be associated to the genetic factors, as well as to the differences of environmental temperature where they were conducted. According to Cahaner et al. (1995), the nutritional requirements of broilers are influenced by the environmental temperature and genetics. No effects of lysine levels over the ration intake (RI) were observed for the broilers. Mendes et al. (1997) worked with 21 to 42 days old broilers kept under high temperature (25.5 to 33.3°C) and also did not verify such effect. Considering that in general the animals, when exposed to high temperature, reduce the ration intake to avoid an increase of heat production (Baziz et al., 1996), it is possible to deduce that under this condition the capacity to adjust the intake is committed due to the nutrient concentration of the ration.

The lysine levels of ration had a quadratic effect ($P<0.01$) over the food

conversion (FC), which improved up to the level 1.03% of total lysine (Table 4), what corresponded to 0.93% of digestible lysine and an estimate total lysine consumption of 24.5 g. A quadratic effect of the lysine level over the food conversion in 22 to 42 days old broilers was also recorded by Barboza (1998) and Conhalato (1998).

Levels of lysine had a quadratic effect ($P < 0.01$) over the weight gain (WG), which improved up to 1.05% of total lysine (Table 5).

Results obtained by Trindade Neto et al. (2011) in a similar study with broilers from the same lineage and age verified that the weight variation occurred according to the equation $\hat{Y} = -1622.36 + 6602.5422X - 3019.1834X^2$, $r^2 = 86.76$ and 1.09% of lysine was indicated as optimal. As observed for final weight, weight gain ($\hat{Y} = -2489.08 + 6596.5735X - 3028.5724X^2$, $r^2 = 83.87$) and feed conversion ($\hat{Y} = 8.55 - 12.4257X + 5.6735X^2$, $r^2 = 89.56$) also had quadratic responses ($P < 0.05$) and estimated the same lysine level (1.09%) as the best model. Considering that the protein deposition is more efficient than fat deposition since it aggregates more water, the increase of PDR in the carcass up to 1.05% of total lysine justifies the improvement observed for the WG and FC of broilers.

Table 4. Dietary lysine level and feed: gain ratio (g/g) of broilers from 22 to 42 days old under high environmental temperature (25.6°C).

Food conversion	Total lysine of ration (%)	Ideal total lysine (%)	Quadratic equation
1.96	0.00	1.03	$\hat{Y} = 7.63131 - 11.2593X + 5.45108X^2$ $r^2 = 0.98$
1.93	0.88		
1.90	0.94		
1.87	1.00		
1.84	1.06		
1.81	1.12		

r^2 = coefficient of determination.

Table 5. Weight gain (g) and lysine levels of rations for 22 to 42 days old broilers kept under high temperature (25.6°C).

Weight gain	Total lysine of ration (%)	Ideal total lysine (%)	Quadratic equation
1215	0.00	1.05	$\hat{Y} = 21.7717 - 66.5417X + 3179.67X^2$ $r^2 = 0.96$
1235	0.88		
1255	0.94		
1275	1.00		
1295	1.06		
1315	1.12		

Table 6. Lysine levels of ration and ideal ration intake for the protein deposition of 22 to 42 days old broilers at high temperature (25.6°C).

Total lysine of ration	Absolute value of ration for protein deposition (g)	Linear equation
0.00	25	$\hat{Y} = -1.23113 + 24.9651\text{lys}$ $r^2 = 0.99$
0.88		
0.94		
1.00		
1.06		
1.12		

The total lysine intake increased linearly ($P < 0.01$) due to the improvement of the lysine levels in the ration according to the equation $\hat{Y} = -1.23113 + 24.9651\text{lys}$ ($r^2 = 0.99$). This result is justified by the fact that the ration intake did not vary among treatments. Although there was a linear increase of total lysine intake, the intake of 25 g provided the highest absolute values of gain and deposition of protein in the carcass (Table 6).

Regarding the carcass composition, a quadratic effect ($P < 0.03$) of the total lysine level was observed over the protein deposition rate (PDR), which increased up to the level 1.05% (Table 7). This result corroborates what was found by Summers et al. (1992) and Deschepper and Groote (1995), who also verified alterations in the chemical composition of 42 days old broiler carcasses when low protein rations supplemented with essential amino acids in thermo-neural environments.

According to Trindade Neto et al. (2010), determining the peak of protein deposition allows estimating the maximum efficiency of amino acids used for the synthesis and accumulation of muscle mass in broiler carcasses. The effect of increase on protein deposition is characterized by the increase of water on body composition. Similarly, the reduction of body fat suggests an increase of protein synthesis efficiency and favors the accumulation of muscle mass. The deposition of protein in the carcass may be related not only to the lysine content of the diet, but also to the genetic strain and to the age of the birds utilized, as well as to thermal- environmental factors, immunologic challenge, among others de Oliveira et al. (2013).

Table 7. Protein deposition rate (g) and levels of lysine in rations for 22 to 42 broilers kept at high temperature (25.6°C).

PDR *	Total lysine of ration (%)	Ideal total lysine for PDR (%)	Quadratic equation
180	0.00	1.05	$\hat{Y} = 428.074 - 1154.76X + 550.523X^2$ $r^2 = 0.81$
176	0.88		
172	0.94		
168	1.00		
164	1.06		
160	1.12		

*Protein deposition rate.

Table 8. Absolute and relative weights of carcass, prime cuts and abdominal fat of 42 days old broilers under high environmental temperature.

Parameter	Lysine levels					CV (%)
Absolute weight (g)	0.88	0.94	1.00	1.06	1.12	-
Weight after fasting	1,720	1,765	1,794	1,857	1,789	5.24
Carcass	1,371	1,412	1,439	1,474	1,407	5.07
Breast with bone	349	390	403	398	405	6.70
Legs	369	368	389	397	382	4.87
Drumstick	183	184	188	194	187	4.93
Thigh	186	183	202	202	193	6.38
Abdominal fat	19	16	14	16	18	16.7

The lysine levels of ratio influenced ($P<0.03$) the carcass absolute weight and yield, which increased up to the levels 1.01 and 0.96% of lysine, respectively, according to the quadratic equations $\hat{Y} = -3340.89 + 9392.72X + 4627.2X^2$, $r^2 = 0.83$ and $\hat{Y} = 27.5967 + 109.332 - 56.907X^2$, $r^2 = 0.96$ (Table 8). Contrarily, Moran Jr. and Bilgili (1990), Kidd et al. (1997) and Conhalato (1998) did not verify any influence of lysine levels over the carcass yield of 21 to 42 days old broilers created during hot months. Barboza (1998) also did not observe effect of lysine levels over the carcass yield of 22 to 40 days old broilers in thermal-neutral environment.

There was a quadratic effect of lysine levels over the absolute weight of breast with bone ($P<0.01$), which increased up to the level 1.06% (Table 8), and a linear effect ($P<0.01$) over the relative weight of breast with bone, which increased according to the equation $\hat{Y} = 16.81124 + 10.6134\text{lys}$ ($r^2=0.67$).

Barboza (1998) observed an increase of 2.8% in the breast yield as the lysine levels increased from 0.95 to 1.15% and 0.8 to 0.98%, respectively. Results obtained in the present work are coherent since according to Baker (1991) the lysine is greatly important for the composition of muscle protein. Furthermore, Moran Jr and Bilgili (1990) state that the lysine supply in adequate levels is fundamental for the production of meat and breast. Moreover, according to Kidd et al. (1997), the requirement of essential amino acids for the maximum yield of breast is higher than what is considered adequate for the maximum growth.

The lysine levels had a quadratic effect ($P<0.07$) over the absolute weight of legs (Table 8), which increased up to the level 1.06% of total lysine according to the equation $\hat{Y} = -497.899 + 1673.27\text{lys} - 788.216\text{lys}^2$ ($r^2=0.80$). Similarly, Conhalato and Barboza (1998) did not observe effect of lysine levels over the yield of legs from 22 to 42 and 22 to 40 days old broilers,

respectively. On the other hand, Mendes et al. (1997) verified an influence of increasing levels of lysine over the yield of legs from 21 to 42 days old broilers created at cyclic high temperature (25.5 to 33.3%).

Levels of lysine influenced ($P<0.07$) the absolute weight of thigh, which had a quadratic increase up to the level 1.02% according to the equation $\hat{Y} = -713.3 - 1773.73\text{lys} - 866.164\text{lys}^2$ ($r^2 = 0.63$) (Table 8). Moreover, levels of lysine influenced ($P<0.01$) the absolute weight of abdominal fat (Table 8), which presented a quadratic reduction up to the level 1.00% according to the equations $\hat{Y} = -315.5 - 602.109\text{lys} + 301.385\text{lys}^2$ ($r^2=0.98$) and $\hat{Y} = 25.6898 - 49.1016\text{lys} + 24.4444\text{lys}^2$ ($r^2=0.99$). Mendes et al. (1997) also verified effects of the lysine levels over the abdominal fat content of 21 to 42 days old broilers created at high temperature, however, Barboza (1998) did not observe effect of levels from 0.80 to 1.10% over the abdominal fat in 22 to 40 days old broilers when kept in neutral environment.

Conclusion

Lysine is pronounced the second greatest restrictive amino acid in broiler nutrition. The synthetic amino acid lysine it is used as the orientation amino acid in poultry for the reason that it is mainly consumed for protein synthesis. The synthetic amino acid lysine it is used as the orientation amino acid in poultry for the reason that it is mainly consumed for protein synthesis.

Lysine levels had a quadratic effect over the food conversion (FC), which increased up to the level 1.03% of total lysine. Lysine levels had a quadratic influence over the weight gain (WG), which increased up to the level 1.05% of total lysine. The total lysine intake increased linearly due to the improvement of lysine content in the ration. An effect of total lysine was

observed over the protein deposition rate (PDR), which presented a quadratic increase up to the level 1.05%.

Lysine levels of the ratio influenced the absolute weight and carcass yield, which presented a quadratic increase up to the level 1.01 and 0.96%, respectively. There was a quadratic effect of lysine levels over the absolute weight of breast with bone, which increase up to the level 1.06%, and a linear effect over the relative weight of breast with bone. Lysine levels had a quadratic effect over the absolute weight of legs, which increased up to the level of 1.06%. Lysine levels influenced the absolute weight of thigh, which increased up to the level 1.02%. Lysine levels influenced the absolute weight of abdominal fat, which presented a quadratic decrease up to the level 1.00%.

Conflict of interest

The authors have not declared any conflict of interest.

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III. Chapter 3

The influence of essential amino acid in the chick's diet interferes in the weight gain

Aurélio Ferreira Borges

Marco Túlio Silva Borges

Raquel Nogueira de Moraes

Abstract

One of the attributes of amino acid lysine is to manufacture muscle protein, as it is a physiologically essential amino acid for immunity, development and production in birds. This study evaluates the influence of several levels of the essential amino acid lysine for chicks, with age of about 504 hours, maintained at a temperature of approximately 29 degrees Celsius. The basal diet contained 21.0% crude protein and 3,000 kcal of energy of biological transformations/kg. This formula was supplemented with levorotatory-lysine acid monohydrochloride, resulting in diets with 1.040 to 1.280% of total lysine for five experimental treatments. The treatments were found to have a mathematical expression squared influence on animals' weight gain, conversion of diet, total liver mass and protein synthesis index. The effect of the five experimental treatments was not observed in diet consumption, fat synthesis index, absolute weights of intestines and heart, and percentage of liver mass. It was concluded that the total lysine requirements for chicks aged up to 504 h were 1.240%.

Key words: Thermal environment, dietary lysine, performance, rearing setting.

Introduction

According to Zaboli et al. (2016), temperature control in sheds for slaughter chickens may induce thermo tolerance, possibly by modifying physiological parameters during the first days of chronic heat stress. This is due to the importance of cardiovascular and respiratory systems in the thermoregulation of these birds. In the respiratory system of the bird, blood circulation can weaken control of body temperature in a housing environment that is not stable. There is also the potential challenge, given that due to the challenges posed by environmental degradation, the global temperature will increase approximately from 0.7 to 2.6°C of at least more than 52 years or more.

In the tropical regions of Brazil State, temperature variation in the range of 34 to 45°C can occur between the months of August and May. This promotes a significant reduction in the performance of chicks. The growth of these modern birds over the years, owing to continued genetic progress and the economic growth of the poultry industry in hot climates, requires ways to mitigate thermal stress. Therefore, unequal approaches, such as sheds controlled by the artificial climate, the low population density, nutritional screening of chicks, and the decrease in weight gain have been experienced. However, many of these practices are overestimated and inefficient (Zaboli et al., 2016; Borges, 2017). High amounts of heat of tropical origin still reduce the weight of digestive and respiratory organs in chicks, to decrease the percentage metabolic rates in birds.

This promotes the reduction of metabolic heat production (Lara and Rostagno, 2013; Muheisen et al., 2017). High temperatures can be reduced with Levorotatory-Lysine Acid Monohydrochloride upplementation, without modifying the performance of chicks. Consequently, by ratifying the different responses related to the effect of high temperatures, it is evident that the requirements of chicks are modified not only according to genetics, but also as a consequence of the thermal environment to which these animals are placed, in the different stages of development (Belloir et al., 2017).

This author adds that the dietary level of rations for chicks raised at elevated temperatures may be reduced by the addition of lysine-levorotatory acid monohydrochloride without any changes in body development. Therefore, the reason for the various responses to the phenomenon of high temperatures means that the diets of chicks can be modified not only in relation to genetics but also in the thermal adaptation in which these animals are reared.

The amino acid lysine-levorotatory acid mono- hydrochloride in the diet has an essential function in protein turnover in musculature of chicks, as it promotes protein synthesis. The deficiency of this amino acid promotes compact protein synthesis, especially in the pectoralis major muscle, which is more sensitive to this amino acid than to the wings and thigh muscles. Lysine has been described as influencing the muscular development of the carcass, since it improves the performance of the musculature and decreases the fat of the carcass (Cruz et al., 2016).

Glutamate indexes found in the muscle of birds are influenced by the natural process of lysine decomposition. The results of the decomposition of lysine, piperidine carboxylic acid, DL- α -Aminoadipic Acid and saccharopine dehydrogenase were increased in broiler diets by about 150%. These results suggest that the short-term diet rich in lysine-levorotatory acid

monohydrochloride can improve the meat flavor of broilers in various environmental situations (Watanabe et al., 2015). Nutritional protein for chicks is the high-priced dietary nutrient in the market, yet the use of amino acids consisting of crystals offers several advantages. Its use provides reductions in crude protein concentrations in the diet and the ejection of this nitrogen into the environment, which favors the reduction of environmental pollution. Most broiler diets are formulated with all essential amino acids, where they are fed in optimal proportions to the lysine concentration in the diet (Franco et al., 2017).

The Lysine-levorotatory acid monohydrochloride from vegetable fermentation, such as sugarcane, was as efficient as the lysine offered in the consumer market, potentially being offered in commercial dimensions (Tabassum et al., 2015). Lysine is the amino acid used as an example in chicks because it is used in the production of proteins. It means that it is the first most limiting amino acid in diets for these birds (Belloir et al., 2017).

When chicks are kept in warm environment, there is reduction in the size of the heart, liver and small intestine, to compensate for the heat load to be dissipated in the environment (Borges, 2017). Chicks up to 504 h old, raised in a heat stress environment, represented by a temperature of approximately 29 °C, have lower weights of metabolically active tissues (heart, liver and intestine). The author adds that abdominal fat deposition, noble cut yield and slaughter carcass weight are influenced by the ambient temperature. The objective of this article is to evaluate the effects of several levels of lysine for chicks up to 504 h of age, when maintained in representative heat stress at approximately 29°C; thus, simulating the natural conditions in Brazil State. The research problem arises from the question: what are the effects of different level of lysine in the diets of chicks up to 504 hours of age, when kept in heat

stress representative of natural conditions in Brazil State? The overall conclusion of the study is that the levels of amino acid lysine on the diet on absolute (dg) and relative weight of the heart, liver, gizzard, intestine and carcass of chicks subjected to a temperature of approximately 29°C, which were slaughtered within 504 hours of age.

Materials and methods

Chicks

Experimentation with chicks was carried out in a climatic chamber at a Laboratory of Climatic Effects and Environmental Factors on cutting chicks in Brazil State. In all, 400 male chicks were used, with a mean initial weight of 350 ± 1.5 dg, and received the vaccines against neurolymphomatosis and Newcastle diseases. The choice of male chicks was justified by the greater absolute weight of breast and legs meat compared to females, in order to verify the tendency of rations to influence increases in the deposition of breast meat and thighs. The chicks remained at 24 to 504 h of life in the experiment under conditions of heat stress at the temperature of approximately 29°C. The statistical model of the experiment it was totally random, consisting of five treatments and eight replicates per treatment. Ten chicks were housed in each replicate.

Feeding regime in the experiment

The experimental recommendations used the spreadsheet with the set of digestibility values developed by Ajinomoto Heartland Llc (2014). In experimental diets, the animals ingested the same amount of protein and

energy several times a day; that is, with diets of the same number of calories and proteins, formulated with corn, roasted soybean meal and corn gluten. They were formulated to meet the nutritional requirements, phosphorus, protein, amino acids, energy and calcium, with the exception of lysine. The basal diet was supplemented with 78.40% of Levorotatory-Lysine Acid Monohydrochloride, resulting in diets with 1.040 to 1.280% total lysine. The total amino acid values of the nutritional components of the basal diet were corrected for digestible amino acids.

Planned experimentation

The 15 metal troughs, each with an area of 72.0 dm², received the chicks. Each compartment is a representative of an experimental unit. The objective of this study was to estimate the range indicating representative thermal stress at a temperature of approximately 29°C for the initial phase of 504 h-old chicks during the artificial warm-up period. The heat and the water vapor in the experimental unit were monitored by a minimum and maximum dry rounded temperature evaluator and humid and black rounded globe. The thermometers were seated at an intermediate height relative to the central battery compartment. Temperatures were recorded daily on two occasions, at 8:30 and 6:30 p.m., performed throughout the experimental period.

The thermal environment was demonstrated in terms of the black globe moisture code (BGMC) and calculated by the following equation: $BGMC = Bgt + 0.36 Dpt - 330.08$, where Bgt is the black globe temperature in degrees Kelvin and Dpt is the dew point temperature in Kelvin degrees (Table 1). The diet and water were freely offered; the water was changed once a day to avoid temperature rise. An uninterrupted flow chart of 24 h of artificial light was used throughout the experimental occasion. The variables that were evaluated

were: total lysine intake, diet conversion, carcass performance, protein placement, full weight and percentage of primary thigh and thigh cuts, breast weight gain and amount of feed consumed. The weight yield of the chicks that were cushioned was obtained by distinguishing between the weight at the end and the beginning of the experiment. The feed conversion was evaluated for the period of 24 to 504 h, based on information on dietary intake and weight gain. The calculation of the food intake during the experimental period was acquired using the difference between the counting of foods provided and despised by the animals and the remnants of the diets supplied. The diets were weighed at the beginning and at the end of the experiment (Alhotan and Pesti, 2016).

Table 1. The heat and the water vapor in the experimental unit.

Stage (h)	Atmosphere (°C)	Qualified atmosphere wetness	BGMC
24	33.21±0.211	53.41±1.791	82.11±0.271
48	32.41±0.721	56.31±2.440	81.51±0.831
72	31.50±0.781	57.31±1.741	80.31±1.090
96	30.51±0.501	54.90±2.210	78.71±0.441
120	30.21±0.210	55.81±1.960	78.51±0.181
144	29.50±0.241	55.51±4.020	77.51±0.761
168-504	29.10±0.391	59.71±3.160	77.40±0.591

Evaluation of the physical formation and constitution of crude protein in carcass of the chicks

Four chicks of each replicate were chosen to be slaughtered, considering the mean weight of the experimental unit, and the weight deviation to + 5% and to - 5%. Then the chicks were slaughtered and plucked, the mass of gutted skeletons was determined. Following, the fatty acids of the chest were excluded and the mass determined. Two whole skeletons, including head

and foot, of each repetition were sprayed in 16 minutes, one at a time, on a commercial horsepower with 1.78 rotations per minute (RPM); and after homogenization a sample was collected. The chicks were weighed after 12.5 h of fasting and then at the end of the experiment. Considering the high fat content of the carcasses, the carcass fragments were oven dried at $\pm 60^{\circ}\text{C}$ for 73 h and the fat removed in the extractor for 4 h. After this, the samples were ground and placed in chalices for further evaluation. Crude protein evaluations were performed in an animal nutrition laboratory. An additional set of chicks of 24 to 504 h of age were slaughtered to determine the body composition of the animals at the beginning of the experiment. Protein deposition in the animal skeleton was measured by the difference between estimates of carcass composition between 24 and 504 h of age of the chicks.

Evaluations using experimental statistics

The numerical data capture for analysis was developed with the scientific support of the computer program, Statistical and Genetic Analysis (SGA). The approximate calculations of total determination of lysine were determined with the aid of the linear or quadratic regression models and the Linear Response Plateau (LRP), observing the adaptation with smaller error.

Results and discussion

The animals received diverse stages of lysine in the diet and were kept in a high temperature environment (29.10°C). The results of performance, specified by feedstuff eating and feed transformation, mass increase, entire lysine ingesting, protein and fat statement rates in the slaughter chick's

carcass of 24 to 504 h grow old were obtained (Table 2). The Levorotatory-Lysine Acid Monohydrochloride indexes of the diet influenced the gain in animals' weight of the chicks, increasing in quadratic equation form until it gets to 1.20% indexes (Table 3); corresponding to an estimated consumption of 99.01 dg of the total lysine.

Due to the increased use of Levorotatory-Lysine Acid Monohydrochloride, no effect of lysine levels on feedstuff eating (FE) was observed. These results were similar to those obtained by those who, grow old working in high temperature conditions, did not find the effect of the stages of Levorotatory-Lysine Acid Monohydrochloride in relation to the initial consumption of chicks. However, it is different from the one pointed out by those who observed a significant modification in the consumption of chick's ration of 24 to 504 h and subjected to warmth pressure. Dietary lysine result ($p < 0.01$) in the feed conversion (FC) was verified for the chicks, which varied in the quadratic form and improved to 1.24% level, corresponding to an estimated consumption of lysine of 100.7 dg (Table 4).

Considering report that male chicks at 37°C up to 504 h of age does not require a higher level of lysine in the feed than those kept at 24°C, it is inferred that the variation of the results of this work can possibly be associated with differences in experimental environmental conditions.

Table 2. Entire consumption of lysine and aliquots of fat and protein account in the skeleton of male chicks of 24 to 504 h.

Parameter	Total lysine level (%)					RSD
	1.04	1.10	1.16	1.22	1.28	
Weight gain (dg)	5,390	5,700	5,890	5,720	5,790	0.0479
Feed intake (dg)	8,070	8,250	8,460	8,180	8,220	0.0452
Feed: gain ratio	1.50	1.45	1.43	1.43	1.42	0.0148

Total lysine intake (dg)	85	91	98	99	106	0.0461
Carcass deposition rate						
Fat (dg)	440	460	470	430	460	0.0555
Protein (dg)	950	1,000	1,070	1,020	1,070	0.0449

RSD = Relative Standard deviation

Table 3. Gain in animals’ weight (dg) of chicks from 24 to 504 h old, underneath 29.1°C.

Weight gain	Lysine complete in diets (%)	Lysine complete doss (%)	Mathematical expression
<u>squared</u>			
5,390	1.04	1.20	$\hat{Y} = -1853.67 + 4067 X - 1695.88 X^2$ $r^2 = 0.77$
5,700	1.10		
5,890	1.16		
5,720	1.22		

Table 4. Lysine starting point in the ration for chicks of 24 to 504 hours of age, at a 29.10 °C.

Feed: gain ratio	Lysine complete in diets (%)	Lysine complete doss (%)	Quadratic equation
1.50	1.04	1.24 $r^2 = 0.96$	$\hat{Y} = 4.10087 - 4.31539 X + 1.73764 X^2$
1.45	1.10		
1.43	1.16		
1.43	1.22		
1.42	1.28		

Table 5. Lysine factor and coefficient of protein constitution of chicks from 24 to 504 h of phase, below 29.10°C.

Deposition rate (dg/day)	Total lysine of ration (%)	Ideal total lysine (%)	Quadratic equation
950	1.04	1.26 $r^2 = 0.71$	$\hat{Y} = -256.843 + 577.436 X - 229.628 X^2$
1,000	1.10		
1,070	1.16		
1,020	1.22		
1,070	1.28		

Cemin et. al. (2017) explains that the requirements of the fundamental amino acids add up with the addition of protein. This suggests that, deamination and excretion of excess protein nitrogen may contribute to the excretion of the first limiting amino acid of lysine. This fact would increase your requirement. The performance results evidenced that early-stage chicks require a higher lysine level to achieve a better FC ratio than a higher gain. The action of the lysine amino acid coefficients in the diet ($p < 0.01$) on the protein deposition rate (dg/daytime) of chicks from 24 to 540 h of age was verified. The coefficients of the amino acid lysine of the diet prompted the protein clarification aliquot (PCA) ($p < 0.08$), which was quadratically added until the coefficient corresponds to 1.26% (Table 5). These results are similar to those obtained by those who verified the efficiency of the coefficients of the amino acid lysine in the PCA in a thermo-neutral environment. It can be inferred that, the requirement of total lysine for protein deposition (1.26%) was higher than that required for weight gain (1.20%).

Table 6. Complete (dg) and relation masses of heart, liver, gizzard and intestine of 504 hours old of chicks subjected to 29.10°C.

Total lysine level (%)						
Parameter	1.04	1.10	1.16	1.22	1.28	RSD (%)
Absolute weight(dg)						
Heart	40	40	50	40	40	13.96
Liver	140	150	160	150	150	11.42
Intestine	240	260	250	250	250	13.04
Relative weight (%)						
Heart	0.95	0.95	0.97	0.95	0.97	15.34
Liver	3.33	3.23	3.30	3.38	3.24	11.45
Intestine	5.53	5.61	5.38	5.42	5.47	11.96

r^2 = is portion statistical model adjustment to the detected values. The r^2 varies between 0 and 1, indicating how much the model explains the observed values. The higher the r^2 , the better it fits the sample.

It was verified that, in absolute values (dg), the 1.16% lysine factor harmonized the ascending fat building (FB) effects. Considering the constitution of the carcass, the consequence ($p>0.10$) of the coefficients of the lysine amino acid of the ration on the FB was not emphasized. These results defer from those achieved by those who found a mathematical expression squared result of the lysine coefficients in the FB of chicks between 24 to 504 h of age, when they worked in a thermo-neutral atmosphere. The absolute (dg) and relative (expressed as percentage of carcass) weights of heart, liver, gizzard and intestine of 24 h old of chicks subjected to high temperature in 29.10°C. The total lysine coefficients of the diets did not influence the integral and rational weights of the organs constituted by the small and large intestines and the heart (Table 6). Absolute liver weight of the chicks varied in a quadratic form, being the highest weight found in the level of 1.17% lysine. The increase in absolute liver weight occurred due to the increase in the body weight of the birds, since the relative weight of the liver did not vary between treatments.

Conclusion

In this study, it was found that the coefficients of the essential amino acid lysine present in the diet influenced the yield of chick's weight gain. This yield by weight increased in a quadratic fashion up to 1.20% coefficient. This corresponds to the estimated consumption of 99.1 dg of the total amino acid lysine of the feed. The effect of the lysine coefficients of the diet is confirmed for the feed conversion, which occurs in the form of a quadratic mathematical equation. There is feed efficiency for feed conversion up to the coefficient

1.24%, which corresponds to the estimated lysine consumption of 100.7 dg. It is verified that the effect in the form of a quadratic equation of the lysine coefficients in the diet ($p < 0.01$) on the rate of protein deposition in dg/day, occurs with feed efficiency of about 1.26% coefficient. The conclusion is that there was absolute (g) and relative (expressed as percentage of carcass) weights gain in gizzard, liver, heart and intestine of 504 h old chicks subjected to high temperature (29.10°C). At least in the subject of the lysine coefficients here tested, the consequence of the total lysine coefficients of the diets on the integral and unrestricted weight of the intestine and the heart was not highlighted in this paper. The absolute liver weight of the birds varied in a quadratic manner, with the highest weight found at 1.17% lysine level. The increase of the absolute weight of the liver occurs due to the increase of the body weight of the birds, since the percentage mass related to the liver does not vary in experiments.

Conflict of interests

The author has not declared any conflict of interests.

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IV. Chapter 4

Environmental performance of aquaculture in Brazil

Aurélio Ferreira Borges

Marco Túlio Silva Borges

Raquel Nogueira de Moraes

Abstract

The objective of this study was to analyze the environmental performance of aquaculture in Colorado of the West City, Rondônia State, Brazil. Fifteen fish farmers were interviewed. For data collection, structured interviews were carried out, using a questionnaire based on information supplied by the United Nations Food and Agriculture Organization (FAO). The questionnaire considered 12 items, organized into three main topics: a) social and legal standards b) environmental standards c) standards of food safety and hygiene. Aquaculture in the city of Colorado do Oeste, Rondônia presents two fish production systems: extensive and semi-intensive. In the semi-intensive system, stocking rate was one fish per m³, on average; tambaqui (*Colossoma macropomum*), tilapias (*Oreochromis spp.*), pirarucu (*Arapaima gigas*) and pintado (*Pseudoplatystoma spp.*) were the species farmed at the largest number. The rate of water renewal was due to the greater availability of natural food in this system. Water renewal was constant in the ponds (1,500 liters per minute). In the semi-intensive system using dug ponds, alevins were stocked and fed during the entire rearing time with natural and exogenous food. The extensive system relied on the natural production of the pond, with stocking

density limited by the production of natural food. The little renewal of water made the cultivation tank itself acted as a decantation lake, with the occurrence of oxidation and sedimentation of residual organic matter, consisting of feces, debris and organic fertilizer. Production of reduced effluent volume took place in the extensive system, compared to the cultivation area. In addition, there was high water turbidity, caused by high concentration of planktonic organisms, and low concentrations of dissolved oxygen in the water. Data showed that nine estates of the interviewed fish farmers had critical environmental performance (less than 30.0%). Six estates of fish farmers had bad environmental performance (between 30.0 and 50.0%) (Coefficient of sustainability = green square x 100 ÷ Total Questions less the yellow squares).

Keywords: Amazonian aquaculture, environmental aquaculture management, sustainable development.

Resumo

Desempenho ambiental da aquicultura no Brasil

Com o presente estudo objetivou-se analisar o desempenho ambiental da aquicultura no município de Colorado do Oeste, estado de Rondônia, Brasil. Foram entrevistados 15 piscicultores. Para a coleta de dados foram realizadas entrevistas estruturadas, utilizando-se de questionário elaborado a partir das indicações dadas pela Organização das Nações Unidas para a Agricultura e Alimentação (FAO). O questionário considerou 12 itens, organizados em três assuntos principais: a) padrões sociais e legais; b) padrões ambientais; c)

padrões de segurança alimentar e higiênicos. A aquicultura no município de Colorado do Oeste, Rondônia, apresenta dois sistemas de produção de peixes: um extensivo e outro semi-intensivo. No semi-intensivo a taxa de estocagem foi em média de um peixe por m³; as espécies mais criadas foram o tambaqui (*Colossoma macropomum*), as Tilápias (*Oreochromis spp.*), o Pirarucu (*Arapaima gigas*) e o Pintado (*Pseudoplatystoma spp.*). A taxa de renovação de água se deveu à maior disponibilidade de alimento natural neste sistema. Houve constante renovação de água nos viveiros (1.500 litros por minuto). No sistema semi-intensivo em viveiro escavado os alevinos foram estocados e alimentados durante todo tempo de criação com alimento natural e exógeno. O sistema extensivo foi dependente da produção natural do viveiro, com densidade de estocagem limitada pela produção natural de alimento. A pequena renovação de água fez com que o próprio tanque de cultivo atuasse como lagoa de decantação, ocorrendo oxidação e sedimentação da matéria orgânica residual, composta por fezes, organismos mortos e adubo orgânico. No sistema extensivo ocorreu produção de reduzido volume de efluente em relação à área de cultivo; houve elevada turbidez da água, causada pela alta concentração de organismos planctônicos; na água havia baixa concentração de oxigênio dissolvido. Os dados mostraram que nove propriedades de piscicultores entrevistados apresentaram desempenho ambiental crítico (inferior a 30,0%). Seis propriedades de piscicultores apresentaram desempenho ambiental péssimo (entre 30,0 e 50,0%) (Coeficiente de sustentabilidade = quadros verdes x 100 ÷ Total de questões menos os quadros amarelos).

Palavras-chave: gestão ambiental da aquicultura, desenvolvimento sustentável, aquicultura amazônica.

Introduction

Continental water resources are an essential component of all terrestrial ecosystems. The general shortage of water, the gradual destruction of freshwater resources and aggravation of pollution in many regions of the world, with the progressive encroachment of incompatible activities, have demanded increasingly planning and integrated management of these resources (Ostrensky *et al.*, 2008). In Brazil, the Executive Group of the Fishing Sector (EGFS) was created by Decree 1697/1995, aiming at reconciling the political aspirations of the government sector. Its objective was to require the development of the sector, proposing to the Board of Natural Resources Policies, a national policy for Aquaculture and Fisheries and coordination of the design of their actions.

From 2003, a more definitive structure focused on socioeconomic theme adopted by the public sector was used and developed. It was created by the issue of Provisional Measure (PM) 1038/2003, the Special Secretariat of Aquaculture and Fisheries (SSAF / PR), linked to the Presidency. This Provisional Measure, in its article 23, states that fishing should be developed respecting environmental legislation, maintaining the division of skills historically established. Federal Legislation (Law 9433 of 1997 (Brazil, 1997)) clearly linked the issues of Water Resources with environmental issues. In this principle, it cannot be disregarded that water is also the raw material of agriculture and livestock productive system.

Sustainable development is the reasoned and responsible use of natural resources without harming the economic value of the natural asset for future generations. Responsible fish farming and sustainable aquaculture are often used as synonyms, but responsible is more acceptable due to the

great importance that this word implies for sustainability. Responsible aquaculture is to make fish farming profitable, with consciousness (Eler & Millani, 2007). Aquaculture currently presents itself as an emergent economic activity in the competition for water resources. It faces the challenge of shaping up the concept of sustainability, which implies to aggregate new values to the technology and to the management and commercialization practices of the sector. The previous presentation of the aquaculture sector contributes to define the context of the research; however, it is not enough to establish its framework. There is a concept lacking, which is sustainable development (Lorenzo, 2010).

With respect to this author, the term sustainable development was used by the Brundtland Commission to designate the development that meets the current needs without compromising the ability of future generations to meet their own needs (United Nations, 1987). Therefore, sustainable development involves a pattern of resource use, which aims to meet human demands while preserving the environment so that these needs can be met not only in the present, but also by future generations. This author comments that sustainable development is conceptually regarded as the intersection of three constituent parts (Figure 1). These three dimensions refer to environmental sustainability, economic sustainability and socio-political sustainability.

Encouraging aquaculture sustainable development in Brazil to reconcile environmental preservation with the distribution of social and economic benefits generated by it is the task for those who compose the national aquaculture scope. Transforming the immense Brazilian potential, often released as a result of the country's continental dimensions, its unique water availability and its unrivaled diversity of fish species cultivated in real

competitive advantage, will not be possible without structural information and strategic planning. The objective of this work is to analyze the environmental performance of aquaculture in the city of Colorado do Oeste in Rondônia State.

Aquaculture Certification Council (ACC) is an international non-governmental organization that grants a stamp to certify the application of management practices ensuring social and environmental responsibility, standards of food safety and hygiene and traceability on chain production of aquaculture businesses. It is an educational and volunteer program that allows businesses to meet national and international requirements. The certification program considers 12 items, which were organized into three main issues: a) social and legal standards, composed by items of property rights and compliance with laws, community relations and worker safety; b) Environmental Standards, consisted of conservation items of protected areas, management of effluents, sediment management, soil and water conservation, origin of post-larvae and fry, disposal of inputs and wastes; c) Safety and hygienic standards for food consisted of management of drugs and chemicals, microbial sanitation, collection and transportation.



Figure 1. Scheme of the three dimensions of sustainable development

Source: Lorenzo (2010).

Material and methods

The study was carried out in Colorado do Oeste, Rondônia State. Fifteen fish farmers were interviewed. The population of fish farmers used to calculate environmental performance in Colorado do Oeste in 2011 was 23 and the sample size used to calculate the environmental performance was fifteen. Structured interviews were used for data collection.

The questionnaire for conducting the assessment of environmental performance in the fish properties sought a theoretical approach to the level of sustainability that has producers in order to appraise their environmental performance. The questionnaire was formulated from information given by *Food and Agriculture Organization* of the United Nations (FAO, 1995) to develop a sustainable aquaculture through its Code of Conduct for Responsible Aquaculture (CCRA).

The questionnaire was generated to give assistance to each of the previous items, placing the study at the level of international requirements (Carrasco, 2006). The fifty-six questions were grouped into twelve items. During the questionnaire application, different dimensions and forms of sustainable crop management were exposed (FAO, 1995). In addition to applying the questionnaire, explanatory talks on current trends of sustainable development and how those rules are applied were held every day, necessary and compulsory, in the case of products exported. The time spent was at least one hour of interview with each fish farmer.

There were three types of answers: Yes, No and Not Applicable. The green color indicated the action in favor of environmental performance, the red one indicated detriment of the environmental performance and the yellow indicated the non-applicability of the question. The color was independent of

the “Yes and No” answer. In some cases, it would be positive to say “yes” and in others, it would be negative. Comparing the feedback with the marked tables, the coefficient of sustainability was determined. The questionnaire was analyzed independently for each farm and through the following equation, the level of sustainability (Banco do Nordeste, 1999) was determined:

Coefficient of sustainability = $\frac{\text{green square} \times 100}{\text{Total Questions} - \text{yellow squares}}$.

All questionnaires were scanned and converted into a spreadsheet file. Data analysis was performed using a statistical package for Windows SPSS (Statistical Package for Social Science) software version 19.0 (2010) and Microsoft Office Excel 2007 software.

For data collection, structured interviews were carried out. The interviews were conducted by applying questionnaires elaborated from Table 1.

Fish farming in the city of Colorado do Oeste, Rondônia State, has two fish production systems: extensive and semi-intensive. In the extensive system, commercial diets are rarely used and fish are fed traditionally with agricultural by-products or animal waste. The product does not exceed 4,500-5,000 kg/ ha/year. However, production costs are low and profits are high. This system is characterized by the use of species such as *Colossoma macropomum*, *Oreochromis* spp., *Arapaima gigas* and *Pseudoplatystoma* spp.

Fish farmers use extruded feed with four different safety levels. The size of the feed pellets is 8-10 mm for a level of assurance 1, 15-20 mm for the level of assurance 2, 10-15 mm for the level of assurance 3 and 6.4 mm for the level of assurance 4. All three feeds with assurance level with 28% of crude protein (CP) are used in omnivore fish in the fattening stage. The feed with 32% CP is used in omnivorous fish in the juvenile phase.

Tambaqui farming in the city of Colorado do Oeste has shown great results. The technology package used in the production of Tambaqui by the semi-intensive system is the pond/dam, where production is divided into two phases: growing phase, with 60-day cycle and fattening phase with 240 to 300-day cycle. In general, semi-intensive system can be classified as one with costs between low and average and production also classified within these magnitudes. Although they are small producers, they use diets, inputs and a density of 1 to 4 fish per m². In semi-intensive system, fish are also cultivated in dug ponds, where alevins are stocked and fed throughout the growing season. Water is enriched with organic or inorganic fertilizers to increase the number of natural foods such as phytoplankton and zooplankton, and with products available in the farm such as cassava, corn, fruits and vegetables that supply the fish. Most of health problems that occurred in the experiment city are related to diseases caused by fungi and bacteria.

Far from being a problem, semi-intensive production can be seen as positive, since, in most cases, the major producers of cultivated aquatic organisms are countries whose production is based on small properties (Valenti *et al.*, 2000).

Table 1. Classification of the environmental performance in Colorado doe Oeste, 2011

Criterion	Classification
Less than 30%	Critical
Between 30 and 50%	Bad
Between 50 and 70%	Adequate
Between 70 and 90%	Good
More than 90%	Excellent

Source: adapted from Carrasco (2006)

In both systems, the production was sold to two fishing industry, one in State of Mato Grosso, and the other located in Vilhena, Rondônia State. The viscera of the fish, whether not sent to the fishing industry, are given as feed to pigs, poultry and carnivorous fish in the farm. The technology used is practically null. Some owners and staff have conducted training courses offered by Emater in Rondônia State. Regarding associations, this does not exist. The way of fish production in the study city is based on the principles of family aquaculture, a way of production dominated by the interaction between management and labor. The establishment members of the family farming in the study city are those managed by the farmer who uses more family labor than hired. It is the family aquaculture that has great capacity to absorb labor and generate income, but not to create jobs.

Regarding size of the enterprises, a division by the extension of the water on the farms used for fish rearing was registered. For classification purposes, this study considered as small producers those who have less than 2000 m² of water on the property; medium producers are those with between 2000 and 10,000 m² of water and large producers are those who have above 10,000 m² of water. The area of water per individual fish farmer in Colorado do Oeste in 2010 was 344,700 m²; the fish produced per year was 193,100 kilograms; the fish sold was 161,00 unit per year; the number of fries purchased was 169,000 units; the feed consumed was 321,950 kilograms; the average cost of feed per cycle was R\$ 310,760.00 and the production estimate was 274,500 ton/year.

Results and discussion

Cultivation of *Colossoma macropomum* in Colorado do Oeste has

shown satisfactory results. The technological package used in the semi-intensive production of *Colossoma macropomum* was ponds dam where the production was divided into two phases: growing phase, 60-day cycle and fattening phase, with 240-300-day cycle. Productivity did not exceed the range of 4,500 - 5,000 kg of fish/ha/year.

The semi-intensive fish cultivation can be classified as the one with costs ranging from low to medium and the production classified within these magnitudes (New, 2003). Although they are small producers, they used feed, other inputs and density in the range of 1-4 fish per m². The cultivation of fish in ponds where alevins were stocked and fed over cultivation period was also developed in this semi-intensive system. The water was nutritionally enriched with inorganic fertilizer, composed by urea simple formulations and formulations associated with nitrogen, phosphorus and potassium. The objective of using those formulations were to increase the amount of natural food in the water of the tanks such as phytoplankton and zooplankton. Fish farmers provided to fishes natural byproducts available in the rural properties such as remains of cassava, corn, fruits and vegetables.

The most occurred sanitary issues in the experiment city are related to diseases caused by fungi and bacteria (Table 2).

Table 2. Drug Use Source.

Premix	Suplement	Pillay (1992)
Malachite green	Desinfectant	Pillay (1992)
Formol	Desinfectant	Pillay (1992)
Terramycin/Oxytetracycline	Antibiotic	Vinatea (1999)
Cloramina/Amoxilina	Antibiotic	Vinatea (1999)
Sodium chloride	Desinfectant	Pillay (1992)
Copper sulfate	Anti-algae	Vinatea (1999)
Potassium permanganate	Desinfectant	Pillay (1992)
Vitamins	Food suplement	Pillay (1992)

Dipterex	Pest control	Pillay (1992)
Sodium hydroxide	Desinfectant	Vinatea (1999)
Sodium hypochlorite	Desinfectant	Vinatea (1999)
Hormone	Reproduction	Pillay (1992)

Source: Adapted from Eler & Millani (2007)

The semi-intensive production can be classified as positive, since in most cases the major world producers of cultivated aquatic organisms are from countries whose production is based in small rural properties (Valenti *et al.*, 2000).

Evaluation of environmental sustainability and environmental performance are based on legal issues, giving priority to the development of an administrative and legal landmark suitable to ensure the introduction and application of environmentally responsible aquaculture practices, represented by the compliance with legal obligations, relations with the community, worker safety, conservation of protected areas, management of effluents from the ponds, management of sediment in the fattening and spawning tanks, management of medicines and therapy that each batch of fish received (Table 3).

Table 3. Answers to the most significant question groups.

Question	% yes	% no
Attending to legal obligations	100	0.0
Relationships with the community	0.0	100
Worker safety	0.0	100
Conservation of protected areas	60.0	40.0
Management of effluents from the ponds	0.0	100
Management of sediment in the tanks	33.3	66.7
Management of banned drugs and chemicals	6.7	93.3
Therapeutic which each batch of fish received	6.7	93.3

Source: survey data

In 100% of the cases studied, it occurred attendance to legal obligations by the fish farmers (Table 3). They stated that they obey the environmental legislation pertinent to environmental licensing for fish activity, according to legal requirements established by Conama (1986) and Conama (1997). On the State Planning, the responsibility for the environmental licensing is by State Environmental Agency, obeying state laws in force, which cannot be more permissible than the Federal Law provisions, which regulates the environmental licensing.

Relationships with the community faced problems as 100.0% of the interviewed fish farmers said that the rural property does not contribute to the community for their well-being and environmental development (health, recreation and education).

These results may be related to the fact that much of the fish production in the city of Colorado do Oeste comes from family producers and a small portion comes from aquaculture business. The lack of technological and managerial qualification in these two production processes is still fragile. There is a lack of mechanisms of public participation. Government mechanisms should not allow political interests protrudes towards technic-scientific and sociocultural issues. This leads to a latent conflict between the need for environmental protection, within the limits required by the forest legislation, with rural production required by the social function of the rural estate.

Regarding the worker safety, and relationships among them, 100.0% of the interviewed fish farmers said they provide training on general safety, personal hygiene and first aid to the employees on the property (Table 3).

Fish farming in Colorado of the West City is funded on resources derived from other productive sectors. Most of the projects is not idealized in balance with the environment. Most enterprises seek to curtail the hiring of

skilled labor and they are not concerned with rural communities. Principles of semi-intensive fish farming of low-impact, environmentally responsible, productive, sustainable and profitable requires the adoption of weighted tactics of production and diagrams responsible for the management of effluent emissions. The adequacy of stocking density of fish in the production systems helps to slow down the accumulation of metabolites and reduce the need for therapeutic agents. The waste management requires the reduction of nitrogen, phosphorus and fecal solids dissipated in the aquatic environment.

Figure 2 shows the distribution of fish farmers in the city of Colorado do Oeste and the present approach to the coefficients of environmental performance in the sample analyzed ($n = 15$) and the distribution of the production area (%) as well as it shows that the first nine estates of the interviewed fish farmers had critical environmental performance (less than 30.0%). The other six estates of fish farmers had poor environmental performance (between 30.0 and 50.0%). In general, the situation of environmental performance is not in favor to all estates, regardless of the area of water of the estate, therefore farmers should get more involved with environmental issues. Issues have been found in community relationships, worker safety and relationships among them, conservation of protected areas, problems in the management of effluent from the ponds, disposal of inputs and waste, management of chemicals and drugs and traceability. Environmental sustainability analysis starts with legal issues, giving priority to the development of a suitable legal and administrative mark to ensure the introduction and application of environmentally responsible aquaculture practices.

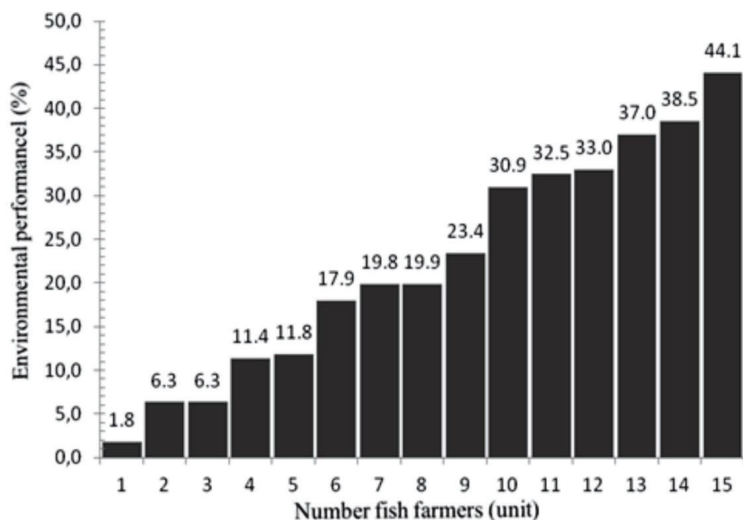


Figure 2. Approach to the coefficients of environmental performance of estates in the analyzed sample, Colorado do Oeste, 2011. Source: survey data and Borges *et al.* (2013).

Conclusions

In semi-intensive system, the average of stocking rate was one fish per m^3 . There was constant renewal of water in the ponds (1,500 liters per minute). In the semi-intensive system in dug ponds, alevins were stocked and fed during the entire rearing period with natural and exogenous food.

The extensive system relied on the natural production of the pond, with stocking density limited by the production of natural food. The little renewal of water made the cultivation tank itself act as a decantation lake. In the extensive system, occurred the production of reduced effluent volume,

compared to the cultivation area; there was high water turbidity caused by high concentration of planktonic organisms and water had low concentrations of dissolved oxygen. Data showed that nine estates of the interviewed fish farmers had critical environmental performance (less than 30.0%). Six estates of fish farmers had bad environmental performance (between 30.0 and 50.0%). The current Brazilian environmental legislation, which considers the preservation of permanent areas in aquaculture is inapplicable, even after being reformulated. It does not meet the specific needs of the Amazonian ecosystem and of fish farmers and it does not consider scientific knowledge and customs of Brazilian Amazonia. Brazilian environmental legislation should be regionalized to become applicable. The percentage coefficients of achieved environmental performance showed that aquaculture in Rondônia State is environmentally unsustainable.

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