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PHYSICAL-CHEMICAL AND MICROBIOLOGICAL QUALITY OF SWINE AND POULTRY DRINKING WATER FROM DIFFERENT SOURCES IN RIO GRANDE DO SUL/ BRAZIL AND ITS RELATIONSHIP WITH CONSUMPTION OF ACIDIFIERS AND BIOCIDAL AGENTS

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Abstract: Water is fundamental in the production of swine and poultry and must be supplied in sufficient quality, in sufficient quantity an adjusted pH to maintain physiological functions. The objective was to evaluate the physical-chemical and microbiological quality and consumption of a commercial acidifier and three chlorine biocidal agents in water from rural properties located in Vale do Taquari, RS, Brazil, by means of pH, free residual chlorine, redox potential (ORP), total alkalinity, total hardness, apparent color, turbidity, total dissolved solids, nitrates and nitrites, total coliform count, *Escherichia coli* and *Salmonella* spp.. Changes in nitrate concentrations, presence of microbial contamination, very strong correlation between alkalinity and acidifier consumption and low consumption of calcium hypochlorite. It was concluded that there are no relevant physical-chemical changes, highlighting the presence of bacterial contamination, reinforcing the need for water treatment.

Keywords: Microbiological contamination, pH, poultry, swine, water.

INTRODUÇÃO

Water is an essential natural resource for the maintenance of life on the planet, performing important biological functions in living organisms. Water sources for animal drinking can come from rivers, creeks and streams, lakes and ponds, springs, wells, rainwater harvesting and water offered by sanitation companies (Palhares, 2014). It is widely reported that the lack of water quality in animal production triggers a series of diseases in pigs and poultry, such as diarrhea and urinary tract infections, which negatively affects the sanitary and zootechnical performance of the animals (Padilha *et al.*, 2013; Palhares, 2014).

Despite its great importance, water is

still considered a forgotten nutrient when compared to feed, genetics, vaccination and management, due to the wide availability of water in production regions (Palhares & Kunz, 2011; Manu & Baidoo, 2020). Water quality is determined through physical, chemical and biological parameters (Mutlu & Kurnaz, 2018). In Brazil, bodies such as the National Health Surveillance Agency (ANVISA) and the National Council for the Environment (CONAMA) establish standards for water potability. According to Ordinance GM/MS No. 888/2021 (Brazil, 2021) and Resolution No. 396/2008 (Brazil 2008), water used concomitantly between animals and humans must be free of total coliforms and *Escherichia coli*. However, there is no concrete information regarding the frequency of water analyzes on rural properties in the legislation. Nevertheless, this periodicity of analyzes depends on the health and biosecurity policies of the integrating companies to which pig and poultry producers are linked.

For water decontamination, the use of chlorine-based biocidal agents is indicated, due to their low cost, high germicidal action (bacteria, algae and fungi) and wide availability (Rodrigues & Scalize, 2019). Added to this, chlorine provides a free residual effect, which helps maintain the potability of water during storage and distribution, that's why it is the most used process on rural properties (Otenio *et al*., 2010; Rodrigues & Scalize, 2019).

Chlorine-based biocides, in contact with water, form hypochlorous acid (known as a strong biocide, with high decontamination power), hypochlorite ion (weak biocide, contributes to decontamination, but to a lesser extent) and a by-product, related to the type of chlorine and inputs used in its production (Silva & Valentini, 2020). Among the agents used for water decontamination, chlorine gas, calcium hypochlorite, sodium hypochlorite, sodium dichloroisocyanurate (dichlorine) and trichloroisocyanuric acid (trichlor) can be highlighted (França & Santos, 2019). Chlorine has an excellent disinfectant action in water, however, at pHs above 8.0 the action tends to decrease, due to the reduction in the formation of hypochlorous acid (Rossi-Fedele *et al*., 2011). Thus, the use of acidifiers in the animals' drinking water is an important tool for regulating the pH, aiming at the best functioning of the chlorine.

The use of acidifiers has been intensifying with the growing ban on the use of antibiotics as growth promoters (Xu *et al*., 2022). An acidification process promotes improvements in nutrient absorption, feed conversion, feed intake, microbiota balance, potentiates the action of pepsin and trypsin, and inhibits the multiplication of pathogenic enterobacteria along the gastrointestinal tract (Busser *et al.,* 2011; Khan & Iqbal, 2015; Xu *et al.,* 2022). However, when acidification is performed manually, the pH of the water fluctuates throughout production and generates losses related to the suppression of beneficial bacteria, diarrhea, infections, reduced digestion and reduced absorption of nutrients (Escuredo *et al.,* 2016; Firrman *et al*., 2022).

The use of acidifiers occurs basically in two ways: via water or feed. The administration of acids via feed is a challenge, due to a possible interaction with the other components of the formula (Xu *et al.,* 2022). In addition, as a rule, animals ingest more water than feed, which makes the acidification of drinking water more interesting and efficient, even facilitating control by the producer, since the dosage can be changed at any time, according to the life stages of pigs and poultry (Escuredo *et al.,* 2016). Acidified water promotes increased consumption compared to drinking water with a high pH (Escuredo *et al.,* 2016). Consequently, it increases feed intake, improves zootechnical performance, reduces the fecal excretion of pathogenic bacteria, improves intestinal modulation of the intestinal microbiota, liver health and action of thyroid hormones, ensuring the homeostatic balance of the organism (Escuredo *et al.,* 2016; Hajati 2018; Luise *et al.,* 2020; Pearlin *et al.,* 2020). Generally, commercially available acidifiers are composed of a blend of organic acids with or without an inorganic acid. Among the most common organic acids, we can highlight products based on formic acid, acetic acid, propionic acid and lactic acid (Escuredo *et al*, 2016; Xu *et al*., 2022).

Bearing in mind the importance of water in poultry and swine production, this study aimed to evaluate the physical-chemical and microbiological parameters of different waters from poultry and swine breeding properties in the region of Vale do Taquari/RS, as well as the influence on the consumption of a commercial acidifier and three commonly used chlorine-based biocides commonly used in water treatment, according to previously published studies and practices found in the field.

MATERIAL AND METHODS

STUDY AREA AND WATER COLLECTION

Raw water collections were carried out from September 9 to October 27, 2022 in 13 rural properties producing poultry and swine located in five municipalities in Vale do Taquari, in the state of Rio Grande do Sul/RS, Brazil, as shown in Figure 1. All properties are linked to a cooperative in the analysis region, and were selected among the swine and poultry integration properties due to pH variability, ranging from pH 6,0 to pH 9,0, and sources (artesian wells and water source/springs) diagnosed by the cooperative's technicians with the monthly visits. Water samples were collected directly from water sources, containning zero free residual chlorine. As there are no specific laws for water quality in animal production, this study used water quality parameters for humans, according to ANVISA (Brazil, 2021) and CONAMA (Brazil, 2008).

At each point, approximately 76.1 liters of water were collected, whicht were placed in 15 5-liter drums to carry out acidification and chlorination curves, since 5 liters of water were used in each curve, the remainder of the water volume it was stored in 5 specific bottles for physical-chemical and microbiological analyses. The specific flasks were packed in isothermal boxes and sent for analysis.

All the following were registered: name of the property, date and time of collection, source of water, depth of the well, drilling time of the artesian well, ambient and water sample temperature, average rainfall for the month, description of the surroundings of the area regarding the presence of sheds, woods, other properties with livestock, type of livestock (pigs and/or poultry). The monthly average rainfall information was obtained from the National Institute of Meteorology (INMET), which accounts for meteorological data through stations.

PHYSICAL − CHEMICAL ANALYZES

The following physico – chemical analyzes were carried out in triplicate: pH – benchtop pHmeter method (Digimed, São Paulo, Brazil), free residual chlorine – quantification by portable free chlorine doser total chlorine MW-11 Milwaukee (Akso, São Leopoldo, Brazil), turbidity – method that measures turbidity in liquids (Akso, São Leopoldo, Brazil), apparent color – Platinum-Cobalt method (Akso, São Leopoldo, Brazil), ORP – pocket ORP meter method (Akso, São Leopoldo, Brazil), total alkalinity by titrimetry (SMWW 23 0 2320 B), total hardness by ICP (spectrometer) and calculation (SMWWW 23 0 2340 B and SMWWW 23 0 2340 C),

Figure 1. Geographic map of Brazil (Figure A), Rio Grande do Sul (Figure B) and Vale do Taquari Region with the location of the 13 swine and poultry producing properties (Figure C) where samples of water for physical-chemical and microbiological analyses and for carrying out acidification and chlorination curves were collected.

Caption: properties I to VI located in the municipality of Teutônia/RS, VII to IX in Westfália/RS, X and XI in Poço das Antas/RS, XII in Colinas/RS and XIII in Bom Retiro do Sul/RS.

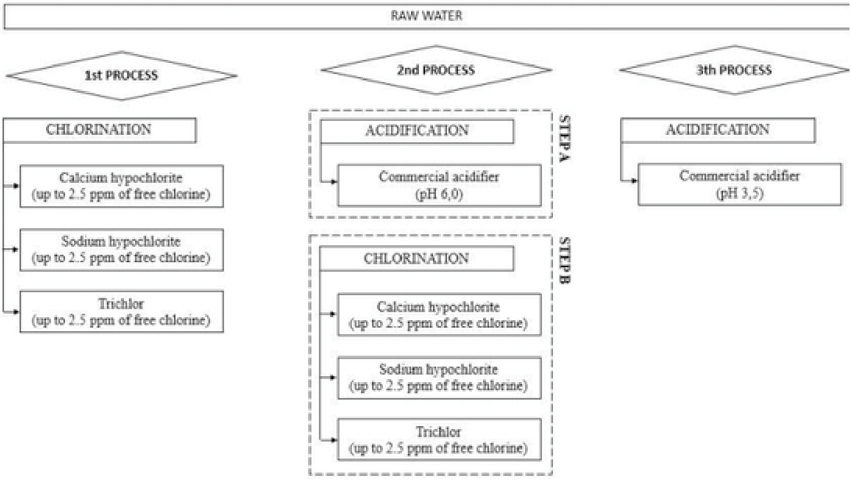

Figure 2. Raw water treatment processes of the 13 analyzed properties.

nitrates and nitrites (as nitrogen) by ion chromatography (EPA 300.1:1997) and total dissolved solids by conductivimetry (SMWWW 23 0 2510 A and B; SMWWW 230 2540 C) (INMETRO, 2019). The analyzes of total alkalinity, total hardness, nitrates, nitrites and total dissolved solids were carried out in an external laboratory.

MICROBIOLOGICAL ANALYZES

Microbiological analyzes of total coliforms, *Escherichia coli* and *Salmonella* spp. were performed in triplicate. To determine total coliforms and *Escherichia coli* in water, the SMWW 23 9221 C and 23 9223 B methodology was used (IMETRO, 2019). Analysis of *Salmonella* spp. it is not indicated in the reference in Ordinance GM/MS No. 888/2021, but it was evaluated as being a pathogen of public health importance that can be transmitted to animals and humans through contaminated food and water (Evangelista & Luciano, 2021). For the determination of *Salmonella* spp. the SMWW 23 9260 B methodology was used (IMETRO, 2019). Due to the methodology for evaluating the most probable number (MPN) of microorganisms in the broths, it was not possible to determine the mean and standard deviation, therefore the most expressive results obtained in the three replications were used for compilation in this work.

QUANTIFICATION OF CONSUMPTION OF ACIDIFIERS AND BIOCIDAL AGENTS

The raw water samples underwent three treatment processes, as shown in Figure 2, with the aim of quantifying the consumption of commercial acidifier commercial acidifier (based on ascorbic acid, citric acid, phosphoric acid, flavor, monosodium phosphate and water) and biocidal agents - 65% calcium hypochlorite ($Ca(ClO)_2$), 90% trichlor (trichloro-s-triazine-trione) and 12% sodium hypochlorite (Na(ClO)2. Trichlor and calcium hypochlorite, as they are solids, were previously diluted in drinking water with a pH close to 7.0 to obtain a 1% saturated chlorine solution.

All processes were carried out in duplicate in the laboratory with pH monitoring (portable pH meter - Digimed, São Paulo, Brazil) and ORP (pocket ORP meter - Akso, São Leopoldo, Brazil). In the chlorination processes, free residual chlorine was also measured (Free and total chlorine meter - Akso, São Leopoldo, Brazil). The chlorides and acidifier were dosed using an insulin syringe weighed on a model M214A precision scale (BEL ENGINEERING, Piracicaba, Brazil).

In the 1st PROCESS, three plastic water jugs with a capacity of 5 liters were filled with the collected water sample and chlorinated to 2.5 ppm free residual chlorine, without changing the pH of the sample. Each jar was chlorinated with a different type of chlorine. In the 2nd PROCESS, again, three jars filled with 5 liters of water were used, which were previously acidified to pH 6.0 (2nd PROCESS STEP A), followed by the chlorination process (2nd PROCESS STEP B) equal to the 1st PROCESS. In the 3rd PROCESS, a jar filled with 5 liters of water was acidified until reaching pH 3.5. Water chlorination with 2.5 ppm of chlorine was established to work with an intermediate value in accordance with Ordinance GM/MS No. 888/2021, which indicates the chlorine range between 0.2 and 5.0 ppm. The choice of pH 6.0 and pH 3.5 is due to the fact that pH 6.0 is recommended for poultry (Farias et al., 2016) and pH 3.5 for pigs (Escuredo et al., 2016).

STATISTICAL ANALYZES

Data referring to physical-chemical and microbiological analyses, consumption of biocidal and acidifying agents were analyzed using descriptive statistics with the calculation of the mean and standard deviation. In order to verify the degree of correlation between the variables well depth, nitrates/nitrites, alkalinity, hardness, pH, acidifier consumption up to pH 3.5 and pH 6.0, the Pearson correlation test was used. To interpret the degree of correlation, r = 0 was considered null, between 0 and 0.3 = weak, between 0.3 and 0.6 = regular; 0.6 and 0.9 = strong, between 0.9 and 1 = very strong and 1= full or perfect (Callegari-Jacques, 2007). The consumption of different chlorine-based agents were compared using Analysis of Variance (ANOVA) and as posts hoc tests Hol-Sindak's was used for multiple comparisons. All analyzes were performed considering a significance level of 5% ($p<0.05$) using GraphPad Prism software version 6.0.

RESULTS AND DISCUSSION

DATA RECORDED IN THE COLLECTION

Of the assessed properties, 30.77% (4/13) produced nursery pigs, 7.69% (1/13) broilers, 15.38% (2/13) laying birds, 7.69% (1/13) of laying and broilers, 15.38% (2/13) of pigs in the nursery phase and broilers and 23.08% (3/13) had a production unit of piglets. In 85% (11/13) the water was collected from artesian wells (PO) and 15% (2/13) from water source/springs (VE). The artesian well is an efficient way to capture deep water through drilling. Water from water source/springs naturally flow from the aquifer, intercepting the surface and originating a body of surface water (Hirata *et al*., 2019). Both water from wells and water source/springs generally present a low risk of contamination, are located within the boundaries of the property and their quality depends on the producer's management, protection and isolation of the springs from human and animal contact, wells built in accordance with the technical recommendations and well maintained, as well as conscious use of fertilizers and agrochemicals (Palhares, 2014). This study covered artesian wells with a depth of 60 to 600 meters, and construction time from 6 months to 30 years. The average rainfall during collections was 97 mm in September and 60.4 mm in October (National Institute of Meteorology, 2022). In general, the surroundings of the water sources had sheds for raising poultry, pigs and cattle, animal waste deposits, residences and fields. Among the properties, 53.85% are pig producers, 30.77% are laying or cutting poultry and 15.38% are poultry and pig producers. As predicted, most properties (84.62%) use the same water intended for animal consumption, also for human consumption.

PHYSICAL – CHEMICAL ANALYZES

Mean results and standard deviation of physical-chemical analyses, Table 1, from water collected from 13 rural properties, were confronted with the standards of potability of water for human consumption, considering that most properties use the same water for human and animal consumption.

The data collected from each property helped in the interpretation of the results found, since the water quality is influenced by several external factors, such as residential and industrial sewage, areas for planting and raising animals, fertirrigation with manure, among others (Bortoli *et al.,* 2018). In Rio Grande do Sul, groundwater originates from the Guarani Aquifer, which is part of the Serra Geral Aquifer System (Quaggio *et al.,* 2018), and, according to Reginato *et al.* (2012), in the state the waters are mainly composed of

Points[1]	pH	Turbidity[2]	Apparent color[2]	Total alkalinity	Total hardness[2]	ORP[4]	Total dissolved solids[2,3]	Nitrites[2,3]	Nitrates[2,3]
PO 01	7.05 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	100.8 ± 0.00	115.70 ± 0.00	426 ± 152	142.00 ± 1.00	>0.02 ± 0.00	6.66 ± 0.10
PO 02	7.12 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	97.33 ± 0.60	99.77 ± 5.37	420 ± 116	109.67 ± 0.76	>0.02 ± 0.00	2.69 ± 0.01
PO 03	8.16 ± 0.2	0.00 ± 0.00	0.00 ± 0.00	60.07 ± 1.70	36.97 ± 0.76	450 ± 145	89.80 ± 1.65	>0.02 ± 0.00	1.96 ± 0.11
PO 04	8.95 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	99.97 ± 0.55	24.93 ± 0.50	336 ± 29	151.00 ± 1.00	>0.02 ± 0.00	0.14 ± 0.01
PO 05	7.32 ± 0.04	2.21 ± 2.00	0.00 ± 0.00	132.57 ± 0.55	103.30 ± 0.44	237 ± 7	147.33 ± 2.08	>0.02 ± 0.00	0.50 ± 0.07
PO 06	7.15± 0.03	0.14 ± 0.24	0.00 ± 0.00	74.97 ± 7.93	84.7 ± 2.22	262 ± 4	105.33 ± 0.40	0.03 ± 0.01	3.44 ± 0.01
PO 07	6.57 ± 0.02	0.86 ± 0.15	0.00 ± 0.00	91.33 ± 1.29	118.10 ± 1.08	295 ± 16	149.67 ± 0.58	>0.02 ± 0.00	11.10 ± 0.32
PO 08	7.24 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	131.10 ± 2.16	217.60 ± 3.75	307 ± 40	243.33 ± 3.06	>0.02 ± 0.00	14.99 ± 0.86
PO 09	8.82 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	190.10 ± 1.65	21.63 ± 0.25	242 ± 10	218.33 ± 1.53	>0.02 ± 0.00	0.22 ± 0.02
PO 10	8.20 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	140.50 ± 2.94	56.27 ± 0.15	471 ± 146	173.33 ± 3.79	>0.02 ± 0.00	2.87 ± 0.02
PO 11	6.99 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	92.3 ± 1.31	95.97 ± 0.75	410 ± 28	100.80 ± 1.05	>0.02 ± 0.00	2.67 ± 0.06
VE 01	6.36 ± 0.16	0.10 ± 0.14	0.00 ± 0.00	17.37 ± 3.95	20.80 ± 0.17	349 ± 2	34.43 ± 0.38	>0.02 ± 0.00	3.26 ± 0.03
VE 02	8.42 ± 0.38	3.79 ± 0.36	8.00 ± 0.60	34.20 ± 0.46	38.43 ± 0.67	313 ± 29	42.97 ± 0.76	>0.02 ± 0.00	1.38 ± 0.03

Table 1. Results of physical-chemical analyzes (pH, turbidity, apparent color, total alkalinity, total hardness, ORP, total dissolved solids, nitrites and nitrates) of water collected from different poultry and/pig farming properties in Vale do Taquari/RS region.

*NOTE: Reference values according to legislation: [1]Points, PO means water from artesian wells and VE water source /springs. [2] BRAZIL. CONAMA Resolution No. 396 of 2008: Total Dissolved Solids (>1,000,000 µg.L-1), Nitrites (1,000 µg.L-1); Nitrates (10,000 µg.L-1). [3]BRASIL. GM/MS Ordinance No. 888, of May 4, 2021: Turbidity (5 uT); Apparent color (15 uH); Total hardness (300 mg/L); Total dissolved solids (500 mg/L), Nitrites (1 mg/L); Nitrates (10 mg/L). [4]Unit of measurement: mV. **Mean values and standard deviation obtained through the analysis of physico – chemical parameters in triplicate.

basaltic rocks, with calcium or magnesium bicarbonate water, neutral and slightly alkaline. Quaggio *et al.* (2018) in a study evaluating variations in the composition of groundwater in the Serra Geral Aquifer System found a pH variation between 6.3 and 10.8, which characterizes the water as predominantly alkaline. These results are in line with what was observed in the present study, where there was a pH variation between 6.36 + 0.16 to 8.95 + 0.02.

All water samples analyzed showed values allowed both by CONAMA Resolution No. 396/2008 and by Ordinance GM/MS No. 888/2021 for turbidity, apparent color, hardness, total dissolved solids and nitrites. The turbidity levels in the samples ranged from 0 to 3.79 + 0.36 NTU. Turbidity corresponds to the presence of suspended particles present in the water, which in turn can protect microorganisms against the action of chlorine (Silva Junior *et al.*, 2021).

The turbidity evidenced in the two samples of water sources/springs may have occurred due to agricultural use in the vicinity, since, second Donadio *et al.* (2005), this fact can influence the turbidity, apparent color and alkalinity, as it can facilitate the contamination of water with suspended particles.

The apparent color originates from the reflection of organic particles in the water and can also be the result of the presence of iron and manganese compounds, so it can originate from the decomposition of organic matter present in the soil and from discharges and domestic or industrial effluents and soil leaching (Libânio, 2010). In this work, the apparent color ranged from 0 to 8.0 + 0.6, and only the VE 02 sample showed alteration in the apparent color and the highest water turbidity value. This fact may have occurred due to the presence of trees in the surroundings and the decomposition processes that occur in the environment (Oliveira *et al.*, 2020) and according to Hernandes *et al.* (2010), because the water source is close to the shed that houses the pigs in the nursery phase, facilitating water contamination.

Hardness ranged from 20.80 + 0.17 to 217.60 + 3.75 mg/L, and refers to the concentration of calcium and magnesium ions expressed as carbonates and, to a lesser extent, iron, manganese, strontium and aluminum in the water (Souza & Sousa, 2020). Hardness is related to the geological nature of the watershed, and in this study the waters come from a region with a predominance of basaltic rocks (Libânio, 2010), which may have influenced the results of moderate hardness (50-150 mg/L) in 53.85% (7/13) of the samples. The total dissolved solids/water salinity ranged from 34.43 + 0.38 to 243.33 + 3.06 mg/L, and consist of a set of organic and inorganic substances. Its presence in water comes from erosive processes, organisms and organic remains, or the incorrect disposal of garbage and sewage (Souza & Sousa, 2020), among the samples, water from water sources/springs presented the lowest values of total dissolved solids, and low hardness values.

Regarding nitrite and nitrate, their occurrence is related to soil leaching or runoff of water that has been exposed to materials with high levels of nitrogen, such as animals' waste, fertilizers and decaying organic matter (Caner & Tiecher, 2017; Capoane *et al.,* 2017; Costa *et al.,* 2018;). The Vale do Taquari region occupies the top of the ranking for the production of pig manure and the second place in the production of poultry manure, so it has a great polluting potential (Schmitz, 2021). The nitrite values found were in accordance with current legislation. Nitrate levels ranged from 0.14 + 0.01 to 14.99 + 0.86 mg/L, and at points PO 07 (358 meters deep) and PO 08 (124 meters deep) they were above 10 mg /L (legislation parameter). It is believed that these results may be related to the irresponsible management of waste in the vicinity of water sources, as evidenced in loco during collection, as waste from poultry and pigs, but mainly from pigs, is rich in nitrogen. This nitrogen, in turn, in excess in the soil, increases the chance of contamination of groundwater resources with nitrate (Caner & Tiecher, 2017; Capoane *et al.,* 2017). Excessive intake of nitrates and nitrites through contaminated water and food can cause poisoning, and the clinical signs are changes in blood color and death due to oxygen deficiency (Palhares, 2014).

Total alkalinity ranged from 17.37 + 3.95 to 190.10 + 1.65 mg/L, relative to the concentration of hydroxides, carbonates and bicarbonates present in the water. These components can react with acids and generate a buffering action (Saidelles *et al.,* 2014; Mendonça *et al.*, 2019). The alkalinity of water can be of natural or anthropogenic origin. Alkalinity of natural origin occurs by the dissolution of rocks and the reaction of CO_2 with water, and the CO_2 may have originated from the atmosphere or from the decomposition of organic matter. The one of anthropogenic origin by industrial dumps (Von Sperling, 2014). The water collected from water sources/springs, obtained low alkalinity values, 17.37 + 3.95 and 34.20 + 0.46, in VE 01 and VE 02, respectively, this may have occurred due to the fact that the water sources/springs were close to the forest and susceptible to contamination by organic matter, but far from industrial areas and not suffering from the action of rock dissolution. Water with high levels of alkalinity becomes improper for consumption, as it may contain high levels of calcium and magnesium (Neto *et al.*, 2016), and this excess in the animals' bodies interferes with the absorption of other minerals such as zinc and iron, and negatively affects the feed efficiency of poultry and swine (Horwat *et al.,* 2019).

It is known that waters with changes in apparent color and turbidity also have microbial contamination (total coliform count and *Escherichia coli*). Water turbidity indicates the presence of suspended solids, such as inorganic particles of sand, silt, clay, organic debris, algae and bacteria (Américo-Pinheiro & Benini, 2018). Well depth was correlated with nitrate levels in water samples from artesian wells, and no significant relationship was found ($p = 0.59$). However, the correlation coefficient (r) shows a tendency towards a weak correlation between these variables, and because it is negative ($r = - 0.189$), it indicates that the greater the depth, the lower the levels of nitrate in the water, since the shallower the the well, the greater the chance of nitrogen particles present in the soil reaching the water. For the variables well depth and alkalinity, no significant relationship was observed either ($p = 0.2618$). However, there is a tendency that the deeper the well, the more alkaline the water is. ($r = 0.3925$). Regarding well depth and total hardness ($p = 0.1817$, $r = -0.4594$) it was also verified that, even without a significant difference, it can be suggested that the deeper the well, the less hard will the water be.

For alkalinity and total hardness, it was found that, despite not having a significant relationship ($p = 0.4143$), there is a tendency that the higher the water hardness, the higher the total alkalinity ($r = 0.2478$). This corroborates with Blumberg and Azevedo Netto (1956) who describe that alkalinity and hardness have a close relationship, because alkalinity is the result of calcium and magnesium bicarbonates, in this sense, alkalinity is equal to hardness, since hardness originates from the salts of these mines. When sodium and potassium bicarbonates contribute to alkalinity without interfering with hardness, alkalinity will exceed hardness. In studies by Silva and Valentini (2020) alkalinity exceeded hardness values at several points. In this work, 5 samples obtained higher alkalinity values when compared to hardness. The relationship established between alkalinity and hardness does not match the relationships established between these parameters and well depth, as there is a significant relationship between alkalinity and hardness, but an inverse relationship between well depth and hardness, and a positive relationship between depth and alkalinity. Perhaps a larger number of samples is needed to confirm these results. On the other hand, total hardness and time in the well showed a tendency towards a strong correlation between the variables, and being positive ($r = 0.6605$) indicates that the older the well, the greater the total hardness of the water, even without showing a significant correlation ($p = 0.0528$). The hydroxides and carbonates, characteristic of alkalinity, promote the elevation of the water pH (Mendonça *et al.,* 2019). This can be observed in the correlation between both parameters, where $r = 0.3549$ indicates a regular and positive trend that the higher the pH, the higher the alkalinity, although not significant (p = 0.2341).

There was no significant correlation between the variables total hardness and pH (p = 0.0823). However, the negative correlation coefficient (r = -0.4994) suggests that the higher the pH, the lower the hardness. On the contrary, Pereira *et al.* (2010) observed a positive correlation between water hardness and pH, as hardness is expressed in calcium carbonate, which acts to increase pH (Vasconcelos, 2015).

MICROBIOLOGICAL ANALYZES

Table 2 demonstrates the results of the microbiological analyzes. No water samples contaminated by *Salmonella* spp.

Points[1]	Total coliforms count[2,3]	*Escherichia coli* count[2,3]	*Salmonella* spp.
PO 01	>2.3x10^1	1.1x10^0	Absent
PO 02	1.1x10^0	<1.1x10^0	Absent
PO 03	5.1x10^0	2.2x10^0	Absent
PO 04	3.6x10^0	<1.1x10^0	Absent
PO 05	<1.1x10^0	<1.1x10^0	Absent
PO 06	>2.3x10^1	<1.1x10^0	Absent
PO 07	1.2x10^1	1.1x10^0	Absent
PO 08	>2.3x10^1	>2.3x10^1	Absent
PO 09	<1.1x10^0	<1.1x10^0	Absent
PO 10	>2.3x10^1	1.1x10^0	Absent
PO 11	<1.1x10^0	<1.1x10^0	Absent
VE 01	>2.3x10^1	6.9x10^0	Absent
VE 02	>2.3x10^1	>2.3x10^1	Absent

Table 2. Results of analysis of total coliforms, *Escherichia coli* and *Salmonella* spp. of raw water collected from different poultry and/or pig farms in Vale do Taquari/RS region.

NOTE: Reference values according to legislation: 1Points, PO is understood to be water from artesian wells and VE water source/strend. 2BRAZIL. CONAMA Resolution No. 396 of 2008: Total coliforms (Absence in 100 mL); Escherichia coli (Absence in 100 mL). 3BRASIL. Ordinance GM/MS No. 888, of May 4, 2021: Total coliforms (Absence in 100 mL); Escherichia coli (Absence in 100 mL).

It was decided to evaluate the raw/original water, as many rural properties are still unaware of the importance of chlorination and acidification of water. In general, there was a challenge in relation to water quality, since 76.92% (10/13) had bacterial contamination. According to Ordinance GM/MS No. 888/2021 (Brazil, 2021) and Resolution No. 396/2008 (Brazil, 2008), water used for human and animal consumption must be free of total coliforms and *Escherichia coli* in 100 mL. Of these, 23.07% (3/13) had total coliforms and 53.85% (7/13) total coliforms and *Escherichia coli* together. Of the waters with changes in microbiological parameters, 60% (6/10) are from pig producers, 20% (2/10) from poultry and 20% (2/10) from pigs and poultry. *Salmonella* spp. and *Escherichia coli* are easily eradicated from water with the use of acidifiers and chlorine-based biocides (Busser *et al.*, 2011; Li *et al.*, 2018; Abnavi *et al.*, 2022). The analysis region stands out in the production of pigs and poultry, which generates a large amount of waste that can be used in soil fertigation. However, the waste from these animals becomes potential water polluters, leading to the contamination of water with coliforms, *Escherichia coli* and other pathogens (Pahl *et al.*, 2018; Cazarotto *et al.*, 2021; Schmitz, 2021). Therefore, it is believed that the presence of bacterial contamination occurred because 84.62% (11/13) of the properties are located in regions close to animal husbandry sheds, weeds, manure beds and crops.

Bacteria belonging to the total coliform group originate from the gastrointestinal tract of humans and warm-blooded animals, and indicate the microbiological quality of the water, the integrity and cleanliness of the water distribution system, boxes and pipes, and possible contamination after treatment in reservoirs or distribution networks, as they develop in pipes under appropriate conditions (water temperature above 13ºC, available nutrients and zero residual chlorine). The genera *Escherichia, Citrobacter, Klebsiella* and *Enterobacter* belong to the total coliform group (Pahl *et al.,* 2018; Silva *et al.,* 2021; Paixão *et al*., 2022).

Escherichia coli causes diarrhea in pigs and localized or systemic infections in poultry, triggering outbreaks and carcass condemnation (Mcvey *et al*., 2013; Dawangpa *et al*., 2021). Its detection in water is indicative of fecal contamination of human and/or animal origin, and may also mean the presence of other pathogens (Libânio, 2010; Macedo *et al.,* 2021).

QUANTIFICATION OF CONSUMPTION OF ACIDIFIERS AND BIOCIDAL AGENTS

Water pH regulation is essential for the intestinal health of poultry and swine, for the modulation of the intestinal microbiota and balance between pathogenic and nonpathogenic microorganisms (Pearlin *et al*., 2020; Xu *et al*., 2022). The acidification curves were performed up to pH 3.5 (3th PROCESS) and pH 6.0 (2nd PROCESS STEP A), according to species recommendations. For pigs, a pH range for drinking water close to 4.0 is indicated, as under these conditions there is enhanced digestibility, especially of proteins (Escuredo *et al*., 2016). The supply of acidified water (pH 3.4 to 3.8) increases the water intake of pigs (Escuredo *et al.,* 2016). For poultry, the recommended pH range is close to 6.0 to improve digestibility and inhibit the growth of pathogenic bacteria, resulting in improved performance (Bailey, 2010; Farias *et al*., 2016).

The acidification and chlorination curves allowed quantifying the average consumption of products in water from different sources. To reach pH 3.5, acidifier consumption was 319.39 + 127.06 g/1000L. and to reach pH 6.0 the average consumption was 156.89 + 80.21 g/1000L. Statistical analysis indicated a significant correlation with a tendency to regular correlation between pH and acidifier consumption both for pH 3.5 (r = 0.3747) and for pH 6.0 (r = 0.4649) (p<0.05). These results indicate that the higher the pH, the higher the acidifier consumption. However, when there is an increase in acidifier consumption, this can be seen in PO 03 and VE 02. On the other hand, there are samples with a high initial pH that consume more acidifier, such as PO 09. Table 3 portrays the acidifier consumption in each property and the relationship with the initial pH of the samples.

A significant relationship (p>0.0001) was observed between alkalinity and acidifier consumption, Figure 3A and 3B, and a tendency towards a very strong correlation (r = 0.9848 for pH 3.5; r = 0.9600 for pH 6.0), so that the higher the alkalinity, the higher the acidifier consumption. Alkalinity represents the ability to neutralize acids and buffer water, so high alkalinity values indicate high buffering capacity (Mendonça *et al*., 2019).

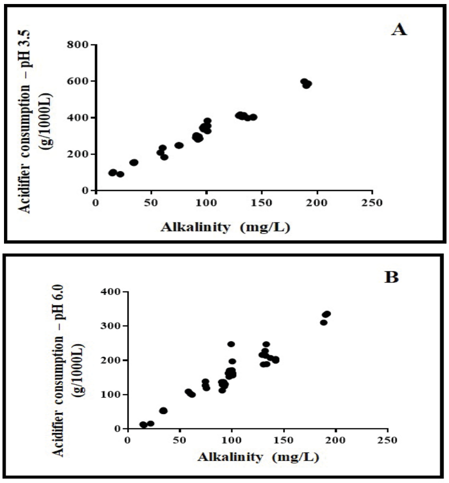

Figure 3. Correlation between acidifier consumption (g/1000L) and alkalinity (mg/L) in raw water to obtain pH 3.5 – 3th PROCESS (figure A) and pH 6.0 – 2nd PROCESS STEP A (figure B) of waters with different poultry and/or pigs breeding properties in Vale do Taquari/RS region.

Points[1]	pH result of physico – chemical analyzes	Acidifier consumption up to pH 3.5 (g/1000L) – 3th PROCESS	Acidifier consumption up to pH 6.0 (g/1000L) – 2nd PROCESS STEP A	Relationship between acidifier consumption up to pH 3.5/ pH result of physico – chemical analyzes	Relationship between acidifier consumption up to pH 6.0/ pH result of physico – chemical analyzes
PO 01	7.05	355.36	159.35	50.41	22.60
PO 02	7.12	345.18	161.66	48.48	22.71
PO 03	8.16	209.28	103.98	25.65	12.74
PO 04	8.95	353.08	205.36	39.45	22.95
PO 05	7.32	405.06	229.65	55.34	31.37
PO 06	7.15	248.30	128.00	34.73	17.90
PO 07	6.57	297.66	124.75	45.31	18.99
PO 08	7.24	414.46	197.93	57.25	27.35
PO 09	8.82	587.94	326.61	66.66	37.07
PO 10	8.2	399.19	203.48	48.68	24.81
PO 11	6.99	285.77	132.14	40.88	18.90
VE 01	6.36	96.13	13.15	15.11	2.07
VE 02	8.42	154.60	52.99	18.36	6.29

Table 3. Relationship between the pH result of the physico – chemical analyzes of the 13 water samples collected in rural properties producing swine and poultry in Vale do Taquari/RS and acidifier consumption (g/1000L) in the acidification curves up to pH 3.5 and pH 6.0.

NOTE: [1]Points, PO water from artesian wells and VE water from spring/strength.

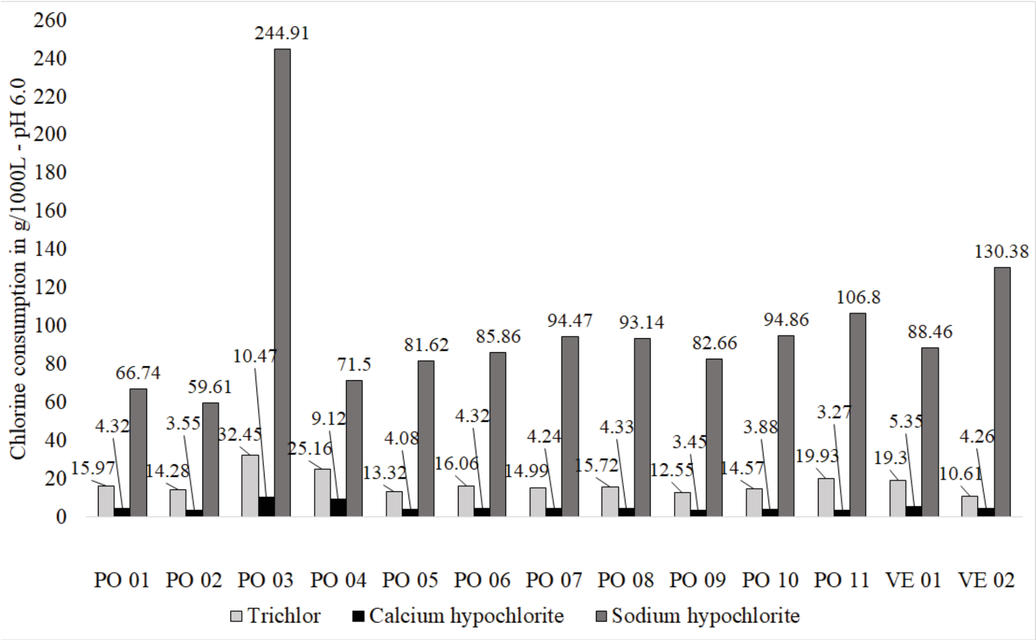

Figure 5. Average consumption (g/1000L) of chlorine-based biocidal agents (trichlor, calcium hypochlorite and sodium hypochlorite) in water with initial pH adjusted to 6.0, 2nd PROCESS, from waters from different poultry farming properties and/ or swine from Vale do Taquari/RS region.

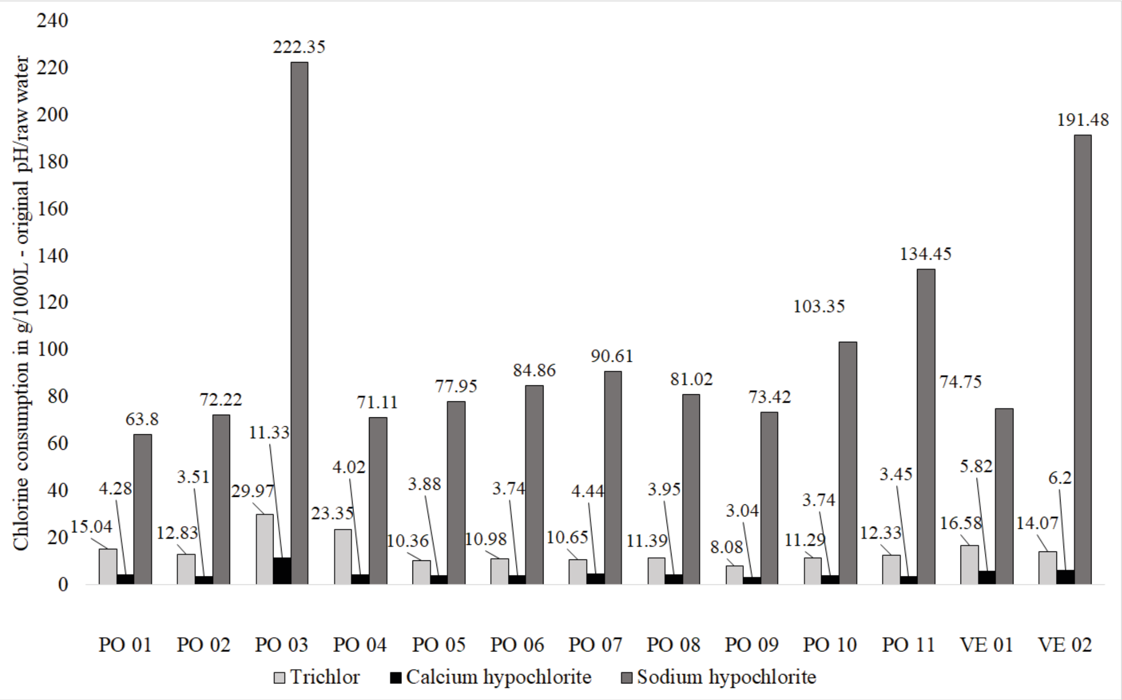

Figure 5. Average consumption (g/1000L) of chlorine-based biocidal agents (trichlor, calcium hypochlorite and sodium hypochlorite), in water with original initial pH/ raw water from water from different poultry farming properties and/ or swine from Vale do Taquari/RS region.

Points¹	1st PROCESS			2sd PROCESS STEP A	2sd PROCESS STEP B			3th PROCESS
	Chlorination up to 2.5 ppm of free residual chlorine			Acidification up to pH 6.0	Water acidification to pH 6.0, followed by chlorination to 2.5 ppm of free residual chlorine			Acidification up to pH 3.5
	Calcium hypochlorite	Trichlor	Sodium hypochlorite	----------	Calcium hypochlorite	Trichlor	Sodium hypochlorite	----------
PO 01	699	840	705	426	749	896	750	530
PO 02	689	877	695	492	749	892	748	550
PO 03	656	877	623	423	762	921	761	662
PO 04	460	846	455	431	749	898	707	578
PO 05	635	738	626	461	710	740	736	443
PO 06	666	842	660	499	739	880	736	480
PO 07	695	841	697	488	741	864	706	502
PO 08	670	846	657	533	735	871	704	533
PO 09	472	768	498	628	707	850	723	730
PO 10	574	828	545	591	725	889	728	664
PO 11	683	770	693	525	715	804	732	512
VE 01	743	891	728	495	755	890	745	500
VE 02	631	774	624	463	701	802	719	546

Table 4. Redox potential (ORP) mV of each treatment.

NOTE: ¹Points, PO water from artesian wells and VE water from water sources/springs.

For chlorination of 2.5 ppm of free chlorine (2nd PROCESS STEP B), water samples with pH adjusted to 6.0 were used. Figure 4 shows the variations in consumption at each collection point. The average consumption of calcium hypochlorite was 4.97 + 2.22 g/1000L and of trichlor was 17.30 + 5.87 g/1000L, representing a consuption 3.48 times more trichlor comared to calcium hypochlorite. Finally, sodium hypochlorite consumed an average of 100.08 + 47.08 g/1000L, symbolizing the highest consumption, 20.14 and 5.83 times more than calcium hypochlorite and trichlor, respectively.

Figure 5 represents the variation in chlorine consumption in water samples considering the original pH/raw water (1st PROCESS). In this process, the average consumption of calcium hypochlorite was 4.72 + 2.18 g /1000L, of trichlor was 14.38 + 6.02 g/1000L, and of sodium hypochlorite was 103.17 + 49.86 g/1000L. The relationship between biocidal agents was once again confirmed, highlighting sodium hypochlorite with 21.86 and 7.18 times more consumption in relation to calcium hypochlorite and trichlor, respectively.

The comparison between the consumption of different chlorines in water previously acidified up to pH 6.0 (2nd PROCESS) and in raw water (1st PROCESS) showed that there was no significant difference between the consumption of trichlor and calcium hypochlorite for both acidified and chlorinated water. However, there was a significant variation between the consumption of trichlor and sodium hypochlorite, and between calcium hypochlorite and sodium hypochlorite regardless of the type of water, Figure 6A and 6B.

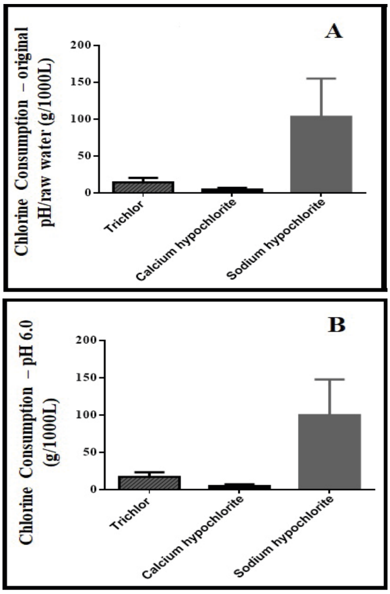

Figure 6. Average consumption of trichlor, calcium hypochlorite and sodium hypochlorite (g/1000L) in raw water/without acidification at the original pH of the physical-chemical analysis – 1st PROCESS (figure A) and water with pH 6.0 – 2nd PROCESS (figure B).

On average, there was a consumption 3.27 times greater of trichlor and 21.00 times greater of sodium hypochlorite, in relation to calcium hypochlorite. These results indicate that the concentration of active content of biocides should not support the final consumption calculation. This is due to the formation of by-products that are generated after adding chlorine to water, for example, each 10 ppm of 90% trichlor forms 9.1 ppm of cyanuric acid, and due to the formation of this by-product its use is discouraged in some countries (World Health Organization, 2022).

The chlorine-based biocidal agent in contact with water dissociates into hypochlorous acid and hypochlorite ion. Hypochlorous acid is primarily responsible for water disinfection, as hypochlorite ion has low disinfection power. The pH interferes with the disinfection power of chlorine, with a pH lower than 7.0 being recommended so that the reaction of chlorine with water forms approximately 78%

of hypochlorous acid (strong biocide). At pHs close to 8.0, the concentrations of the formed components are inverted, with formation of only 28% of hypochlorous acid (Rossi-Fedele *et al*., 2011). With the decrease in the disinfecting power of chlorine, it is necessary to increase its dosage to obtain the desired efficiency, or to increase the contact time. This reinforces the need to acidify the water in conjunction with chlorination.

In this study, sodium hypochlorite was the most consumed biocidal agent compared to other chlorine-based products. It should be noted that this compound has a low active content, is unstable and has a short shelf life, and its stability is influenced by storage conditions (Gomes *et al*., 2020). Its use is a challenge in rural properties due to product storage conditions and exposure to sunlight, which significantly alter its concentration (Freitas *et al.,* 2021). Calcium hypochlorite is a solid, safe and stable product (Mohammed, 2019), easy to administer using tablets and dosing equipment, or in the form of a concentrated solution for dosing using electric pumps. With regard to trichlor, it releases cyanuric acid as a by-product, which reacts with hypochlorous acid, decreasing the concentration of the strong biocide and increasing the contact time required to inactivate pathogens in the water. In addition, trichlor imparts a strong odor, impairing the palatability of the water (Wahman, 2018; Falk *et al*., 2019).

EVALUATION OF REDOX POTENTIAL (ORP)

ORP is a measure of the oxidation and reduction activity of substances used to evaluate water disinfection as an indirect parameter of antimicrobial potential. The oxidation-reduction potential generated by the movement of electrons (in mV), between 650 and 700 mV, is sufficient to eliminate most pathogenic bacteria from the water in 30 seconds, as it causes the oxidation of the cell membranes of microorganisms, culminating in their death (Cano & Carrera, 2020). In raw water samples, the ORP was below the recommended level, therefore untreated water (chlorination or acidification) is more conducive to the development of microorganisms.

The process of acidification up to pH 3.5 increased the final ORP of the water, but only three samples (PO 03, PO 09 and PO 10) obtained values above 650 mV. In the acidification process up to pH 6.0, only one sample (PO 09) obtained a value greater than 650 mV. And the same situation was repeated, when the waters of original pH/raw water were only chlorinated up to 2.5 ppm.

The Table 4 presents the ORP results of water samples with treatments with biocidal agents. It is observed that, in all waters, regardless of the type of chlorine used, the combination of two treatment processes (acidification and chlorination) guarantees ORP results above 650 mV and consequent optimization of the decontamination process of poultry water and swine.

CONCLUSIONS

The results found in the present study demonstrated that the physical-chemical quality of the water in poultry and/or pig breeding properties in Vale do Taquari/RS region can be considered, in general, satisfactory, despite being a region fertirrigated with manure. Within the same region and the same type of source, it was observed that the composition of the waters varied between the properties, which proves that the waters are not equal. The relationship observed between alkalinity and acidifier consumption serves as a subsidy to reinforce that prior analysis of the water to start the acidification and chlorination process is fundamental, in order to guarantee greater assertiveness. In addition, it was possible to prove that in order to obtain a greater efficiency of chlorination, a previous acidification of the water is necessary so that the ORP reaches levels that favor decontamination. This study also proved that calcium hypochlorite was the most efficient biocidal agent in the water chlorination process, offering the best cost-benefit ratio, consuming, on average, 3.27 times less than trichlor and 21 times less than sodium hypochlorite. Therefore, it is recommend using calcium hypochlorite in conjunction with the acidifier. The data obtained in this study are unprecedented and can be used as a decision-making tool in the field in order to improve the quality of drinking water for poultry and swine.

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