Experiments on the Pond Lime Requirement Test and the Effect of Organic Matter on Dissolution of Agricultural Limestone in Aquaculture Ponds

by

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Abstract

Three investigations were performed related to limestone in aquaculture.

The first investigation studied the lime requirement of ponds can be determined by direct titration with standard sulfuric acid of the amount of alkalinity neutralized by the exchangeable acidity displaced from a bottom soil sample equilibrated with a solution of 1.0 N in potassium ion and 0.04 N in bicarbonate (2 mg $CaCO_3$ equivalent mL^{-1}). This procedure, called the K-bicarbonate method here, provided precise estimates of lime requirement that were 9.6% to 27.2% (average = 12.9%) greater than those obtained by the method currently recommended for use on pond soils. It is likely that the K-bicarbonate method neutralizes more of the exchangeable acidity in pond bottom soil samples than does the current method recommended for pond bottom soils and thereby provides a more reliable estimate of lime requirement. Moreover, the K bicarbonate method does not require a hazardous chemical, a mechanical shaker, or a pH meter as does the current method for pond lime requirement. The cost per analysis also is cheaper by the K-bicarbonate method than with the method currently recommended for pond soils.

The second study assessed the influence of single, initial chicken manure applications of 0, 1,000, 2,000, and 4,000 kg/ha on the solubility of agricultural limestone in ponds fertilized with chemical fertilizers. The two higher manure application rates resulted in greater alkalinity ($P < 0.05$) than achieved in control ponds. However, control ponds with alkalinity concentrations of about 40 mg/L did not differ ($P > 0.05$) in chlorophyll *a* concentration from the 2,000 and 4,000 kg/ha manure treatments. Although net yield of tilapia (*Oreochromis niloticus*) was numerically higher in ponds of the 2,000 and 4,000 kg/ha manure treatments, it did not differ from the control ($P > 0.05$)

Laboratory soil-water systems to which organic matter concentrations in soil were increased by 0.0, 0.5, 1.0, 2.0, and 4.0% were established by adding dry, chopped ryegrass (*Lolium perenne*) shoots to sandy soil containing 0.06% organic matter. Agricultural limestone was added to the systems, and pH, alkalinity, and hardness was monitored for 65 d. The pH, alkalinity and hardness of water increased with increasing soil organic matter concentration. The amounts of carbon dioxide released by microbial respiration increased as soil organic matter concentrations increased. Greater carbon dioxide concentrations resulted in more rapid and greater solubility of agricultural limestone. Although organic matter in pond soils would not decompose as rapidly as ryegrass, results indicate that greater soil organic matter concentrations in ponds would favor the rate and extent of agricultural limestone dissolution.

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CHAPTER I. – INTRODUCTION AND LITERATURE REVIEW

1.1 Rationale

Aquaculture ponds often are located in areas with highly-leached, acidic soils. Ponds in such areas typically have less than 20 mg L^{-1} of total alkalinity and bottom soil pH <6 (Boyd 2017). Such waters are poorly buffered against daily changes in pH resulting from varying rates of carbon dioxide removal by photosynthesis and return of carbon dioxide to the water by respiration. There also is a limited amount of carbon dioxide for photosynthesis in low alkalinity water, and phosphate added in fertilizers will be strongly absorbed by acidic bottom soil. Agricultural limestone often is applied to such ponds to raise the pH of bottom soil and increase the total alkalinity of pond water (Boyd and Tucker 1998).

The determination of liming rate in ponds has traditionally been problematic, because of the lack of a simple means of determining the amount of liming material needed to provide adequate alkalinity (Boyd 2017). Ponds differ greatly in bottom soil properties across and among regions, and these differences affect the liming rate. Methods for determining lime requirement of bottom soils have been developed for agricultural soils, and these methods were modified for use in pond aquaculture (Boyd 1974; Pillai and Boyd 1985). However, the pond lime requirement procedures have not been widely adopted in aquaculture, because they require specialized and hazardous chemicals, expanded-scale pH meters, and other items typically unavailable at aquaculture farms or aquaculture laboratories.

Agricultural limestone is not very soluble in water, and its solubility is closely related to the presence and concentration of carbon dioxide. In water bodies where atmospheric carbon dioxide is the main source of dissolved carbon in the water, the equilibrium alkalinity in water at equilibrium with atmospheric carbon dioxide is around 60 mg L^{-1} and up to 60 days may be required for attainment of this equilibrium concentration (Sá and Boyd, 2017). Boyd and Tucker (1998) suggested that organic matter applications at the time of liming should accelerate the rate and extent of agricultural limestone dissolution, but experimental data to verify this hypothesis are not available. One also might expect, based on Boyd and Tucker’s hypothesis, that agricultural limestone might dissolve faster in ponds with higher concentrations of organic matter in bottom soils.

1.2 Objectives

The objectives of the present study were as follows:

- Develop a simple lime requirement test for aquaculture ponds based on an acid-base titration technique.

- Determine if chicken manure added to fertilized ponds would allow a higher concentration of alkalinity following application of agricultural limestone and be beneficial to tilapia production.

- Determine in a laboratory study whether greater concentration of organic matter in bottom soils increased the amount of alkalinity following agricultural limestone application.

1.3 Pond Production of Tilapia

Tilapia are native to Africa and the Middle East. These species live primarily in shallow streams, ponds, rivers and lakes, but they also are found in brackishwater. Tilapia is a relatively new aquaculture product in the Americas. Aquaculture of tilapia in South and Central America began with small-scale culture for subsistence farming in the late 1960s and 1970s (Fitzsimmons 2000). The climate in the continental United States is not conducive to tilapia culture, but it is cultured at a few locations under the cover of greenhouses or in indoor, water-recirculating systems (Boyd and Tucker 2014).

Tilapia remains a popular product in the retail sector, and recently whole, fresh tilapia can increasingly be found in supermarkets. National Marine Fisheries Service (NMFS) trade statistics reported that there were 226,000 tonnes of whole fresh tilapia imports to the United States during the first quarter of 2015, up from 83,000 tonnes during the same time period the previous year. The major suppliers were Myanmar, Bangladesh, Pakistan, and China. Mexico was the largest supplier from Latin America. Global tilapia production exceeded 4.85 million metric tons in 2014 (ftp://ftp.fao.org/FI/STAT/summary/default.htm).

Tilapia are considered ideal candidates for aquaculture because of their tolerance of wide pH fluctuations (Reite et al., 1973; George 1975), extreme temperatures (Li Kuang Cong et al.

1961; Denzer 1967), high ammonia and nitrite concentrations (Redner and Stickney 1979) and low dissolved oxygen concentrations (Maruyama 1958; Lowe-McConnell 1959). Some species can even be cultured at high salinities (Chervinski and Hering 1973; Watanabe et al. 1997). Tilapia are comparatively more resistant to most fish diseases and parasites than other common aquaculture species (Plumb 1997).

Manure has a long tradition of use for fertilizing tilapia and other aquaculture ponds. However, large applications of manure to ponds may result in water quality problems (Boyd and Tucker, 2014). The term manure applies to livestock dung, plant wastes and by-products from agriculture, and cut fresh grass and dry hay. The composition of manure varies greatly. Midwest Plan Service (2004) reported nutrient concentrations in manure for many animal types and manure storage and handling systems (Table 1.1). Phosphorus is the primary nutrient that impacts on surface freshwater resources such as streams, lakes and wetlands. Excess phosphorus will increase algae growth which decomposes and consumes oxygen. Phosphorus is needed in ponds to stimulate phytoplankton growth (Boyd and Tucker 2014), but too much phytoplankton can lead to dissolved oxygen depletion. Fish cannot live without enough oxygen in the water. Nitrogen is important in pond aquaculture because it is a major component of the protein of plants and animals and influences productivity. The major forms of inorganic nitrogen are ammonia, nitrate, and nitrite. Excess ammonia nitrogen can be toxic to fish (Boyd and Tucker 2014). Nitrate which results from the nitrification of ammonia is not toxic to fish, but nitrate in drinking water can cause severe illness in infants and livestock. Nitrite in ponds can be toxic to fish. Animal feed lots, agricultural fertilizers, manure, industrial waste waters are common sources of excess nitrogenous compounds reaching lakes and streams.

Total fertilizer nutrient concentration rarely exceeds 10% in most manure sources and frequently is a fraction of that. For example, nitrogen, phosphate and potash are approximately 8.5% of the weight of poultry litter, 1.5% of the weight of hog slurry and 0.2% of the weight of hog lagoon effluent. Most commercial grade fertilizers exceed 30% nutrient concentration by weight (Boyd and Tucker 2014). Low nutrient concentration increases the time and cost of transportation and land application of manure (Lory et al. 2006). Organic manures, being less expensive compared to chemical fertilizer, contain almost all the essential nutrient elements (Jana et al. 2001). Thus, they can be effective pond fertilizers.

Fish production is an important sector of the aquaculture industry. The use of manure in aquaculture supports the production of protein using inputs of little nutrient value to man or livestock (Wohlfarth and Hulata 1987). Manures stimulate both primary and secondary productivity (Schroeder 1978) and may be more available or cheaper than commercial fertilizers and formulated diets. Animal manure is often used in semi-intensive systems to improve the primary production of ponds and increase fish growth (Boyd 1982). Knud-Hansen et al. (1991) reported that organic manure contributed to fish production by supplying inorganic phosphorus, nitrogen, and carbon for algal growth, and by stimulating detrital production and heterotrophic utilization. The manure is directly consumed by fish, and the released nutrients support the growth of mainly photosynthetic organisms (Moav et al. 1977; Little and Edwards 1999). Additionally manures are applied in terrestrial agriculture to increase soil organic matter concentration which improves soil texture and when it decomposes it releases plant nutrients (Sloan et al. 2003).

High fish yields are possible in manured ponds (Moav et al. 1977; Wohlfarth and Schroeder 1979). Thus, it is not surprising that many studies on manuring have been conducted across a wide range of pond culture systems (McGeachin and Stickney 1982; Burns and Stickney 1980; Behrends et al. 1982; Hopkins and Cruz 1982). Stickney et al. (1979) indicated that growth of *T. nilotica* was most rapid in ponds that had a history of relatively high manuring rates. Green et al. (1989), Zhu et al. (1990), Dhawan and Kaur (2002), and Stickney et al. (1979) reported an increase in fish yields as a result of organic fertilization. Terziyski et al. (2007) reported ponds treated with cattle manure demonstrated significantly higher productivity than the controls

Boyd (1982) reported that poultry manure triggers more production of phytoplankton in ponds than any organic fertilizers including chemical fertilizer. The poultry industry is one of the largest and fastest growing sectors of livestock production in the world, with meat and egg production currently growing at an annual rate of approximately 5% (Sims and Wolf 1994). Utilizing this large quantity of poultry manure could bring many benefits to aquaculture management. Poultry manure has a higher fraction of biodegradable organic matter than other livestock wastes (Hill 1983; Jewell and Loehr 1977; Morris and Casler 1977). Wastes from poultry tend to have higher concentrations of nitrogen, phosphorus, and potassium compared with other sources of organic animal manures. Chicken litter has been used successfully as an organic fertilizer for tilapia production in many parts of the world (Miller 1975; Lovshin 1977; Rappaport and Sarig 1978; Green et al. 1989). Different kinds of manure can be utilized; cow, swine, poultry, sheep, etc. Among manures used, chicken manure is preferred because of its ready solubility and high level of phosphorus (Knud-Hansen et al. 1991). Kang'ombe et al. (2006) suggest that the use of chicken manure produces better results than cattle and pig manure treatments in ponds.

Most farmers use manure to ponds without adequate management, and while fish production may increase, manure may also lead to pollution both within the ponds and into waters which ponds drain. It is necessary to keep water quality at an acceptable range in ponds to benefit the production of fish. Qin et al. (1995) investigated the increased oxygen consumption and water-quality deterioration at varying doses of organic fertilizer. Similar negative effects during the first month of manuring were also reported by Lin et al. (1999) when green weed material was applied. Nevertheless, it is clear that manuring can result in as much or more fish production than possible with commercial (N-P-K) fertilizers.

1.4 Effects of Liming

Aquaculture ponds are seldom built where pH is extremely low, but ponds with acidic bottom soils and low alkalinity water are common. Neutralization of bottom soil acidity leads to better growth of benthic food organisms for fish (Bowling 1962), and it increases the rate of microbial recycling of nitrogen and phosphorus from organic matter (Boyd and Pipoppinyo 1994). Phosphorus from pond bottoms is most available to plants in the water column at soil pH 6.5-7.0 (Boyd and Scarsbrook 1974). Liming increases alkalinity, improving the buffering capacity of water and providing more inorganic carbon for phytoplankton photosynthesis (Boyd et al. 2016). Liming increases calcium or calcium and magnesium concentrations in pond water to facilitate flocculation and sedimentation of suspended particles (Boyd and Tucker 1998) and to raise the pH to favor microbial degradation of dissolved humic substances (Hasler et al. 1951). These effects lessen turbidity to improve light penetration for photosynthesis. The effects of liming allow greater aquacultural production than possible without liming in fertilized ponds with low alkalinity water. Liming alone usually will not increase production, but the combination of liming and fertilization typically increases production by 25 to 200% over that possible with fertilization alone (Mortimer 1954; Boyd and Tucker 1998; Hickling 1962; Arce and Boyd 1975).

Fish and shrimp production in ponds often is increased with manufactured feed (Boyd and Tucker 1998; Boyd and McNevin 2015; Murad and Boyd 1991). Such ponds often are limed and fertilized at the beginning of the production cycle to enhance natural food availability for newly-stocked postlarvae or fingerlings that cannot use feed as effectively as can larger life stages (Boyd and Tucker 1998). Nutrients from uneaten feed and feces of the culture animals result in dense phytoplankton blooms. In low alkalinity water, wide daily fluctuations in pH result from phytoplankton photosynthesis and community respiration (Boyd and Tucker 1998). High afternoon pH may stress aquatic animals directly (Boyd 2015). Nitrogen added in feed but not assimilated into biomass of culture animals enters the water as ammonia (NH_3) (Boyd and Tucker 1998). Aqueous ammonia and ammonium (NH_4^+) occur in a pH and temperature dependent equilibrium (Trussell 1972):

$$NH_3 + H^+ = NH_4^+. \tag{1.1}$$

Ammonia is potentially toxic to aquatic animals, and as pH rises, ammonia, in un-ionized form) increases in proportion to the ionized form ammonium. The analytical procedure for ammonia nitrogen measures both ammonia and ammonium (total ammonia nitrogen or TAN). High pH (≥8.5) in waters with elevated TAN concentration can cause NH_3 toxicity and stress or possibly kill culture animals (Hargreaves and Kucuk 2001; Hargreaves and Tucker 2004; Zhou and Boyd 2015).

Liming increases buffering capacity to minimize the pH increase when photosynthesis is proceeding rapidly (Boyd and Tucker 1998).

Burnt or hydrated lime application to bottoms of empty ponds can increase soil pH enough to kill unwanted organisms including vectors of disease (Flamm et al. 2016; Suresh et al. 2006; Sills 1974). When dissolved oxygen concentration is dangerously low, burnt or hydrated lime can be applied to remove carbon dioxide (Hansell and Boyd 1980) that interferes with oxygen absorption by aquatic animals (Boyd 2015; Hansell and Boyd 1980). Furthermore, many farmers believe that small, frequent doses of lime precipitate phosphate, remove carbon dioxide, and reduce phytoplankton growth to maintain pH between 7.8-8.2. Shrimp farmers also believe that calcium favors molting of shrimp (Boyd et al. 2017). The benefits of frequent applications of lime are dubious.

In highly-intensive aquaculture – such as plastic-lined ponds and water recirculating systems – liming may be necessary to replace alkalinity neutralized by nitrification (Avnimelech

2012; Boyd and Tucker 2014). Sodium carbonate (Na_2CO_3) or sodium bicarbonate ($NaHCO_3$) often are used because of their high solubility (Boyd et al. 2016).

1.5 Source of Liming Materials

The common source of liming materials is limestone, a mineral comprised of calcium carbonate ($CaCO_3$) or a mixture of calcium carbonate and magnesium carbonate ($MgCO_3$) (Bliss et al. 2012). Substances similar to limestone in composition are chalk (a fine-grained, marine limestone), marl (calcium carbonate mixed with clay that precipitated from lakes), and seashells (Bliss et al. 2008).

Agricultural limestone is made by crushing limestone rock, chalk, marl, or seashells. At least 50% of particles in most agricultural limestones will pass a screen with 0.25-mm apertures (Brady and Weil 2007). Agricultural limestone is sometimes mixed with 8-10% of lignosulfonate and pelletized. The pellets react no faster than ground limestone of similar particle size (Murdock 1997). Liquid agricultural limestone is made by suspending in water limestone particles about 90% of which pass a screen with 0.075-mm apertures. Attapulgite clay is sometimes added to maintain particles in suspension. This product reacts faster than traditional or pelletized agricultural limestone (Wurts 2014), but it has less acid-neutralizing capacity per unit weight than does dry agricultural limestone. Pelletized and liquid agricultural limestone are more expensive than normal agricultural limestone.

Limestone can be heated at 900-1,000°C in a kiln to drive off carbon dioxide (Wingate 1985) to produce burnt lime as illustrated below for conversion of calcium carbonate to calcium oxide (CaO):

$$\mathrm{CaCO_3} \underset{\Delta}{\rightarrow} \mathrm{CaO} + \mathrm{CO_2}. \tag{1.2}$$

If limestone is not pure calcium carbonate, both calcium oxide and magnesium oxide (MgO) will be in the product. Burnt lime is also called quick lime or unslaked lime.

Burnt lime can be treated with water to produce hydrated lime as illustrated below for the hydration of pure calcium oxide:

$$\mathrm{CaO} + \mathrm{H_2O} \rightarrow \mathrm{Ca(OH)_2}. \tag{1.3}$$

Hydrated lime also may contain magnesium hydroxide ($Mg(OH)_2$). It also is known as builder's lime or slaked lime.

1.6 Lime Requirement

It may seem that the liming rate for ponds could be calculated from the difference in the desired alkalinity and the initial alkalinity. For example, if a 10,000 m^3 pond has an alkalinity of 10 mg L^{-1} (as $CaCO_3$), and the target alkalinity after liming is 50 mg L^{-1}, 400 kg $CaCO_3$ [10,000 $m^3 \times (50 - 10)$ g $CaCO_3$ $m^{-3} \times 10^{-3}$ kg g^{-1}] must be added to the water. This amount of agricultural limestone would be neutralized by acidity in the bottom soil without increasing alkalinity.

Liming rates can be determined in pond experiments in which different amounts of liming material are applied and the alkalinity measured. Hickling (1962) reported results of such an experiment in ponds at a research station at Malacca, Malaysia as follows:

Agricultural limestone (kg/ha)	Alkalinity (mg/L as $CaCO_3$)
0	12
500	20
1,000	30
1,500	35
2,000	40
4,000	55

Alkalinity reached the expected equilibrium concentration at the highest agricultural limestone application rate. Such data are site specific, and ponds with different bottom soil properties would respond differently.

The Adams-Evans buffer solution method (Adams and Evans 1962) for determining liming rates for agricultural crop soils in the southeastern United States was modified for application to sportfish ponds (Boyd 1974). The procedure, called the Alabama pond method (AP method), gave the lime requirement for lowering base unsaturation of soil to 0.25 and consistently increasing alkalinity to 20-30 mg L^{-1}. The AP method has been widely used for sportfish ponds in the southeastern United States. However, because of regional differences in soil properties and the need for greater alkalinity in ponds for food fish and crustaceans than in sportfish ponds, the AP method is not widely applicable. The authors are aware that the AP method has been tried in several other areas of the United States and in Europe and Asia. Some of these trials proved successful while others did not.

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Table 1 Estimated nutrient concentration in manure for selected animal types and manure storage and handling systems. Data is adapted from *Manure Characteristics* (MWPS-18, 2004)

Livestock system	Units	Total Nitrogen (N)	Ammonium N	Phosphate (P_2O_5)	Potash (K_20)
Pig, nursery, pit slurry	lbs/1000 gal.	25	14	19	12
Pig, grow-finish, deep- pit slurry	lbs/1000 gal.	50	33	42	30
Pig, farrow-finish, pit slurry	lbs/1000 gal.	28	16	24	23
Dairy cow, pit slurry	lbs/1000 gal.	31	6	15	19
Layer hen, pit slurry	lbs/1000 gal.	57	37	52	33
Pig, grow-finish, lagoon water	lbs/acre-in	113	113	56	85
Pig, farrow-finish, lagoon water	lbs/acre-in	127	113	81	102
Dairy cow, lagoon water	lbs/acre-in	114	102	47	82
Broiler, dry litter	lbs/ton	46	12	53	36
Turkey, dry litter	lbs/ton	40	8	50	30

CHAPTER II. K BICARBONATE METHODS FOR LIME REQUIREMENT

2.1 Introduction

Agricultural limestone or other liming materials often are applied to sportfish ponds in the United States and to food fish and crustacean ponds worldwide to neutralize bottom soil acidity and maintain an adequate concentration of total alkalinity in water (Boyd and Tucker 2014). As there was not a reliable way of determining the amount of liming material needed in ponds, Boyd (1974) slightly modified the Adams-Evans (AE) buffer solution method (Adams and Evans 1962) often used for establishing liming rates for agricultural crop soils in the southeastern United States for application to sportfish ponds. However, because of regional differences in soil properties and the need for greater total alkalinity in ponds for culture of food fish and crustaceans than in sportfish ponds, the Boyd (1974) modification for ponds of the AE lime requirement method was not applicable outside the southeastern United States. Pillai and Boyd (1985) made changes in the lime requirement method for sportfish ponds that allows its use for pond soils worldwide irrespective of their properties provided they are not pyritic (potential or active acid-sulfate soils).

The lime requirement procedure for sportfish ponds was shown to consistently estimate adequate liming material to raise pH of bottom soil to 6.0 and total alkalinity to 20-30 mg L^{-1} (Boyd 1974), and it has been widely used in the southeastern United States. The more general method of Pillai and Boyd (1985) – called the PB method hence forth – raised soil pH in ponds to 7.0-7.5 and provided total alkalinity of 40-50 mg L^{-1}. However, the PB method has not been adopted widely; its use is hampered by the necessity for a specialized and hazardous chemical (*p*-nitrophenol or 4-nitrophenol) and a pH meter capable of measuring to the nearest 0.01 pH unit. There are several other buffer methods in addition to the AE method for use on agricultural soils that could be modified for ponds (Shoemaker et al. 1961; Lierop and Westerman 1990; Godsey et al. 2007; Pagani and Mallarino 2011), but the determination of lime requirement by buffer methods requires an accurate measurement of the pH change in the buffer solution caused by the soil sample.

The principle of buffer methods such as the AE method and its modifications for ponds is rather simple. Potassium (K^+) or some other basic cation in the buffer solution exchanges for acidic ions – mainly aluminum ion (Al^{3+}) – held on cation exchange sites in the soil as illustrated below:

$$\text{Soil} - Al^{3+} + 3K^+ \rightleftharpoons \text{Soil} - 3K^+ + Al^{3+}. \quad (2.1)$$

Aluminum ion is displaced from the soil and hydrolyses in solution resulting in acidity (H^+):

$$Al^{3+} + 3H_2O \rightleftharpoons Al(OH)_3\downarrow + 3H^+. \quad (2.2)$$

Hydrogen ion released by hydrolysis causes the pH of the buffer to decline in direct proportion to the quantity of exchangeable aluminum ion and other acidic ions (H^+ and Fe^{3+}) on the soil. By measuring the pH change of the buffer, the amount of exchangeable acidity in the soil can be determined.

The pH of soil is related to its base unsaturation that is the ratio of exchangeable acidic ions to the cation exchange capacity (CEC) of the soil (Boyd 1995). If the graphical relationship of base unsaturation to pH is obtained for soils in a particular region, the base unsaturation at any given pH may be determined (Adams and Evans 1962). The pH change of the buffer provides an estimate of the exchangeable acidity of the soil, and the amount of exchangeable acidity that must be neutralized to give a particular pH – and a specific base unsaturation – may be calculated:

$$\mathrm{EA_n} = \mathrm{EA_t} \times \frac{\mathrm{BUS_i}}{\mathrm{BUS_i} - \mathrm{BUS_d}} \qquad (2.3)$$

where EA_n = exchange acidity to be neutralized (meq kg^{-1}); EA_t = total exchangeable acidity estimated from pH change in the buffer (meq kg^{-1}); BUS_i = initial base unsaturation; BUS_d = desired base unsaturation for desired soil pH. The quantity $BUS_i – BUS_d$ represents the fraction of EA_t that must be neutralized to provide the desired soil pH.

The acidity to neutralize (EA_n) can be converted to a liming rate (mg $CaCO_3$ kg $soil^{-1}$) by multiplying by the factor 50 mg $CaCO_3$ meq^{-1}. The results may be expanded for the weight of soil to be limed to give a lime requirement. In agriculture, the lime requirement typically is reported as the amount of calcium carbonate necessary to raise the pH of the upper 15-cm layer of soil that weighs about 2,240,000 kg ha^{-1} to the desired pH (Brady and Weil 2008). The acid neutralizing value of liming materials in percentage $CaCO_3$ (pure $CaCO_3$ = 100%) must be known in order to adjust the lime requirement to a liming rate for the particular liming material to be applied (Boyd and Tucker 2014).

Agricultural crops differ in pH preference, and buffer methods allow liming rates necessary to attain specific soil pHs. In pond aquaculture, there is no known reason to attain a specific pH in the bottom soil. The objective is to increase soil pH and allow greater total alkalinity in the water (Boyd 1995). The PB method was designed to neutralize a large amount of exchangeable acidity and lower base unsaturation as much as possible. This approach has a considerable advantage, because a graphical relationship between base unsaturation and pH of soils is not needed. The weight of pond soils is typically less than that of agricultural soils; the average dry weight of the 1 ha × 15 cm layer of pond soil is about 1,500,000 kg ha^{-1} (Boyd and Cuenco 1980; Boyd et al. 2010).

The basis for the new lime requirement method proposed and tested in the present study involves use of a potassium chloride-potassium bicarbonate solution rather than the buffer solution containing *p*-nitrophenol of the AE (and PB) methods. The concentration of potassium ion in the solution for the proposed method (called the K-bicarbonate method hence forth) is almost twice that of the buffer solution for the PB method to displace as much exchangeable aluminum ion from the soil sample as possible by exchange for potassium. The solution for the

K-bicarbonate method is 0.04 N in bicarbonate (equal to 2 mg mL^{-1} total alkalinity as $CaCO_3$ equivalent). The principle of the method is to displace aluminum ion by potassium ion (as in the AE and PB methods). However, in the K-bicarbonate method, hydrogen ion from hydrolysis of aluminum ion displaced from soil will neutralize bicarbonate, and the decrease in the total alkalinity of the solution – that can be measured by acidimetry – allows a direct estimate of the amount of liming material (as $CaCO_3$ equivalent) necessary to neutralize soil acidity and lower base unsaturation as much as possible. The pH of pond bottom soils with very low base unsaturation typically is 7.0 to 7.5 (Pillai and Boyd 1985).

2.2 Materials and Methods

2.2.1 Soil samples

Fifteen soil samples (5-cm diameter × 15-cm deep cores) for use in this study were collected from ponds on the E. W. Shell Fisheries Center (SFC) at Auburn University by a procedure described by Munsiri et al. (1995). Thirty samples from sites representing major physiographic regions and soil groups in Alabama were available from a previous study (Ou 2013). The soils from the SFC were dried in a mechanical oven at 60 °C and pulverized to pass a sieve with 0.85-mm apertures – the same procedure that was used for the other samples. Dry samples were stored in plastic bags until used in analyses.

The pH of all soil samples was determined in 1:1 mixtures of dry pulverized soil and distilled water (Thunjai et al. 2001). The organic carbon concentration also was determined by sulfuric acid-potassium dichromate digestion (Walkley-Black method) described by Nelson and Sommers (1982).

2.2.2 Proposed K-bicarbonate lime requirement method

<u>Analysis</u>

1. Prepare a solution 1.0 N in potassium ion and 0.04 N in bicarbonate (2 mg $CaCO_3$ equivalent mL^{-1}) by diluting 71.6 g L^{-1} potassium chloride (KCl) and 4.044 g L^{-1} potassium bicarbonate ($KHCO_3$) to 1 L with distilled water.

2. Mix 20.0 g of dry, pulverized soil sample and 40.0 mL of the bicarbonate solution in a 250-mL Erlenmeyer flask. Place on an oscillating platform shaker (200 rpm) for 30 min.

3. Remove the flask from the shaker and let it stand for particles to settle. Filter the supernatant through a Whatman No. 1 filter paper.

4. Pipet 5.00 mL of the filtrate into a 250-mL beaker and dilute to 50.0 mL with distilled water. Titrate to the methyl orange endpoint with 0.02 N sulfuric acid (H_2SO_4).

Calculation of lime requirement

1. The initial $CaCO_3$ equivalence of the bicarbonate solution is:

$$\mathrm{I_{CaCO_3}} = (\mathrm{V})(\mathrm{C}) \tag{2.4}$$

where $\mathrm{I_{CaCO_3}}$= initial $CaCO_3$ equivalence of the solution (mg $CaCO_3$); V = volume of bicarbonate solution mixed with soil sample (mL); C = concentration of bicarbonate solution (mg $CaCO_3$ mL^{-1}).

2. The final $CaCO_3$ equivalence ($\mathrm{F_{CaCO_3}}$) of the bicarbonate solution is calculated from titration data:

$$F_{CaCO_3} = (N)(T)(50)\,\frac{V}{v} \tag{2.5}$$

where F_{CaCO_3}= final $CaCO_3$ equivalence of the solution (mg $CaCO_3$); N = normality of sulfuric acid (meq mL^{-1}); T = titration volume for sulfuric acid (mL); 50 = mg $CaCO_3$ meq^{-1}; v = volume of aliquot of the filtrate of the soil-bicarbonate mixture taken for titration (mL).

3. Estimate the $CaCO_3$ equivalence of the sample:

$$S_{CaCO_3} = \left(I_{CaCO_3} - F_{CaCO_3}\right)\frac{1{,}000}{W} \tag{2.6}$$

where S_{CaCO_3} = sample $CaCO_3$ equivalence (mg $CaCO_3$ kg^{-1}); 1,000 = mg kg^{-1}; W = weight of soil sample (g).

4. Calculate the lime requirement of the pond bottom soil. The upper 15 cm of soil within which lime typically reacts has an average weight of 1,500,000 kg ha^{-1}; thus the factor relating S_{CaCO_3} to lime requirement is based on the relationship that 1.0 mg $CaCO_3$ kg^{-1} is equivalent to 1.5 kg $CaCO_3$ ha^{-1}:

$$LR = S_{CaCO_3} \times 1.5 \tag{2.7}$$

where LR = pond bottom soil lime requirement (kg ha^{-1}).

Terms in the equations used above can be combined as follows:

$$\mathrm{LR} = \left[[(\mathrm{V})(\mathrm{C})] - [(\mathrm{N})(\mathrm{T})\frac{\mathrm{V}}{\mathrm{v}}(50)]\right]\frac{1{,}500}{\mathrm{W}}. \tag{2.8}$$

In the case where lime requirement is determined using 20.0 g soil, 40.0 mL of 0.04 N (2 mg $CaCO_3$ mL^{-1}) bicarbonate solution, and 5.0 mL of filtrate taken for titration with 0.02 N sulfuric acid, the terms in Equation 2.8 may be further combined:

$$\mathrm{LR} = (80 - 4\mathrm{T})75 \tag{2.9}$$

2.2.3 PB lime requirement method

1. Prepare the modified AE buffer solution by dissolving 10 g *p*-nitrophenol, 37 g potassium chloride, 7.5 g boric acid (H_3BO_3), and 5.25 g potassium hydroxide (KOH) in 1,000 mL distilled water and adjusting pH to 8.00 ± 0.10 if necessary (Pillai and Boyd 1985). A pH change of 0.1 unit in the buffer equals 0.16 meq acidity.

2. Weigh 20.0 g dry, pulverized pond soil, put into a 125-mL Erlenmeyer flask, add 40 mL buffer solution, and place on a mechanical shaker (200 rpm) for 30 min.

3. Measure the pH of the soil-buffer mixture with a glass electrode.

4. Calculate the lime requirement by the equation:

$$LR = (8.00 - \text{pH of soil-buffer mixture}) \times 6{,}000. \tag{2.10}$$

2.2.4 Evaluation of K-bicarbonate method

The shaking time necessary to assure complete reaction of the bicarbonate solution with a soil sample was established by shaking triplicate samples of a soil on the mechanical shaker for 10 min, 20 min, 30 min, 1 hr and 2 hr and determining lime requirement. Five replicates of four samples were analyzed by both the PB method and the bicarbonate method to determine if means and variances differed between the two methods. All samples were analyzed for lime requirement by the K-bicarbonate method and the PB method, and the results of the two methods were compared by regression analysis. Sets of three replications each of four samples were treated with the K-bicarbonate solution. One set was agitated for 30 min of the mechanical shaker. Other sets were shaken by hand for 30 sec, let stand for 30 min, 1 hr, or 2 hr, and shaken again for 30 sec. The lime requirement of the samples was determined by the K-bicarbonate method to ascertain if mechanical shaking is necessary.

2.3 Results

Soil samples ranged in pH from 3.89 to 6.45 and had organic carbon concentrations of 0.11 to 2.5%. They also varied in texture from sandy loams to clays. Samples were fairly representative of the range in soil properties expected for ponds with acidic bottom soils and low alkalinity waters (Boyd 1995; Boyd and Tucker 2014).

There were no differences ($P > 0.05$) in lime requirement related to shaking time. But, after shaking for 30 min, the lime requirement by the K-bicarbonate method reached a stable level (Table 2.1). The shaking or stirring times normally recommended in lime requirement protocols are 30 min or less (Pillai and Boyd 1985; Hue and Evans 1986; Sikora 2006).

The mean lime requirements for five replicates of four samples were numerically greater by the K-bicarbonate method than with the PB method (Table 2.2), but only two samples differed ($P < 0.05$) in lime requirement between the two methods. Considering all four samples, the lime requirement averaged 11.1% higher by the K-bicarbonate method.

The precision of both methods was good with coefficients of variation for the five replicates of each of four samples ranging from 2.3 to 5.8% for the K-bicarbonate method and from 1.5 to 9.1% for the PB method (Table 2.2). Analysis of homogeneity of variances revealed that variances by the two methods did not differ ($P > 0.05$) for any of the four samples.

A plot (Fig. 2.1) of the results of single analyses of the entire collection of samples by the K-bicarbonate method (X variable) and the PB method (Y) revealed no apparent difference in the relationship between the pond water samples from ponds at SFC and the surface soils from different areas in Alabama. Therefore, results from all samples were regressed. The correlation coefficient had an R^2 of 0.976 ($P < 0.01$) and the regression equation was $Y = 0.9278X – 142.26$. However, the slope of the regression line differed from 1.0 ($P<0.05$) and the slope of the regression line differed from 0.0 ($P<0.05$). The greatest lime requirements were for six of the samples from ponds at the SFC. These samples had organic carbon concentrations greater than other samples. Organic matter in soil increases the CEC leading to a greater lime requirement in acidic soil (Boyd 1995).

The K-bicarbonate method gave a greater lime requirement than the BP method across the entire range of lime requirement represented by the samples, but the difference between methods was greater for lower lime requirements. Solving the regression equation (Fig. 2.1) for 1,000, 2,000, 3,000, 4,000, 5,000, and 6,000 kg ha^{-1} by the K-bicarbonate method predicted the following lime requirements in respective order for the PB method: 786, 1,713, 2,641, 3,569, 4,497, and 5,425. The differences decreased from an overestimation of 27.2% at 1,000 kg ha^{-1} by the K-bicarbonate method to 9.6% at 6,000 kg ha^{-1}. The average of the difference was 12.9% – similar to the average difference of 11.1% found in the replicated comparisons of four samples by the two methods (Table 2.2).

Of four samples shaken by hand, two samples allowed to stand for only 30 min had lower lime requirements ($P<0.05$) than samples shaken for 30 min on the mechanical shaker (Table 2.3). Samples allowed to stand for 1 hr or 2 hr did not differ in lime requirement from those that were shaken mechanically for 30 min.

The pH of the K-bicarbonate solution was 8.3, and the pH remained above 7.0 after exposure to the soil. The pH of the buffer solution for the PB method is 8.0, and if the pH is below 7.0 at the end of the procedure, the protocol must be repeated with a smaller soil sample. The higher pH for the K-bicarbonate solution likely increases the charge (CEC) of the soil slightly (Pratt 1961), but it would not change the amount of exchangeable acidity initially present.

2.4 Discussion

In soil-testing laboratories, the decision on liming agricultural soils is made based on soil pH and optimum pH for the crop species to be cultivated in the soil. Thus, the farmer sends the sample to the laboratory to find out if liming is required; and, if it is, how much liming material to apply. In aquaculture, the procedure followed is different. The pond manager or a person rendering advice on pond management measures the total alkalinity of the pond water. The pH often is measured also, but it may vary by 1 to 3 units during a 24-hr period because of biological activity (Boyd and Tucker 2014). Because of this variation, water pH, unlike soil pH in agriculture, is not a reliable indicator of the need for liming (Boyd 1974). If the total alkalinity of the water is below 40 mg L^{-1}, liming materials should be applied to increase the total alkalinity (Boyd and Tucker 2014).

In a pond with a total alkalinity of 15 mg L^{-1}, the alkalinity concentration should be raised by 25 mg L^{-1}. Alkalinity typically is reported in terms of equivalent $CaCO_3$, but a liming treatment equal to 25 mg L^{-1} $CaCO_3$ (375 kg ha^{-1} in a 1-ha pond of 1.5 m average depth) would not result in the desired increase in alkalinity. The liming material would be expended quickly in neutralizing bottom soil acidity and the increase in alkalinity would be small and brief. Thus, in pond liming, the bottom soil acidity must be neutralized so that the alkalinity of the pond water can be increased to the minimum desired concentration of 40 mg L^{-1} or above (Boyd 2012).

When pond bottom soils are sent to a soil testing laboratory, the task of the laboratory is not to make a decision on the need for liming but to determine how much liming material should be applied to the pond. There is no specific target pH for liming based on the aquaculture species as there is for agricultural crops. As an alternative, Pillai and Boyd (1985) suggested completely neutralizing the exchangeable acidity of the bottom soil. The exact pH of 0.0 base unsaturation – no soil acidity present – is not known for most soils and varies among soils of different types. Thus, the approach taken in the PB method (a modification of the AE method) and also for the K-bicarbonate method described in this study was to neutralize as much exchangeable acidity as possible and thereby affect a very low base unsaturation albeit possibly not 0.0.

There is no lime requirement standard against which to compare the accuracy of lime requirement methods in predicting true lime requirement. The reliability of a method must be determined by the effectiveness of the predicted liming rate in raising the pH of agricultural soil or the pH of pond soil and total alkalinity of pond water. As mentioned earlier, the PB method will raise pH of pond soil above 7.0, and the total alkalinity of pond water to 40-50 mg L^{-1}. In aquaculture, a total alkalinity concentration of 60-80 mg^{-1}, or even greater in crustacean ponds, is desired (Boyd and Tucker 2014).

The greater lime requirement obtained by the K-bicarbonate method may have resulted from the higher concentration of potassium ion (1.0 N) in the K-bicarbonate solution than in the PB solution (0.59 N) resulting in greater replacement of exchangeable acidic ions on the soil by potassium. The use of a 1.0 N potassium solution for replacing aluminum ions on soil in the K-bicarbonate method should not be considered unusual, because solutions for extracting exchangeable cations from soil for analysis or for CEC determination often have a concentration of ammonium or some other cation of 1.0 N (Jackson 1958; Tan 2005; Carter and Gregorich 2008).

Estimation of a slightly higher lime requirement is actually a desirable feature of the K-bicarbonate method, because using somewhat more liming material than determined by the PB method should result in greater total alkalinity in pond water and a longer time before another application of liming material is necessary. Those wanting a result comparable to the PB method can adjust the K-bicarbonate result using a regression equation in Fig. 2.1. But, we do not believe that the PB method is widely used, and it is unlikely that anyone would be concerned about the difference.

The K-bicarbonate method appears acceptable for use in aquaculture. It contains two common laboratory chemicals, potassium chloride and potassium bicarbonate, neither of which is hazardous. The protocol can be done with or without a mechanical shaker, and pH measurement is unnecessary.

In the protocol for the K-bicarbonate method used in this study, 40 mL of bicarbonate solution contains 80 mg or 1.6 meq of equivalent $CaCO_3$. A 20-g soil sample that would yield enough exchangeable acidity to react exactly with all of the bicarbonate in 40 mL of the bicarbonate solution would have a lime requirement of 6,000 kg ha^{-1} (from Equation 2.9). Of course, if there is no alkalinity left in solution, one would only know that the lime requirement was 6,000 kg ha^{-1} or greater. Thus, the protocol given here should only be used for soils with lime requirements up to 5,000-5,500 kg ha^{-1}. For greater lime requirement, a smaller soil sample should be added to the 40-mL aliquot of bicarbonate solution. Of course, Equation 2.8 rather than Equation 2.9 would have to be used to calculate the lime requirement of the smaller soil sample.

Most application rates of liming materials for aquaculture ponds are based on reference to literature such as Hickling (1962), Boyd and Tucker (1998), and Wudtisin and Boyd (2006) about liming rates that were successful in specific experiments or proven effective in particular regions through experience. The K-bicarbonate method is simple enough that it could provide a means of determining liming rates for individual aquaculture ponds worldwide.

There is another advantage of the K-bicarbonate method that might make the method appealing for even some agricultural applications. Buffers commonly used in soil testing were developed before implementation of laws regulating disposal of hazardous waste (Sikora 2007). In the United States, the Resource Conservation and Recovery Act was passed by Congress in 1976 to improve waste management (Horinko 2002). This act was expanded in 1980 to include chemicals hazardous because of ignitability, corrositivity, reactivity (explosion risk), and toxicity (US Environmental Protection Agency 1980a,b). The compound *p*-nitrophenol used in the original AE buffer solution and the PB buffer solution is a hazardous compound because of toxicity. Another widely used lime requirement procedure (Shoemaker et al. 1961) contains *p*-nitrophenol plus potassium chromate that is a carcinogen. Adherence to hazardous waste laws requires following specific protocols that include toxic waste disposal resulting in greater cost of laboratory operation.

Sikora (2006) developed a buffer solution in which potassium chromate and *p*-nitrophenol used in the Shoemaker lime requirement procedure were replaced by 2-(Nmorpholino) ethanesulfonic acid monohydrate and imidazone. Huluka (2005) used monobasic potassium phosphate to replace *p*-nitrophenol in the Adams-Evans buffer solution. This modification could be applied to the PB method to allow removal of the *p*-nitrophenol. Liu et al. (2005) recommended discontinuing the AE buffer method and determining lime requirement by direct calcium hydroxide titration.

These modifications in lime requirement determination are acceptable for large soil-testing laboratories in the United States, but they are not as attractive in aquaculture. Modification of the Shoemaker method requires expensive and specialized organic chemicals. Methods based on alteration of the AE method or its replacement by calcium hydroxide titration still rely on a pH meter. The K-bicarbonate method presented here would be easily adaptable in laboratories associated with aquaculture water quality investigations or even in agricultural soil-testing laboratories. Of course, the disadvantages of the K-bicarbonate method for use in agricultural soil-testing are that it does not allow lime requirement determinations for obtaining a specific soil pH, and titration is more time consuming than pH measurement.

Costs of chemicals for making the K-bicarbonate solution and PB buffer solution with and without *p*-nitrophenol (Huluka 2005) are provided (Table 2.4). With *p*-nitrophenol, reagent cost for the PB method is greater than for the K-bicarbonate method. Removing *p*-nitrophenol from the PB method buffer by its replacement with monobasic potassium phosphate, reduces reagent cost to slightly less than for the PB method.

In comparing the two methods, 20.0 g soil samples and 40 mL of buffer solution were used. Of course, the weight and volume could each be halved without affecting the results of the test and thereby cut the chemical cost in half. If reduced quantities of soil and buffer solution are used, lime requirement must be calculated with Equation 2.8 rather than Equation 2.9.

2.5 Conclusions

The K-bicarbonate method for lime requirement of pond bottom soils is simpler and less expensive to conduct than the PB method. Moreover, the K-bicarbonate method does not require the hazardous chemical, *p*-nitrophenol, necessary in the PB method. The PB method could be modified by replacing *p*-nitrophenol with monobasic potassium phosphate (Huluka 2005) eliminating the hazardous chemical and reducing reagent cost below that of the K-bicarbonate method. Nevertheless, the PB method would still require a pH meter.

The K-bicarbonate procedure estimates a higher lime requirement than does the PB method – about 20% greater up to 3,000 kg ha^{-1} and 10% greater at higher lime requirements. This difference is an advantage because the PB method does not raise total alkalinity in pond water as high as desirable in some types of aquaculture.

The PB lime requirement method was never widely used – possibly because of the need for a specialty (and hazardous) chemical and a pH meter capable of 0.01-unit sensitivity. Most liming rates for ponds currently are approximations based on the literature or experience, and a more accurate method for determining lime requirement of individual ponds would be greatly beneficial. The K-bicarbonate method could provide a convenient means of estimating pond lime requirements based on the exchangeable acidity of pond soil samples. Hopefully, it will be adopted for widespread use.

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Table 2 Effects of time of mechanical shaking on the lime requirement of a soil sample as determined by the proposed K-bicarbonate procedure.

Shaking time	Lime requirement (kg ha^{-1})
10 min	672 a
20 min	696 a
30 min	720 a
1 hr	720 a
2 hr	720 a

[a] Entries indicated by the same letter did not differ at P = 0.05 as determined by Tukey’s Studentized Range (HSD) tests.

Table 3 Means ± standard deviations and coefficients of variation (CV) for lime requirements determined by two methods on five replications of each of four soils. Means were compared by *t*-tests and homogeneity of variances was tested by *F*-tests.

K-bicarbonate method		PB method			
Lime requirement (kg/ha)	CV (%)	Lime requirement (kg/ha)	CV (%)	*t*	*F*
868 ± 39	4.5	845 ± 23	2.7	1.75	1.23
1541 ± 82	5.3	1264 ± 116	9.1	3.82*	1.81
2160 ± 126	5.8	1848 ± 29	1.5	4.19*	2.51
4315 ± 101	2.3	4196 ± 91	2.1	1.75	1.23

*Significant at *P* = 0.05.

Table 4 Means ± standard errors for lime requirement (kg ha^{-1}) following initial shaking for 30 sec by hand of soil-potassium bicarbonate solutions and different lengths of standing time as compared to shaking continuously on a mechanical shaker for 30 min.

	Standing time for shaking by hand			Mechanical
Sample	30 min	1 hr	2 hr	shaker
1	1,380 ± 29 a	1,440 ± 13 a	1,513 ± 12 a	1,540 ± 92 a
2	1,760 ± 151 a	2,220 ± 135 b	2,220 ± 87 b	2,120 ± 125 b
3	320 ± 138 a	400 ± 92 a	620 ± 69 a	340 ± 92 a
4	4,020 ± 158 a	4,120 ± 69 b	4,280 ± 37 b	4,320 ± 120 b

*Means indicated by the same letter do not differ (P = 0.05) according to Tukey's Studentized Range (HSD) test. Horizontal comparisons only.

Table 5 Cost comparisons for chemicals used in the Pillai and Boyd (1985) method and the new K-bicarbonate method for determining lime requirement of pond bottom soils.

				Cost sample^{-1} ($ US)	
Chemical	Source	Grade	Price ($US kg^{-1})	PB method	K-bicarbonate method
Boric acid	VWR	ACS	95.02	0.028	---
Potassium bicarbonate	Sigma-Aldrich	ACS	104.46	---	0.017
Potassium chloride	VWR	ACS	86.10	0.127	0.247
Potassium hydroxide	VWR	ACS	67.00	0.014	---
p-nitrophenol	Sigma-Aldrich	Reagent	630.00	0.252	---
Total	---	---	---	0.421	0.264

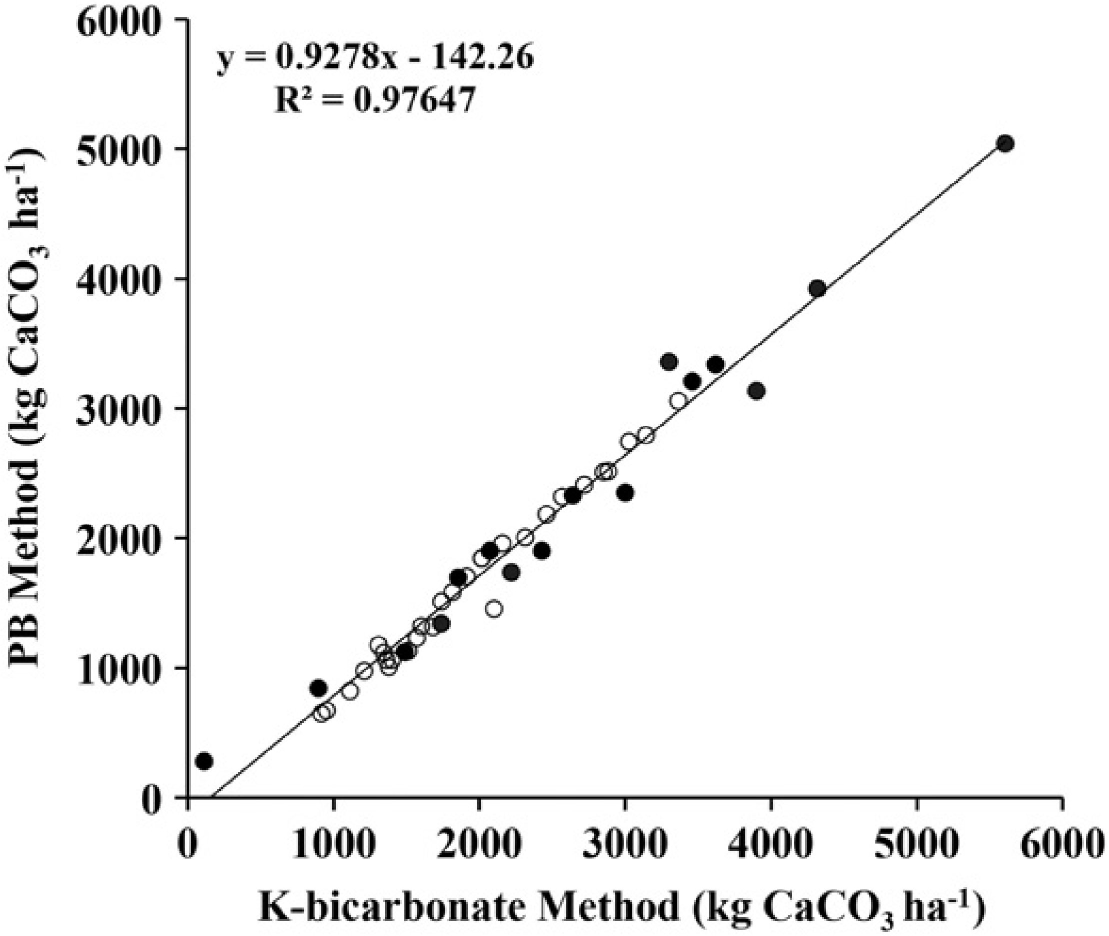

Fig. 1 Regression equation for relationship between bottom lime requirement by the proposed K-bicarbonate method and the method currently recommended for 45 soil samples. Solid dots are for samples from ponds on the Shell Fisheries Center at Auburn, Alabama. Open dots are for samples from major physiographic regions and soil associations in Alabama.

CHAPTER III. – EFFECTS OF A SINGLE APPLICATION OF CHICKEN MANURE ON AGRICULTURAL LIMESTONE SOLUBILITY AND TILAPIA PRODUCTION IN LOW-ALKALINITY PONDS

3.1 Introduction

Primary productivity and fish production in fertilized ponds is usually less in ponds with acidic bottom soils and low-alkalinity waters than in those with neutral to slightly alkaline soil and higher alkalinity water (Boyd and Tucker, 2014). Liming materials are applied to increase soil pH and alkalinity in aquaculture ponds (Mortimer, 1954; Hickling, 1962; Boyd, 2017).

Agricultural limestone is the most common liming material in agriculture (West and McBride, 2005) and in aquaculture (Boyd, 2017). Agricultural limestone is made by pulverizing limestone until most of its particles pass a screen with 0.25-mm apertures. Agricultural limestone is a mixture of calcium and magnesium carbonate in which more than half of the carbonate is combined with calcium (Bowles, 1956; Thunjai et al., 2004).

Limestone is not highly soluble in water, but its solubility is increased by dissolved carbon dioxide (Stumm and Morgan, 1996) as illustrated below using calcium carbonate to represent limestone:

$$CaCO_3 + CO_2 + H_2O \rightleftharpoons Ca^{2+} + 2HCO_3^{-}. \qquad (1)$$

Magnesium carbonate reacts with carbon dioxide in the same manner as does calcium carbonate. Chemically equivalent quantities of bicarbonate and calcium plus magnesium result from the reaction of limestone with water and carbon dioxide. The bicarbonate imparts alkalinity to water, while calcium and magnesium cause water hardness. The common way of reporting alkalinity and hardness is to express concentrations of bicarbonate and calcium plus magnesium as their equivalent calcium carbonate concentrations (Boyd, 2015).

In a container with solid phase limestone and distilled water open to the atmosphere, the equilibrium alkalinity and hardness concentrations for several agricultural limestones from different sources were around 60 mg $CaCO_3$/L (Boyd et al., 2016; Sá and Boyd, 2017). In ponds with low-alkalinity water, treatment with agricultural limestone usually will not increase alkalinity and hardness above 40 mg/L (Arce and Boyd, 1975). A minimum alkalinity of 20 mg/L is considered an acceptable level in sportfish ponds in which fish biomass usually is 200-500 kg/ha, but a higher minimum alkalinity of 50-60 mg/L is considered desirable in tilapia and other food fish aquaculture ponds with greater fish biomass (Boyd and Tucker, 2014).

As seen in Eq. 1, increasing carbon dioxide concentration will increase the dissolution of agricultural limestone and greater alkalinity and hardness concentrations will result. Kumar et al. (2005) found greater alkalinity in ponds treated with manure than in those treated with chemical fertilizers. Nevertheless, the influence of manure application on alkalinity concentration in ponds treated with agricultural limestone is poorly understood and deserves investigation.

3.2 Materials and Methods

3.2.1 Ponds

This study was conducted during the 2015 growing season in earthen ponds at the E. W. Shell Fisheries Center (SFC) of Auburn University located at Auburn, Alabama USA (coordinates 32.60 N, 85.48 W). Ponds were constructed on soils of the Piedmont Plateau that are acidic, fine-loamy, kalonitic, thermic Typic Kanhapludunts (McNutt, 1981). Eight study ponds were of the E-Series (400 m^2 × 1 m average depth) and four were of the R-Series (200 m^2 × 0.75 m average depth). Ponds are filled and water levels maintained by pipe flow from a reservoir with a protected, wooded watershed. Reservoir water contains 12-18 mg/L alkalinity and hardness, and it also is low in nutrient and organic matter concentrations (Somridhivej and Boyd, 2016). Ponds for the study were refilled with water following draining for fish harvest in November 2014.

3.2.2 Pond liming and fertilization

Chicken manure obtained from the Poultry Research Unit at Auburn University was applied by broadcasting over pond surfaces on 10 April 2016. A control and three chicken manure treatments (Table 1) each replicated three times were assigned to ponds by drawing lots. Pond waters contained 15-20 mg/L alkalinity on 12 April, and treatments of 0, 1,000, 2,000, and 4,000 kg/ha were applied over pond surfaces on 16 April.

No additional manure was applied to ponds, but they were treated with urea (45% N) and commercial, liquid fertilizer (10% N, 34% P2O5) at rates of 6 kg N and 3 kg P2O5 at 3-wk intervals beginning on 23 April and continuing until 10 October for a total of nine applications. This fertilization rate was found to be optimum for production of bluegill (Lepomis macrochirus) in ponds at the SFC (Wudtisin and Boyd, 2005; Boyd et al., 2008), and it also has been used at the SFC to fertilize ponds stocked with tilapia and other species.

3.2.3 Fish

Female tilapia (*Oreochromis niloticus*) were obtained from breeding ponds on the SFC. They were held for 1 wk in indoor tanks and transferred to ponds. Average individual fish weight was 26 g, and fish were stocked on 14 May at a rate of 4,000/ha. Ponds were drained and fish harvested between 12 and 14 October for an average grow-out period of 152 d. At harvest, the total weight of fish in each pond was determined.

3.2.4 Water quality measurements

Water samples were collected weekly from 5 May to 16 September using a 90-cm water column sampler (Boyd and Tucker, 1992). At the time of sampling (0800-0830 h), water temperature at the water surface was measured with a mercury thermometer. Samples were placed into 1-L plastic bottles, transported within 10-15 min to the nearby water quality laboratory, and analyses initiated immediately. Total alkalinity concentration was measured by titration to pH 4.5 with 0.02 N sulfuric acid, while total hardness and calcium hardness analyses were made by titration of separate aliquots with 0.01 M ethylenediaminetetraacetic acid disodium salt (Na-EDTA) to the eriochrome black T and murexide endpoints, respectively

(Eaton et al., 2005). Electronic meters were used to measure pH (Orion 3 Star pH Benchtop, Thermo Scientific, Singapore) and specific conductance (Orion 3 Star Conductivity Benchtop; Thermo Scientific, Singapore).

Every other week, samples also were analyzed for chlorophyll *a* concentration by acetone extraction and spectrophotometry (Boyd and Tucker, 1992), soluble reactive phosphorus by membrane filtration and ascorbic acid finish (Eaton et al., 2005), total phosphorus by sodium perchlorate digestion and ascorbic acid finish (Gross and Boyd, 1998; Eaton et al., 2005). Total ammonia nitrogen was determined by the salicylate procedure (Le and Boyd, 2012), and nitrate nitrogen analysis was by the NAS reagent method (van Rijin, 1993).Concentrations of chlorophyll *a*, nitrate nitrogen (NO_3–N), total ammonia nitrogen (TAN), total phosphorus (TP) and soluble reactive phosphorus (SRP) were determined for the samples every other week. The TAN concentration was measured by the salicylate method (Le and Boyd 2012); NO_3–N nitrogen was determined by the NAS reagent method (van Rijn 1993); total phosphorus analyses were made according to Gross and Boyd (1998). Other analysis followed protocols recommended by Boyd and Tucker (1992).

3.2.5 Data analysis

Data were analyzed using the general linear model (GLM) after checking for normality with the Shapiro-Wilk test for homogeneity. When the null hypothesis was not satisfied in the GLM, data for the particular variables were transformed. Duncan’s range test was used to detect whether there were differences between group means at the 0.05 significant level. Statistical procedures were performed by SAS (version 9.1), and graphs were made using Excel (version 2013).

3.3 Results

3.3.1 Water quality

Water temperatures measured at 0800-0830 h were usually around 27 °C in May, and they increased to 30-31 °C in August (Fig. 3). Temperatures decreased to 29-30 °C at the end of August, but increased again to 30-31 °C in early September. Water temperature was 28-29 °C in mid-September. Study means (average for all sampling dates) were around 29 °C did not differ ($P > 0.05$) among control ponds and treatment ponds (Table 6).

Average weekly pH for controls and treatments were 7.5-9.5. Weekly means above 8.5 were recorded mostly during the first 8 wk, and averages were usually around 7.8-8.0 afterwards (Fig. 1). Control and treatment ponds had similar pH ($P > 0.05$), but the numerical values of control pond pH were about 0.2-pH unit less than pH of treatment ponds.

Weekly averages for specific conductance were 105-165 μs/cm (Fig. 1) with individual ponds having values of 90-186 μS/cm. Weekly values for specific conductance usually increased in order control, low, high, and medium chicken manure treatments. Study means for specific conductance did not differ ($P > 0.05$) among control and different treatments because of high variation.

Total alkalinity reached a fairly constant concentration in control ponds after the first sampling date (Fig. 3). Alkalinity was relatively constant after early August in the low chicken manure treatment, but it appeared to still be rising on the last sampling date in the medium and high treatments. Study averages for alkalinity did not differ between control and low treatment or between the medium and high treatment (Table 7), but the two groups differed from each other ($P < 0.05$). Alkalinity was only 1 mg/L greater in the high treatment than in the medium treatment.

Total hardness followed a pattern similar to total alkalinity, and as with alkalinity, study averages for hardness were not different between control and low treatment or between medium and high treatment, but hardness concentrations differed between the two groups (Table 7). In the control and low treatment, alkalinity and hardness concentrations had similar study means, but average hardness was about 4 mg/L less than alkalinity in the medium treatment and around 6 mg/L less in the high treatment.

Calcium hardness concentrations followed the same trends seen in total hardness (Fig. 3; Table 7). The difference in total hardness and calcium hardness represents hardness caused by magnesium. Calcium and magnesium concentrations calculated from their respective hardness concentrations (Boyd, 2015) in order of increasing chicken manure inputs were: calcium; 9.94, 10.54, 13.21, and 12.42 mg/L and magnesium; 3.64, 3.65, 4.06, and 4.28 mg/L.

Weekly average concentrations of total ammonia nitrogen gradually increased from around 0.15 mg/L to 0.2 mg/L, and concentrations were similar among control and treatment ponds on all dates (Fig. 8). There was more variation in nitrate nitrogen concentration (Fig. 8) than for total ammonia nitrogen concentration, and weekly means for nitrate nitrogen were 0.10-0.15 mg/L in May and 0.12-0.25 mg/L in September. Study means for total ammonia nitrogen and nitrate nitrogen did not differ among the control and the different chicken manure treatments (Table 7). It should be mentioned here that urea used as nitrogen fertilizer in the ponds hydrolyzes within a few days to ammonia in soil and water (Overrein and Moe, 1966). Also, ammonia nitrogen water is quickly removed by aquatic plants and oxidized to nitrate by nitrifying bacteria (Hargreaves, 1998). Nitrate also is lost from pond water when it is reduced to gaseous dinitrogen (N_2) in anaerobic zones by denitrifying bacteria (Hargreaves, 1998). Thus, high total ammonia nitrogen and nitrate concentrations are not common in fertilized ponds.

Weekly concentrations of total phosphorus and soluble reactive phosphorus tended to increase slowly until September and then decrease slightly (Fig. 5). Concentrations were 0.18-0.28 mg/L total phosphorus and 0.06-0.13 mg/L for soluble reactive phosphorus. There were no differences among control pond and the different chicken manure treatments in total phosphorus and soluble reactive phosphorus concentrations (Table 7). Phosphorus concentrations in fertilized ponds usually are rather low in spite of high inputs because of phosphorus adsorption by bottom soil (Boyd and Tucker, 1998).

Chlorophyll *a* concentrations steadily increased from around 15-18 μg/L in May to 40-45 μg/L in August (Fig. 6). They declined in late August and early September to 30-35 μg/L. There was little difference among control ponds and the different treatment ponds in the weekly chlorophyll *a* concentrations, and study means were also similar ($P > 0.05$).

3.3.2 Fish production

The fish stocked were supposed to be all females, but this proved untrue and small fish were present at harvest. Survival was not measured, but ponds were checked almost every day for dead fish, but none were seen. Fish originally stocked reached average individual weights of 107-126 g, but there were no differences in control and treatment means. Gross yields were 1,073-1,261 kg/ha, and net yields were 832-1,000 kg/ha. Although there were no differences ($P > 0.05$) in production among control and treatments, production was numerically greater in the 2,000 and 4,000 kg/ha manure treatments.

3.4 Discussion

Survival and yields of fish in ponds were products of environmental suitability, nutrient inputs, management effectiveness, and random chance. General environmental conditions and management were reasonably suitable as evident from the fact that dead fish were not observed. Water temperature was within the range of 26-34 °C necessary for good growth of *O. niloticus* (Azaza et al., 2008).

Optimum pH for tilapia growth under controlled conditions in the laboratory was 7-8, and growth was less at pH 6 and at pH 9 (El-Sherif and El-Feky, 2009). The pH in ponds depends upon the dissolved carbon dioxide concentration. The pH increases when carbon dioxide uptake rate by plants for use in photosynthesis exceeds the rate of carbon dioxide release in respiration by the pond biota and *vice versa* (Boyd et al., 2016). Average weekly pH exceeded 9 in ponds of the control and treatments on two dates and individual ponds had pH above 9 on several sampling dates. Ponds on the SFC and elsewhere typically have pH above 8.5 in the afternoon, and pHs of 9.0-10.0 are not uncommon (Boyd and Tucker, 2014). The pH in ponds of this study may have been higher than optimum at times, but pH was no higher than usually reported in fertilized aquaculture ponds.

Specific conductance of ponds was low, and based on the usual relationship between specific conductance and salinity (Walton, 1989), salinity in ponds apparently ranged between 75 and 125 mg/L. Iqbal et al. (2012) found *O. niloticus* to grow better at 1,600 and 4,000 mg/L salinity than at 800 mg/L salinity, while Azevedo et al. (2015) reported that *O. niloticus* grew best at 7,000 mg/L salinity. Growth of tilapia in ponds at the SFC was likely less than could have been achieved in water of greater salinity.

Phytoplankton blooms in ponds were not great enough to cause low nighttime dissolved oxygen concentration as evident from the chlorophyll *a* concentration- nighttime dissolved oxygen relationship for tilapia ponds described by Romaire et al. (1978). Although dissolved oxygen concentration was not monitored, fish were not seen piping at the surface, and low dissolved oxygen concentration was not considered to have negatively impacted fish. Total ammonia nitrogen concentrations were much lower than the minimum concentration expected to negatively affect tilapia (Hargreaves and Kucuk, 2001).

Tilapia production in ponds was controlled almost exclusively by availability of food resulting from primary productivity and from suspended manure particles which tilapia reportedly can eat (Boyd and Tucker, 2014). Primary productivity in ponds was supported by nutrients from the single chicken manure application and periodic chemical fertilizer applications. Liming material increases total alkalinity which increases the availability of inorganic carbon for photosynthesis and provides pH buffering when photosynthesis rates are high (Boyd et al., 2016).

Tilapia yields in the present study were at the low end of the ranges of 1,000-4,000 kg/ha from ponds fertilized with livestock manure and 1,000-3,000 kg/ha in ponds treated with chemical fertilizers reported in a review by Boyd (2018). This review also indicated that manure usually was applied to tilapia ponds at average rates of 50-150 kg/ha/d (dry weight basis). On average, a rate of 50 kg/ha/d of chicken manure for the 152-d duration of the present study would be a total input of 206 kg N/ha and 230 kg P_2O_5/ha – twice the total inputs made to ponds of this study (Table 1). Chemical fertilizer application rates for tilapia ponds often are twice or more the rate used in this study (Boyd, 2018). Thus, low yields in the present study resulted from comparatively low nutrient inputs.

The inputs in manure made in this study were greater than those from chemical fertilizer in the high chicken manure treatment, and they were around 60% and 30% of chemical fertilizer inputs in the medium and low manure treatments, respectively. The input of P_2O_5 was greater than from chemical fertilizer in the medium and high treatments and around 65% of chemical fertilizer P_2O_5 input to the low treatment. Smaller quantities of fertilizers applied frequently are more effective in stimulating and maintaining phytoplankton blooms than are single applications of larger amounts (Boyd and Tucker, 1998). This results because ammonia from fertilizer is lost by diffusion to the air and by nitrification to nitrate with subsequent denitrification to dinitrogen gas (Hargreaves, 1998), and phosphorus is rapidly adsorbed by sediment (Boyd and Tucker, 1998). Chemical fertilizer applications were probably more important to continued phytoplankton productivity than were the initial chicken manure applications. This opinion is supported by the observation that chlorophyll *a* concentrations were similar among ponds. In fact, there was no difference among control and treatment ponds in chlorophyll *a* concentration on the first sampling date (5 May) which was 25 d following manure applications.

Chicken manure does not contain substances that would increase alkalinity directly, but ammonia mineralized by microbial action on manure will titrate as alkalinity (Eaton et al., 2005). Total ammonia nitrogen concentration was usually less than 0.25 mg/L, and at the temperature and pH of the ponds, no more than 20% of the ammonia nitrogen was present as ammonia (www.hbuehrer.ch/Rechner/Ammonia.html). The contribution of ammonia to alkalinity in the ponds was insignificant. Biological nitrification process reduces alkalinity, and oxidation of 1 mg/L of ammonia nitrogen results in enough acidity to neutralize 7.14 mg/L of alkalinity (Hunt and Boyd, 1981). Ponds had similar total ammonia nitrogen concentrations, and the effect of nitrification on alkalinity would have been similar among all ponds. The alkalinity concentrations in ponds resulted from the initial alkalinity and alkalinity resulting from dissolution of agricultural limestone.

The main premise that adding manure would supply additional carbon dioxide to increase agricultural limestone solubility and alkalinity was verified for initial manure applications of 2,000 and 4,000 kg/ha (wet weight). Although phosphorus concentrations were somewhat greater in these ponds, neither phytoplankton productivity (as indicated by similar chlorophyll *a* concentration) nor fish yield were greater ($P > 0.05$) in medium and high treatments than in the control and low treatment.

It is interesting that fish production was numerically greater in the medium and high treatments. Also, a scatter plot of pond averages of alkalinity concentrations versus net fish production (Fig. 6) revealed that with exception of two ponds with average alkalinity values around 40 mg/L, there was a general trend of increase in net yield of fish between 40 and 60 mg/L alkalinity. This study did not provide decisive results for fish yield because of the usual problem of inadequate ponds at research stations for sufficient treatments and replication (Boyd and Tucker, 1998). Of course, researchers could accept a lower level of probability for declaring differences among means, but the tradition is 0.05 (Cohen, 1994).

3.5 Conclusions

This study revealed that initial treatments of 2,000 or 4,000 kg/ha (fresh weight) of chicken manure to ponds fertilized with chemical fertilizer increased total alkalinity by about 12 mg/L as compared to ponds into which no chicken manure was applied. However, there was no benefit of initial manure application on primary productivity (based on chlorophyll *a* concentration) or fish production ($P > 0.05$). Improvement in agricultural limestone solubility resulting from carbon dioxide generated by microbial action on manure should, nevertheless, make this treatment useful in ponds with low alkalinity water.

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Table 6. Total nitrogen and phosphorus inputs made to ponds with initial chicken manure and continuing chemical fertilizer applications.

	Total input (kg/ha)					
	Nitrogen (N)			Phosphorus (P_2O_5)		
	Manure	Chemical[1] fertilizer	Total	Manure	Chemical[1] fertilizer	Total
0	---	54	54	---	27	27
1,000	16.3	54	70.3	18.1	27	45.1
2,000	32.5	54	86.5	36.2	27	63.2
4,000	65.0	54	119.0	72.4	27	99.4

[1] Calculated from average dry matter and dry weight concentrations of nitrogen and phosphorus for 1672 samples of chicken manure (Brown 2013).

Table 7. Study means (average of weekly means) and standard deviations for water quality variables in chemically-fertilized ponds receiving single, initial applications of fresh chicken manure at 0 kg/ha (control), 1,000 kg/ha (low), 2,000 kg/ha (medium), and 4,000 kg/ha (high).

	Chicken manure treatment			
Variables	Control	Low	Medium	High
Water temperature (C)	29.3 ± 1.6^{a}	29.3 ± 1.5^{a}	29.2 ± 2.3^{a}	28.6 ± 0.9^{a}
pH (standard units)	8.2 ± 0.1^{a}	8.0 ± 0.1^{a}	8.0 ± 0.1^{a}	8.0 ± 0.2^{a}
Alkalinity (mg/L)	41.6 ± 2.7^{a}	41.9 ± 1.8^{a}	53.4 ± 4.8^{b}	54.3 ± 5.6^{b}
Hardness (mg/L)	38.9 ± 1.6^{a}	41.4 ± 6.4^{a}	49.2 ± 6.3^{b}	48.7 ± 6.1^{b}
Calcium hardness (mg/L)	24.8 ± 1.6^{a}	26.4 ± 2.7^{a}	33.0 ± 3.3^{b}	31.1 ± 2.3^{b}
Specific conductance (μScm)	120 ± 5^{a}	129 ± 25^{a}	154 ± 17^{a}	143 ± 24^{a}
Total ammonia nitrogen (mg/L)	0.17 ± 0.03^{a}	0.18 ± 0.05^{a}	0.19 ± 0.02^{a}	0.19 ± 0.08^{a}
Nitrate nitrogen (mg/L)	0.12 ± 0.10^{a}	0.15 ± 0.10^{a}	0.18 ± 0.01^{a}	0.17 ± 0.01^{a}
Total phosphorus (mg/L)	0.18 ± 0.001^{a}	0.20 ± 0.004^{a}	0.21 ± 0.016^{b}	0.25 ± 0.002^{b}
Soluble reactive phosphorus (m/L)	0.06 ± 0.001^{a}	0.06 ± 0.003^{a}	0.07 ± 0.002^{b}	0.10 ± 0.002^{b}
Chlorophyll *a* (μg/L)	30.3 ± 5.1^{a}	31.8 ± 4.7^{a}	31.3 ± 3.5^{a}	32.3 ± 4.6^{a}

Table 8. Means and standard deviations for initial and final fish weights and gross and net yields of tilapia (*Oreochromis niloticus*) in chemically-fertilized ponds receiving single initial applications of fresh chicken manure at 0 kg/ha (control), 1,000 kg/ha (low), 2,000 kg ha (medium), and 4,000 kg/ha (high).

	Chicken manure treatment[1]			
Performance indicator	Control	Low	Medium	High
Initial weight (g)	26.2 ± 0.02	26.2 ± 0.03	26.2 ± 0.02	26.2 ± 0.4
Final weight (g)	107 ± 20.1	110 ± 28.7	126 ± 15.2	121 ± 2.5
Gross yield (kg/ha)	1073 ± 201	1102 ± 287	1260 ± 152	1214 ± 25
Net yield (kg/ha)	823 ± 201	8.52 ± 287	1010 ± 152	964 ± 25

[1] No difference (horizontal comparisons only) in any of the performance indicators as determined by Duncan’s range test at *P* = 0.05.

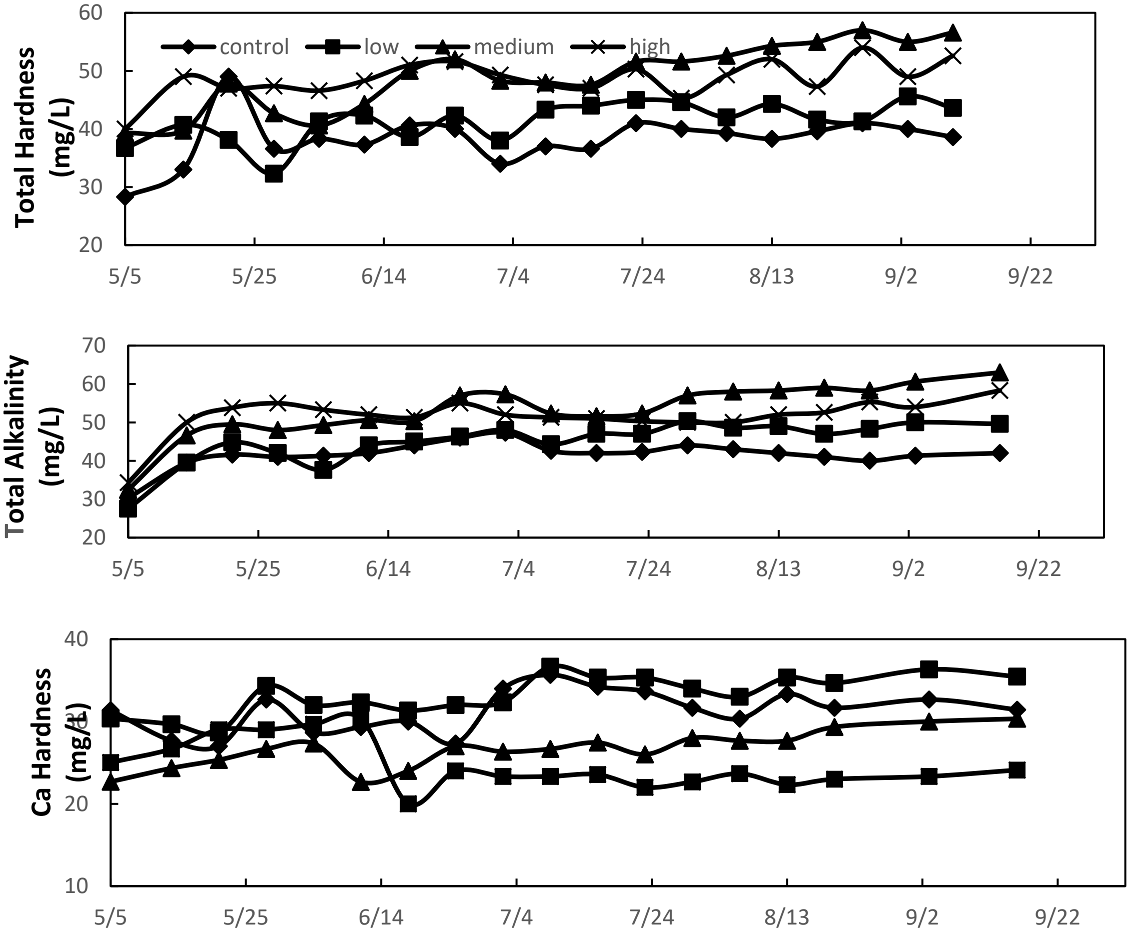

Fig. 2. Weekly averages (n = 3) for water temperature, pH, and electrical conductivity in tilapia ponds treated with 2,000 kg/ha agricultural limestone. Ponds received single, initial treatments of 0 (control), 1,000 kg/ha (low), 2,000 kg/ha (medium), and 4,000 kg/ha (high) of fresh chicken manure.

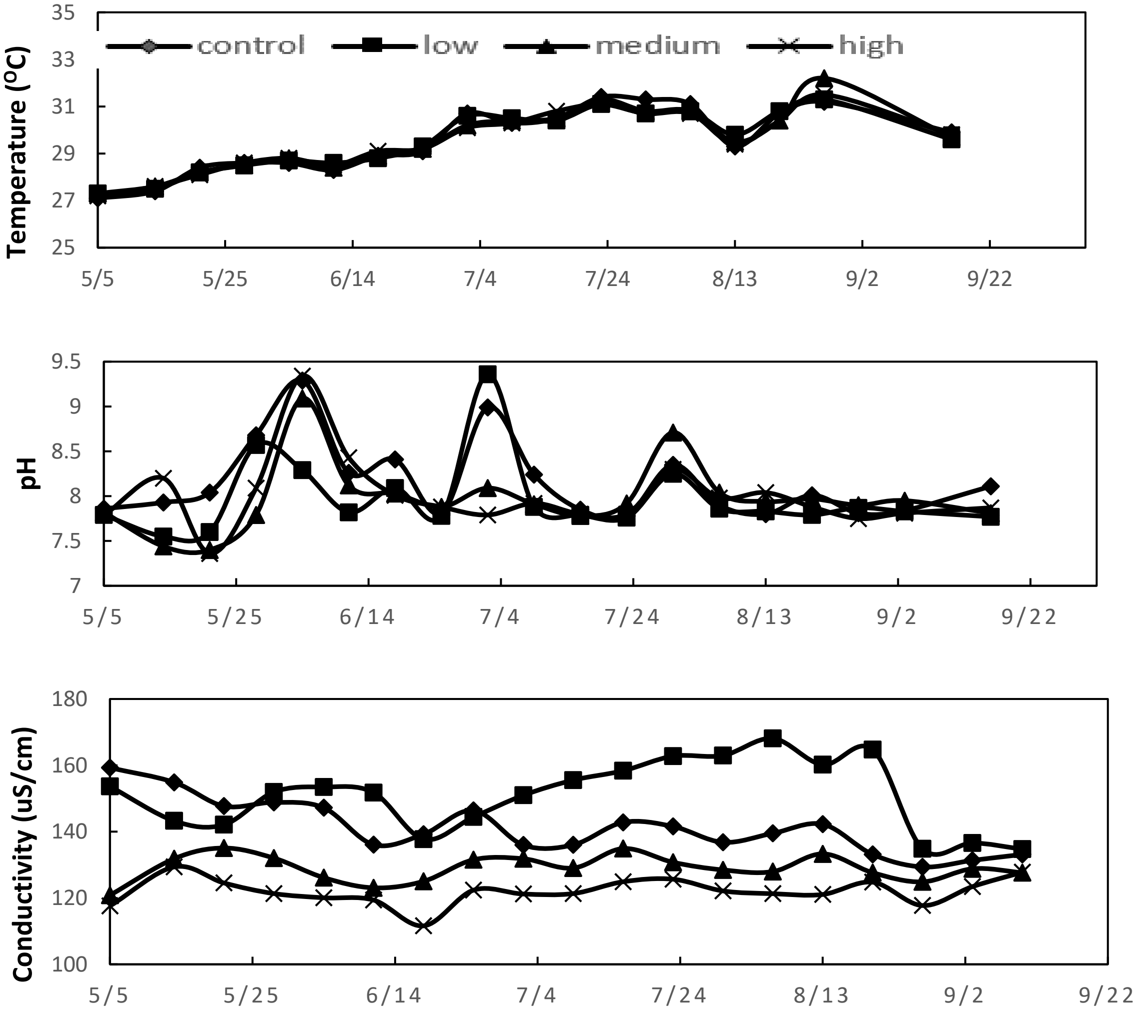

Fig. 3. Weekly averages (n = 3) for total alkalinity, total hardness, and calcium (Ca) hardness concentrations in tilapia ponds treated with 2,000 kg/ha agricultural limestone. Ponds received single, initial treatments of 0 (control), 1,000 kg/ha (low), 2,000 kg/ha (medium), and 4,000 kg/ha (high) of fresh chicken manure.

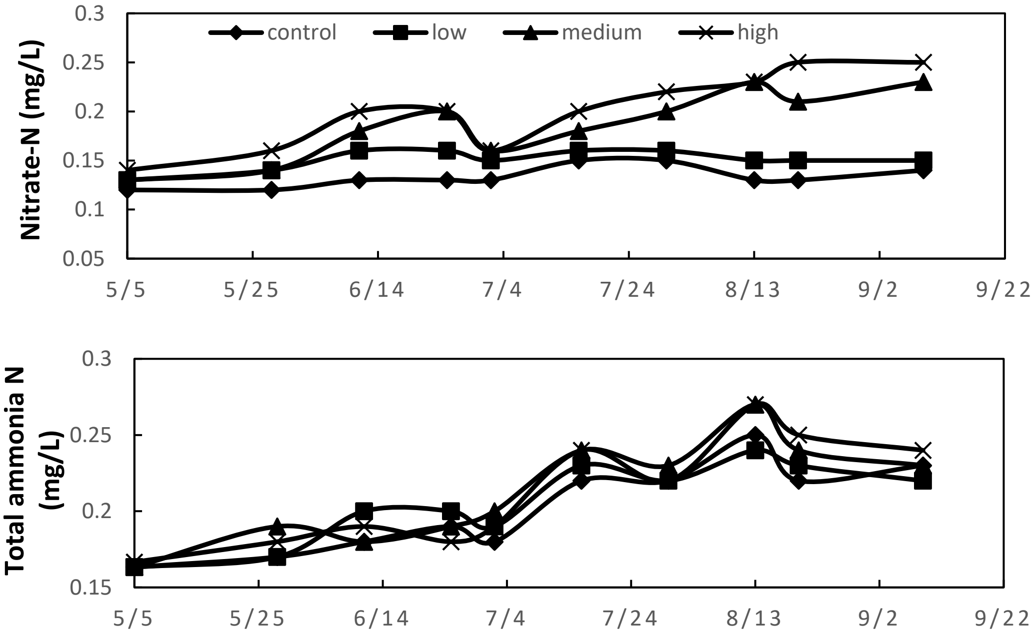

Fig. 4. Biweekly averages (n = 3) for total ammonia N (nitrogen) and nitrate-N concentrations in tilapia ponds treated with 2,000 kg/ha agricultural limestone. Ponds received single, initial treatments of 0 (control), 1,000 kg/ha (low), 2,000 kg/ha (medium), and 4,000 kg/ha (high) of fresh chicken manure.

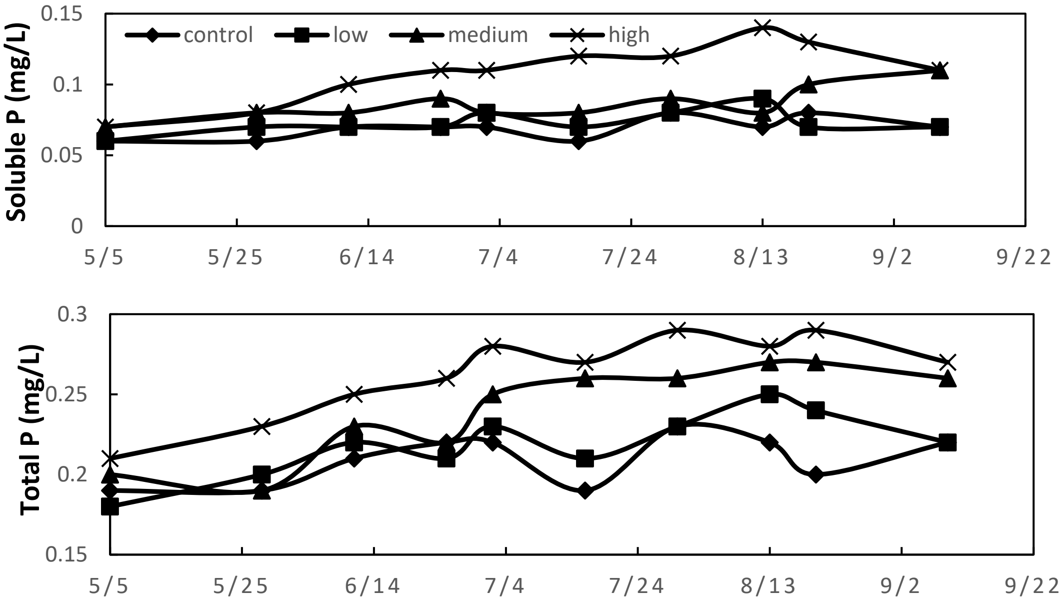

Fig. 5. Biweekly averages (n = 3) for soluble P (phosphorus) and total P concentrations in tilapia ponds treated with 2,000 kg/ha agricultural limestone. Ponds received single, initial treatments of 0 (control), 1,000 kg/ha (low), 2,000 kg/ha (medium), and 4,000 kg/ha (high) of fresh chicken manure.

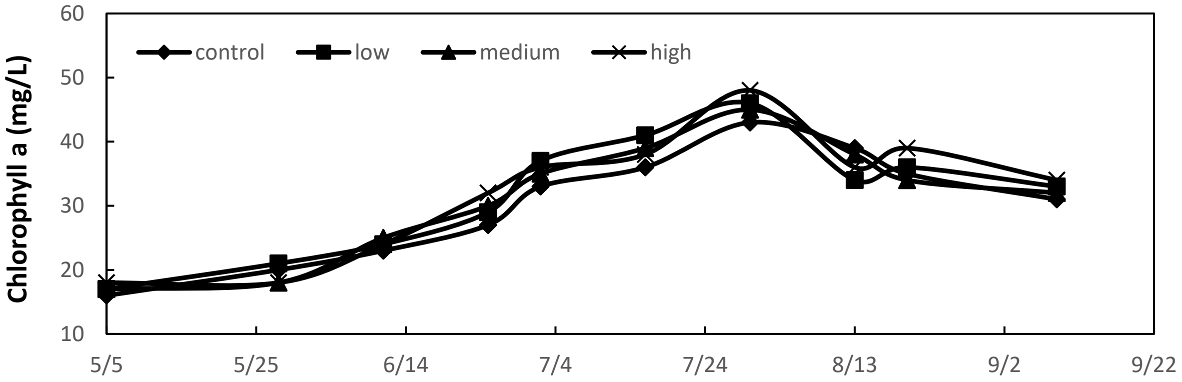

Fig. 6. Biweekly averages (n = 3) for chlorophyll *a* concentrations in tilapia ponds treated with 2,000 kg/ha agricultural limestone. Ponds received single, initial treatments of 0 (control), 1,000 kg/ha (low), 2,000 kg/ha (medium), and 4,000 kg/ha (high) of fresh chicken manure.

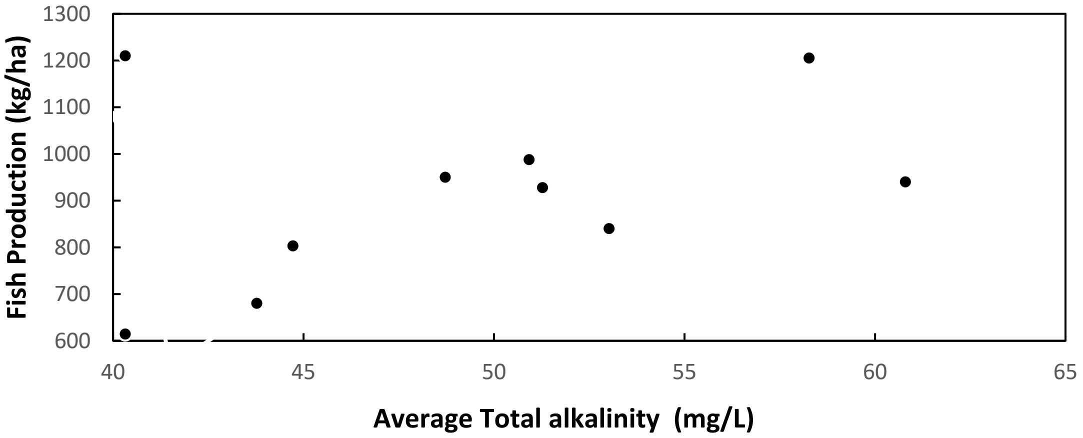

Fig. 7. Scatter diagram between pond study averages for alkalinity and fish production in ponds

CHAPTER IV. – EFFECT OF ORGANIC MATTER CONCENTRATION ON AGRICULTURAL LIMESTONE DISSOLUTION IN LABORATORY SOIL-WATER SYSTEMS

4.1 Introduction

Agricultural limestone dissolves according to the following reaction in which calcium carbonate ($CaCO_3$) represents agricultural limestone:

$$CaCO_3 + CO_2 + H_2O \rightleftharpoons Ca^{2+} + 2HCO_3^-. \tag{4.1}$$

Limestone is seldom pure calcium carbonate (Bowles 1956); it usually also contains magnesium carbonate ($MgCO_3$). But, magnesium carbonate dissolves in exactly the same manner as does calcium carbonate (Boyd et al. 2016). The bicarbonate ion (HCO_3^-) imparts alkalinity to the water, while calcium and magnesium ions provide hardness in chemically equivalent amounts to alkalinity.

Carbon dioxide is an atmospheric gas that dissolves sparingly in water – its equilibrium concentration is about 0.6 mg L^{-1} at the present atmospheric carbon dioxide concentration (Somridhivej and Boyd 2017). This is enough to provide an equilibrium concentration of about 60 mg L^{-2} with most limestones (Sá and Boyd 2017). However, in aquaculture ponds, there are other sources of carbon dioxide. This gas is released into the water by the respiration of aquatic plants and animals and also by the decomposition of dead organic matter by heterotrophic microorganisms (Boyd and Tucker 1998). One major source of carbon dioxide in ponds is the decomposition of bottom soil organic matter. Boyd et al. (2010) measured soil organic matter in bottom soil samples from 233 ponds for different types of aquaculture in nine countries, and bottom soils contained from less than about 1% organic carbon to 7% organic carbon. A factor of 1.8 often is used to convert organic carbon to organic matter (Nelson and Sommers 1982). Agricultural limestone likely dissolves faster and more completely in ponds with large accumulation of soil organic matter than in ponds with less soil organic matter.

The organic matter in pond soils can be divided into two fractions. Fresh organic matter (labile organic matter) is easily decomposed, while organic matter residues that have been in ponds for months or years (refractory organic matter) decompose more slowly (Munsiri et al. 1995; Sonnenholzner and Boyd 2000). Ponds have different ratios of labile organic matter: refractory organic matter (Munsiri et al. 1995). This likely would affect the relationship between soil organic matter concentration and decomposition rate. Nevertheless, it seems logical that a relationship between soil organic matter concentration, carbon dioxide evolution, and the dissolution of agricultural limestone that often is applied to the bottoms of empty ponds. Most of the particles of agricultural limestone applied over the water surface of ponds settle to the bottom before dissolving (Boyd and Tucker 1998).

The hypothesis that agricultural limestone dissolves better in ponds with higher organic matter concentrations was tested in a laboratory study. In order to avoid the complication of the ratio of labile organic matter: refractory organic matter in the sediment, fine sand that did not contain a measurable concentration of organic matter was augmented with pulverized dry grass to achieve different organic matter concentrations. Sand-organic matter mixtures were placed in aquaria, low alkalinity water added, and agricultural limestone applied.

4.2 Materials and Methods

This study was conducted at the water quality laboratory at the E.W. Shell Fisheries Center (SFC), Auburn University, Auburn, Alabama, USA. The “synthetic” bottom soils of different organic matter concentrations were prepared from fine sand obtained from a sandy area on the SFC that passed a screen with 0.085-mm apertures. Ryegrass (*Lolium perenne*) was cut from an area of the SFC where it had been planted for erosion control. The grass was cut into 2-cm long segments and air-dried. It required 1,682 cm^3 of the sand to cover the bottoms of the 29 cm × 25 cm deep aquaria used in this study to a depth of 2 cm. This volume of sand had a weight of 2,820 g.

The dry pulverized hay was placed in an oven for 16 hr at 105°C to drive off the remaining moisture. Triplicate, 100-mL aliquots of the hay were transferred to tared evaporating dishes, the dishes and hay were weighed, and then placed in a muffle furnace at 550°C for 2 hr. The dishes and residues remaining after ignition were cooled to room temperature in a desiccator and weighed. The weight loss in ignition was determined as follows:

$$\text{Weight loss on ignition (g mL}^{-1}\text{)} = \frac{(\mathrm{V}-\mathrm{A})-(\mathrm{C}-\mathrm{A})}{\mathrm{V}} \tag{4.2}$$

where A = tare weight of dish (g); B = weight of dish plus hay pre-ignition (g); C = weight of dish plus residue post-ignition (g); V = volume of hay added to dish pre-ignition (g). This weight loss was assumed to be organic matter.

The “synthetic” soils of different organic matter concentrations were established in triplicate in the aquaria as shown in Table 4.1. The appropriate quantities of grass were mixed into the sand that had already been measured into the aquaria. Treatments and controls were assigned to the aquaria at random so that the trial could be conducted as a completely random design. The sand-grass mixture was wetted and allowed to stand undisturbed for 3 days in a temperature-controlled room (26-28°C) so that the organic matter would begin to decompose and stick to the sand particles. This was necessary to prevent the organic matter from floating out of the sand after the aquaria were filled with water.

Agricultural limestone was sprinkled uniformly over the soil in each aquarium at a rate of 5 g aquarium^{-1}. Twenty liters of municipal water (from Auburn, Alabama, USA) containing 22.0 mg L^{-1} total alkalinity and 20.2 mg L^{-1} total hardness were added to each aquarium. Evaporation losses were replaced with the well water as necessary.

The agricultural limestone and water were added to the aquaria on 9 January 2015. Water samples (100 mL) were removed from each aquarium at weekly intervals until termination of the study on 16 March 2015. The pH was measured with an electronic pH meter and glass electrode (Orion 3 Star pH Benchtop, Thermo Scientific, Singapore, Singapore). Total alkalinity was measured by titration to pH 4.5 with 0.02 N sulfuric acid. Total hardness was determined by titration with 0.01 M ethylenediaminetetraacetic acid to the eriochrome black-T endpoint. These analyses followed standard protocol (Eaton et al. 2005).

Data were subjected to analysis of variance (ANOVA) using a general linear model (GLM). Differences among treatments and control were further assessed by the Duncan's range test. The significance level was set at $P<0.05$. All statistical analyses were carried out using SAS 9.3 (SAS Institute, Cary, North Carolina, USA).

4.3 Results

The concentrations of the major nutrients in the ryegrass are listed (Table 2). The material had a high organic carbon content (55.29%) and a carbon:nitrogen ratio of 47 which is rather typical of foliage sometimes used as pond fertilizers (Boyd, 2018). The concentrations of other major elements also were similar to those found in plant foliage (Morrison, 1961).

Average total alkalinity concentrations in control aquaria increased from 22.0 and 25.8 mg L^{-1} to about 45.0 mg L^{-1} after 30 d (Fig. 1); but, at the end of the trial, control alkalinity was still below equilibrium alkalinity of around 60 mg L^{-1} reported for several agricultural limestone products (Boyd et al., 2016; Sá & Boyd, 2017). Those solubility tests were conducted in flasks vigorously mixed with a magnetic stirrer, while water in aquaria of the present study were mixed more gently. In the 0.5% organic matter treatment, alkalinity appeared to still be rising on day 65 when its concentration reached 61.8 mg L^{-1} (Fig. 1; Table 3). In aquaria with soils to which more organic matter had been added, alkalinity increased faster than in the control and 0.5% organic matter treatment, and it reached average concentrations of 75.3 to 124.6 mg L^{-1} after 65 d (Fig. 1; Table 3). The experiment was terminated after 65 d, because attainment of equilibrium alkalinity in control aquaria would have necessitated either greater mixing or continuation of the trial for much longer with gentle mixing.

Total hardness concentrations (Fig. 1; Table 3) were 18.1-20.2 mg L^{-1} at the beginning of the experiment, and changes in hardness followed the general pattern observed for alkalinity. This similarity resulted because dissolution of agricultural limestone yields chemically equivalent concentrations of bicarbonate and of calcium and magnesium which are the respective ions that impart alkalinity and hardness to water (Boyd et al., 2016). Aquaria containing soil with 2% organic matter had numerically greater hardness than alkalinity concentration, and aquaria of the 4% organic matter treatment had a greater hardness than alkalinity concentration ($P < 0.05$).

The pH in water in aquaria of control and treatments were similar (7.74 and 7.79) at the beginning and increased slowly as the trial progressed (Fig. 1). After 65 d, average pH exceeded 8.0 in all groups. The pH values were averaged directly which is the acceptable way of obtaining average pH for nearly all aquaculture applications (Boyd, Tucker, & Viriyatum 2011). Average pH values after 65 d (Table 3) were different ($P < 0.05$) among control and treatments. Lowest pH was 8.09 in the control and 8.49 for the 4% organic matter treatment.

4.4 Discussion

Calcium and magnesium released into water by microbial decompositions increased hardness, but these ions did not influence alkalinity. Moreover, increases in calcium and magnesium concentrations by liming are usually of little benefit to freshwater aquaculture production, because neither phytoplankton nor fish require more than 1-2 mg L^{-1} of these two cations (Boyd et al., 2016).

Ammonia was released by decomposition of ryegrass, and ammonia titrates as total alkalinity (Boyd et al., 2016). Ammonia nitrogen in water exists as ammonia and ammonium (NH_4^+) in a pH and temperature dependent equilibrium:

$$NH_3 + H^+ = NH_4^+. \qquad (2)$$

Ammonia is favored by high pH, but at pH 8.5 and 27°C, only 17% of the total ammonia nitrogen ($NH_3 + NH_4^+$) would be in NH_3 form (Emerson, Russo, Lund, & Thurston, 1975). Ammonia is lost from water by volatilization to the air, nitrification, and uptake by aquatic plants (Hargreaves, 1998). Aquaria developed planktonic algae blooms, nitrification has been shown to occur rapidly in open containers of water with high ammonia nitrogen concentrations (Hunt & Boyd, 1981), and gentle mixing of water favored volatilization of ammonia. The investigators lacked foresight to measure total ammonia nitrogen concentrations, but it seems doubtful that the alkalinity increase was appreciably influenced by titration of ammonia as alkalinity.

Other components of ryegrass decomposition would not influence alkalinity in unlimed aquaria. If a treatment in which organic matter was added but limestone was not applied had been included in the study, carbon dioxide would not have been used to dissolve limestone. Accumulation of carbon dioxide would have decreased pH and not increased alkalinity. The observed alkalinity increases in organic matter treatments resulted from greater dissolution of agricultural limestone through the effect of carbon dioxide from microbial action on organic matter.

Compared to alkalinity concentrations in the control, final, average alkalinity concentrations of treatments were 36.4%, 66.2%, 111.5%, and 175% greater in the 0.5%, 1.0%, 2.0%, and 4.0% organic matter treatments, respectively. Corresponding increases in hardness were 26.2%, 55.9%, 124.1%, and 225.2%. The greater increase in hardness than in alkalinity for the 4% organic matter treatment could have resulted from release of calcium and magnesium ions from ryegrass decomposition. Based on calcium and magnesium concentrations in the ryegrass (Table 3), the hardness equivalence of these two cations in the 4.0% organic matter treatment was 207 mg L^{-1}. But, the ryegrass did not completely decompose in 65 d. In cropland soil, about one-third of fresh ryegrass organic matter might decompose in this length of time (Jenkinson & Ayanaba, 1977; Saggar, Parshotam, Sparling, Feltham, & Hart, 1996).

Ryegrass decomposition also released ammonia nitrogen. Biological oxidation of 1 mg L^{-1} ammonia nitrogen produces acidity equivalent to 7.14 mg L^{-1} of calcium carbonate or alkalinity (Hunt & Boyd, 1981). Nitrogen in ryegrass of the 4% organic matter treatment could have neutralized up to 9.423 mg of alkalinity (equal to 471 mg L^{-1} alkalinity in the aquaria), but as mentioned above, only a portion of the ryegrass decomposed. Nevertheless, nitrification occurred in the aquaria and neutralized bicarbonate (alkalinity) in water while hardness cations remained. Nitrification was likely the main reason for greater hardness than alkalinity.

Equilibrium pH in a calcium carbonate-water system open to the air is 8.3 and waters with higher pH do not contain carbon dioxide (Boyd et al., 2016). Carbon concentrations in the ryegrass was 55.29% (Table 2). Assuming that one-third of ryegrass added to soils in aquaria decomposed, carbon dioxide released would have been equivalent to 472 mg L^{-1} in the 0.5% organic matter treatment and 3776 mg L^{-1} in the 4% organic matter treatment. Some of this carbon dioxide went to dissolve agricultural limestone. One-half of carbon in bicarbonate resulting from limestone dissolution is from carbon dioxide and the other half is from carbon in limestone as can be seen by the equation:

$$CaCO_3 + CO_2 + H_2O \leftrightarrow Ca^{2+} + 2HCO_3^- \qquad (3)$$

Because 1 mg L^{-1} of alkalinity is equal to 1.22 mg L^{-1} of bicarbonate (61 mg HCO_3^- meq^{-1} ÷ 50 mg $CaCO^3$ meq^{-1}), bicarbonate is 19.67% carbon and equivalent to 72.1% carbon dioxide [19.67% C × (44 g CO_2 $mole^{-1}$ ÷ 12 g C $mole^{-1}$)], and half of the carbon in bicarbonate is from carbon dioxide (Eq. 3), it follows that 1 mg L^{-1} alkalinity contains carbon from 0.36 mg L^{-1} of carbon dioxide. Increases in alkalinity observed in organic matter treatments required (in order of increase organic matter level) 119, 216, 364, and 571 mg L^{-1} of carbon dioxide. The corresponding amounts of carbon dioxide released by decomposition likely were around 472, 943, 1888, and 3776 mg L^{-1}. This equates to 25.2, 22.9, 19.3, and 15.1% of the carbon dioxide released in order of increasing organic matter treatment rate.

Relatively high pH values in aquaria were indicative of low carbon dioxide concentrations. The apparent expression for reaction of carbon dioxide in water is:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H+ \qquad K = 10^{-6.35}. \tag{4}$$

The reaction in Eq. 4 was used to calculate carbon dioxide concentration in aquaria on the final sampling date. There was no carbon dioxide in the 4.0% organic matter treatment because pH was above 8.3. The control contained 0.55 mg L^{-1} of carbon dioxide, and concentrations for the three remaining treatments were 0.76-0.78 mg L^{-1}. Algae growing in aquaria removed some carbon dioxide, but the light intensity of 350 lux was not sufficient for rapid photosynthesis. The majority of carbon dioxide diffused into the air. The observation of pH above 8.5 at times in some aquaria resulted from phytoplankton utilizing bicarbonate as a carbon source in absence of free carbon dioxide (Boyd et al., 2016).

Increases in alkalinity were strongly correlated with amounts of organic matter added to the soil (Fig. 2). Ryegrass was a fresh organic matter source with a high labile:recalcitrant organic matter ratio. In aquaculture ponds, the ratio of labile to recalcitrant organic matter in bottom soils would be less than it was in fresh ryegrass, and the ratio also would vary among pond soils with similar organic matter concentrations. Nevertheless, the study provides evidence that effectiveness of agricultural limestone in increasing alkalinity would be favored in ponds with higher concentrations of bottom soil organic matter. Findings also support the opinion expressed by Boyd et al. (2016) that application of livestock manure or other organic fertilizer at the time of liming would lead to a more rapid increase and greater alkalinity than otherwise would be attained.

4.5 Conclusions

The relationship between soil organic matter and alkalinity concentration found during this study suggests that the amount of organic matter in pond bottoms will effect total alkalinity concentration. The effect on alkalinity by a particular concentration of dry ryegrass mixed in soil would be greater than for the same concentration of more complex and partially decomposed organic matter in a pond soil. Nevertheless, findings of this study provide a reason why the same agricultural limestone rate often results in different total alkalinity concentrations. They also suggest that reaction of agricultural limestone applied at the beginning of grow-out periods could be accelerated by application of organic fertilizers at the time of liming.

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Table 9. Soil organic matter additions used in laboratory soil-water systems.

Organic matter addition rate (%)	Dry, chopped ryegrass addition (g)	Sandy soil (g)	Agricultural limestone addition (g)
0.0	0.0	2820	5.0
0.5	14.1	2820	5.0
1.0	28.2	2820	5.0
2.0	56.4	2820	5.0
4.0	112.8	2820	5.0

Table 10. Composition of dry ryegrass (*Loliumperenne*) used as organic matter source.

Element	Concentration (% dry matter)
Carbon	55.29 ± 0.08
Nitrogen	1.17 ± 0.15
Phosphorus	0.3 ± 0.01
Potassium	1.36 ± 0.16
Calcium	0.79 ± 0.07
Magnesium	0.43 ± 0.005
Sulfur	0.46 ± 0.02

Table 11. Average and standard deviations for water pH and concentrations of total alkalinity and total hardness after 65 d in control and different soil organic matter concentrations in laboratory soil-water systems treated with agricultural limestone.

Organic matter addition rate (%)	pH[1] (standard units)	Total alkalinity[1] (mg CaCO$_3$ L^{-1})	Total hardness[1] (mg CaCO$_3$ L^{-1}
0.0	8.1 ± 0.04 a	45.3 ± 1.15 a	48.3 ± 2.36 a
0.5	8.1 ± 0.03 a	61.8 ± 1.52 b	61.0 ± 1.00 b
1.0	8.2 ± 0.01 b	75.3 ± 4.04 c	75.3 ± 1.25 c
2.0	8.3 ± 0.02 c	95.8 ± 7.22 d	108.3 ± 12.90 d
4.0	8.5 ± 0.05 d	124.6 ± 4.01 e	157.2 ± 7.11 e

[1] Vertical entries with the same letter do not differ at *P* = 0.05 according to Duncan's range test.

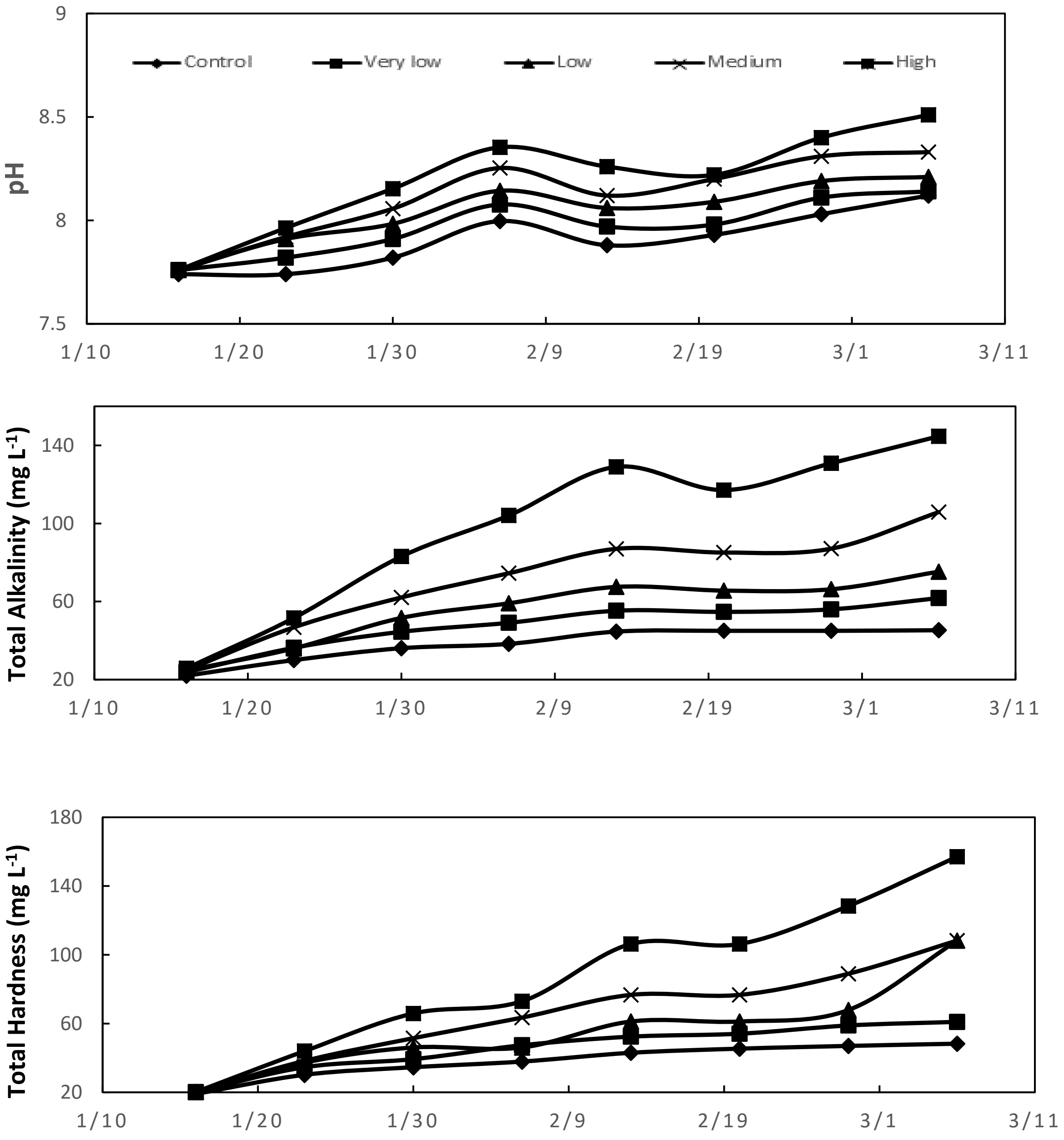

Fig. 8. Weekly average water pH, alkalinity, and hardness concentrations in laboratory soil-water systems with different soil organic matter concentrations and to which agricultural limestone was applied.

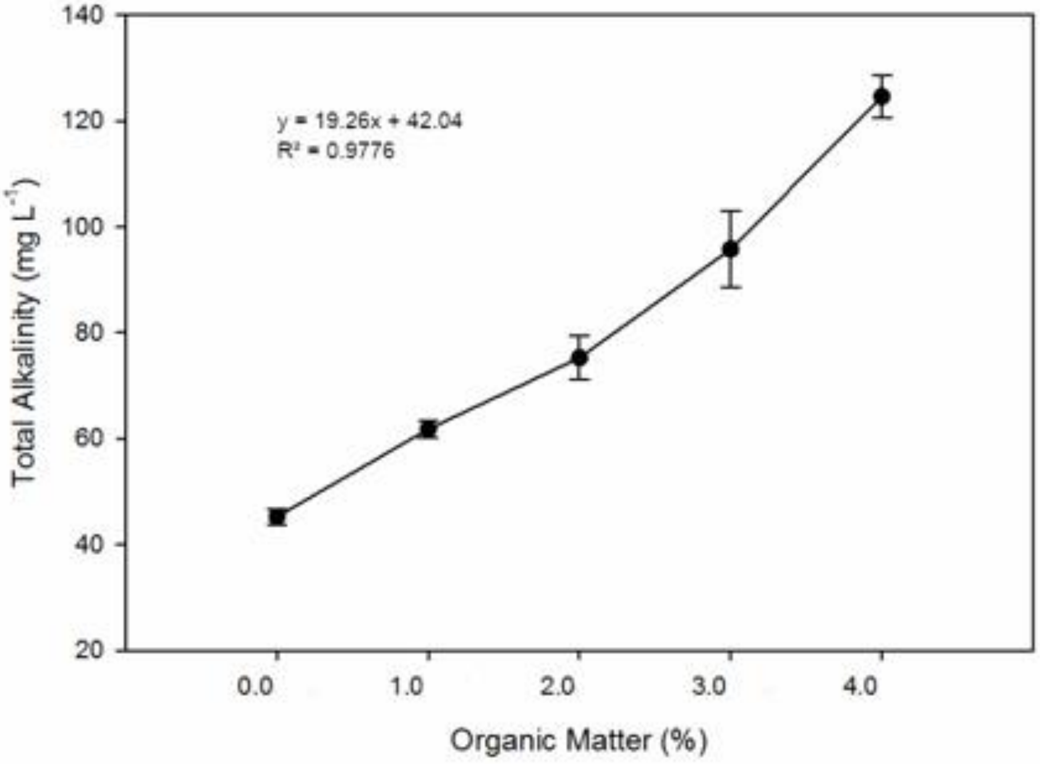

Fig. 9. Regression of soil organic matter concentrations versus total alkalinity in laboratory soil-water systems to which agricultural limestone was applied.