Blended Organic Acids and Palm Vinegar (Acetic Acid) as Performance Enhancers and Inhibitor to Salmonella spp. in Poultry

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***Abstract*—In study 1, the vitro susceptibility testing of Salmonella typhimurium using disk diffusion was conducted. The treatments were: -T1- Plain Water; T2- 1ml Acetic Acid/50ml water; T3-1ml Blended Acidifiers /75ml water; T4- 1ml Hypochlorite/100ml water. The growth of S. typhimurium was affected by the extremely acid and moderately alkaline (*P*<0.01) and hypochlorite treated water (*P*<0.05). Infected samples were found to be negative to S. Typhimurium after the trial. For Study 2, a total of 224 heads of Lohmann LSL white, 115 week- old hens were used and distributed in four treatments with seven replications in completely randomized design such as: T1- Control; T2- 3.0% hypochlorite; T3-3.5% Blended Organic Acids; T4- 5.0% Acetic Acid. The production performance of the old hens did not influence by the treatments except for the yolk color, 4.71-4.93, pale yellow (Roche Yolk Color Fan). The acidifiers (*P*<0.01) improved the FCR of the 119-123 week-old layers with significant effect on additive and feed cost during the first month of the study. This study proved that acetic acid is economically viable and practical alternative to maintain layers health conditions and performance.**

***Index Terms*—Salmonella typhimurium, acidifiers, acidic acid, hypochlorite**

I. Introduction

Food safety is a global concern that raised the consciousness of all the players in the poultry meat and eggs. The awareness led them to implement strict food safety that will guarantee that all their products are free from the harmful organisms that can threaten public health. One of these harmful organisms is Salmonella, [1].

Salmonella remains to be a major hazard to poultry products (e.g. meat and eggs). In fact, there are estimated six million cases of illness from it across European Union each year, majority of which are linked to poultry products, indicates that a multifaceted research approach will be required to control *Salmonella* and *Campylobacter* during poultry production, [2]. (According to Professor O'Brien, the development of several protocols in reducing the risks of Salmonella both in livestock and humans resulted to the dramatic decline of its cases from more than 18,000 in 1993 to just 459 in 2010. Despite this, measures are minimally employed in many developing countries such as the Philippines [3].

Majority of livestock and poultry farms in the Philippines, particularly small scale poultry and livestock production do not employ biosecurity measures on feeds, drinking water, sanitation, vaccination, visitors, predators and domesticated animals. These conditions are categorically below under food safety standard. Most of these small -animal holders rely on their poultry for a living and because they lack knowledge on bio-security and preventive measures for infections, majority of the animal holders only utilize practical solutions like antibiotics usage for any microbial influx in their animal activity.

The main risks for *Salmonella* infections in poultry flock are introduction via several routes of the contaminated water and feed systems, farm housing, manures, insufficient biosecurity and management practices [2]. In chicken, contamination occurs via beak into the intestinal tract. Infection can easily develop and spread through the intestinal tract. Once infected, animals may develop into seeder birds that excrete high numbers of *Salmonella* via faeces, thereby infecting other birds [4]. The widespread use of antimicrobials in veterinary medicines promotes development of resistant strains that can infect humans via the food chain like the Extended Spectrum Beta-Lactamase (ESBL) enzyme being produced by bacteria that blocks the function of antibiotic known to be widely distributed in poultry and poultry products [5], [6].

Organic acids have found to be a successful replacement to antibiotics against *Salmonella* spp. which can be applied via the drinking water and feed. Further, organic acids also influenced egg quality through the increased egg weight and albumen height with organic acids in drinking water in high temperatures [7]. Moreover, the efficacy of palm vinegar consisting mainly of acetic acid (CH_3COH) with pH between 5 and 6 were

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investigated against *Salmonella* spp. and performance enhancers for chicken layer [6]. The researchers at the Food Biotechnology Department, Instituto de la Grasa in Seville, Spain observed that used of vinegar (5% acetic acid) slowed the bacterial activity against all strains tested in several food products as attributed to its acidity, [8]. Thus, the objectives of the study were to evaluate and compare the efficacy of blended acidifiers, organic vinegar and chlorine (acetic acid) in inhibiting the growth of *Salmonella* in poultry (1), to determine the production performance and egg quality of layers as affected by acidifiers and organic vinegar in drinking water (2), to test growth inhibitory activity of blended organic acids, palm vinegar (acetic acid) and hypochlorite against *S. typhimurium* (3), and to evaluate the presence of *Salmonella* in manure (4).

II. Materials and Methods

A. Study 1. In Vitro Growth Inhibitory Activity of S. Typhimurium against Blended Organic Acids, Palm Vinegar (Acetic Acid), and Hypochlorite

The growth inhibition test for *S. tyhpimurium* using disk diffusion was performed. The strain of was purchased from Philippine National Collection of Microorganism. The culture was maintained on nutrient agar slants and petri dishes to keep viability until needed. Working cultures were obtained by inoculating 2ml of pure culture into 250ml 1% peptone (Oxoid Limited), 0.05% yeast extract (Oxoid Limited) and 1% glucose (PYG) media at room temperature for 16-24 h with shaking (150 rev/min) until reaching cell count of 10ϭ to 10ϭ per ml. Bacterial counts were verified using Neubauer slide (hemocytometer, Boekel instruments) at 40 X magnification). Growth inhibition is present if final absorbance minus initial absorbance was ≤0.05 [9], [10]. The study used the single factor experiment consisted of four treatments and three replications per treatment. The data was analyzed in LSD to determine the significant difference among treatment means.

B. Efficacy of the Hypochlorite, Blended Organic Acids, and Palm Vinegar (Acetic Acid) on Production Performance of the Old Lohmann LSL White Layer

Experimental design: The study used the single factorial experiment in Completely Randomized Designed. A total of 224 birds were randomly distributed in four treatment groups with seven replicates per treatment consisting of 56 layers per treatment and 8 layers per replicate. All data were analyzed using analysis of variance for CRD in LSD to determine the significant difference among the treatment means. The treatments are T1-Control; T2-hypochlorite at recommended rates); T3-3.5% blended organic acids; T4-5.0% natural vinegar (acetic acid).

Experimental birds: The existing stocks, 115 week-old Lohmann LSL white of the chicken layer were used in the study. Production record of the flock, egg quality, mortality rate and initial weight of the birds were taken before the start of the trial. This was conducted at the Egg production Farm of BPSU Abucay Campus from November 2011 to March 2013.

Laboratory analysis of cloaca swabs for S. thypimurium: Cloaca swabs from the experimental birds were analyzed for *Salmonella* before the start of the trial. The cloaca swabs were again collected and analyzed for two months after the start of the study.

Methods of application of the experimental materials: The study used the liquid form blended organic acids (SELKO), Palm vinegar and hypochlorite. The experimental old laying hens were provided with separate drinker to measure their water intake. Daily water consumption was measured.

pH analysis: The drinking water with varying levels of the additives was analyzed for pH content.

Data analysis: Inhibition test of the *S. typhimurium* using the blended organic acids (SELKO), palm vinegar and hypochlorite were done to evaluate the inhibiting ability for *S. typhimurium.* The study also evaluated the 3 -week production performance of the old hens such as hen-day egg production, feed consumption, water intake, feed conversion ratio (kg feed/kg egg, and mortality/culled. For egg quality parameters include; egg weight, egg mass per hen-day, egg mass per hen-housed, yolk color and second class eggs. Both data on production and egg quality parameters were analyzed on a weekly basis.

III. Results and Discussion

A. In Vitro Growth Inhibitory Activity of S. Typhimurium against Blended Organic Acids, Palm Vinegar (Acetic Acid), and Hypochlorite

Table I showed the highly significant inhibitory effect (P<0.01) of the blended organic acids, and hypochlorite on *S. thipymurium*, while the acetic acid also significantly affected the growth of the test organism (*P*>0.05). [2], [11] reported that besides probiotics, the continued use of organic acids remains to be an alternative preventive program to reduce and maintain low levels of Salmonella using 1ml natural acetic acid/50 ml drinking water against the growth of *S. thypimurium*. The findings revealed the inhibiting ability of blended acidifiers and hypochlorite.

TABLE I. Growth Inhibition of *Salmonella Thypimurium* as Affected by Organic Acids and Hypochlorite (mm)

Treatments	Mean
T1- Plain Water	0.000
T2-1ml Acetic Acid/50 ml water	8.89*
T3- 1ml Blended Acidifiers /75ml water	19.82**
T4- 1 ml Hypochlorite/ 100 ml water	17.79**
G. Mean	11.62

CV-28.95 %; LSD .05 = 6.34; LSD .01 = 9.22; *significant at *P*>0.05; **significant at *P*>0.01

B. Efficacy of the Hypochlorite, Blended Organic Acids and Palm Vinegar (Acetic Acid) on Production Performance of the old Lohmann LSL White Layer

The laboratory test of the cloaca swabs for Salmonella indicate the occurrence of the said bacteria in the

experimental birds. The assigned hens at the replicates I and III of the treatment IV were confirmed for *Salmonella* spp. infection.

The application of hypochlorite, acetic acid and blended acidifiers in drinking water did not influence the feed consumption of the old lohmann hens (Table II). The results showed that acidifying and chlorinating of drinking water did not depress the normal feed intake of the old laying hens. [12] Dietary acidification with organic acids has been shown to contribute to environmental hygiene by preventing feed from microbial deterioration. It also supports a healthy immune function, which can improve concurrent pathogen resistance of the poultry [13].

TABLE II. AVERAGE DAILY FEED CONSUMPTION OF LOHMANN LSL WHITE CLASSIC FROM 115-127 WEEKS OF AGE

Treatments	Feed consumption, g		
	115-119 weeks	119-123 weeks	123-127 weeks
T1-control	0.117	0.114	0.114
T2-3.0% Hypochlorite	0.115 ns	0.1139 ns	0.114 ns
T3-3.5% Blended acidifiers	0.115 ns	0.1149 ns	0.115 ns
T4-5.0% natural acetic acid	0.115 ns	0.1134 ns	0.115 ns

The FCR of layers at the second month of the study (119-123 weeks old) was highly significantly affected by the acetic acid treated drinking water while the hypochlorite and blended organic acids significantly improved the hens FCR (Table III). Organic acids are claimed to be a valuable substance to prevent pathogens from decontaminating feeds, improved growth and efficiency [12]. The FCR of 123-127 week old hens were not affected by the additive supplementation in drinking water.

TABLE III. FEED CONVERSION RATIO (FCR) OF LOHMANN LSL WHITE CLASSIC FROM 115-127 WEEKS OF AGE

Treatment	FCR		
	115-119 weeks	119-123 weeks	123-127 weeks
T1-control	1.67-	1.71	1.65
T2-3.0% Hypochlorite	1.50ns	1.64*	1.64ns
T3-3.5% Blended acidifiers	1.64ns	1.64*	1.64ns
T4- 5.0% natural acetic acid	1.62ns	1.62**	1.65ns

The results show that the laying percentage of the old Lohmann layers ranging from 56.35-69.85 was not affected by the acidification and chlorination of drinking water from 115- 127 weeks of age. The results suggest that for economic reason, old hens should not be kept when egg production percentage drops to 70% and below.

The monthly egg productions of the old Lohmann hens were comparable to each other from 115-127 weeks of age. Nevertheless, the egg production of the treated groups were consistently higher than the control showing that the acidification of the drinking water slightly influence the egg performance of the old Lohmann hens during the three months study period. This can be attributed with that organic acids activate enzymes, serve as energy source for the gastro intestinal tract, help the chelation of minerals and stimulate intermediate metabolism [12].

There was no significant difference (P>0.05) on egg mass of the old Lohmann LSL white hens from the period of the study (Table IV). However, the mean value of the control (53.75) and 61.49 for the blended organic acids showed that acidification of the drinking water increased the egg mass of the old Lohmann layers. Further, organic acids also influenced egg quality through the increased egg weight and albumen height with organic acids in drinking water in high temperatures [14].

TABLE IV. AVERAGE EGG MASS AND MORTALITY RATE OF LOHMANN LSL WHITE CLASSIC FROM 115-127 WEEKS OF AGE

Treatment	Average egg mass, g	Mortality rate (%)
	115-127 weeks	115-127 weeks
T1-control	53.75	8.03
T2-3.0% Hypochlorite	58.83 ns	7.14
T3-3.5% Blended acidifiers	61.49 ns	10.71
T4-5.0% natural acetic acid	58.06 ns	4.46

TABLE V. AVERAGE YOLK COLOR, SHELL THICKNESS AND WEIGHT OF EGG SHELL OF LOHMANN LSL

Treatment	Egg Quality		
	Yolk color (Roche yolk color fan)	Shell thickness, mm	Weight of egg shell, g
T1-control	5.71	0.34-	5.90
T2-3.0% Hypochlorite	5.14ns	0.32 ns	5.21ns
T3-3.5% Blended acidifiers	4.93*	0.31 ns	5.39ns
T4-5.0% natural acetic acid	4.71**	0.32 ns	5.30ns

Meanwhile, despite of the treatments insignificant effect, the addition of the acetic acid in drinking water for three months reduced the mortality rate. The decrease of the water pH, buffer the capacity and prevent the growth of undesirable microbes [12], [15].

The supplementation of organic acids and acetic acid (palm vinegar) in drinking water affected (P<0.01) the egg yolk color intensity (Roche yolk color fan) (Table V). The egg shell thickness was not affected by the treatments and fall within the acceptable range. The weight of egg shell was not affected proving that acidifying of the drinking water did not alter the egg shell quality of the old Lohmann hens.

IV. CONCLUSION AND RECOMMENDATIONS

The study showed the inhibition of *S. typhimurium* growth using the blended organic acids (19.22mm), hypochlorite and acetic acid. The results confirmed the work of [11], [12], [16] that the use of organic acids can be used as alternative preventive measure against *Salmonella* spp. The findings were attributed from the extremely acid drinking water with the pH levels of acetic

acid treated water and blended acidifiers (<4.0) and extremely alkaline for hypocsshlorite treated water (8.44).

For Study 2, the feed consumption was not significantly affected by the treatments while the FCR of the 119-123 week-old layers were significantly improved by the acidifiers. The rate of lay and monthly egg production were not affected. The cost of additive and feed were higher during the first month of the study and comparable in the rest of the study period. The treatments did not influence the mortality rate, egg mass, shell thickness and weight of egg shell, except for the yolk color (affected by acetic and blended organic acids).

This study showed the potential of organic acids and acetic acids to improve health and optimum poultry performance and food safety. It is recommended to use 80-100 week- old birds with an egg production rate of 70 percent for the trial to evaluate the effect of the additives in flock performance and productivity.

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Hermogenes M. Paguia currently is Professor II and Director for Extension Services of the Bataan Peninsula State University, Philippines. In his twenty years of being a faculty, researcher and extensionist, he looks back and remembers that day. If he would be given another plenty of years, He would gladly take it. His government service began in 1994 when he was employed as School Farm Demonstrator of the Bataan National Agricultural School. On the same year, he pursued his graduate study at Pampanga Agricultural College taking up Master of Science in Agriculture major in Animal Science. He completed the program on May 1996.

He took and completed his Doctor of Philosophy on Agricultural Sciences major in Animal Science at Pampanga Agricultural College in 2006 degree. During his years in the university, He has seen that many households are enthusiastic on the propagation of poultry and livestock. This made him want to study poultry nutrition further. His dissertation titled "The Laying Performance of Chicken Layer (*Gallus domesticus* L.) as Affected by Organic Acids in Combination with Organic Additives on Egg Quality" has received a dissertation grant from CHED and has been presented to the international conference held at CLSU Nueva Ecija in April 2007. He started to immerse himself in research work on that year when his first institutional research proposal on "Effects of Multi-Blend Organic Additives on Three Strains Chicken Layer on Laying Performance and Egg Quality" has been approved and implemented. From then on, he conducted different researches and development projects on poultry nutrition, farming systems and policy on agricultural programs and was made a reality through the initiatives and devoted support of his colleagues. In his twenty years of experience in the field of Agriculture, He is still passionate for more solutions for the common, small scale farmer and grower.

He is actively engaged on extension, he is optimistic that the technology to be developed from the proposed study can be of aid to those who are in need of practical solutions to poultry Salmonella infestation. It is timely that the way the small poultry farms are managed to be improved not only to produce safe and quality products, but for assurance of a profitable enterprise.