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EFFECT OF A PLANT EXTRACT IN SEVERAL TRAITS OF PLYMOUTH ROCK BARRED HENS AND PULLETS CHALLENGED WITH *SALMONELLA TYPHIMURIUM* IN A RURAL VILLAGE IN CENTRAL MEXICO

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ABSTRACT
The effect of Chrysactinia mexicana Gray extract on poultry challenged with Salmonella typhimurium, was evaluated: 1) The aim of the survey was to understand the status quo of backyard poultry production in a rural area, 2). A field study with forty Plymouth Rock Barred Laying hens were used to test the effects of C. mexicana, and 3) 160 day old Plymouth Rock Barred pullets, were assigned to: T1 control; T2 control + S. typhimurium challenge; T3 control + S. typhimurium + C. mexicana; and T4 control + S. typhimurium + antibiotic. Crop, gizzard, proventriculus and duodenum colony forming units (CFU) were measured, and leukocyte and erythrocyte counts. In addition, weight gain and feed intake was measured. The liver, bursa, thymus and spleen were weighed. Results show that 75% of farmers in the community have hens. The main diseases in their fowl: respiratory 45%; diarrhea 35% and parasites 20%. 90% of farmers have no access to veterinary services. Results from the field study show differences (P<0.05) between the treated group with C. mexicana and the control group with no treatment. Feed intake, total weight gain and final body weight was higher (P<0.05) for control group among the other treatments. Treatment challenged plus antibiotic showed lower CFU counts than treatment with S. typhimurium and C. mexicana. Thymus, bursa and spleen weights were similar (P>0.05) for the C. mexicana and antibiotic treatments. Leukocyte and erythrocyte counts were lower (P<0.05) in control group. C. mexicana extract could be a tool to diminish bacteria in hens.

Keywords: *Chrysactinia mexicana*, Salmomella typhimurium, Poultry, Backyard system.

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Contribution/ Originality
This study is one of very few studies which have investigated the use of *Chrysactinia mexicana* extract on poultry performance challenged with *Salmonella typhimurium*

1. INTRODUCTION
Village poultry can be found in all developing countries and play a vital role in many poor households providing scarce animal protein in the form of meat and eggs [1]. It has been estimated that 80 per cent of the global poultry population occurs in traditional family-based production systems [2]. Livestock, especially poultry species, have been shown to provide a practical and effective first step in alleviating rural poverty as a ready source

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of quality nutrients in the human diet [3]. In Mexico more than 85% of the farmers practice it. Animal diets basically consist of kitchen-leftovers, worms and when available, some maize grain. Labor is provided by the household and the poultry represents an important source of protein from meat and eggs, especially for children [4, 5]. The backyard poultry production system is highly affected by several diseases; among them are the gastrointestinal bacteria like *Salmonella typhimurium* that leads to high morbidity and mortality [6]. Some researchers [7] in a study of rural poultry production in Tetiz, Yucatan ,Mexico found that 97.3% of the families with poultry had chickens and showed high mortality and low production traits. It has been shown that the incorporation of herbs and their associated essential oils into their diet may provide beneficial effects on poultry performance and health due to the antimicrobial activity of their phytochemical components [8]. Reports from around the world include exhaustive lists of plants that have been reported to have medicinal properties [9, 10]. *Chrysactinia mexicana* Gray, commonly known as false Damiane is a small shrub distributed throughout the southwest United States and central and northern Mexico [11]. The major chemical components of *C. mexicana* are: eucalyptol (41.3%), piperitone (37.7%) and linalyl acetate (9.1%) [12-15]. A group of researchers [16] studied the *in vitro* antimicrobial effects of *C. mexicana*, which have demonstrated some bactericidal activity. Other group [17] evaluated the effect of *C. mexicana* in maize weevil (*Sitophilus zeamais* Motsch) and found that the leaf powder totally prevented F1 progeny from emerging. In another experiment [18] it was demonstrated that *C. mexicana* aqueous extract induced an antidepressant effect in mice. In another laboratory two experiments were conducted and found that *Chrysactinia mexicana* showed antiprotozoal acitivity against *Entamoeba histolytica* and *Giardia lamblia*, also had an effect on *Trichomonas vaginalis trophozoites* [19, 20]. Moreover, other researchers [21] found that *C. mexicana* showed the greatest antimicrobial activity against the drug resistant strain of *Mycobacterium tuberculosis*. An experiment was performed [22] to test the effect of *C. mexicana* ethanolic extract on 21 week old laying hens challenged with *Salmonella typhimurium*, and showed the *C. mexicana* extract as having a bactericide effect. No research has been done elsewhere with *C. mexicana* extract and its effects on poultry. Because of the high cost of antibiotics and the low income of these types of backyard poultry producers, it makes the treatment of their poultry a real problem. The objective of the present study was to perform a survey about the status quo of backyard poultry production of a rural community; perform a field study with Plymouth Rock Barred hens to test the effect of *C. mexicana* through a spot agglutination test for Salmonella typhimurium; and assess the effect of *C. mexicana* Gray in a controlled experimental trial with Plymouth Rock Barred pullets challenged with *S. typhimurium*.

2. MATERIALS AND METHODS

2.1. Survey

The study was performed in the community of San Diego municipality of Rioverde S.L.P. Central Mexico. Located 21° 54´ N and 100° 10´ W, and 980 m above sea level. Climatic conditions include annual average temperature of 21 ° C and rainfall of 479.5 mm [23]. There were 326 families in the community. A survey using a random sample of 50 families, representing 15.3% of total population, was conducted to investigate back-yard poultry production issues. A questionnaire was designed to obtain information including; production, nutrition, health and reproductive aspects, facilities and equipment for poultry production, as well as meat and egg consumption.

2.2. Plant Extract

Plant collection was made from Guadalcazar village, located in a semi-desert area in the center zone of México. Leaves were separated from the plants, placed on plates and dried for three weeks at room temperature. The leaves were then ground and the extract was obtained by common extract methods, such as heat extraction, gravity column or percolation technique with ethanol [24, 25]. Two hundred g of leaf ground powder samples were placed in a column by gravity or percolation and the solvent was added and sat for 48 h, using about 5 L of ethanol solvent. The samples were then dried in an extraction chamber. The obtained extract was then concentrated at reduced pressure to 29 °C with a rotavapor (R-210/R-215 Buchi) [26, 27]. Finally, the extract was dried by freeze-drying process (cryodesicattion).

2.3. Field Study

The field study was performed in the same village as mentioned above. In order to describe the effect of *C. mexicana* extract under no controlled conditions, one poultry farmer was selected. 40 Plymouth Rock Barred hens were chosen, at day one of the trial and a rapid whole blood agglutination test for *Salmonella typhimurium* was applied to all of the hens. Then two groups of 20 hens each were created. Group 1 (G1) control group was managed as usual; group 2 (G2) same management as G1 but hens in this group received *C. mexicana* extract in the water for 15 days. After the end of the study the agglutination test for *Salmonella typhimurium* was performed again. Briefly, a clean white tile marked into squares of 3 x 3 cm was used. One drop (about 0.02 ml) of crystal-violet-stained antigen was placed in the center of each square. One sample of fresh whole blood was obtained from the wing vein of each hen using a needle with triangular point. An equal size drop of fresh whole blood was placed to a drop of antigen and mixed using a fine glass rod, the drops kept agitated for up 2 minutes. A positive reaction in indicated by easily visible clumping of the antigen within 2 minutes [28].

2.4. Experimental Study

A complete randomized design was used. 160 one day old Plymouth Rock Barred laying pullets were allocated to individual cages, 40 pullets per treatment: T1: control, T2: control + *S. typhimurium* challenge, T3: control + *S. typhimurium* + *C. mexicana* extract and T4: control + *S. typhimurium* + antibiotic. The *C. mexicana* extract was administered orally via an esophageal cannula during 15 days with a dose of 0.5 ml of the extract according to previous research in our laboratory [22]. To test the plant extract a standard suspension of *S. typhimurium* (ATCC 14028) was prepared to meet the 0.5 Mc Farland standard equivalents to 10^8 CFU/ml concentration [29].

S. typhimurium challenge was given in an identical manner at days two and six of the experiment [30]. The antibiotic used was enrofloxacin 5 mg (Baytril 0.05 %, Bayer, Mexico) the dose was 1 mL/1L water every 24 hours. Feed and water was offered *ad libitum* and, pullets were not vaccinated. Feed was formulated to meet or exceed the NRC [31] requirements for laying pullets (Table 1). Measured variables were initial body weight, weight gain, final weight, feed intake and quantification of colony forming units per ml (CFU/ml) in gizzard, duodenum, proventriculus, and crop of hens which were slaughtered 15 days after *S. typhimurium challenge*. In addition, Leukocyte and erythrocyte counts were determined by Natt and Herrick´s Stain method [32]. Briefly, a standard red blood cell diluting pipette was used to dilute whole anticoagulated blood with the Natt & Herrick´s solution at the rate of 1:200 the diluted blood was allowed to mix for two minutes before it was discharged into the hemacytometer counting chamber. Then using the high dry (40X) objective of the microscope, the total number of red and white cells were counted. Also, the liver, bursa, thymus, and spleen were removed and weighed. For the Colony Forming Units (CFU), from the different organs and using sterile scissors a small (approximately 1 cm) hole was cut, two milliliters of sterile PBS were pipetted into each organ. Only 2 to 4 mL of liquid was recovered. One milliliter was used for a culture. The colony counting procedure used was the membrane filter technique [33].

2.5. Data Analysis

Analysis of the survey was analyzed using PROC UNIVARIATE and PROC FREQ procedures of statistical analysis system (SAS) software [34]. The field study data were analyzed by logistic regression (LOG REG) analysis of SAS software [34]. For the experimental study, a complete randomized design was used to assess the extract activity. Analysis of variance was performed with PROC GLM of SAS, and Tukey means with SAS Institute [34] software program. Bacterial numbers were converted to log CFU for statistical analysis.

Table-1. Basal diet composition[1]

Ingredient	g/kg diet
Yellow corn 8%	566.33
Soybean meal 46%	357.08
Calcium	15.80
Vegetable oil	28.35
Phosphate	17.94
Sodium chloride	4.00
Vitamin mix[2]	2.50
Mineral mix[2]	2.50
DL-Methionine	2.38
Threonine	0.30
L-Lysine	1.75
Choline	1.07
Chemical composition	
Metabolisable energy, Kcal/kg	2975
Crude protein, %	21.73
Fat ,%	5.30
Fibre, %	2.91
Ash, %	6.62
Methionine, %	0.64
Lysine, %	1.33
Calcium, %	1.00
Phosphorus,%	0.75
Threonine, %	0.86

[1]Diet was offered ad libitum for the duration of the trial, and was formulated to meet or exceed all requirements for growing pullets [31].

[2]Vitamin mix provided (per kg final diet): thiamin, 1.8 mg; riboflavin, 3.6 mg; pantothenic acid, 11.5 mg; niacin, 35 mg; pyridoxine, 3.5 g; folic acid, 0.6 mg; biotin, 0.2 mg; vitamin B-12, 10 µg; retinyl palmitate, 0.9 mg; cholecalciferol, 50 µg, all-*rac*-α-tocopheryl acetate, 36.8 mg; menaquinone, 5 mg. Mineral mix provided (per kg final diet) selenium, 0.2 mg; copper, 8.1 mg; zinc, 40.7 mg; manganese, 62 mg; iron, 105.4 mg; iodine, 0.35mg.

3. RESULTS

3.1. Survey

Results from survey show that average family size is 5 ± 2 people with an average age of 25 ± 15years old. The level of education was 3rd year of elementary school. Results also showed 70% of the people in the village own their houses and that 75% of farmers have poultry. All farmers have a backyard where they have different species of animal, such as rabbits, pigs, hens, chickens, turkey, sheep, cows and horses. Of these species, poultry represents 33% of the total animals. The diseases present in their fowl were: respiratory 45%; diarrhea 35% and parasites 20%. In addition mortality was due to: respiratory 35%, diarrhea 55% and predators (human, dogs and fox) 10%. Poultry breeds were divided among Creole 50%, Rhode Island Red 30% and Plymouth Rock Barred 20%. Table 2 shows the results of a portion of the questionnaire. It is divided into three columns based on the farmer´s income; very low income, low income and regular income. It can be seen that most of the items have lower values for the very-low income farmers; low egg and meat consumption as well as small flock size. The same is true for feed source, poultry housing and knowledge about diseases. Moreover, the very-low income farmer does not typically access to veterinary services.

3.2. Field Study

The results from the field study show differences ($P<0.05$) between the treated group with C. mexicana and the control group with no treatment. From the 20 hens positive to S. *typhimuriun* at the beginning of the study, only two were positive to *S. typhimuriun* at the end of the study, with a morbidity of 10% and 0% mortality (Table 3). The 20 hens in the untreated group remained positive to *S. typhimuriun*, with 100% morbidity and 10% mortality at the end of the study.

Table-2. Backyard Poultry production situation of San Diego Village, Rioverde, SLP.

Item	Very-low income	Low income	Regular income
Egg Consumption	1 egg per week 80%	1-3 egg /week 15%	5 eggs /week 5%
Meat Consumption	1 time a week 80%	2 times /weeks 17%	3 time/week 3%
Flock Size	1-5 Hens 50%	6-10 hens 30%	11-20 hens 20%
Access to Veterinary Services and Pharmaceuticals	Never 90%	Sometimes 3%	Yes (frequently use private service providers) 7%
Feed Source	kitchen-leftovers, insects, worms 45%	Crop By-products, Corn grain 45%	Balanced commercial ration 10%
Poultry Housing	None 85%	Sometimes, usually from used local materials 12%	Cages 3%
Training	None 96%	Moderate: control of ND, Gumboro, fowl cholera; breed selection, supplememnatary feeding, appropriate housing 3%	Considerable: wide ranging control; breed selection, used of balanced ration, good housing 1%

Results from survey from 50 families of San Diego Village community, Rioverde, SLP

Table-3. Spot Agglutination test for *Salmonella typhimurium*, mortality and morbidity of hens

Item	C. Mexicana	Control
Day 0	20 hens positive/Salmonella	20 hens positive/Salmonella
Day 15	2 hens positive/Salmonella^{b}	20 hens positive/Salmonella^{a}
Morbidity %	10	100
Mortality %	0	10

^{ab}Means within columns with different letter are significantly different (P<0.05)

3.3. Experimental Trial

Feed intake, total weight gain, final body weight and feed conversion rate was higher (P<0.05) for control group (T1) among the other treatments (Table 4). Treatment 2 with *S. typhimurium* challenge had the lowest trait performance. Treatment with challenge and *C. mexicana* extract (T3) and treatment with challenge and antibiotic (T4) had similar (P>0.05) feed intake, total weight gain, final body weight and feed conversion rate response. The control group had lower (P<0.05) CFU for crop, gizzard, proventriculus and duodenum compared with the other treatments (Table 4). The highest (P<0.05) content of CFU for all four organs was for the T2. The treatment challenged with *S. typhimurium* and *C. mexicana* extract had lower (P<0.05) CFU for the organs than T2. Treatment challenged with antibiotic showed lower CFU counts than treatment challenged with *S. typhimurium* and *C. mexicana* extract. Thymus, bursa and spleen weights were lower (P<0.05) for the control group than other treatments. Thymus, bursa and spleen weight were similar (P>0.05) for control T3 and T4. Treatment 2 had the highest (P<0.05) weight values among the rest of treatments. Leukocyte and erythrocyte counts were lower (P<0.05) in control group compared with the other treatments, the highest values (P<0.05) were for the treatment challenged with *S. typhimurium*. Treatment with challenge plus *C. mexicana* and treatment with challenge plus antibiotic had similar (P>0.05) weights.

Table-4. Means of Plymouth Rock Barred pullets performance, organ weights, blood cells and colony forming units [log CFU/ml] on crop, gizzard, duodenum and proventriculus with different treatments

Treatment	T1	T2	T3	T4	SEM
Performance					
Initial body weight [g]	33.05	32.47	33.18	32.57	0.918
Final body weight [g]	203.75ᵃ	181.92ᶜ	200.05ᵃ	206.62ᵃ	2.015
Total weight gain [g]	170.07ᵃ	149.45ᵇ	166.87ᵃ	174.05ᵃ	4.021
Average daily gain [g]	8.12ᵃ	7.1ᵇ	7.9ᵃ	8.2ᵃ	0.112
Feed intake [g] Feed Conversion Rate	315.28ᵇ 1.85a	395.18ᵃ 2.60c	351.24ᶜ 2.10b	348.12ᶜ 2.00b	7.172 0.024
Colony Forming Units [log CFU/ml]					
Crop	5.20 ᵈ	5.44ᵃ	5.33ᵇ	5.30ᶜ	0.013
Gizzard	1.90 ᵈ	2.90ᵃ	2.70ᵇ	2.50ᶜ	0.024
Proventriculus	3.58 ᵈ	4.23ᵃ	3.91ᵇ	3.81ᶜ	0.018
Duodenum	3.77 ᵈ	4.44ᵃ	4.06ᵇ	3.84ᶜ	0.039
Organ weights					
Thymus [g]	0.435ᶜ	1.178ᵃ	0.822ᵇ	0.805ᵇ	0.036
Bursa [g]	0.689ᶜ	0.960ᵃ	0.789ᵇ	0.720ᵇ	0.055
Spleen [g]	0.268ᶜ	0.359ᵃ	0.300ᵇ	0.299ᵇ	0.012
Blood Cells					
Leukocyte/ mm3	5.01ᶜ	5.46ᵃ	5.33ᵇ	5.25ᵇ	0.010
Erythrocytes/mm3	5.84ᶜ	6.34ᵃ	6.27ᵇ	6.19ᵇ	0.021

[a,b,c,d] Means within columns with different letter are significantly different ($P<0.05$). T1=Control basal diet; T2=Control + challenge with *S. typhimurium;* T3=Control + *S. typhimurium* + *C. mexicana* extract; T4= control + *S. typhimurium* + antibiotic.

4. DISCUSSION

The survey shows that 75% of farmers in the community have hens in their backyard. This is consistent with a group of researchers [35] who carried out a study in a rural community of Veracruz, Mexico which found that 63 % of farmers have hens in their backyard. The results in the present study demonstrate that hens are very important in rural communities. The survey results illustrate that feeding and nutrition of the birds is still a problematic issue, with a high incidence of disease increasing the problem. Another study performed by other group [4] in the state of Puebla, Mexico showed that several respiratory diseases and attacks by predators were mentioned as the main causes of mortality, and the lack of quality feed ingredients and practically no veterinary advice were identified as the most important constraints of household poultry production. Moreover, It is clear that the field study performed in the rural community of San Diego Village demonstrated the problems of hens with the diseases mentioned above. With these results it's very likely that the morbidity caused by *S. typhimurium* affects most of the fowl in the rural community, and the use of *C. mexicana* in these cases was very helpful in decreasing the presence of *S. typhimurium*. For the experimental trial the effect of *C. mexicana* in pullets performance was evident; the total weight gain for the treatment challenged plus *C. mexicana* and the group challenged with antibiotic were similar. This was probably due to the effects of the flavonoids of plant extract working to cope with the effects of the infection [22, 36]. As mentioned by other authors [37] who reported *C. mexicana* to have antimycobacterial activity, it also has anti-diarrheic activity [38]. Eeucalyptol and 26 other diterpenes have been reported to decrease cytokines IL-2 (Th1) and IL-10 (Th2) that are anti-inflammatory inhibiting the response of T cells [39]. Finally, it has been reported that the essential oil from *Cymbopogon proximus* contains piperitone as the largest compound (73.8%). This compound antagonizes the actions of serotonin and histamine, by the interaction of its receptors [40]. According to the results of this experiment, the use of *C. mexicana* extract could be a good alternative, especially for low-income families in rural areas that cannot afford to purchase antibiotics for their hens.

5. CONCLUSION

Backyard poultry production in rural communities in Mexico remains a very important activity, and production is limited by several factors; nutrition, and diseases among others. The *Chrysactinia mexicana* extract showed good performance traits and could be a tool to increase poultry production in rural areas.

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