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Surfactants against Ectoparasites Associated with ZamPen Native Chicken

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Abstract

The economically important ectoparasites in chicken includes lice and mites that serves as vector of zoonotic diseases to humans. This study was conducted to determine the effects of natural surfactant and synthetic surfactant in dislodging ectoparasites from the breed of ZamPen native chickens procured randomly and acclimatized in a housing pen. Chickens were bathed with surfactants such as commercial shampoo, commercial powdered soap, formulated tobacco liquid soap, commercial liquid soap and water through three tub method. The physicochemical parameters of liquid soap was evaluated within the range. Results showed that there was a significantly high number of ectoparasites dislodged after the first and second applications of the treatments except those bathed with water only. However, after the third application of treatments, a significant number of ectoparasites were dislodged from chickens treated with commercial powdered soap and commercial liquid soap but significantly a low number of ectoparasites were dislodged from chickens treated with formulated tobacco liquid soap. This suggests that the remaining ectoparasites were not able to recover its population in chickens treated with formulated tobacco liquid soap after the third treatment application. The clinging ability of the ectoparasites may have been rendered weak within seven days after the third application. Dislodged ectoparasites include three species of lice such as *Menopon gallinae*, *Menacanthus stramineus*, *Lipeurus caponis* and two species of mites such as *Ornithonyssus bursa* and *Megninia* sp. Hence, the formulated tobacco liquid soap is effective and a potential alternative organic treatment in reducing ectoparasites on infested chickens.

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Introduction

Consumers prefer native chicken meat over that of broiler because of its unique taste, distinct flavor and texture, presence of nutraceutical compounds (functional food), and lower fat content (DOST-PCAARRD, 2016). The ZamPen native chicken is a purified breed from the mixed breed of native chickens in Zamboanga Peninsula, Philippines with faster growth than other native chickens. It is suitable in free range conditions as they are naturally foragers which entails lesser feeding cost and appropriate for commercial free range poultry rearing (PCAARD-WESMAARRDEC, 2017). However, Lay *et al.* (2011) emphasized that the health condition of ZamPen native chicken can be jeopardized by various parasites since free ranging birds have a greater chance of acquiring diverse parasites population. Njunga (2003) also mentioned that parasites infesting free ranged chicken are either in interaction with other infected animals or acquired in a scavenge environment, with a prevalence of 100% frequency of occurrence. In free range system, chickens are rendered susceptible to ectoparasitism resulting to debilitating health condition (Portugaliza and Bagot, 2015) manifested as itching, annoyance, loss of sleep, general weakness, loss of appetite, restless, allergy (Al-Saffar and Al-Mawla, 2008), pediculosis and mange (Tamiru *et al.*, 2014) leading to weight loss. Another symptoms is the decreasing animal production (Portugaliza and Bagot, 2015) such as drop of egg production in layers and anemia, significant decrease in %PCV (packed cell volume), TRBC (Total Red Blood Cells) and Hb (Hemoglobin) concentration in chicken, leukocytosis with increase in heterophils, and eosinophils. Damage and harm caused by the ectoparasites depends on the number of parasites, nutritional status of the host and intercurrent diseases (Al-Saffar and Al-Mawla, 2008).

Among the economically important species of ectoparasites in chicken includes species of biting lice (shaft louse, wing louse, feather louse and fluff louse), tick, fleas and mites (Bala *et al.*, 2011). Ectoparasite infestation can be either mixed or single infestation (Al-Saffar and Al-Mawla, 2008; Mirzaei *et al.*, 2015). In addition, ectoparasites serves as a vector of zoonotic and economic diseases (Portugaliza and Bagot, 2015) and according to Wolf (2010), lice transmit and serve as reservoir of pathogens like swinepox and *Rickettsia*. Ticks and tick-borne diseases can transmit zoonotic bacteria, viruses and protozoa (Ahmed *et al.*, 2007). Fleas serve as carrier of known human and animal pathogenic microorganisms such as *Yersinia*, *Rickettsia* and *Bartonella* (Wall and Shearer, 2001; Dieme *et al.*, 2015). Hence, proper management, control and prevention of ectoparasite infestation in chicken is vital to ensure maximum productivity. The epicuticle of ectoparasites is typically waterproof. It consist of waxy layer of lipid molecules, making it very resistant to chemical attack and insoluble to ordinary solvents. These sclerotized cuticle prevents the body of the ectoparasite from being soaked with water and from excessive drying (Metcalf and Flint, 1962). However, surfactants have been used as wetting and emulsifying agents in ectoparasites (Burgess *et al.*, 2015). Its molecule have amphiphilic structure including an affinity for water and oil that allows surfactant activity of the compound to emulsify some of the lipid protective waterproofing layer of the cuticle of the insects resulting to dispersion, foaming, flocculation and decontamination of the substance (Yuan *et al.*, 2014). As the material is rinsed off, the emulsified lipids would be taken with it, leaving the insects susceptible to dehydration and the protective layer on the surface of its cuticle is damaged. Due to the amphiphilic action of surfactants such as shampoos, soaps and dishwashing liquid can be utilized to reduce the ectoparasites in chickens (Burgess *et al.*, 2015).

The action of surfactants can be enhanced by incorporating the ethno veterinary plants such as tobacco. The tobacco plants have been used to treat skin of livestock afflicted with external parasites (Guarrera, 1999). Dried leaves of tobacco had been found out by Dannang (2013) to have a highly significant effect in controlling lice and mites in hen at egg laying period. Since 2003, tobacco plant application in controlling ectoparasites have just existed in the form of powder, dust, and liquid spray (Njunga, 2003; Dannang, 2013) and not as a surfactant based product. The incorporation of tobacco with a surface active agent called surfactants may enhance the effectivity of surfactant in minimizing the ectoparasite infestation in ZamPen native chickens hence the objective of the study. Specifically, this study aims to produce a formulated tobacco liquid soap capable of dislodging ectoparasites in the chicken and to determine the number and identify the species of ectoparasites such as lice and mites dislodged from ZamPen native chicken applied with the different treatments of surfactants.

Materials and methods

Procurement of the ZamPen Native Chicken

The breed of native chicken developed in Zamboanga Peninsula is called ZamPen native chicken. The male or rooster has a pea combed with brown red-orange feathering with streaks of black. The female or hens are black and has a brown-black hackle feathers. Both sexes of ZamPen native chicken have black-tail feathers and have a bright red earlobes and faces. It is a heavy breed of native chicken and can stand upright with gray shanks. A total of 45 ZamPen native chickens about 360 days old were procured randomly from San Ramon Prison and Penal Farm Zamboanga City, Philippines and acclimatized for 15 days in the setup pens.

Housing of the ZamPen Native Chicken

The ZamPen native chickens were provided with a housing shade constructed inside a pen as shown in Fig. 1. Each pen measures 9 × 8 meters fenced with a net. The housing shade measures about 3 × 3 meters and constructed out of nipa for its roofing and wood for its structural support and roosting. Commercial feeds were provided as nourishment for the ZamPen native chicken. Feeding management, maintenance and sanitation were sustained.

Labelling the ZamPen Native Chicken with Tags

Each ZamPen native chicken was tagged using a 1 × 2 centimeters sheet of colored hard folder attached to the releasable cable ties at the base of the left wing of the chicken. The labelling tags were laminated to prevent it from mechanical damage. The numbers 1 to 45 are labelled according to Color Hole Number of Folder (CHNF) scheme whereby the color of the cut folder piece represents the Treatment (T) as follows: green (T1), red (T2), yellow (T3), violet (T4), and blue (T5). The number of hole/s punched in the piece stands for the replicate number and the number of the cut folder piece shows the sample number as illustrated in Fig. 2.

Preparation of Tobacco Extract

The method of Willson (2018) was used for the extraction of tobacco with some modifications. About 125 grams of dry tobacco leaves were chopped and soaked in 3 liters of water within 24 hours. The mixture was strained using cheese cloth. The extract was stored in plastic screw capped container.

Preparation of Tobacco Liquid Soap

For the preparation of the liquid soap, the method of Maslowski (2018) was adapted with some modification. A volume of 487.9 ml of olive oil and 207 ml of coconut oil were mixed in a 1000 ml beaker and heated in a low flame. In a separate beaker, a 487.9 ml of water and 155.9 ml Potassium Hydroxide (KOH) were mixed to be a lye-water solution. When the lye-water solution appears clear, it is added to the heated oils and stirred for 5 minutes until all the oils were evenly mixed. After homogenizing the mixtures, this was stirred for 30 minutes and continue for about 30 minutes with the lid open. The stirring goes every 30 minutes for 3 to 4 hours until the trace (soap paste) is achieved with a soap jelly-like appearance. Once the trace is ready, 1182.9 ml of distilled water was boiled to a large stainless pot with a lid, then the soap paste were added. The heat was turned off once the mixtures were well combined and stirred after 1 hour. When the mixture is smooth, 28.3 grams of borax were dissolved in a 60 ml of boiling water. A volume of 60 ml borax solution was added to the soap base to neutralize the mixtures. Prior for the formulated treatment, a volume of 90ml tobacco extract was added to the liquid soap. This was placed in labelled container and allow to sit for 7 days without disturbing to allow the sediments to settle at the bottom of the bottle. This was then transferred to a dispensing bottle and store in a room temperature. The treatments with different surfactants used are Treatment 1 (commercial shampoo); Treatment 2 (commercial powdered soap); Treatment 3 (formulated tobacco liquid soap); Treatment 4 (commercial liquid soap with anti-ectoparasite) serve as positive control; and Treatment 5 (water) serve as negative control.

Physicochemical Parameters of the Formulated Tobacco Liquid Soap

For the evaluation of physicochemical parameters, the method of Afsar and Khanam (2016) was adapted in order to establish the quality of the (T3) formulated tobacco liquid soap. The physicochemical parameters evaluated are (a) clarity, color and odor, (b) pH, (c) foam height, and (d) foam retention capacity.

Clarity, Color and Odor

The clarity and color of the formulated tobacco liquid soap were assessed by its appearance in a white background and the odor was determined in a drop on the palm through smelling.

Potential Hydrogen or pH

The pH is a chemical parameter which measures the degree of acidity or alkalinity of the resulting liquid soap (Widyasanti *et al.* 2018). The pH of the tobacco liquid soap was determined by placing 5 grams of the soap on a 50 ml beaker filled with distilled water. The tip of the calibrated digital pH meter was then immersed in the beaker containing the soap. This was performed three times in order to obtain the average pH value of the formulated tobacco liquid soap.

Foam Height

Foamability is a dynamic property of the foam-generating power of a liquid which refers to foam volume generated by supersaturating a liquid with a gas under pressure and then releasing the pressure by either forcibly injecting or beating a gas into liquid to determine the immediate stabilization of foam (Sakai and Kaneko, 2004). A 0.5 grams of the tobacco liquid soap was dispersed in a beaker of 25 ml distilled water. The solution was transferred to a 100 ml graduated cylinder and was given 25 times back and forth stroke. The solution was allowed to stand still and the foam height was measured above the volume of the soap solution.

Foam Retention Capacity

The measure of the time a foam film exists without bursting is foam retention. This is used to establish the average foam lifetime or the rate of foam decay (Sakai and Kaneko, 2004). A volume of 25 ml tobacco liquid soap was taken into a 100 ml graduated cylinder covered with and was shaken 10 times. The volume of foam was recorded at 1 minute interval in every 4 minutes.

Application of the Treatments

The procedure of Damerow (2016) was adapted using three tub method for the application of the treatments. The first tub containing 6 liters of water was added with the designated volume of the treatment while the second and the third tub contained only six liters of water for rinsing. As shown in Fig. 3, the chicken was slowly immersed in the first tub (tub 1) up to the neck. Then the body of the chicken was thoroughly soaked in the first tub by raising lowering and drawing back and forth. The extra lather in the tail and vent was rubbed from the dorsal part of the body towards the tail and the ventral part down to the legs. The head and neck was applied by getting an ample amount of water with soap and gently massaging from the head towards the feather. The rinsing was done in the second tub (tub 2) and the final rinse in the third tub (tub 3) for approximately 1 minute following the tub 1 procedure. Each chicken was dried by gently squeezing excess water from the body and gently wrapping the chicken using a fabric cloth then it was returned to its corresponding pen. The procedure were followed for the rest of the chickens. The treatments were applied every after twenty one days for two consecutive months considering the life cycle of the ectoparasites. Each treatment was replicated three times with three sample chickens in each of the replicate. The first, second, and third applications of the treatments with different surfactants were recorded.

Collection and Preservation of the Dislodged Ectoparasites

The water remains of each of the chicken after bathing were filtered using a sieve with a cloth on top shown in Fig. 4. The filtrate were disposed and the filter along with residue was collected and brought to the laboratory for segregation and identification of the ectoparasites. Visible ectoparasites were picked using a forceps and placed in a vial containing 70% ethyl alcohol. The remaining residues and filter were examined further for presence of microscopic ectoparasites.

Quantification, Processing and Examination of Ectoparasites

Total number of mites were quantified according to Leigh *et al.* (1984) with slight modification by making a transparent 37 grid measuring 1 mm^2 (18 vertical and 18 horizontal) intersecting at the middle and placed in a 90 mm diameter petri plate. The number of mites observed in the 37 grids are multiplied by the total area of petri plates (6361.74 mm^2). The total number of lice was counted in each petri plate. The processing of dislodged ectoparasites for slide mount were done based on Sabuni *et al.* (2010) and Bala *et al.* (2011), by dehydrating the ectoparasites in a series of alcohol concentration of 80%, and 95% for about 30 to 40 minutes. The dehydrated specimen was cleared in 10% KOH for 2 days and mounted on a slide for identification. The processed ectoparasites were examined using stereomicroscope and was documented on its dorsal surface. Important morphological features in arachnids were documented such as the position of head and mouthparts termed as the capitulum that is relative to the thorax and abdomen on dorsal surface and shape of the body.

Characterization and Identification

Each species of ectoparasites were characterized based on the external morphological features on its dorsal surface and appendages. Dichotomous keys of Price and Graham (1997); Wall and Shearer (2001); and Yevstafieva (2015) of lice and mites on chicken and poultry were used in identification and classification of the ectoparasites.

Statistical analysis

Using SPSS 17.0 version, the One-Way Analysis of Variance (ANOVA) was used to determine if there is a significant difference on the number of dislodged ectoparasites from ZamPen native chicken treated with different surfactants. Post-hoc analysis using Duncan's Multiple Range Test (DMRT) was used to determine which among the surfactants dislodged a significantly high number of ectoparasites from the chicken. Results with $p<0.05$ were considered statistically significant.

Results and discussion

In this study, the physicochemical parameters of the formulated tobacco liquid was evaluated in order to determine its quality and efficacy properties. As presented in Table 1, the physicochemical parameters of the formulated tobacco liquid soap for Treatment 3 (T3) exhibited good appearance characteristics.

Table 1. Physicochemical parameters of the formulated tobacco liquid soap (T3).

Clarity	Color	Odor	pH	Foam Height	Foam Retention
Clear	Yellow orange	Aromatic	9.6	3.7 ml	5.0 ml

The pH is alkaline with a value of 9.6 that falls within the accepted pH range of 8.5 to 10.5 for liquid soap. These pH values might be due to the process of saponification resulting to decrease the harshness of the formulated liquid soap (Warra *et al.*, 2011). Furthermore, higher pH values make the soap basic and lather easily (Habib *et al.*, 2016).

One of the main parameters in the cleansing efficacy of a liquid soap is the foam height and foam retention capacity (Ordoyo and Sepe, 2019). As presented also in Table 1, the foam height is 3.7 ml and the foam retention capacity of the soap is 5 ml. This may influence the lathering and cleansing properties of liquid soap (Omwoyo *et al.*, 2014). The effect of the tobacco liquid soap in dislodging a significantly higher number of ectoparasites can be attributed to the actions of the chemical property of the formulated soap and the active compound nicotine which is an extremely fast-acting nerve toxin that mimics acetylcholine in the electron density at the carbonyl and/or the ethereal oxygen, and the distance between both oxygen and the quaternary nitrogen, making it an autonomic blocking agent at ganglia and neuromuscular junctions of the insects (Yamamoto, 1969).

Table 2. The One-way Analysis of Variance (ANOVA) on the total number of ectoparasites dislodged from ranged ZamPen native chickens after the first, second and third applications of the treatments with different surfactants.

Application	Treatment (T)	Sum of Squares	df	Mean Square	F	Sig.
Application 1	Between Groups	2864586.28	4	716146.57	11.73	0.000**
	Within Groups	2443189.96	40	61079.75		
	Total	5307776.23	44			
Application 2	Between Groups	667774.31	4	166943.58	4.59	0.004**
	Within Groups	1454809.69	40	36370.24		
	Total	2122584.00	44			
Application 3	Between Groups	997599.27	4	249399.82	4.41	0.005**
	Within Groups	2263256.04	40	56581.40		
	Total	3260855.31	44			

*Significant if $p<0.05$

**Highly Significant if $p<0.01$.

The nicotine binds to acetylcholine receptors at nerve synapses causing uncontrolled nerve firing resulting to disruption of normal nerve impulse activity which leads to rapid failure of body systems that depend on nervous input for proper functioning of the insects (El-Wakeil, 2013). By the aid of soap's chemical properties, the amphiphilic structure that is said to have an affinity for water hydrophilic group -COOH,-SO_3H polyoxyethylene chain and an affinity for oil -Si,-CF,-CF_2 polyoxypropylene chain group (Yuan *et al.*, 2014), this tend to adsorb gas-liquid and solid-liquid interfaces when in contact (Bajpai and Tyagi, 2007) forming a spherical with a hydrophilic exterior and hydrophobic interior colloidal solution known as micelle (Dias, 2015).

This solution binds to the structural framework of the insect's exoskeleton, the chitinous cuticle that is apparently impregnated with lipoprotein lipids (Metcalf and Flint, 1962). With the body system immobilized by the nicotine and the protective coat layer disrupted, rendering the insects and arachnids prone to dislodgement from the body of the ZamPen native chicken.

The mean number of dislodged mites and lice after the first, second and third applications of the Treatments (T) with different surfactants on the ranged ZamPen native chickens are illustrated in Fig. 5. After the first treatment application, most of the mites and lice were dislodged from the body of the chicken except the use of water (T5). Chickens treated with commercial shampoo (T1) had the highest mean number of dislodged mites while formulated tobacco liquid soap (T3) had the highest mean number of dislodged lice followed by commercial powdered soap (T2) and commercial liquid soap with anti-ectoparasite (T4). The use of water (T5) had the least number of mites and lice dislodged. It is also shown that there are lesser number of mites and lice dislodged after the second treatment applications, while on the first and third treatment applications there were an increased in number of mites and lice dislodged in the chickens treated with commercial shampoo (T1), commercial powdered soap (T2) and commercial liquid soap with anti-ectoparasite (T4), but no increase in the chickens treated with formulated tobacco soap (T3). The chickens bathed with water (T5) did not show ectoparasites dislodged.

Table 3. Post-hoc analysis using Duncan's Multiple Range Test (DMRT) on the number of ectoparasites dislodged from ranged ZamPen native chickens after the first, second and third applications of the treatments with different surfactants.

Application	Treatments (T)	Subset for alpha = 0.05		
		1	2	3
Application 1	T5	2.330		
	T3		289.260ᵃ	
	T4		299.930ᵃ	
	T2			615.030ᵇ
	T1			705.270ᵇ
Application 2	T5	0.110ᵃ		
	T4	26.93ᵃ		
	T3		132.920ᵇ	
	T2		256.290ᵇ	
	T1		308.500ᵇ	
Application 3	T5	0.000ᵃ		
	T3	1.330ᵃ		
	T4		137.78ᵇ	
	T1		297.97ᵇ	
	T2		361.34ᵇ	

Means having the same letter are not significantly different from each other.

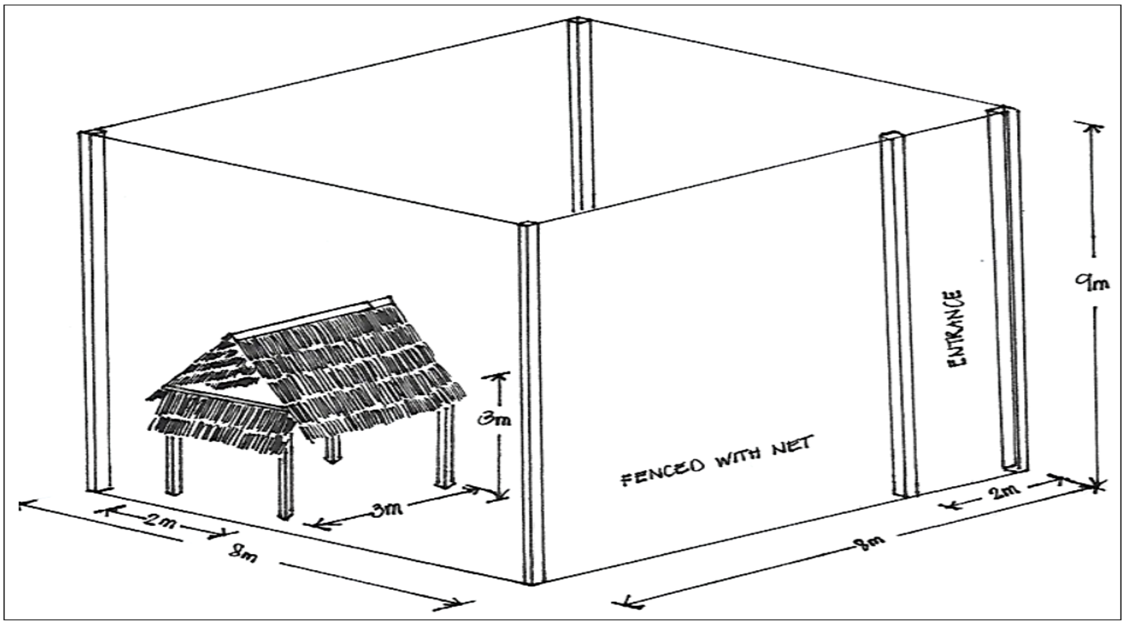

Fig. 1. The 9 × 8 meters pen with a 3 × 3 meters housing shade.

Generally, as shown in Fig. 5, the ZamPen native chickens treated with commercial shampoo (T1) had a significantly higher number of ectoparasites dislodged after the first and second treatment applications. This is because commercial shampoo used contained four types of surfactants that act for the dislodgement of the ectoparasites. Present in the ingredients are sodium lauryl sulfate and sodium laureth sulfate as anionic surfactants, trimethylalkylammonium chlorides as cationic surfactant, betaines as amphoteric surfactants and phenoxyethanol as non-ionic surfactant (Dias, 2015). These anionic surfactants are negatively-charged hydrophilic polar in nature that act on increasing electrical negative charges, causing a deep cleansing capability on surface on the hair. The cationic surfactant contain a quaternary ammonium ion that is characterized by a positively-charged hydrophilic polar group attached by salt bonds to neutralize the negatively charged net of the hair surface and minimize frizz while along with the amphoteric surfactants which take in both positive and negative charges to regulate and control the pH during washing (Trueb, 2007; Yuan *et al.*, 2014; Dias, 2015).

The non-ionic surfactant serves as a good cleansers, dispersion and emulsification properties in the commercial shampoo because it does not possess charged polar group (Trueb, 2007).

Those ZamPen native chickens treated with commercial powdered soap (T2) might be due to the anti-redeposition agents present in the powdered soap that prevents loosened soil from redepositing on a cleaned fabric. The capability of anionic polymers of the surfactant to adsorb the soils and substrates and give them negative charges are utilized by the anti-redeposition agents by stabilizing the rolling up mechanism dispersion and augmenting the reduction in oil/water interfacial tension (Bajpai and Tyagi, 2007).

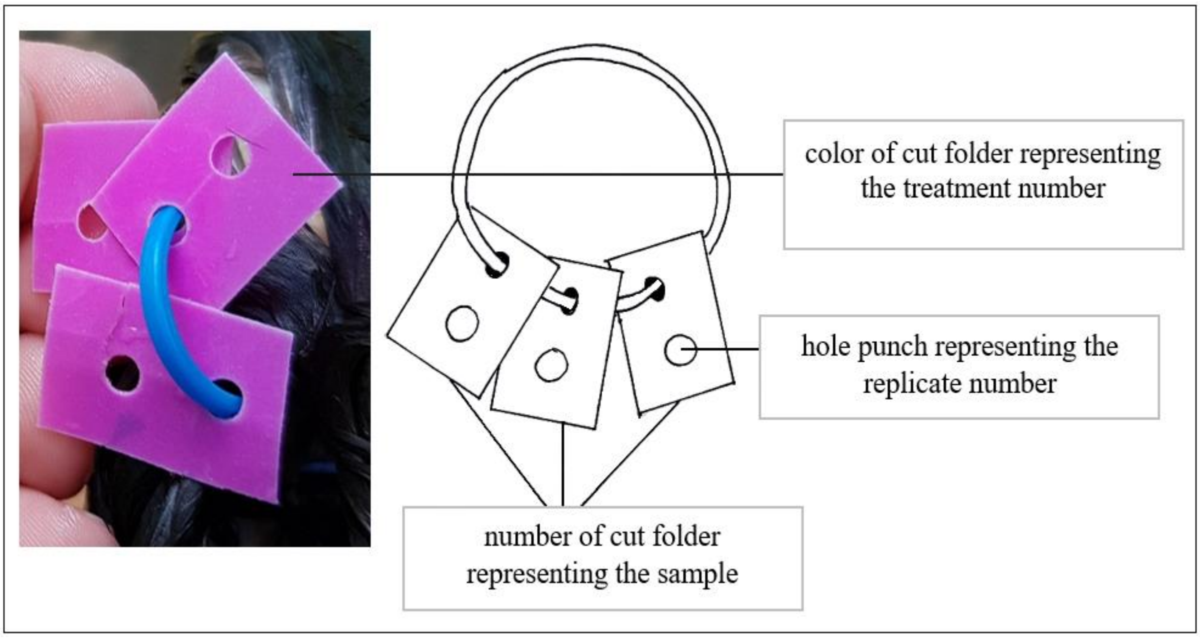

Fig. 2. The CHNF scheme used for tagging the ZamPen native chicken.

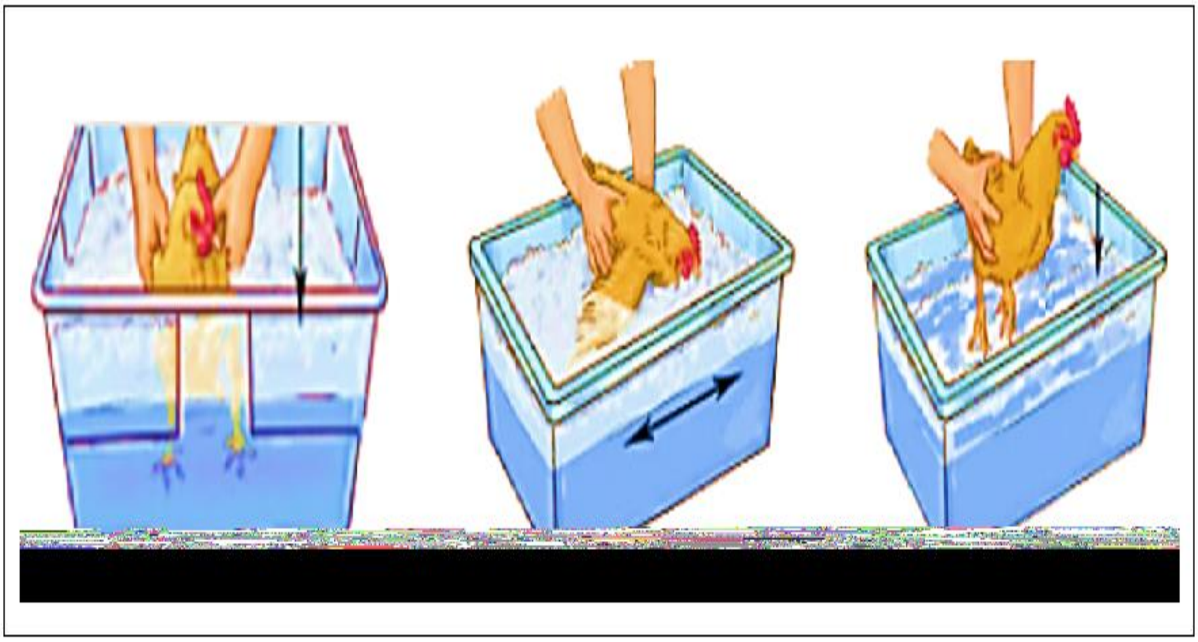

Fig. 3. The three tub method of bathing the chicken (Damerow, 2016).

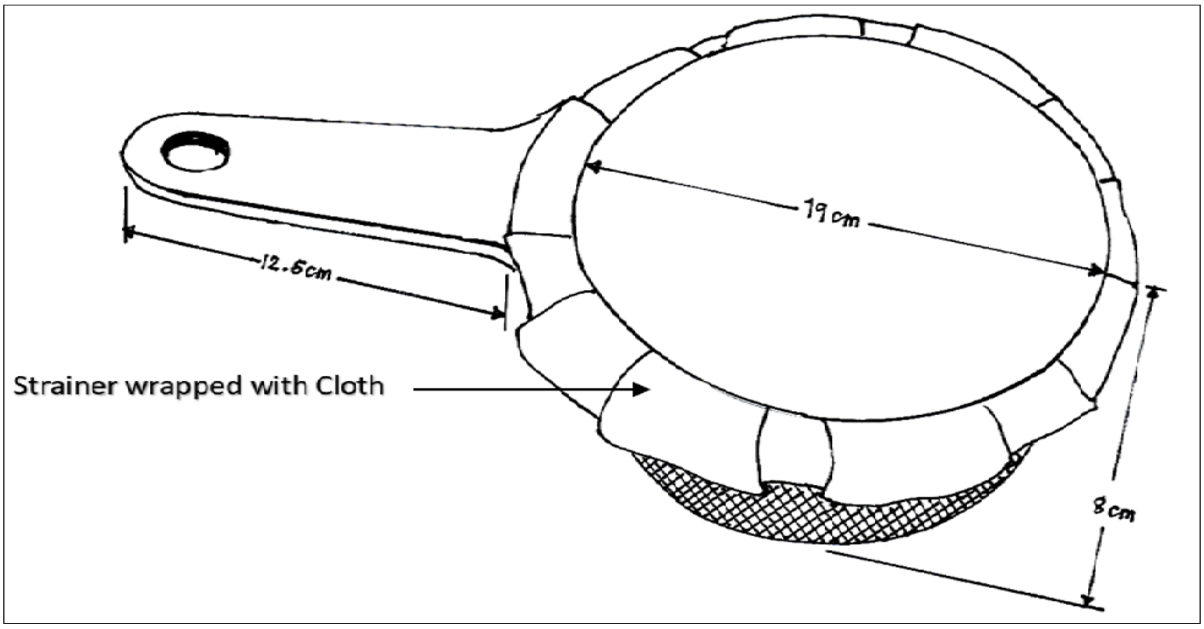

Fig. 4. A sieve as a tool for the collection of the dislodged ectoparasites.

The enzymes found in the powdered soap had a high tendency adherence that are capable of removing fatty stains, lipids and other deposits such as protein stains grass and blood (Hasan *et al.*, 2010). Similar event could result to the detachment of the mites from the body of the chicken by emulsifying the fluid injected on the surrounding adjacent tissues of the mites for attachment (Wall and Shearer, 2001) and some of the lipid protective waterproofing layer of the cuticle (Burgess *et al.*, 2015) leaving the ectoparasites susceptible to drowning.

The synergy between permethrin and piperonyl butoxide might be responsible for the dislodgement of mites and lice in the chickens treated with commercial liquid soap with anti-ectoparasite (T4). The piperonyl butoxide enhances the potency of permethrin in keeping the sodium channels of the ectoparasites opened for prolonged periods, thus delaying repolarization leading to paralysis (Kester *et al.*, 2012). However, Sungkar *et al.* (2014) found out that cure rate of scabies treatment using permethrin was high, but the recurrence rate was also high. Chickens applied with water (T5) had the minimal or almost no effect on the dislodgement of ectoparasites. The ectoparasites dislodged from the chicken is likely due to the force of the water and the bather during the bathing process causing the ectoparasites to fall.
The identification and classification of the lice and mites present in ZamPen native chickens were based on the dichotomous keys of Price and Graham (1997); Wall and Shearer (2001); and Yevstafieva (2015). Fig. 6 shows the identified mites associated with ZamPen native chicken dislodged from different surfactant with the gnathosome and opisthosomal body region.

The species of *Meginia* sp. are feather mites and *Ornithonyssus bursa* are from the species of blood sucking mite. The identified lice associated with ZamPen native chicken dislodged from different surfactant are show in Fig. 7. The species of *Menopon gallinae* known as the shaft louse had a triangular head wider than it is long, forehead with flatly curved sides in the middle vaguely angular. The abdomen is elongated and conically narrowed posteriorly. The species of *Menacanthus stramineus* or body louse had a head which is wider than long, parabolically rounded forehead. The abdomen is oblong-oval, the last segment had angular dorsal and rounded ventral surfaces covered with fine hairs. The species of *Lipeurus caponis* or wing louse had long slender body with a grayish color found mainly in the inner part of wing tail and head feathers of the chicken. It only feeds on parts of the feathers, but so intensively that is also called the depluming louse. Based on the study of Odenu *et al.* (2016), the most prevalent species of lice identified was *M. stramineus* not including *L. caponis* whilst the only species of mites was *Knemidocoptes mutans* not *Meginia* sp. and *O. bursa*. Also, they indicated that the overall prevalence rate of 87.90% for lice infestation was higher than that of mites with 7.26% in domesticated chickens in contrast to the results of the present study in ZamPen native chickens having a higher infestation of mites compared to lice. According to Proctor (2003), the action of commercial shampoo reduces the clinging ability of the *Megninia sp.* on legs I and II with apophyses on its long legs used for clasping plumaceous barbules and on tarsal ambulacra of *Ornithonyssus bursa* which they used to adhere to the feather and skin of the chicken.

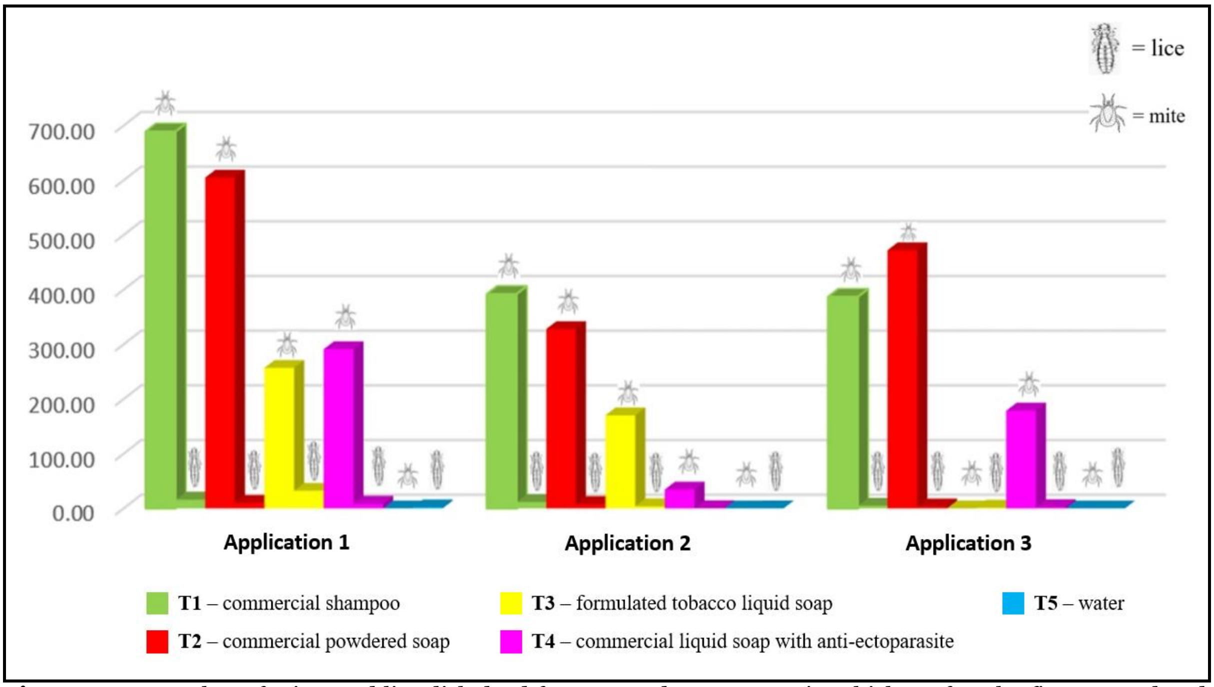

Fig. 5. Mean number of mites and lice dislodged from ranged ZamPen native chicken after the first, second and third applications of the treatments.

The opisthosomas of the two mites may also be affected by the action of the four different surfactant since they had a poorly sclerotized opisthosomas and also exhibit fairly weak body sclerotization thus, rendering them prone to dislodgement. On the other hand Dimethiconol, a silicon based content of the commercial shampoo, is the one responsible for the dislodgement of lice in the chicken.

The Dimenthiconol penetrates and coat, occluding the internal surfaces of the spiracles and permanently blocking the opening of the trachea. The permanent physical block of the outermost sections of the louse respiratory tract by Dimenthiconol, prevents water excretion leading to gut rupture and irreversible immobilization, resulting to death of the lice (Burgess, 2009). The One-way ANOVA as presented in Table 2, revealed that formulated tobacco liquid soap and commercial surfactants had a highly significant effect on the number ectoparasites dislodged on the chicken after the first, second and third treatment applications. The Post-hoc analysis using Duncan's Multiple Range Test (DMRT) is shown in Table 3. After the first application of the treatments, it reveals that the ranged ZamPen native chickens treated with formulated tobacco liquid soap (T3) and commercial liquid soap with anti-ectoparasite (T4) had a significantly lesser number of ectoparasites dislodged compared to commercial shampoo (T1) and commercial powdered soap (T2) but significantly higher than those chickens bathed with water (T5). After the second application of the treatments, the ranged ZamPen native chickens treated with commercial shampoo (T1), commercial powdered soap (T2) and formulated tobacco liquid soap (T3) had a significantly higher number of dislodged ectoparasites than those chickens treated with commercial liquid soap with anti-ectoparasite (T4) and those chickens bathed with water (T5). After the third treatment application of the treatments, the ranged ZamPen native chickens treated with formulated tobacco liquid soap (T3) and those chickens applied with water (T5) had a significantly lesser number of ectoparasites compared to chickens treated with commercial shampoo (T1), commercial powdered soap (T2) and commercial liquid soap with anti-ectoparasite (T4).Table 2 further indicates that among all different treatments of surfactants, the formulated tobacco liquid soap is the most effective in dislodging the economically important ectoparasites in ZamPen native chicken specifically the lice and mites that serves as vector of zoonotic diseases to humans. The results of the present study supports the previous findings of Dipeolu and Ndungu (1991), Iqbal *et al.* (2006), and Avinash *et al.* (2017) that have demonstrated the acaricidal efficacy of the leaves of tobacco (*Nicotiana tabacum*) used in the ethno-veterinary practices and tobacco leaves was found to be effective in reducing the numbers of ectoparasites.

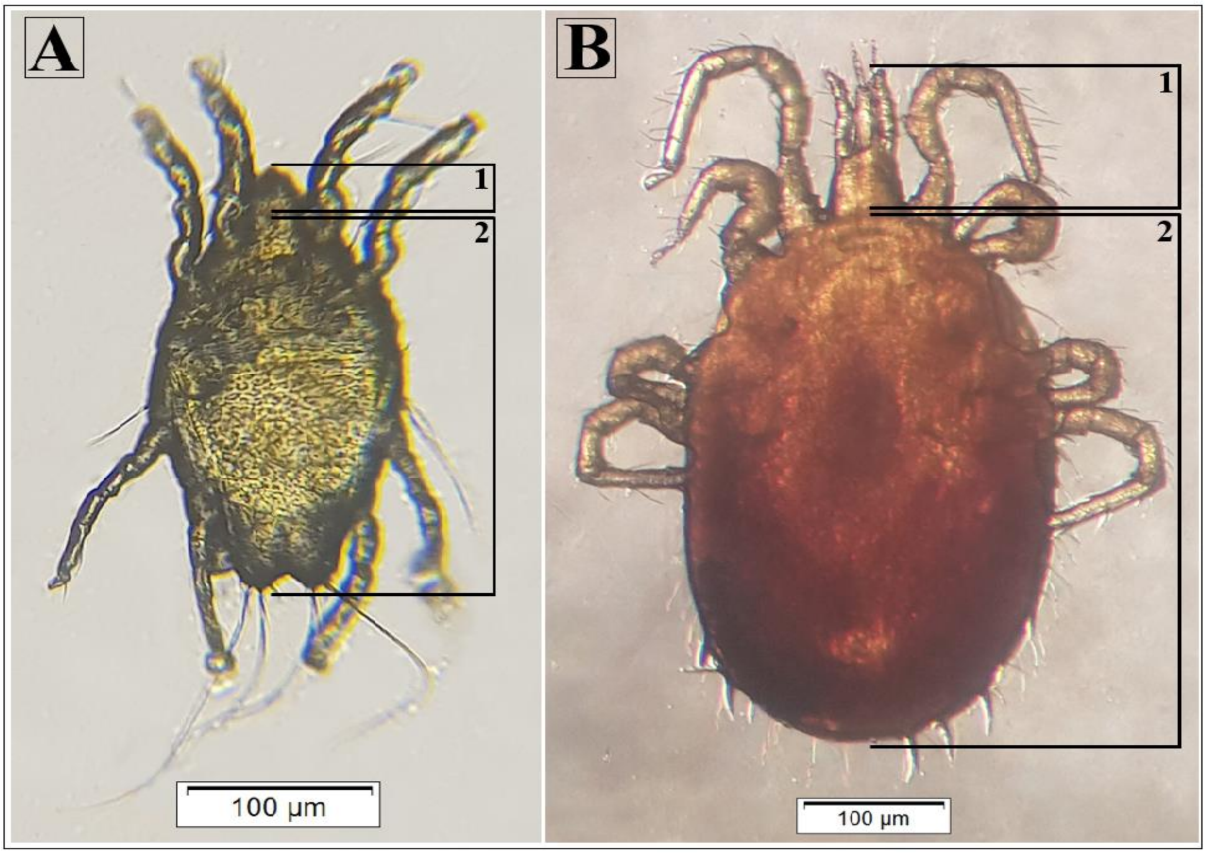

Fig. 6. The identified species of mites dislodged from ranged ZamPen native chickens under 80X stereomicroscope are (A) *Megninia* sp. and (B) *Ornithonyssus bursa* showing its 1- capitulum in the gnatosome and 2- opisthosome.

Overall results in the third application of treatment revealed that chickens treated with commercial shampoo (T1) had only a minimal decrease in number of dislodged ectoparasites while those treated with commercial soap (T2) and chicken anti-ectoparasite (T4) showed an increase in number of dislodged ectoparasites compared to the number of ectoparasites after the second treatment application. This could be attributed to the fact that most of the ectoparasites are dislodged during the first application. It also suggest that there is a reduction in the population of ectoparasites after the second and third applications. However, the formulated tobacco liquid soap (T3) significantly reduce the number of ectoparasites infesting the body of ranged ZamPen native chicken after the first and second application of treatments. The significantly low count of dislodged ectoparasites after the third application indicates that the ectoparasites were not able to recover its population. It is also possible that formulated tobacco liquid soap had a gradual effect on the clinging ability of the ectoparasites, thus the effect of nicotine on the ectoparasites may not be immediately after the bathing but it may have weakened the ectoparasites after sometime causing them to fall or get dislodged from the chicken within seven days resulting to fewer ectoparasites dislodged after the third application. This study indicates that the use of tobacco as a liquid soap is effective in eliminating the presence of ectoparasites in a ranged ZamPen native chicken.

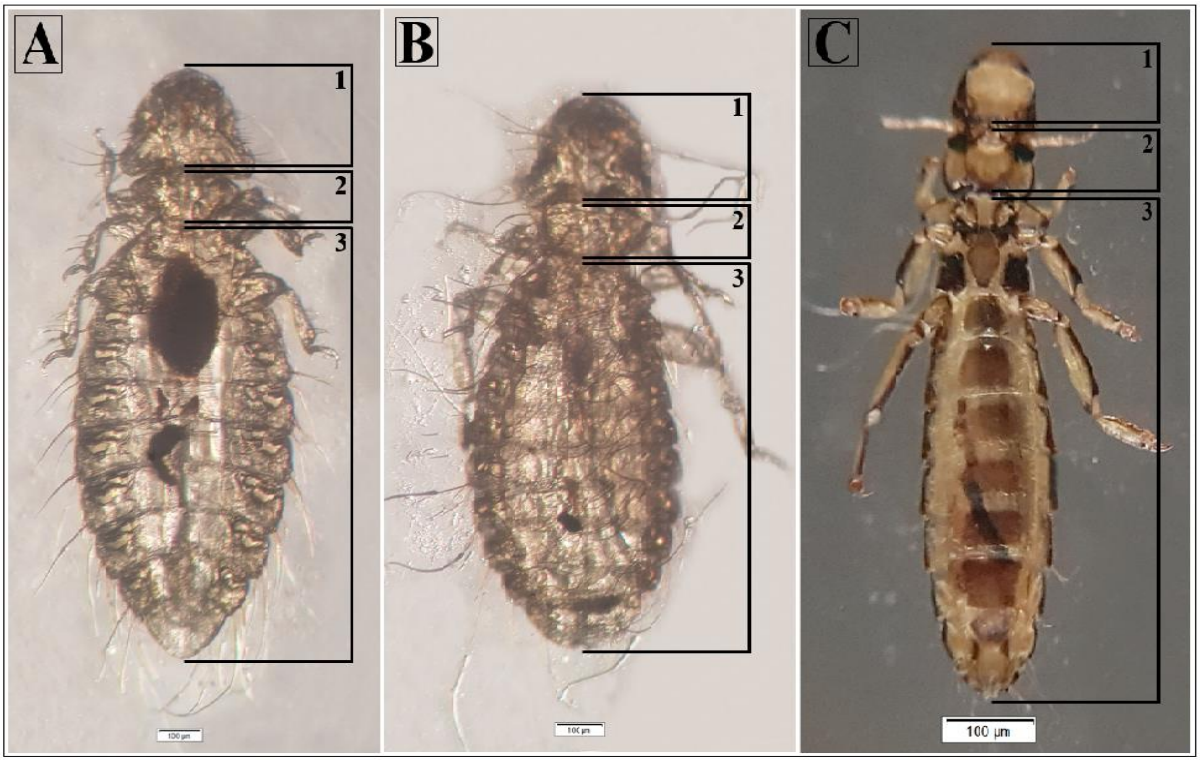

Fig. 7. The identified species of lice dislodged from ranged ZamPen native chickens under 80X stereomicroscope are (A) *Menopon gallinae*, (B) *Menacanthus stramineus* and (C) *Lipeurus caponis* showing its 1- head, 2- thorax and 3- abdomen.

Conclusion

The application of formulated tobacco liquid soap, commercial shampoo and commercial powdered soap significantly dislodged higher number of ectoparasites from the body of ranged ZamPen native chickens. Results indicates that tobacco liquid soap was formulated capable of dislodging ectoparasites of chickens.

The ectoparasites found to be dislodged using different surfactants includes three species of lice such as *Menopon gallinae* (shaft louse), *Menacanthus stramineus* (body louse) and *Lipeurus caponis* (wing louse) and two species of mites such as *Ornithonyssus bursa* (blood sucking mite) and *Megninia* sp. (feather mite). This study suggest that the formulated liquid tobacco soap has potential to be an alternative organic treatment against ectoparasites infesting ranged native chickens and to other domesticated farm animals.

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