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ARTICLE

Effects of Thyroidal Disturbance on the Behavior of Domestic Dogs (Canis lupus familiaris)

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ABSTRACT

Hypothyroidism is not uncommon in dogs, but it is actually very often diagnosed in elderly dogs. When and how does the disease start? What are the first recognizable signs? The first symptoms are usually changes in the behavior. First, these changes are quite subtle, but as the illness progresses, they can get very grave. We do often hear from the worried owners, that their report of a behavioral change to their vet is often ignored, not taken seriously or simply interpreted as unsteady or insufficient dog training/ education. This not taking seriously of the first signs is very concerning and a big problem in many ways. It is delaying the finding of the right diagnosis and treatment, which leads to suffering of the animal and the owner. In some cases, it leads to giving the dog up as an unbearable danger to the family. So the dog, who is only ill and could be back to normal with the right medical treatment, finally ends up in a dog shelter or a new family. The common understanding is, that hypothyreoidism is an illness solely occurring in the elderly dog. In contrast to this, the authors found out, that thyroidal problems occur already at relatively young ages. This is a very important finding, considering that many clinically practising veterinarians expect hypothyreoidism only in the aged or elderly dog and will not run any diagnostics in relatively young or middle-aged animals. The authors also found significant differences in the personality traits of emotional stability and extraversion. Therefore, we would like to expand the existing studies, so that this widely underestimated topic finally comes to the fore and hopefully, in the future the right diagnostcal steps can be taken at an early stage of the disease.

1. Introduction

The connection between thyroid diseases and behavioral changes has been sufficiently documented in human medicine and should be ruled out in all cases of seemingly problematic or unusual behavior before starting behavioral therapy [1].

Unfortunately, our experience showed us, that the situation is still different in veterinary medicine: many veterinary colleagues only think of a thyroid problem

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when the clinical picture is already more than obvious and clear.

Hypothyroidism is not uncommon in dogs (Canis lupus familiaris). We therefore think it should be mandatory that the clinically active veterinary colleagues start diagnostics much earlier in order to recognize the beginning of hypothyroidism. This is the only way that the animal (and also the owner) can be spared a lot of suffering at an early stage, and that is exactly what we should expect from clinical veterinarians.

Also, as connections to brain metabolism have already been investigated in other species (see below), and the Domestic dog is getting more and more common as a model for human psychiatry as Overall found out [2], investigations in this regard should also lead to a better understanding of brain mechanisms and behavioral physiology in general.

In the BSAVA Manual of Canine and Feline Behavioral Medicine by Horwitz and Mills [3], hypothyroid aggression has been presented as a separate form of aggression, which appears to be similar to the aggression in social conflict or fear. The authors point out that the clinical signs of hypothyroidism can be mild or absent.

Fatjo and Bowen [4] report that hypothyroidism lowers the threshold for aggressive behavior. It is seen as a multifactorial event, an influence on the serotonin metabolism is also mentioned.

The effects on behavior can be explained physiologically by the effects of TSH, which acts as a neurotransmitter [5]. Panic attacks have been reported in human psychiatry at elevated levels [6]. TRH has an antidepressant effect and is known for the following interactions in human psychiatry: poor concentration and anxiety with a reduced thyroxine alpha 1 receptor level, in the case of depression in humans, T4 is increasingly converted to the non-functioning kT3 [7].

Findings from gynecology also support our concern: Interactions between TRH and prolactin are known: an increased TRH level leads not only to an increase in the TSH level, but also to an increase in the amount of prolactin in the body. This in turn results in a drop in LH and FSH, which slows cycle activity. Estrogens also increase the amount of thyroglobulin, which in turn increases T4 levels [8].

Interactions with other neurotransmitters have been demonstrated in laboratory animal studies: in the case of hypothyroidism, the serotonin and dopamine receptors are broken down more quickly. Furthermore, it was found that thyroxine also directly increases serotonin levels and increases the activity of dopamine receptors [9,10].

In cases of rats with hypothyroidism, the number of norepinephrine receptors in the brain increases. T3 and T4 prevent the re-uptake of GABA, one of the most important transmitters in the brain's arousal-cushioning circuits. Therefore, GABA remains longer in the synaptic cleft at higher levels of thyroxine. Thyroid hormones also affect gene expressions of behavioral receptors. A reduced thyroxine level leads to reduced metabolism and therefore to a protracted presence of cortisol. This explains why the behavioral symptoms of the hypothyroid dog are similar to those of a dog under stress, loss of control or cortisone treatment [11,12].

Well balanced or increased serotonin levels lead to a reduction in/ of aggressiveness [13].

Studies by Aronson, Dodmann and Doddser showed that in a sample of 200 dogs (Canis lupus familiaris) with behavioral problems, 61% of the animals were hypothyroid or at least had a suboptimal thyroid parameters [11,12].

Neutered dogs (male and female) had at significantly higher risk of hypothyroidism [14].

Huber recognized an increased level of T3 in dioestrous female dogs and an increase of T4 during estrus [8].

Unfortunately, in our experience, the first signs of incipient thyroid disease usually went unnoticed for a very long time.

Most dogs get the diagnosis of a thyroidal disease, when they already show unmistakable clinical symptoms like obesity, lethargy, coat and skin changes, further weight gain despite small or normal amounts of food and the so-called "sad face". The first symptoms usually appear as changes in behavior. We do often hear from the worried owners, that their report of a behavioral change to their vet is often not taken seriously or interpreted as unsteady dog training/education.

In our behavioral pet consultancy in Germany, Austria and Switzerland we have lately seen some cases of a new and very disturbing tendency in some vets – supplying these dogs without further diagnostics with unneeded pharmacotherapeutic drugs like fluoxetine. Fluoxetine is an antidepressant that increases serotonin levels in the brain. The most common side effects of fluoxetine in the body are lethary, change in appetite, weight changes, runny nose, dry mouth, drowsiness, weakness, sore throat, nausea or diarrhea. The biggest problems in our case are the behavioral side effects of the drug: Behavioral side effects of fluoxetine include anxiety, panic attacks, trouble sleeping, irritability, agitation, hostility, aggression, restlessness, hyperactivity, or increased depression. These side effects are often the problems, which the drug was administered to decrease in the first place. Dogs with changes in their behavior due to a thyroidal illness will of course not respond well to this medical treatment. In some

cases, the animals experience dramatic side effects.

Therefore, we would like to expand the existing studies with further relevant data collection, so that this widely underestimated topic finally comes to the fore.

2. Material and Methods

We evaluated all cases from our consultancy in Germany, Austria and Switzerland in which there was a thyroid problem in the processed cases from 2010 to 2018. There are a total of 559 dogs (Canis lupus familiaris) with thyroid problems or hypothyroidism.

In order to be able to create an individual report and recommendations for an animal with behavioral problems, we work with 4 different questionnaires and ask for current blood values, which the owners provide.

The first questionnaire was created by us to collect general information on anamnesis, signalement, living conditions etc., in which we ask about the individual life and the current living conditions of the animal: We ask for information about origin, breed, age, other animals and persons in the household, living environment, such as the daily routine including occupation of the animal, the living situation, feeding, handling, rules for the animal, upbringing, sleeping habits, fears, unwarranted hunting behavior, aggression towards other dogs, towards familiar and/or unfamiliar humans, noise sensitivity etc.

A detailed behavioral anamnesis is also carried out, which clarifies at what age and in what situations the unwelcome behavior first occurred and what the possible causes are. We inquire about previous measures and their effects.

In this connection, a detailed medical history is also taken, for which we also request current blood values and medical findings that have already been collected. This is absolutely essential so that we can rule out "euthyroid sick syndrome". Not every change in the thyroid levels has its cause in the thyroid itself. Very often, there is a secondary change in the thyroid levels because of another primary serious or chronic disease. The thyroid reacts to that condition. A precise definition is important here so that no misdiagnosis is made. That is why we look into all existing medical data of the dog. If the report of the owner or the medical data requires further diagnostics or treatments, we ask to do and deliver us those. Very common reasons to a change in the behavior can be pain. This can be pain resulting from old injuries, skeleton problems, joint diseases and so on. Sometimes it is necessary to rule this out by asking the owner to see a physiotherapists with their dog. But also other chronic diseases can lead to a general feeling of being unwell in the animal, which can lead to unexpected reactions of the dogs. So the medical history is very important in our research.

The 2nd questionnaire we use was developed by Turcsan et al. [15], who intended by using owner reported assessments to characterize dog breeds on 4 complex behavioral traits: Trainability, boldness, calmness and dog sociability [15]. Instead of the terms "boldness" and "calmness" we used the terms "extraversion" and "stability" to distinguish these qualities from the supertrait model of shy vs bold [16,17].

In this questionnaire, emotional stability and sociality to dogs both score from zero to eight, openness (= trainability) scores between zero and ten, and extraversion between zero and six points. For details see Turcsan et al. [15] .

The 3rd questionnaire relates to the emotional disposition and is a translation of Sheppard & Mills' questionnaire [18].

The 4th questionnaire assesses impulsivity based on the work of Wright et al. [19].

After evaluating the data collected from 2010 to 2018 we found a total of 559 animals with possible thyroidal cause versus non diseased animals.

Data were analysed by Excel and SPSS, by means of X^2-test. All tests were two-tailed, and the significance level is set at < 0.05.

3. Results

There are castrated and uncastrated males and females of different age groups, see Figures 1, 2 and 3:

In Figure 1, we see that thyroidal problems occur already at relatively young ages, in the mean in about 3 year old dogs, partly already with the beginning of their youth. Nevertheless there is a significant difference (p=0,0003 x^2).

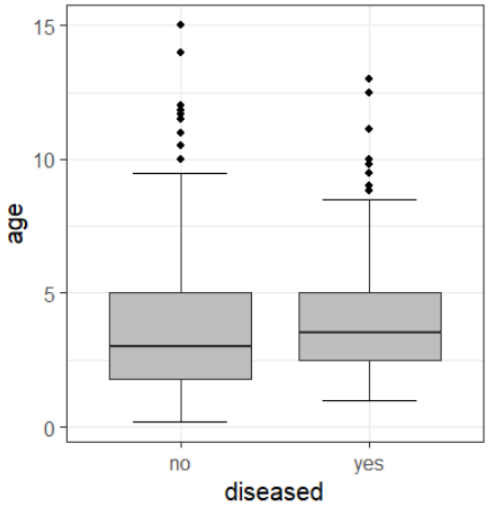

Figure 1. Age groups (Age distribution of diseased vs. healthy dogs (Canis lupus familiaris) in a sample of behavioral consultancy clients)

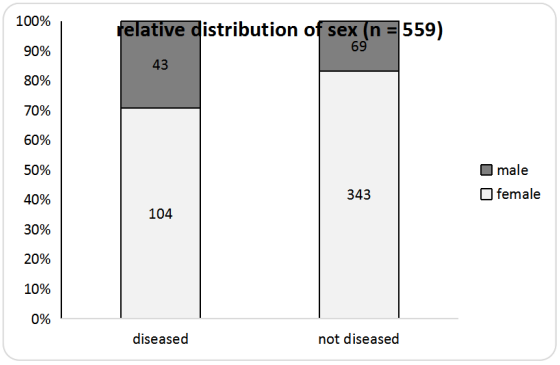

Figure 2. Sex (Sex distribution of diseased vs. healthy dogs (Canis lupus familiaris) in a sample of behavioral consultancy clients)

In Figure 2, we see that thyroidal problems occur both in female and male dogs, but in a significant difference (p=0,017 x²). Relatively more males than females seem to have thyroid problems.

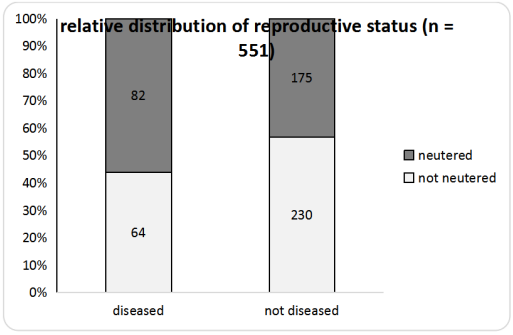

Figure 3. Reproduction status (Reproduction status distribution of diseased vs. healthy dogs (Canis lupus familiaris) in a sample of behavioral consultancy clients)

Thyroidal problems occur more often in neutered dogs than in intact ones (p= 0,0095 x^2).

In Figures 4-7 our results on the effect of disease on the behavior, evaluated on the basis of a questionnaire by Turcsan et al. [1], are shown:

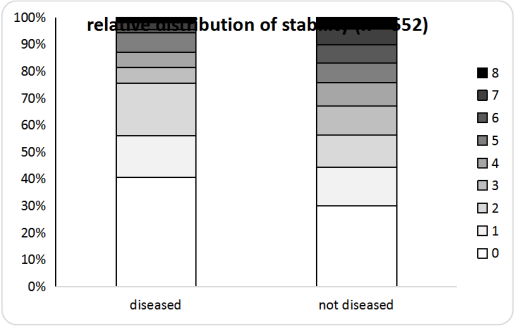

Figure 4. Emotional stability (Emotional stability distribution of diseased vs. healthy dogs (Canis lupus familiaris) in a sample of behavioral consultancy clients):

We found out, that the thyroid disease seems to have a significant effect on the emotional stability of the dog. The scores for emotional stability are lower for diseased dogs (p= 0,0001 x^2).

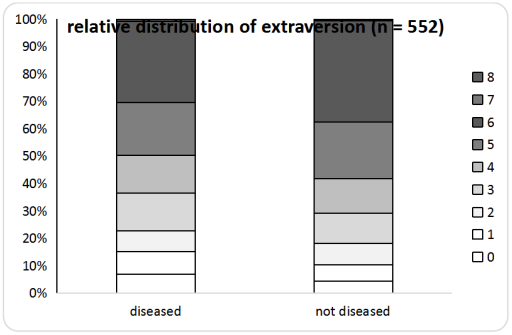

Figure 5. Extraversion (extraversion distribution of diseased vs. healthy dogs (Canis lupus familiaris) in a sample of behavioral consultancy clients)

In the area of extraversion, a tendency can be seen in animals with thyroid disease. Diseased dogs have lower scores and more non-diseased than diseased are found in the categories with higher scores (p= 0,0539 x^2).

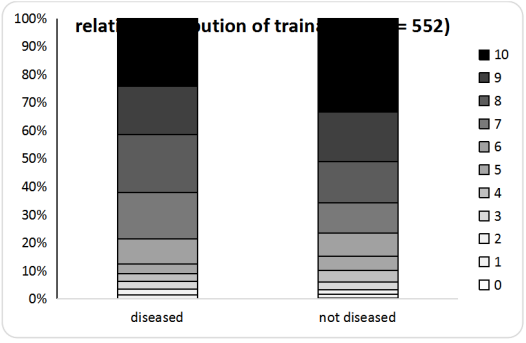

Figure 6. Trainability (=openness) (trainability (=openness) distribution of diseased vs. healthy dogs (Canis lupus familiaris) in a sample of behavioral consultancy clients)

No obvious differences can be seen in the area of trainability (= openness) (p= 0,1439 x^2).

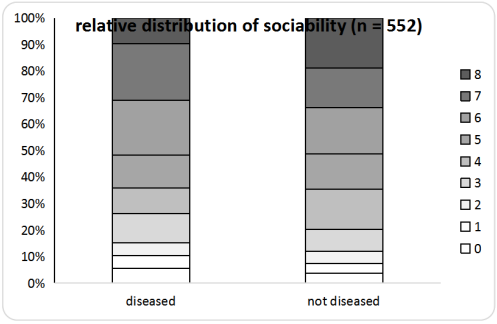

Figure 7. Sociability (sociability distribution of diseased vs. healthy dogs (Canis lupus familiaris) in a sample of behavioral consultancy clients)

No statistical significance can be seen in the area of sociability (0,02735 x^2).

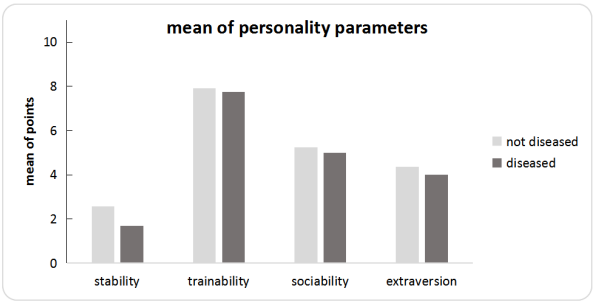

Figure 8. Mean of personality parameters (Mean of personality parameters distribution of diseased vs healthy dogs (Canis lupus familiaris) in a sample of behavioral consultancy clients)

Figure 8 shows a comparison of the "super-traits": shy and bold did not find a significance (p= 0,1813x^2); both shy and bold type dogs seem to have thyreoid problems at similar rates.

4. Discussion

We see, that in all 4 areas of stability, trainability, sociability and extraversion the non diseased group is ahead of the dogs with thyroidal disease.

In figure 1, we see that thyroidal problems occur already at relatively young ages, in the median in about 3-year-old dogs. This is a very important finding, considering that many clinically practising veterinarians expect hypothyreoidism only in the aged or elderly dog!

The occurrence of behavioral signs of hypothyreoidism at rather early age may be the result of the necessity of sufficient levels of thyroidal hormones for the development of the brain [20].

In Figure 3, we see that thyroidal problems occur more in neutered dogs. This may be, because the reproductive cycle and thyroid hormones are connected [8]. An increased frequency of thyreoideal problems was also found e.g. by Sundburg et al. [21].

We found, that the thyroid disease has a significant effect on the emotional stability of the dog.

In the area of extraversion, a clear tendency can be seen in animals with thyroid disease. We can conclude that a reduction of thyroid hormones seems to lead to lower scores in this personality trait.

No statistical significance can be seen in the area of trainability. This differs from our personal experience, which showed that the ability to concentrate and to be inventive in finding solutions on their own and also process learning units is significantly higher in non diseased dogs.

No statistical significance can be seen in the area of sociability. This also differs from our personal experience, which seems to show that a non diseased dog is friendlier and more open towards previously unknown conspecifics and also shows less aggressivity.

Considering all of this, it would make sense to initiate appropriate thyroidal diagnostics at the first signs of behavioral change. If a dog, that has been completely well-behaved to date, suddenly shows unusual behavior, this should be taken seriously and should lead to further diagnostic. This could avoid a lot of suffering and grief, for the animal as well as for the owner. Often the one who suspects correctly an existing hypothyroidism is the animal trainer, not the vet! Only when the animal trainer gives the advise, the vet then examines on the owners wish. In our opinion, the goal of our veterinary work should be to think in the right direction before medical laymen recognize the correct diagnosis.

There are thousands of family dogs in many countries whose behavior, as well as veterinary data, are routinely observed, collected, evaluated and treated. Using them in comprehensive and comparative studies not only for curative medical purposes but also as a data pool for basic research could lead to a better understanding. Brain metabolism and behavioral physiology researchers could thus create and analyze a huge data pool from non- or minimally invasive studies for mammalian behavioral physiology and perhaps (see Overall 2000) [2] also as a model for human psychiatry, The Domestic dog due to life history, cognitive and social capabilities is much easier to compare to humans than e.g. laboratory rodents are.

5. Conclusions

Future research plans: In the second step of our work, we want to continue to investigate the consequence of a low thyroxine for animals with behavioural problems that are adjusted to medication. For this purpose, after the medication has been correctly adjusted, which has to be tested in a blood sample, regular inquiries are made about the change in behavior since the therapy. We will report about it in a second part of the publication.

Conflict of Interest

There is no conflict of interest.

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ARTICLE

Insecticidal Activity of Crude Extracts of *Hyptis suaveolens* (Bush Mint) on *Anopheles* Mosquitoes Collected from Lafia, Nasarawa State, Nigeria

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ABSTRACT

Anopheles gambiae is a vector that is responsible for the transmission of malaria parasites which causes high morbidity and mortality in Nigeria and the world at large. Human-vector contact can be reduced by the use of conventional repellents being sold in the market, though some of these repellent are not environmentally friendly and *An. gambiae* have developed resistance to some of these repellents. To this end, the phytochemical constituents and insecticidal activity of crude extracts of *Hyptis suaveolens* (bush mint) was determined on adult *An. gambiae* mosquitoes collected from Lafia, Nasarawa State, Nigeria to evaluate its effect in controlling them. Here, 70% ethanolic and diethyl ether fat crude extracts were made from *H. suaveolens* dried leaves and used to carry out the experiment. The phytochemical screening of the ethanol extract revealed the presence of alkaloids, flavonoid, saponins, tannins, steroids and reducing sugar. Glycoside was not detected in the ethanol extract. Only steroids was detected in diethyl ether extract the rest of the phytochemical tested were absent. *An. gambiae* larvae were collected from the field and were raised to F_1 progeny adults that were used for the study. WHO protocol for carrying out human bait repellency cage test was used. Human hand treated with the extracts was exposed to a cage containing 30 female mosquitoes for each of the extracts respectively. The ethanolic crude extract treatment proved to be more effective in repelling mosquitoes with 0% (0/30) mosquito landing than diethyl ether extract which had 40% (12/30) mosquitoes landing and the control hand (untreated left hand) had the highest landing rate 63.3% (19/30) which showed very high significant difference (χ^2 = 27.2619, df = 2, P <0.00001) in relation to the treatments. After 24 hours holding period, the mortality rate of exposed mosquitoes was observed to be highest in the ethanolic treatment 73.3% (22/30). In conclusion, *H. suaveolens* extracts have repellency potential in controlling adult *An. gambiae.*

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1. Introduction

Malaria is one of the world's most important mosquito borne disease because of it great morbidity and mortality rate. It was estimated that about 300 million new cases of malaria occur every year worldwide that results in 1-2 million deaths [1]. Mosquitoes are responsible for transmitting malaria and other diseases such as dengue fever, yellow fever, filariasis and other fevers [2]. There are different type of mosquito species living in tropics and sub tropics region of the world such as *Anopheles, Aedes* and *Culex*. *Anopheles* is one of the most common mosquitoes in Nigeria and it is the primary vector that transmit malaria parasite [3].

The control of mosquito is becoming increasingly difficult because of the development of resistance by the vector against the synthetic inorganic insecticide and environmental hazard caused by the insecticide as a result of persistent use [4] in the absence DDT and other insecticides for effective control of mosquitoes, wide variety of plant species from various ecosystems that have a range of acute and chronic toxic effect against mosquitoes were used locally to control mosquitoes in communities [5]. Currently more than 2000 plants species have been identified as having insecticidal and repellant properties. About 344 plants are known to possess anti-mosquito characteristics and this includes *Hyptis suaveolens* [6].

Hyptis suaveolens (L. Poit) also known as bush mint, bush tea, pignut, or chan is a very common plant found along roadsides and farmsteads in different parts of the world mainly in the tropics and subtropics, the leaves are opposite and ovate, about 2.5 cm to 10 cm long [7].

Hyptis suaveolens is used in rural areas as repellent for biological control of mosquitoes [8]. Abok *et al.* [9] opined that *Hyptis suaveolens* exhibited larvicidal activity against *Anopheles gambiae* larvae. In Kenya an ethnobotanical studies carried out on *H. suaveolens* showed that the plant was able to repel mosquitoes effectively after burning overnight in a room [10]. Similarly, fieldworks carried out with *H. suaveolens* crude extract showed that the effect of a solution containing 8% of the plant oil persisted and repelled up to 97% of mosquitoes by 5 hours after application [11]. The development of such alternative repellent will go a long way considering the health challenges that the poor and less privileged individuals, mostly in tropical Africa and Nigeria in particular are facing to control mosquitoes. The effectiveness of the currently used organochlorine (DDT and Lindane), orgnophosphorus (malathion), carbamates (carboxyl) and pyrethroid insecticides against the vector is reducing everyday [4,12-14].

Based on the above stated challenges and burden caused by mosquitoes, this study was designed to determine the phytochemical constituent of *Hyptis suaveolens* dried leaves crude extracts and the effect of the plant extract in controlling adult *An. gambiae* mosquitoes with the hope that a novel and more effective products may be developed from the plant for controlling mosquitoes in the tropics where the disease is more endemic.

2. Materials and Methods

2.1 Study Area

Nasarawa State is located in the middle belt region of Nigeria. It lies between latitude: 8°34'13.8544″ N and longitude: 8°18'31.8388″E (using DMS) the state shares boundary with Benue and Kogi States at the South. It shares the north boundary with Kaduna State. The west boundary it shares with the Federal Capital Territory, Abuja. And the east boundary it shares with Taraba State and Plateau State. The plant sample and mosquito larvae used for this study were collected from Mararraba-Akunza area, Lafia, Nasarawa State.

2.2 Sample Collection

H. suaveolens sample was collected from Mararraba-Akunza area, Lafia, Nasarawa State. The plant was transported to the Botany laboratory of Federal University of Lafia for identification after which it was air-dried under shade at room temperature.

The *Anopheles* mosquito larvae that was raised to adults and used for the study were collected from Mararraba-Akunza area, Lafia, Nasarawa State, thereafter transported to Zoology laboratory of Federal University of Lafia for sorting and identification then raised to adult F1 progeny for the study.

2.2.1 Plant Sample Preparation and Extraction

The leaves of the test plant were rinsed with water to remove dirt and was spread out on a clean surface and allowed enough time to air-dry under shade at room temperature. The extraction was carried out in the department of Chemistry laboratory of Federal University of Lafia.

The cold organic method of extraction by Harborne [15] was employed for the plant extraction. The ratio of plant to solvent is 1:5 i.e. for every 1 gram of plant sample it was diluted with 5 milliliters of the organic solvent.

Firstly to defat, the 200 gram of the pulverized plant sample was weighed into a Winchester bottle and 1000 milliliter of diethyl ether was poured into the bottle. The solution was kept for 24 hours and was shaken periodically to enable thorough mixture of the solution. After 24

hours, the solution was filtered, the plant extract was kept in a fume cabinet to allow the diethyl ether to evaporate and the residue was shade dried.

Secondly, to obtain the 70% ethanol extracts. The dried residue was weighed into a clean Winchester bottle after which 1000 milliliter of 70% ethanol was introduced into the bottle. 700 milliliter of ethanol was diluted with 300 milliliter of distilled water to obtain the 1000 milliliter of 70% ethanol. The solution was kept for 24 hours and was shaken periodically to enable thorough mixture of the solution. After 24 hours the solution was filtered and the plant extract was kept in a fume cabinet for evaporation.

Furthermore after the evaporation of the solvents, the fat extract and the 70% ethanol extract was kept in a refrigerator to maintain the potency of the extract before the experiment was carried out.

Qualitative Phytochemical Screening

Phytochemical screening of the plant extracts was carried out employing standard procedures and tests [16-18]. The procedures used for the screening process are briefly described below.

Test for Alkaloids

Alkaloids content of the extracts was determined using techniques by Trease and Evans [18], 5 mL of the extract was added to a test tube and 2 mL of 1% HCl was added and boiled for 10 minutes on a steam bath. The mixture was cooled and filtered. After which, the filtrate was treated with Mayer's, Wagner's and Dragendroff's reagents. The turbidity of the resulting precipitate was an indication of the presence of alkaloids.

Test for Flavonoids

Flavonoids content of the extract was determined using techniques by Trease and Evans [18], 0.5 g of the extract was added into a test tube and 10 mL of ethylacetate was added then boiled in a water bath for 5 minutes. To five (5) mL of the extract, 1 mL of diluted aqueous ammonia was added and shaked vigorously. The layers were allowed to separate and the colour of the ammonia layer observed for a yellow colouration in the aqueous ammonia layer which indicates the presence of Flavonoids.

Test for Saponins

Saponins content of the extract was determined using the techniques by Vishnoi [16] and Sofowora [17], here 2 mL of the extract was added into a test tube and a few volume of distilled water added. The solution was shaked vigorously. The presence of a stable froth (Foaming) indicates the presence of Saponins.

Test for Tannins

Tannins content of the extract was determined using techniques by Trease and Evans [18], here 2 mL of the extract was added into a test tube and 1-2 drops of diluted ferric chloride solution was added. A blue-black green or blue-green precipitate indicates the presence of tannins.

Test for Steroids

Steroids content of the extract was determined using the technique of Sofowora [17], here 5 mL of the extract was added into a test tube and 2 mL of chloroform was added. After which 2 mL of concentrated sulphuric acid was carefully added to form a lower layer. A red-brown colour of the interphase indicates the presence of Steroidal ring.

Test for Reducing Sugar

Reducing sugar content of the extract was determined using techniques by Trease and Evans [18], here 5 mL of the extract and 3-4 drops of Fehling reagents (I and II) was added and the mixture heated on a water bath to boil. A red precipitate indicates the presence of reducing sugars.

Test for Glycosides

Glycosides content of the extract was determined using the techniques by Vishnoi [16] and Sofowora [17], here 2 mL of the extract was added into a test tube and 5 mL of Fehling's reagents (I and II) added into the test tube the mixture was boiled in a water bath for 5 minutes (this is to remove any reducing sugar present in the sample). After boiling it was allowed to cool and filtered. To the filtrate, 2 mL of diluted sulphuric acid was added. The mixture was reheated, cooled and neutralized with an equal volume of sodium hydroxide. To this, another 5 mL of Fehling's reagents (I and II) was added and the mixture reheated on a water bath for 10 minutes. A brick red precipitate indicates the presence of glycosides.

2.2.2 Rearing of the *Anopheles* Larvae to Adult

The field caught larvae of *Anopheles* mosquito used for this research, were reared to adults in cages in the Department of Zoology Laboratory of Federal University of Lafia, Nasarawa State. The adult mosquitoes were fed on sugar solution for continuous maintenance of mosquito colony.

Test for Repellency

The repellency test was carried out using the cage test of World Health Organization (WHO) [19]. The cage test is the most common way of testing the effectiveness of mosquito repellency. Three cages with a slot for inserting an arm were used as described by WHO [19].

After inserting the various arms into different cages, the data on the number of landing on the hands were collected. According to the WHO protocol, if within 3 minutes there is no mosquito landing on the untreated hand then the test will stop, because the volunteer might be naturally repellent to mosquitoes [19].

Three cages were used, each containing 30 mosquitoes, one cage for the control arm, second cage for the arm having diethyl ether extract and the last cage arm having the ethanol crude extract was inserted. The mosquitoes were starved for 48 hours before the test [19].

During the experiment the volunteer who most refrain from smoking and use of scented product 12 hours before the experiment, inserted his arm without the plant extract in the control cage [20]. Numbers of mosquitoes landing was recorded within the 30 minute test time. After 24 hours the mortality rate was recorded [19].

Two tests were carried out. The first test was for the 70% ethanol extract and the second test was for the diethyl ether extract. This was done in other to compare the two *H. suaveolens* extracts which is more effective on repelling mosquito.

2.3 Statistical Analysis

Data obtained was analyzed using R Console software version 3.2.2. Pearson Chi-square test was used to compare the proportion of *An. gambiae* that landed on the hand in relation to extracts of *H. suaveolens* treatments. The P-value < 0.05 was considered statistically significant.

3. Results

Qualitative Phytochemical Constituents of Crude Extracts of *H. suaveolens* Leaves

The qualitative phytochemical result (Table 1) depicts the presence of alkaloids, flavonoid, saponins, tannins, steroids, reducing sugar in the 70% ethanolic crude extract of *H. suaveolens*. Only steroids was detected in diethyl ether extract the rest of the phytochemical tested were absent. Glycosides was not detected in the plant extracts.

Table 1. Qualitative Phytochemical Constituents of Crude Extracts of *H. suaveolens* Leaves

Secondary metabolite	*H. suaveolens* Ethanolic extract	*H. suaveolens* Diethyl ether extract
Alkaloids	+	–
Flavonoid	+	–
Saponins	+	–
Tannins	+	–
Steroids	+	+
Reducing Sugar	+	–
Glycosides	–	–

Key: + = Present
– = Not detected

Repellency Activity of *H. suaveolens* Ethanol and Diethyl Ether Extracts on Treated Baited Arm against Adult *Anopheles gambiae* Landing

The total number of *Anopheles gambiae* that landed on the hand was highest in the control 19 (63.3%) followed by those exposed to diethyl ether extract 12 (40%) while none 0 (0%) landed on crude ethanol extract treatment as shown in (Table 2). Therefore, the number of *An. gambiae* that landed in relation to different extracts of *H. suaveolens* showed a very high significant difference ($\chi^2 = 27.2619$, df = 2, P = 0.000001202).

Table 2. Repellency Activity of *H. suaveolens* Ethanol and Diethyl Ether Extracts on Treated Baited Hands against Adult *Anopheles gambiae* Landing

Treatment	No. of *An. gambiae* Exposed	No. of *An. gambiae* that landed (%)
Control arm (untreated arm)	30	19 (63.3)
Ethanol extract arm	30	0 (0.0)
Diethyl ether extract arm	30	12 (40.0)

Mortality of Adult *Anopheles gambiae* in Relation to 24 Hours Holding Period

Mortality was highest in the crude ethanol extract 22 (73.3%) while only 19 (63.3%) died in diethyl ether extract and control 19 (63.3%) respectively (Table 3). However, there was no significant difference ($\chi^2 = 0.9$, df = 2, P = 0.6376) in mortality rate across extracts.

Table 3. Mortality of Adult *Anopheles gambiae* in Relation to 24 Hours Holding Period

Arms Exposed	No. *An. gambiae* Exposed	No. Dead (%)
Control Arm	30	19 (63.3)
Ethanol extract Arm	30	22(73.3)
Diethyl ether extract Arm	30	19 (63.3)

4. Discussion

The result obtained clearly showed that ethanolic crude extract of *H. suaveolens* was made up of 6 phytochemical constituents with the exception of glycosides. Also, only steroids were present in screened diethyl ether extract. The phytochemicals recorded in both crude extracts is in agreement with study done by Dakum *et al.* [21] who documented the presence of same phytochemicals in the phytochemical analysis of *H. suaveolens* methanolic and aqueous extracts. Also, the presence of steroid in the two crude extracts in this study is in accordance with the finding of Shenoy *et al.* [22] who also reported the presence of steroids in phytochemical screening of *H. suaveolens* diethyl ether extract.

During the bioassay, the control group had the highest number of mosquitos landing 19 (63.3%) and there were more than 2 mosquitoes that landed on the volunteer con-

trol arm within 3 minutes, which indicated that the volunteer was not naturally repellent to mosquitoes as reported by WHO [19] and Anuar and Yusof [20].

The repellency exhibited by 70% ethanol extract which yield 0% landing on the treated arm as against the diethyl ether extract in which 40% (12/30) *Anopheles gambiae* landed possibly suggest that ethanolic solvent tends to be effective in extracting high number of active ingredients present in plant products that can serve as repellants. This agrees with Shaalam *et al.* [5] in a review of botanical phytochemicals with mosquitocidal potential reported that ethanolic extract had the highest efficacy on mosquito repellency compared to diethyl ether (fat) extract.

The mortality rate was very high for those exposed to the 70% crude ethanol extract 22 (73.3%) and low in those exposed to fat extract. This might be as a result of the phytochemicals present in each of the extract making the ethanol extract very effective and having very high repellency and mortality ability compare to the diethyl ether fat extract as reported by Shaalam *et al.* [5] and Hemen *et al.* [8]. The 63.3% (19/30) mortality recorded in the control might be as a result of the 48 hours starvation observed in order for the test to be conducted, although sugar solution was placed immediately after the test on top of the cage for the 24 hours holding period before mortality rate was recorded.

From the result obtained in this bioassay, *H. suaveolens* crude extracts have proven to be as effective as DEET (N, N-dimethyl-3-methylbenzamide) for personal protection against adult An. *gambiae* mosquitoes bite as reported by Hemen *et al.* [8] and as reported by Abgali and Alavo [11] that the plant is having mosquitocidal ability.

5. Conclusions

Results from this study have proven that *H. suaveolens* crude extracts can serve as alternative repellent to synthetic insecticides in the control of *An. gambiae,* the causative agent of malaria in Nigeria and this will also help reduce the burden caused by the vector to humans. The plant is abundantly available and the processing of it to obtain crude extract is very easy and cost effective, hence could serve as a more favorable option in the control of mosquitoes in our environment since it is biodegradable, environmentally friendly and affordable.

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Conflict of Interest

There is no conflict of interest.

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ARTICLE

Effect of Additional Feed Supplement Fermentation Shrimp Waste Extract on Digestibility in Sentul Chicken Growth Phase

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ABSTRACT

This study aims to determine the effect of adding feed supplements of fermented shrimp waste extract in the ration on the digestibility of local chicken rations in the growth phase. The research was carried out in Jatinangor District as well as the Laboratory of Ruminant Animal Nutrition and Animal Feed Chemistry, Faculty of Animal Husbandry, Padjadjaran University, Sumedang. The method used in this study was experimental with a Complete Randomized Design (RAL). The data were analyzed by fingerprint test (ANOVA) with further tests using the Dunnet test. The object of this study consisted of 20 Sentul chickens raised from the age of 1 day to 12 weeks, divided into 5 treatments and 4 tests. The treatment consists of P0 = Basal ration without the addition of feed supplement fermented shrimp waste extract, P1 = Basal ration + 0.5% feed supplement fermented shrimp waste extract, P2 = Basal ration + 1.0% feed supplement fermented shrimp waste extract, P3 = Basal ration + 1.5% feed supplement fermented shrimp waste extract, and P4 = Basal ration + 2.0% feed supplement fermented shrimp waste extract. The changes observed are the digestibility of dry matter, the digestibility of organic matter, and the digestibility of proteins. The results showed that the addition of feed supplements for fermented shrimp waste extract had a significantly different influence on the digestibility of dry matter, the digestibility of organic matter, and the digestibility of protein. This study concludes that the addition of a feed supplement of 2% fermented shrimp waste extract in the ration can produce a high ration digestibility value for Sentul chicken in the growth phase.

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1. Introduction

Sentul chickens are one of the local chickens from Ciamis that has quite good ability and productivity compared to other local chickens. Sentul chickens have good productivity in their meat and egg production so Sentul chickens are dual-purpose chickens. Along with the increasing demand and consumption of local chickens, Sentul chicken has the potential to be developed, so there need to be improvements in the feed that support its products so that it can produce optimally [1-3].

Feed is one of the factors affecting livestock productivity in addition to genetics and maintenance management. The feed given must have a complete nutritional content so that the feed can meet the needs of livestock for basic living, production, and reproduction. Growth is the process of increasing the number and size of cells in all parts of the body. Good quality feeding will result in a good growth response. Good feed is feed that is formulated to meet all the nutritional needs of livestock, has good digestibility, and does not hurt livestock [3-5].

Digestibility is the number of nutrients absorbed by the body. Digestibility is a nutrient part of the feed that is not excreted in the faeces. Food substances present in faeces are considered undigested food substances. Digestibility is based on the assumption that nutrients not present in the faeces are depleted and absorbed. Feed that has a high digestibility value indicates that it is of good quality. High digestibility shows that the livestock can absorb well the nutrient content in the feed needed by the livestock so that if the digestibility of the feed is high, livestock productivity will be optimal [6-8].

The addition of feed supplements to the feed can complement the nutrient content in the feed. A feed supplement is a feed additive that has nutritional value that serves to meet the nutritional content in the feed. The content contained in the feed supplement is amino acids, vitamins, and minerals. Feed supplements can be obtained from waste that still has nutritional value. Shrimp waste is fishery waste that can be used as a feed supplement [9,10] GIT morphometry, and microbiota populations. Four hundred one-day-old Ross 308 chicks were randomly distributed to four dietary treatments (10 replicates, 10 birds each. This shrimp waste has the disadvantage of chitin which can bind protein so that its digestibility is low. The treatment carried out on shrimp waste to optimize its potential is by fermentation using the bacteria *Bacillus licheniformis, Lactobacillus* sp., and yeast *Saccharomyces cerevisiae* [11-14]. This fermented shrimp waste is then extracted so that astaxanthin content is obtained which is beneficial for livestock productivity. Astaxanthin is a carotenoid derived from aquatic organisms such as shrimp. Several studies have shown that astaxanthin has antioxidant activities that can help maintain the body's health from free radicals. Antioxidant activity derived from astaxanthin is expected to maintain intestinal health in chickens which affects the digestibility of the chicken so that chickens can digest nutrients optimally and produce optimally as well [15]. The addition of feed supplement fermented shrimp waste extract can increase the digestibility value of feed in local chickens.

2. Materials and Methods

2.1 Experimental Livestock

The livestock used as the object of study was Sentul chickens raised from DOC with as many as 100 heads with an average body weight of 37.25 ± 1.95 grams. DOC was obtained from the Poultry Breeding Development Center (BPPTU) Jatiwangi, Majalengka. The livestock used as the study sample was a growth phase Sentul chicken (age 12 weeks) with as many as 20 heads which were not distinguished by sex (unsexed). Sentul chickens are kept in an individual cage and begin to be given treatment at the age of 2 to 12 weeks.

2.2 Trial Cage

The cages used for maintenance are 20 individual cage units with a size of 70 × 80 × 80 centimeters, while the cages used for sampling are 20 individual cage units with a size of 40 × 30 × 40 centimeters. The cage is equipped with a feed bin and a place to drink. Chickens are randomly placed according to the layout of the experiment.

2.3 Trial Ration

The rations used at the treatment stage in the study were made based on the need for metabolizable energy and proteins. The arrangement of feed ingredients that make up the ration used in the study was corn, soybean meal, MBM, rice bran, coconut oil, $CaCO_3$, premix, bone meal, NaCl, and fermented shrimp waste extract. The feed ingredients and the content of nutrients and metabolizable energy used in the study can be seen in Table 1.

The percentage of use of feed ingredients in the composition of the research ration is presented in Table 2 as follows.

Table 1. Nutrient content and metabolizable energy of feed ingredients constituents of rations

Feed Ingredients	Nutritional Content								
	ME	crude protein	crude fat	crude fibre	calcium	cystine	lysine	methionine	phosphorus
	Kcal/kg	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Yellow corn	3370	8.60	3.90	2.00	0.02	0.18	0.20	0.18	0.10
Soybean meal	2240	44.00	0.90	6.00	0.32	0.67	2.90	0.65	0.29
Meat bone meal	2300	48.00	9.40	1.40	10.30	0.00	1.77	0.49	5.10
Rice bran	1630	12.00	13.00	12.00	0.12	0.37	0.71	0.27	0.21
Coconut oil	8600	-	100	-	-	-	-	-	-
$CaCO_3$	-	-	-	-	38.00	-	-	-	-
Premix	-	-	-	-	80.00	-	-	-	-
Bone meal	-	-	-	--	22.00	-	-	-	19.00
NaCl	-	-	-		0.10	-	-	-	-
FSWE*	2614	39.29	-	7.79	6.81	0.54	3.04	1.46	2.83

*Fermented shrimp waste extract

Table 2. Basal ration formula

Feed Ingredients	Composition of the Ration
	(%)
Yellow corn	58.65
Soybean meal	25.00
Meat bone meal	1.50
Rice bran	10.21
Coconut oil	1.00
$CaCO_3$	0.40
Premix	0.50
Bone meal	2.39
NaCl	0.35

As for the content of nutrients and metabolic energy contained in the basal ration, it is shown in Table 3.

Table 3. Nutrient content and metabolizable energy of basal ration

Nutrient and metabolizable energy	Content
ME (Kcal/kg)	2,807
Crude protein	17.90
Lysine (%)	0.93
Methionine (%)	0.30
Methionine + cystine (%)	0.61
Threonine (%)	0.68
Calcium (%)	0.98
Nonphytate P (%)	0.49
Na (Sodium) (%)	0.20
Na+K-Cl (mEq)	239.58

Fermented shrimp waste extract is not included in the formula because of the purpose of its use as a feed supplement. Fermented shrimp waste extract is used as a feed additive added to the ration. The rate of addition of fermented shrimp waste extract in the ration is in the range of 0.5% ~ 2%. Rations that are not given additional fermented shrimp waste extract are used as control rations to be used as a comparison with treatment rations.

2.4 Research Procedure

Process of making fermented shrimp waste extract

The process of making fermented shrimp waste extract begins with washing shrimp waste in running water, shrimp waste that has been thoroughly washed is put into a stainless jar for fermentation using *B.lincheniformis* inoculants at a dose of 2% and incubated into an auto-shaker-bath machine for 2 days in a temperature of 45 °C turns 120 rpm. After obtaining the deproteination product, inoculation is carried out using *Lactobacillus* sp. at a dose of 2% and incubated into the auto-shaker-bath machine for 2 days with a temperature of 35 °C at 120 rpm. After obtaining the demineralization product, fermentation is carried out using *S. cerevisiae* as much as 3% and incubated into an auto-shaker-bath machine for 2 days at a temperature of 30 °C revolutions of 120 rpm. The bioprocess product (Astaxanthin 26.75%) was extracted with mineral supplementation Se (selenium) 0.15 ppm (73 ppm in the form of selenite) and then added filler / compacting agent. The final process is carried out by grinding until it becomes flour with a particle size of 100 mash to be mixed with other ration constituent ingredients.

Maintenance and sampling process

As many as 100 Sentul chickens are kept from DOC up to 12 weeks of age. At the rearing stage, chickens are placed in individual cages measuring 90 × 80 × 85 centimeters. Chickens are given rations and drink adlibitum, the ration of each cage is different according to the treatment. At the age of 0 to 2 weeks, chickens are given a mash-shaped commercial ration on each cage. Commercial rations are intended as a preliminary stage, the treatment ration is carried out after the chickens are 2 weeks old until the age of 12 weeks. During maintenance, the ration is given in the form of mash and given as much as 2 times a day.

Measurement of digestibility is carried out at the age of chickens of 12 weeks. On the measurement of digestibility of chickens, Sentul is placed into individual cages. The administration of the treatment ration is carried out in the morning. Drinking water is given adlibitum. The sampling technique was carried out by taking the fecal mass from the cecum [16]. The method of determining the digestibility uses an internal indicator (lignin) as a comparison [17]. After 6 hours, the chicken is slaughtered and its colon is removed to obtain a sample of faeces. The faecal sample is then dried and so on analyzed the content of dry matter, organic matter, and crude protein, while its indicators (lignin ration and faeces) are analyzed by the Van Soest method.

Experimental Design and Statistical Analysis

This research was carried out using the Random Design Technique. Consists of five (5) treatments, namely without and with the addition of feed supplement fermented shrimp waste extract with five (4) repeats. Rations without the addition of feed supplements of fermented shrimp waste extract are used as control rations. As for the treatment, namely P0 = Basal ration (which is not added fermented shrimp waste extract), P1 = Basal ration + 0.5% fermented shrimp waste extract, P2 = Basal ration + 1% fermented shrimp waste extract, P3 = Basal ration + 1.5% fermented shrimp waste extract, P4 = Basal ration + 2% fermented shrimp waste extract.

The changes observed in this study were the digestibility of dry matter, the digestibility of organic matter, and the digestibility of protein. The data that is role-picked are tested with fingerprints. If the results of the fingerprints differ markedly, then to find out the difference between the treatment and the control ration, a Dunnet test was carried out.

3. Results and Discussion

The addition of various levels of feed supplement of fermented shrimp waste extract in the ration to the digestibility value of the dry matter, digestibility of organic matter, and protein ration efficiency, based on the results of the study can be seen in Table 4.

Table 4. Average digestibility of dry matter, digestibility of organic matter, and digestibility of protein rations

Parameters	Treatment				
	P0 (0%)	P1 (0.5%)	P2 (1.0%)	P3 (1.5%)	P4 (2.0%)
Digestibility of dry matter	63.80[a]	67.90[a]	68.24[a]	72.24[b]	73.56[b]
Digestibility of organic matter	65.85[a]	69.84[a]	70.11[a]	73.00[b]	74.65[b]
Digestibility of protein	66.03[a]	74.22[b]	75.29[c]	79.58[d]	79.73[d]

The results of the fingerprint test (ANOVA) showed that the addition of various levels of feed supplement of fermented shrimp waste extract in the ration had a significant effect on the digestibility value of the dry matter, and digestibility of organic matter, and the ration protein density ($P<0.05$). Based on the results obtained, it shows that the higher the level of adding feed supplements to fermented shrimp waste extract, the value of digestibility of dry matter, digestibility of organic matter, and digestibility of ration proteins will increase. Dunnet test results showed that the addition of 1.5% ~ 2% feed supplement of fermented shrimp waste extract had a markedly different effect on the digestibility value of dry matter and organic matter compared to the control ration. Whereas in protein digestibility, the addition of 0.5% ~ 2% exerts a markedly different influence compared to the control ration.

The digestibility of a feed ingredient is a reflection of the high low value of the benefits of the feed ingredient. If the digestibility is low, the benefit value is low, on the other hand, if the digestibility is high, the benefit value is also high [4,8,18]. Digestibility that has a high value reflects the large contribution of certain nutrients in livestock, while feed that has a low digestibility value indicates that the feed is less able to supply nutrients for basic living and production [19,20].

The digestibility of dry matter of the ration is measured to determine the number of nutrients digested by chickens. The digestibility of dry matter will be one of the indicators to determine the quality of the ration. The higher the digestibility of dry matter, the higher the nutritional opportunities used by livestock for their growth [18,21]. The addition of a feed supplement of fermented shrimp waste extract in the ration has a good effect so that it results in a

higher average digestibility value of dry matter compared to basal rations that are not fed feed. The increase in digestibility value is partly due to the different compositions in the ration so that the value of food substances in the ration is different, in this study the composition of the rations was different due to the addition of various levels of fermented shrimp waste extract. The normal amount of digestibility value of dry matter is influenced by various factors, including the content of food substances, the amount of ration consumed, and the rate of digestion in the digestive tract. In addition to the composition of different rations, the treatment of feed ingredients can affect the digestibility value. In this study, the feed supplement added to the ration was a fermented product.

Shrimp waste that has a limiting factor in the form of chitin which is difficult to digest is fermented using *B. licheniformis, Lactobacillus* sp., and *S. cerevisiae* which can produce chitinase and protease enzymes. Chitinase and protease enzymes produced during the fermentation process can catalyze and degrade chitin complex compounds by cutting the glycosidic bond between N-acetyl glucosamine (the monomer that makes up chitin) to make it simpler and easier to digest. Shrimp waste treatment with the fermentation method increases the protein content and reduces some of the chitin content with the help of the chitinase enzyme. The final product of fermentation usually contains compounds that are simpler and easier to digest than the original material [12].

The fermented process in feed ingredients, in addition to increasing nutrient content and digestibility, can also increase palatability. Feed ingredients undergoing a fermentation process produce good feed physical quality and high palatability. Palatability of the ration affects the consumption of dry matter of the ration. Consumption of dry matter rations is influenced by several factors including the quality, quantity, and palatability of the ration. The consumption of dry matter greatly affects the digestibility value of dry matter [22,23].

The ration added to fermented shrimp waste extract contains astaxanthin which is an antioxidant. The fermented shrimp waste extract contains Astaxanthin as much as 436 ppm [7]. The use of Astaxanthin derived from fermented shrimp waste as much as 10 mg/mL ~ 20 mg/mL or equivalent to 10 ppm ~ 20 ppm can show strong antioxidant activity. Astaxanthin has benefits for poultry digestion, which can reduce structural damage to the poultry digestive system under certain stress conditions [15]. Digestive health in chickens is something that must be considered, considering the process of absorption of nutrients contained in feed occurs in the digestive tract, namely in the small intestine. Disturbance and damage to the digestive tract will cause problems in the process of absorption of nutrients in the feed consumed by livestock.

The digestibility of organic matter is closely related to the digestibility of dry matter because some dry matter is organic matter consisting of crude protein, crude fat, crude fibre, and no nitrogen extract. The digestibility of organic matter shows the number of nutrients such as fats, carbohydrates, and proteins that can be digested by livestock [6,19,24]. The digestibility value of organic matter has a higher value than the digestibility value of dry matter. The digestibility value of dry matter is lower than the digestibility of organic matter, this is because organic matter does not contain ash, while dry matter still contains ash. Ash content can slow or inhibit the dry matter digestibility of rations [19].

The digestibility value of the organic matter of the ration is related to the digestibility value of the dry matter in the ration. The increase in the digestibility of organic matter is in line with the increase in dry matter digestibility because most of the dry matter components consist of organic matter, so the factor that affects the high and low dry matter digestibility is the digestibility of organic matter [6]. The increase in the digestibility value of dry matter rations caused by the addition of feed supplements of fermented shrimp waste extract influences the digestibility of organic matter rations. In this study, the addition of a 2% feed supplement of fermented shrimp waste extract provided the highest digestibility value of organic matter. This is related to the high digestibility value of the dry matter in the ration to which a 2% feed supplement of fermented shrimp waste extract is added, the high digestibility of dry matter describes the high content of organic matter because most of the dry matter components are composed of organic matter.

Protein digestibility depends on the protein content contained in the ration. Rations that have low protein content generally have low digestibility and vice versa [6]. In this study, the supplementary feed of fermented shrimp waste extract ration had a higher protein digestibility value than the basal diet. This is caused by the addition of shrimp waste extract that has undergone bioprocess, namely the fermentation process by *B. licheniformis, Lactobacillus* sp., and *S. cerevisiae*. Shrimp waste has a fairly high protein content but shrimp waste has a limiting factor in the form of chitin which causes the protein to be difficult to digest. The fermentation process uses *B. licheniformis, Lactobacillus* sp., and *S. cerevisiae* which have the enzymes protease and chitinase so that they can reduce the chitin content and increase protein digestibility. The fermentation process in shrimp waste with microorganisms *B. licheniformis, Lactobacillus* sp., and yeast *S. cerevisiae*

can improve the protein quality of shrimp waste containing chitin by increasing the completeness and balance of essential amino acids and having optimal digestibility [11].

In addition to having protein content, shrimp waste contains Astaxanthin which is an antioxidant. Shrimp waste contains protein and minerals, as well as Astaxanthin, which illustrates the potential for use as a feed additive in poultry rations [11]. Astaxanthin has an effective role in increasing the proportion of beneficial microorganisms in the intestine, astaxanthin works to inhibit pathogenic bacteria that compete for food as well as maintain the balance of beneficial microbes in the intestine [25]. Astaxanthin can increase the activity of beneficial microbial organisms and digestive enzymes so that the digestibility of and absorption of nutrients contained in the feed increase. The addition of fermented shrimp waste extract in the ration can increase the ability of the intestines to digest and absorb nutrients so that the digestibility value increases.

Based on the results of the study, the average value of basal ration digestibility was in the range of 63-66% while the average value of digestibility of the treatment ration was in the range of 67-79%. The quality of feed based on the level of digestibility is divided into 3 categories, namely digestibility values ranging from 50-60% indicating low feed quality, moderate digestibility levels ranging from 60-70%, and high digestibility of more than 70% [26]. This indicated that the treatment ration had better quality than the basal ration which was not added with fermented shrimp waste extract. This increase in feed quality was due to the addition of various levels of fermented shrimp waste extract feed supplements in the ration.

4. Conclusions

Based on the results of the study, it was concluded that the addition of feed supplements fermented shrimp waste extract in the ration influences the digestibility of dry matter, the digestibility of organic matter, and the digestibility of protein rations of Sentul chicken. The addition of a feed supplement of fermented shrimp waste extract in the ration of as much as 2% provides the digestibility value of dry matter digestibility, digestibility of organic matter, and the highest digestibility of ration protein in the Sentul chicken growth phase.

Conflict of Interest

There is no conflict of interest.

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ARTICLE

Composition and Distribution of Mosquito Vectors in a Peri-Urban Community Surrounding an Institution of Learning in Lafia Metropolis, Nasarawa State, Central Nigeria

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ABSTRACT

Vector surveillance is very key in solving mosquito-borne health problems in Nigeria. To this end, the composition and distribution of mosquito vectors in a peri-urban community surrounding an institution of learning in Lafia metropolis, Nasarawa State, Central Nigeria was carried out between December 2016 and June 2017. The Prokopack Aspirator was used to collect indoor resting mosquitoes between 6:00 a.m. and 9:00 a.m. from 30 randomly selected houses. Mosquitoes collected were knocked down and transferred into a well labelled petri-dish and taken to the laboratory for processing. A total of 664 mosquitoes were collected which spread across *Culex quinquefasciatus* 572 (86.14%), *Anopheles gambiae* 88 (13.25%) and *Aedes aegypti* 4 (0.60%). The abundance of mosquitoes in relation to seasons, species, sex, abdominal conditions as well as transmission indices across seasons significantly varied ($P < 0.05$). But, the distribution of mosquito in relation to house types showed no significant difference ($P > 0.05$). The inhabitants of the area should ensure that all drainages flow through so as to reduce mosquito breeding grounds. Also, members of the community should always protect themselves by sleeping under insecticide treated bed nets.

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1. Introduction

Mosquitoes are small insects with piercing and sucking mouthparts belong to the order Diptera (True flies), belongs to the family Culicidae. There are 3 subfamilies; Anophelinae, Culicinae and Toxorhynchitinae [1]. Only Culicinae and Anophelinae mosquitoes are able to spread diseases [2]. According to Service (1993) [3], various species of mosquitoes transmit some of the most harmful human and livestock diseases. Viral diseases, such as yellow fever, dengue fever, and chikungunya, transmitted mostly by *Aedes aegypti* [4]. The parasitic disease collectively called malaria, caused by various species of *Plasmodium*, carried by female mosquitoes of the genus *Anopheles*. Lymphatic filariasis (the main cause of elephantiasis) which can be spread by a wide variety of mosquito species. West Nile virus is a concern in the United States, but there are no reliable statistics on worldwide cases [5]. Tularemia, a bacterial disease caused by *Francisella tularensis*, is variously transmitted, including by biting flies. *Culex* and *Culiseta* are vectors of tularemia, as well as arbovirus infections such as West Nile virus [6]. The recent global health issue on alert is the emergence of mosquito-borne arbovirus, the Zika Virus which is transmitted by *Aedes* mosquitoes [7-9].

Mosquitoes have a worldwide distribution, occurring throughout the tropical and temperate region and are only absent in Antarctica [10]. However, some genera have restricted distribution and may be confined to certain areas, for instance, the genus *Haemagogus* is only found in central and South America [11]. The report by Bates (1949) [12] showed that certain mosquito species are ubiquitous but are more widespread in the tropical and sub-tropical regions and are also found in temperate climates and even in the Arctic during summer. Several factors which includes habitats, weather, physico-chemical parameters accounts for the distribution of mosquitoes [13,14]. Amusan *et al.* (2004, 2006) [15,16] opined that mosquitoes breed in rice field, ground pools and artificial containers.

Mahesh and Jauhari (2004) [17] found *An. subpictus* abundant in wet season and less in early dry season in Doiwala area of Uttaranchal. Also, Husainy (1986) [18] reported more number of *An. annularis* in rainy season (68%) than dry season in old Bastar district of Chhattisgarh in Central India. *Anopheles culicifacies*, the major vector of malaria in India was high during wet season and rarely observed in dry season [19]. Similar observation was made by Bansal and Singh (1993) [20] in Bikaner district of Rajasthan on the prevalence and seasonal distribution of anopheline fauna. Malakar *et al.* (1995) [21] reported high density of *A. culicifacies* during early wet season between April and May in the foot-hills of Darjeeling district.

Homes within a community are not often uniform in their designs, therefore occupants have heterogeneous exposure levels based on the quality or completeness of their primary residence. This micro-level heterogeneity in human-vector contact has been shown to be facilitated by several factors, including lack of window screening [22], open eaves [23], or the failure to use personal protective measures such as bed nets or insect repellants [24,25]. Paul and Dave (2006) [26] did a research in two countries between June 2002 and April 2003 on the influence of house construction in Trinidad and the Dominican Republic on the indoor abundance of mosquitoes using xenomonitoring surveys and found out that the mean number of *Culex quinquefasciatus* was greater in cement homes than in either wood or other poorer quality homes.

According to Micheal (2014) [27] in a study on the abundance of indoor resting mosquito populations and *Plasmodium falciparum* infection of *Anopheles* species in settlements around the Zaria dam, Zaria, Kaduna State found out that female mosquitoes were higher than males and this may be associated with the fact that the females seek for blood meal for ovarian development. Unlike studies done by Ideozu (1987) [28] and Nendangtok (1991) [29] where more males were collected than females based on outdoor collection.

Ebenezer *et al.* (2013) [30] showed that unfed female mosquitoes were the highest 2098 (45.9%), followed by fed 1888 (41.3%), then gravid 478 (10.5%) and the least was half gravid 102 (2.2%). This may be due to the established position that species with blood meals are strongly anthropophilic and those without blood in the abdomen had not fed by the time they were collected [31].

The study on spatial distribution and indoor-resting density (IRD) of mosquito species in the lowland rainforest of Bayelsa State, Nigeria by Ebenezer *et al.* (2013) [30] revealed the predominant species to be *Culex quinquefasciatus* (45.6%) followed by *An. gambiae* (24.2%), *Ae. aegypti* (18.1%), *An. funestus* (8.6%) while the least was *An. nili* (3.5%). Also, Arum (2021) [32] recorded very high abundance of indoor resting adult *Anopheles gambiae* over *An. funestus, An. coustani* and *An. pharoens is* in a semi-arid ecosystem of Baringo district, Kenya. Tiwari *et al.* (1997) [33] while working on indoor resting *Anopheles* in stone quarry area of Allahabad district, Uttar Pradesh state of India encountered 14 *Anopheles* species with *An. subpictus* being dominant followed by *An. culicifacies*, *An. annularis* and *An. pallidus*.

The indoor resting density of mosquitoes in Bayelsa State ranged from 2.9 ~ 38 mosquito/room/night [30]. Umar *et al.* (2014) [34] recorded high IRD (9.10 ~ 14.0 mosquito per house) of female *Anopheles* mosquitoes in

wet season but low IRD (5.5 mosquito per house) during the dry season. The trend of IRD of mosquito species in decreasing order according to Ebenezer *et al.* (2013) [30] was *Cx. quinquefasciatus* (38.6/room), *An. gambiae* (20.5/room), *An. funestus* (7.3/room) and *An. nili* (2.9/room). Baghel *et al.* (2009) [35] recorded high incidence of mosquitoes during the late wet season (58.27 mosquito/man per hour) as compared to early wet season (41.72 mosquito/man per hour) and late dry season (35.65 mosquito/man per hour). Ebenezer *et al.* (2013) [30] showed that the overall man biting rate (MBR) of mosquitoes in Bayelsa State was 19.10 bites/person/night. Also, the overall MBR was 10.61 bites/person/room, though species specific MBR was 9.8 bites/person/night for *Cx. quinquefasciatus* while *An. gambiae* 8.7 bites/person/night.

Survey of mosquitoes in academic environment [36,37] and surrounding environs [38] is of very high significance. The presence of mosquitoes in peri-urban areas pose a great threat to the well-being of people living there most especially children [39]. To this end, an assessment on the composition and distribution of mosquito vectors in a peri-urban community surrounding a higher Institution of learning in Lafia metropolis, Nasarawa State, Central Nigeria was carried out in order to generate mosquito species checklist, determine their abundance across seasons, house types, sex, abdominal conditions as well as entomological transmission indices.

2. Materials and Methods

2.1 Study Site

The study was conducted in Mararraba-Akunza, with the coordinates 8°28'11.6004"N- 8°28'25.3524"N and 8°35'3.8364"E - 8°34'48.774"E in Lafia Local Government Area of Nasarawa State, Nigeria (Figure 1). Average temperature is about 26.8 °C, humidity is relatively high and rainfall is 456 mm [40]. The site is a peri-urban settlement where agricultural activities take place.

2.2 Ethical Consent

The chief, elders and head of households in Mararraba-Akunza community were visited and adequately informed on the importance of the research. Thereafter, the people consented and the chief granted permission for the research to be carried out by endorsing the ethical consent form.

2.3 Duration of the Study

The study period was between December 2016 and June 2017.

2.4 Sample Collection

Mosquito day catch was carried out in line with the World Health Organization procedure. Prior to the day of sampling, thirty (30) houses were randomly selected and the head of households were informed to keep their doors and windows closed during the morning hours until the rooms were sampled. Selected houses were revisited throughout the period of this study. The Prokopack Aspirator is a battery powered equipment for insects collection [41]. The Prokopack Aspirator operation is simple. It was used in the collection of mosquitoes early in the morning from 6:00 a.m. to 9:00 a.m. for three days, ten households per day. The battery in the outer compartment of the backpack was attached to the power cord (red indicates positive polarity and black indicates negative polarity). The aspirator was turned on with a rotary switch located by the handle. After collection was made, the lid on the collection cup was properly covered before turning off the unit. Afterwards, cotton wool soaked with chloroform was used to knockdown mosquitoes for a period of five minutes and then samples in the collection cup were transferred into a well labeled petri dish and transported to Zoology laboratory of Federal University of Lafia for morphological identification. Collection of mosquitoes spread across early dry (December 2016), late dry (March 2017) and early wet (June 2017) seasons.

2.5 Morphological Identification and Preservation

All collected mosquitoes in petri dishes were later sorted out and morphologically identified using a dissecting microscope and identification keys by Gillies and Coetzee (1987) [42] and Kent (2006) [43].

2.6 Statistical Analysis

The indoor resting density of female *Anopheles* per structure per night was calculated using the formula by Williams and Pinto (2012) [44]:

$$\text{Indoor Resting Density (IRD)} = \frac{\text{Total number of female vectors collected}}{\text{Total number of houses}}$$

All the females (F) collected are separated by species and counted. The total number of collected females of each species was then divided by the total number of occupants (W) who spent the previous night in the rooms that were used for the collection [44].

$$\text{Man Biting Rate (MBR)} = \frac{\text{No.of female mosquitoes collected (F)}}{\text{Total number of occupants (W) in the houses}}$$

Data obtained were analyzed using R Console version 2.9.2. One way analysis of variance was used to compare the mean abundance of mosquitoes in relation to seasons as well as house types. Pearson's Chi-square test was used

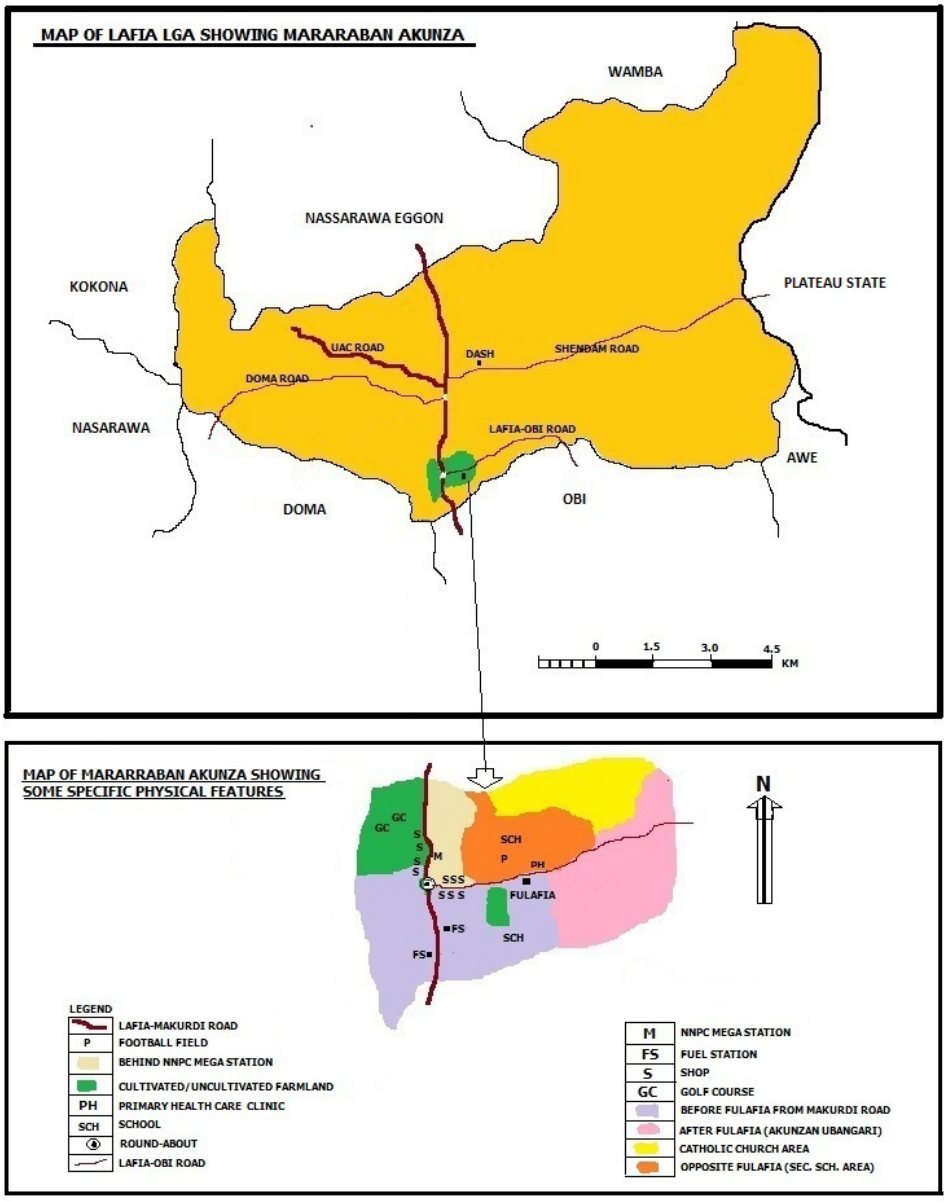

Figure 1. Map of Lafia L.G.A. of Nasarawa State, Nigeria showing Mararraba-Akunza

to compare proportions of mosquitoes in relation to seasons, species, house types, sex, and abdominal conditions. Level of significance was set at $P < 0.05$.

3. Results

3.1 Composition of Mosquito Species in Mararraba-Akunza Community

Mosquito species checklist generated at the end of this study is shown in Table 1. An overall total of 664 mosquitoes were caught resting indoors. The 664 mosquitoes spread across a family, two sub-families, three genera and three species (Plate 1). The highest number of resting indoors mosquitoes was *Culex quinquefasciatus* 572 (86.14%), followed by *Anopheles gambiae* 88 (13.25%) and the least was *Aedes aegypti* 4 (0.60%). Therefore, the abundance of mosquitoes in relation to species showed a very high significant difference ($\chi^2 = 849.3$, df = 2, $P < 0.00001$).

3.2 Abundance of Mosquitoes in Relation to Seasons

The abundance of mosquitoes in relation to seasons showed a very high significant difference ($F_{87} = 20.42$, Adjusted $R^2 = 0.3038$, $P = 0.0000000535$, Figure 2). *Culex quinquefasciatus* was the most abundant in each seasons, the early wet season had the highest number of mosquitoes 573 (86.30%) then late dry 64 (9.64%) and early dry 27 (4.07%) respectively.

Table 1. Composition of mosquitoes in Mararraba-Akunza, Lafia LGA, Nasarawa State

Species	Seasons			Total (%)
	Early Dry	**Late Dry**	**Early Wet**	
Aedes aegypti	0(0.00)	0(0.00)	4(100.00)	**4(0.60)**
Anopheles gambiae	5(5.68)	1(1.14)	82(93.18)	**88(13.25)**
Culex quinquefasciatus	22(3.85)	63(11.01)	487(85.14)	**572(86.14)**
Total (%)	**27(4.07)**	**64(9.64)**	**573(86.30)**	**664**

Comparison of mosquitoes abundance between species: χ^2 =849.3, df = 2, P < 0.00001

a. *An. gambiae* b. *Cx. quinquefasciatus* c. *Ae. aegypti*

Plate 1. Mosquitoes species collected indoors from Mararraba-Akunza area, Lafia L.G.A., Nasarawa State, Central Nigeria

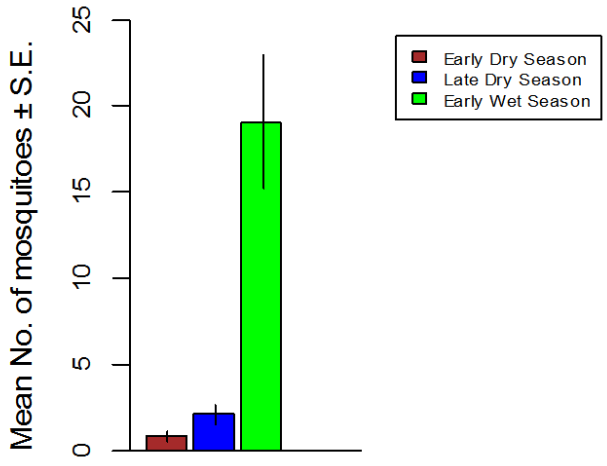

Figure 2. The mean abundance of mosquitoes in relation to seasons

3.3 Abundance of Mosquitoes in Relation to House Types

The cement block house had the highest number of indoor resting mosquitoes 412 (62.05%) followed by mud house 239 (35.99%) and was least 13 (1.96%) in house built with both cement block and mud. However, there was no significant difference (F_{87} =0.0886, Adjusted R^2 = –0.02091, P = 0.9153, Figure 3) in the abundance of mosquitoes in relation to house types.

3.4 Abundance of Mosquitoes in Relation to Sex

A very high significant difference (χ^2 = 97.163, df = 1, P < 0.00001) was observed in the abundance of mosquitoes in relation to sex. The female indoor resting mosquitoes were higher 459 (69.13%) than the males 205 (30.87%) as shown in Table 2.

3.5 Abundance of Mosquitoes in Relation to Abdominal Conditions

Table 3 shows that the fed female mosquitoes were the most abundant 308 (67.10%) followed by unfed ones 65 (14.16%), gravid 45 (9.80%), while the least were the half gravid (8.93%). Therefore, the abundance of mosquitoes in relation to abdominal conditions showed a very high significant difference (χ^2 = 436.82, df = 3, P < 0.00001).

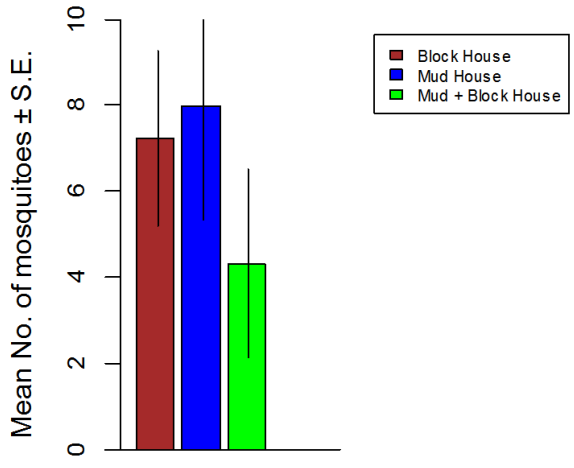

Figure 3. Mosquitoes abundance in relation to house types

3.6 Entomological Transmission Indices of Mosquitoes in Relation to Seasons

Figure 4 shows the entomological transmission indices of mosquitoes in relation to seasons. The overall indoor resting density (IRD) of mosquitoes per house was highest in early wet 13.23 followed by late dry 1.3 whereas it was least in early dry season 0.77. Thus, a very high significant difference (χ^2 =19.468, df = 2, P = 0.00005924) in IRD of mosquitoes in relation to seasons was recorded. Similar trend was observed for man-biting rate (MBR) in the order 4.09, 0.40, and 0.24 per man/night in each household in early wet, late dry and early dry season respectively (Figure 4). Also, MBR in relation to seasons showed a significant difference (χ^2 =6.0178, df = 2, P = 0.04935).

Table 2. Abundance of mosquitoes in relation to sex

	Sex (%)	
Species	**Female**	**Male**
Aedes aegypti	3(0.65)	1(0.49)
Anopheles gambiae	76(16.56)	12(5.85)
Culex quinquefasciatus	380(82.79)	192(93.66)
Total (%)	**459(69.13)**	**205(30.87)**

Comparison of mosquitoes abundance between sex: χ^2 = 97.163, df = 1, P < 0.00001

Table 3. Blood digestion stages of female mosquitoes

Species	**Abdominal Conditions (%)**				**Total (%)**
	Unfed	**Fed**	**Half Gravid**	**Gravid**	
Aedes eagipti	0(0.00)	3(100.00)	0(0.00)	0(0.00)	**3(0.65)**
Anopheles gambiae	5(6.58)	68(89.47)	3(3.95)	0(0.00)	**76(16.56)**
Culex quinquefasciatus	60(15.79)	237(62.37)	38(10.00)	45(11.84)	**380(82.79)**
Total (%)	**65(14.16)**	**308(67.10)**	**41(8.93)**	**45(9.80)**	**459**

Mosquitoes abundance between abdominal conditions: χ^2 = 436.82, df = 3, P < 0.00001

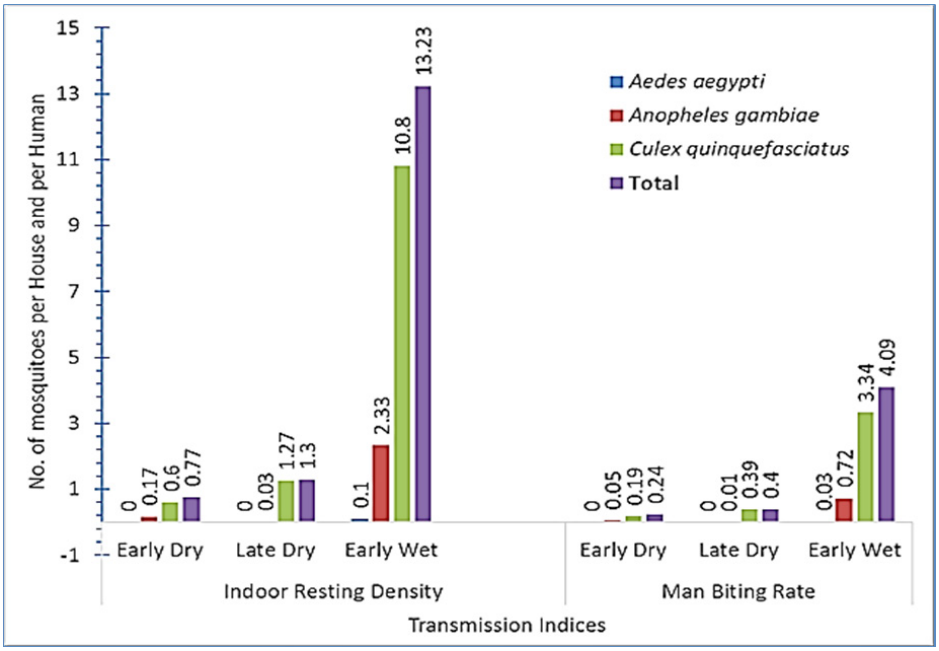

Figure 4. Entomological transmission indices of mosquitoes in relation to seasons

4. Discussion

4.1 Mosquito Species Composition in Peri-Urban Area Surrounding an Institution

The abundant and diverse mosquito species recorded in the study area which cut across a family, two sub-families, three genera and three species probably suggest that the area is a good breeding site for mosquito vectors that may likely transmit disease if they bite any infected individual within Mararraba-Akunza community. This is similar with the finding of Ebenezer *et al.* (2013) [30] who recorded a family, two sub-families, three genera and five species in a study of spatial distribution and indoor resting density of mosquito species in lowland rainforest of Bayelsa State, Nigeria. Also, Ifeyinwa and Tochi (2012) [45], in a study of the distribution and occurrence of mosquito species in the municipal areas of Imo State, Nigeria recorded three genera and eight species.

The observed variation in the abundance of *Culex quinquefasciatus* over other indoor resting mosquito species in the study site possibly suggest a likelihood for transmission of filariasis if they bite any infected person by chance. This agrees with the finding of Gillet (1972) [46] who described *Culex quinquefasciatus* as the commonest nuisance mosquitoes in most parts of Africa and it is a potential vector of filariasis. Also, the indoor resting populations of mosquitoes in some selected areas of Kaduna metropolis was surveyed by Suleiman (2012) [47] who recorded higher number of *Culex* species 99.74% over other species 0.26%. On the contrary, Adeleke (2010) [48] in a study on population dynamics of indoor sampled mosquitoes and their implication in disease transmission in Ikenne area, Abeokuta, Ogun State, South-western Nigeria observed that *Ae. aegypti* was generally predominant indoors.

Furthermore, the preponderance of *Culex quinquefasciatus* over other species clearly shows that the study area has a lot of polluted water bodies that supports breeding success of *Culex quinquefasciatus* in Mararraba-Akunza community. Similarly, Adebote *et al.* (2006) [49] and Afolabi *et al.* (2010) [50] observed that the larvae of culicine mosquito was the most predominant in Zaria. The low proportion of the anophelinae to the culicine mosquito in this present study could be attributed to the location and seasons covered [51,52]. On the other hand, the study by Ebenezer et al. (2014) [53] caught more *An.gambiae* s. l. and implicated it as the vector of malaria parasite in Bayelsa State. Previous study by Umar (2014) [34] on the molecular characterization of *Anopheles* and *Plasmodium* species and retrospective study of malaria in Katsina State, Nigeria showed excess of *Anopheles gambiae* over other species of mosquitoes.

The very low number of *Aedes* species suggests that they are diurnal and mostly feed outdoor. This is similar with the finding by Amusan *et al.* (2005) [54] who caught few *Aedes* species and has a ubiquitous mode of feeding.

4.2 Mosquitoes Distribution in Relation to Seasonality

The high proportion of mosquitoes during early wet

season could be associated to the increase of breeding sites created by rainfall which may possibly account for more vector-borne diseases during the wet season. Similarly, Malar *et al.* (2015) [55] in a study on influential inflicts of monsoon and agricultural practices among the population density of mosquitoes in the agro-rural villages of Madurai recorded higher number of mosquitoes during the wet season. Also, Overgaard *et al.* (2002) [56] showed that the abundant occurrence of mosquitoes are more diverse in the rainy season than during the dry season. The decrease in mosquito abundance in dry season could be attributed to the flushing of larvae in breeding sites due to prolonged rainfall which leads to a decrease in mosquitoes population and will require sometime to resurge. This is in agreement with the finding of Amusan *et al.* (2005) [54] who observed a decrease in mosquitoes larvae due to continuous rainfall which spans into the early dry season causing high larval flush. The variation in mosquitoes abundance across seasons is similar to the studies by Afolabi *et al.* (2006) [57] who recorded variation in the abundance of mosquitoes in relation to seasons in different ecological zones in Nigeria. This study corroborates with the findings of Amusan *et al.* (2005) [54] and Malar *et al.* (2015) [55] who showed that rainfall, relative humidity and temperature accounts for fluctuations in abundance of mosquitoes. In some cases, increased rainfall may increase larval habitat and vector population by creating new habitats, while excess rain would eliminate habitats through flooding, which decreases the vector population [54,55,58,59]. Relative humidity is a critical factor affecting the life cycle pattern of mosquitoes [60].

4.3 Mosquitoes Association with House Types

The mosquitoes caught showed no preference for a particular house type. Although the observed variation in mosquitoes proportions across house types possibly suggests that cement block house type provides a very smooth surface for endophilic mosquitoes to rest. This in line with the finding of Paul and Dave (2006) [26] on the study of the influence of house construction on the indoor abundance of mosquitoes who recorded homes that are made of cement significantly had the highest abundance of mosquitoes resting indoors compared to mud and mud/block house.

4.4 Sex-Wise Mosquitoes Abundance

The high variation in the abundance of mosquitoes in relation to sexes favoured females which possibly suggest that females easily access human host blood meal at indoor point in large volume. This is in agreement with Micheal (2014) [27] who recorded high number of female mosquitoes than males in a study of the abundance of indoor resting mosquito populations and *Plasmodium falciparum* infection of *Anopheles* species in settlements around the Zaria dam, Zaria, Kaduna State. Also, Suleiman (2012) [47] found that the sex ratio of mosquitoes favoured females with 78.68% while males constituted 21.32%. On the contrary Ideozu (1987) [28] and Nendangtok (1991) [29] reported that there were more males collected than females which can be attributed to the collection made outdoors.

4.5 Mosquitoes Physiological Conditions

The number of fed mosquitoes showed high variation over unfed, gravid and half gravid physiological conditions thereby suggesting high indoor human-vectors contact and transmission risk of diseases. This is expected due to the fact that a large proportion of resting females are usually fed. This is in concordance with the finding of Suleiman (2012) [47] who found more fed female population 94.31% while the unfed was 5.69%. This disagrees with the finding of Ebenezer *et al.* (2013) [30] on the study of distribution and indoor resting density of mosquito species in the lowland rainforest of Bayelsa State who recorded the unfed mosquitoes as the highest followed by fed, gravid and half gravid. The proportion of fed *Anopheles* and *Culex* were higher than *Aedes aegypti*. This was not surprising because they are anthropophilic [48]. The high number of female mosquitoes caught shows that they are strongly anthropophilic and those without blood in the abdomen had not fed as at the time they were collected.

4.6 Entomological Transmission Indices

The indoor resting density of mosquito changed significantly in favor of early wet season which suggests that households are more likely to be infected with mosquito-borne diseases. The early wet season in the area showed that each house will have thirteen mosquitoes. This is in agreement with the finding of Oyewole *et al.* (2005) [61] who found variation in IRD across seasons. The high indoor resting density of *Culex* mosquito is line with the study by Adeleke *et al.* (2008) [62], and Okiwelu and Noutcha (2012) [63] who recorded a high IRD due to number of emerged adult as a result of wide distribution of breeding sites during the early wet season.

Man-biting rate of *Anopheles* and *Culex* was four mosquitoes per individual in a room during the early wet season. This is in accordance with the report by Mboera *et al.* (2010) [64] who recorded high IRD during wet season than dry season.

5. Conclusions

This study clearly shows that Mararraba-Akunza area has got good breeding sites for mosquito vectors. The population of mosquito in the area across seasons was dynamic in favour of the early wet season. *Culex quinquefasciatus* was predominant throughout the season. The mosquitoes in the area showed no preference for a particular house type. The sex ratio of the mosquito was in favour of females. Most of the female mosquitoes collected were blood fed, which implies a high likelihood of transmission of mosquito-borne infections. The transmission indices peaked during the early wet season. The inhabitants of the area should maintain a high sense of hygiene by clearing all blocked drainage water ways. Also, they should sleep under insecticide treated bednets (ITNs). Lastly, the government and non-governmental organizations (NGOs) concerned in Nasarawa State should distribute more ITNs to the people.

Conflict of Interest

There is no conflict of interest.

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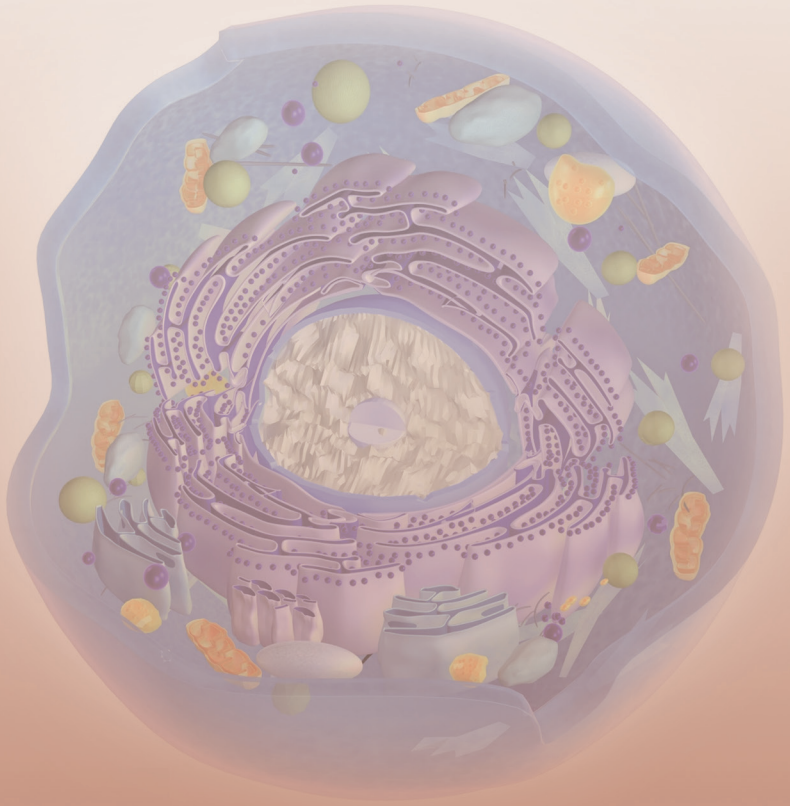

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