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Influence of Diet on Population Growth and Allergen Production in Cultured House Dust Mites - *dermatophagoides Farinae* and *dermatophagoides Pteronyssinus*

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INFLUENCE OF DIET ON POPULATION GROWTH AND ALLERGEN PRODUCTION IN CULTURED HOUSE DUST MITES - *DERMATOPHAGOIDES FARINAE* AND *DERMATOPHAGOIDES PTERONYSSINUS*.

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Swetha Avula Poola ENTITLED ___ Influence of Diet on Population Growth and Allergen Production in Cultured House Dust Mites - *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

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Dermatophagoides farinae and *Dermatophagoides pteronyssinus* culture extracts are used for skin testing, immunotherapy and research studies. There is a need to develop an effective culture method so that standardized extracts and allergens can be produced. The purpose of this study was to examine if different diets effect mite population growth and the amount of allergen produced in cultures. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* were cultured on diets A and B. The density of mites, life stage composition and allergen concentration was determined at two week intervals. Enzyme-linked immune sorbent assays (ELISA) were used to assess group 1 and group 2 allergen concentrations. This study showed that diet is an important factor for culturing of mites. Diet B was found to be better suited to culture *Dermatophagoides farinae* whereas diet A was better for culturing of *Dermatophagoides pteronyssinus.* Diet A cultures of *Dermatophagoides pteronyssinus* produced greater concentrations of group 2 allergen than diet B.

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I. INTRODUCTION

Asthma is a disease that affects about 300 million people worldwide and around 50% of this population lives in developing countries with poor health care. Approximately 20 billion US dollars are spent globally in the treatment of allergic rhinitis, including medication, physicians' consultations and loss of productive work (Pawankar et al., 2008). Increased cases of perennial asthma due to indoor allergens is of major concern as people are spending more and more time indoors (Platts-Mills et al., 1997). A survey conducted by the National Center for Health Statistics in US showed that 27.5% of the patients (from ages 6-59 yrs) showed a positive skin test to house dust mite allergens. Prevalence of house dust mite sensitivity was the highest of all the indoor and outdoor allergens tested (Arbes et al., 2005). House dust mites are prevalent in homes in humid regions of the world and cause allergies worldwide (Arlian and Morgan, 2003). In the U.S., about 30 per cent of the population is sensitive to at least one species of dust mite (Arlian and Platts-Mills, 2001). People sensitive to house dust mites have perennial rhinitis, atopic dermatitis and asthma.

Dermatophagoides pteronyssinus (DP) and *Dermatophagoides farinae* (DF) are two major dust mites found in house dust. Two other species of house dust mites that cause allergies are *Euroglyphus maynei* and *Blomia tropicalis* but they occur in more limited geographic areas (Arlian et al., 1998). DF and DP are the source of about 21 allergens (Thomas et al., 2007). Group 1 (Der f 1 and Der p1) and Group 2 (Der f 2 and Der p 2) are the most characterized and are known to induce reaction in more than 90% of mite sensitive patients (Arlian and Platts-Mills, 2001; Arias-Irigoyen et al., 2007).

These mites are grown commercially for making extracts that are used for research studies, skin testing and immunotherapy. It is necessary to understand the mite biology, origin of the allergens and the nature of the allergens in order to develop an effective culture method so that standardized extracts and allergens can be produced for immunotherapy or skin prick tests.

I. Background

A. Classification of Mites, Life Cycles and Identification:

Dermatophagoides pteronyssinus (DP) and *Dermatophagoides farinae* (DF) belong to Phylum Arthropoda, Subphylum Chelicerata, Class Arachnida, Order Acari, Suborder Astigmata and Family Pyroglyphidae. The subphylum Chelicerata has chelate mouth parts. House dust mite chelicerae have a fixed and a moving digit that form a pincer for grasping (Arlian, 1989; Arlian and Platts-Mills, 2001). Astigmatid mites lack an organized respiratory system and stigmata (external openings).

The mite body is divided into an idiosoma and gnathosoma. The gnathosoma is the anterior projecting mouth part that consists of paired pedipalps and pincher-like chelicerae. Pedipalps have a sensory function while the chelicerae are used to break down and handle food particles. The food mixes with the salivary secretions in the pre-oral cavity and then a pharyngeal pump transfers this semi-solid mixture into the pharynx and esophagus. Food is then digested in the ventriculus of the gut. The undigested food, enzymes and sloughed brush border are packaged in a peritrophic membrane to form fecal pellets. Fecal pellets pass through the posterior intestine and out the anus (Arlian and Morgan, 2003). The enzymes in the fecal pellets along with the mite bodies are the

sources of potent allergens and can cause sensitization or induce an allergic response in already sensitized individuals (Arlian and Platts-Mills, 2001).

Life stages of house dust mite development include egg →larva → protonymph → tritonymph → adult. Each life stage goes through an active phase and a quiescent phase after which it molts into the next stage. Life stages of mites can be distinguished based upon specific morphologic characters that are visible under a microscope (Arlian, 1989):

Larva – This stage has six legs. The genital papillae, external genital morphology and internal reproductive structures are absent. They also lack ventral and genital setae and some dorsal and lateral body setae.

Protonymph – This stage has eight legs. Anal, genital, dorsal and lateral setae are present. They have one pair of genital papillae. The genital opening between legs four is absent.

Tritonymph – This stage has eight legs. Two pairs of genital papillae are exhibited between legs four and the genital opening is absent.

Adult – This stage has eight legs. The females have genital openings between legs three and males have opening between legs three and four. A bursa copulatrix, duct and seminal receptacle complex are seen in females and males have anal suckers, a penis and sclerotized structures that are associated with it.

Dermatophagoides farinae: Detailed description of *D. farinae* can be found in Arlian 1989; Colloff and Spieksma 1992; Colloff 1998; Fain 1965, 1966. The DF female is about 360 μm in length. Females have transverse circular striations over the entire length

of the dorsal surface of the body. The anterior genital apodeme is slightly arc shaped and the posterior edges do not extend beyond the most anterior part of the genital opening. The bursa copulatrix has a funnel shaped external opening the wall of which is chitinized and pitcher shaped. The apex of tarsi I and II have chitinized pointed processes. The male is 290 µm in length. First pair of legs is the largest and the third pair is slightly larger than the second and fourth pairs. The dorsal posterior shield roughly forms a square and does not extend anterior to coxae IV. Epimeres of coxae I are fused medially to form a V or Y shape. Aedeagus and paired basal genital sclerites form a triangular pattern.

When life stages of DF were studied at 75% RH and 23 ^{0} C it was seen that : egg took 10.1±0.8 days to develop, larvae took 9.2±1.3 days with a quiescent phase of 3.8±0.8 days, protonymphs needed 8.6±2.8 days with a quiescent phase of 3.7±1. and tritonymphs needed 7.7±1.3 with a quiescent phase of 3.6±0.7 days (Arlian and Dippold, 1996).

***Dermatophagoides pteronyssinus*:** Detailed description of *D. pteronyssinus* can be found in Arlian 1989; Colloff and Spieksma 1992; Colloff 1998; Fain 1965, 1966. DP is smaller in size. The female is 300 µm in length. Cuticular striations run transverse on the anterior part and longitudinally on the posterior half of dorsal body surface. The anterior genital apodeme is more sharply arched in females of DP than in DF. The vestibule of bursa copulatrix is not heavily chitinized the base of the seminal receptacle bowl has a central pedicle in lateral view or flower shaped in ventral view. Males are 275 µm in length. Legs I and II are similar in size when compared to legs III which are slightly enlarged (Arlian, 1993). The dorsal posterior shield is longer than wide and extends

anteriorly beyond coxae IV and the genital apparatus. Epimeres of coxae I usually do not touch medially. The aedeagus and paired basal genital sclerites form a sharp arch pattern.

A study conducted by Arlian et al. (1990) on DP shows that at relative humidity (RH) 75% and 23^0 C, Eggs need 8.1±0.9 days to develop. Larval stage needs about 10.4±3.2 days with a quiescence period of 4.0 ± 1.4 days. Protonymph stage needs 6.9±2.0 days with a quiescence period of 3.2±1.0. Tritonymph needs 8.3±3.5 with a quiescence period of 3.4± 1.5 and then it emerges into adult stage.

B. **Where are house dust mites found and what do they feed on?**

The dust mites feed on skin scales shed by humans. Therefore, the greatest densities of dust mites in homes are in locations where the most skin scales collect. In the United States, dust mite density is highest on bed room carpets, living room carpets, couches and carpets in homes (Arlian et al., 1982; Arlian et al., 1992).

C. Importance of Humidity and Temperature with respect to House Dust Mites

The house dust mites, DF and DP have a normal water content of 71-76% of their total body weight (Arlian, 1992). The water content varies according to the relative humidity and their physiologic conditions. According to a study conducted by Arlian (1977), mites obtain 4.1% of their required water from food and the rest is absorbed from the atmosphere at 75% RH. Hence, the major source of water for these mites is from the atmosphere and this is why RH is as important and determines their geographical prevalence. DF and DP require a RH of 75% and a temperature of 25^0C for optimal growth. A higher temperature such as 35^0C increases the development rate but decreases the fecundity and longevity of dust mites (Arlian et al., 1990). Temperature differences

within a house vary with respect to carpeted and uncarpeted floor, couches in living room and mattresses. Therefore, depending on RH, temperature and availability of food the density of dust mites varies between locations within a house and between houses.

In a mite, the absorption of water from ambient air occurs as a result of the secretion of a hyper-osmotic solution by the supracoxal gland (Wharton and Furumizo, 1977). This hyper-osmotic solution picks up water by diffusion and then it is ingested. A study by Arlian et al. (1982) showed that there is an increase in consumption of food causing an increase in the fecal pellet production by mites at higher RH. At lower RH the food consumption decreased and so did the amount of fecal pellets produced. At 85% RH, DF consumed an amount of food equivalent to about 42.8% of its body weight and DP consumed 50.9% of its body weight per day. At 75% RH, the food consumed by DF was about 8.4% and DP was about 10.3% of their body weights respectively. Hence, by reducing the RH from an 85% to a 75% there is a considerable reduction in the quantity of food consumption and fecal pellet formation. Therefore, less allergen is produced when RH is lower. The critical equilibrium humidity (CEH) for mite survival at 25^0 C is found to be 70% for DF and 73% for DP. They produce less fecal pellets at RH below the CEH.

In temperate climates, there is a decrease in number of live mites that occur in homes in the winter compared to summer (Arlian et al., 1982). The reason for this decrease in number is associated with the lower RH and temperature during the winter that forces the mites into a motionless quiescent phase or causes them to die from dehydration. In the quiescent phase, the mite bodies are glued on to the substrate and

their metabolic rate slows down. The mites remain in this state until favorable conditions return in the spring. During spring in temperate climates when the outside temperature increases, heaters that dry air are turned down and indoor RH increases in parallel with outdoor RH. The mites become active again and reproduce (Arlian et al., 1983). Geographically, mite populations are prevalent and dense in houses in humid regions and there is a diminished population of mites and mite density in homes in dry climates (Arlian et al., 1982, 1983, 1992, 1998). In a study conducted by Arlian et al. (1998), it was found that DF and DP can thrive at a temperature of 20^0 C and 65, 70 and 75% RH. The mites need a RH greater than 65% periodically to hydrate themselves. The study also showed that *Euroglyphus maynei* was unable to survive under similar conditions. If they do not get this (greater than 65% RH) periodically they become dormant and die.

A landmark five-year study was conducted by researchers from eight different geographic areas of the United States to determine the density, species prevalence of mites and levels of antigen Der p 1 to which the patients were exposed over the course of the study. A total of 252 houses were sampled in Cincinnati, Ohio; New Orleans, La; Memphis, Tenn; Galveston, Texas; Greeneville, N.C; Delray Beach, Fla; San Diego, Calif and Los Angeles, Calif. The study showed that both DF and DP were prevalent in greater than 74% of homes. But one of the species (either DP or DF) dominated over the other and made up for more than 75% of the total mite population in the house. Less than 25% of homes were inhabited only by DF or only by DP. More than 90% of homes had an average mite density of 100 mite/gm of dust (clinically significant value) over time, except in Memphis and Los Angeles. *Euroglyphus maynei* was found in 35.7% of houses in New Orleans, Memphis, Galveston, Delray Beach, and San Diego. *Blomia tropicalis*

occurred in the same places but in lower numbers. Hence, the study found that DF and DP are the most wide spread and prevalent species in the United States. As a result of this study, allergists now test for sensitivity and treat for the two species.

Temperature: During Fall and winter along with fluctuation of RH fluctuation of temperature occurs. To see the effects of temperature on DF (Arlian and Dippold, 1996) and DP (Arlian et al., 1990) mite population growth studies were conducted with a constant 75% RH and different temperatures - 16 ^{0}C, 23^{0}C, 30^{0}C and 35^{0}C. Females were randomly collected from thriving lab cultures and caged separately in glass tubes that were closed at the ends with 35μm nylon mesh for ventilation. Initial amount of culture medium was added to the glass tubes for the mites to feed and was added as required later throught the study. When the female deposited an egg, the female was taken out of the cage and the egg observed until it developed into an adult. The cages were observed everyday and twice a day at 35^{0}C the results were recorded. As a part of the same study to determine the fecundity of DF, one hundred cages containing males and tritonymphs were held at 75% RH and 23^{0}C. Only females that emerged from the tritonymphs were kept along with the males. When the female started to deposit eggs the male was removed from the cage and the number of eggs produced by the female was recorded. After which the egg was removed from the cage and the process was continued until the egg production by the female ceased and the female died.

It was seen in these two studies that to develop from egg to adult stage DF and DP took 35.6±4.4 and 34.0±5.9 respectively at 23^0C. At 30^0C, DF took 17±1.2 and DP took 19.3±2.5. DF did not grow at 16 ^{0}C and 35^0C. DP took 122.8±14.5 at16 ^{0}C and 15.0±2.0 days at 35^0C.Therefore, DP grows better in spite of fluctuations in temperature.

Developmental times were seen to be inversely related to temperature. DF developed better at 23^0C and 30^0C. At 16 ^{0}C and 35^0C only 2 and 15% of DF eggs became adults, respectively. Fifty nine and 87% of eggs developed into adults at 16 and 35^0C, respectively for DP (Arlian et al., 1990). These results led to the inference that DP developed better in cooler and hotter temperatures than DF and DF has a narrow range of tolerance towards fluctuations in temperature.

D. How do house dust mites act as allergens?

House dust mite allergens are proteins that have epitopes that are recognized by T-cell and B-cell receptors and to which IgE can bind. Dust mite allergens induce a type 2 T-lymphocyte response. The development of atopic disease is due to production and circulation of IgE. Initial sensitization involves B cell activation by mite allergen that results in a clone of plasma cells that produce IgE and memory B cells. The IgE binds to the surface of mast cells that reside in various tissue including the skin and mucosa of the nose, lungs, intestine etc. Subsequently, an allergic reaction occurs when the allergen enters the body and binds to IgE on mast cells and basophils that are bound by the high-affinity Fc receptors (FcεR1). The crosslinking of FcεR1- bound IgE molecules by the allergen leads to release of pharmacologically active mediators that are responsible for tissue damage. These mediators include histamines, prostaglandins, leukotrienes and cytokines and are responsible for the symptoms associated with allergies. IgE also

mediates allergen presentation with FcεRI and FcεRII (CD 23) receptors on Langerhan cells and monocytes (Shakib et al., 1998). Subsequent exposure to the same or cross-reactive allergens causes an inflammatory reaction.

The allergic reaction is T-cell dependent. The B cells are activated to proliferate and develop into antibody secreting plasma cells by physical interaction with and cytokine signals from helper Th2 cells. The APC (antigen presenting cell) processes the antigen and displays it on the surface in association with the MHC class II molecule. The Th2 cell recognizes this and is activated; it then produces cytokines (IL-4, IL-5, IL-6, IL-10 and IL-13) that help in the activation of B cells and their maturation to IgE producing plasma cells. Th1 cells produce IL-2, IFN-γ, and TNF-α/β, that down regulate IgE synthesis, so Th1 and Th2 polarization occurs. The polarization is maintained by Th1 cytokines trying to suppress the production of Th2 cytokines and vice-versa.

E. Different types of allergens found:

There are currently 21 groups of dust mite allergens (IUIS nomenclature). The HDM allergens can be classified as proteins belonging to enzymes, ligand binding proteins, tropomyosin and calcium binding (Chapman et al., 2007). Most of the mite allergens have been characterized and their biological activity has been identified to some extent.

Group 1- Cysteine Protease,

Group 2- ML domain binding protein

Group 3- Trypsin

Group 4- Amylase

Group 6- Chymotrypsin

Group 8- Glutathione -S-Transferase

Group 9- Serene Protease

Group 10-Tropomyosin

Group 11- Paramyosin

Group13- Fatty acid binding protein

Group14- Large lipid transfer protein

Group 15- Chitinase

Group 16 &17- Gelsolin and EF hand binding proteins

Group 18- Chitinase like

Group 19- Anti-microbial peptide

Group 20- Arginine kinase

Others: 5,7,12, 21 (Angus et al., 2004; Thomas et al., 2007).

A study conducted in Australia by Hales et al. (2006) showed that the most important allergens are Group 1 and 2 allergens. Group 1 and 2 allergens bound 50 to 65% of IgE and allergens belonging to groups 4, 5 and 7 bound to the remaining 30-50% of IgE in mite allergic individuals.

F. Allergic reactions and Importance of Group 1 and Group 2 Allergens:

Patients sensitive to house dust mites have diseases such as asthma, perennial rhinitis and atopic dermatitis. Mite bodies and mite feces are the source of allergens that

cause allergic reactions (Arlian et al., 1987). The Group 1 allergens originate from the fecal matter and are believed to be the enzymes produced by the digestive tract of the mite (Arlian and Platts-Mills, 2001). Group 1 allergens (Der f 1 and Der p 1) are glycoproteins that have a molecular weight of 25 kDa and have cysteine protease activity. Fecal pellets have an average diameter of 20 μm and can become airborne. The pellets can be inhaled into the lungs or come in contact with skin and mucous membranes (Tovey et al., 1981). Group 2 allergens (Der f 2 and Der p 2) have a molecular weight of 14 kDa and are homologus to primate epididymus protein (Arlian and Platts-Mills, 2001). That is, more than 90 percent of patients produce with allergies to house dust mites produce IgE to Group 1 and Group 2 allergens (Platts-Mills et al., 1997).

Dust mite allergens have the ability to cleave a number of immunologically related and protease activated receptors (PARs) (Ashoknathan et al., 2002; Shakib et al., 1998). Der p 1 is believed to cleave α1- antitrypsin (a serine protease inhibitor) which provides innate immunity on mucosal surfaces (Kalsheker et al., 1996). After entering the mucosa, it activates mast cells, resulting in secretion of IL-4 and IL-13 that stimulate increased IgE synthesis. Cleavage of CD23 on B cells prevents negative IgE regulatory signals and thereby enhances IgE synthesis. Der p 1 also cleaves CD25 (α subunit of Il-2 receptor) on peripheral blood T-cells causing a decrease in T cell proliferation and IFN-γ production. Therefore, Der p 1 causes a shift in Th1/Th2 subsets in favor of Th2 and in turn increases recruitment and activation of eosinophils (Shakib et al., 1998).

Cysteine protease activity of Der p 1 increases mucosal permeability of the bronchial mucosa. Specifically, it proteolyses the tight junction proteins occludin and claudin, resulting in transepithelial delivery of the allergen (Wan et al., 1999). Cell

surface zymogen is activated by Der p 1 which then cleaves tight junctions. Intracellular processing occurs due to tight junction perturbation or through signal transduction pathways that finally affects tight junctions.

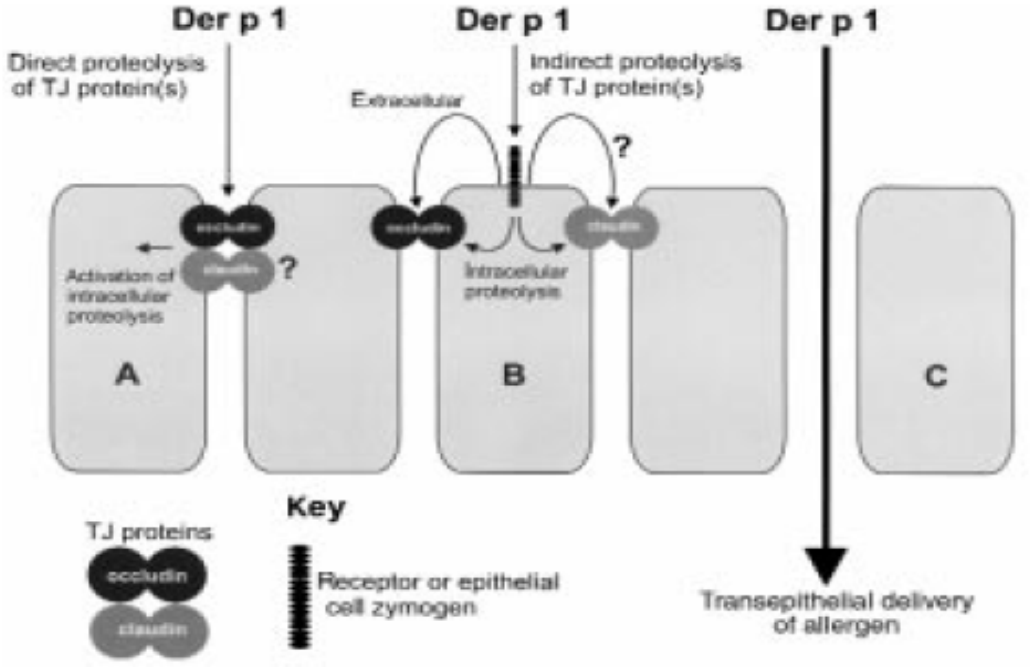

Figure 1: ***Adapted from Wan et al., 1999*****. Schematic summary of how Der p 1 might open TJs in lung epithelium.**

Der p 1 also cleaves protease activated G-protein receptors (PAR), found on respiratory epithelium resulting in the release of the pro-inflammatory cytokines Il-6 and Il-8 (Deary et al., 1998; Ashokanathan et al**.,** 2002; Kauffman et al., 2006).

Kauffman et al., (2006) studied the mechanism by which purified Der p 1 and recombinant allergens rDer p1, rDer p 2 and rDer p 5 affected the biochemical properties of airway derived epithelial cells. The involvement of protease activated receptors was analyzed using mouse fibroblasts that expressed human PAR-1, PAR-2 or PAR-4. They found that the allergens activated the human airway-derived epithelial cells in at least two

ways. First, a protease independent way that boosted production of proinflammatory cytokines without affecting cell morphology. Second, a protease dependent activation that caused cell shrinking, cell-desquamation and production of proinflammatory cytokines. They demonstrated that a functional PAR2 was present on airway epithelial cells by using specific PAR2 agonists that induced production of IL-6 and IL-8. Conflicting results have been seen by investigators in this matter with studies indicating that Der p 1 is not responsible for activating the PAR2 signaling cascade (Ashokanathan et al., 2002). Kauffman et al. (2006) suggested that contamination of purified Der p 1 with Der p 3 was the cause of this conflict. These two mechanisms act synergistically to aggravate the inflammatory response in asthmatic patients. Der p1 and Der f1 have 80% sequence homology and have cross-reactive epitopes (Arlian and Platts-Mills, 2001). Hence their immunology should be closely related.

G. Cross-reactivity between group 2 allergens:

The group 2 allergens are found to be proteins of ML (MD-2 related lipid-recognition) domain family. They are related to the Toll-like receptor 4 (TLR-4) co-receptor MD2. ML proteins are involved in innate immunity and lipid metabolism (Inohara and Nunez, 2002). The exact immunologic effect of mite ML proteins is not very clear. Der f 2 and Der p 2 show 88% sequence homology. The tertiary structure of the allergen is stabilized by three disulfide bonds and is responsible for its IgE binding (Smith and Chapman, 1995).

Homologs of group 2 allergens are seen in closely related mites like *Euroglyphus maynei* and nonpyroglyphid mites of the families Glyciphagidae (e.g. *Lepidoglyphus*

destructor and *Glycyphagus domesticus*) and Acaridae (e.g. *Tyrophagus putrescentiae*) (Gafvelin et al., 2000; Arlain and Platts-Mills, 2001).

Cross-reactivity occurs when antibodies react with similar epitopes of different allergenic proteins. There is 85% sequence homology between Der f 2, Eur m 2 and Der p 2. Lep d 2 shows 40% homology and Tyr p 2 shows 41% sequence homology to Der p 2 (Smith et al., 2001). Higher sequence homologies indicate that there is a close structural similarity. Due to structural similarity, antibodies cross-react to these group 2 allergens.

H. Cross-reactivity between house dust mites and stored product mites:

Many patients are sensitive to multiple species of mites. It is not clear if this is because the patients are sensitized to many species specific allergens or if they are sensitive to many cross-reacting allergens. For example, IgE cross-reactivity between DP and *Glycyphagus domesticus* (storage mite) makes it hard to find monosensitized patients. Arias-Irigoyen et al., (2007) studied in Huelva, Spain, the allergenic importance and sensitivity of patients to *G.domesticus* and the IgE cross reactivity between the more ubiquitous DP, *Lepidoglyphus destructor* and *Tyrophagus putrescentiae*. Species of mites present in this area were identified by examining the house dust samples collected from patient's houses. Skin prick (following standard procedure) and conjunctival provocative tests (by applying a drop of *G.domesticus* extract at 10BU/ml in conjunctival sac of one eye and saline in the other eye) were performed on patients. RAST (Radioallergosorbent test) assays detected specific IgE. RAST inhibition assays compared cross-reactivity between different mite species found. Their study indicated a low cross-reactivity between DP and *G.domesticus* and suggested that *G.domesticus* can act as a primary

sensitizing mite. However, the wide spread distribution of DP makes it difficult to find patient's monosensitized to *G.domesticus*. Hence cross reactivity between DP and *G.domesticus* makes it difficult to diagnose and treat patients who are allergic to *G.domesticus* only. Arias-Irigoyen et al., (2007) suggest that cross-reactivity could be one of the reasons for the unsuccessful immunotherapy in patients.

Yadav et al., (2006) conducted a study in southwestern Ohio to determine the serum IgE present in the population to the storage mites, *L. destructor* and *A. siro*. The extent of co-sensitization to other astigmatid mites was also studied. An earlier survey had shown that 6.5% of the same population in southwestern Ohio had serum IgE to another storage mite, *T. putrescentiae* (Kondreddy et al., 2006). In this study, SDS-PAGE (Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis) gels were loaded with 100µg of the mite extract proteins (*A.siro* and *T.putrescentiae*) and electrophoresed at 200V. The separated proteins were transferred to ProBlott membranes, which were then washed, blocked and placed in slot blot apparatus. The individual lanes were loaded with patient serum samples that were diluted in the ratio of 1:5. After overnight incubation, the blots were washed and removed from the apparatus and incubated in 0.7µCi of 125 I-labeled anti-human IgE overnight. Blots were then washed and dried and placed on film for varying periods up to 21 days. Selected serum samples were also used to probe blots containing extracts of all 9 mite species. Two µg of protein of each mite extract along with one lane of molecular weight standards were loaded on 10 well 12% gels. Each blot was then incubated with a single serum sample diluted in ratio of 1:5. Three additional slot blots containing 10 µg of extract from the three foods that were used to grow the mites on were tested on 21 of the 30 samples. They found that of the 600 samples tested

3.3% and 2.3% had IgE to proteins of the stored product mites *L.destructor* and *A. siro* respectively. Hence, storage mite allergenicity exists in a significant number in this population. Another important finding of this study was the association between storage mite sensitization and house dust mite sensitization. Of 30 selected serum samples, 90% had IgE to proteins of at least 2 of the species of storage mites tested and 87% had IgE to at least 1 species of storage mite and to 1 or more of the pyroglyphid mites. Therefore, this study does not exclude the possibility that the house dust mites and storage mites have a few cross-reactive allergens. But in the present study the investigators believed that patients were exposed to and sensitized to both storage and dust mites because the allergens of sterile mites were higher molecular weight proteins compared to the low molecular weight proteins for house dust mites. They suggest more studies to confirm that IgE cross-reactivity was responsible for storage and house dust mite allergenicity seen in this study. Since storage mites are capable of inducing allergic reactions in patients the authors suggested that these mites should be included in routine diagnosis and treatment of allergy.

I. Allergen profiles during growth of mite cultures.

Martinez et al., (2000) showed that differences in intra and inter species cross-reactivity exist. They studied inter and intra-species allergenic cross-reactivity based on qualitative and quantitative differences existing throughout growth curves. For the mite culture a 1:1 autoclaved mixture of rat and mouse meal and dried yeast extract was used to culture DF and DP. The cultures were incubated at 23-25 ^{0}C and 75-80% RH (maintained by saturated NaCl solution).Twelve cultures per species were made from the

same inoculum and they were examined microscopically at a regular basis (14 days) for mite count (50-100mg) and contamination. Extracts were then obtained from cultures. Extracts were prepared from the three different growth phases: F1- Latency (0-8 wks for DF and DP), F2- Exponential growth phase (10-18 wks for DP and 8-14 wks for DF) and F3- Death phase (20-24 wks for DP and 16-20 wks for DF) by agitating the culture at 4-8 ^{0}C in PBS. After this it was centrifuged at 3,000g for 30 min and the supernatant filtered. The extracts were then dialyzed in membranes with a 5,000Da (cut-off at 4 ^{0}C with distilled water for 24 hrs), centrifuged at 12000 g for 30 min and then lyophilized. Human sera from 37 mite sensitive individuals with persistent allergic symptoms and with no history of immunotherapy or other sensitizations were collected. This serum was used for cross-reactivity evaluations by RAST-inhibition technique. Four dilutions of the allergen (0.01-10 μg) and 50 μl of pooled sera from patients sensitized to house dust mites were added to solid phase antigen. Solid phase antigen was obtained by coupling the extract solution to 6mm diameter CNBr-activated paper discs using a previous method. Phadezym-RAST (an enzyme marker) was used to measure antibody activity. Inhibition curves were plotted with percentages of binding of specific antibodies to solid phase versus logarithm of extract concentration of added inhibitor. This study indicates that the F2 allergens are the most potent allergens and are the best source for cross-reactive study. The F3 extracts had low allergenic potency and inhibition percentage. Therefore, allergenicity and cross-reactivity varies within an allergen depending on growth of the culture and extract of mites.

PURPOSE OF THIS RESEARCH & SPECIFIC AIMS

Dust mites are commercially cultured to obtain materials to make extracts for use in diagnostic tests, immunotherapy and research studies. The source and purity of mite allergen is important as it can affect the results of immunotherapy, diagnostic skin prick tests and cross-reactive studies. Immunotherapy treatments can induce severe anaphylactic reactions in patients and crude whole mite extracts could introduce new allergenic sensitivity. As Gafvelin et al. (2001) suggested, we should look to replace mite extracts with recombinant allergens. However, mites are the source of many allergens and few allergens have been produced by recombinant methods. Also, allergens we use should be identical to the isoform most commonly found in natural mite material. Studies must be conducted to find the growth stage of mites that produces the most potent and complete set of allergens this information can be used to produce recombinant allergens for all testing purposes.

My research helps us understand the ideal stage of allergen production in a mite population. In this study, I examined if population density and different diets had any effect on the amount of allergen produced. Like RH and temperature, diet is an important factor for the mite population growth. Different diets can influence mites to grow at different rates and allergens produced by mites. Based on this study we can develop standardized allergen extracts that can be used in diagnosis, treatment and cross-reactive studies.

Specific aims:

1. Determine the population growth rates of mites grown on specific diets.
2. Determine the life stage composition of the population at multiple times as the population grows (e.g. 2, 4, 6, 8….etc weeks).
3. Determine the concentration of the allergens, Der f 1, Der f 2, Der p 1 and Der p 2 and their ratio over time of culture.

METHODS:

A. Stock laboratory Mite cultures:

Cultures of *Dermatophagoides farinae* (DF) and *Dermatophagoides pteronyssinus* (DP) for this study were started from thriving cultures that are maintained on diet A (yellow food) at 75% RH and room temperature in the laboratory of L.G.A. at Wright State University (Arlian et al., 1990). For my research, these laboratory stock cultures were used to start a single mature culture of mites that was grown on a specific food (either diet A or diet B) and maintained at 75% RH and room temperature. The stock cultures for my studies were allowed to grow until they consisted mostly of mites and little food and served as a source of mites for starting the test cultures. So, the new test cultures for this project were started with mostly mites and little contaminating food.

B. Experimental cultures:

Cultures were set up for *Dermatophagoides farinae* (DF) and *Dermatophagoides pteronyssinus* (DP) on two different foods - Diet A and Diet B to determine the influence of diet on mite population growth and allergen production. Five replicate cultures were started for each mite species and each diet. Each food (diet) was equilibrated at 75% RH for at least 2 days prior to setting up the 5 cultures for each food and mite species. The test cultures were maintained in one cup glass jars (Ball corp. U.S.A) at room temperature. For diet A, each test culture jar was started with 30 grams of food and 6 grams of mites (from the stock culture). For diet B the test food was 60 grams of food inoculated with 6 grams of mites (from the stock culture) per test culture jar.

The jars with mites and food were placed on a raised (7 cm) plastic platform (with holes in the platforms for ventilation) in two round 6 qt buckets with dimensions 21cm × 20 cm (both platforms and buckets were from Rubbermaid, Winchester, VA). The buckets had airtight lids and contained saturated salt solution (NaCl) at the bottom to maintain 75% relative humidity. The glass jars used had screw-on tight-fitting lids. To provide ventilation, the lids of the glass jars and buckets had a 2 to 2.5mm hole covered with 36 μm pore size nylon monofilament screen (Small Parts Inc. U.S.A).

Samples of the test culture material in the jars were collected every 2 weeks starting from t=0 through t=12 weeks or greater until the cultures matured and the population size, life stage and allergen concentrations were determined in these samples.

C. Population size, life stage composition and allergen concentration of the samples collected every two weeks was determined by the following methods:

1. Life Stage

Two 50 mg samples (a and b) were removed from each of the 5 cultures and collected in 15ml-sterile disposable centrifuge tubes (Fisher brand) along with 3ml of 95% ethanol. After 1-2 days, slides of the mite material from each of the tubes were made using the following method

- Using a 5 $^{3/4}$ inch pasture pipette (VWR), 2-3 drops of the mite- alcohol mixture were placed on 25×75 mm, 1mm thick plain microscope slides (VWR plain micro slides) that were labeled.
- The alcohol was left to evaporate until just dry.

- 2-3 drops of lactic acid (Sigma Life Science, St. Louis, Mo) was added to the mites on the slide and then mixed using a counting probe.
- A glass cover slip, 18×18 mm was placed over the mites
- This procedure was repeated for all of the samples.
- The slides were then warmed in an oven (Blue M, Blue Island, IL) at 55 ^{0}C for 2-3 days so that the mite bodies became clear and the morphology necessary to determine the life stages could be distinguished when observed under the microscope.
- A minimum of 250 mites was counted for each of the five cultures for each sampling time. The life stages- eggs, larvae, protonymphs, tritonymphs, males and females were counted and recorded using a compound microscope (OPTIPHOT-2,Nikon,Japan).
- At each time interval, the mean number of eggs, larvae, protonymphs, tritonymphs, males and females was calculated for duplicate samples of all five cultures.

2) Population size:

- Two weighed 10-50 mg samples (lesser quantity is taken out when the population density is higher) were removed from each culture and placed in a gridded petri dish (9x9 cm). The mites were then counted using a dissecting microscope.
- The culture was thoroughly mixed. A gridded petri dish (9x9 cm) was placed on the weighing balance and zeroed.
- 10-50 mg of the culture media was weighed and the amount noted. With the help of a probe the culture material was then uniformly distributed over the bottom of the petri dish.

- The number of live mites (larvae, protonymph, tritonymph and adults) in the petri dish was then counted using a dissecting microscope (Wild Heerbrugg-M8) and the number recorded. Eggs were not counted.
- If the initial weight of the sample was less than 50 mg the number was extrapolated to 50 mg so that all the results could be compared.
- The second sample was also counted by the method described above. After, both the samples from the first culture were counted and the numbers recorded following the same method I began counting the next culture. The next four cultures were counted following the same method.

3) Allergen concentration:

Two samples (a and b) of about 100mg each of well mixed culture were removed from each of the five culture jars and stored at -20 ^{0} F (in 15-ml centrifuge tubes). These were used later to estimate the amount of allergens (Der f 1, Der p 1, Der f 2 and Der p 2) present in each of the samples over time. Allergen concentration was determined by ELISA (Enzyme-Linked Immuno Sorbent Assay) using mouse monoclonal antibodies.

D. Determination of the most efficient mite extracting solution:

Mite extracts were made so that all the allergens in the mite bodies and the food could be obtained in a solution form to run ELISA's. Previous observations in our laboratory indicated that the extracting solution used could influence the amount of allergen obtained from mite material. Therefore, PBS, (Phosphate Buffer Saline), PBST (PBS+1% Tween) and B-PBST (PBS+1% Bovine Serum Albumin+ 1% Tween) were tested to

determine which solution would give the best yield of allergen. Spent culture mite material from cold storage was used.

Replicates of three fifty milligram samples were collected in 15-ml centrifuge tubes. Ten mls of the three extracting solutions being tested were added to their respective tubes and vortexed for 15 sec. The samples were then shaken (Orbit, Lab Line) overnight at 100-150 rpm. Then each tube was vortexed for 20 sec and placed in a sonicator for another 10 min (with ice). Then the 15ml tubes containing the samples were centrifuged for 15 min at 27000rpm (1000 g) (Marathon 10K, Fischer Scientific). One ml was then pipette out into micro-centrifuge tubes which were then spun for 10 min at 14,500 rpm (14,000 g) in a micro centrifuge (MiniSpin plus, Eppendorf). The supernatant (extract) was collected in bullet tubes and the pellet discarded. The sample (extract) from the bullet tubes was used for ELISA. These sample extracts were assayed for Der p 1 and Der p 2 by ELISA. Samples with PBS solution showed the least amount of allergen whereas BPBST showed the highest amount of allergens from the same samples. Thus, BPBST was used for extracting allergens from the test culture material.

E. ELISA analysis of samples for Der 1 and Der 2:

1. Sample Extraction:

The two 100mg samples from section C 3 were labeled and stored at -20^{0}C until all samples for each species on both foods were collected. The ‘a’ samples were taken out of the -20^{0}C freezer and kept at 4^{0}C for 2 days so that the samples could thaw. The ‘b’ samples remained stored in the freezer for further testing. Before adding the extracting solution, the samples were taken out of the refrigerator and allowed to reach room

temperature for about an hour. The weighing balance, Denver Instrument company (TR-403) was calibrated and the first sample was 'zeroed' To this sample, 10 ml of B-PBST(1% BSA & 1% Tween) was added as this was found to be the best extracting solution (Section D). The sample (100 mg sample+ 10ml B-PBST) weight was noted to eliminate any errors in dispensing the B-PBST and each vortexed for 15 sec. The samples were the placed on a shaker overnight at 100-150 rpm.

The next day, using the above mentioned method (section D) the samples were taken off the shaker, vortexed, centrifuged and micro-centrifuged. The 1ml of supernatant was pipetted out into new micro-centrifuge tubes and the rest of the supernatant from the 15 ml tubes were pipetted into another set of tubes that were labeled and stored at -80^0C for future use. The micro-centrifuge tubes containing 1ml of the sample were then spun for 10 min in a micro-centrifuge. The supernatant was pipetted into new bullet tubes and the pellet discarded. Hence each sample had a separate bullet tube that was labeled and stored at -20^0C. The sample from the bullet tubes was used for ELISA to determine the concentration of Der 1 and Der 2 allergen.

2. Procedure for ELISA:

ELISA dust mite kits from Indoor Biotechnologies (Charlottesville, Va) were used to determine the allergen content of group 1 and group 2 (lot 31024) allergens. 96-well ELISA Plates (Costar 3590, Fisher scientific) were made for extracts of mite material from both Diet A and Diet B samples at the same time. Samples from each culture cup had a group 1 and a group 2 plate. 100 µl of the mouse monoclonal antibody to Der f 1, 6A8 (Lot 31022) was added to 100 ml of carbonate-bicarbonate buffer (0.2mg/ml dilution), pH 9.6 used to coat all group 1 plates. For group 2 plates, 100 µl of

1D8 mouse monoclonal antibody (Lot 30031) was diluted by adding it to 100 ml of carbonate-bicarbonate buffer. The plates were then covered and incubate overnight at 4^0C or until used. Plates were then washed three times with PBST (Dulbecco's phosphate buffered saline with 0.05% Tween 20) and blocked at least for 1 h at room temperature with B-PBST (1% bovine serum albumin in PBST). Plates were washed three times with PBST Universal standards diluted according to manufacturer's instruction (1:10 dilution) in a single set of tubes (1:10 dilution) from which they were added to the first row of all the group 1 and group 2 plates.

Bullet tubes were arranged in a bullet box according to the ELISA map where each row represented mite extraction samples from each time. For example, row B column #1 was t=0, row C column#1 was t=2 so on and so forth until row H column #1that had t=12 week mite extraction samples. Doubling dilutions of mite extract samples were made with the first column containing 200µl of sample with 200µl of B-PBST (1% BSA). The next column (#2) had 200µl of the sample from column #1 and 200µl of B-PBST. The process of dilution was repeated until column #12. Samples t=0 were loaded in row B, t=2 in C and so on t=12 was loaded in row H for each of the cultures. Standards (Lot 31012) and samples were added at 90 µl/ well on both group 1 and group 2 plates and incubated for one and a half hours. After washing the plates three times with PBST, 100 µl/ well of the mouse monoclonal biotinylated antibody 4C1 for group 1(Lot 30068) and 7A1 for group 2 plates (Lot 31008) were added. Plates were washed three times and then the 100 µl/ well of Streptavidin-Peroxidase (1:1000 dilution, Lot 18604-ZK64C) we added and the incubated for 30min-1hr. The plates were then washed twice with PBST and twice with PBS. The assay was developed by adding 100 µl of 1 mM ABTS (2, 2'-

azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) in 70mM citrate phosphate buffer (pH 4.2) and 1:1000 dilution of 30% hydrogen peroxide. The plates were read when the optical density at 405 nm reached 2.0-2.4 on a plate reader (Universal Microplate reader, Bio-Tek Instrument).

F. Data Analysis (For DF and DP on both diets):

1. Population growth:

The number of mites per 50mg of food at every 2-week interval was counted for all five replicates (two samples per replicate). The means ±SEM were then plotted against time. The population growth best fit an exponential growth curve. As described by Arlian et al. (1998), the $P_t = P_0\, e^{kt}$ equation was used to calculate population growth rates and correlation coefficients. Where, P_t represents population density at any given time, P_0 is the population density at time (t) 0, k is the rate constant for increase and decrease in population and t was the time. The population doubling times (t_2) was equal to $ln\ 2/k$ or 0.69/k and half time (t ½) was $ln\ 0.5/k$ or -0.69/k. Also, in order to see a liner relationship in the form of y = mx+ b natural log of the each of the data points (means) were taken and plotted against time and the slope of the equation was equal to the growth rate constant.

2. Allergen concentration:

For all the replicates at 2 week intervals (one sample per replicate), allergen concentrations (ng) per 100mg of the culture material were calculated. The Means ± SEM were plotted and the resulting curve best fit an exponential growth curve. Using the same method as described above the rate of increase in allergen concentration and correlation coefficients were calculated.

3. Life stage:

The life stage composition of all five replicates (two samples per replicate) at 2 week intervals were counted. The number of eggs, larvae, protonymphs, tritonymphs, males and females per 1000 mites at each time was plotted.

RESULTS

I. *Dermatophagoides farinae*

1. Population size, life stage composition and allergen concentration of *Dermatophagoides farinae* (DF) cultured on diet A.

a) Population growth:

Population growth of DF on Diet A showed an exponential increase (Figure 2). Mean initial population density was 631.5 ± 50 mites per 50 mg of culture material at time 0 and grew until 12 weeks at which time the mean population density was 3255.7 ± 403.9 mites per 50 mg of cultured material (Table 1). The cultures had matured by 10-12 weeks and population size started to plateau as the diet was depleted. The growth rate of the population was 20.2% per week and the population doubled in 3.41 weeks (Figure 3, Table 2).

b) Life stage composition:

Eggs were the dominant life stage during the 12 week culture period except at week 2 (Figure 4, Table 3). Eggs constituted of 28-35% of the total life stages (Figure 4) at all times, except at week two when larvae dominated (Figure 4, Table 3). The density of eggs and larvae present at any time seemed inversely related to each other. As the number of larvae increases the number of eggs dropped and vice versa. Protonymphs were the least numerous life stage and represented < 6% of the total population at all times (Table 3). Tritonymphs constituted 12-16% of the population during weeks 0-8, but at 10 weeks both the tritonymphs and the larvae dropped to 9% each (Figure 4). Nymphs were the least numerous of all the life stages (Table 3, Figure 4). Both male and female populations grew equally and comprised 13-21% and 14-23%, respectively of the total population. As the cultures

matured and depleted the food supply, the adults became the dominating life stage compared to nymphs and larvae (Table 3, Figure 4).

c) Group 1 and group 2 allergen content:

Der f 1 and Der f 2 allergen contents increased exponentially as the mite population grew. The allergen content was lowest at week zero, where the Der f 1 was 2.1 ± 0.2 and Der f 2 was 1.7 ± 0.2 micrograms per 100 mg of culture material (Table 4, Figure 5). The allergen content was the highest at 12 weeks and was 31.66 ± 9.1 and Der f 2 was 12.55 ± 2.97 (±SEM) micrograms per 100 mg of culture material (Table 4, Figure 5). The amount of Der f 1 became greater than Der f 2 after about 6 weeks. The concentration of Der f 1 increased more rapidly than the concentration of Der f 2 (Figure 6). Accumulation rate constant for Der f 1 was 0.22 and doubling time was 3.14 weeks. Der f 2 had an accumulation rate constant of 0.15 and doubling time of 4.63 weeks (Table 5, Figure 6). Der f 1 for diet A was 1.22 times more than Der f 2 at week zero (Table 5, Figure 7). The highest ratio was at week 8, where Der f 1 was 2.55 times more than Der f 2 concentrations. Der f 1 accumulated at a rate of 12.8% per week more than Der f 2 accumulated (Table 5, Figure 7).

Table 1: Mean *D. farinae* population density (mites per 50 mg of culture material) grown on Diet A. The population density at each time is the average of 5 replicate cultures. Data represents active life stages (larvae, protonymphs, tritonymphs and adults).

Time (Weeks)	0	2	4	6	8	10	12
Average number of mites per 50mg of culture material	631.5	349.4	558.5	1058.3	2270.7	3073.8	3255.7
Std Error of Mean	50.4	32.6	73.0	156.7	323.3	372.3	403.9

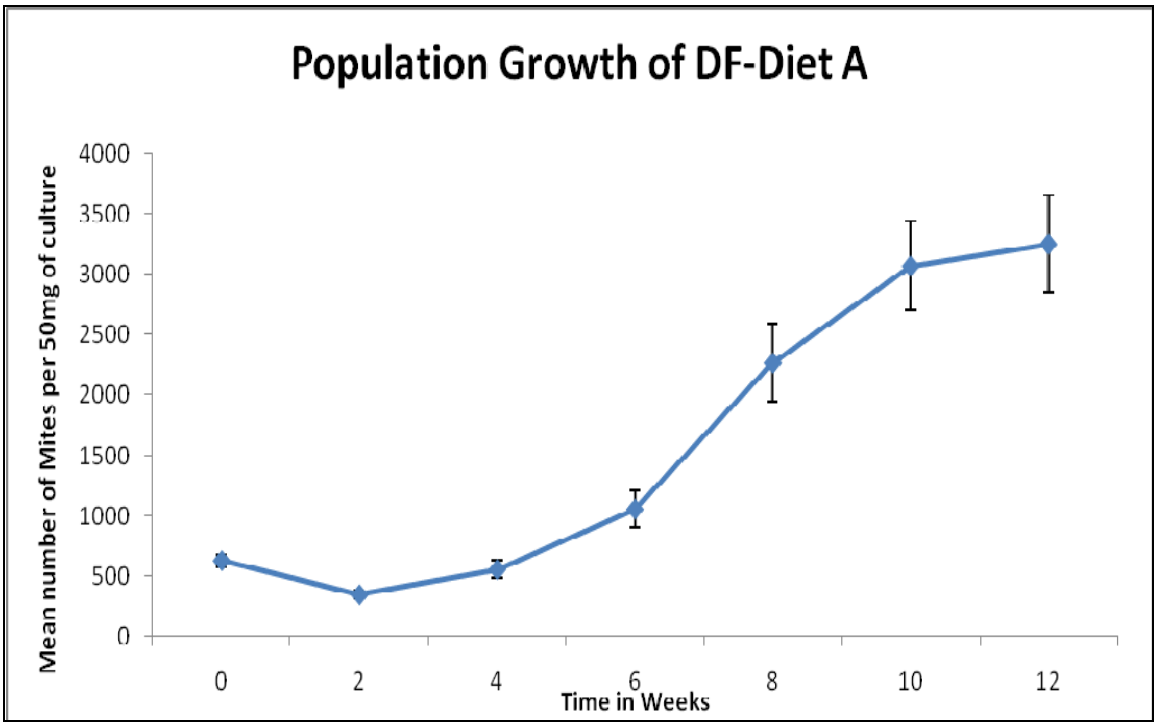

Figure 2: Population growth of *D. farinae* grown on Diet A. Live mites were counted at two week intervals starting from t=0 until the cultures matured and were out of food. Error bars represent ±SEM (Data from Table 1).

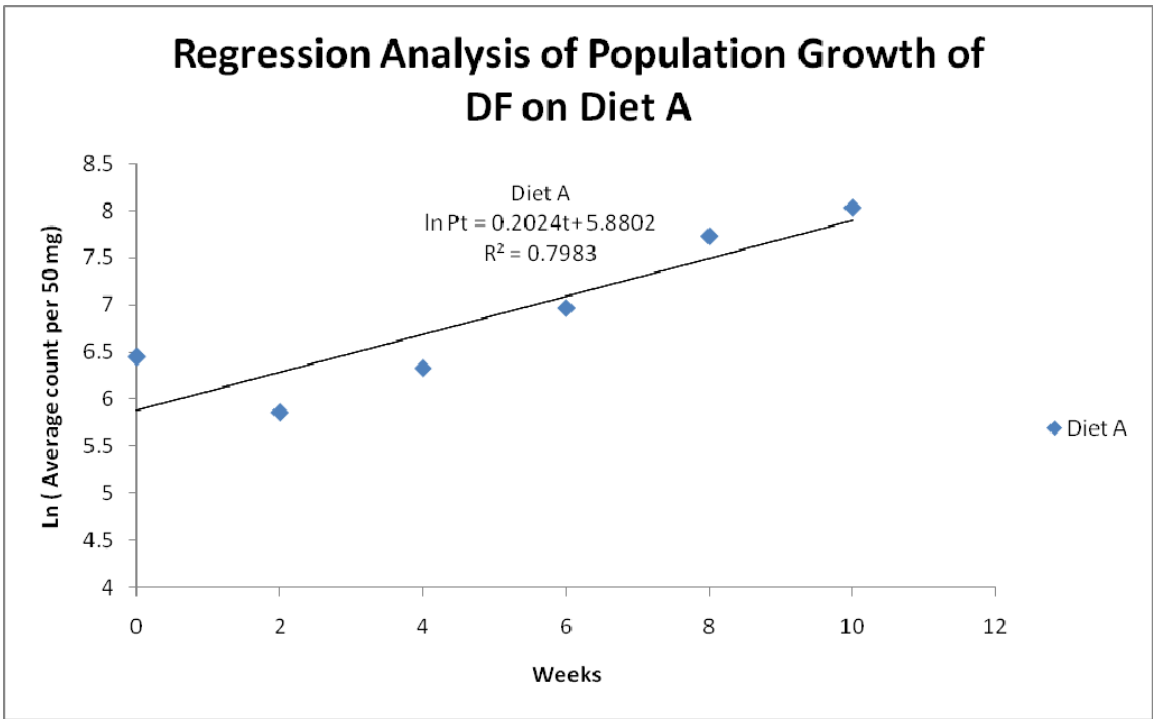

Figure 3: Natural logs of the mean population densities for *D. farinae* grown on diet A. Plots are natural logs of data from Table 1.

Table 2: Correlation coefficient (r^2), growth rate constant (k) and doubling time for *D. farinae* grown on diet A.

Diet	Correlation coefficient (r^2)	Growth rate constant (k /wk)	Doubling time
Diet A	0.7983	0.2024	3.41 wks

Table 3: Life stage composition* of *D. farinae* during population growth on Diet A. Data are mean number (±SEM) of each life stage per 1000 mites examined.

Time	Eggs	Larva	Protonymph	Tritonymph	Male	Female
0	389.7 ±31.1	181.1 ±12.8	17.6 ±4.5	118.1 ±19.9	150.3 ±10.0	143.3 ±24.4
2	189.6 ±22.9	343.5 ±21.9	66.2 ±7.7	122.7 ±18.5	145.6 ±6.4	132.4 ±8.9
4	343.4 ±60.7	216.8 ±23.3	36.3 ±5.8	137.8 ±24.1	123.1 ±14.6	142.7 ±20.9
6	361.9 ±59.9	263.0 ±50.4	7.8 ±2.8	111.5 ±17.0	124.0 ±8.8	131.8 ±10.2
8	291.6 ±31.3	190.9 ±22.5	39.0 ±9.3	158.3 ±15.8	143.3 ±21.5	176.9 ±32.8
10	357.0 ±33.5	89.6 ±8.1	31.8 ±9.6	86.0 ±19.5	209.8 ±21.2	225.8 ±21.7

*At least 250 mites per culture were counted for each culture. Each life stage was extrapolated to a 1000 and then the averages for all the five replicates were taken. Average ±SEM of all the five replicates at each time interval were then calculated.

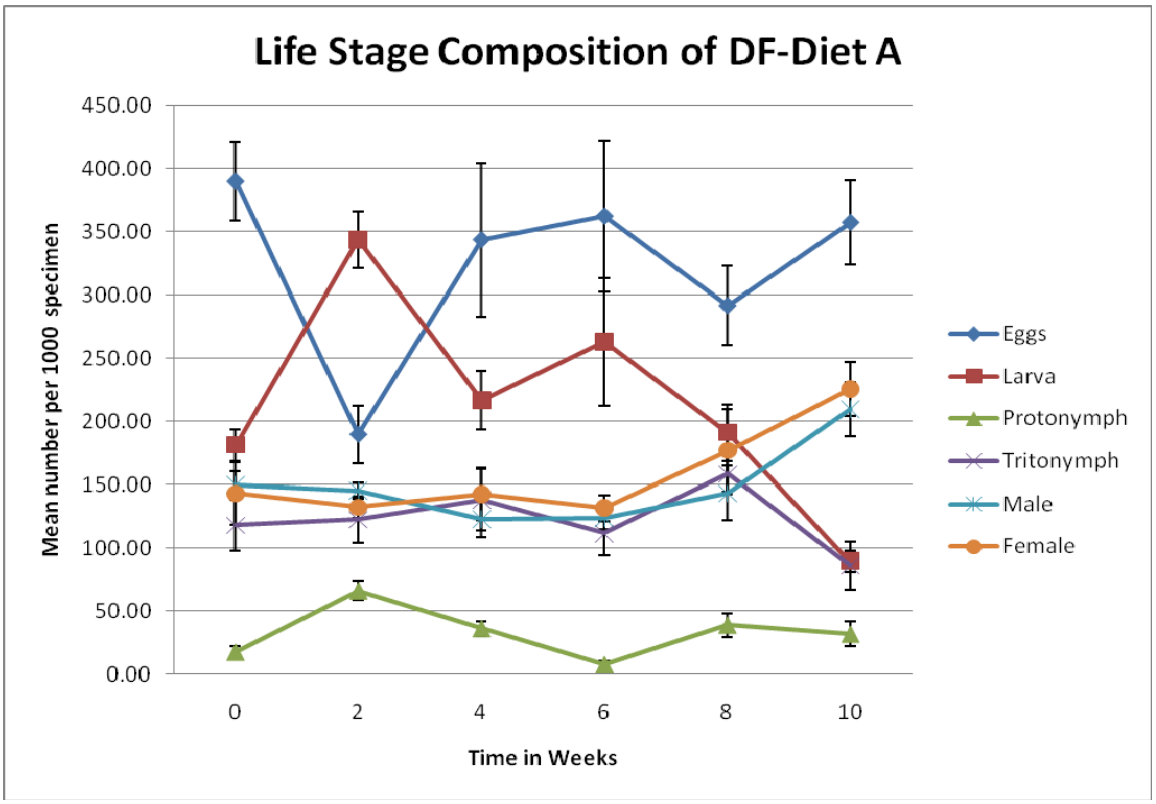

Figure 4: Life stage composition of *D. farinae* grown on Diet A. At least 250 mites (consisting of all life stages) per culture were counted for each culture. Each life stage

was extrapolated to a 1000 and then the averages ±SEM of all five replicates at each time interval were calculated (Data in Table 3).

Table 4: Mean micrograms of Der f 1 and Der f 2 allergen per 100 mg of culture material and their ratio (±SEM) at two week intervals during the population growth of *D. farinae* (shown in fig 1) on diet A.

Weeks	Der f 1	Der f 2	Der f 1/ Der f 2
0	2.10±0.20	1.72±0.23	1.22±0.88
2	2.48±0.17	1.98±0.43	1.26±0.4
4	3.63±0.22	2.27±0.31	1.6±0.69
6	5.41±0.40	2.5±0.24	2.17±1.72
8	8.00±0.55	3.15±0.42	2.55±1.29
10	13.65±0.48	5.66±0.66	2.41±0.73
12	31.64±9.12	12.55±3.0	2.52±3.07

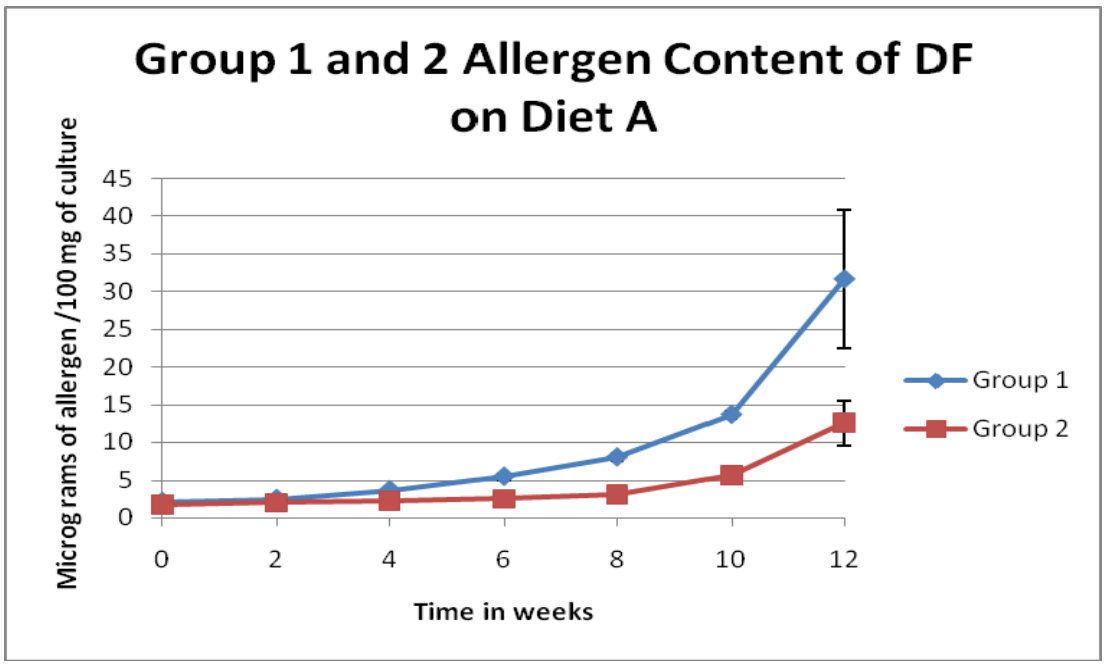

Figure 5: Der f 1 and Der f 2 allergens increase exponentially in cultures of *D. farinae* grown on diet A. After week 4 the amount of Der f 1 produced is greater than the amount of Der f 2 produced. Data are from Table 4.

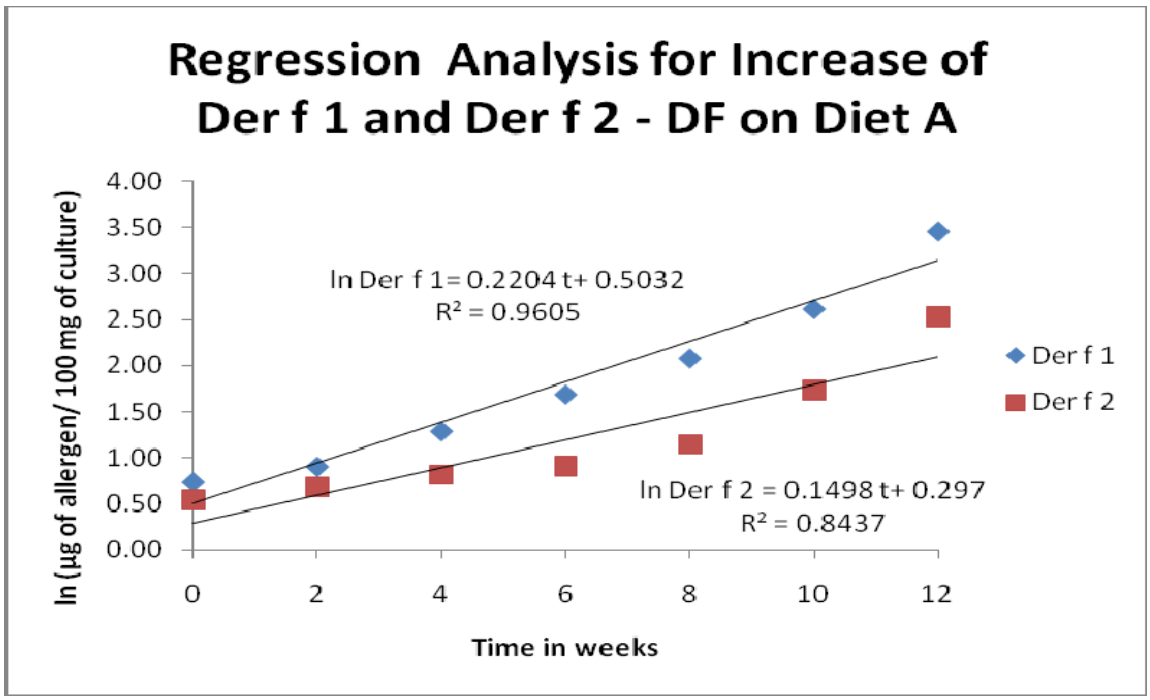

Figure 6: Natural log of the average (±SEM) of the Der f 1 and Der f 2 allergen concentration present in cultures of *D. farinae* grown on Diet A. Data are ln of values from Table 4.

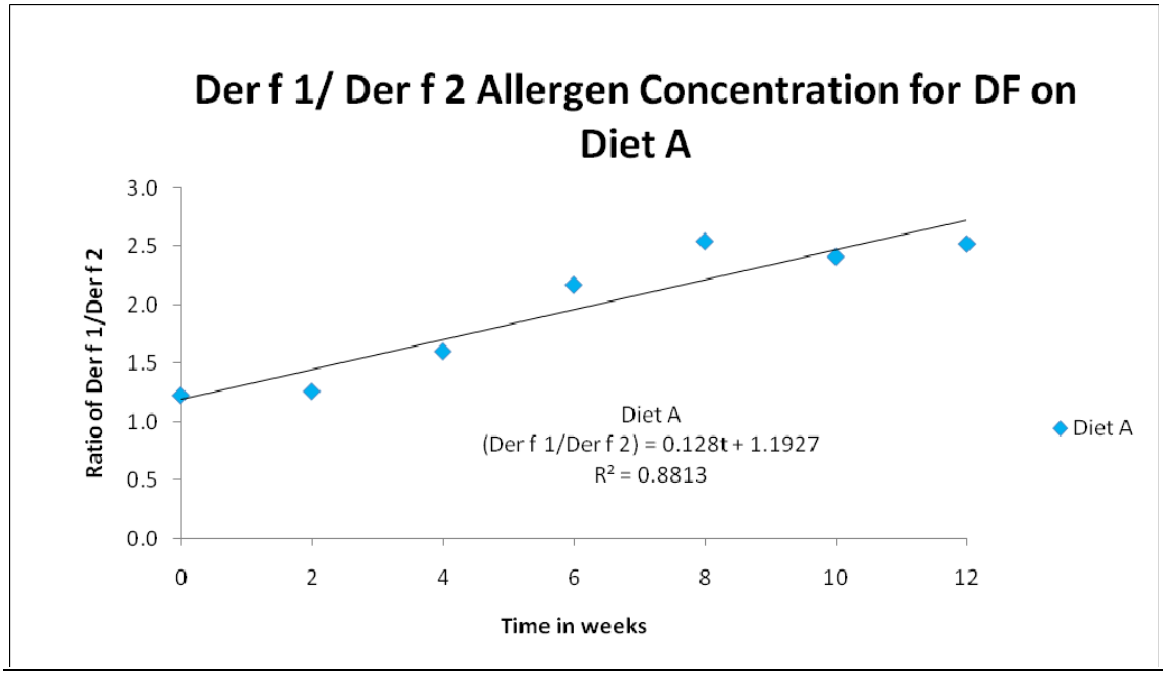

Figure 7: Ratio of Der f 1/ Der f 2 in cultures of *D. farinae* grown on Diet A. Data is from Table 4.

Table 5: Rate constant (k), correlation coefficient (r^2) and doubling time for accumulation of allergens Der f 1 and Der f 2 in *D. farinae* cultures grown on diet A.

Allergen	Correlation coefficient (r^2)	Growth rate constant (k / week)	Doubling time
Der f 1	0.9605	0.2204	3.14 wks
Der f 2	0.8437	0.1498	4.6 wks
Der f 1/ Der f 2	0.8813	0.128	

2. Population size, life stage composition and allergen concentration of Dermatophagoides farinae (DF) cultured on Diet B.

a) Population growth:

Population growth of DF on Diet B showed an exponential increase (Table 6, Figure 8). The initial population density was 146.6 ± 6.8 per 50 mg of culture material at time 0 and it grew until 10 weeks at which time the population was 2143.9 ± 75.5 per 50 mg (Table 6). The population density dropped from 2565.7 ± 151.9 to 2143.9 ± 75 mites from week 8 to week 10. The cultures had matured at week 8 and the population density started to decline by 10 weeks as the diet was depleted. Cultures grew at a rate of 38.5% per week and took 1.79 weeks to double (Table 7, Figure 9).

b) Life stage composition:

Eggs were the dominant life stage during the ten week culture period except at week ten, when adults dominated (Table 8, Figure 10). Eggs constituted of 45% and 70% of the total life stages at week one and two, respectively (Figure 10). The density of eggs and larvae present at any time seemed inversely related to each other. As the number of larvae increased the number of eggs dropped and vice versa. Larvae were the highest in density at week 6 (24.6%). Protonymphs were the least numerous life stage and represented < 2% of the population at all times (Table 8). Tritonymphs constituted 6-14.5% of the population during weeks 0-8, but at 10 weeks both the tritonymphs and the larvae dropped to 12% and 13%, respectively (Table 8). Nymphs were the least numerous of all the life stages except at week 2 and week 10 when the larvae were the lowest in number (Table 8, Figure 10). Both male and female populations grew equally and comprised 7.7-23% and 9.3-20%,

respectively of the total population. As the cultures matured and depleted the food supply, the adults became the dominating life stage compared to nymphs and larvae (Table 8, Figure 10).

c) Group 1 and group 2 allergen content:

Der f 1 and Der f 2 allergen contents also grew exponentially. The allergen contents were lowest at week zero, where the Der f 1 was 1.79 ± 0.50 and Der f 2 was 0.42 ± 0.08 micro grams per 100 mg of culture material, respectively (Table 9, Figure 11). The allergen contents were the highest at 10 weeks. Der f 1 was 39.45 ± 10.55 and Der f 2 was 4.32 ± 0.9 (±SEM) micro grams per 100 mg of culture material (Table 9, Figure 11). The concentration of Der f 1 was greater than the concentration of Der f 2 for the entire 10 week growth period. Concentration of Der f 1 increased at a rate of 31.83% per week and took 2.17 weeks to double (Table 10, Figure 12). By comparison, Der f 2 concentrations increased at a rate of 25% per week and took 2.76 week to double. Therefore, Der f 1 accumulated at a rate of 45.3% more than Der f 2 (Table 10, Figure 13).

Table 6: *D. farinae* population density (mites per 50 mg of culture material) grown on Diet B. The population density at each time is the average of 5 replicate cultures. Data represents active life stages (larvae, protonymphs, tritonymphs and adults).

Time in Weeks	0	2	4	6	8	10
Average Count per 50mg	146.6	291.7	1078.8	2108.0	2565.7	2143.9
SEM	6.9	14.3	33.8	142.4	151.9	75.7

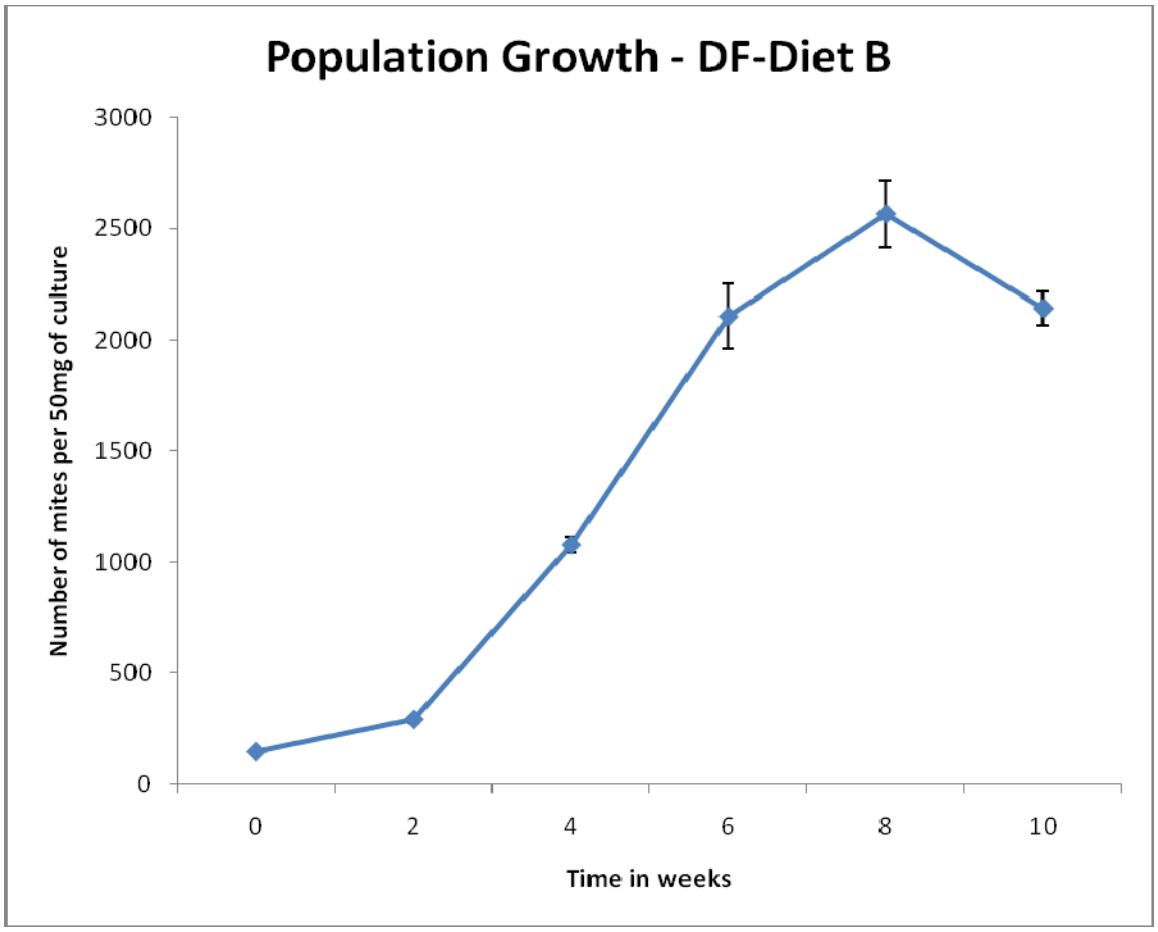

Figure 8. Population growth of *D. farinae* grown on Diet B. Live mites were counted at two week intervals starting from t=0 until the cultures matured and were out of food. Data are represented as Mean ±SEM (Data from Table 6).

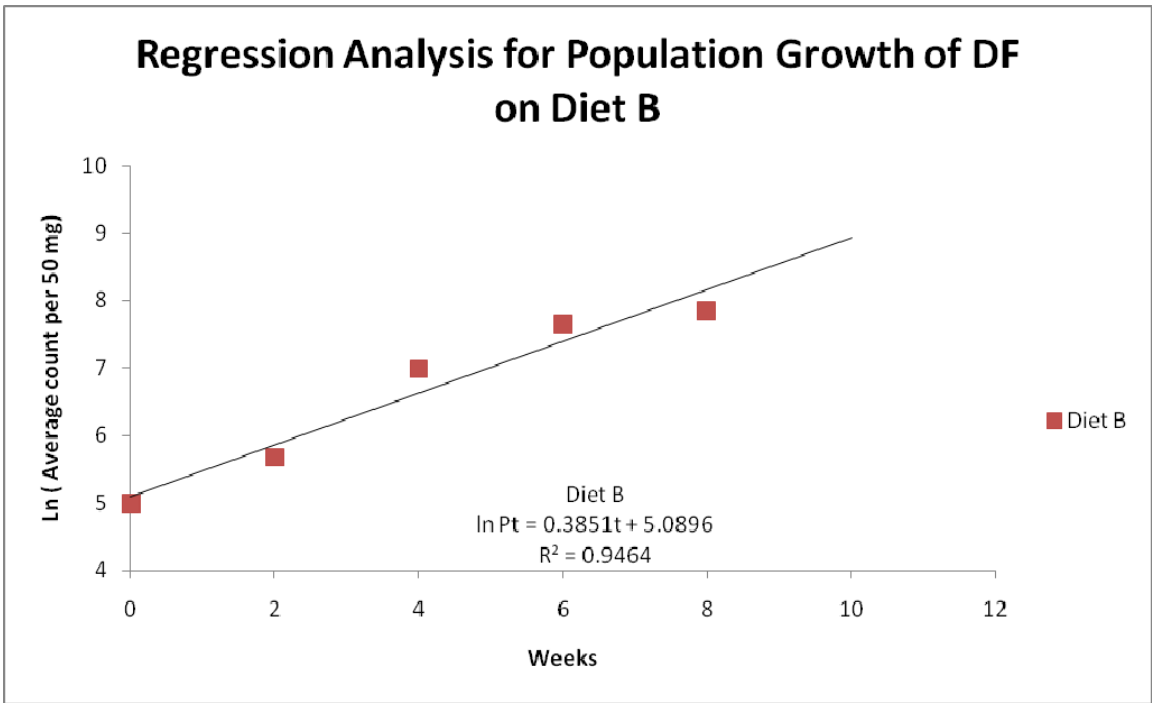

Figure 9: Natural logs of the mean population densities for *D. farinae* grown on diet B. Plots are ln of data in Table 6.

Table 7: Correlation coefficient (r^2), growth rate constant (k) and doubling time for *D. farinae* grown on diet B.

Diet	Correlation coefficient (r^2)	Growth rate constant (k / week)	Doubling time (weeks)
Diet B	0.9464	0.3851	1.79

Table 8: Life stage composition* of *D. farinae* during population growth on Diet B. Data are mean number ±SEM of each life stage per 1000 mites examined.

Time	Eggs	Larva	Protonymph	Tritonymph	Male	Female
0	441.4 ±30.3	142.3 ±17.4	7.8 ± 3.3	116.3 ± 13.1	164.7 ± 22.8	127.5 ± 15.2
2	706.7 ± 12.4	52.5 ± 9.8	8.6 ± 4.7	61.1 ± 4.2	77.9 ± 2.4	93.2 ± 10.8
4	539.4 ± 33.8	174.5 ± 24.5	18.3 ± 3.0	61.3 ± 2.1	105.6 ± 18.6	100.9 ± 17.6
6	353.1 ±23.7	248.3 ±26.7	20.2 ± 6.2	145.3 ± 22.7	133.5 ± 11.6	99.6 ± 12.5
8	408.4 ± 41.0	137.2 ± 21.0	14.1 ± 4.0	75.6 ± 3.9	179.5 ± 12.6	185.2 ± 20.8
10	281.7 ± 30.4	130.8 ± 12.5	16.0 ± 6.1	121.9 ± 12.9	239.8 ± 19.8	209.8 ± 18.8

*At least 250 mites were counted for each culture. Each life stage was extrapolated to a 1000 and then the averages for all the five replicates were taken. Mean ± SEM of all the five replicates at each time interval were then calculated

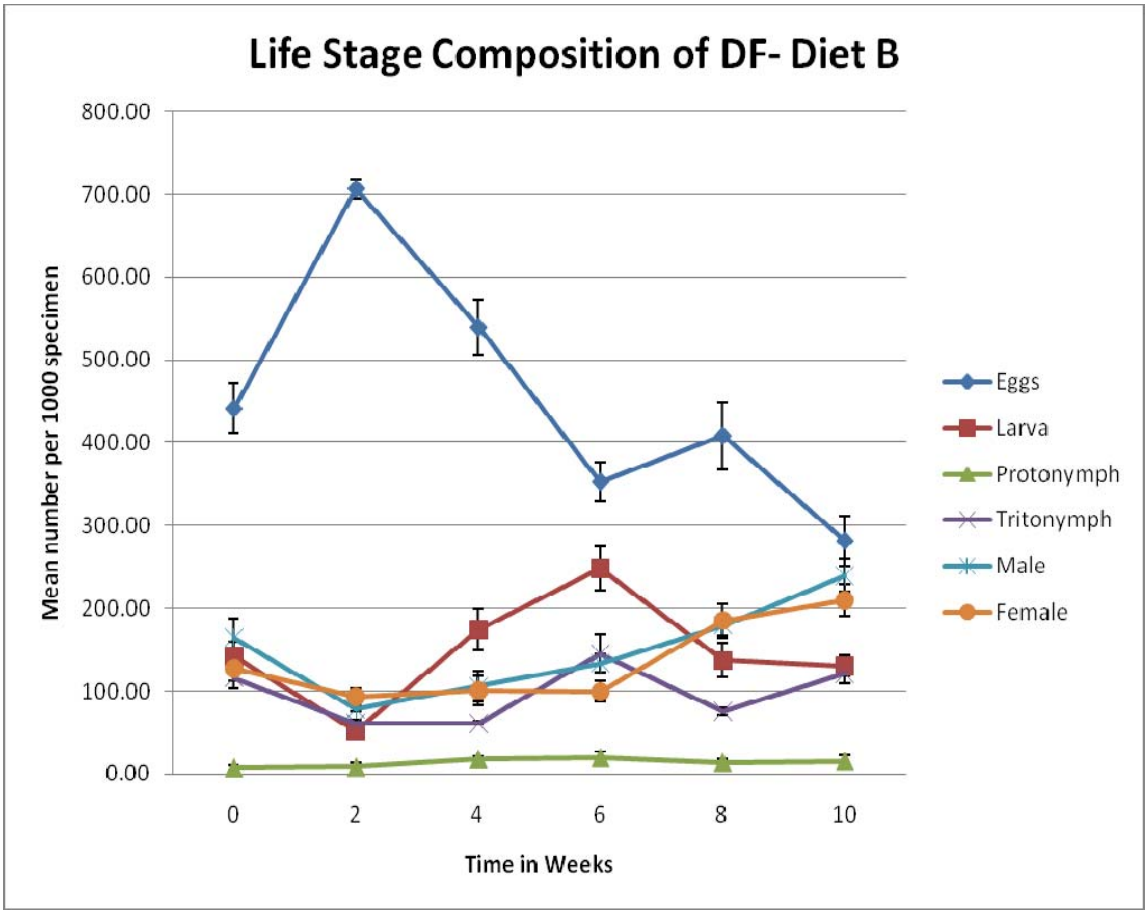

Figure 10: Life stage composition of *D. farinae* grown on Diet B. About 250 or more mites (consisting of all life stages) were counted for each culture. Each life stage was extrapolated to a 1000 and then the Mean ± SEM of all the five replicates at each time interval were then calculated. Plotted data are from Table 8.

Table 9: Mean micrograms of Der f 1 and Der f 2 allergen per 100 mg of culture material and their ratio (±SEM) at two week intervals during the population growth of *D. farinae* (shown in Fig 7) on diet B.

Weeks	Der f 1	Der f 2	Der f 1/ Der f 2
0	1.79±0.51	0.42±.08	4.28±6.57
2	3.25±1.02	0.50±0.08	6.46±12.72
4	6.49±2.02	0.83±0.16	7.85±12.61
6	13.53±3.87	1.54±0.18	8.76±21.35
8	24.71±7.90	2.85±0.58	8.64±13.49
10	39.45±10.54	4.32±0.90	9.13±11.72

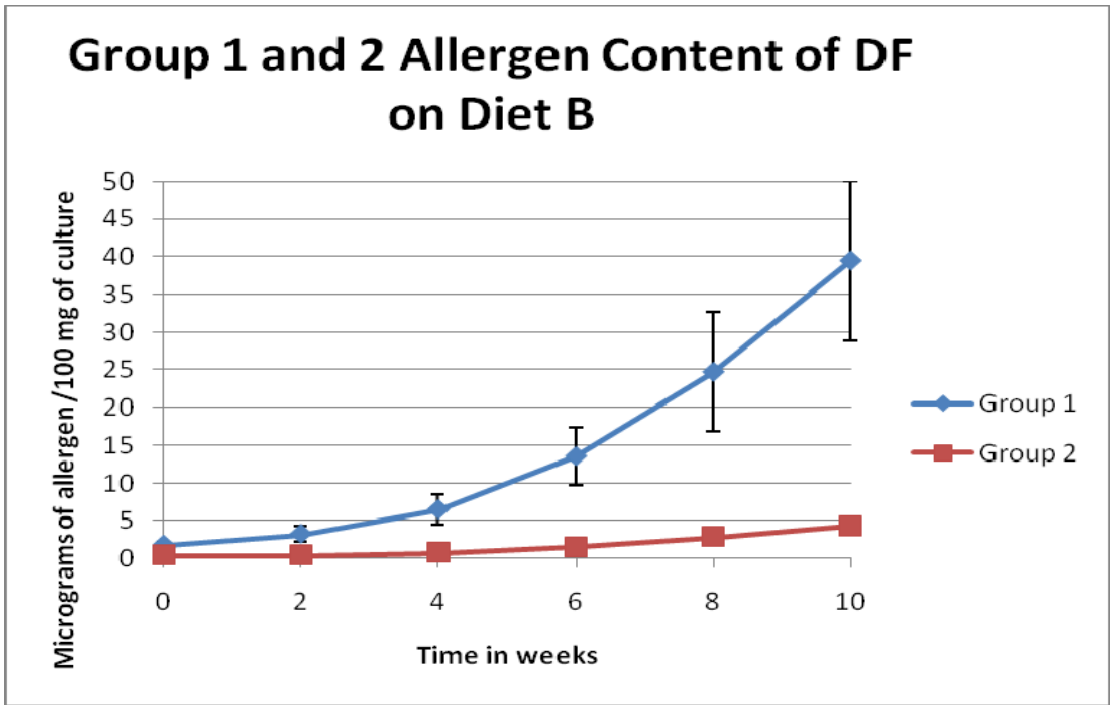

Figure 11: Der f 1 and Der f 2 allergens increase exponentially in cultures of *D. farinae* grown on diet B. The amount of Der f 1 produced is greater than the amount of Der f 2 produced. Data is from Table 9.

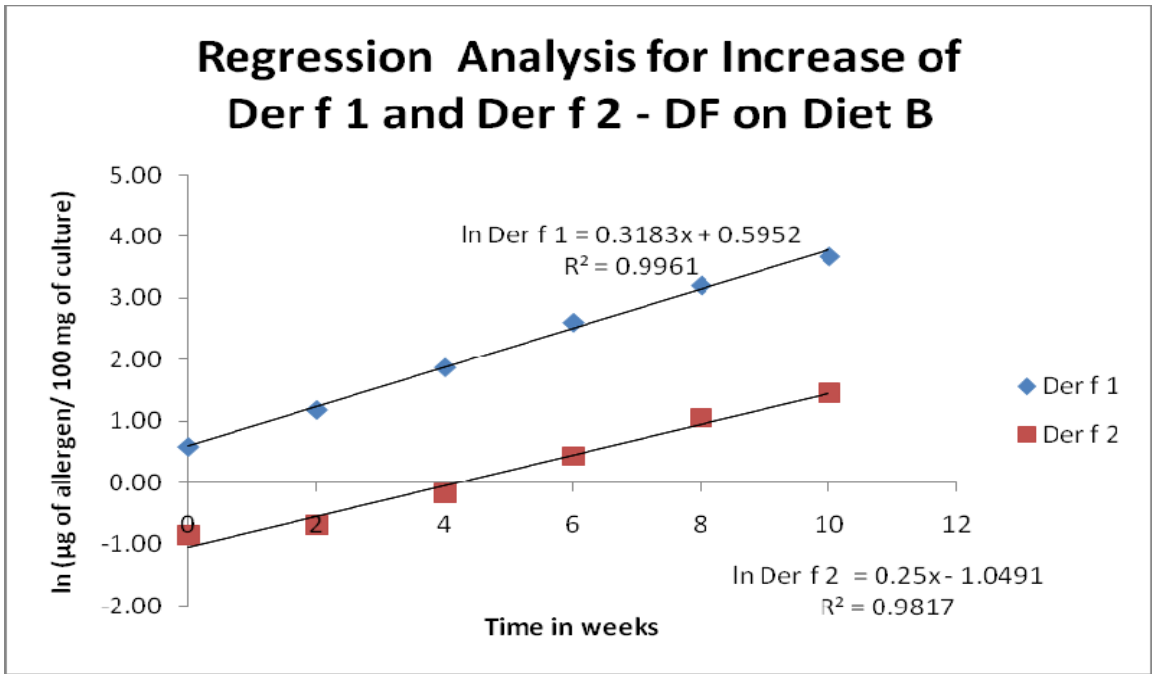

Figure 12: Natural log of the average (±SEM) of the Der f 1 and Der f 2 allergen concentration present in cultures of *D. farinae* grown on Diet B. Plots are ln of data from Table 9.

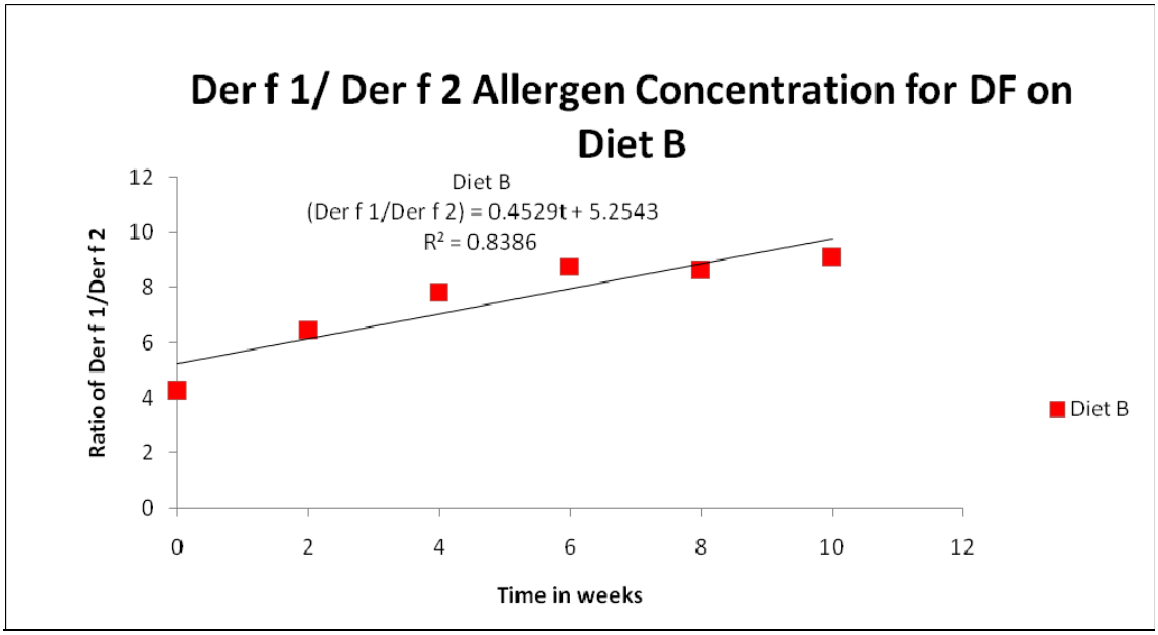

Figure 13: Ratio of Der f 1/ Der f 2 ratio in cultures of *D. farinae* grown on Diet B. Data is from Table 9.

Table 10: Rate constant (k), correlation coefficient (r^2) and doubling time for accumulation of allergens Der f 1 and Der f 2 in *D. farinae* cultures grown on diet B.

Allergen	Correlation coefficient (r^2)	Growth rate constant (k / week)	Doubling time
Der f 1	0.9961	0.3183	2.17 wks
Der f 2	0.9817	0.25	2.76 wks
Der f 1/ Der f 2	0.8368	0.4529	

3. Comparison of population growth and allergen content of D. farinae grown on diet A and diet B.

a) Population growth:

Population growth of *D. farinae* on both diet A and diet B showed an exponential growth (Figure 14 and Table 11). Regression analysis comparisons of the natural logs of the population density averages for each time are represented in Figure 15 and Table 12. *D. farinae* on diet B started with a lesser population than *D. farinae* started on diet A, but overtook the population on diet A at 4 weeks. The diet A population caught up with the diet B population at week 8 when food is becoming limited in both diets. The growth rate constant for diet B was 0.39 while for diet A it was 0.20 per week (Figure 15, Table 13). Doubling time of the population for diet A was 3.41 weeks and diet B was 1.74 weeks (Table 13). Hence *D. farinae* grown on diet B had a shorter doubling time and a faster growth rate than *D. farinae* grown on diet A.

b) Allergens:

Der f 1- *D. farinae* grown on diet B produced more group 1 allergens than it did when grown on diet A (Table 14, Figure 16 and 17). The allergen concentration on both the diets started with values that were similar, 2.10 and 1.79 (µg per 100 mg of culture material). At 4 weeks the allergen concentration of diet B had increased to 1.78 times more than that of diet A, not statistically significant (*p*-value > 0.05) (Table 14). Diet B reached its maximum allergen concentration of 39.45 (µg per 100 mg of culture material) by week 10 but diet A reached its maximum concentration of 31.66 (µg per 100 mg of culture material) at week 12.Therefore, DF cultured on diet A took about 2 weeks longer to reach its maximum group 1

allergen content. Diet A had an accumulation rate of 22.04% per week and took 3.14 wks to double (Table 15, Figure 17). Diet B had an accumulation rate of 31.82% per week and took 2.17 wks to double (Figure 17, Table 15). Although *D. farinae* on diet A took a longer time to reach its maximum allergen concentration than on diet B it was not a statistically significant difference (p-value > 0.05)

Der f 2- The initial concentrations of Der f 2 was 1.72 and 0.42 µg per 100 mg of culture material for diet A and B, respectively (Table 16, Figure 18). During the first 6 weeks of culture diet A had more group 2 allergen than diet B, but at 8 weeks diet B allergen content was about equal to that of diet A. Diet B reached its maximum allergen concentration of 4.3 µg per 100 mg of culture material at 10 weeks and diet A reached its maximum concentration of 12.55 (µg per 100 mg of culture material) at 12 weeks. Although the concentration of group 2 allergens produced by mites on diet A were significantly higher (p-value < 0.05), mites on diet B had a better accumulation rate and doubling time.

Diet A had a Der f 2 accumulation rate of 14.97% per week and took 4.63 wks to double this concentration. Overall, mites on Diet A produced more group 2 allergens than those on diet B. Diet B had an accumulation rate of 25.01% for Der f 2 and took 2.76 wks to double (Table 17, Figure 19).

Der f 1/ Der f 2 ratio- The Der f 1 for diet A culture was 1.22 times more than for Der f 2 cultures at week zero (Table 18, Figure 20). The highest ratio was at week 8, where Der f 1 was 2.55 times more than Der f 2 concentrations. Der f 1

accumulated at a rate of 12.8% per week more than Der f 2 accumulated (Table 18, Figure 21).

The Der f 1 for diet B was 4.28 times more than Der f 2 at week zero (Table 17, Figure 19).The highest ratio was at week 10, where Der f 1 was 9.13 times more than Der f 2 concentration. For diet B, Der f 1 accumulated at a rate of 45.3% per week more than Der f 2 accumulated (Table 18, Figure 20).

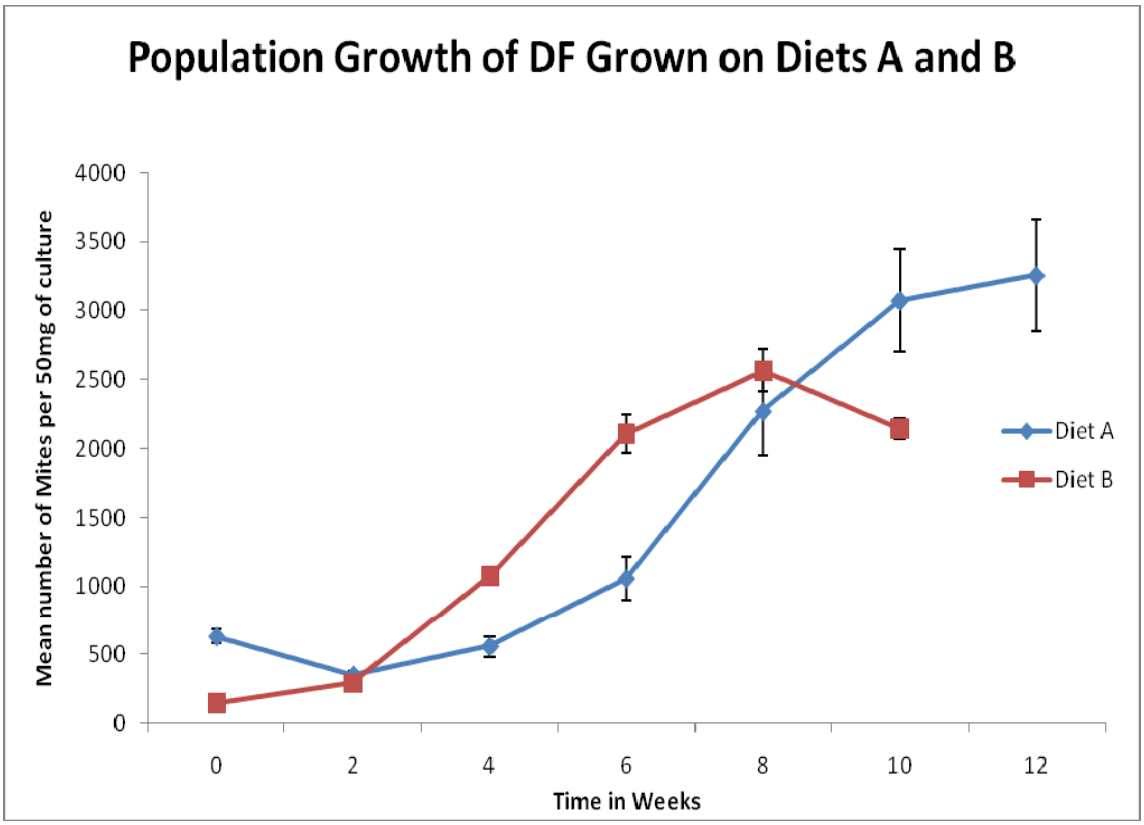

Figure 14: Comparison of the mean population densities for *D. farinae* grown on diets A and B. Data are shown in Table 11**.**

Table 11: *D. farinae* population density (mites per 50mg of culture material) grown on Diet A. The population density at each time is the average of 5 replicate cultures. Data represents active life stages (larvae, protonymphs, tritonymphs and adults).

Time (Week)	0	2	4	6	8	10	12
Diet A	631.5 ±50.4	349.4 ±32.6	558.5 ±73	1058.3 ±156.7	2270.7 ±323.3	3073.8 ±372.3	3255.7 ±403.9
Diet B	146.6 ± 6.9	291.7 ±14.3	1078.8 ±33.8	2108.0 ±142.4	2565.7 ±151.6	2143.9 ± 75.7	

Table 12: Comparison of population growth of *D. farinae* cultured on Diets A and B. Data in Table is the natural log of mite population densities shown in Tables 1 and 6 for diet A and B, respectively.

Time in Weeks	0	2	4	6	8	10
Diet A	6.44	5.85	6.32	6.96	7.72	8.03
Diet B	4.98	5.67	6.98	7.65	7.85	

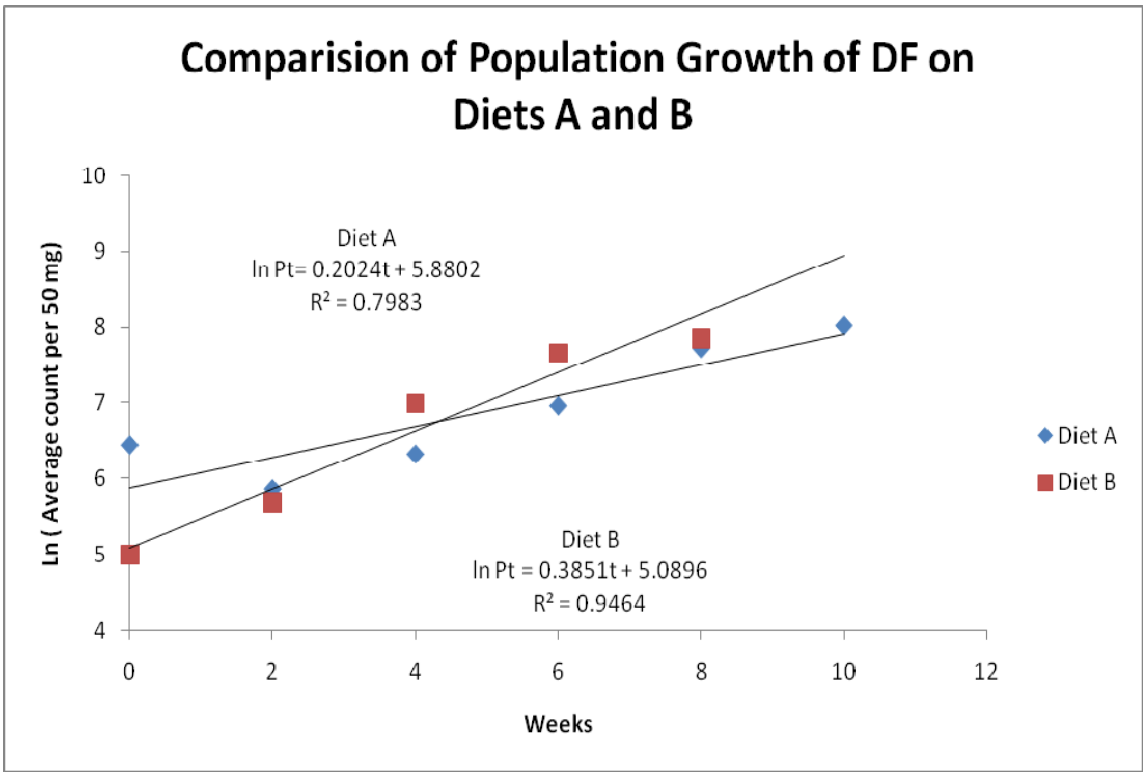

Figure 15: Natural logs of the mean population densities for *D. farinae* grown on diets A and B. Data are shown in Table 12.

Table 13: Comparison of population growth rate constant (k), correlation coefficient (r2) and doubling time for *D. farinae* at 75% RH and room temperature grown on diets A and B.

Diet	Correlation coefficient (r^2)	Growth rate constant (k / week)	Doubling time
Diet A	0.7983	0.2024	3.41 wks
Diet B	0.9464	0.3851	1.79 wks

Table 14: Comparison of Der f 1 allergen concentration during *D. farinae* population growth on diets A and B. Data are mean micrograms of Der f 1 per 100 mg of culture (±SE). Data are from Tables 4 and 9.

Weeks	Diet A	Diet B
0	2.1±0.20	1.79±0.51
2	2.48±0.17	3.25±1.03
4	3.63±0.22	6.49±2.02
6	5.42±0.41	13.53±3.88
8	8.0±0.55	24.71±7.9
10	13.65±0.48	39.45±10.55
12	31.66±9.12	

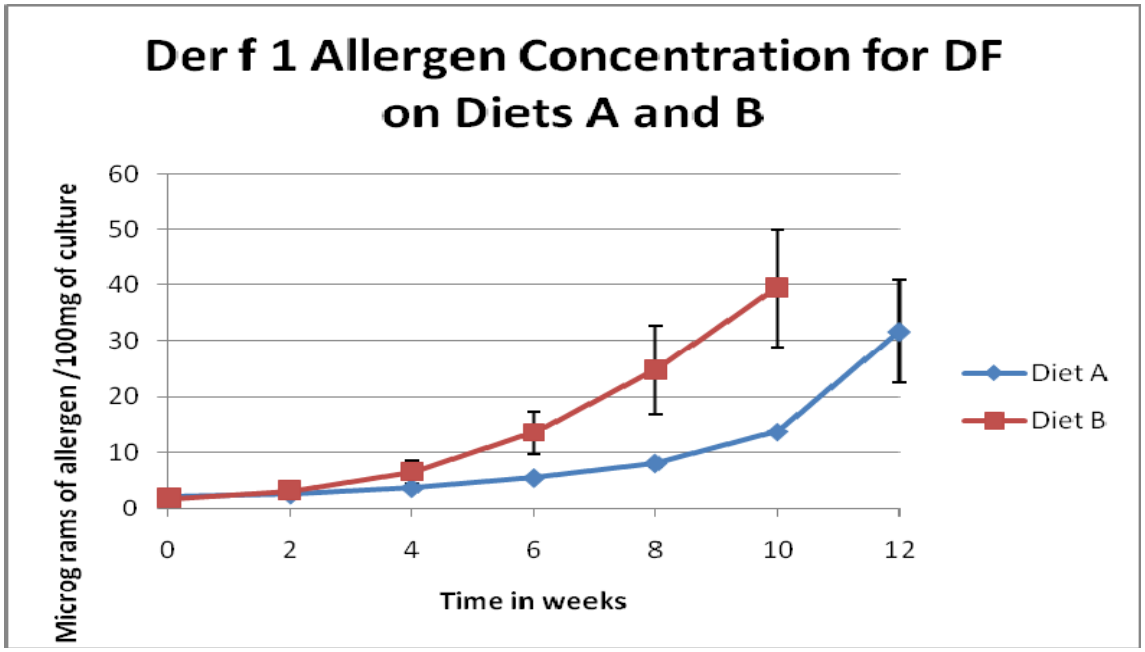

Figure 16: Comparision of Average (± SEM) concentration of Der f 1 allergen present in cultures of *D. farinae* on Diet A and Diet B. Data from Table 14.

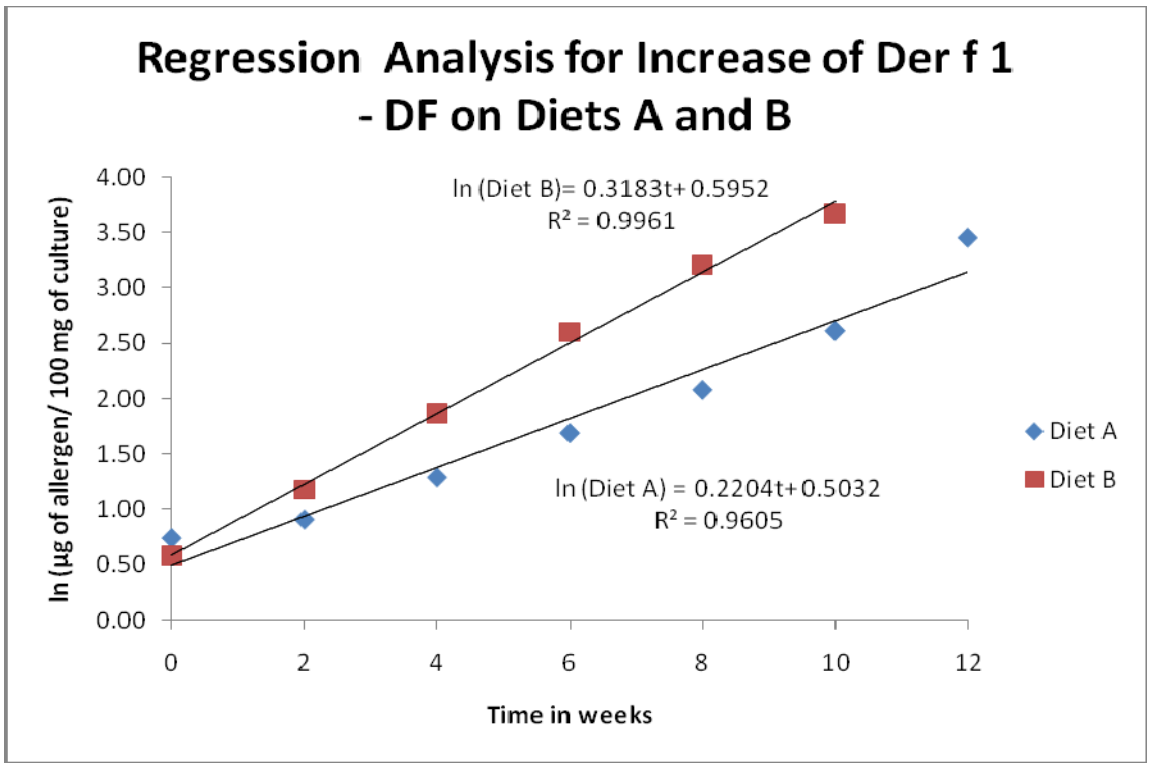

Figure 17: Natural log of the average ± SE of the Der f 1 allergen present in cultures of *D. farinae* on diets A and B. Plots are ln of data shown in Table 14.

Table 15. Der f 1 allergen rate constant (k), correlation coefficient (r^2) and doubling time for *D. farinae* cultured on diets A and B.

Diet	Correlation coefficient (r^2)	Accumulation rate constant (k /wk)	Doubling time (wks)
Diet A	0.9605	0.2204	3.14
Diet B	0.9961	0.3183	2.17

Table 16: Comparison of the averages concentration of Der f 2 for *D. farinae* grown on diet A and B. Data are micrograms ± SEM of Der f 2 allergens per 100 mg of culture.

Weeks	Diet A*	Diet B
0	1.72±0.23	0.42±0.08
2	1.98±0.42	0.50±0.08
4	2.27±0.31	0.83±0.16
6	2.5±0.24	1.54±0.18
8	3.15±0.42	2.86±0.59
10	5.66±0.66	4.32±0.9
12	12.55±2.96	

P-value <0.005, significantly different

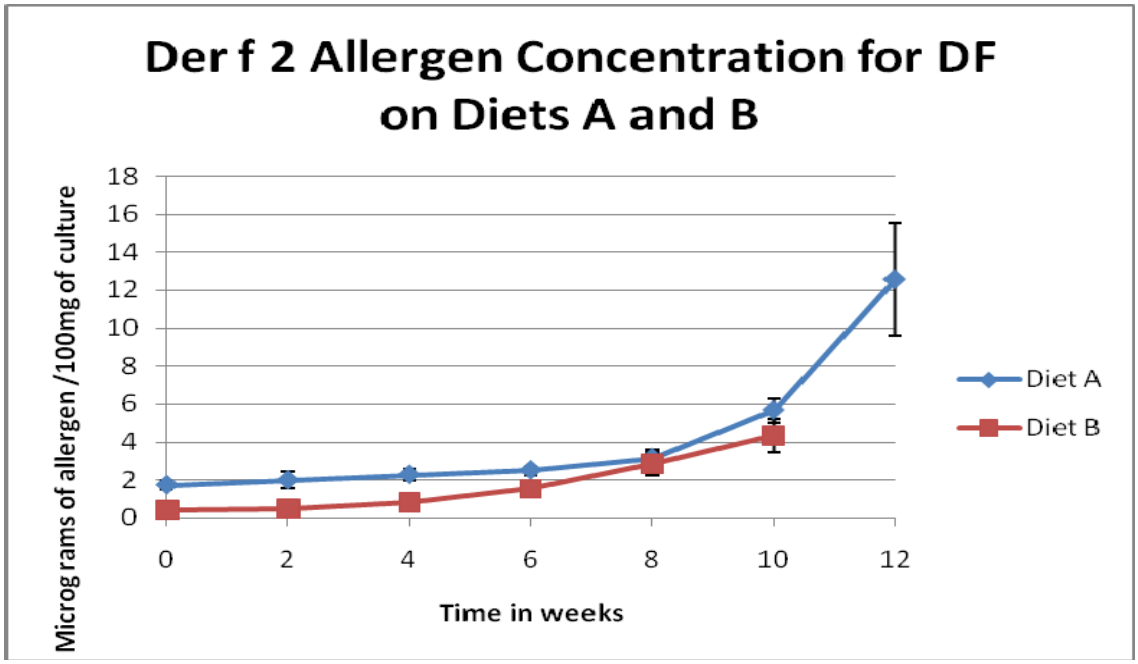

Figure 18: Average ± SEM of Der f 2 allergen present in cultures of *D. farinae* on Diet A and Diet B. Data from Table 16.

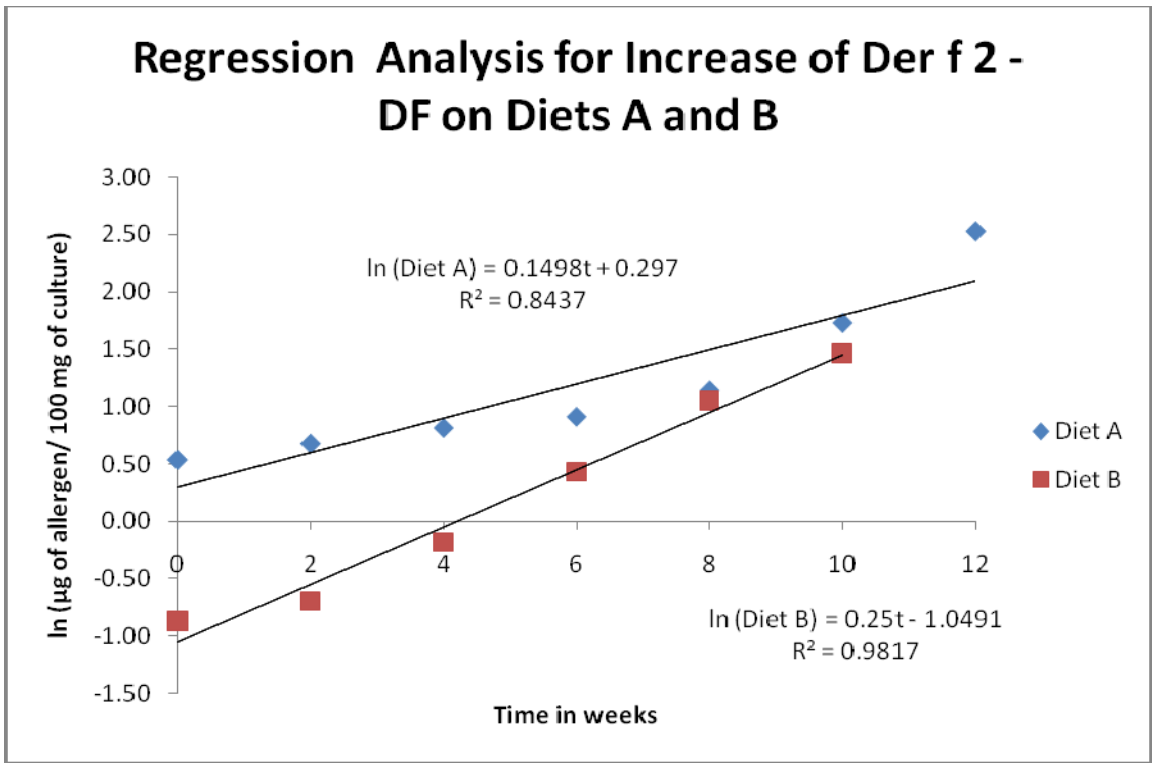

Figure 19: Natural log of the data from Table 16 are plotted for Der f 2 allergen concentration present in cultures of *D. farinae* on diets A and B.

Table 17: Comparison of Der f 2 accumulation rate constant (k), correlation coefficient (r^2) and doubling time in cultures of *D. farinae* grown on diets A and B.

Diet	Correlation coefficient (r^2)	Accumulation rate constant (k / wk)	Doubling time (wks)
Diet A	0.843	0.15	4.63
Diet B	0.9818	0.25	2.76

Table 18: Ratio of Der f 1/Der f 2 for *D. farinae* grown on diet A and diet B. Data is ratio ± SE.

Weeks	Diet A	Diet B
0	1.22±0.88	4.28±6.57
2	1.26±0.4	6.46±12.72
4	1.60±0.69	7.85±12.61
6	2.17±1.72	8.76±21.35
8	2.55±1.29	8.64±13.49
10	2.41±0.73	9.13±11.72
12	2.52±4.14	

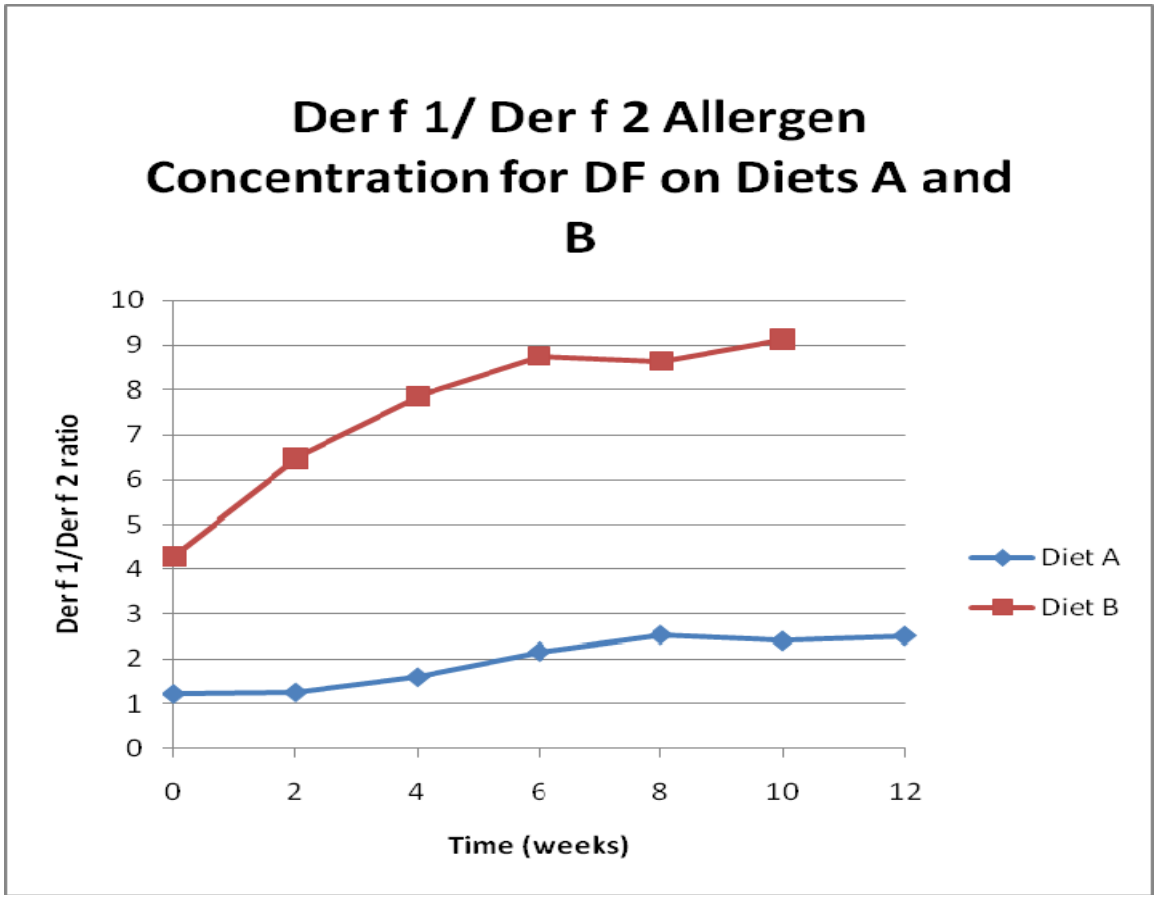

Figure 20: Ratio of Der f 1/ Der f 2 in cultures of *D. farinae* grown on diet A and diet B.Data is from Table 18.

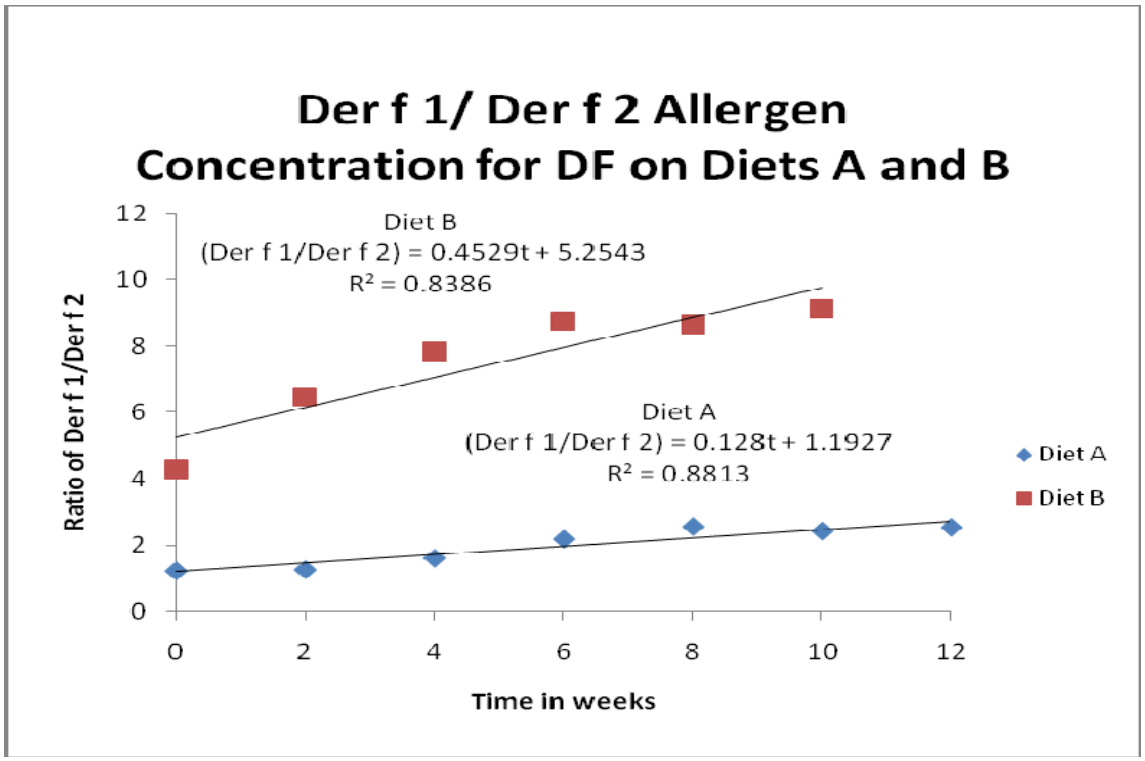

Figure 21: Ratio of Der f 1/ Der f 2 of *D. farinae* on Diet A and Diet B. Data from Table 18 are plotted.

Table 19: Comparison of ratio of Der f 1 to Der f 2 allergen accumulation rate constants (k) and correlation coefficients (r^2).

Diet	Correlation coefficient (r^2)	Accumulation rate constant (k / wk)
Diet A	0.8813	0.128
Diet B	0.8386	0.4529

II. *Dermatophagoides pteronyssinus (DP)*

4. Population size, life stage composition and allergen concentration of *Dermatophagoides pteronyssinus* cultured on diet A.

a) Population growth:

Population growth of DP on Diet A showed an exponential growth (Figure 22). Initial population count was 676.2±116.5 per 50 mg of culture material at time 0 and grew until 12 weeks at which time the population density was 6311.2± 323.7 per 50 mg of cultured material (Table 20). The cultures had matured by 10-12 weeks and population size started to plateau as the diet was depleted. The growth rate of the population was 32% per week and the population doubled in 2.15 weeks (Figure 23, Table 21).

b) Life stage composition:

The eggs, males and the females started out as the dominant life stages at week zero, consisting of 35, 30 and 25% of the life stages respectively (Table 22, Figure 24). The egg population was the highest at week 6 and constituted of 65% of the total life stages (Figure 24). Density of larvae was the highest at week 8 (21.9%). Protonymphs were the least numerous life stage and their density was highest at week 4 constituting of 6.3% of the total life stages. Tritonymph population was the highest at 10 weeks (23.3%) (Table 22). Larvae and the nymphs remained the least numerous of all the life stages (Table 22, Figure 24). Both males and females were the dominant life stages during the 12 week culture period except at week 6 (Table 22, Figure 24). The numbers of males were slightly higher than the number of females (about 45-64 mites) at weeks 0, 2 and 4.

c) Group 1 and group 2 allergen content:

Der p 1 and Der p 2 allergen contents increased exponentially as the mite population grew. The amount of Der p 1 was less than the amount of Der p 2 produced by DP cultured on diet A. The allergen content at week zero was 1.75 ± 0.15 µg per 100 mg of cultured material, the lowest for Der p 1. The Der p 1 allergen concentration was at a maximum of 13.96 ± 1.7 µg, at week 12 (Figure 25, Table 23). The Der p 2 allergen content started out with a higher concentration of 4.52 ± 0.4 µg than Der p 1, at week 0 and was at a maximum level remained at higher concentrations than Der p 1 at all times during the 12 week culture period. Accumulation rate constant of Der p 1 was 0.20 and doubling time was 3.51 weeks. Der p 2 had an accumulation rate of 0.18 and a doubling time of 3.88 weeks (Table 24, Figure 26). Since the amount of Der p 2 allergen concentration was high for diet A, the ratio of Der p 1/ Der p 2 was always below 1. The ratio was highest at week 8, when the Der p 1/ Der p 2 ratio was 0.6 ± 0.39 (Table 23, Figure 27). Group 1 accumulation rate was negative.

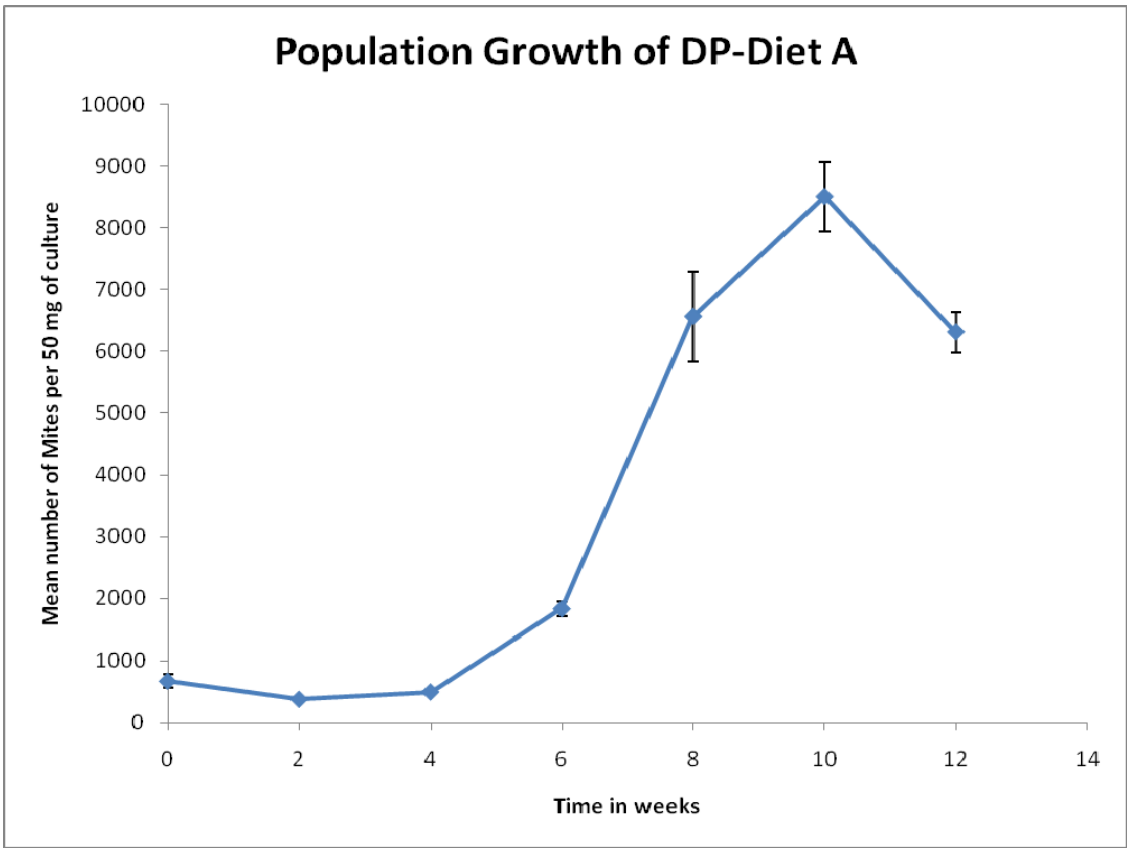

Figure 22: Population growth of *D. pteronyssinus* on Diet A. Live mites were counted at two week intervals starting from t=0 until the cultures matured and were out of food. Error bars represent ±SEM (Data from Table 20).

Table 20: Mean *D. pteronyssinus* population density (mites per 50mg of culture material) grown on Diet A. The population density at each time is the average of 5 replicate cultures. Data represents active life stages (larvae, protonymphs, tritonymphs and adults).

Time (Weeks)	**0**	**2**	**4**	**6**	**8**	**10**	**12**
Average Count per 50mg	676.2	385.1	502.8	1831.2	6560.7	8499.2	6311.2
SEM	116.5	25.9	31.4	119.7	721.1	562.4	323.7

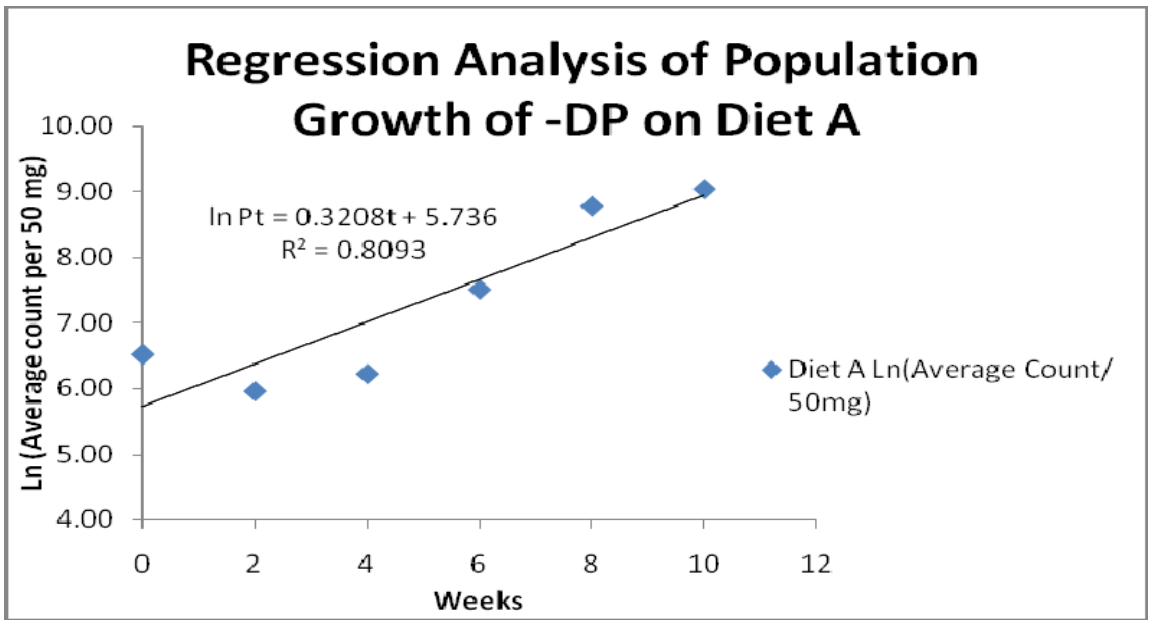

Figure 23: Natural logs of the mean population densities for *D. pteronyssinus* grown on diet A. Plots are natural logs of data from Table 20.

Table 21: Correlation coefficient (r^2), growth rate constant (k) and doubling time for *D .pteronyssinus* grown on diet A.

Diet	Correlation coefficient (r^2)	Growth rate constant (k /wk)	Doubling time
Diet A	0.8093	0.3208	2.15wks

Table 22: Life stage composition* of *D.pteronyssinus* during population growth on Diet A. Data are mean number (±SEM) of each life stage per 1000 mites examined.

Time	Eggs	Larva	Protonymph	Tritonymph	Male	Female
0	355.6 ±25.1	41.1 ±5.1	8.6 ±3.9	28.6 ±7.9	310.6 ±17.41	255.6 ±14.5
2	69.0 ±25.6	53.8 ±23.5	20.2 ±5.0	56.3 ±9.4	432.3 ±18.8	368.4 ±25.3
4	195.2 ±18.5	144.4 ±24.6	63.2 ±13.4	80.0 ±13.9	285.7 ±18.7	231.5 ±22.7
6	656.8 ±21.7	77.3 ±22.1	35.1 ±4.6	33.4 ±3.4	96.0 ±10.9	101.4 ±11.1
8	266.7 ±22.0	219.7 ±10.3	59.5 ±7.3	152.8 ±12.8	157.8 ±6.7	143.5 ±9.1
10	129.7 ±11.7	174.7 ±14.8	61.4 ±4.2	233.6 ±4.9	206.9 ±9.7	193.7 ±7.6

*At least 250 mites per culture were counted for each culture. Each life stage was extrapolated to a 1000 and then the averages for all the five replicates were taken. Average ± SEM of all the five replicates at each time interval were then calculated.

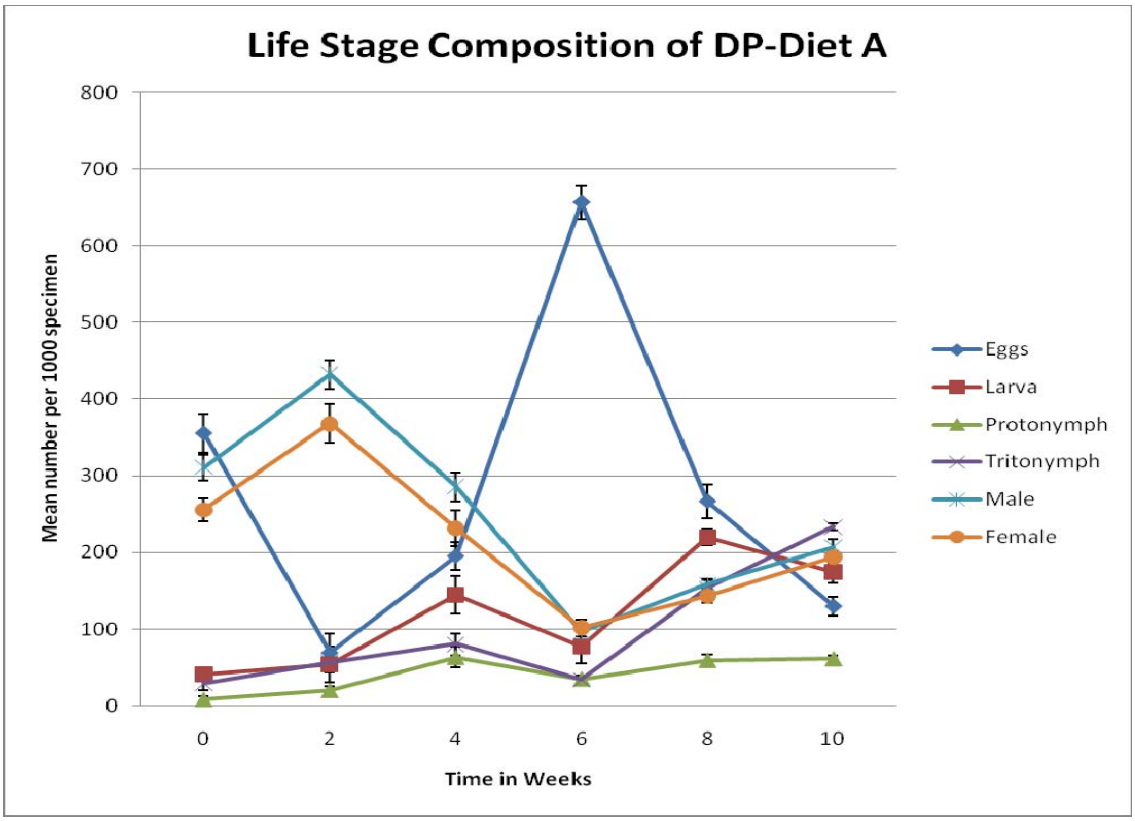

Figure 24: Life stage composition of *D.pteronyssinus* grown on Diet A. At least 250 mites (consisting of all life stages) per culture were counted for each culture. Each life

stage was extrapolated to a 1000 and then the averages ±SEM of all five replicates at each time interval were calculated (Data in Table 22).

Table 23: Mean micrograms of Der p 1 and Der p 2 allergen per 100 mg of culture material and their ratio (±SEM) at two week intervals during the population growth of *D.pteronyssinus* (shown in fig 21) on diet A.

Weeks	Der p 1	Der p 2	Der p 1/ Der p 2
0	1.75±0.15	4.52±0.4	0.39±0.37
2	2.13±0.09	4.16±0.41	0.51±0.21
4	3.03±0.12	5.56±0.56	0.54±0.21
6	4.97±0.64	9.45±1.43	0.53±0.45
8	7.74±0.56	12.83±1.45	0.60±0.39
10	11.43±1.15	24.94±2.22	0.46±0.52
12	13.96±1.7	34.89±6.1	0.4±0.28

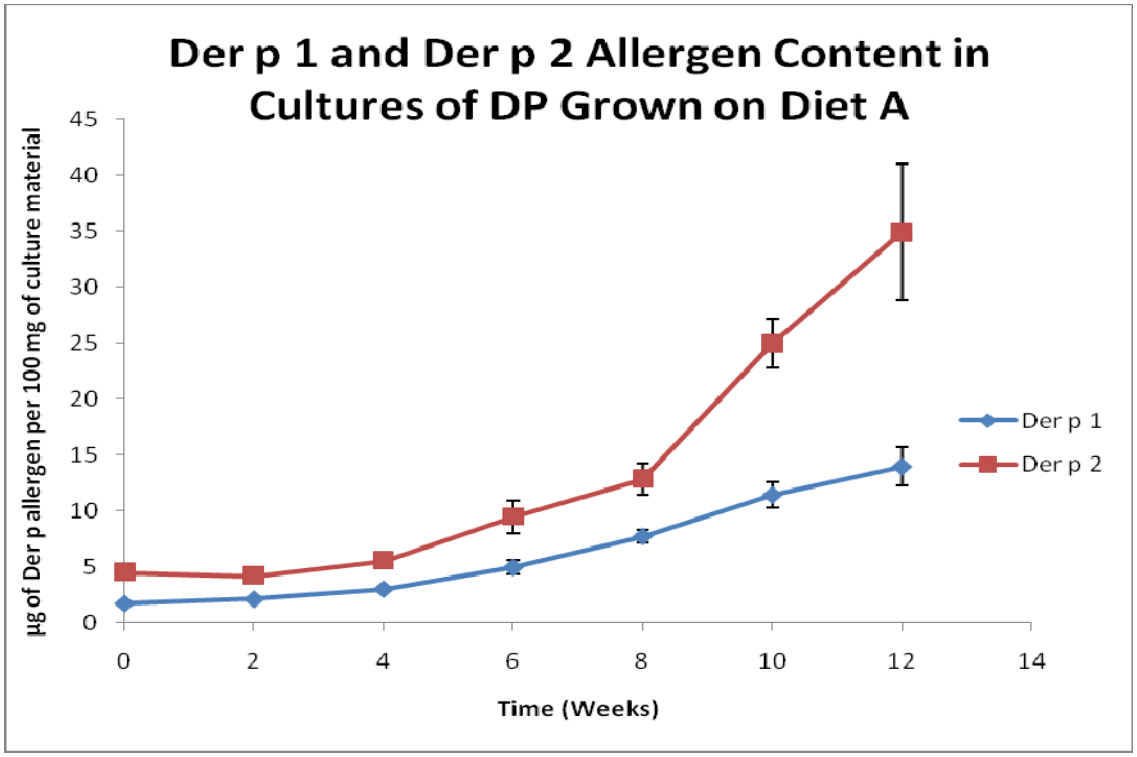

Figure 25: Der p 1 and Der p 2 allergens increase exponentially in cultures of *D.pteronyssinus* grown on diet A. The amount of Der p 2 produced is greater than the amount of Der p 1 produced. Data are from Table 23.

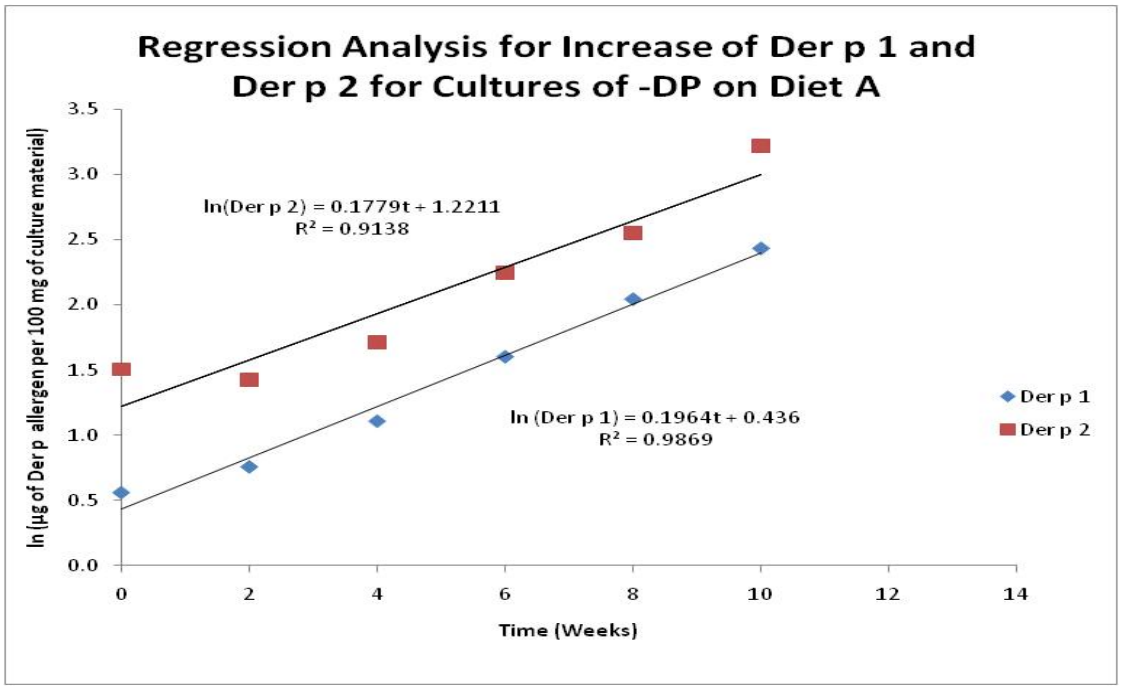

Figure 26: Natural log of the average (±SEM) of the Der p 1 and Der p 2 allergen concentration present in cultures of *D. pteronyssinus* grown on Diet A. Data are ln of values from Table 23.

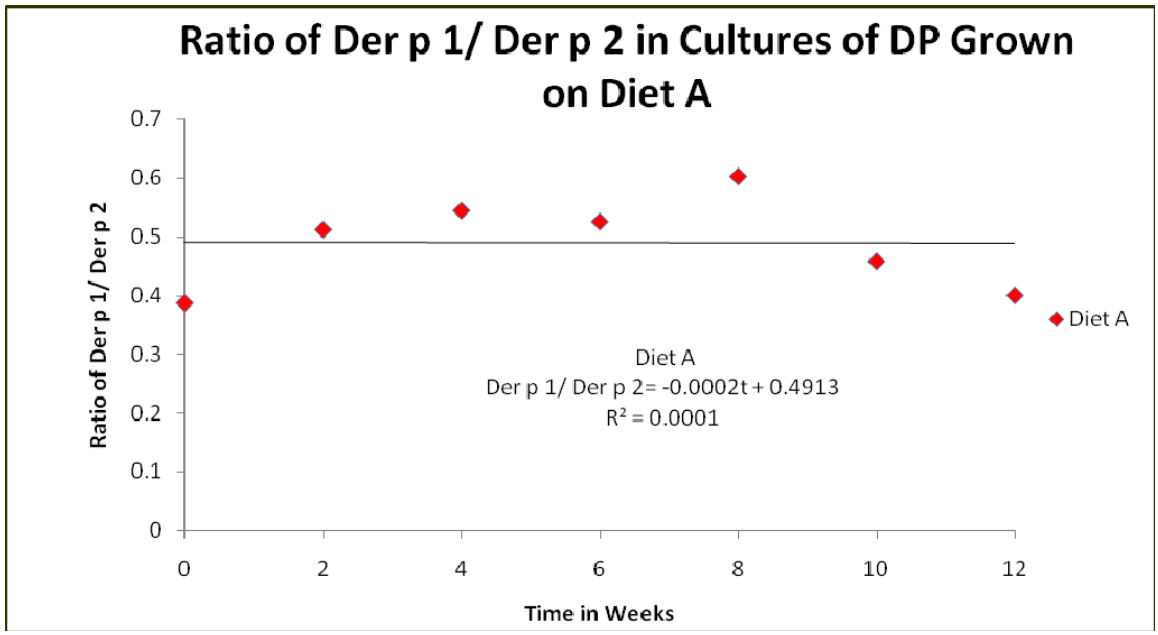

Figure 27 : Ratio of Der p 1/ Der p 2 in cultures of *D. pteronyssinus* grown on Diet A. Data are values from Table 23.

Table 24: Rate constant (k), correlation coefficient (r^2) and doubling time for accumulation of allergens Der p 1 and Der p 2 in *D. pteronyssinus* cultures grown on diet A.

Allergen	Correlation coefficient (r^2)	Accumulation rate constant (k / week)	Doubling time
Der p 1	0.9869	0.1964	3.51 wks
Der p 2	0.9138	0.1779	3.88 wks
Der p 1/ Der p 2	0.0001	-0.0002	

5. Population size, life stage composition and allergen concentration of Dermatophagoides pteronyssinus (DP) cultured on Diet B.

a) Population growth:

Population growth of DP on Diet B showed an exponential growth (Figure 28, Table 25). The initial population density was 305.95±15.7 per 50 mg of culture material at time 0 and it grew until 12 weeks at which the population density was 3319.32±20 per 50 mg of culture material (Table 19). The cultures had matured at week 10 and started to decline by 12 weeks as the diet was depleted. Cultures grew at a rate of 48.4% and took 1.42 weeks to double the population (Figure 29, Table 26).

b) Life stage composition:

The eggs were the dominant life stage thought the growth of the culture making up at least 50% of the population until week 10 when it dropped to 40% (Table 27, Figure 30). The density of eggs peaked at week 6 and consisted of 59.5% of the total life stages (Figure 30). Larvae were the highest in density at week 8 (25.2%). Protonymph were the least numerous population at all times, at week 10 their population density was the highest consisting of 8.1% of the total life stages (Table 27). Tritonymph population was the highest at 10 weeks and consisted of 17.7% of the total population (Table 27). Larvae and the nymphs remained the least in number when compared to all the life stages. The male and female population distribution was equal and was the highest at week 4 and was, 14.1% for males and 13.3% for females (Table 27, Figure 30).

c) Group 1 and group 2 allergen content:

Der p 1 and Der p 2 allergen contents also grew exponentially. The allergen contents were lowest at week zero, where the Der p 1 was 1.38±0.73 and Der p 2 was 1.8±0.28 µg per 100 mg of culture material, respectively (Table 28, Figure 31). The allergen contents were the highest at week 14. Der p 1 was 38.76±1.77 and Der p 2 was 15.38±0.94 µg per 100 mg of culture material. Group 1 and group 2 allergens started out at almost equal concentrations until week 2 after which group 1 allergens started accumulating at higher concentrations. Der p 1 accumulated at a rate of 26% per week and took 2.66 wks to double where as Der p 2 accumulated at a rate of 18.5% per week and took 3.71 weeks to double in concentration (Figure 32, Table 29). Therefore, Der p 1 accumulated at a rate of 10.2% more than Der p 2 (Figure 33, Table 29).

Table 25: Mean *D.pteronyssinus* population density (mites per 50mg of culture material) grown on Diet A. The population density at each time is the average of 5 replicate cultures. Data represents active life stages (larvae, protonymphs, tritonymphs and adults).

Time in Weeks	**0**	**2**	**4**	**6**	**8**	**10**	**12**
Average Count per 50 mg of culture	305.95	555.75	986.17	1147.08	2852.15	3300.43	3319.32
SEM	15.71	24.96	45.64	27.66	94.10	121.95	207.04

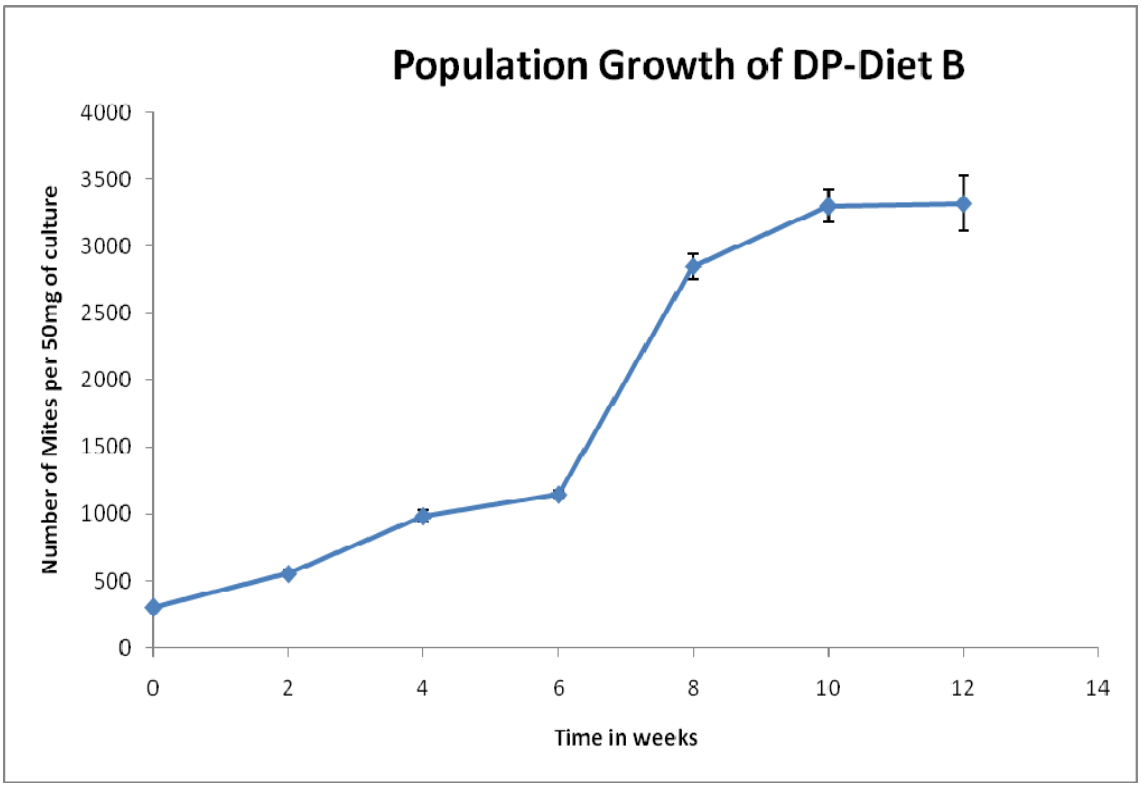

Figure 28: Population growth of D.pteronyssinus on Diet B. Live mites were counted at two week intervals starting from t=0 until the cultures matured and was out of food. Data is represented as Mean ±SEM (Data from Table 25).

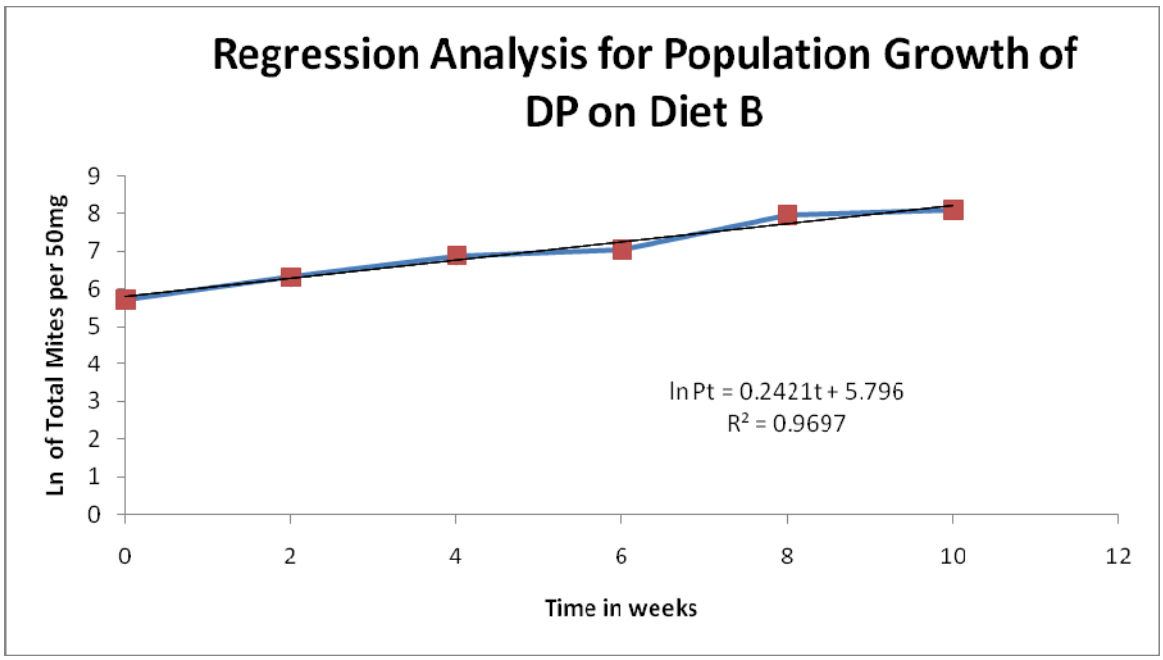

Figure 29. Natural logs of the mean population densities for *D. pteronyssinus* grown on diet B. Plots are ln of data in Table 25.

Table 26: Correlation coefficient (r^2), growth rate constant (k) and doubling time (t_2) for *D. pteronyssinus* grown on diet B.

Diet	Correlation coefficient (r^2)	Growth rate constant (k / week)	Doubling time
Diet B	0.9697	0.2421	2.85 wks

Table 27: Life stage composition* of *D.pteronyssinus* during population growth on Diet B. Data are mean number ±SEM of each life stage per 1000 mites examined.

Time (weeks)	Eggs	Larva	Protonymph	Tritonymph	Male	Female
0	505 ±30.6	252.7 ±41.4	6.4 ±3.3	20.3 ±4.2	117.6 ±21.1	98.1 ±15.6
2	568.9 ±31	121.7 ±11.3	47.9 ±6.9	89.1 ±14.9	92.2 ±9.4	80.3 ±8.4
4	479.3 ±26.6	101 ±13.6	14.3 ±8.1	130.9 ±7.2	141.3 ±18.5	133.2 ±21.1
6	595.0 ±26.2	57 ±9.4	26.6 ±7.3	55.5 ±3.4	133.8 ±7.8	132.2 ±17.6
8	488.5 ±30	195 ±22.7	20 ±5.4	99 ±14.3	111.7 ±6.7	85.8 ±10.6
10	365.1 ±21.2	145.2 ±11.1	81.4 ±513.9	177.3 ±4.9	139.4 ±11.6	91.6 ±16.3

*At least 250 mites per culture were counted for each culture. Each life stage was extrapolated to a 1000 and then the averages for all the five replicates were taken. Average ± SEM of all the five replicates at each time interval were then calculated.

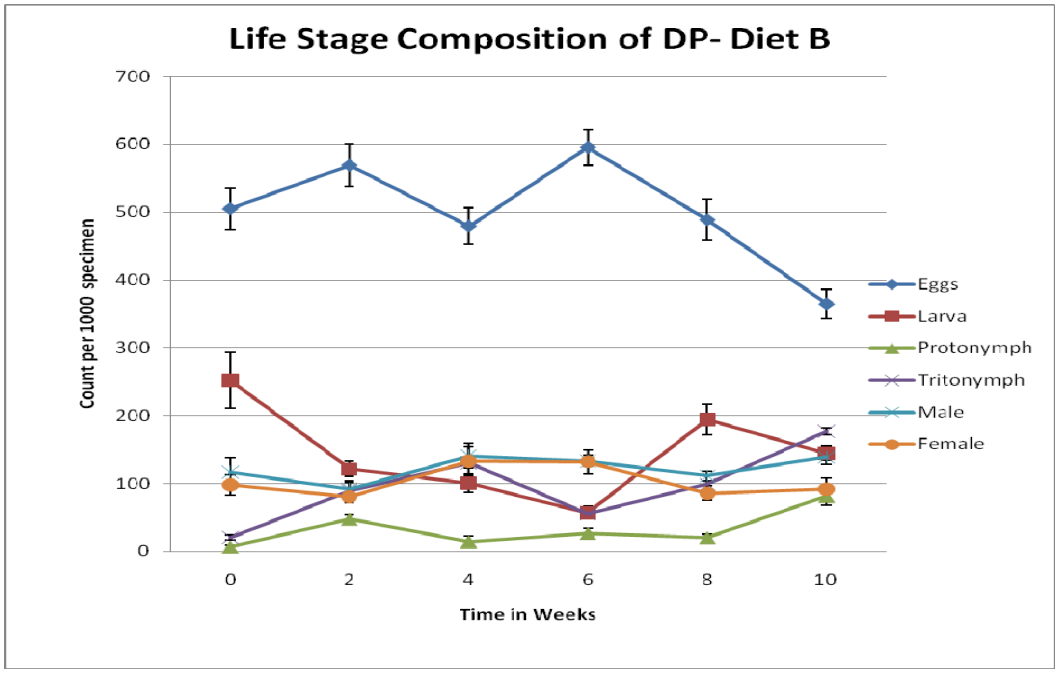

Figure 30: Life stage composition of *D. pteronyssinus* grown on diet B. About 250 or more mites (consisting of all life stages) were counted for each culture. Each life stage was extrapolated to a 1000 and then the Mean ± SEM of all the five replicates at each time interval were then calculated. Plotted data are from Table 27.

Table 28: Mean micrograms of Der p 1 and Der p 2 allergen per 100 mg of culture material and their ratio (±SEM) at two week intervals during the population growth of *D .pteronyssinus* (shown in Fig 21) on diet B.

Weeks	Der p 1	Der p 2	Der p 1/ Der p 2
0	1.38±0.73	1.8±0.28	0.77±2.65
2	2.28±0.11	2±0.11	1.14±1.07
4	4.35±0.41	2.33±0.24	1.87±1.72
6	7.11±0.51	3.69±0.09	1.93±5.48
8	11.22±1.12	5.26±0.77	2.13±1.46
10	20.23±2.16	10.39±1.42	1.95±1.52
12	30.06±2.42	14.79±1.54	2.03±1.57
14	38.76±1.77	15.38±0.94	2.52±1.88

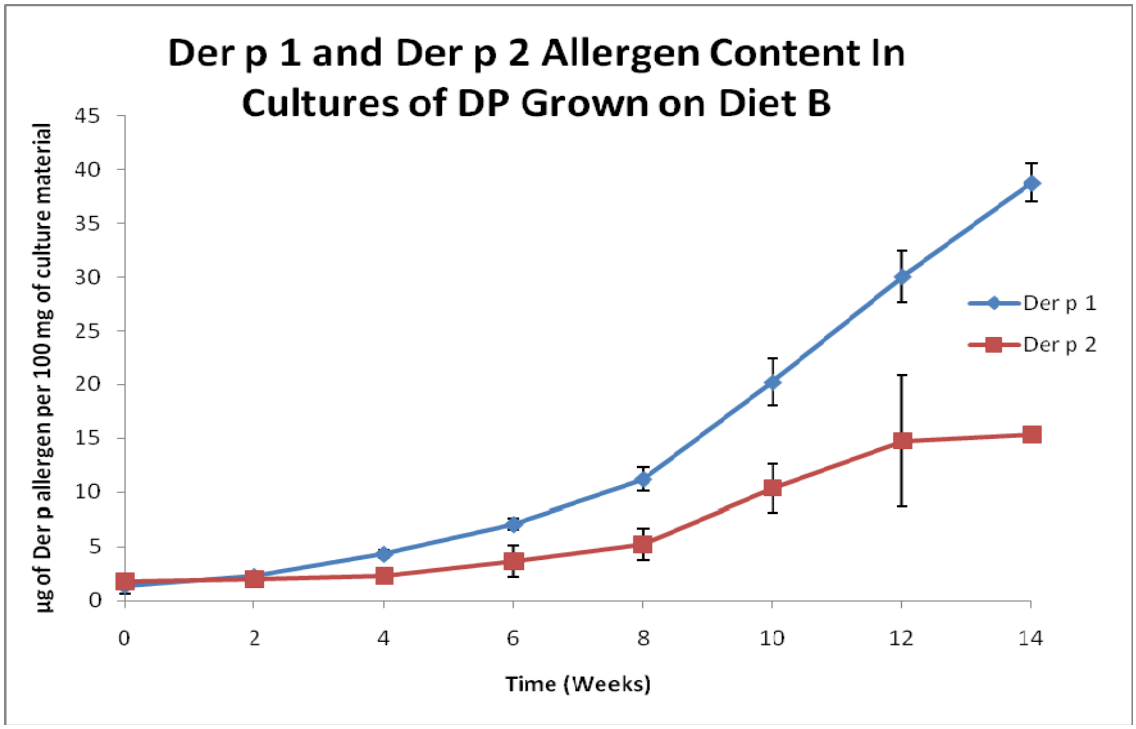

Figure 31: Der p 1 and Der p 2 allergens increase exponentially in cultures of D.pteronyssinus grown on diet B. The amount of Der p 1 produced is greater than the amount of Der p 2 produced. Data are from Table 28.

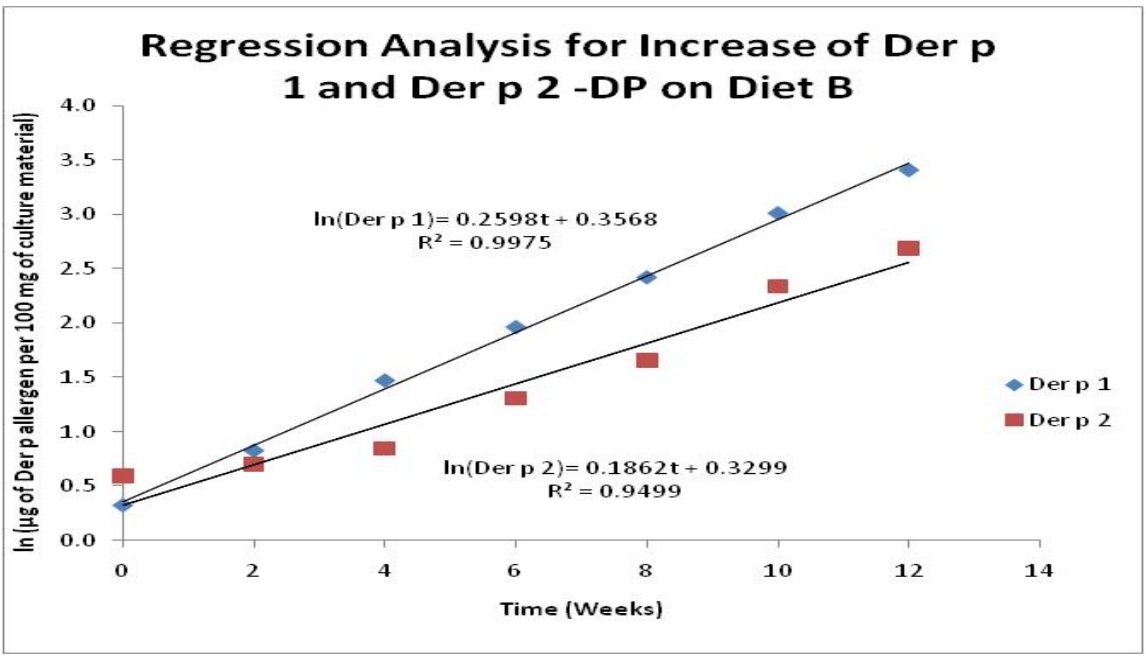

Figure 32: Natural log of the average (± SEM) of the Der p 1 and Der p 2 allergen concentrations present in cultures of D. pteronyssinus grown on diet B. Plots are ln of data from Table 28.

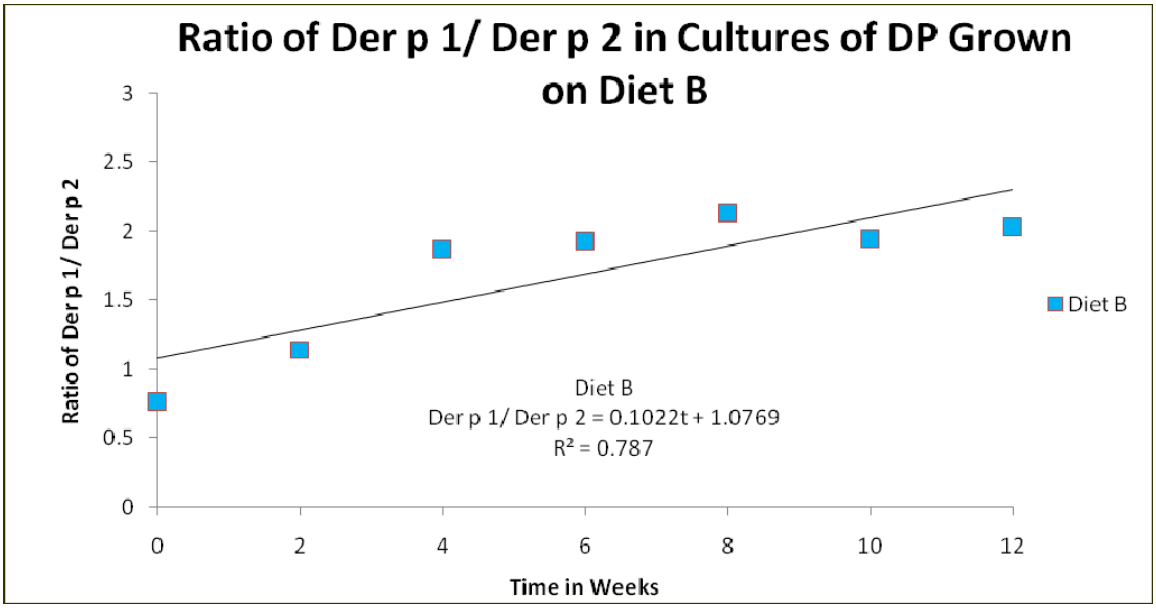

Figure 33: Der p 1/ Der p 2 ratio in cultures of *D. pteronyssinus* grown on diet B. Data is from Table 28.

Table 29: Rate constant (k), correlation coefficient (r^2) and doubling time for accumulation of allergens Der p 1 and Der p 2 in *D. pteronyssinus* cultures grown on diet B.

Allergen	Correlation coefficient (r^2)	Accumulation rate constant (k / week)	Doubling time
Der p 1	0.9975	0.2598	2.66 wks
Der p 2	0.9499	0.1862	3.71 wks
Der p 1/ Der p 2	0.787	0.1022	

6. Comparison of population growth and allergen content of *D. pteronyssinus* grown on diet A and diet B.

a) Population growth:

Population growth of *D. pteronyssinus* on both diet A and diet B showed an exponential growth (Figure 34 and Table 30). Regression analysis comparison of the natural logs of the population density averages for each time are represented in figure 35 and Table 31. *D. pteronyssinus* on diet B started with a lesser population than *D. pteronyssinus* started on diet A at week 0, but at weeks 2 and 4 the diet B population over took the diet A population. This difference from 0 to 4 weeks was not significantly different (p- value > 0.05). From week 6 until week 12 the diet A population growth was significantly (p- value < 0.05) more than the Diet B population growth. The growth rate constant for diet B was 0.24 while for diet A it was 0.32 per week (Figure 35, Table 31). Doubling time of the population for diet A was 2.15 weeks and diet B was 2.85 weeks (Table 31). Hence *D. pteronyssinus* grown on diet A had a shorter doubling time and a faster growth rate than *D. pteronyssinus* grown on diet B.

b) Allergens:

Der p 1- *D. pteronyssinus* grown on diet B produced more group 1 allergens than it did when grown on diet A (Table 32, Figures 36 and 37). The allergen concentration on both the diets started with values that were similar, 1.75 and 1.38 for diet A and diet B respectively (µg per 100 mg of culture material). At 6 weeks the allergen concentration of diet B had increased to 1.43 times more than that of diet A (Table 32). Diet B reached its maximum allergen concentration of 38.76 (µg per 100 mg of culture material) by week 14 but diet A reached its maximum

concentration of 13.96 (μg per 100 mg of culture material) at week 12.Therefore at 12 weeks, group 1 allergen produced by *D. pteronyssinus* cultured on diet B was 2.15 times more than that produced by diet A. Diet A had an accumulation rate of 19.64% per week and took 3.51 wks to double (Table 33, Figure 37). Diet B had an accumulation rate of 26% per week and took 2.66 wks to double (Figure 37, Table 33). *D. pteronyssinus* on diet A produced less group 1 allergen than on diet B and but it is not a statistically significant difference (*P*-value > 0.05).

Der f 2- The initial concentrations of Der f 2 was 4.52 band 1.8 μg per 100 mg of culture material for diet A and B, respectively (Table 34, Figure 38). During the 12 week culture period diet A had more group 2 allergen than diet B. Diet B reached its maximum allergen concentration of 15.38 μg per 100 mg of culture material at 14 weeks and diet A reached its maximum concentration of 34.89 (μg per 100 mg of culture material) at 12 weeks. The concentration of group 2 allergens produced by mites on diet A were significantly higher (*P* > 0.05), but mites on diet B had a slightly better accumulation rate and doubling time

Diet A had a Der p 2 accumulation rate of 17.8% per week and took 3.88 wks to double this concentration. Diet B had an accumulation rate of 18.6 % for Der p 2 and took 3.71 wks to double (Table 35, Figure 39).

Der p 1/ Der p 2 ratio- The Der p 1 concentration for diet A culture was 0.39 times of Der p 2 concentration at week zero (Table 36, Figure 40). The highest ratio was at week 8, where Der p 1 concentration was 0.6 times of Der p 2 concentrations. Der p 1 accumulated at a rate less than zero per week than Der p 2

(Table 37, Figure 41). Therefore, the ratio was constant all through the growth of culture.

The Der p 1 for diet B was 0.77 times of Der p 2 at week zero (Table 36, Figure 40). The highest ratio was at week 14, where Der p 1 was 2.52 times more than Der p 2 concentrations. For diet B, Der p 1 accumulated at a rate of 10.2% per week more than Der p 2 accumulated (Table 37, Figure 41).

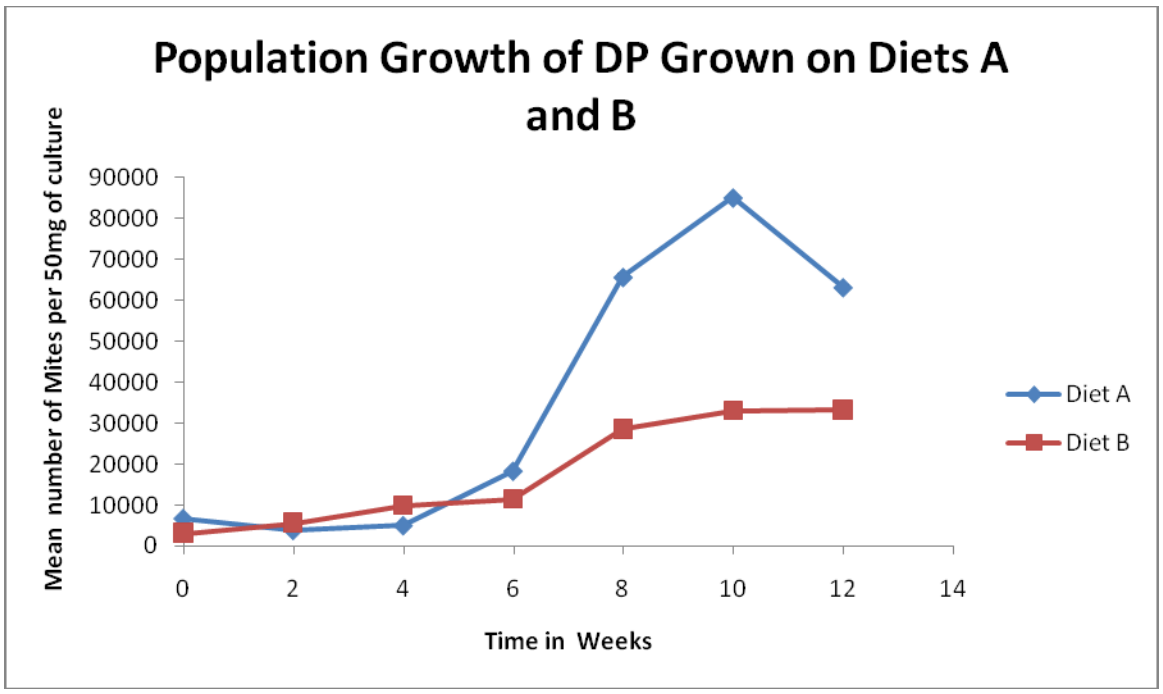

Figure 34: Comparision of the mean population densities for *D. pteronyssinus* grown on diets A and B. Data are shown in Table 30.

Table 30: *D. pteronyssinus* population density (mites per 50mg of culture material) grown on Diet A. The population density at each time is the average of 5 replicate cultures. Data represents active life stages (larvae, protonymphs, tritonymphs and adults).

Time (weeks)	0	2	4	6	8	10	12
Diet A	6762.1 ±116.5	3850.8 ±25.9	5027.7 ±31.4	18312 ±119.7	65607.4 ±721.4	84992.2 ±562.4	63111.7 ±323.7
Diet B	3059.6 ±15.7	5557.5 ±25	9861.7 ±45.6	11470.9 ±27.7	28521.6 ±94.1	33004.4 ±122	33193. 3 ±207

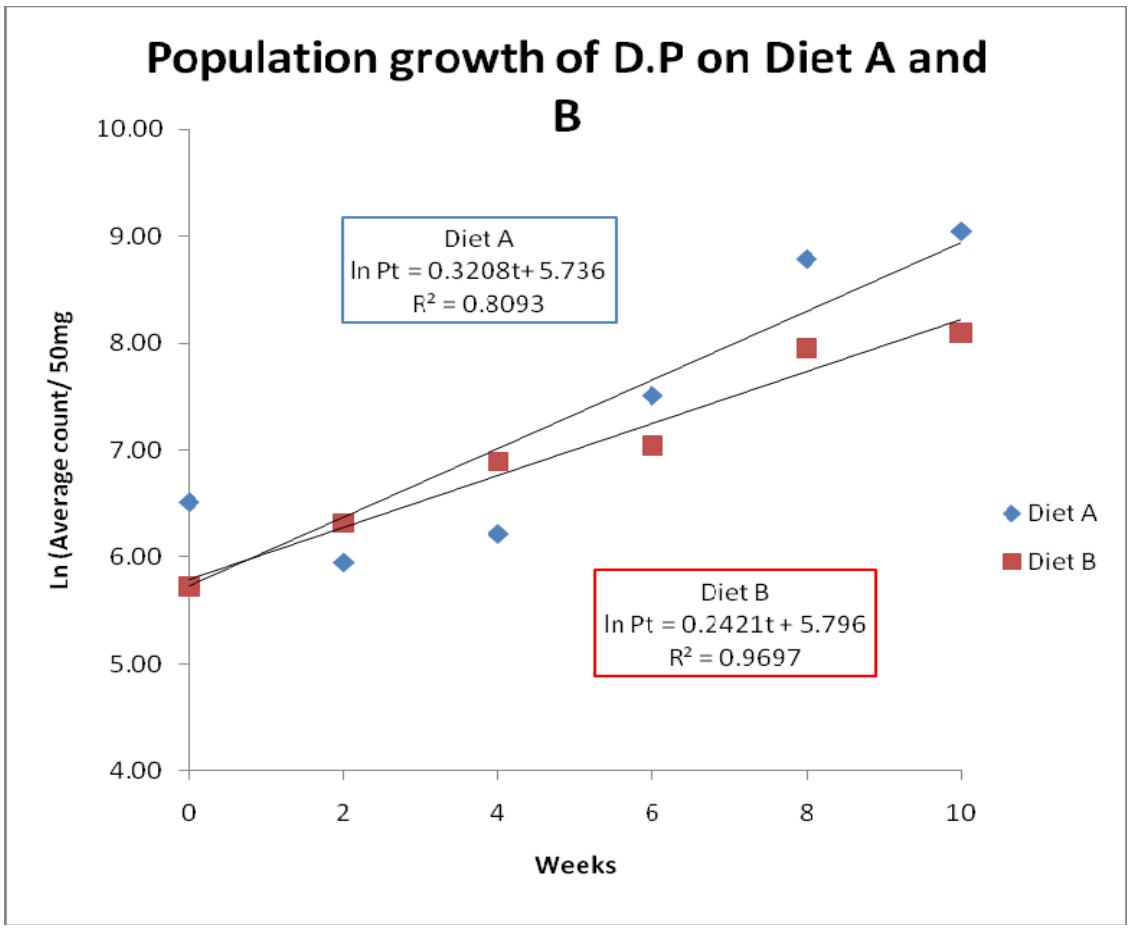

Figure 35: Natural logs of the mean population densities for *D. pteronyssinus* grown on diets A and B. Data are shown in Table 30.

Table 31: Total population growth rate constant (k), correlation coefficient (r^2) and doubling time for DP at 75% RH and room temperature.

D. pteronyssinus	Correlation coefficient (r^2)	Growth rate constant (k / week)	Doubling time
Diet A	0.8093	0.3208	2.15 wks
Diet B	0.9697	0.2421	2.85 wks

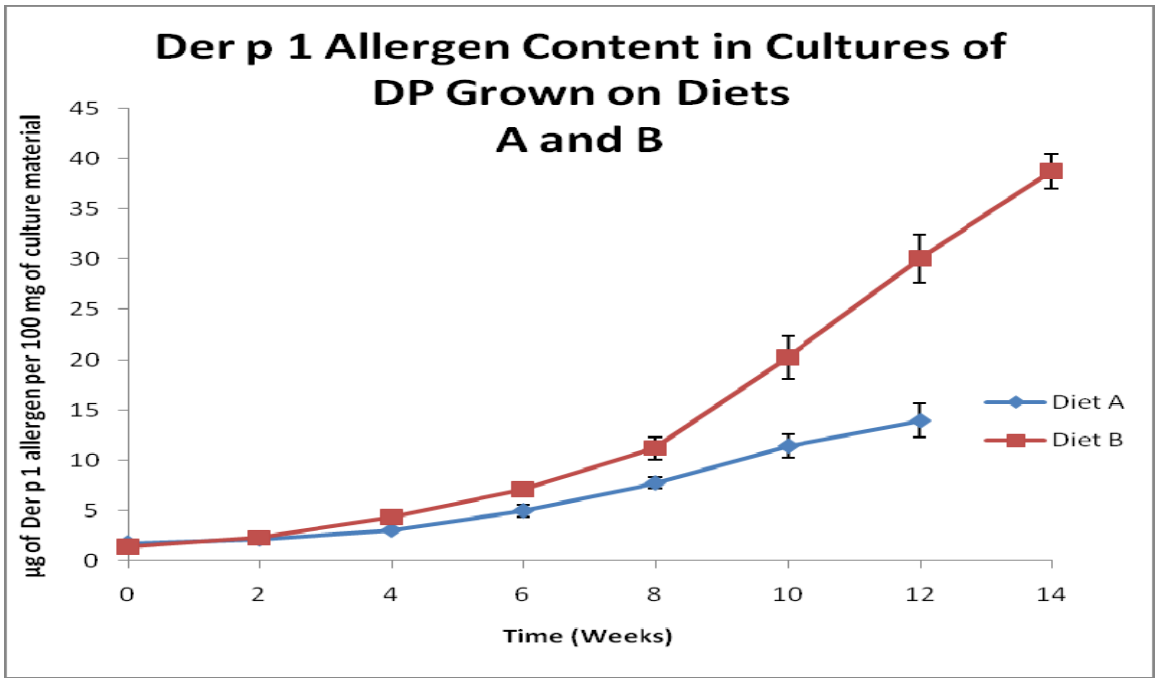

Figure 36: Comparison of Average (±SEM) concentration of Der p 1 allergen present in cultures of *D. pteronyssinus* on diet A and B. Data are from Table 33.

Table 32: Comparison of Der p 1 allergen concentration during *D. pteronyssinus* population growth on diets A and B. Data are mean micrograms of Der p 1 per 100 mg of culture (±SE). Data are from Tables 23 and 28.

Weeks	Diet A	Diet B
0	1.75±0.15	1.38±0.73
2	2.13±0.09	2.28±0.11
4	3.03±0.12	4.35±0.41
6	4.97±0.64	7.11±0.51
8	7.74±0.56	11.22±1.12
10	11.43±1.15	20.23±2.16
12	13.96±1.7	30.06±2.42
14		38.76±1.77

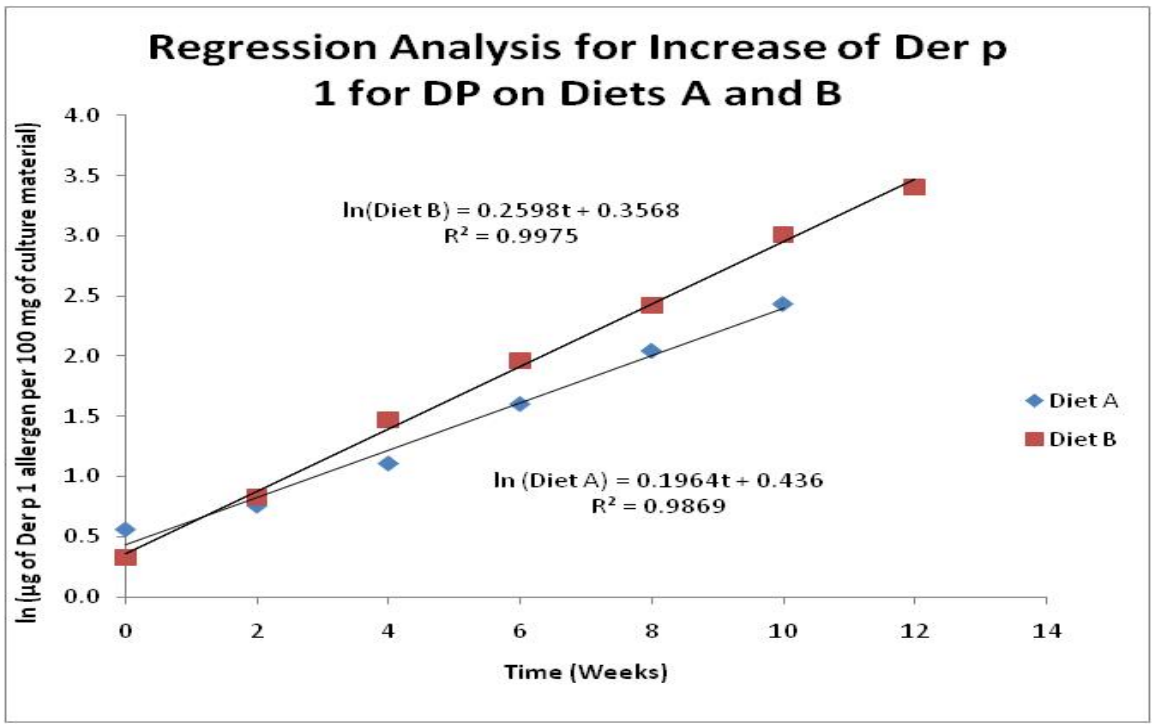

Figure 37: Natural log of the average ± SE of the Der p 1 allergen present in cultures of *D. pteronyssinus* on diets A and B. Plots are ln of data shown in Table 33.

Table 33: Der p 1 allergen rate constant (k), correlation coefficient (r^2) and doubling time for *D. pteronyssinus* cultured on diets A and B.

D. pteronyssinus	Correlation coefficient (r^2)	Accumulation rate constant (k / week)	Doubling time
Diet A	0.9869	0.1964	3.51 wks
Diet B	0.9975	0.2598	2.66 wks

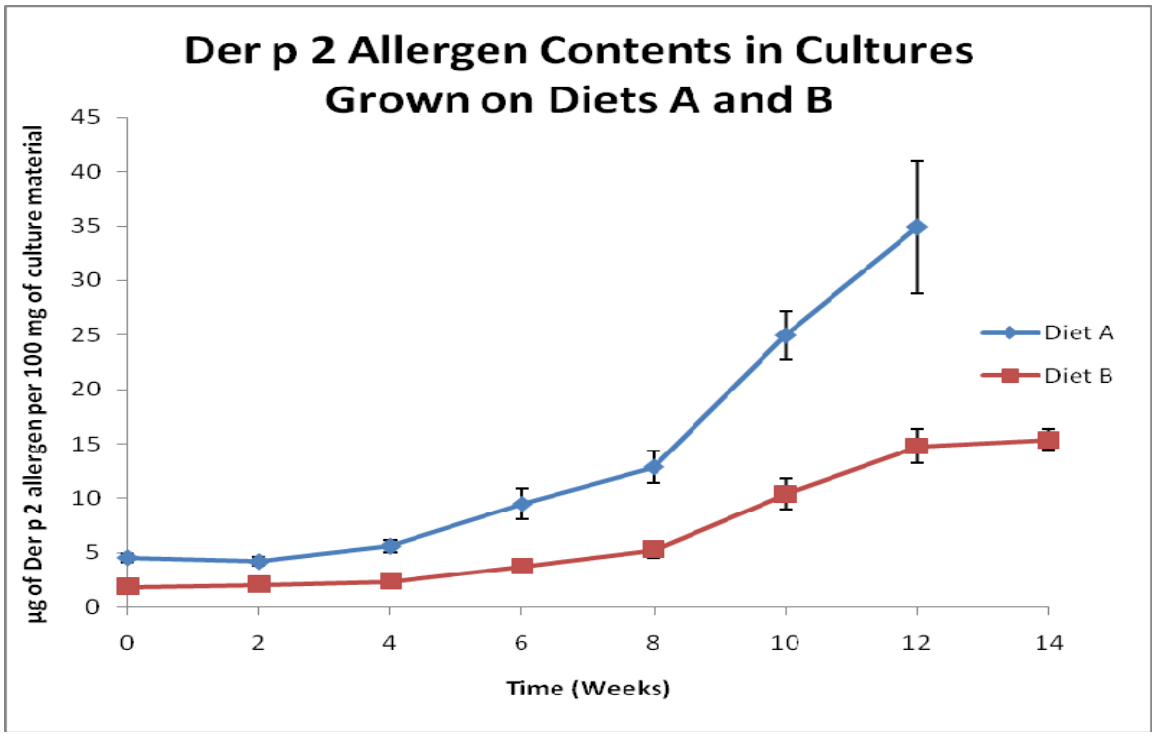

Figure 38: Comparison of Average (±SEM) concentration of Der p 2 allergen present in cultures of *D. pteronyssinus* on diet A and B. Data are from Table 35.

Table 34: Comparison of Der p 2 allergen concentrations during *D. pteronyssinus* population growth on diets A and B. Data are mean micrograms of Der p 2 per 100 mg of culture (±SE). Data are from Tables 23 and 28.

Weeks	Diet A	Diet B
0	4.52±0.4	1.8±0.28
2	4.16±0.41	2±0.11
4	5.56±0.56	2.33±0.24
6	9.45±1.43	3.69±0.09
8	12.83±1.45	5.26±0.77
10	24.94±2.22	10.39±1.42
12	34.89±6.1	14.79±1.54
14		15.38±0.94

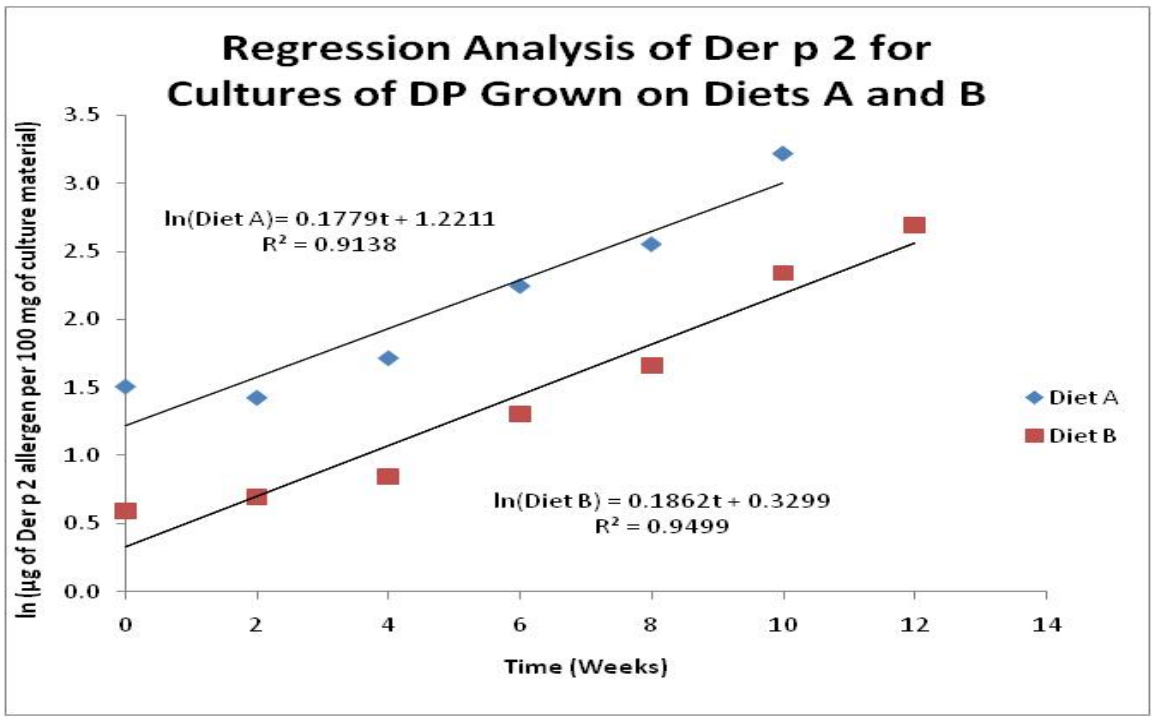

Figure 39: Natural log of the average ± SE of the Der p 2 allergen present in cultures of *D. pteronyssinus* on diets A and B. Plots are ln of data shown in Table 35.

Table 35: Comparison of Der p 2 allergen rate constant (k), correlation coefficient (r^2) and doubling time for *D. pteronyssinus* cultured on diets A and B.

D. pteronyssinus	Correlation coefficient (r^2)	Accumulation rate constant (k / week)	Doubling time
Diet A	0.9138	0.1779	3.88 wks
Diet B	0.9499	0.1862	3.71 wks

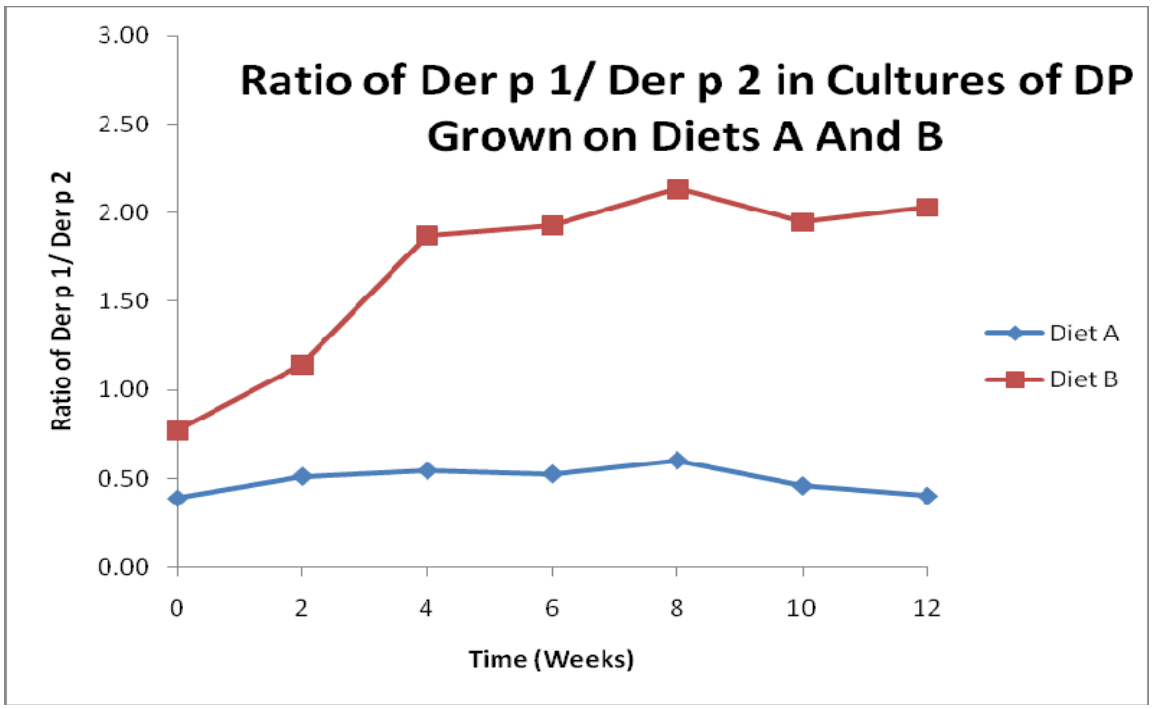

Figure 40: Ratio of Der p 1/ Der p 2 in cultures of *D.pteronyssinus* grown on diet A and B. Data are from Table 37.

Table 36: Ratio of Der p 1/ Der p 2 for *D.pteronyssinus* grown on diet A and B. Data is ratio ±SE.

Weeks	Diet A	Diet B
0	0.39±0.37	0.77±2.65
2	0.51±0.21	1.14±1.07
4	0.54±0.21	1.87±1.72
6	0.53±0.45	1.93±5.48
8	0.60±0.39	2.13±1.46
10	0.46±0.52	1.95±1.52
12	0.4±0.28	2.03±1.57
14		2.52±1.88

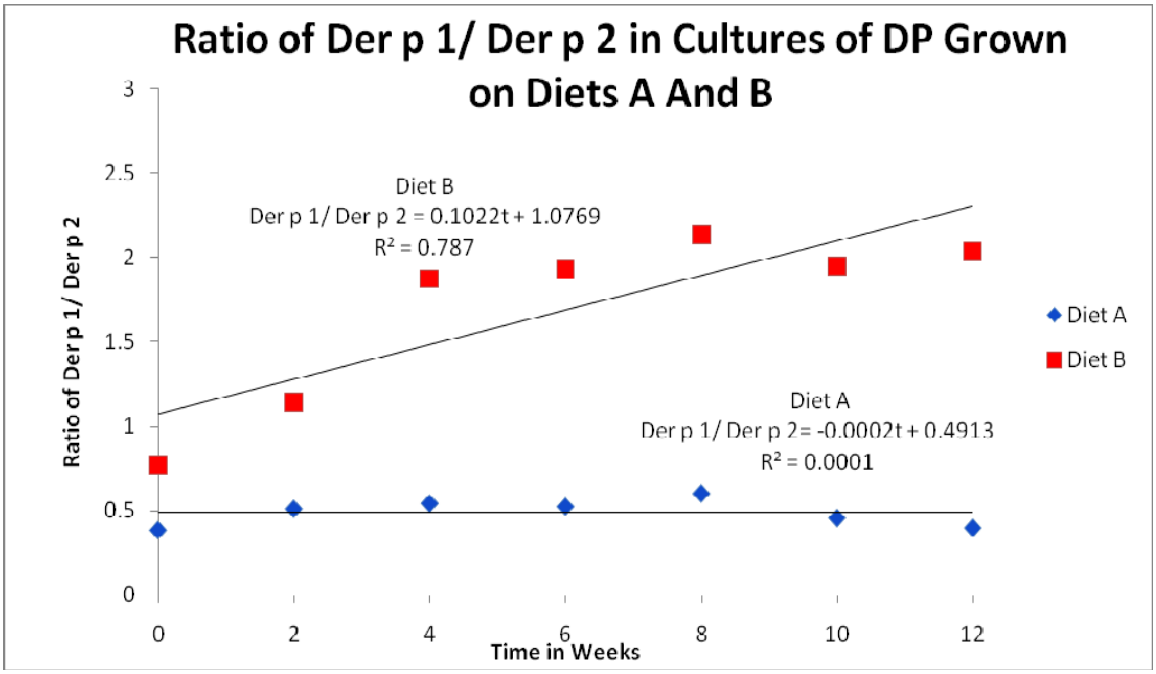

Figure 41: Ratio of Der p 1/ Der p 2 in cultures of *D.pteronyssinus* grown on diet A and B. Data from Table 37.

Table 37: Comparison of the ratio of Der p 1 to Der p 2 allergen accumulation rate constant (k) and correlation coefficient (r^2).

D. pteronyssinus	Correlation coefficient (r^2)	Accumulation rate constant (k / week)
Diet A	0.0001	-0.0002
Diet B	0.787	0.1022

DISCUSSION

General

House dust mite cultures are rich in eggs, larvae, nymphs, adults, exoskeleton and feces. Extracts made out of these cultures contain bioactive molecules, including digestive enzymes excreted in feces, molting proteins and other enzymes. These bioactive molecules found in the cultures are identical to the ones found in natural mite habitats, but more concentrated due to the high number of mites in a culture. Humans have been shown to react immunologically to these substances. The allergens found in homes can become air borne and inhalation of this causes asthma. Skin contact with the allergens can cause atopic dermatitis. Allergic rhinitis can occur when mucosal surfaces of the nose and eyes come in contact with airborne allergens or contaminated hands. Although some activities of some mite allergens have been studied (e.g: cysteine protease) more research needs to be done to fully understand the biochemical properties of the different allergens and their physiologic action in humans. Hence, it is very important to produce potent and standardized allergens for pursuing such studies.

Mite extracts are also used in diagnostic skin prick tests and in immunotherapy of patients. House dust mites are commercially cultured for these purposes. It has been seen in previous studies that extracts made from different phases of the mite culture gave different results. Previously, extracts from the exponential growth phase (F2) were found to have a higher allergenic potency and concentration of major allergens (Eraso et al., 1997; Martinez et al., 2000). So, there is a need to optimize the culturing methods of the dust mites in order to produce the most potent allergens and reduce the variations due to different culture conditions. The purpose of this study was to determine the ideal stage of

mite culture that contains the highest allergen concentration. We also wanted to see if *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* had different diet requirements and if diets influenced the population growth and the allergens produced by these house dust mites.

Mite Population Growth

The mite population is affected by temperature and relative humidity (Arlian et al., 1990; Arlian et al., 1992). In this study we wanted to see if diet had an effect on the population growth of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. Both cultures of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* on diets A and B grew exponentially over time. This result is consistent with previous studies (Arlian et al., 1998; Eraso et al., 1997).

Dermatophagoides farinae on diet B had a doubling time of 1.79 weeks and growth rate of 38.5% per week. This doubling time is 1.62 weeks less with an 18.3% faster growth rate than on diet A (Figure 15, Table 12). The doubling time was 3.09 weeks and growth rate was 35.1% for *Dermatophagoides farinae* cultures that had an initial population of 40 females in a previous study conducted by Arlian et al. (1998). So, the growth rate and doubling time of diet A is similar to this study. By contrast, *Dermatophagoides farinae* cultures grown on diet B had a growth rate 18.3% faster than cultures grown on diet A. The mite population cultured on diet A took 1.67 weeks longer to double than it did on diet B (Figure 15, Table 12). These results indicate that diet B meets the nutritional requirements of *Dermatophagoides farinae* better than diet A.

Overall, when both the species are compared, *Dermatophagoides farinae* on diet B had the best growth rate, 38.5% and doubling time 1.79 weeks. *Dermatophagoides pteronyssinus* on diet A had a growth rate of 32% and a doubling time of 2.15 weeks. So

Dermatophagoides pteronyssinus grows better on diet A whereas *Dermatophagoides farinae* grows better on diet B. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* probably have different dietary requirements to grow optimally. Diet could be one of the factors affecting the mite population in its natural habitat and could explain the greater prevalence of *Dermatophagoides farinae* in some homes and *Dermatophagoides pteronyssinus* in others from the same geographic area.

Dermatophagoides pteronyssinus population cultured on diet A had a growth rate of 32% per week. Previous studies showed that *Dermatophagoides pteronyssinus* had a growth rate range of 30.8-35.1% per week (Arlian et al., 1998). Hence, results from this study are consistent with previous studies. *Dermatophagoides pteronyssinus* grown on diet A had a growth rate 7.8% higher than the mites grown on diet B. Hence, diet B cultures took 0.7 weeks longer for their population to double than the diet A cultures (Figure 35, Table 31). The population growth was significantly higher on diet A cultures after the fourth week (P-value < 0.05). Therefore it can be said that diet A is better suited for culturing *Dermatophagoides pteronyssinus.*

Both *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* cultured on diet A showed a drop in population at two weeks. When the mites are mixed with food at week zero, there is much food and few mites. The powdery nature of the food could make movement difficult for the mites. Mites crawl out to the surface of the food probably to absorb water from the air (by observation). Therefore, during the (initial) early culture time (t=0 to t=2 wks) when the mite population is low physical and chemical properties of the food may cause some stress on the mites to survive; hence a drop in the population is seen.

When compared at eight weeks *Dermatophagoides pteronyssinus* on diet A had the highest population of mites, 65,607.4 ± 721.4 mites per 50 mg of culture material (Figure 22). The population of *Dermatophagoides pteronyssinus* on both diets was higher than for *Dermatophagoides farinae*. Similar results were found in a study conducted by Eraso et al. (1997), *Dermatophagoides pteronyssinus* cultures had higher population density than *Dermatophagoides farinae* cultures. The reason for this is unclear. *Dermatophagoides pteronyssinus* reproduces faster and has a higher growth rate than *Dermatophagoides farinae* (Arlian et al., 1998). However, there may be other contributing factors like temperature, physical and chemical properties of the diet.

This is the first study to assess the life stage distribution along with the population and allergen content in a mite culture. Here we observed the life stage composition for each time interval when the population and allergen samples were collected. It gave us an insight of the population life stage structure at each time interval. Any association between life stage distribution, population growth and allergen content can be assessed with this information. The life stage composition for both species cultured on diets A and B showed similarities. Eggs were the most dominant life stage except for *Dermatophagoides pteronyssinus* on diet A where the adults dominated all the life stages. Density of eggs and larvae were inversely related. Protonymphs were the least numerous of all life stages. All the cultures had a high initial egg population, except *Dermatophagoides pteronyssinus* on diet A.

The egg population depends on the number of fertile males and females. At 23^0C *Dermatophagoides farinae* requires about 10 days for eggs to develop into larvae and 9 days for larvae into protonymph (Arlian and Dippold, 1996). Hence, if at week 2

the number of eggs is high then at week 4 all the eggs would have developed into larvae. The percentage of eggs is a direct indicator of the population growth. It was seen that a fall in egg percentage indicated a drop in population, while increase in eggs caused spike in the population growth. At two weeks, *Dermatophagoides farinae* -diet B cultures had 70% of total life stages were eggs followed by a spike in population growth at week 4. Towards the end of culturing period the percentage of eggs dropped. Interestingly at two weeks, diet A cultures had 34.3% larvae and 18.9% eggs followed by a meager growth in population at week 4. The drop in population could indicate that the larval stage of *Dermatophagoides farinae* is more susceptible to temperature as the mites on diet A had a drop in population at week two and did not recover until week 6.The density of larvae and eggs seemed to be inversely related for *Dermatophagoides farinae* on both diets. As the number of eggs increased the number of larvae decreased and vice versa. It again relates to the fact that eggs develop into larvae in 10 days. This shows that diets A and B did not affect the life cycle of the mite. Both of the diets consistently produced eggs that developed into larvae after 10 days.

The protonymphs were the least numerous life stage for *Dermatophagoides farinae*. They represented less than 2% for diet B population and less than 6% of mites on diet A. Protonymphs take 8.6 ± 2.8 days to develop at 23 ^{0}C and 27 ± 5.2 days at 16^{0}C (Arlian and Dippold, 1996). The slight increase in protonymph percentage seen in diet A cultures could be because the mites were taking a longer time to develop into tritonymphs. For diet B, the larvae were going through the protonymph life stage and developing into tritonymphs much faster. In general the nymphs were the least numerous and adults the most numerous life stage (Tables: 3, 8; Figures: 4, 10). Towards the end of

the culturing period the adult population was the highest when compared to larvae and nymphs, indicating that the adults are not producing as many eggs due to shortage of food. Since there are not as many eggs being produced, the number of larvae is also reduced, making adults the largest portion of the population.

Dermatophagoides pteronyssinus on diet A had 21.5% higher adult population when compared to eggs at time zero. It could be one of the reasons for the high population seen in this culture. By 2 weeks, they had 80% adults and only 5% larvae (Figure 24, Table 22).Therefore, the high number of adults could have helped the population growth to recover faster than the *Dermatophagoides farinae* cultured on diet A, where the larvae were 34.3% and adults 27.7% of the total life stages. Cultures of *Dermatophagoides pteronyssinus* on diet B had high density of eggs, 50.5% at time zero. Eggs remained as the dominant life stage all throughout the culture period. Larvae and eggs showed an inverse relationship in diet B culture that was not seen very clearly in the diet A culture. The high number of eggs produced by *Dermatophagoides pteronyssinus*, as this culture had more adults, could have masked the inverse relationship between eggs and larvae.

Allergen Accumulation in Cultures Over time

Group 1 allergen is a digestive enzyme produced by the gut of the mite and is also excreted in feces (Tovey et al., 1981). Hence, along with live and dead mite bodies feces also contribute to the allergen content in a culture as well as in the mite's natural habitat. In order to obtain maximum allergen concentration, it is important to include the culture medium along with mite bodies to make allergen extracts.

As a result of exponential increase in mite population and accumulation of fecal particles the group 1 allergen accumulation was also exponential in cultures from this study. Osterberg et al. (2008), did a similar study and used purified mite bodies without the culture material. Der f 1 was seen to decrease linearly where as the Der f 2 content increased through the first part and then decreased towards the end of the cultivation period. Therefore cultures should not be extracted towards the end of the culturing period. The use of pure mite bodies as compared to the whole culture in this study could be the reason for this difference. Hence, fecal particles and dead mites add to the allergen concentration of a culture.

Group 1 allergens for *Dermatophagoides farinae* cultured on diet A and B reached their maximum concentrations by week 12 and 10, respectively (Table 14, Figure 16, 17). The difference between both the maximum concentrations was not statistically significant (*P*-value > 0.05). Cultures on diet A took 1.62 weeks longer than cultures on diet B for its allergen concentration to double. The allergen accumulation rate for cultures on diet A was 9.8% slower than cultures on diet B. It was consistent with the population growth, as *Dermatophagoides farinae* on diet A took 1.67 weeks longer to double in size compared to the mites on diet B. Therefore, it can be said that for *Dermatophagoides farinae,* population growth rate and group 1 allergen accumulation rate are directly proportional. As the mite population increased exponentially, the group 1 allergen concentration in the culture media also increased exponentially.

Group 1 allergen of *Dermatophagoides pteronyssinus* increased exponentially in parallel with the increase in mite population directly on both diets A and B. It was interesting to see even though *Dermatophagoides pteronyssinus* on diet A had the highest

mite density at 12 weeks (when compared to all cultures) the amount of group 1 allergen produced was 13.96 ± 1.7 µg per 100 mg at 12 weeks whereas, *Dermatophagoides pteronyssinus* on diet B produced 38.76 µg per 100 mg at 14 weeks (Figure 31). Therefore, for *Dermatophagoides pteronyssinus* cultures, diet B produced more group 1 allergens than diet A. Hence, diet can be a major factor in the production of an allergen.

When both species *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* were compared on both the diets A and B, it was seen that mites on diet A produced less group 1 allergen. Between both the species, *Dermatophagoides farinae* produced the maximum of group 1 allergen concentration of 39.45 ± 10.55 µg per 100 mg of culture material at 10 weeks on diet B (Table 14, Figure 15). *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* cultures on diet B produced almost similar amounts of group 1 allergen indicating that diet has an important role in the amount of group 1 allergen produced. So even if *Dermatophagoides pteronyssinus* cultures had greater population density than *Dermatophagoides farinae* the amount of group 1 allergen produced was not very high. But a study conducted by Eraso et al. (1997) showed that *Dermatophagoides pteronyssinus* cultures had high density of mites as well as allergen content when compared to *Dermatophagoides farinae.* One possible reason for the difference could be the diet and differences in culturing method. In the Eraso et al.(1997) study, food was added to the cultures whenever the cultures were low on food, where as food was a constant in this study. Therefore diet B was best suited for culturing *Dermatophagoides farinae.*

Group 2 allergens belong to the ML (MD-2 related Lipid recognition) domain lipid binding family (Inohara et al., 2002). The ML domain lipid domain family

includes mammalian epididymal HE-1 like protein, Neimann-Pick type C2 (NPC2) proteins, myeloid differentiation type 2 (MD-2) protein and lipo-phosphate transfer protein of many fungi (Thomas et al., 2007). The exact function of group 2 allergens is unknown, but it may be associated with the reproductive tract of the male mite (Thomas and Chua, 1995). There would be an increase or decrease in the allergen content based on the male mite population. In contrast studies conducted by Jeong et al. (2002), showed that Der f 2 is synthesized in the epithelium, secreted in lumen of the anterior midgut and mixed with the digestive juices of both male and female mites. Fecal pellets were also found to be concentrated with the group 2 allergen. Therefore, group 2 allergens cannot be associated only with male mites. Life stage and allergen results from this study show us that there is a constant increase in allergen concentration and it cannot be associated only with the male mite population.

ML domain proteins are associated with lipid metabolism and LPS signaling. MD-2 proteins act as an extracellular binding partner for Toll like receptor 4 (TLR-4) and are essential for triggering the innate immune response in mammals to bacterial LPS (Inohara et al., 2002).

Also, recent studies on human NPC2 have indicated that it plays an important role in sterol transportation. The ML domain of NPC2 is associated with cholesterol binding. A mutation in the NPC2 gene causes an autosomal recessive lipid storage disorder (Berger et al., 2005). The study also shows that the NPC2 mediated lipid transportation has been conserved thought evolution from yeast to mammals. NPC2 and Der p 2 have structural similarities and therefore could have similar functions (Friedland et al., 2002). In this study, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* cultures

on diet A produced significantly higher amounts of group 2 allergens suggesting that diet A has more lipid content causing the mites to produce more Der 2.

The physiologic function of Der 2 in a mite is still unclear. But evidence from previous studies indicates that it binds to lipid. In a mite, Der 2 could either have an immunologic or a lipid transportation function. Since in this study we see Der 2 being produced in larger quantities on diet A than on diet B it makes us wonder if the high lipid content in diet A is causing the mites to produce more Der 2. Therefore, Der 2 could be involved in lipid transportation rather than having a protective function in a mite.

Independent of the diet, the species of mite itself can produce differences in the amount of allergens produced. *Dermatophagoides pteronyssinus* cultured on both diets A and B produced more group 2 allergens than *Dermatophagoides farinae* cultured on diets A and B. However, diet can influence the amount of group 2 allergen produced. Cultures of both species on diet A produced more group 2 allergen than cultures on diet B. This makes us wonder if higher lipid content in diet A was causing the mites to produce more group 2 allergen. The lipid content of both diets is unknown. These results suggest more studies need to be done to understand the relationship between diet and the quantity of allergen a mite produces. One way to test it would be to increase the lipid content in diet B and see if it increases the amount of group 2 allergen produced.

Conclusions

This study shows that the mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are affected by diet. It can influence the population dynamics, the amount

of the major allergens (group 1 and group 2) and their ratio produced by these mites. My study shows that at 8 weeks, *Dermatophagoides farinae* diet B cultures produced 3.08 times more Der f 1 than diet A cultures (µg/100 mg), with a rate of 31.8%**.** *Dermatophagoides pteronyssinus* diet A cultures produced 2.44 times more Group 2 allergen than diet B cultures (µg/100 mg) with a rate of 17.8%. Diet B is suited for culturing *Dermatophagoides farinae* and diet A is suited for culturing *Dermatophagoides pteronyssinus*. Hence, diet is an important factor to be considered while culturing mites for skin prick tests, immunotherapy and research studies.

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