ADRENAL STEROID, BLOCKING AGENT, AND SOCIAL STRESS EFFECTS ON NORTHERN FOWL MITE POPULATION DEVELOPMENT ON LEGHORN CHICKENS AND TOXICOLOGICAL EVALUATION OF SELECTED ACARICIDES (ACARINA: MACRONYSSIDAE)

by

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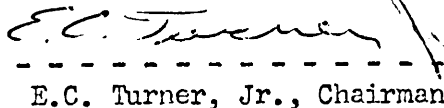

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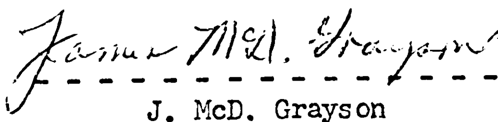

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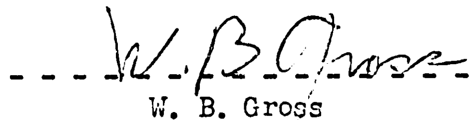

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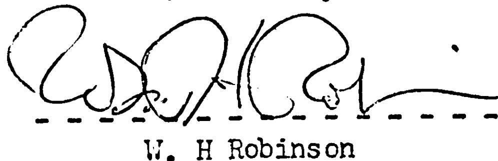

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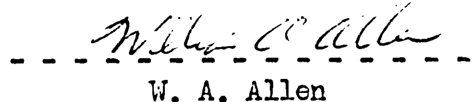

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INTRODUCTION

The northern fowl mite, _Ornithonyssus sylviarum_ (Canestrini and Fanzago, 1877), is considered by many specialists to be the most damaging poultry ectoparasite currently present in the United States. These blood-feeding mites are capable of completing their life cycle without leaving the body and plumage of the host. Infestations occurring in large flocks may eventually affect the majority of birds, but Payne (1930) observed that occasional chickens remain mite-free. Loomis et al. (1970) noted this phenomenon and theorized that an immune response or hormone activity might be responsible. Matthysse et al. (1974) demonstrated the formation of circulating, precipitating, and skin-sensitizing (reagin-like) antibodies in chickens in response to prolonged northern fowl mite infestation, and indicated a difference in mite susceptibility between lines of chickens selected for varying dietary arginine requirement. Hall and Gross (1975) proved that Leghorn chickens genetically selected for high plasma corticosterone response to a 2-week period of social stress were significantly more resistant to mite infestation than birds selected for a low corticosterone response. In these experiments, additional social stress concurrent with parasitic challenge was shown to modify slightly the mite susceptibility of the 2 lines.

Many acaricides and insecticides have been evaluated for control effectiveness against northern fowl mites. While the majority of such studies have involved direct application of toxic compounds to birds, several precise toxicological laboratory techniques have been designed.

Only four materials are currently registered in Virginia for direct application to commercial poultry; of these, malathion (diethyl mercaptosuccinate, S-ester with O,O-dimethyl phosphorodithioate) has been reported the subject of mite resistance in California (Rodriguez and Riehl, 1963). Reports of acaricide effectiveness against northern fowl mites have appeared with decreasing frequency in recent years. Hall et al. (1975) demonstrated that chlordimeform (N'-(4-chloro-o-tolyl)-(N,N-dimethylformamidine)) was extremely effective against northern fowl mites in a caged layer environment. In 1976, all uses of this compound were suspended by the manufacturer. Synthetic pyrethroid insecticides have shown promise as effective arthropod control agents in a variety of situations, but use against poultry ectoparasites has not been reported. Availability of compounds with different modes of action is important in the maintenance of control programs.

Housing large numbers of chickens in wire cages has largely supplanted floor-managed birds in egg-production operations. The advantages of the former system with respect to overall efficiency cannot be disputed, but the attendant effect on parasite burdens has not been fully investigated. The environment in such facilities is conducive to relatively high egg output and the elimination of contact with litter limits the opportunity for infestation with many species of parasites. The popularity of this system may be responsible for the increase in northern fowl mite populations. However, certain additional management techniques may help to reduce mite populations

without relying solely on acaricide applications.

This report concerns the effect on population development of the northern fowl mite on 1) chickens subjected to intravenously- or orally-administered adrenal steroids and blocking agents, 2) chickens from lines genetically selected for high or low plasma corticosterone response to social stress, 3) roosters genetically selected for differing antibody production, and 4) birds subjected to extremes of social interaction. The effect of host sex was investigated with respect to the involvement of female sex hormones and the time of rapid mite population development during the early life of female birds.

In addition, studies were conducted to determine: 1) the acute toxicity to northern fowl mites of those compounds currently registered in Virginia for direct application to poultry, 2) the possibility of malathion resistance in mites collected from southwestern Virginia, 3) the effectiveness of synthetic pyrethroid insecticides as mite-control agents on chickens, and 4) those techniques which might be implemented easily as part of a pest management program in large caged-layer poultry operations.

REVIEW OF LITERATURE

The northern fowl mite is an important ectoparasite of poultry in temperate climates and is the most severe pest of caged laying hens in these regions. First recorded attacking poultry in the U.S. by Wood (1920), it has been reported subsequently from most sections of the country, especially the south. These mites are almost invariably encountered upon the vent region of infested birds (Cameron, 1938; Loomis et al., 1970).

Several studies have examined various aspects of northern fowl mite biology, life history, and economic impact (Cameron, 1938; Combs, 1963; Foulk, 1964). Cameron (1938) studied the biology of _O. sylviarum_ in Canada and concluded that the average duration of the egg stage is 30.4 hours and the larval stage 8.3 hours in an artificial environment of 100-104 F (37.8-40.0 C) and 90 to 100 percent relative humidity. He stated also that the mite does not enter a summer dormant period, has an upper thermal death point between 104.2 and 108.5 F (40.1-42.5 C), and will not breed on very young chicks. The longest period of survival away from a suitable host was recorded to be 11 days at 70 F (21.1 C).

Sikes and Chamberlain (1954) observed northern fowl mites in a laboratory environment and determined the egg and larval stages to last less than one day each. The protonymph required two feedings before moulting to a non-feeding deutonymph in one to one and one-half days. The transformation from deutonymph to adult required less than one day; therefore, the cycle from egg to adult required four

to five days.

Combs and Lancaster (1965) found that at temperatures of 102 F (38.9 C) or above, northern fowl mites died before laying eggs. In this study, the optimum temperature for shortest pre-oviposition times was 90 F (32.2 C) at 73 to 76 percent relative humidity. The time required for the life cycle from egg to egg ranged from 140 to 162.5 hours (5.8 to 6.7 days). The maximum number of life stages were found in the feather area from one-half to one inch (1.27 to 2.54 cm) from the host's body. The microenvironmental temperature measured at a distance of one inch from the skin surface was 86 F (30 C), and the observation made that the humidity was high. Unfed protonymphs were recorded to survive for as long as 32 days away from the host. Based on the lethal effects of temperature above 102 F (38.9 C), they postulated that high summer temperatures were responsible for observed declines in mite populations during that season.

Matthysse et al. (1974) found that northern fowl mites exist in an extremely dessicating environment in the plumage during colder months, and Combs (1964) stated that the mite is a "cold weather" parasite. Furman and Loomis indicated the northern fowl mite to be less severe on poultry in the humid coastal region of California than in the dry interior valley (in Matthysse et al., 1974). J.G. Matthysse (Professor of Entomology, Cornell University, Ithaca, New York; in conversation) suggested that during cool months the northern fowl mite feeds as much for the water as for the nutrients provided by blood. He also indicated that cold air carries very little ambient humidity

and that northern fowl mites probably do not conserve moisture well. Hall (1975) extrapolated this line of reasoning to conclude that blood meals taken primarily for their moisture content provide incidentally increased raw materials necessary for growth and reproduction, thus leading to the increased mite populations commonly reported during the winter months.

Kirkwood (1963) reported the maximum unfed lifespan of _O. sylviarum_ to be 21 days. Caesar (1923) maintained mites alive in a bottle for 18 days; Abasa (1969) reported 25 percent survival after 13 days at a temperature of 25 C (77 F) and relative humidities ranging from 75 to 80 percent.

Chamberlain and Sikes (1950) demonstrated the first successful laboratory colonization of northern fowl mites. The host animals used were baby chicks, thus apparently refuting the assumption by Cameron (1938) that mites would not live on immature birds. Matthysse et al. (1974) developed an efficient procedure for mass rearing mites in laboratory colonies using paper containers and restrained baby chicks.

Furman et al. (1953) indicated that egg production was reduced in chicken flocks heavily infested with northern fowl mites. They also reported retarded growth of baby chicks. Loomis et al. (1970) did not find significant differences in egg production means between eight different family lines of laying hens rated from negatively to heavily infested with northern fowl mites. They stated that mean protein values from serum analysis of non-infested and heavily-infested birds were not statistically different, but that a sub-optimal protein diet

possibly complemented the proliferation of mites on hens. The packed red blood cell volume of blood from heavily infested hens was not lower than that of mite-free hens, suggesting that anemia is not a symptom of heavy mite infestation. A subsequent study by Matthysse et al. (1974) showed microcytic normochromic anemia in roosters heavily infested with northern fowl mites. Hall (1975) demonstrated the development of anemia in roosters from a mite-susceptible line which had supported mite populations in excess of 25,000 per bird for several weeks. He was unable to confirm reduction in egg output in commercial layers by measuring egg production between birds selected for either negative or large mite populations. It is possible that this experiment failed to deal adequately with the physiological differences in birds demonstrating large differences in mite susceptibility when faced with essentially identical parasitic challenge. Gross and Siegel (1974) demonstrated considerable difference in egg production between two lines of chickens genetically selected for differing plasma corticosterone response to social stress; Hall and Gross (1975) subsequently confirmed significant differences in mite susceptibility between the two lines. Matthysse et al. (1974), in a large-scale laboratory experiment, were able to show egg production reduction attributable to northern fowl mite infestations. The difficulty of assessing actual impact of northern fowl mites on egg production is a reminder of problems which are concomitant with usage of large, naturally-infested poultry flocks; this phenomenon may extend to other host-parasite relationships as well.

Hosts of the northern fowl mite include numerous domestic and wild birds. Hall and Townsend (1977) indicate this pest to be the most commonly encountered parasite in wild bird's nests in Virginia. Cameron (1938) reported Dicrostomyx hudsonius (Pallas) and man as accidental mammalian hosts. Strandtmann and Wharton (1958) listed records of O. sylviarum from several small mammals, including Mus musculus (L.), and W.V. Miller (Shell Chemical Co., Modesto, California; personal communication) reported collection of northern fowl mites from house mice living in chicken quarters. Hall and Turner (1976) collected adult and nymphal mites from Rattus norvegicus (Berkenhout) in a mite-infested chicken house and theorized that these rodents might hasten mite spread in the immediate vicinity. Attempts to induce mites to propagate successfully with albino laboratory rats as the sole host indicated that species of Muridae are probably only temporary carriers of O. sylviarum.

Many researchers believe that mite infestations in commercial poultry flocks stem from wild birds. This phenomenon has been investigated by Hoyle (1938) and the mite has been found in wild birds' nests during the summer (Maw et al., 1935). The rapidity of infestation was studied by Cameron (1938), but not in a caged layer situation. Matthysse et al. (1974) found that mite populations on chickens initially increase in exponential fashion, reach a peak population in nine to ten weeks, and subsequently taper off. Additional peaks of mite population may reoccur on the same chickens, but typically never attain the density of the initial infestation explosion. Once severely

by northern fowl mites, birds seldom regain the immaculate vent plumage of never-infested animals, but may support only minor infestations for varying periods of time. These workers also believe believe that population peaks may result from physical limitations within the feather coat, in that large mite densities result in excessive feather matting, and that build-up of mite excreta and cast skins may render the substrate less optimal for mite development. Periodic moulting of feathers may alleviate these limitations and permit additional aggressive population expansion.

Despite the cyclic nature of mite populations on a single bird, it has been noted in many studies that even in heavily-infested flocks, some birds continually remain free of parasites (Payne, 1930; Cameron, 1938; Loomis et al., 1970). Loomis et al. (1970) suggested that an immune response on the part of the birds or an intrinsic factor such as hormone activity might be responsible for the occurrence of mite-resistant birds. Matthysse et al. (1974) demonstrated the presence of circulating, precipitating, and skin sensitizing (reagin-like) antibodies following northern fowl mite infestation and postulated that antibody-mediated immunity might be responsible for the decline of mite populations following the initial large peak. It was noted during this study that it required approximately nine weeks to sensitize a majority of the test hens; the correlation between antibody production and mite population curves led to the conclusion that at least the immediate skin sensitizing system was a functional defense against mites. Evaluation of their test results pointed out chicken

strain, sex and individual bird differences in immunocompetence. It is interesting that the differences in mite population development recorded by Hall and Gross (1975) occurred during the initial 8 weeks of infestation; therefore, it seems improbable that the immune system postulated by Matthysse et al. (1974) could account for the different infestation levels.

The study of immunity to ectoparasites has involved few controlled experiments with respect to resistance to infestation. Numerous observations have indicated strain and individual resistance to various external parasites, but little data is available to demonstrate actual mechanisms of resistance. Trager (1939a, 1939b) and Riek (1958, 1959) indicated that acquired immunity can be developed to infestations of slow-feeding ticks. This immunity can be associated with circulating antibodies which are postulated to accelerate local cellular reactions which result in the prevention of the arthropod from obtaining its blood meal.

Nelson and Bainborough (1963) discussed resistance of sheep to the ked Melophagus ovinus (L.). Histologic studies of skin obtained from sheep following infestation showed arteriolar vasoconstriction. The subepidermis was edematous and showed eosinophilic and lymphocytic cellular infiltrations. The upper dermis of resistant sheep contained large numbers of empty capillaries; the capillaries of susceptible sheep contained many erythrocytes. The conclusion was that acquired immunity to the ked was caused by cutaneous arteriolar vasoconstriction which cut off the capillary blood flow to the upper dermis, preventing

the insects from obtaining requisite blood meals.

From the preceeding examples, Benjamini and Feingold (1970) theorize that acquired immunity to several species of haematophagous arthropods can be developed, with antibodies directed against antigens in the oral secretions of the arthropod. The interaction of these antigens with antibodies leads to histopathologic changes which result in blocking the blood supply to the arthropod. They question whether a similar immunity can be developed against arthropods which obtain their blood meals within a short time.

Social stress and adrenal corticosteroids can affect the resistance of chickens against various infectious diseases (Colmano and Gross, 1971; Gross, 1972, 1974; Gross and Siegel, 1975). High levels of corticosterone have in some instances been shown to increase the effectiveness of phagocytic defense (Vernon-Roberts, 1969). High levels of social interaction or other stresses such as cold, as well as manipulations such as selective breeding for high levels of plasma corticosterone, injection of adrenocorticotrophic hormone, or the injection of corticosterone increase the resistance of chickens to _Escherichia coli_ septicemia (Gross and Colmano, 1970, 1971). High levels of steroids or social stress increase resistance of chickens to _E. coli_ septicemia, _Staphylococcus aureus_ infection, _Streptococcus fecalis_ infection (Gross and Colmano, 1967, 1969), _Trichinella spiralis_ (Davis and Reed, 1958), and _Plasmodium berghei_ (Friedman et al., 1969).

Lodmell et al. (1970), working with amputee male mice infested with the louse _Polyplax serrata_ (Burm.), noted that large numbers of lice

developed on mice housed in disturbed hierarchies where mutual grooming was inhibited. A 50 percent depression of circulating eosinophils in mice under stress compared to those in stable hierarchies provided evidence of increased corticosteroid secretion.

High levels of circulating corticosteroids have been noted to affect skin histology. Chapman and Bassett (1970) found that in sheep, epidermal and dermal thickness is decreased, as are acid mucopolysaccharide and mast cell concentration. Collagen strands appeared to coalesce and glucose utilization was depressed so that the tissues could not proliferate normally. Nelson et al. (1975) believe that such changes, by themselves, might make feeding easier for blood-sucking ectoparasites in addition to inhibiting acquired resistance.

Nelson (1962), studying ectoparasites on sheep, indicated that any stress sufficiently acute and continuous to cause an increase in corticosteroid secretion can effect major changes in the normal cyclic population curves of sheep keds. Injection of ACTH, cortisone, or oral administration of acetylsalicylic acid was shown to decrease resistance of sheep to infestation with this insect. Applegate (1970) and Applegate and Beaudoin (1970) found that daily injection of corticosterone into English sparrows and house sparrows caused increased numbers of circulating avian malarial parasites in birds with latent infections. Hall and Gross (1975) demonstrated that Leghorn chickens genetically selected for high plasma corticosterone response to a 2-week period of social stress were significantly more resistant to northern fowl mite infestation than birds selected for a low corticos-

terone response. In these experiments, social stress was shown to modify slightly the mite susceptibility of the two lines.

Gross and Siegel (1974) outlined the importance of steroids to the defense strategy of chickens. They indicated that steroids offer a mechanism by which animals can use physical and sensory inputs to improve adaptability to changing environments. The latter, plus population pressures and infections result in increased steroid levels and favor individuals which respond with high levels of steroids. Birds with increased steroid levels lay more eggs, have lower body weights, are more active, have better sensory discrimination, and have stimulated phagocytic cells. A main function of lymphocytes is to supply protein required for growth. High steroid levels result in reduced lymphocyte numbers, and increased availability of ingredients for anaerobic energy. The decreased lymphocyte mass decreases lymphoid defense ability as well as the ability to respond to unfamiliar antigen.

Higher populations of parasites on male animals compared to females have been recorded frequently. Ali and Sweatman (1966) found that female laboratory mice, or males injected with estrogen, were more resistant than other categories to infestation with *Rhipicephalus sanguinens* (Latr.). In Hereford cattle, bulls tend to carry more lice than do cows (Haufe and Shemanchuk *in* Nelson et al., 1975). Nelson, Bell and Clifford (*in* Nelson et al., 1975) showed that individually-housed male mice harbor more lice than do females. E.C. Turner, Jr. (Professor of Entomology, VPI&SU; personal communication) indicates

that most breeder beef cattle bulls are more severely infested with horn flies than are steers or cows.

Nicol et al. (1964) termed estrogen the natural stimulant of body defense in both male and female animals, with the reticuloendothelial system more active in females and under the control of estrogen. Mathies (1962) stated that estradiol treatment of male mice reduced the numbers of adult Aspiculuris tetraptera parasitizing the animals, and Bailenger et al. (1964) showed that treatment of normal male mice with testosterone lowered resistance to Hymenolepis nana while treatment of normal females with estrogens enhanced host resistance as measured by the size and number of parasites. Katz (1963) found that gonadectomized animals always had more worms than sham-operated animals. The temptation was to explain this in terms of the effect of estradiol, but it was concluded that an intricate balance of hormones may play a more important role than either of the sex hormones. Roosters are invariably reported to maintain higher populations of northern fowl mites than are hens (Maw et al., 1935; Abasa, 1965; Matthysse et al., 1974; Hall and Gross, 1975).

In an intriguing series of experiments, Rothchild and Ford (1964) found that external application of hydrocortisone and corticosterone would cause ovarian maturation in rabbit fleas (Spilopsyllus cuniculi Dale) fed solely on castrated male or virgin female rabbits. Under normal circumstances, rabbit flea females can mature their ovaries only by feeding on a doe rabbit during the last ten days of pregnancy or on new-born baby rabbits during the first five or six days of life

when artifacts of the mother's hormone complement are still present.

Chemical control of northern fowl mites has received considerable attention from entomologists, parasitologists, and poultry specialists. Furman (1953) listed the requirements for a good poultry acaricide: 1) effective control, 2) simple and rapid applicability, 3) residual control effect, 4) absence of tainting qualities, 5) low toxic hazard to poultry, and 6) low cost. The choice of acaricides may be influenced largely by the management system employed; birds in wire cages must be treated directly while floor-managed flocks can contact materials applied to roosts and litter. Chemical effectiveness is generally evaluated via applications targeted at infested birds, but Furman (1953) and Foulk and Matthysse (1964) described precise laboratory techniques for assessing acaricide toxicity to replicated mite samples.

Nicotine sulfate alone and in combination with other compounds was a standard poultry acaricide for many years (Payne, 1929; Cameron, 1938; Moore and Schwardt, 1954; Foulk and Matthysse, 1963). The toxicity of this material to poultry necessitated application as a roost, litter, or premise treatment and limited its usefulness in caged layer operations. Sulfur as a dust or an emulsion has been effective in reducing poultry mite populations, but because birds must be handled individually the practicality of this approach is reduced in flocks containing many thousands of birds (Furman, 1953; Foulk and Matthysse, 1963).

The advent of synthetic organic insecticides permitted evaluation of numerous compounds for poultry ectoparasite control. DDT (1,1,1-trichloro-2,2-bis=(p-chlorophenyl) ethane) is relatively ineffective

against northern fowl mites (Povar, 1946), as are lindane (1,2,3,4,5,6-hexachlorocyclo=hexane, 99 percent or more gamma isomer), and methoxychlor (1,1,1-trichloro-2,2-bis=(p-methoxyphenyl) ethane) (Ritcher and Insko, 1948; Peterson, 1949; Furman, 1953), although Hoffman (1956) found that in some instances lindane could provide adequate control. Other chlorinated hydrocarbond showed initial promise as poultry acaricides. Neotran (bis-(p-chlorophenoxy) methane) 10.0 percent dust was effective, but this material performed only erratically when applied as 0.5 to 2.0 percent sprays. Toxaphene (bicyclic terpene camphene chlorinated to 67-69 percent) applied as 10.0 percent dust produced satisfactory mite control (Furman, 1953) but was undesirably toxic to poultry.

Acaricides such as Sulphenone® (parachlorophenyl phenyl sulfone), Dimite® (di-(p-chlorophenyl) methyl carbinol), Aramite® (2-(p-tert-butylphenoxy)-1-methyl ethyl 2-chloroethyl sulfite), and ovex (p-chloro phenyl p-chlorobenzenesulfonate) exhibited limited effectiveness when applied directly to birds (Furman, 1953).

Of organophosphorous compounds, malathion (diethyl mercaptosuccinate, S-ester with O,O-dimethyl phosphorodithioate) has received perhaps the widest attention for control of northern fowl mites. Initial reports of its effectiveness (Vincent et al., 1954; Furman and Coates, 1957) indicated that the compound exhibited numerous advantageous characteristics and residual effect for approximately 30 days in either dust or spray form. Rodriguez and Riehl (1958, 1959, 1960a, 1960b) demonstrated the utility of malathion for mite control on caged poultry; Harding

(1955) and Simco et al. (1962) for dust treatments to litter and nests, and Hoffman (1956) for dusts applied to flocks housed on wire. However, Foulk and Matthysse (1963) indicated that malathion failed to give satisfactory control of northern fowl mites in northern New York beginning in 1960. Rodriguez and Riehl (1963) reported mite resistance to malathion in California with cross resistance extending to other organophosphorous compounds, but not to carbaryl.

Reports of the effectiveness of carbaryl (1-naphthyl-N-methylcarbamate) (Hoffman, 1956, 1960; Kraemer, 1959; Simco et al., 1962; Foulk and Matthysse, 1963; Furman and Lee, 1969) show it to be an effective mite control agent when applied as spray or dust to litter or directly to birds. Carbaryl has also been shown to provide northern fowl mite control systemically when fed to chickens (Kraemer and Furman, 1959; Furman and Peiper, 1962).

Coumaphos (O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl)-O,O-diethyl phosphorothioate) also has been shown to be an excellent acaricide for northern fowl mite control (Kraemer, 1959; Hoffman, 1960; Knapp and Krause, 1960; Knapp, 1962; Simco et al., 1962; Foulk and Matthysse, 1963). Shaw et al. (1964) studied coumaphos residues in poultry tissue and eggs with the conclusion that birds exposed to dust and fog treatments retained only minute quantities of the compound.

Ronnel (O,O-dimethyl O-2,4,5-tri=chlorophenyl phosphorothioate) has been investigated with respect to toxicity to poultry mites (Linkfield and Reid, 1958; Hoffman, 1960; Knapp and Krause, 1960;

Foulk and Matthysse, 1963). Smith et al. (1965) demonstrated that treatment of penned birds with 5.0 percent granular formulations produced low residues in eggs which affording excellent mite control.

Dicapthon (O-(2-chloro-4-nitrophenyl) O,O-dimethyl phosphorothioate), dioxathion (S,S'-p-dioxane-2,3-diyl O,O-diethyl phosphorodithioate, cis and trans isomers) (Hoffman, 1960), tetradifon (p-chlorophenyl benzene=sulfonate), diazinon (O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate), naled (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), dichlorvos (2,2-dichlorovinyl dimethyl phosphate)(Foulk and Matthysse, 1963), and deet (N,N-diethyl-m-toluamide) (Kraemer, 1959) have been evaluated for acaricidal or repellent properties against the northern fowl mite.

A compound which has recently come into widespread use is stirofos (2-chloro-1-(2,4-dichloro=phenyl) vinyl diethyl phosphate), initially reported by Furman and Lee (1969) to be highly effective against poultry ectoparasites. Hall et al. (1975) found that chlordimeform (N'-(4-chloro-o-tolyl)-(N,N-dimethyl formamidine))afforded protection equal to that of stirofos, but at only one-tenth the concentration. Chlordimeform works against amino acids; this is a different mode of action than that employed by organophosphates.

At least two relatively non-toxic materials have been tested for control effectiveness against the northern fowl mite. Santocel C®, a powdery silica aerogel designed to act as a dessicant and abrading agent was found by Kraemer (1959) to produce insufficient penetration of poultry plumage. Preliminary tests conducted by E.C. Turner, Jr.

(VPI&SU) indicate that Exxon BPRL-5344-11 and BPRL-5344-16, light viscosity mineral oils, were moderately effective in controlling northern fowl mites when applied in the pure form to poultry vents at the rate of 10 to 20 ml per bird. Control was unsatisfactory when the materials were sprayed as emulsions using high pressure equipment.

Furman and Stratton (1963, 1964) reported an intriguing series of experiments in which sulfaquinoxaline (N-(2-quinoxalinyl) sulfanilamide), a drug used for control of poultry diseases such as coccidiosis, fowl cholera, and fowl typhoid, was noted to decrease mite populations following low-level administration of the compound in the feed. Experimental use of 0.033 percent sulfaquinoxaline in the feed reduced mite populations to zero within 5 weeks. Removal of the treated feed with birds subject to reinfestation resulted in mite populations reestablishing their former level in an additional 5 weeks. Unfortunately, various field trials showed sulfaquinoxaline to be unsuitably dangerous, causing internal hemorrhage in birds; therefore, its use has largely been curtailed.

Staal (1975) stated that insect growth regulators have not been effective against Acarina at moderate doses. It was postulated that these arthropods may rely on mechanisms for reproduction and metamorphosis different from insects, and that insect-active chemical structures are not appropriate for mites. Kitaoka (1970) found that ponasterone A, beta-ecdysone, and inokosterone application to _Ornithodoros_ _moubata_ (Murray) manifested oviposition-inhibitory, super-moulting, and killing effects on engorged adults at doses

ranging from 0.5 to 10.0 ug per ml of blood ingested through an artificial membrane. The compounds also caused moulting in non-moulting nymphs fed an insufficient amount of blood, or a non-blood meal.

At present, malathion, carbaryl, coumaphos, and stirofos are the only compounds registered in Virginia for northern fowl mite control on commercial poultry (Anon., 1977).

MATERIALS AND METHODS

Laboratory studies on host resistance to northern fowl mites.

Test animals. This work was conducted from 1975 to 1977 in isolation rooms at the Veterinary Science Disease Research Area, VPI&SU, Blacksburg. In all cases, birds tested were specific pathogen free Cornell random bred Leghorn chickens. Three lines were used: 1) birds genetically selected for a high (HPC) or low (LPC) level of plasma corticosterone response to social stress; 2) birds selected genetically for high (HP) or low (LP) persistence of antibody titer in response to sheep red blood cells; and 3) birds from non-selected colonies. The average age of the birds at the beginning of each experiment was between 12 and 16 weeks except where noted otherwise.

All experimental chickens (except in the extreme high stress environment) were given feed (a specially prepared drug-free mash) and water *ad lib*. Temperature and relative humidity were continually monitored with calibrated recording hygrothermographs. Large windows in each room allowed all cages to receive equal illumination; no supplementary lighting was used.

Cages.

Intravenous steroid administration. The cages used in this work consisted of eight in-line plywood units 40 cm deep, 30 cm high and 12.5 cm wide each. Birds were placed singly into these cages so that their heads protruded through a slot at the front where feed and water were easily reached. Lateral movement was limited by solid partitions and a steel rod prevented the occupants from backing out.

Fecal matter was deposited over the rear margin of the units and removed daily. Chickens could be held restricted in this device for indefinite periods.

High and low stress environments. The caging system used for evaluation of low stress environments was arranged in an isolation room and consisted of 32 in-line plywood cages each 23 cm wide, 45 cm deep and 30 cm high. The units were divided equally into 2 tiers. The front, back and floor of all cages consisted of 2.5 cm mesh wire screen; neighboring units were separated by solid plywood walls extending forward to the front margin of the feed cups, thus insuring visual isolation of each chicken. Feed and water were provided to each cage via stainless steel cups situated at the front and accessible through openings in the wire screen. A supplementary automatic watering system was installed also; however, water cups were used because isolated chickens often fail to learn proper use of automatic waterers. The low stress caging system is illustrated in Figure 1.

The high stress environment (Figure 2) was constructed in an isolation room adjacent to the low stress system. Consisting of seven linearly-arranged plywood units each 80 cm deep, 50 cm wide, and 50 cm high, the front, back and floor of each cage was constructed of 2.5 cm mesh heavy wire screen, and the partitions between individual units were solid plywood. Feed was provided via individual troughs at the front of each cage and was accessible via portals cut in the wire. An automatic watering system was furnished with two separate cups at the rear of each pen. Manure from each cage was allowed to accumulate

Figure 1. Low stress environment cages at the Veterinary Science Department. Chickens were caged singly in these units.

Figure 2. High stress environment cages at the Veterinary Science Department. Several chickens were confined in each cage.

on the concrete floor of the room and was removed at periodic intervals.

Steroids and blocking agents. Corticosterone (Kendall's Compound B; delta^4-pregnen-11-beta,21-diol-3,20-dione), desoxycorticosterone (Riechstein's Substrate Q; delta^4-pregnen-21-ol-3,20-dione), and dexamethasone (a synthetic adrenal cortical steroid analog; 9-alpha-fluoro-16-alpha-methylprednisolone) were obtained in 1.0 to 10.0 gram lots from the Sigma Chemical Co., St. Louis, Missouri.

Metyrapone (Metopirone®; 2-methyl-1,2-di-3-pyridyl-1-propanone), an adrenal steroid blocking agent, was obtained in kilogram lots from the Ciba-Geigy Corp., Greensboro, North Carolina.

Perthane® (Q-137; 1,1-dichloro-2,2-bis (p-ethylphenyl) ethane) and Rhothane® (DDD, TDE; 1,1-dichloro-2,2-bis=(p-chlorophenyl) ethane), chlorinated hydrocarbon insectcides of the DDT family, were obtained in technical form from the Rohm and Haas Co., Philadelphia, Pa.

Estrogen (ECP®, estradiol cypionate; 17-(beta)-cyclopentylpropionate ester of alpha estradiol) was obtained in 10.0 ml lots each containing estradiol cypionate (2.0 mg), chlorobutanol anhydrous, and cottonseed oil. The source of supply was The Upjohn Co., Kalamazoo, Mi.

Methods of administering steroids to test animals.

Intravenous administration. Glass 10.0 ml syringes were automated by placing them in a holding bracket so the plungers were gradually depressed by sliding bars driven by a long threaded rod turned by a small 36 revolution per day synchronous electric motor. The pitch of the threading was such that during a 24-hour period the

plungers would be depressed sufficiently to expel 10 ml of liquid. Six syringes could be operated simultaneously by this device.

Teflon® and polyethylene tubing was used to convey the liquid from each syringe to the appropriate bird. This was accomplished by first penetrating the brachial vein on the undersurface of the wing with an 18 ga. hypodermic needle and subsequently inserting the thin-wall Teflon® tube through the needle and as far as possible into the proximal reaches of the vein. Holding pressure over the incision to prevent hemorrhage, the needle was withdrawn over the free end of the tubing and a stainless steel coupler was affixed to permit attaching the Teflon® tube to identically-sized polyethylene tubing connecting to the appropriate syringe. Surgical tape was pressed to the tube at the point where it exited the brachial vein, and this was sutured to the undersurface of the wing. The treated wing was then furled against the body and held in place with surgical tape.

Experience with the above method of affixing the tubing to the wing demonstrated that tubes often pulled free. Therefore, on the second experiment using this technique, the Teflon® tube was secured in a different manner. After the needle had been withdrawn, it was passed subdermally for approximately 1.0 cm in front of and at right angles to the axis where the tubing exited the brachial vein. By running the free end of the tubing through the needle, it could be looped under the skin. When the needle was withdrawn a second time, the tubing was sutured into the fold of skin where it crossed over itself at a right angle. The needle was then inserted through the top of the

wing near the base, and the tubing passed upward through the orifice. When the wing was furled with surgical tape there were no additional cases of tubing loss. Using this technique, 10 ml of liquid could be administered daily directly to the blood system.

Corticosterone was prepared for administration via this system by first weighing appropriate quantities on an electronic balance and then dissolving the material in small quantities of ethanol. Physiological saline was added to bring the volume to the point where 10 ml of the final liquid preparation contained the requisite amount of steroid. The small amounts of ethanol used were readily metabolized by the birds, and no ill effects were noted.

Dietary administration. The following materials were added to feed at various times during the course of these experiments: corticosterone, desoxycorticosterone, dexamethasone, metyrapone, Perthane® and Rhothane®. In all cases, the following direct technique was applied: sufficient material for 8 to 10 kilogram lots of final feed was weighed on an electronic balance and subsequently dissolved in 10 ml of acetone. This solution was then added to approximately 250 ml ethanol and sprinkled over weighed amounts of feed spread in thin layers on a concrete surface. The feed was thoroughly mixed during the application procedure, thus insuring even distribution of the steroid or blocking agent solution. After the ethanol evaporated, the material was fed to the appropriate chickens.

Administration of estrogen. The estradiol cypionate solution previously described was intended for injection and was used in this

manner. Roosters were injected using tuberculin syringes according to a weekly regimen, alternating between intramuscular placement deep in the breast or thigh.

<u>Topical application of corticosterone</u>. A stock solution was prepared by dissolving 20.0 mg corticosterone in 2.0 ml acetone and adding this to 10.0 ml absolute ethanol. The total volume was then brought up to 200 ml with distilled water for a concentration of 100 ppm corticosterone. One hundred ml of the stock solution was diluted with an equivalent amount of distilled water to produce a 50 ppm solution, and serial dilutions were continued in this fashion until sequential doses ranging down to 12.5 ppm were obtained. A control spray was prepared by adding 2 ml acetone and 10 ml ethanol to 88 ml of distilled water.

Application of the corticosterone and control material to the test birds was accomplished by atomizing the liquid with a glass misting device. A measured amount of material was placed into the device, and all birds receiving identical treatment were sprayed until the vent area was thoroughly wet. Measurement of the remaining liquid revealed that an average of 4.4 ml of fluid was applied per chicken.

<u>Mites</u>.

<u>Source of infestation and identification</u>. Hens severely infested with northern fowl mites were procured from a commercial egg-production establishment in Montgomery County, Virginia, in early 1974. Samples of mites from these hens were mounted on glass slides in Hoyer's Medium and identified as *Ornithonyssus sylviarum*. Confirmation of this

identification was made by D.E. Johnston (Professor, Acarology Laboratory; Ohio State University, Columbus, Oh.).

Roosters from the LPC line were infested with northern fowl mites obtained in the above manner and maintained at the Price's Fork Research Center in ventilated Horsefall units. Mites from this population served as infestation foci for all subsequent experiments.

Transfer of these mites to experimental birds was accomplished by brushing approximately 75 live mites from an infested feather onto the upper and lower vent region of each experimental bird. In certain experiments, identified later, a larger innoculum was used to promote rapid population expansion. Care was taken to make the transfers as uniform as possible.

Methods for counting and estimating mite populations. Mite populations were recorded at periodic (typically 5-day) intervals as actual numbers of parasites. All counts were made by the principal investigator using bright artificial light or a battery-operated headlamp and holding the bird so that the vent region could be inspected easily (Figure 3). Counts were confined to the region directly above and below the vent. Each bird was examined also over the rest of the body, though mites were seldom found away from the vent area. The counting procedure was to record each mite singly up to a total of 50, to the nearest five from 50 to 100, to the nearest 25 between 100 and 500, and to the closest 100 thereafter. Only sclerotized or blood-fed mites were visible using this procedure. Subsequent microscopic analysis of mite populations on feathers demonstrated that this tech-

Figure 3. The author examining a rooster for northern fowl mites. Counts were confined to the vent region of the bird.

nique consistently produced values at approximately 40 percent of the true total population of all life stages present. Because of the consistency of measurements obtained via this procedure, no corrections in values were made.

Analysis of life stages. At the termination of Experiments 2 and 3, feathers bearing "typical" mite populations were plucked from various birds under several steroid regimens and subjected to microscopic analysis. The mites were washed from the feathers in 80 percent ethanol and placed in watch glasses divided into 8 equal portions by lines scribed on the bottom. Using a binocular microscope set at 10X magnification, the mites were classified as adult, fed nymph, unfed nymph or larva, or egg. Each sample was assessed 3 times, agitating between observations. Only those mites resting in one of the 8 divisions were counted at each observation.

Additional observations.

Haematocrit readings were made periodically on blood from birds in the various experimental flocks to determine red blood cell volume. This was done by removing a small sample of blood from the brachial vein of each chicken with tuberculin syringes and 27 ga. needles. The blood from individual birds was transferred to heparinized micro-capillary tubes which were then plugged on one end with soft clay. The tubes were processed for several minutes at 10,000 rpm in a haematocrit centrifuge, and the packed red blood cell volume read directly in percent from a graduated scale.

Post-mortem examination was carried out on the chickens from

Experiment 7. At this time the birds were weighed, and the body cavity opened. Weights for the spleen, liver, thymus, bursa of Fabricius, and testes were measured on an electronic balance.

Skin histology. At the time of autopsy, several chickens from all treatments in Experiment 7 were evaluated for differences in skin histology. Small sections of tissue were removed from the vent of each bird directly above the cloaca. These were preserved in Gilson's Fluid (a mercury-base fixative). After one week, the samples were dehydrated by running them through sequentially-increasing concentrations of ethanol. After one hour in absolute ethanol, each section was transferred to xylene, then to baths of 50 percent xylene, 50 percent parafin, and finally to melted purified parafin (M.P. 61 C). Each sample was then embedded in parafin blocks and sectioned in 12.0 micron layers. Several sections from each sample were mounted on glass slides, rehydrated, stained with Mallory's triple-staining process, dehydrated, and cover slips attached. Measurements of skin thickness were made using a constant magnification employing a phase-contrast binocular microscope with a calibrated micrometer eyepiece. At this time, additional histological observations were made.

Experimental procedure.

Experiment 1 (Effect of Intravenous Corticosterone Administration. Four 16-week-old LPC roosters and 4 HPC roosters of equal age were placed in the restraining units and started on a diet containing metyrapone (500 ppm) to inhibit corticosterone synthesis. After several days' stabilization, one LPC and one HPC rooster were started

on an intravenous corticosterone regimen of 1.0 mg per week, and two additional LPC roosters were started on doses of 10.0 mg per week. The remaining birds were left as untreated controls.

After 5 days, each chicken was challenged with approximately 75 northern fowl mites of all stages, and population assessment of mite development was made at 5-day intervals thereafter for 21 days.

Experiment 2 (Effect of Intravenous Corticosterone Administration. Six LPC roosters approximately 40 weeks old were assigned treatments as follows. Two individuals received intravenous administration of corticosterone at the rate of 1.5 mg per day, 3 were provided with feed containing metyrapone (500 ppm) and 1 was left as a control. During the initial stabilization period, all birds were fed metyrapone to suppress corticosterone synthesis. Five days after initiation of intravenous steroids, all birds were challenged with approximately 1500 live mites of all stages. Subsequent population assessments were made at 3-day intervals for the next 20 days.

Experiment 3 (Effect of Corticosterone, Desoxycorticosterone, and Dexamethasone in the Feed). Twenty four LPC-line and 8 HPC-line 16-week-old roosters were placed singly in cages in the low stress environment. Four groups of 6 LPC birds each were randomly selected and assigned one of the following treatments: corticosterone (20 ppm), desoxycorticosterone (30 ppm), dexamethasone (10 ppm), or control (non-treated feed). Eight HPC-line roosters were divided into 2 groups of 4 birds each. One group received desoxycorticosterone (30 ppm) in the feed; the other served as an untreated control. After 5 days'

stabilization, all birds were challenged with approximately 75 live mites of all stages. Subsequent assessment of mite population development was made at 5-day intervals for the next 27 days.

All birds in the experiment were weighed at the beginning of steroid administration and periodically thereafter.

At the termination of the experiment, samples of mite-infested feathers were removed from LPC-line roosters receiving corticosterone, desoxycorticosterone, and control treatments and analyzed for life stages present.

Experiment 4 (Effect of Desoxycorticosterone and Metyrapone in the Feed). Thirty one 12-week-old chickens grouped and treated as follows were used during this test. All were randomly assigned cages in the low stress environment room. LPC-line roosters: 4 birds - metyrapone (500 ppm), 4 birds - desoxycorticosterone (60 ppm), 3 birds - untreated control. LPC-line pullets: 4 birds - metyrapone (500 ppm), 4 birds - desoxycorticosterone (60 ppm), 4 birds - untreated control. HPC-line roosters: 3 birds - metyrapone (500 ppm), 3 birds - desoxycorticosterone (60 ppm), 2 birds - untreated control.

A 12-day stabilization period was allowed between the time when steroid administration was begun and each bird was challenged with approximately 75 live mites of all stages. Subsequent checks for mite population development were made at 5-day intervals for the next 29 days.

Experiment 5 (Effect of Corticosterone and Perthane® in the Feed). Twenty eight 20-week-old LPC-line roosters were randomly

divided into 7 groups of 4 each and assigned communal cages in the high stress environment room. Treatments were assigned randomly as follows: 2 cages received corticosterone (20 ppm), 2 cages received Perthane® (500 ppm), and 3 cages served as controls. The chickens were left 3 weeks to acclimate to the environment and then challenged with approximately 75 live mites each.

The corticosterone administration continued throughout the experiment; however, feeding of Perthane® was terminated after 3 weeks.

Assessment of mite population development was made at 5-day intervals for a period of 29 days.

Experiment 6 (Effect of Graded Dosages of Corticosterone and the Effect of Rhothane®). Twenty six 40-week-old LPC-line roosters were randomly assigned cages in the low stress environment quarters. These chickens had been moved several times during their lifetimes and had spent at least one month in an outside communal ranger. They were randomly assigned steroid treatment in groups as follows: 5 birds - corticosterone (10 ppm), 5 birds - corticosterone (20 ppm), 5 birds - corticosterone (40 ppm), 5 birds - Rhothane® (500 ppm), and 6 birds - untreated control.

The test birds were permitted several weeks in which to adjust to their surroundings, and then steroid treatments were started. One week later all chickens were innoculated with approximately 75 live mites each. Inspections for assessment of mite population development were made at 5-day intervals for the succeeding 29 days.

Experiment 7 (Effect of Graded Doses of Corticosterone and

Desoxycorticosterone). Thirty two 12-week-old cockerels from the Poultry Science random bred Cornell white Leghorn colony were randomly assigned cages in the low stress environment. Treatments were randomized throughout the room. The following numbers of chickens received steroid dosages: 5 roosters each - corticosterone (15 ppm), corticosterone (30 ppm), corticosterone (45 ppm), desoxycorticosterone (30 ppm), desoxycorticosterone (80 ppm); 7 roosters - untreated controls.

At the same time, 56 roosters of the same age and from the same base colony were placed in groups of 8 into the 7 communal cages in the high stress environment. Steroid treatments were randomly assigned to each cage as follows: corticosterone (10 ppm), corticosterone (20 ppm), corticosterone (40 ppm), desoxycorticosterone (30 ppm), desoxycorticosterone (80 ppm). Two cages were initially reserved as controls; however, the automatic watering system malfunctioned in one unit immediately after the chickens were installed. The resulting deprivation severely affected the occupants, and they were not included in the analysis of the experiment.

To insure an extreme amount of social interaction in the high stress environment, moderate feed deprivation was practiced in the following manner. Troughs containing the feed allotment for each cage were allowed to become completely depleted before being refilled. No cage was left without feed for more than 12 hours. The large number of birds per cage and the resultant anxiety over food allocation caused an extreme amount of in-fighting among birds quartered together.

One week was allowed for the chickens to adjust to the experimental

procedure before the steroid regimen was begun. One day later, all birds were challenged with approximately 75 mites each. Assessment of mite population development was subsequently made at weekly intervals.

On the first observation period subsequent to infestation, it was noticed that mite populations among the high stress colony were extremely low. These birds were then reinfested with an additional innoculum equivalent to the original.

At the termination of the experiment, blood samples were taken from all chickens for lymphocyte analysis. At the same time, each bird was innoculated with 0.1 ml of 0.5 percent horse erythrocytes to determine antibody response. One week later all birds were killed and subjected to post mortem examination.

Experiment 8 (Effect of Antibody Persistence). Five HP and 7 LP 24-week-old roosters were divided into groups of 2 or 3 from each line and placed alternately into communal cages in the high stress environment. After a 2-week stabilization period, all birds were challenged with approximately 75 live mites each. Mite population development was assessed at weekly intervals for the ensuing month.

No feed deprivation was practiced during this test, and no artificial steroids were administered.

Experiment 9 (Effect of Estrogen). Forty two 24-week-old LPC-line roosters were divided into 7 groups of 6 birds each and placed at random into communal cages in the high stress environment. Estrogen treatment was randomly assigned to each pen and was identical

for all birds contained therein. The treatments used were 0.1, 0.2, 0.3, 0.4, and 0.5 mg per week. Two cages were reserved for controls; however, several chickens in one control cage died of unknown causes and the entire group was not included in subsequent analysis.

Experiment 10 (Topical Application of Corticosterone). Five groups of 6 LPC-line 30-week-old roosters were housed in communal cages in the high stress environment. All birds were infested with equal numbers (ca. 75 each) of northern fowl mites. After 4 weeks all birds were supporting moderately large mite populations.

Corticosterone was applied topically to all birds from 4 cages as described earlier. The 5th cage served as control and received a spray containing only acetone, ethanol, and water.

Pre-treatment mite counts were made for all experimental birds immediately before the steroid solution was applied. Two days later all birds were retreated with identical solutions. Four days after the final spray each bird was evaluated for mite population and averages calculated for all treatment levels.

Laboratory studies using bed bugs receiving known doses of corticosterone. Numerous attempts to induce northern fowl mite adults, nymphs, and larvae to feed through various artificial membranes were unsuccessful. Materials tested included parafin film, fresh chick skin, and animal intestine. Raising the temperature of the blood reservoir to 104 F and affixing small feathers to the membranes to simulate normal environments did not induce feeding behavior. To determine if corticosterone itself was responsible for reduced ectoparasite

burdens, it was decided to test a blood-feeding insect which would engorge readily through artificial substrates.

Test insects. Sufficient numbers of *Cimex lectularius* L. (Gainesville "normal" strain) to start a colony were procured from the Insects Affecting Man Laboratory, USDA-ARS, Gainesville, Fla. These were placed in quart ice-cream containers from which the tops had been removed and replaced with fine-mesh nylon bolting. The colonies were kept in environmental chambers set to maintain 80 F and 80 percent R.H. and fed weekly on restrained laboratory rabbits.

Preparation of diets. Roosters from the LPC-line maintained at the Price's Fork Research Center for northern fowl mite colonization were used as blood doners for this work. Five ml of blood was drawn from each rooster and placed in a common vessel. Clotting was avoided by insuring that 10 percent of the total volume was comprised of a 2.5 percent sodium citrate solution in distilled water.

The pooled blood sample was then divided into 2 equal portions and one saved as a control. Between uses, all blood diets were frozen at 0 F; rupture of red blood cells was minimized by adding a small amount (2.5 percent) of glycerol to each sample.

Glass 4-dram vials were used to hold the final diet preparations. Corticosterone was added to test vials in the following manner. A stock solution was prepared by dissolving 160 mg of corticosterone in 1.0 liter of distilled acetone. One ml of this solution was then added to 999 ml of distilled acetone; consequently, 1 ml of the final solution contained 160 nanograms (10^{-9}g) of corticosterone. Three test vials

were prepared per dose by adding either 0.25, 0.5, or 1.0 ml of the corticosterone solution and allowing it to evaporate. Subsequently, 4.0 ml of blood were added to each vial, producing final corticosterone concentrations of 10, 20, or 40 nanograms per ml blood. Three control vials were prepared using no corticosterone solution.

The membrane used throughout this experiment was extremely thin lamb caecum. Small squares ca. 2 cm per side were stretched over the mouths of all vials. Rubber bands were used to hold the membrane in place. A small amount of glycerol was rubbed into the membranes thus prepared to insure flexibility.

Heating apparatus for bed bug diets. In order to insure that all blood diets were kept at the approximate body temperature of warm-blooded hosts (ca. 100 F), a device was constructed to hold 12 test diets at constant temperature (Figure 4). A rectangular wooden box 45 cm long, 12.5 cm deep, and 12.5 cm high was provided with 12 holes sufficiently large so that a 4-dram vial could be inserted. The holes were drilled on the side of the box and were slanted obliquely; consequently, liquid contained in the vials collected at the exterior margin. Two plywood arms were screwed to the ends of the box and permitted a wooden holding tray to be raised or lowered in front of the row of holes. This tray was provided with shallow holes into which were placed 12 small bed bug colonies. In this manner, all test colonies could be brought into contact with the appropriate blood vial.

The wooden box was filled with clean sand to the level of the holes drilled along its side and a 140 watt heating tape buried the entire

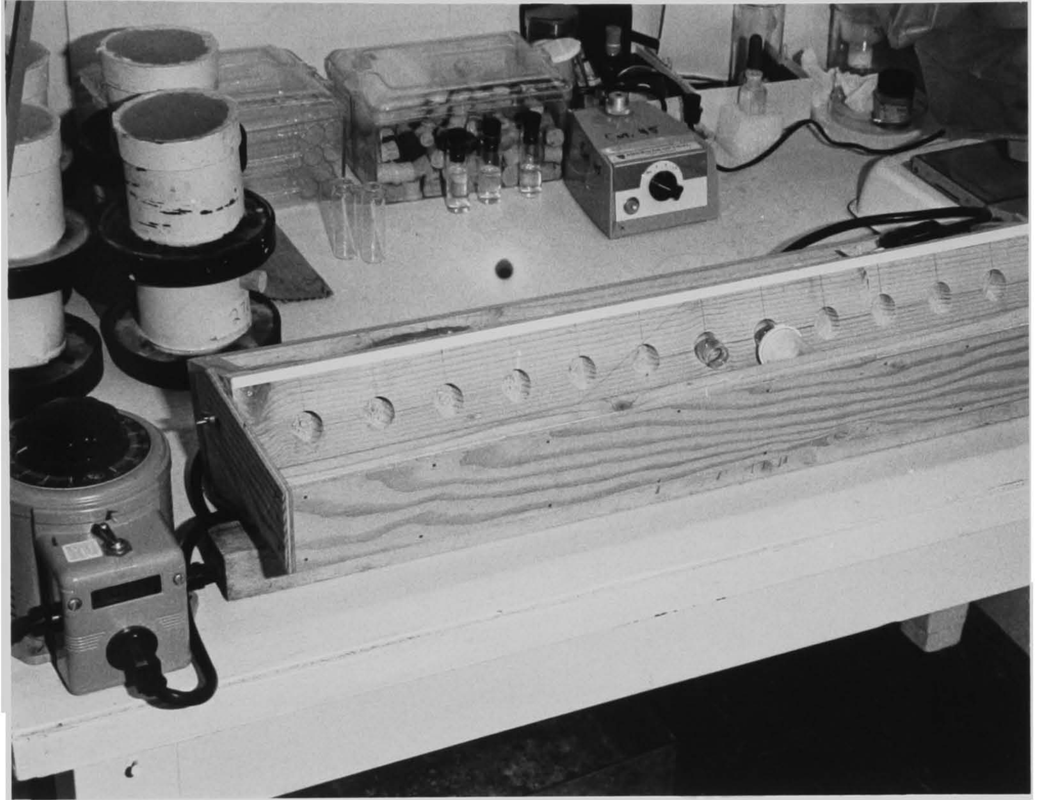

Figure 4. The apparatus used to artificially feed bed bug colonies blood meals containing added amounts of corticosterone. A variable transformer provided precise temperature control.

length of the device. A variable transformer was connected to the heating tape and permitted precise adjustment in temperature. The sand, when warm, provided sufficient mass so that temperature fluctuations were minimal. Proximal ends of the 4-dram blood-filled vials were covered with warm sand and the contents of all vials raised to 100 F via this method.

Test colonies of bed bugs. Fifteen-dram plastic snap-cap vials were used to house bed bugs during the feeding experiments. The caps were removed and 2 cm holes cut through the centers. The caps were then used to hold squares of fine nylon mesh in place across the mouths of the vials. Each vial was provided with stiff paper running around half the inside margin from top to bottom. The paper provided a substrate over which the bed bugs could climb easily.

Experimental procedure. Twenty four bed bug colonies were prepared by placing 1 male and 1 female bed bug adult into each 15-dram vial. The bugs had been starved for 1 week prior to formation of the colonies to minimize oviposition not induced by the test blood meals.

The colonies were divided into 2 groups of 12 each and assigned steroid treatments at random. Three colonies were used per treatment. After all blood diets had stabilized at 100 F, each colony was placed into the holding rack in front of the appropriate vial and the mesh screens brought into contact with the membrane. The slant of the vials caused blood to lie against the membrane and consequently be available to the bugs.

Each colony was permitted to remain affixed to the heated blood diet for 15-minute periods weekly. During the remainder of the time, colonies were returned to the environmental chamber. After 30 days, all bed bugs in the test colonies were killed with ethyl acetate fumes. The number of nymphs hatched and unhatched eggs in each colony at this time was recorded for each steroid treatment.

Field studies relating social stress and plasma corticosterone to northern fowl mite infestations. In the course of several large-scale acaricide effectiveness tests against northern fowl mites in commercial poultry quarters, it was noticed that chickens caged alone tended to support greater mite infestations than neighboring birds housed in groups. This was supported also by laboratory experimental data (Hall and Gross, 1975).

Locations sampled. Field observations were conducted at 2 locations in Virginia during 1975. The first was carried out at Rural Retreat in a shallow-pit building containing approximately 6,300 1-year-old white Leghorn hens housed two per cage in two tiers. Sanitary conditions were poor and a moderate northern fowl mite infestation was spread throughout most of the house.

The second experiment was conducted in Roanoke County. Approximately 16,000 1-year-old Rhode Island hens were housed in 30.5 cm wide three-tier cages over a deep manure pit. The hens were rotated according to row occupied; therefore, only 1 row (approximately 100 m long) contained large mite populations. Hens in the deep-pit house

were housed in groups ranging from 1 to 4; in the shallow-pit house cages were designed to hold a maximum of 2 hens.

Experimental procedure. Counts for northern fowl mite population assessment were made by 2 investigators each using battery-operated headlamps. Each tier of all rows was searched until singly-caged hens were located. These hens were then rated for mite infestation; subsequently, all hens in both adjacent cages were rated. The following scale was used to provide a mite population index for each chicken (Hall et al., 1975):

Index Number	Number of Mites
0	No live mites found
1	1-5 mites
2	6-15 mites
3	16-50 mites
4	51-100 mites
5	101-500 mites
6	500 or more mites

Approximately 15 seconds were required to rate non- or heavily-infested hens. Intermediate values required more time: up to 1 minute was spent searching the feather coat.

At Rural Retreat, blood samples were taken from 10 negatively-infested and 10 severely-infested hens selected at random. These samples were then analyzed for packed red blood cell volume.

Blood samples were obtained from 9 severely-infested hens caged alone and 8 negatively-infested hens in adjacent communal cages at the deep-pit house in Roanoke. These samples were taken with glass syringes and immediately processed by centrifuging in glass tubes at 5,000 rpm for 5 minutes. Dr. W.B. Gross (Veterinary Science Department, VPI&SU)

subsequently analyzed the blood samples for plasma corticosterone levels. The protein-binding analysis technique was employed and compared with standard curves previously derived at the laboratory. Standardized samples were included at random during the procedure as a check.

Evaluation of acaricide effectiveness against northern fowl mites.

Laboratory toxicological evaluations. Acaricides were tested for acute toxicity to northern fowl mites by using the technique described by Foulk and Matthysse (1964). Standard 14.6 cm Pasteur pipettes were prepared by glueing small squares of 100-mesh bolting cloth over the large end with epoxy cement. Because of the time involved completing this operation, several hundred pipettes were prepared in batches.

Acaricides were obtained in technical grade and dissolved in all cases with distilled acetone. Concentrations were recorded in grams per liter and subsequently converted to parts per million. Acaricide residues were established in the test pipettes by placing them in groups of 5 into large (25 X 200 mm) test tubes and filling these with test solution. After pouring excess liquid off, the tubes were drained on paper towels and allowed to dry thoroughly. Tubes were never allowed to remain for more than 1 hour before testing was begun. Tubes washed with distilled acetone alone served as controls in all cases.

Mites were aspirated into the test pipettes using an electric vacuum pump set to deliver a suction of approximately 3 lbs. per square inch. Plastic tubing connected the input nozzle on the pump with a

large glass tube used to hold the pipettes. Each end of the glass tube was closed by corks having holes drilled through the center. A glass rod seated firmly in one cork connected to the plastic suction line. The forward cork served as a holder for the test pipettes, which were inserted point-first into the hole. The cork was then replaced into the end of the large glass tube. Using this method, mites could be aspirated into the test pipettes where they were stopped by the bolting cloth glued on the large end. The narrow tips were then sealed by pushing them into bits of modeling clay.

Mites were procured before each test by bringing an infested rooster from the Price's Fork Research Center colony to the laboratory. Feathers containing large numbers of mites were placed into a sterilized porcelin tray under a fluorescent light bar. Twenty mites were aspirated into each test pipette. Only vigorous adult mites were used; this was accomplished by selecting mites which had actively crawled up the sides of the tray. Control pipettes were always filled first.

Five pipettes were used per concentration, and experiments were always repeated on separate days; therefore, response to each concentration included at least 200 mites.

Pipettes were held in an environmental chamber set to maintain a temperature of 80 F (26.7 C) and 80 percent R.H. for 18 hours after exposure to acaricide residue. Each pipette was then examined under a binocular microscope. Mites were counted as alive if any movement was noted.

Experimental mortalities were corrected for controls in all cases.

Control mortality was always under 5 percent. Dosages were converted to common logarithms and corrected mortalities to probits. Linear regression analysis was then used to derive an LC_{50} value for each compound.

Simulated field application of permethrin. Initial tests on individual mite-infested roosters showed that permethrin ((3-phenoxyphenyl)-methyl-(±)-cis,trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) produced effective control within 24 hours. Imperial Chemical Industries, Ltd., makers of permethrin, had not obtained an Environmental Protection Agency experimental use permit for the compound at the time these investigations were made. To assess the effectiveness of permethrin when applied to commercial flocks, a simulated field experiment was arranged using laboratory chickens. All birds were destroyed at the termination of this investigation.

Birds used. Twenty six LPC-line roosters 30 weeks old were randomly assigned cages in the low stress environment quarters. All birds were infested with northern fowl mites and the populations allowed to build up for 1 month.

Preparation and application of acaricide. Permethrin emulsifiable concentrate containing 4.0 pounds active ingredient per gallon was used to make 2 gallons of 0.5 percent A.I. solution (w/w). This solution was subsequently diluted twice on a 1:1 basis, producing additional concentrations of 0.25 and 0.125 percent permethrin. Six birds were assigned at random to each treatment; 8 roosters served as controls and were distributed randomly throughout the room.

A compressed-air stainless steel sprayer (2 gallon capacity) was used to apply the 3 concentrations to the appropriate chickens. The adjustable nozzle was set to deliver a coarse spray under 40 psi and all test animals were wet to run-off (ca. 1 gallon per 100 birds). Care was used to insure thorough coverage of the vent area.

Pre-treatment mite counts showed that all test groups were supporting equivalent mite populations. Mite populations on all chickens were assessed at weekly intervals for the next 35 days, and at periodic intervals thereafter. After 77 days the birds were destroyed. All counts were made using the mite population index described earlier for field evaluations.

Additional testing of permethrin. Two additional tests were conducted to evaluate the effectiveness of permethrin on chickens in floor-managed flocks. In the first, a small breeder flock containing 10 hens and 2 roosters housed on wire screen inside a large plywood frame building was treated with 0.05 percent permethrin EC. Pre-treatment counts showed that mite populations were equivalent on all birds. One rooster and 5 hens were then marked on the back with red dye and reserved as controls. The remainder of the flock was sprayed with the acaricide solution by holding each bird while the sprayer thoroughly wet the vent region.

The second test was conducted in 2 small plywood houses each containing small breeder flocks of approximately 10 hens and 2 roosters. These houses contained elevated roosting areas over floors covered deeply with wood shavings. Only the roosters in these houses were

treated with 0.05 percent permethrin EC. All roosters indexed 6.0 before treatment was applied.

Mite counts in these experiments were made at irregular intervals for the next 124 days.

Acute toxicity of permethrin and chlordimeform to chickens. To determine the safety margin available when permethrin or chlordimeform were applied to laboratory chickens, 2 tests were designed to evaluate 24-hour acute toxicity of these compounds. This was considered necessary because of the extremely low concentrations of these acaricides recommended by the manufacturers for northern fowl mite control.

Permethrin EC was diluted with water to produce final concentrations (w/w) of 5.0, 2.5, and 1.25 percent A.I. One male and 1 female chicken each were immersed for 30 seconds in one of the 3 concentrations. Only the head was allowed to remain above the surface of the liquid. In addition, a male and a female chicken were administered 10 ml of 1.25 percent permethrin orally, and a single rooster was given 10 ml of 25 percent stock permethrin EC solution in a single oral dose.

Chlordimeform soluble powder (SP) was diluted in water to produce final concentrations of 10.0, 5.0, and 2.5 percent A.I. (w/w). One male and 1 female chicken each were immersed for 30 seconds in one of the 3 concentrations. In addition, 2 female and 2 male birds were dosed orally with 10 ml of 10.0 percent chlordimeform.

All test birds were observed for several minutes post-treatment, and subsequently placed in steel cages. Provided with fresh feed and water, they were left for 24 hours. After this period, all chickens

were examined for overt signs of illness.

Statistical analysis of experimental data.

Laboratory experiments on resistance to northern fowl mites. Mite counts recorded as actual numbers of parasites were converted to logarithms (base 10) to normalize the distribution of data. Statistical techniques employed included analysis of variance (ANOVA), Student's t-test, Student-Newman-Keuls stepwise multiple range test, Chi-square test, and product-moment correlation coefficient.

Field tests. Mite indexes were analyzed using ANOVA, Student's t-test, and Chi-square tests; corticosterone analysis was tested via the Chi-square procedure.

Acaricide evaluations. Log-dose probit-mortality data was analyzed using linear regression and coefficients of determination. Simulated field data was analyzed via ANOVA and Student's t-tests.

RESULTS AND DISCUSSION

Laboratory studies on host resistance to northern fowl mite infestation.

Experiment 1 (Effect of Intravenous Corticosterone Administration). Doses of 1.0 and 10.0 mg of corticosterone per week produced profound systemic effects of roosters from the LPC-line (Table I). Three chickens rapidly weakened and died; symptoms included watery feces, listlessness, and reduction in feed intake. The control bird from the LPC-line demonstrated none of these ill effects.

One HPC-line rooster receiving 1.0 mg of corticosterone per week was able to maintain its homeostasis despite the steroid regimen. It is possible that birds selected for higher levels of plasma corticosterone possess sufficient substrate to utilize adequately the increased levels present during corticosterone therapy.

Northern fowl mite population development was not affected significantly by artificial administration of corticosterone during this experiment. All LPC-line chickens receiving steroid supported larger mite infestations than the single control bird. HPC-line roosters generally supported fewer mites. The difference between the LPC- and HPC-lines with respect to mite development has been demonstrated previously (Hall and Gross, 1975). The failure of steroid administration to reduce mite populations was attributed to inadequate dosage plus unsatisfactory attachment of the plastic tubing carrying the steroid solution. Several times during the course of the experiment, it was noted that the tubes supplying the steroid doses had pulled

Table I

Mite populations for all chickens tested during Experiment 1. Selected birds were given intravenous doses of corticosterone at the rate of 1.0 or 10.0 mg per week.

	BIRD LINE							
	LPC	LPC	LPC	LPC	HPC	HPC	HPC	HPC
CORTICOSTERONE DOSE:								
(mg/week - IV)	1.0	10.0	10.0	0	1.0	0	0	0
MITE POPULATIONS:								
DAYS POST-INFESTATION								
6	65	40	25	6	4	55	85	75
14	2000	2000	425	125	325	900	750	350
17	10000	15000	3000	1000	1800	1000	500	1100
20	dead	dead	dead					
21	-	-	-	3000	5000	350	1500	600

free from their attachment at the brachial vein on the undersurface of the wing. The interrupted flow of test material may have influenced the ultimate physiological effect on mite development.

The average daily temperature recorded during the course of this experiment was 67.3 F (19.6 C) and the average daily relative humidity 65.2 percent.

Experiment 2 (Effect of Intravenous Corticosterone Administration). Intravenous doses of corticosterone at the rate of 1.5 mg per day caused a reduction in northern fowl mite population development (Table II). The more secure method of attaching the plastic tubing into the brachial vein insured an uninterrupted flow of steroid solution during the entire course of the experiment.

Earlier work dealing with the effect of corticosterone administration on parasite burdens (Applegate, 1970; Applegate and Beaudoin, 1970) has involved single doses of steroid on a periodic basis. W.B. Gross (Veterinary Science Department, VPI&SU; personal communication) possesses data which show the *in vivo* half-life of corticosterone in the chicken to be approximately 20 minutes. It is therefore probable that single doses of corticosterone may be sufficient to stimulate the hypothalmus to cease CRF production. This in turn would cause cessation of ACTH output and consequently the synthesis of corticosterone by the adrenal cortex. I theorize that the briefly elevated plasma corticosterone levels resulting from single doses of steroid would be of insufficient duration to evoke the requisite physiological responses necessary for parasite resistance, and that in fact this procedure

Table II

Mite populations for all chickens tested during Experiment 2. Selected birds were given corticosterone (1.5 mg per day IV) or metyrapone (500 ppm in the feed).

TREATMENT	DAYS POST-INFESTATION						
	2	4	7	10	13	16	20
CORTICOSTERONE	15	55	75	250	600	1000	1200
CORTICOSTERONE	0	8	15	150	350	1500	3500
METYRAPONE	50	57	250	1000	5000	11000	16000
METYRAPONE	46	62	175	1200	6000	15000	18000
METYRAPONE	60	75	300	1800	10000	14000	12000
CONTROL	86	110	300	1500	5000	15000	15000

will result in an actual decrease of plasma corticosterone levels over time. The lag time before the biofeedback mechanism stabilizes itself following single corticosterone doses may be long enough so that steroid levels cannot be brought to their former titer before most of the artificially-induced dose has been metabolized.

Metyrapone administered in the feed at 500 ppm was shown to cause a slight increase in mite populations on the restrained LPC-line roosters. LPC-line chickens housed in visually-isolated restraining cages evidently perceive this environment as low-stress, and consequently exhibit very low levels of adrenal steroid production. This would account for the slight difference in mite susceptibility between control birds and those on a metyrapone diet.

Haematocrit readings for all experimental chickens taken midway through the course of this test showed all birds maintaining moderately high packed red blood cell volumes. The average daily temperature recorded during the experiment was 69.5 F (20.8 C) and the average daily relative humidity 56.6 percent.

Experiment 3 (Effect of Corticosterone, Desoxycorticosterone, and Dexamethasone in the Feed). Corticosterone administered in the feed at a concentration of 20 ppm significantly increased resistance to northern fowl mites over birds given a diet containing 30 ppm of desoxycorticosterone (Table III). No overlap was evident with respect to average mite populations on birds receiving corticosterone, desoxycorticosterone, or control treatments (Figure 5). Mite resistance of birds receiving dexamethasone therapy was extremely variable; physio-

Table III

Average mite populations on all LPC-line roosters tested during Experiment 3. Steroids were administered via the feed.

TREATMENT	NR REPS	DAYS POST-INFESTATION 1	6	11	16	21	27
CORTICOSTERONE (20 PPM)	5	18.4 a */	16.8 a	26.8 a	39.0 a	102.8 a	163.2 a
DESOXYCORTICOSTERONE (30 PPM)	4	63.5 b	175.0 b	462.5 b	1012.5 b	3250.0 b	4750.0 b
UNTREATED CONTROL	6	47.7 b	81.7 ab	133.3 a	311.0 a	726.0 ab	1439.2 ab

*/ - Means within columns not showing a common letter are significantly different at the 0.05 level by the ANOVA and LSR tests.

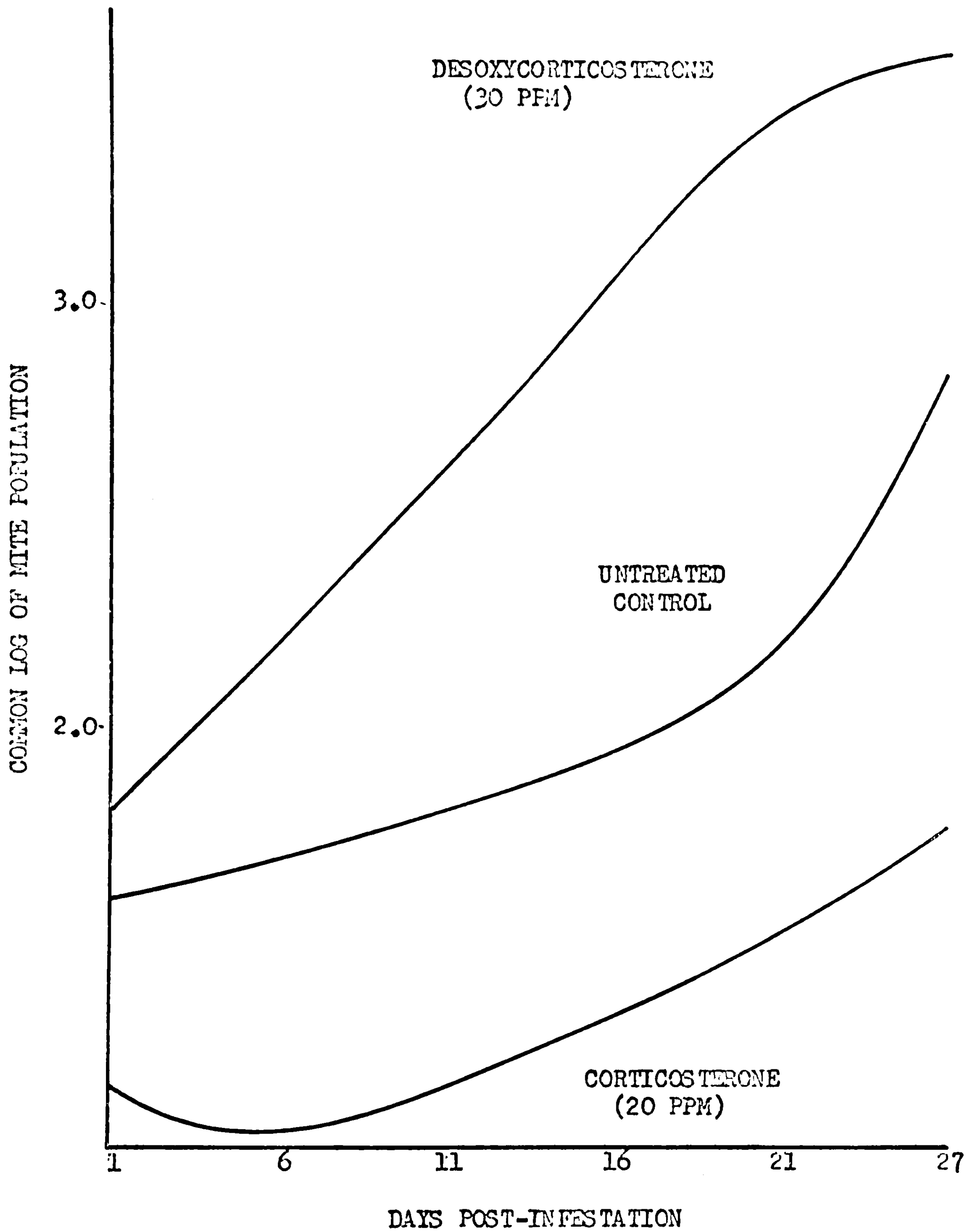

Figure 5. Average mite populations on three groups of LPC-line roosters during Experiment 3.

logical response to this compound included watery feces, listlessness, and reduced feed intake. Because of the severe side-effects induced by dexamethasone, it was not included in the analysis of mite populations or investigated further in subsequent experiments.

No significant differences were noted between control HPC-line roosters and those receiving desoxycorticosterone at 30 ppm in the feed (Table IV). It is interesting that the inherant resistance of this genetic line to infestation with northern fowl mites should be affected less by steroid administration than that of the LPC-line. Because the criterion for genetic selection involved the ability to adapt to factors influencing adrenal output, it is possible that the HPC-line birds can compensate for environmental and artificial factors designed to influence steroid levels.

Analysis of percentage weight gains achieved by all roosters used during this experiment (Table V) shows that only dexamethasone produced an average value different from the untreated control birds. The reduction in weight gain contributed to the decision to avoid further use of this compound. Weight gain is an important factor in determining how chickens are adapting to their environment.

Correlation of mite populations and percent weight gains (Table VI) produced relatively low negative values for all test groups. The overall correlation coefficient for all roosters was -0.43 ($P \leq 0.05$). The slight negative value obtained indicates that the experimental measurements were sensitive enough to measure the deliterious effect of northern fowl mite populations on the physiology of the test birds.

Table IV

Average mite populations on all HPC-line roosters tested during Experiment 3. Steroid treatments were administered via the feed.

TREATMENT	NR REPS	DAYS POST-INFESTATION 1	6	11	16	21	27
DESOXYCORTICOSTERONE (30 PPM)	4	41.5 a */	63.5 a	105.0 a	126.3 a	156.3 a	312.5 a
UNTREATED CONTROL	4	44.5 a	67.8 a	70.8 a	103.1 a	140.0 a	344.0 a

*/ - Means within columns not showing a common letter are significantly different at the 0.05 level by the ANOVA test.

Table V

Analysis of percent weight gains for all LPC-line roosters tested during the course of Experiment 3. Steroids were administered via the feed.

TREATMENT	REPS.	AVERAGE PERCENT WEIGHT GAIN
CORTICOSTERONE	6	24.7 a */
DESOXYCORTICOSTERONE	6	24.3 a
DEXAMETHASONE	6	-10.5 b
UNTREATED CONTROL	6	27.2 a

*/ - Means within columns not showing a common letter are significantly different at the 0.001 level by the ANOVA test.

Table VI

Correlation of mite populations with percent weight gains on the final observation period in Experiment 3. Only the overall correlation produced a significant value.

TREATMENTS							
CONTROL		CORTICOSTERONE		DESOXYCORTICOSTERONE		DEXAMETHASONE	
% WT. GAIN	MITES	% WT. GAIN	MITES	% WT. GAIN	MITES	% WT. GAIN	MITES
28.7	150	36.3	125	30.4	4000	- 5.8	1000
29.2	5000	19.6	10	24.3	150	- 3.2	800
29.5	200	18.2	175	20.0	10000	-12.9	10000
26.7	30	18.1	1000	26.9	6000	-20.2	12000
24.3	3000	13.9	3000	25.6	2500	- 6.8	15000
24.9	10000	39.4	350	20.9	2500	-14.0	200
r = -0.49		r = -0.51		r = -0.27		r = -0.36	

Overall r = -0.43 ($P \leq 0.05$)

Feather samples taken at random from roosters under different steroid regimens and subsequently subjected to microscopic analysis showed important differences in population profiles. At the end of Experiment 2, mite samples taken from both untreated controls and birds receiving corticosterone dosages (Table VII) show mite populations on the former chickens to produce significantly more eggs. The percentage of adult mites in the samples obtained from corticosterone-treated chickens was proportionately higher. This phenomenon was observed also in samples taken from birds at the conclusion of Experiment 3 (Table VIII). Here, untreated controls and birds receiving desoxycorticosterone supported mites laying significantly more eggs than did corticosterone-treated chickens. In this test, differences in population among older mites was most apparent with respect to blood-fed nymphs.

Using percentages to provide numbers for statistical analysis can often lead to erroneous conclusions. This was avoided in the present study by converting life-stage counts from samples of unequal numbers to the common base of percentages. These were then compared using the ANOVA technique. Results point to a general trend toward an older or more mature population on resistant chickens, in contrast to a younger, fast-expanding mite population on susceptible birds.

The greater numbers of eggs layed by mites on susceptible chickens may result from superior nutrition afforded by blood meals from these hosts, or comparatively greater ease with which blood can be obtained from the substrate. It is also possible that corticosterone itself

Table VII

Analysis of northern fowl mite life stages sampled from roosters receiving different steroid treatment at the conclusion of Experiment 2. The corticosterone was administered intravenously.

TREATMENT	NUMBER OF SAMPLES	PERCENT OF LIFE STAGE PRESENT			
		ADULTS	FED NYMPHS	UNFED NYMPHS/LARVAE	EGGS
CORTICOSTERONE	12	23.0 a */	28.9 a	25.3 a	22.8 a
UNTREATED CONTROL	27	16.6 b	25.0 a	23.8 a	34.5 b

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the ANOVA test.

Table VIII

Analysis of northern fowl mite life stages sampled from LPC-line roosters at the conclusion of Experiment 3. The steroids were administered to the test birds via the feed.

TREATMENT	NUMBER OF SAMPLES	PERCENT OF LIFE STAGE PRESENT			
		ADULTS	FED NYMPHS	UNFED NYMPHS/LARVAE	EGGS
CORTICOSTERONE	9	10.8 a*/	22.6 a	18.9 a	47.8 a
DESOXYCORTICOSTERONE	9	7.7 a	10.6 b	13.9 ab	67.9 b
UNTREATED CONTROL	9	11.7 a	13.7 ab	11.0 b	63.7 b

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the ANOVA and LSR tests.

inhibits the reproductive cycle of the mites, or a phase of ecdysis, with the consequent smaller mite populations evident on steroid-treated animals.

The average daily temperature recorded during the course of this experiment was 67.3 F (19.6 C) and the average daily relative humidity 65.2 percent.

Experiment 4 (Effect of Desoxycorticosterone and Metyrapone in the Feed). Metyrapone administered via the feed at a concentration of 500 ppm caused significant increases in northern fowl mite populations on roosters from the LPC-line (Table IX). This difference became apparent 19 days post-infestation and continued throughout the remainder of the experiment. Twenty nine days after infestation, the metyrapone-treated animals supported over 3 times more mites than did the untreated controls.

Desoxycorticosterone in the feed at 60 ppm did not produce results equivalent to Experiment 3 where the dosage used was 30 ppm. In the current experiment, desoxycorticosterone was on no occasion significantly different from control animals with respect to mite populations. While metyrapone-fed and control animals exhibited essentially identical slopes of mite population development (Figure 6), desoxycorticosterone administration produced a flatter curve and the lowest final mite population.

It is possible that at 60 ppm, desoxycorticosterone induces sufficient biological activity in LPC-line roosters to promote a slight degree of mite resistance. At lower doses, this compound may in effect

Table IX

Average mite populations on all LPC-line roosters tested during Experiment 4. The steroid and blocking agent used were administered via the feed.

TREATMENT	NR REPS	DAYS POST-INFESTATION 3	8	14	19	24	29
DESOXYCORTICOSTERONE (60 PPM)	7	59.3 a */	138.3 a	262.5 a	462.5 ab	700.0 a	1062.5 a
METYRAPONE (500 PPM)	7	54.5 a	162.5 a	562.5 a	1325.0 a	3050.0 b	6000.0 b
UNTREATED CONTROL	5	31.0 a	58.3 a	165.0 a	425.0 b	716.8 a	1750.0 a

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the ANOVA and LSR tests.

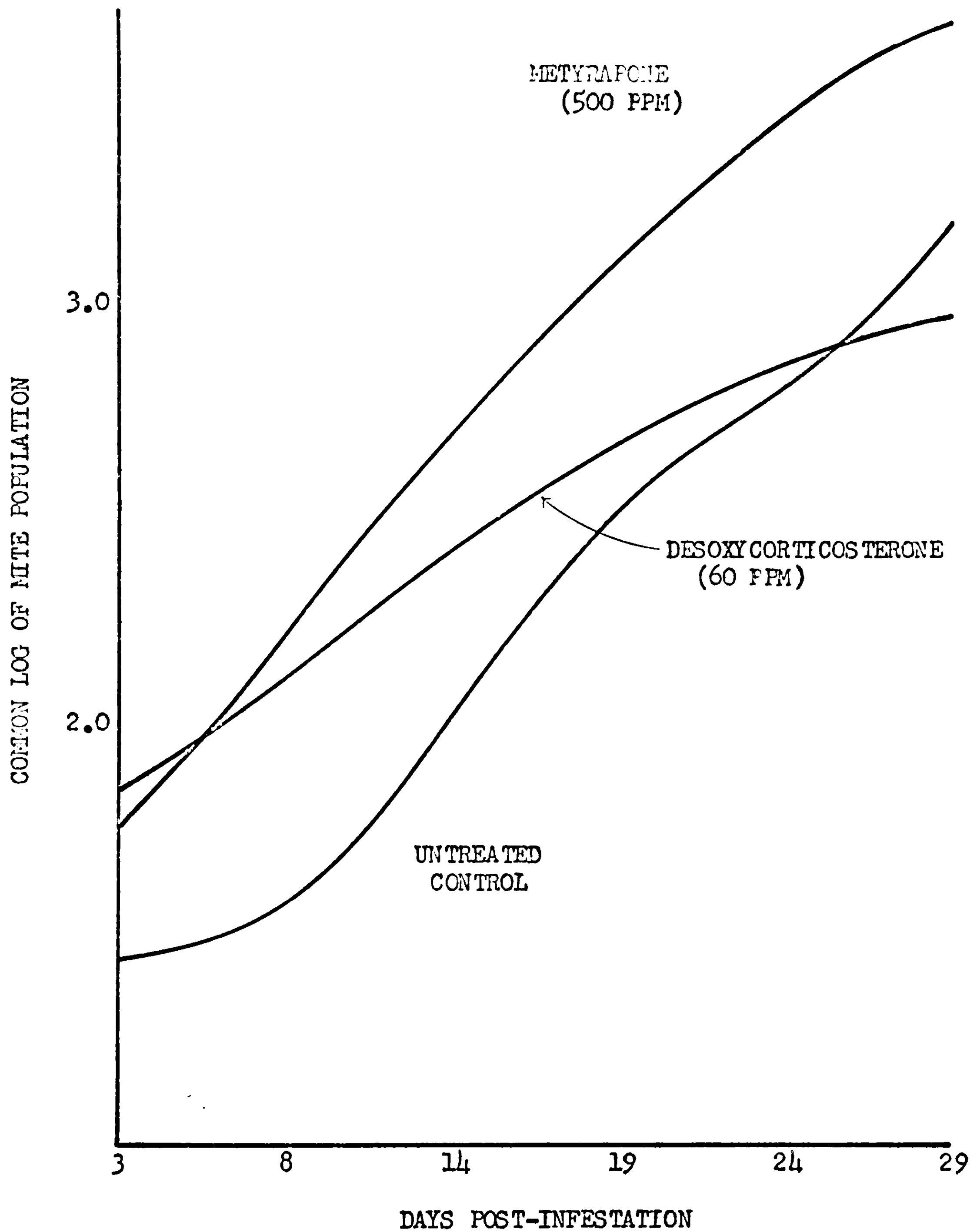

Figure 6. Average mite populations on the LPC-line roosters tested during the course of Experiment 4.

trigger the biofeedback mechanism of the birds and act essentially as a blocking agent.

HPC-line roosters treated with metyrapone and desoxycorticosterone at 500 and 60 ppm respectively did not support mite populations significantly different from control birds (Table X). These results give further evidence to support the conclusion that HPC-line chickens differ from their LPC-line counterparts in adaptability to steroid treatments. It is evidently possible to induce the requisite physiological processes necessary for mite resistance by steroid administration, but difficult to eradicate these phenomena when induced by genetic selection.

LPC-line pullets proved extremely resistant to northern fowl mite infestation. This resistance was noted to decrease markedly at the time of first egg production. Accordingly, infestation levels on all hens evaluated during this experiment were adjusted so that dates of first egg-laying fall at a common point (Figure 7).

The abrupt rise in mite populations following initial sexual maturation of hens may indicate a supplemental role of sex hormones in northern fowl mite resistance; however, the vent feather coat of hens is generally of a finer nature than that of roosters. It is possible that the increasing coarseness of vent plumage on hens at this time provides increased environmental suitability for mite development.

The average daily temperature recorded during this experiment was 69.5 F (20.8 C) and the average daily relative humidity 56.6 percent.

Experiment 5 (Effect of Corticosterone and Perthane® in the Feed).

Table X

Average mite populations on the HPC-line roosters tested during Experiment 4. The steroid and blocking agent used were administered via the feed.

TREATMENT	NR REPS	DAYS POST-INFESTATION 3	8	14	19	24	29
DESOXYCORTICOSTERONE (60 PPM)	3	88.0 a */	188.0 a	597.0 a	1825.0 a	2233.0 a	2275.0 a
METYRAPONE (500 PPM)	3	32.0 a	36.0 a	60.0 a	113.0 a	162.0 a	225.0 a
UNTREATED CONTROL	2	76.0 a	143.0 a	325.0 a	588.0 a	1588.0 a	2588.0 a

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the ANOVA and LSR tests.

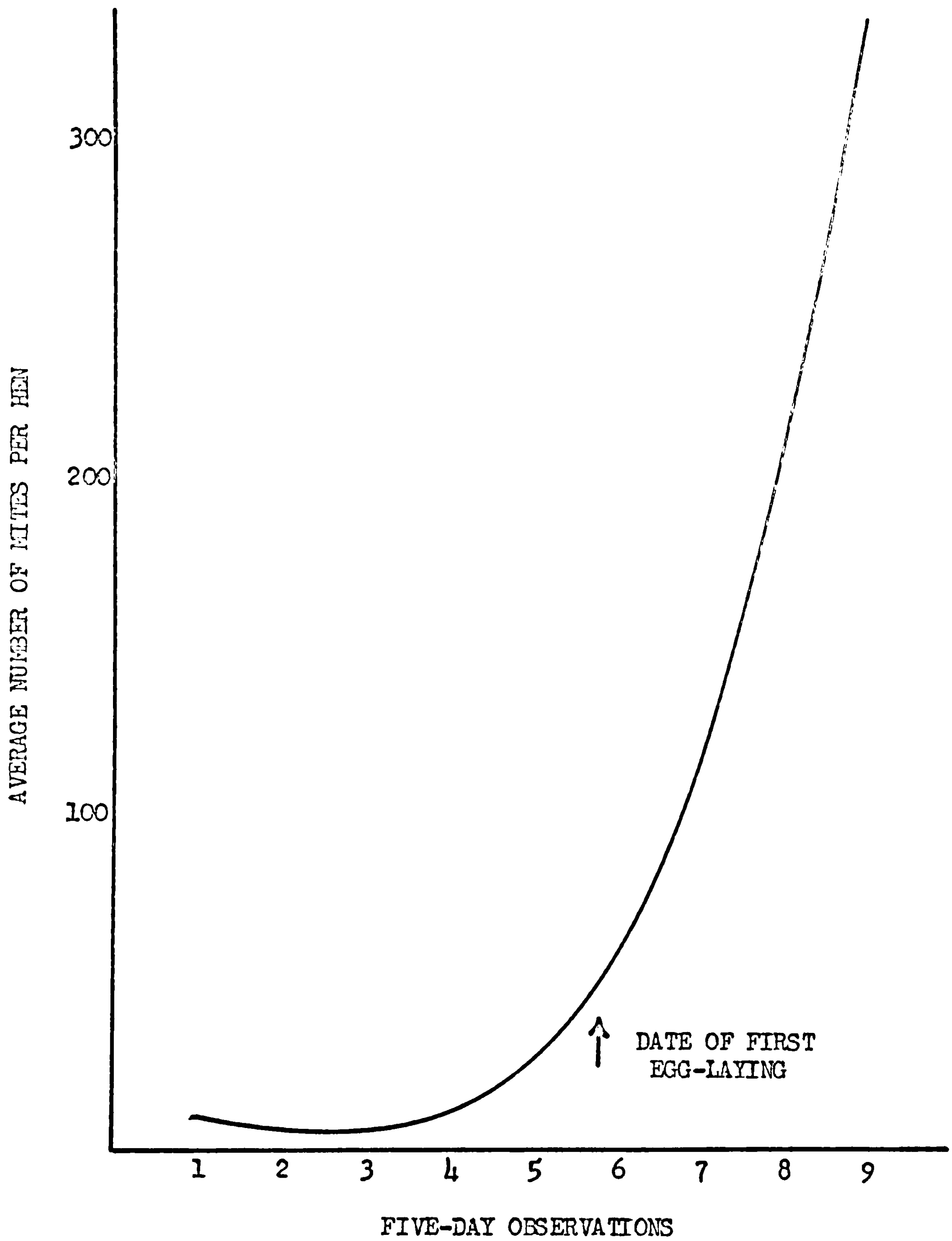

Figure 7. Average mite population development on pullets tested during Experiment 4. The graph was adjusted so that all dates of first egg-laying occur on a common point.

Corticosterone administered in the feed at 20 ppm produced a significant reduction in mite populations over those evident on control birds for the first 5 observation periods (Table XI). On day 29, the average mite population on corticosterone-treated chickens from the LPC-line was 1479 and was 6625 on untreated control roosters.

Perthane ® induced a variable response in LPC-line roosters and this variation was responsible for the non-significance of mite population differentials between control and corticosterone-treated birds on day 29 (Figure 8). The DDD-type insecticide fed to test birds at a rate of 500 ppm did not produce results equivalent to metyrapone at similar dosages. It is possible that a slight systemic effect from the insecticide was responsible for the fact that resultant mite populations were lower than expected. In addition, insecticide-contaminated feces may have allowed the material to affect mite populations developing below the vent.

Experiment 6 (Effect of Graded Dosages of Corticosterone and the Effect of Rhothane ®). Doses of corticosterone administered in the feed at the rates of 10, 20, and 40 ppm produced little effect on mite population development on LPC-line roosters, both among themselves and compared to untreated controls (Table XII and Figure 9). A non-linear response to corticosterone treatment was noted in that doses of 10 and 40 ppm produced mite populations in excess of those evident on control birds and roosters receiving 20 ppm corticosterone in the feed.

The DDD-type insecticide Rhothane ®, effective in reducing adrenal output in experiments on disease resistance (W.B. Gross; personal

Table XI

Average mite populations on the LPC-line roosters tested during Experiment 5. The birds were housed in groups of 4 and steroid and blocking agent was administered via the feed.

TREATMENT	NR REPS	DAYS POST-INFESTATION 3	8	13	19	24	29
CORTICOSTERONE (20 PPM)	7	18.7 a */	42.9 a	93.4 a	225.7 a	540.0 a	1478.6 a
PERTHANE (500 PPM)	8	57.5 b	131.9 b	320.6 b	837.5 a	4909.4 ab	6000.0 a
UNTREATED CONTROL	8	47.8 b	135.0 b	446.9 b	3062.5 b	5225.0 b	6625.0 a

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the ANOVA and LSR tests.

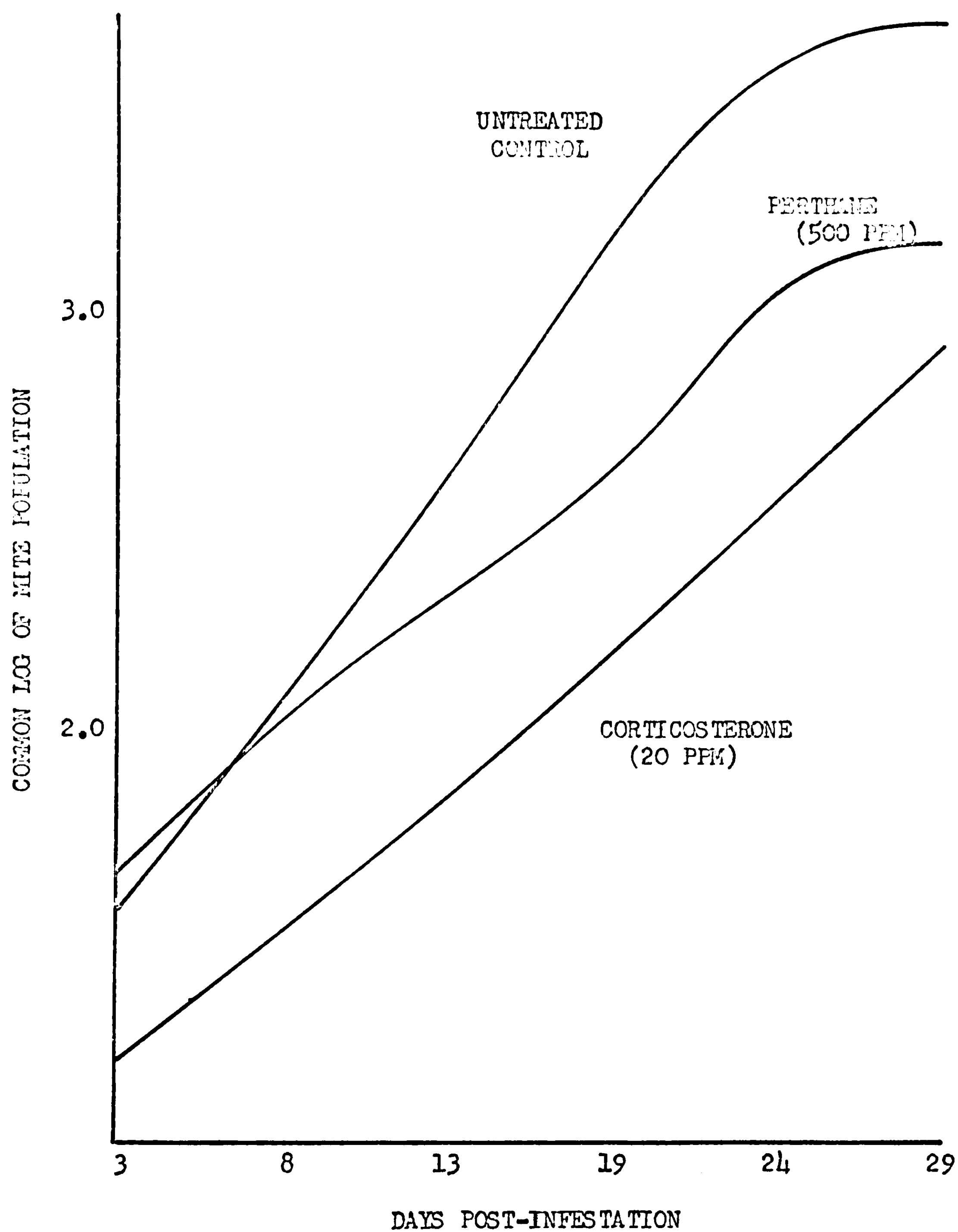

Figure 8. Average mite populations on all LPC-line roosters tested during Experiment 5. The birds were housed in groups of four.

Table XII

Average mite populations on the LPC-line roosters tested during Experiment 6. These chickens were approximately 40 weeks old and had been moved several times during their lives.

TREATMENT	NR REPS	DAYS POST-INFESTATION 3	9	14	19	24	29
CORTICOSTERONE (10 PPM)	5	12.6 a */	158.4 a	692.2 a	5051.0 a	10700.0 a	15100.0 a
CORTICOSTERONE (20 PPM)	5	15.0 a	103.8 a	417.0 a	2210.0 a	7300.0 a	9500.0 a
CORTICOSTERONE (40 PPM)	5	22.8 a	178.0 a	1610.0 a	6260.0 a	14100.0 a	25200.0 a
RHOTHANE (500 PPM)	5	51.8 b	321.0 a	1262.0 a	1690.0 a	3500.0 a	8180.0 a
UNTREATED CONTROL	6	18.5 a	102.8 a	1060.0 a	3408.3 a	8166.7 a	9750.0 a

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the ANOVA and LSR tests.

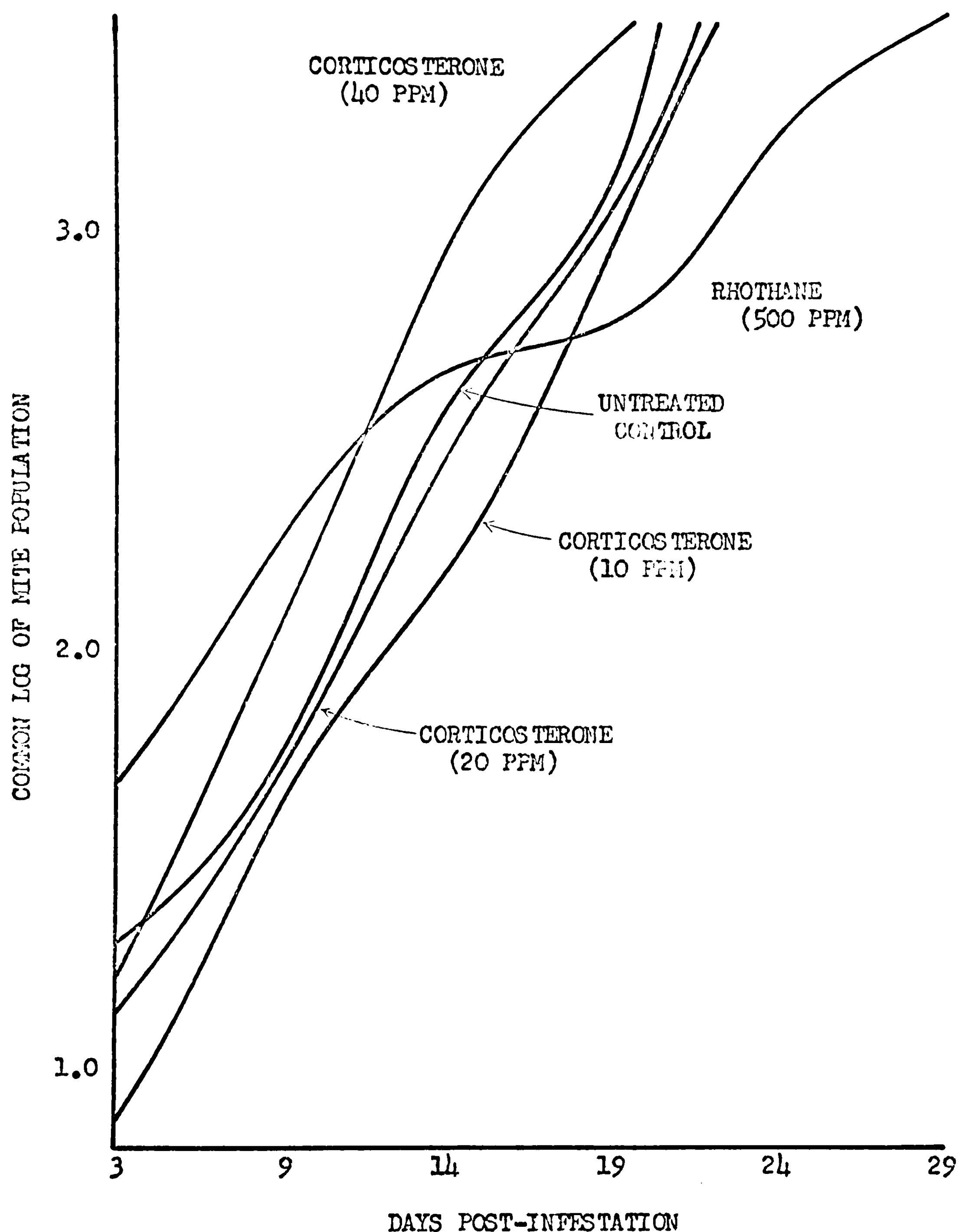

Figure 9. Average mite populations on the LPC-line roosters tested during Experiment 6. These chickens were approximately 40 weeks old and had been moved several times previously.

communication) did not produce results equivalent to metyrapone administration. It is postulated that, like Perthane®, the material exerts a systemic acaricidal activity which precludes the development of maximum northern fowl mite populations.

The results of this experiment with respect to prior history of the experimental birds are extremely important. The chickens used had been moved to different environments several times during their lives and evidently adapted with greater facility than birds raised in more stable surroundings. The lack of significant differences in mite susceptibility even with the administration of steroid compounds is further evidence in support of compensatory adaptations within the physiological and psychological areas of the test animals. The fact that adequate results can be obtained only with test animals which have been reared under stable conditions will be important to future experiments dealing with ectoparasite resistance.

The average daily temperature recorded during this experiment was 72.7 F (22.6 C) and the average daily relative humidity 64.7 percent.

Experiment 7 (Effect of Graded Doses of Corticosterone and Desoxycorticosterone). Chickens from the Cornell random bred colony were shown to be physiologically influenced by manipulation of environmental stress or the administration of adrenal steroids. Control roosters housed to promote extremely low levels of social interaction suffered rapid mite population development (Figure 10).

Corticosterone at the rate of 15 ppm or desoxycorticosterone at

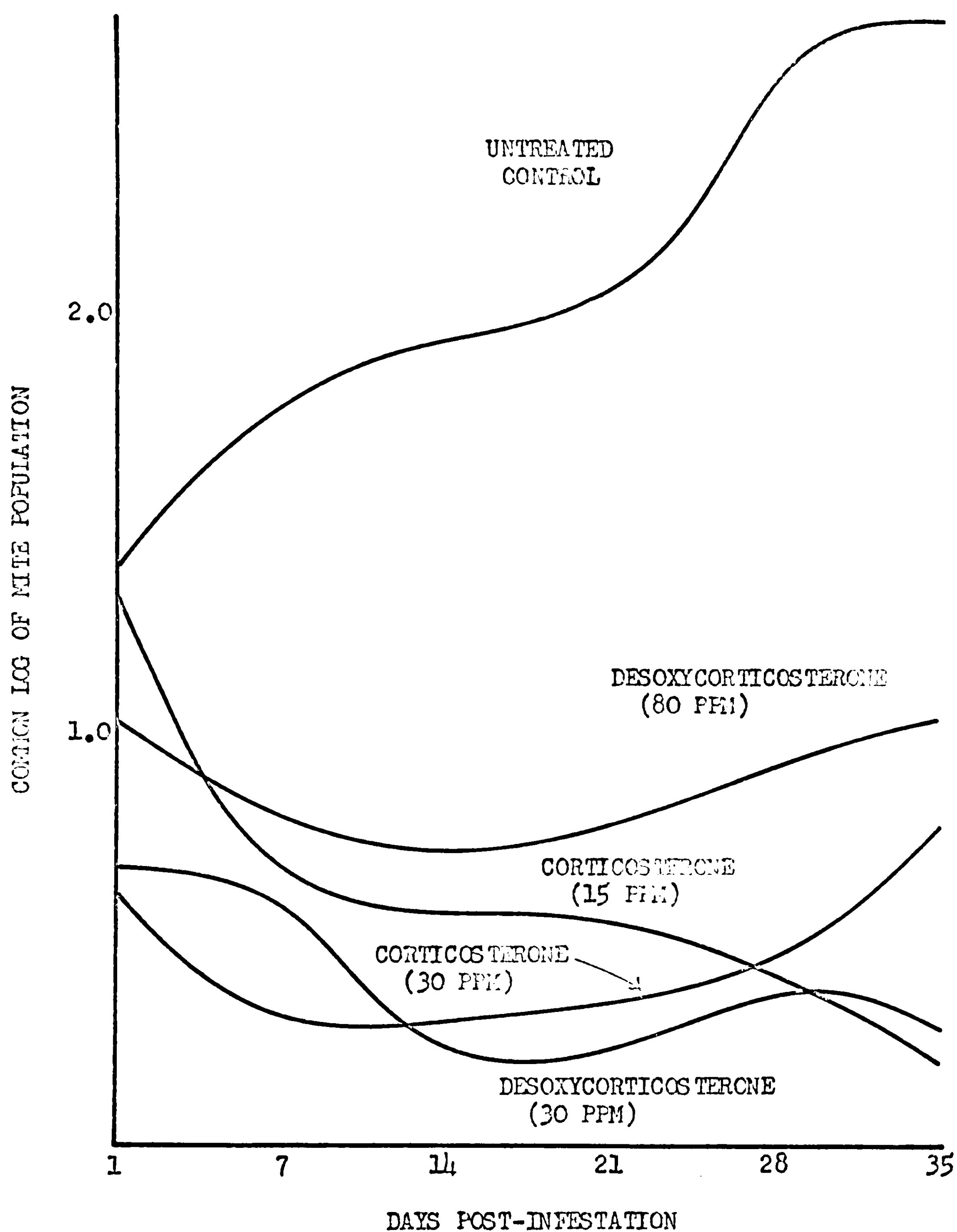

Figure 10. Average mite populations on the roosters housed in the low stress environment during Experiment 7. Each bird was caged alone and disturbed as little as possible.

30 ppm administered via the feed proved to be the most effective doses for induction of mite resistance in this experiment. During 35 days post-infestation, both these steroids had reduced mite levels in their respective test groups to populations significantly lower than those on the control birds (Table XIII). Increasing doses of either steroid above the previously mentioned levels resulted in larger mite populations in the low-stress environment; however, these were not significantly different from other test groups.

Roosters housed in the high-stress environment were extremely resistant to northern fowl mite infestation (Table XIV). No significant differences were noted among any test groups with the exception of those birds receiving corticosterone at the rate of 20 ppm. It is postulated that the increased corticosterone secretion resulting from the high stress levels induced via the social environment combined with the feed intake of steroid to produce overdose levels. The increased mite populations resulting from above-optimum corticosterone levels has been demonstrated both in Experiments 1 and 6, and in the low-stress portion of the current test. In the latter evaluation, corticosterone doses of 45 ppm induced rapid mite population development.

The differences in mite resistance between the high- and low-stress housing systems can be seen in Table XV. On the final observation period, roosters housed alone in visually-isolated cages supported an average mite population approximately 95 times greater than their counterparts in the high stress system. The resistance to mites dis-

Table XIII

Average mite populations for roosters housed in the low-stress environment during the course of Experiment 7. Each rooster was caged alone and disturbed as little as possible.

TREATMENT	NR REPS	DAYS POST-INFESTATION 1	7	14	21	28	35
CORTICOSTERONE (15 PPM)	5	31.4 ab */	24.2 ab	26.2 ab	30.8 a	6.8 a	2.0 ab
CORTICOSTERONE (30 PPM)	5	6.2 b	4.0 ab	9.8 bc	15.8 a	50.8 a	351.6 bc
CORTICOSTERONE (45 PPM)	5	26.6 ab	36.2 bc	233.0 ab	2050.4 a	-	-
DESOXYCORTICOSTERONE (30 PPM)	5	8.8 ab	10.6 ab	2.8 bc	2.8 a	12.6 a	2.6 ab
DESOXYCORTICOSTERONE (80 PPM)	5	15.4 ab	25.6 ab	33.6 ab	46.6 a	210.6 a	420.6 bc
UNTREATED CONTROL	7	30.3 a	85.0 c	286.6 a	794.4 a	3484.2 b	6127.0 c

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the ANOVA and LSR tests.

Table XIV

Average mite populations for roosters housed in the high stress environment during the course of Experiment 7. Moderate feed deprivation was practiced to increase levels of social stress.

TREATMENT	NR REPS	DAYS POST-INFESTATION 1	7	14	21	28	35
CORTICOSTERONE (10 PPM)	7	1.7 a */	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a
CORTICOSTERONE (20 PPM)	6	3.2 a	3.7 a	13.2 b	14.0 b	50.4 b	125.0 b
CORTICOSTERONE (40 PPM)	7	5.7 a	1.6 a	4.9 b	1.7 a	1.9 a	1.1 a
DESOXYCORTICOSTERONE (30 PPM)	7	2.4 a	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a
DESOXYCORTICOSTERONE (80 PPM)	7	4.7 a	1.0 a	1.0 a	1.0 a	1.2 a	11.2 a
UNTREATED CONTROL	13	6.0 a	1.5 a	2.6 c	1.1 a	3.4 a	8.4 a

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the ANOVA and LSR tests.

Table XV

Analysis of mite populations between all roosters tested in both the low- and high-stress environments during the course of Experiment 7. Note the difference in average mite populations.

ENVIRONMENT	NR REPS	DAYS POST-INFESTATION 1	7	14	21	28	35
LOW STRESS (SINGLE CAGES)	32	20.4 a */	34.3 a	110.4 a	509.2 a	853.8 a	1625.7 a
HIGH STRESS (COMMUNAL CAGES)	47	4.2 b	1.6 b	3.6 b	2.9 a	7.8 b	16.6 b

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the Student's t-test.

played by the chickens housed in communal cages was such that even a second innoculation with northern fowl mites failed to produce large populations.

Autopsy of all roosters used during the course of this experiment revealed important physiological differences with respect to environmental stress and steroid treatment. Chickens in the low-stress housing were affected more by steroid administration than the birds under higher stress levels (Table XVI). Corticosterone, plus desoxycorticosterone administered at 80 ppm were shown to decrease lymphatic tissue mass and testes weight significantly. The 2 largest doses of corticosterone (30 and 45 ppm) caused increased deposition of fat in the liver, and doses of 15 and 45 ppm inhibited weight gain.

Birds maintained under high levels of social stress were less affected by steroid administration (Table XVII). Corticosterone doses of 20 and 40 ppm caused a reduction in lymphatic tissue mass, and the latter level was shown to inhibit weight gain. Only corticosterone given at the rate of 20 ppm caused increased fat deposition in the livers of test birds. It is believed that the higher metabolism levels of chickens under large amounts of social stress require additional energy input to preclude excessive fat storage.

Comparison of autopsy results from chickens in both the low- and high-stress housing (Table XVIII) show significant divergence with respect to total body weight and weight of testes. The average weight of birds from visually-isolated cages was approximately 300 g higher at autopsy than that of birds under high levels of social stress.

Table XVI

Average values for measurements made at autopsy of all roosters tested in the low-stress environment during the course of Experiment 7.

TREATMENT	BODY WEIGHT	LIVER	TESTES	SPLEEN	THYMUS	BURSA	ANTIBODY (LOG_2)
			(PERCENT OF BODY WEIGHT)				
CORTICOSTERONE (15 PPM)	1619*	3.3	0.42	0.10*	0.15*	0*	11.8
CORTICOSTERONE (30 PPM)	1804	8.1*	0.05*	0.08*	0*	0*	8.0
CORTICOSTERONE (45 PPM)	1533*	9.0*	0.04*	0.07*	0*	0*	6.5
DESOXYCORTICOS. (30 PPM)	1749	2.0	0.27	0.16	0.37	0.09	13.4
DESOXYCORTICOS. (80 PPM)	1725	2.1	0.17*	0.16	0.40*	0.15*	12.5
UNTREATED CONTROL	1824	2.1	0.43	0.19	0.31	0.07	11.3

* - Denotes significant (P 0.05) difference from control value by the Student's t-test.

Table XVII

Average values for measurements made at autopsy of all roosters tested in the high-stress environment during the course of Experiment 7.

TREATMENT	BODY WEIGHT	LIVER	TESTES	SPLEEN	THYMUS	BURSA	ANTIBODY (LOG_2)
			(PERCENT OF BODY WEIGHT)				
CORTICOSTERONE (10 PPM)	1461	2.1	0.07	0.12	0.20*	0.05	10.3
CORTICOSTERONE (20 PPM)	1476	4.0*	0.15	0.08*	0.06*	0*	13.5
CORTICOSTERONE (40 PPM)	1109*	3.1	0.03	0.08*	0*	0*	11.8
DESOXYCORTICOS. (30 PPM)	1377	2.6	0.04	0.15	0.27	0.10	11.0
DESOXYCORTICOS. (80 PPM)	1542	2.5	0.05	0.12	0.26	0.09	11.6
UNTREATED CONTROL	1415	2.4	0.06	0.14	0.27	0.07	11.2

* - Denotes significant (P 0.05) difference from control value by the Student's t-test.

Table XVIII

Comparison of average values for measurements made at autopsy of all roosters from both the low- and high-stress environments during Experiment 7. Significant differences were apparent with respect to whole body and testes weights.

ENVIRONMENT	BODY WEIGHT	LIVER	(PERCENT OF BODY WEIGHT) TESTES	SPLEEN	THYMUS	BURSA	(LOG_2) ANTIBODY
LOW-STRESS	1710 a */	4.4 a	0.23 a	0.13 a	0.21 a	0.05 a	10.6 a
HIGH-STRESS	1397 b	2.8 a	0.07 b	0.12 a	0.18 a	0.05 a	11.6 a

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the Student's t-test.

Testes weight in the high-stress animals averaged 3 times lower than that produced by birds in the low-stress situation.

Correlation of mite populations from all experimental birds with measurements made at autopsy indicates that testes weight is involved (Table XIX). This, plus evidence that hens change their mite-resistance profile at sexual maturation, lends support to the theory that sex steroids may play a supplemental role in the pattern of mite development on chickens.

Skin samples taken from the region dorsad to the vent show interesting differences when compared with northern fowl mite populations. Skin from chickens supporting extremely large mite burdens contains significantly higher numbers of capillaries per unit area (Figure 11) than skin from birds which were mite-resistant (Figure 12). This phenomenon was shown to hold true for birds in which resistance to mites had been induced both via environmental manipulation and by steroid therapy. A correlation coefficient of 0.63 ($P \leq 0.05$) was produced by comparing mite populations with numbers of capillaries per unit length, 0.74 ($P \leq 0.05$) for comparison of mite populations with thickness of the capillary-bearing subepidermal layer, and 0.71 ($P \leq 0.05$) for mite populations and upper epidermal thickness.

The results of this experiment demonstrate that the probable mechanism of action of elevated adrenal steroid levels in relation to subsequent mite population development is via availability of blood. Both sex adult northern fowl mites obtain moisture, protein, and other materials essential for growth and reproduction solely via

Table XIX

Correlation of measurements made at autopsy with the last observed mite populations for all roosters housed in the low-stress environment during Experiment 7.

MEASUREMENT	CORRELATION WITH MITE POPULATION
WEIGHT	NONE
LIVER	NONE
TESTES	POSITIVE */
SPLEEN	NONE
THYMUS	NONE
BURSA	NONE
ANTIBODY	NONE

*/ - Correlation coefficient significantly positive ($P \leq 0.05$) by the Student's t-test.

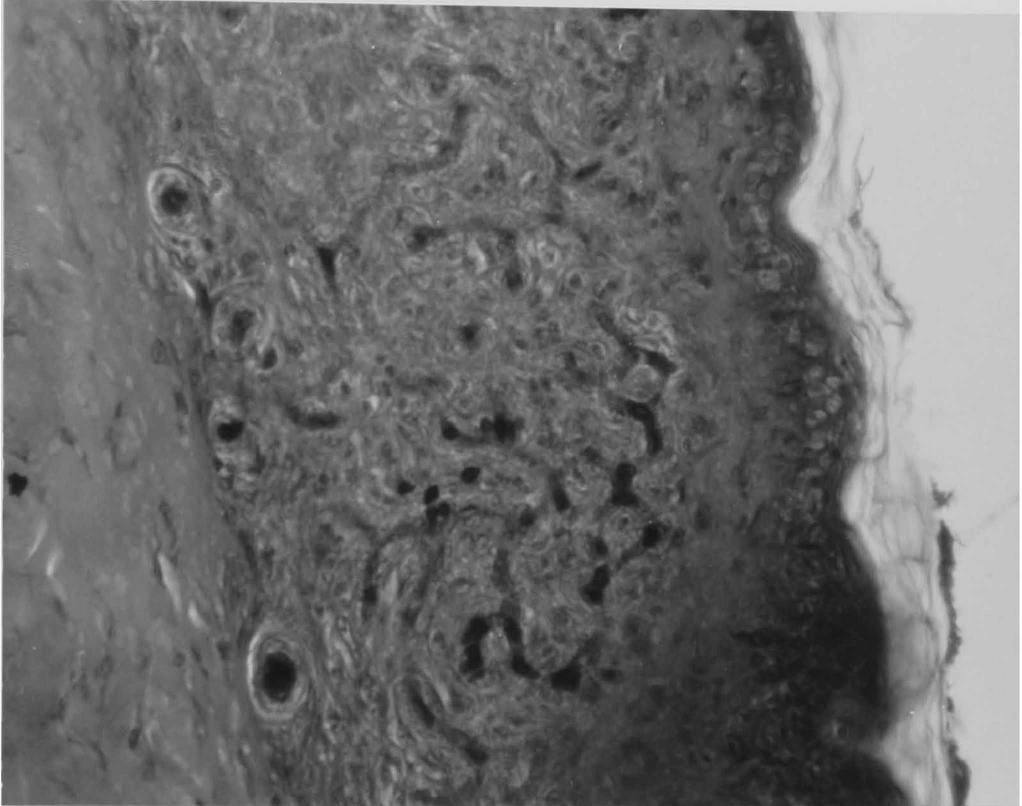

Figure 11. A cross section of skin obtained from an untreated control rooster. This bird was extremely susceptible to mite infestation. Note the high capillary density.

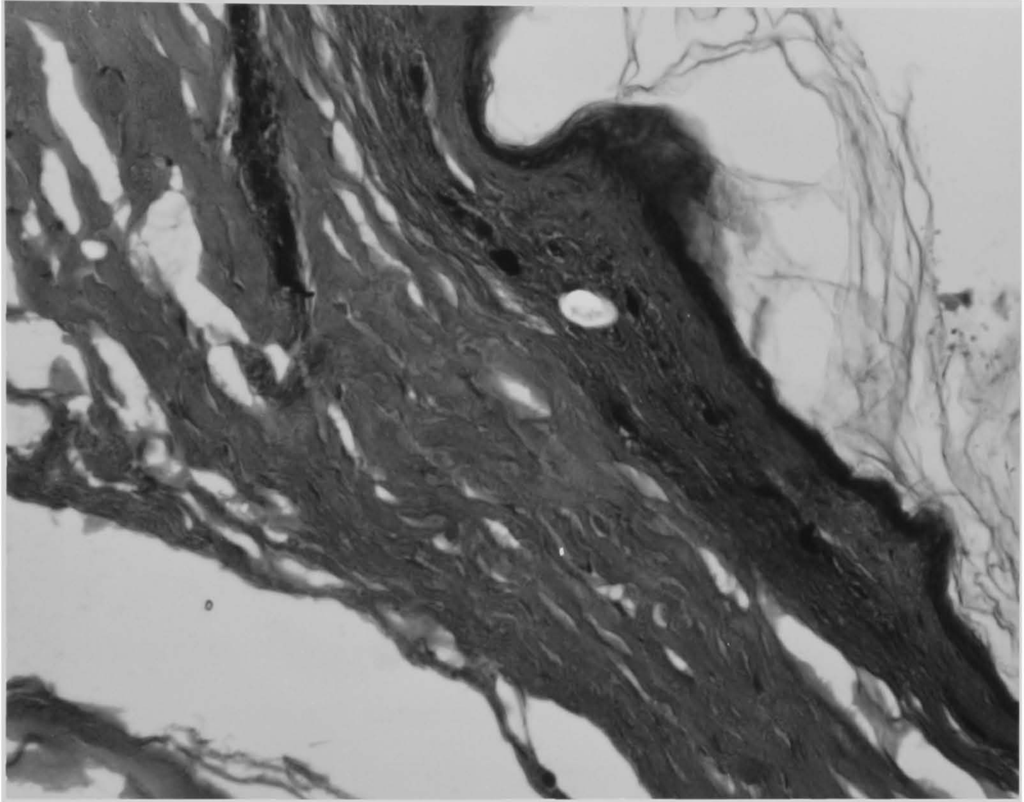

Figure 12. A cross section of skin obtained from the vent region of a rooster fed corticosterone at the rate of 30 ppm. This bird was extremely mite resistant. Note the small number of capillaries in comparison with Figure 11.

intake of host blood. The decreased availability of capillaries at the skin surface of the avian host may decrease the ease with which mites are able to obtain satisfactory blood meals, and would explain the decreased percentage of egg production evident in mite populations located on relatively resistant birds. That this phenomenon is encountered in birds subjected to high levels of social stress was confirmed by examination of skin samples obtained from control animals in this environment. Slide-mounted sections from high stress, mite-resistant birds compare favorably with samples from birds receiving corticosterone or desoxycorticosterone at optimum doses.

Non-preference of northern fowl mites for certain resistant chickens may be linked to subtle temperature differences at the skin surface. The higher capillary density on susceptible birds may produce slightly higher dermal temperatures, and northern fowl mites may be able to detect these minute differentials. Dr. G. Wharton (Professor of Acarology, Ohio State University; personal communication) has demonstrated that at least Trombiculid mites possess sensitive temperature receptors which are able to detect small changes in temperature. It is not known if northern fowl mites are equipped with similar facilities.

The average daily temperature recorded for the low stress environment during this experiment was 61.1 F (16.2 C) and the average daily relative humidity 57.0 percent. The corresponding values obtained in the high stress environment were 65.7 F (18.7 C) and 60.8 relative humidity.

Experiment 8 (Effect of Antibody Persistence). Roosters which had been previously selected genetically for differences in ability to maintain antibody titer were shown to exhibit little difference in mite susceptibility (Table XX). Observation of mite population development for 1 month post-infestation revealed both groups to support identical parasite loads.

The results of this experiment, plus evidence from physiological investigation of the HPC- and LPC-line chickens, indicates that antibody defense if probably not responsible for the resistance of certain chickens to initial mite challenge. Birds from the LPC-line have been shown to produce antibody earlier, maintain antibody longer, and respond to lower doses of antigen than their counterparts in the HPC-line. The consistently greater resistance of the HPC-line to mite infestation also points to the minor role of antibody involvement in mite resistance.

The average daily temperature recorded during this experiment was 64.8 F (18.2 C) and the average daily relative humidity 30.0 percent.

Experiment 9 (Effect of Estrogen). Estrogen alone was not shown to be responsible for the observed differences in northern fowl mite susceptibility between male and female chickens. Weekly intramuscular doses of estradiol ranging from 0.1 to 1.0 mg produced only a slight increase in mite resistance (Table XXI). All estrogenized roosters supported significantly lower mite populations than did control birds for the initial 17 days of the experiment; however, variation within test groups eliminated significant differences on the 22nd day post-

Table XX

Average mite populations recorded on all roosters from the LP and HP lines tested during the course of Experiment 8. These chickens differ in their ability to maintain antibody titer.

BIRD LINE	NR REPS	DAYS POST-INFESTATION 3	7	14	21	28
HP (HIGH PERSISTENCE)	5	75.0 a*/	1600.0 a	5200.0 a	13100.0 a	15000.0 a
LP (LOW PERSISTENCE)	7	52.0 a	842.9 a	6357.0 a	12857.0 a	16714.0 a

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the Student's t-test.

Table XXI

Average mite populations on all roosters subjected to estradiol injection during the course of Experiment 9. Intramuscular doses were given on a weekly basis.

ESTRADIOL (MG/WEEK)	NR REPS	DAYS POST-INFESTATION 2	7	12	17	22
0.1	6	12.0 a */	28.8 a	75.7 a **/	257.0 a	1183.3 a
0.2	6	13.7 a	34.2 a	80.0 a	179.0 ab	565.0 a
0.3	6	16.7 a	38.8 a	115.8 a	354.2 bc	1195.8 a
0.4	6	8.3 a	9.8 a	39.2 a	105.8 ab	279.2 a
0.5	6	8.3 a	10.5 a	40.7 a	103.3 ab	476.7 a
0 (CONTROL)	6	34.7 b	86.7 b	312.5 b	733.3 c	1930.8 a

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the ANOVA and LSR tests.

**/ - Estradiol dosage on this group increased to 1.0 mg/week after day 12.

infestation.

The fact that mite population differentials between test and control chickens in this experiment were not as large as those commonly observed when female and male birds are compared, plus evidence stemming from mite populations versus time of egg-laying in hens and testes weight in roosters, indicates that sex hormones exert a modifying influence over the pattern of ectoparasite resistance. It is possible that the estradiol levels used, plus age of the test roosters, were not optimum with respect to producing the full potential of this compound.

The average daily temperature recorded during this experiment was 71.1 F (21.7 C) and the average daily relative humidity 64.2 percent.

Experiment 10 (Topical Application of Corticosterone). Topical treatments of corticosterone doses ranging from 12.5 to 100 ppm caused moderate reduction in northern fowl mite populations on roosters (Table XXII). The observed mortalities do not correlate well with a linear scale; this probably indicates that the effect of the compound is not that of acute toxicity to mites. The chemical structure of corticosterone is similar to ecdysone, and Kitaoka (1972) found that insect ecdysones produced physiological effects on certain species of ticks. Large doses of corticosterone applied directly to the acarine cuticle may influence internal physiological processes. The level of this steroid contained in the blood of HPC-line chickens is much lower than that applied topically in this experiment.

The average daily temperature recorded during this experiment was

Table XXII

Pre- and post-treatment mite populations and percent population decreases for all rates of corticosterone applied topically to roosters during the course of Experiment 10. Corticosterone treatments were applied with a glass atomizer.

DOSAGE OF CORTICOSTERONE	NR REPS	MITES PRE-TREATMENT	MITES POST-TREATMENT	% DECREASE
12.5 PPM	6	4325.0	2558.3	41
25.0 PPM	6	1545.8	1083.3	30
50.0 PPM	6	3795.8	1391.7	63
100.0 PPM	6	6720.8	2387.5	64
CONTROL	6	1425.0	1391.7	2

65.4 F (18.6 C) and the average daily relative humidity 39.1 percent.

Laboratory studies using bed bugs receiving known doses of corticosterone. In 2 separate experiments, bed bug colonies maintained on blood diets containing known added doses of corticosterone showed no significant differences in relation to numbers of eggs or nymphs produced (Table XXIII). Steroid treatments ranging from 5 to 20 nanograms per ml of blood were designed to approximate and exceed the levels of corticosterone available to northern fowl mites feeding on chickens from the HPC-line.

The non-reactivity of bugs subjected to steroid diets plus histological evidence from resistant and susceptible birds examined during Experiment 7 indicate that corticosterone and desoxycorticosterone themselves are probably not directly responsible for mite resistance in birds. It is believed that the effect of these compounds on the physiology of the avian host induces those differences which increase resistance capability.

Field studies relating social stress and plasma corticosterone to northern fowl mite infestations. Management of commercial laying hens with respect to numbers of birds per cage was shown to be related to northern fowl mite infestation levels. Examination of hens selected on the basis of being caged alone showed that these birds supported higher numbers of mites than did hens housed several per cage in proximate locations (Table XXIV).

In a similar survey, a second commercial egg-production facility

Table XXIII

Average number of *Cimex lectularius* (L.) nymphs and eggs developing in colonies artificially fed blood containing various amounts of added corticosterone.

CORTICOSTERONE ADDED TO BLOOD (NG/ML)	AVERAGE NUMBER OF BED BUG NYMPHS	BED BUG EGGS
TEST 1		
0	15.8 a */	11.8 a
5	19.6 a	13.8 a
10	15.2 a	15.8 a
20	23.0 a	21.3 a
TEST 2		
0	20.0 a	16.2 a
5	25.4 a	14.2 a
10	23.6 a	19.6 a
20	16.2 a	13.4 a

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the ANOVA test.

Table XXIV

Average mite indexes for hens sampled at a commercial egg-production facility in Roanoke, Virginia, in 1975. Hens were selected on the basis of number of birds per cage.

BIRDS PER CAGE	NUMBER OF SAMPLES	AVERAGE MITE INDEX
ONE	5	5.8 a */
TWO - FOUR	24	3.3 b

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the Student's t-test.

was checked in a like manner (Table XXV). In 197 samples, birds housed alone were shown to support larger mite infestations than chickens which had at least 1 cagemate. These results are similar to those obtained in Experiment 7, in which the degree of social interaction influenced subsequent northern fowl mite infestations.

This phenomenon provides the commercial poultryman with a convenient survey tool, in that chickens housed alone may be rapidly identified and examined. The greater susceptibility of these individuals to mite infestations will enable management personnel to spot incipient mite outbreaks and institute control procedures before average mite populations become large enough to reduce egg output or annoy egg-handling personnel.

Analysis of blood samples obtained from commercial hens shows that plasma corticosterone levels differ with respect to housing practices and mite infestations (Table XXVI). Hens selected on the basis of freedom from mites and maintained 2 or more per cage had significantly higher plasma corticosterone levels than hens housed singly and supporting large mite populations. Haematocrit readings taken on hens housed in a like manner showed no significant differences.

Evaluation of cause and effect in relation to steroid levels can be misleading; however, the consistency of laboratory measurements on steroid administration indicates that housing practices probably directly influence the steroid levels, which are in turn responsible for the observed mite population differentials. Parasite infestation itself is undoubtedly a stress factor, but it is probable that chickens

Table XXV

Average mite indexes for hens sampled at a commercial egg-production facility in Rural Retreat, Virginia, in 1975. Hens were selected on the basis of number of birds per cage.

BIRDS PER CAGE	NUMBER OF SAMPLES	AVERAGE MITE INDEX
ONE	65	3.6 a */
TWO	132	1.8 b

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the Student's t-test.

Table XXVI

Plasma corticosterone levels determined via the protein-binding analysis for hens selected for large or negative mite infestation. The hens were housed in a commercial egg-production facility in Roanoke County, Virginia, during 1975.

	PLASMA CORTICOSTERONE (NG/ML)	
	SEVERELY-MITE-INFESTED HENS	NON-MITE-INFESTED HENS
	3.0	4.0
	3.5	4.0
	2.0	4.0
	4.5	2.5
	2.5	4.5
	2.0	5.0
	4.0	6.0
	3.0	4.5
	3.5	
$\bar{Y}$ =	3.1 a */	4.3 b

*/ - Means within rows not followed by a common letter are significantly different at the 0.05 level by the Chi-square test.

which become most heavily infested under circumstances prevailing in commercial establishments are those from the end of the normal distribution scale of steroid competency which are unable to respond adequately to environmental stimuli.

Evaluation of acaricide effectiveness against northern fowl mites.

Laboratory toxicological evaluations. Of 5 compounds tested (Figures 13 - 17), carbaryl proved to be the most toxic material to northern fowl mites of those currently registered in Virginia for direct application to poultry (Table XXVII). The LC_{50} obtained (6.64 $\pm$0.02 ppm) is undoubtedly the reason for the continued use of this chemical; however, the control afforded is typically short-lived, probably because of non-persistence in the plumage.

Malathion, which has been used extensively by the poultry industry for northern fowl mite control, produced an LC_{50} of 146$\pm$1.0 ppm. This value is approximately 21 times higher than that reported by Foulk and Matthysse (1964) using an identical technique. It is believed that this represents the first direct evidence of malathion tolerance by northern fowl mites in the eastern U.S., and may be the cause of increasing consumer dissatisfaction with the performance of the compound.

The synthetic pyrethroid compound permethrin (Ectiban®) produced and LC_{50} essentially similar (21.9$\pm$0.2 ppm) to that of stirofos (18$\pm$0.1 ppm). The latter compound has come into widespread usage as a poultry ectoparasiticide. The superior control afforded by the synthetic

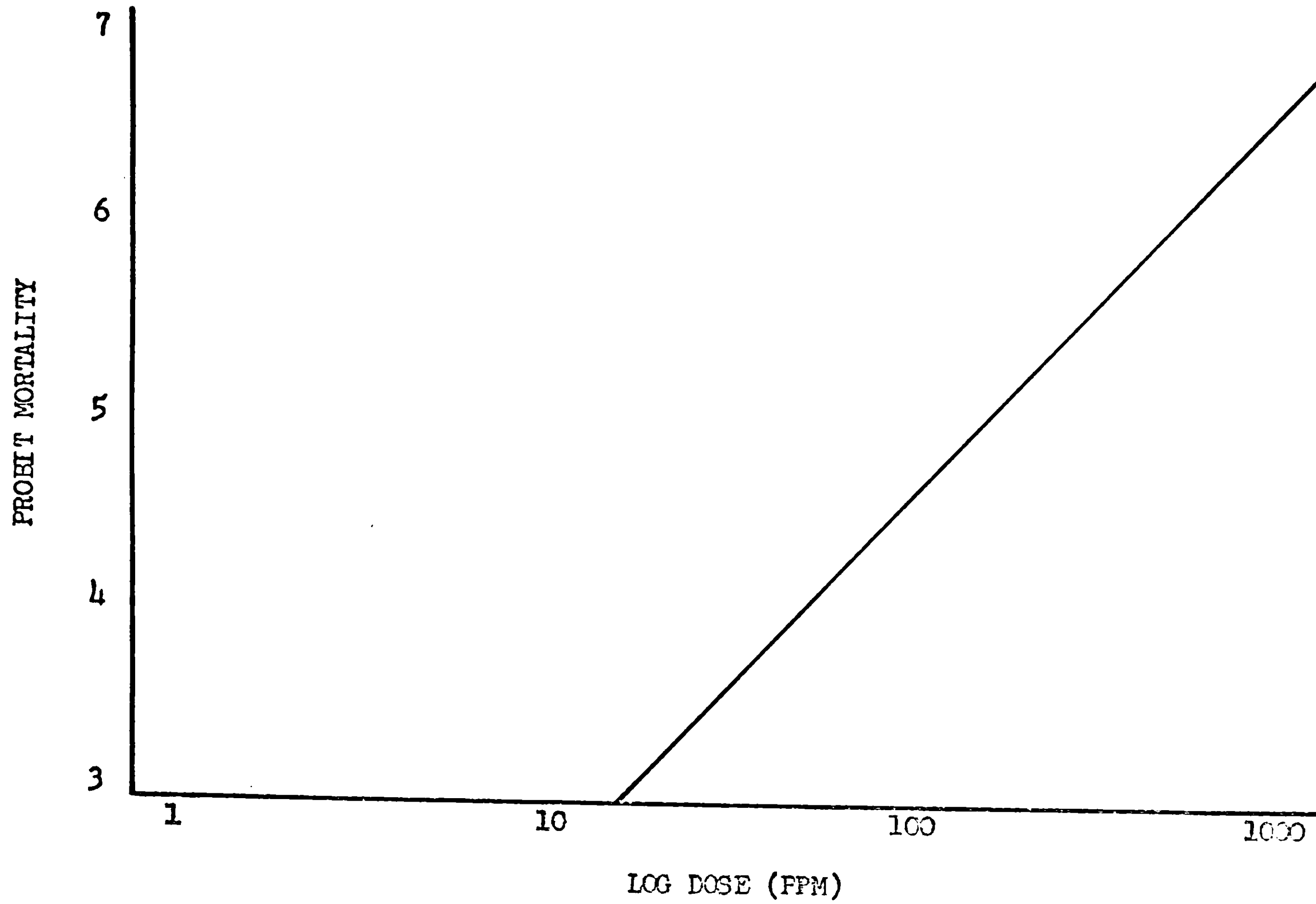

Figure 13. Dose-mortality curve for the 18-hour acute toxicity of malathion to adult northern fowl mites.

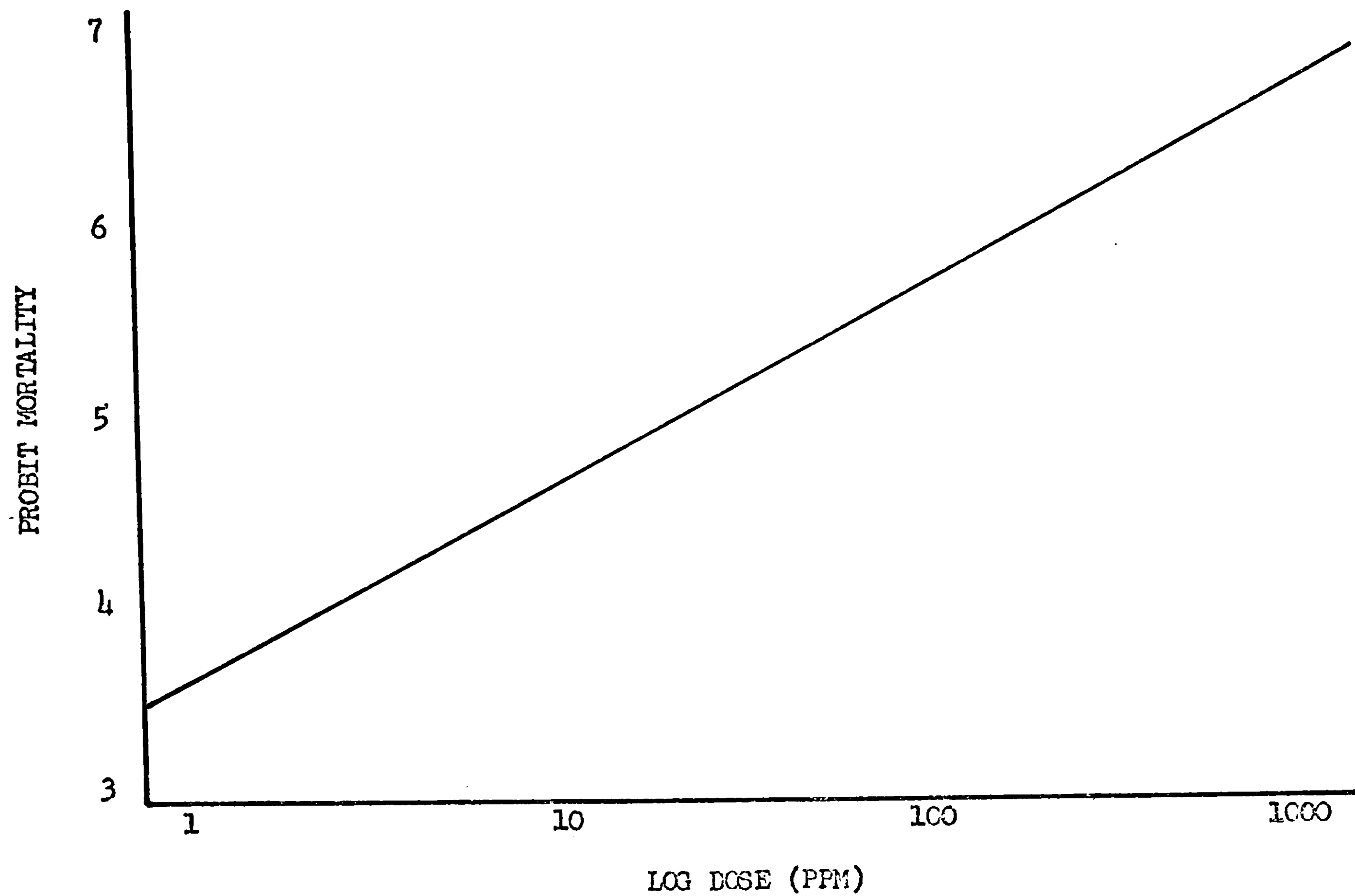

Figure 14. Dose-mortality curve for the 18-hour acute toxicity of permethrin to adult northern fowl mites.

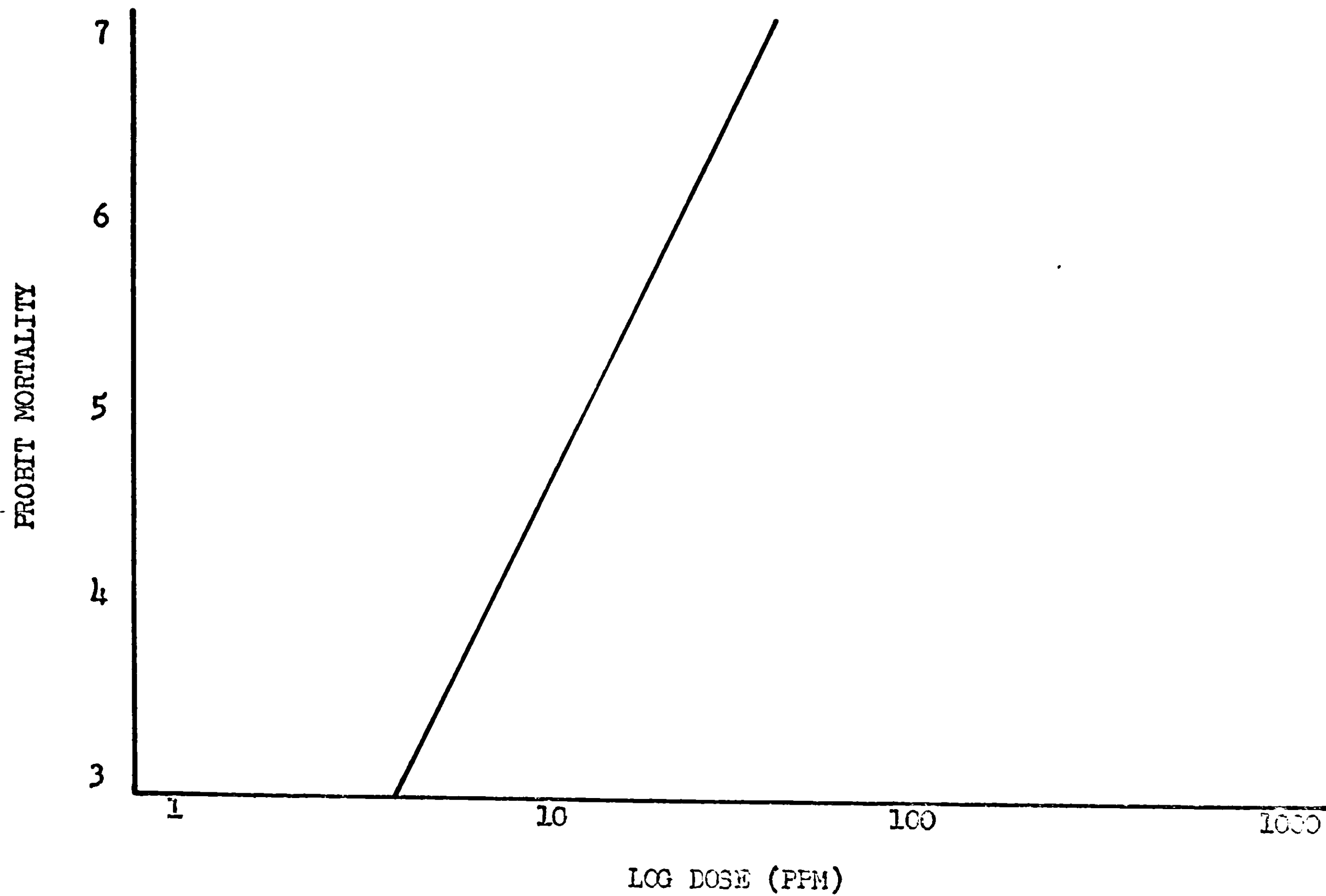

Figure 15. Dose-mortality curve for the 18-hour acute toxicity of stirofos to adult northern fowl mites.

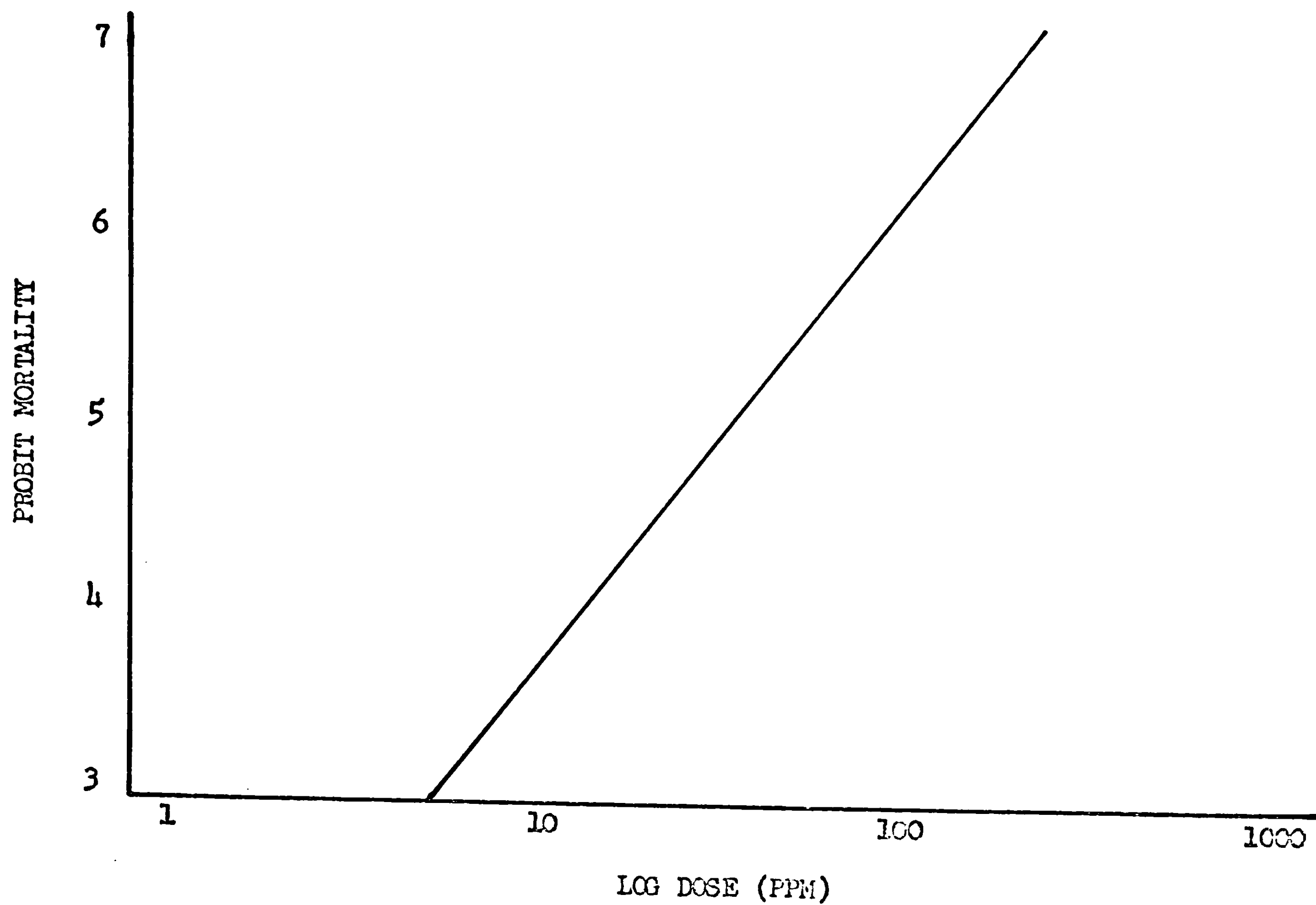

Figure 16. Dose-mortality curve for the 18-hour acute toxicity of coumaphos to adult northern fowl mites.

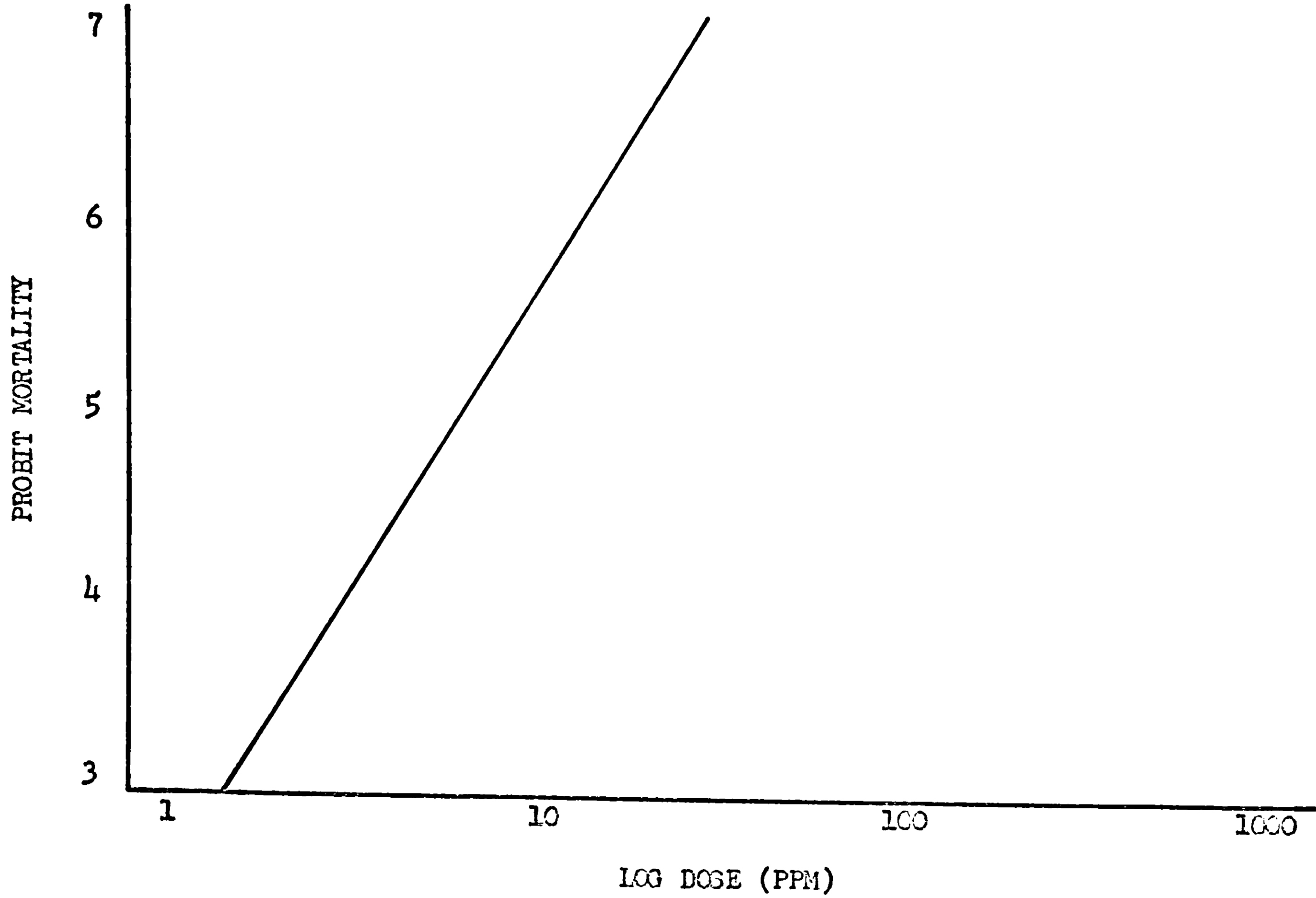

Figure 17. Dose-mortality curve for the 18-hour acute toxicity of carbaryl to adult northern fowl mites.

Table XXVII

LC_{50} values produced by laboratory investigations of acute toxicity of various acaricides to northern fowl mites. The technique employed produced extremely precise values.

ACARICIDE	REGRESSION EQUATION	LC_{50}
MALATHION	Y = 2.21X 2.43	146.0 ± 1.0 PPM
PERMETHRIN	Y = 1.03X 2.59	21.9 0.2 PPM
STIROFOS	Y = 3.90X 0.10	18.0 0.1 PPM
COUMAPHOS	Y = 2.78X 0.97	28.0 0.1 PPM
CARBARYL	Y = 3.43X 2.20	6.6 0.02 PPM

pyrethroid is primarily because of its superior persistence in the environment.

Simulated field application of permethrin. Single-caged Leghorn roosters initially supporting equally large mite infestations were cleared of parasites for 77 days after treatment with doses of permethrin EC ranging from 0.5 percent to 0.125 percent A.I. No significant differences were noted between dosages, and all rates applied afforded 100 percent control during the course of the experiment (Table XXVIII). Chickens assigned to the untreated control group maintained a large average mite population in the closed test room; therefore, permethrin probably does not exert much vapor-phase activity.

The length of time during which treated birds which were continually subject to reinfestation from neighboring control chickens remained free of northern fowl mites is indicative of the long residual life of permethrin.

It is unfortunate that this experiment could not be carried for a longer time period; however, 77 days post-treatment control indicates that synthetic pyrethroid compounds such as permethrin may prove valuable to the poultry industry.

In a separate series of experiments, permethrin EC was applied to floor-managed breeder flocks at the rate of 0.05 percent A.I. A single flock maintained on wire mesh exhibited 100 percent control of northern fowl mites for 74 days following treatment. After 124 days, mite populations on test and control chickens were once again identical (Table XXIX). The gradual decline of mite infestations on the control

Table XXVIII

Average mite indexes for roosters treated with 3 rates of permethrin in a simulated field experiment. Birds were caged singly and conditions of acaricide application were designed to approximate those used when treating commercial poultry.

		DAYS POST-TREATMENT								
ACARICIDE DOSAGE	PRE-TREATMENT	1	7	14	21	28	35	49	63	77
0.5%	6 a */	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
0.25%	6 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
0.125%	6 a	0.2 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
CONTROL	6 a	6.0 b	6.0 b	6.0 b	6.0 b	5.9 b	6.0 b	6.0 b	6.0 b	5.9 b

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the ANOVA and LSR tests.

Table XXIX

Average mite indexes for a floor-managed Leghorn breeder flock treated to run-off with permethrin EC containing 0.05% A.I. The birds were housed on a wire-screen floor.

		DAYS POST-TREATMENT			
TREATMENT	PRE-TREATMENT	7	35	74	124
0.05% PERMETHRIN	3.4 a */	0 a	0 a	0 a	2.8 a
UNTREATED CONTROL	3.2 a	2.6 b	1.4 b	2.0 b	2.8 a

*/ - Means within columns not followed by a common letter are significantly different at the 0.05 level by the Student's t-test.

chickens during the course of this test was produced by the virtual eradication of mites from several control hens. Because treated and untreated birds were allowed to mingle during periods between observations, it is postulated that permethrin-treated roosters servicing mite-infested hens may transfer sufficient acaricidal material to effect adequate parasite control.

Roosters housed on wood shavings with small groups of hens and treated with 0.05 percent A.I. permethrin exhibited reduction in mite populations for 74 days post-treatment (Table XXX). After 124 days, mite infestation levels were approximately equal to those before treatment. Reinfestation with northern fowl mites is accomplished more easily when birds are housed on shavings or sawdust rather than on wire screen because of the increased number of adequate mite harborages away from the host.

Acute toxicity of permethrin and chlordimeform to chickens. Immersion or oral administration of permethrin at doses ranging from 1.25 to 5.0 percent A.I. were noted to produce no overt ill effects after 24 hours (Table XXXI). An oral drench of 10 ml permethrin EC stock solution (4 lb/gal) left the test animals with a disagreeable odor, but no additional symptoms.

Immersion in chlordimeform solutions ranging in strength from 2.5 to 10.0 percent A.I. produced no ill effects after 24 hours. However, an oral drench of 10 ml of 10 percent A.I. chlordimeform evoked immediate retching and convulsions. One day later, all test birds appeared normal. The dissociation of chlordimeform in aqueous solution may

Table XXX

Average mite indexes for roosters treated to run-off with 0.05% A.I. permethrin EC. The roosters were housed with breeder hens on floors of wood shavings.

TREATMENT	PRE-TREATMENT	DAYS POST-TREATMENT 7	35	74	124
0.05% PERMETHRIN	6.0 a	0	0.25	1.5	4.8

Table XXXI

Evaluation of the acute toxicities of permethrin EC and chlordimeform SP to Leghorn roosters and hens. Concentrations were greater than typical dosages by factors of 10 in order to simulate typical errors of acaricide mixing.

ACARICIDE USED	CONCENTRATION	METHOD OF ADMINISTRATION	BIRDS USED	RESULTS NOTED
PERMETHRIN EC	5.0%	IMMERSION	1 MALE 1 FEMALE	NO ILL EFFECTS AFTER 24 HR.
	2.5%	IMMERSION	1 MALE 1 FEMALE	NO ILL EFFECTS AFTER 24 HR.
	1.25%	IMMERSION	1 MALE 1 FEMALE	NO ILL EFFECTS AFTER 24 HR.
	1.25%	10 ML ORALLY	1 MALE 1 FEMALE	NO ILL EFFECTS AFTER 24 HR.
	25.0%	10 ML ORALLY	1 MALE	STRONG CHEMICAL ODOR; NO OTHER OVERT EFFECT AFTER 24 HR.
CHLORDIMEFORM SP	10.0%	IMMERSION	1 MALE 1 FEMALE	NO ILL EFFECTS AFTER 24 HR.

Table XXXI (Con't)

ACARICIDE USED	CONCENTRATION	METHOD OF ADMINISTRATION	BIRDS USED	RESULTS NOTED
CHLORDIMEFORM SP	5.0%	IMMERSION	1 MALE 1 FEMALE	NO ILL EFFECTS AFTER 24 HR.
	2.5%	IMMERSION	1 MALE 1 FEMALE	NO ILL EFFECTS AFTER 24 HR.
	10.0%	10 ML ORALLY	2 MALE 2 FEMALE	VIOLENT RETCHING AND CONVULSIONS; ALL CHICKENS APPARENTLY RECOVERED AFTER 24 HR.

liberate sufficient acidic radicals to produce a severe irritation to mucous membrane, especially when used at 10 percent concentration.

CONCLUSIONS

From the results of this study, the following can be concluded:

1. Artificial administration of adrenal cortical steroids or synthetic blocking agents can influence northern fowl mite population development on Leghorn roosters. Optimum doses of corticosterone or desoxycorticosterone in the feed can significantly bolster resistance of roosters to mite infestation. Conversely, blockage of the adrenal cortex via metyrapone decreases resistance of birds to northern fowl mites. Increasing or decreasing steroid doses beyond those found most effective results in a loss of resistance and often increased susceptibility.

2. High levels of social interaction increase resistance of chickens to northern fowl mite infestation in a manner similar to but more effective than steroid administration. The cause of such resistance is projected to be increased corticosterone secretion by the adrenal cortex.

3. The mechanism of resistance is primarily a decrease in capillary density at the skin surface. This phenomenon can be achieved by either administration of steroids at effective dosages or increasing levels of social stress. The decreased capillary area may present mites with difficulty in obtaining blood meals or may induce subtle temperature differentials to which mites are sensitive.

4. Laboratory and field observations agree with respect to social stress levels, management techniques, plasma corticosterone levels,

and numbers of mites parasitizing chickens. Commercial poultry housed in wire cages are sensitive to number of birds per unit. Chickens caged alone have lower plasma corticosterone levels and support more northern fowl mites than do birds caged in pairs or larger groups. This phenomenon may be used as a convenient survey tool for detecting incipient mite outbreaks, or may form an important part of a poultry pest management program. Keeping cage densities at the maximum level may reduce parasite burdens and increase production efficiency.

5. Chickens which are the best producers of meat and eggs are often those which are most susceptible to mite infestation. Allocation of resources to maintenance of resistance mechanisms draws energy from other physiological processes. The areas which are first to suffer are weight gain and testes mass.

The incompatibility of stress-induced, steroid-initiated, or inbred northern fowl mite resistance with desire for maximum productivity will cause increased reliance on chemical mite control and efficient management. The cost of bolstering mite resistance with laboratory-grade steroids is prohibitive: approximately 200 and 120 dollars per day for supplying 10,000 caged layers with 20 ppm corticosterone or 30 ppm desoxycorticosterone, respectively. It is possible that less refined grades of the latter material, especially, may eventually see short-term use as an anti-stress agent.

6. Modern poultry management practices, particularly caged-layer systems, promote stable, low-stress environments and are conducive to northern fowl mite outbreaks. Genetic selection of chickens for maxi-

mum egg-production may also tend toward producing mite-susceptible birds.

7. Sex hormones play an important supplementary role in determining the pattern of mite resistance in chickens. Estrogen alone is not responsible for the observed difference in mite susceptibility between hens and roosters, but has been shown to influence mite development on roosters. The change in mite susceptibility on pullets at the time of sexual maturation indicates that sex steroids are involved.

8. The effect of administered steroids upon parasite resistance can be demonstrated only using test animals raised under known, stable conditions. Leghorn chickens which have been moved to different environments, reared under unstable conditions, or subjected to extreme water deprivation cannot reliably provide consistent experimental results. Researchers in the field of disease and parasite resistance will want to consider these factors in future experiments.

In addition, experimental differences between test groups can be shown most effectively by stabilizing test environments and using only test animals of known origin. This procedure will assist in minimizing variation within experimental groups.

8. Antibody defense is not the reason for mite resistance in chickens subjected to initial mite challenge. Birds which are most severely mite affected are the most competent antibody producers, and increasing stress levels to a point where mite populations are suppressed results in little change in antibody competency.

9. Malathion tolerance was demonstrated in mites originating from

commercial poultry quarters in southwest Virginia. The LC_{50} value produced by this compound was approximately 21 times greater than that measured 15 years ago in New York using an identical technique. This is believed to be the first documented record of insecticide tolerance by this mite species in the eastern U.S.

10. The synthetic pyrethroid compound permethrin produced excellent, long-lasting northern fowl mite control when applied in simulated field tests. While the acute toxicity of this material is not materially different from stirofos, the superior control afforded is believed to result from persistence in the feather coat.

Roosters treated with permethrin and housed with mite-infested hens in floor-managed flocks reduced mite populations among female birds. This resulted from transfer of acaricidal compound from roosters to hens during mating activity.

The results of this study point to the requirement for an effective system of pest management in relation to poultry husbandry. Caged-layer houses are a special problem. The poultry producer can now be offered 2 alternatives: birds which are mite resistant but inferior producers, or birds which produce at maximum levels but which require high managerial levels to avoid parasite problems. It is probable that most poultrymen will choose the second alternative.

High production chickens will demand intensified sanitation, parasite surveys, and monetary input. Maximizing cage densities, managing birds in floor colonies whenever possible, and adequate

chemical control will help avoid problems relating to northern fowl mites.

Additionally, new chemicals such as chlordimeform and the synthetic pyrethroids may provide poultry husbandrymen with effective tools employing various modes of action for use against poultry ectoparasites.

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VITA

Robert D. Hall was born on March 6, 1947, in Washington, D.C. His parents are David G. Hall and Pauline E.O. Hall of Arlington, Virginia. He graduated from Washington-Lee High School in Arlington, Virginia, in 1965. From 1965 through 1968, he attended the University of Virginia in Charlottesville. In 1968, he entered the U.S. Air Force where he served four years with USAFSS and was discharged with the rank of Staff Sergeant. After completing undergraduate college work at the University of Maryland, College Park, in 1973, he began graduate studies in Entomology at Virginia Polytechnic Institute and State University. He received the M.S. degree in June, 1975, and has held a Graduate Teaching Assistantship in the Department of Entomology since that time.

In 1968, he married Sarah Jane Dinsmore of Vienna, Virginia. She received the M.A. degree in Education from Virginia Polytechnic Institute and State University in 1977 and is currently employed as an elementary school teacher in Giles County, Virginia. Their first child, Emily Dinsmore Hall, was born on June 10, 1977.

In September, 1977, he will assume the position of medical and veterinary entomologist with the Department of Entomology, University of Missouri - Columbia. In addition, he will become a Reserve Preventative Medicine Officer with the 14th Preventative Medicine Unit, United States Army, based at Springfield, Missouri.

Robert D. Hall

ADRENAL STEROID, BLOCKING AGENT, AND SOCIAL STRESS EFFECTS ON NORTHERN FOWL MITE POPULATION DEVELOPMENT ON LEGHORN CHICKENS AND TOXICOLOGICAL EVALUATION OF SELECTED ACARICIDES

by

Robert D. Hall

(ABSTRACT)

Administration of adrenal steroids or blocking agents at optimum doses influenced northern fowl mite development on chickens. Corticosterone at 20 ppm or desoxycorticosterone at 30 ppm in feed were most effective in inhibiting mite infestations. High levels of social stress increased resistance of chickens to mites in a manner similar to but more effective than steroid administration. The mechanism of resistance was a decrease in capillary density at the skin surface. Commercial laying hens caged alone had lower plasma corticosterone levels and supported more mites than hens caged in groups. Stress-induced, steroid initiated, or inbred mite resistance was incompatible with maximum production from chickens. Resistant chickens produced poorer weight gains and testes mass than did susceptible birds. Sex hormones were shown to play a supplementary, and antibody a minor role in mite resistance.

Carbaryl was shown to be the compound most toxic to northern fowl mites of those registered in Virginia for application to poultry. Malathion resistance was noted in mites from a commercial poultry house. The synthetic pyrethroid permethrin was effective against these mites.