



Physiologic and nutritional factors affecting the egg production of the rabbit stomach worm  
*Obeliscoides cuniculi*  
by Charles Alfred Slyngstad

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE  
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**Abstract:**

This study was undertaken to investigate physiological and nutritional factors affecting the rabbit stomach worm *Obeliscoides cuniculi*. This trichostrongylid nematode has been studied for a possible model system for the gastric nematode of cattle, *Ostertagia ostertagi*.

Several similarities between these two nematodes have been found, including the phenomenon of arrested development.

Rabbits infected orally with 14,500 third stage *O. cuniculi* (L3) and subsequently bred showed a significant ( $P < .05$ ) increase in egg production after approximately 20 days of lactation as compared to non-bred female controls. No significant differences in egg counts were recorded during gestation, parturition or early lactation.

Female rabbits infected with 8,000 *O. cuniculi* L3 and later found to have patent infections were given daily injections for 28 days of 0.5, 5.0 or 50 mg of the plant growth hormone, gibberellic acid (GA3). Egg production seemed to be dose dependent. At the level of 5 mg per day, GA3 increased egg production, although not by significant amounts. The 50 mg per day level of GA3 tended to inhibit egg production, while the dose of 0.5 mg per day had no effect. Injections were resumed for 14 days following an 80 day rest-period. No significant differences were noted at any level of administration following this second series of injections.

Wheat sprouts, known to affect reproduction in *Microtus montanus*, were added to the diet of male rabbits which had prior oral inoculations of 750 *O. cuniculi* L3. Sprouts were fed at a rate of 15 grams per day per each individual for 28 days. Worm egg production prior to feeding or after feeding commenced showed no significant difference from the controls. The results of these experiments indicate that certain physiological conditions associated with lactation in rabbits and injections of GA3 at 5 mg daily increase egg production of *O. cuniculi*, while at higher and lower doses the effects were negative or absent.

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PHYSIOLOGIC AND NUTRITIONAL FACTORS AFFECTING THE EGG PRODUCTION  
OF THE RABBIT STOMACH WORM OBELISCOIDES CUNICULI

by

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A thesis submitted in partial fulfillment  
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## ABSTRACT

This study was undertaken to investigate physiological and nutritional factors affecting the rabbit stomach worm Obeliscoides cuniculi. This trichostrongylid nematode has been studied for a possible model system for the gastric nematode of cattle, Ostertagia ostertagi. Several similarities between these two nematodes have been found, including the phenomenon of arrested development.

Rabbits infected orally with 14,500 third stage O. cuniculi (L<sub>3</sub>) and subsequently bred showed a significant ( $P < .05$ ) increase in egg production after approximately 20 days of lactation as compared to non-bred female controls. No significant differences in egg counts were recorded during gestation, parturition or early lactation.

Female rabbits infected with 8,000 O. cuniculi L<sub>3</sub> and later found to have patent infections were given daily injections for 28 days of 0.5, 5.0 or 50 mg of the plant growth hormone, gibberellic acid (GA<sub>3</sub>). Egg production seemed to be dose dependent. At the level of 5 mg per day, GA<sub>3</sub> increased egg production, although not by significant amounts. The 50 mg per day level of GA<sub>3</sub> tended to inhibit egg production, while the dose of 0.5 mg per day had no effect. Injections were resumed for 14 days following an 80 day rest-period. No significant differences were noted at any level of administration following this second series of injections.

Wheat sprouts, known to affect reproduction in Microtus montanus, were added to the diet of male rabbits which had prior oral inoculations of 750 O. cuniculi L<sub>3</sub>. Sprouts were fed at a rate of 15 grams per day per each individual for 28 days. Worm egg production prior to feeding or after feeding commenced showed no significant difference from the controls. The results of these experiments indicate that certain physiological conditions associated with lactation in rabbits and injections of GA<sub>3</sub> at 5 mg daily increase egg production of O. cuniculi, while at higher and lower doses the effects were negative or absent.

## INTRODUCTION

Innumerable morphological and physiological adaptations have contributed to the survival of existing animal species over evolutionary time. This paper deals with one such adaptation: arrested development, also referred to as diapause or dormancy. Arrested development occurs in many unrelated groups of invertebrates and has been well studied especially among members of the Arthropoda. In the Phylum Nematoda, more than 30 species exhibit this phenomenon (Michel, 1968), and among these helminths, arrested development permits the survival of many economically important parasites of domestic animals. Among these is the gastric nematode, Ostertagia ostertagi, of cattle. Martin et al. (1957) found adults of O. ostertagi in cattle several months removed from pasture. Previously this parasite was thought to have an uninterrupted direct life cycle requiring approximately three weeks for completion after the exposure of a susceptible host. The typical life cycle of these nematodes includes the ingestion of infective larvae ( $L_3$ ) by the host, where these larvae then enter a histotropic phase of the life cycle in which they are closely associated with the gastrointestinal epithelium. These larvae ( $L_3$ ) migrate into the gastric mucosa and undergo two molts or ecdyses, arrested development ensuing after the first, when it occurs. The 4th larval stage is capable of remaining embedded in the host's gastrointestinal epithelium for a prolonged period.

Michel (1968) listed several factors which may lead to the induction of arrested larvae:

- A. Environmental effects
- B. Diet
- C. Size of infective larval dose
- D. Presence of adult worms
- E. Host age resistance
- F. Acquired immunity

Other reports have contained similar summaries (Taylor and Michel, 1953; Madsen, 1962; Michel, 1969; Armour, 1970; Jarrett and Urquhart, 1971, and Ogilvie and Jones, 1973). Certain of these mechanisms will be discussed in detail in conjunction with post diapause development of the rabbit stomach worm, Obeliscoides cuniculi.

It was stated in the opening paragraph that physiological adaptations were required for survival through evolution. It has become apparent that the ability of a parasite to stop its development for a prolonged period of time is truly a beneficial adaptation. This mechanism has enabled the parasite to synchronize its life cycle with that of the host by remaining dormant during a time when existence outside the protective environment of the host is unfavorable.

#### Post-diapause Development

If parasites have acquired the ability to cease development during the winter when environmental conditions for free living stages are unfavorable, these same parasites must also have a mechanism

to resume development at a time when the environment is more suitable for survival. Several processes have been proposed but thus far none has adequately described which mechanisms are responsible for resumption of development. The terms usually applied to resumed development are spring rise or post-parturient rise (Crofton 1954, 1958). In using these terms it is often assumed that the rise in fecal egg counts, which is observed in the spring of the year, is the result of resumed development of dormant larvae. In reporting on post-parturient rises in nematode egg counts of ewes, Connan (1967) stated that the generally accepted view is that the rise in egg counts results from maturation of previously dormant larvae. This is the case particularly with Ostertagia spp. which may have accumulated in the host some months previously (Naerland, 1949; Taylor, 1953; Crofton, 1954, 1958; Soulsby, 1957; Field, Brambell and Campbell, 1960; Dunsmore, 1965). The factors or mechanisms which terminate arrested development are not entirely clear. Some of these are listed below.

- A. Maturation of adult worms (recruitment)
- B. Environmentally conditioned larvae
- C. Depression in host resistance
- D. Lactation
- E. Parturition
- F. Diet

A. Adult worms

Michel, Lancaster and Hong (1975, 1976) reported that the resumption of development of O. ostertagi was regulated in some way by the presence of adult worms. If adults were regularly removed, larvae were constantly developing to replace these lost adults. This idea supports the concept of a critical number. Any larvae ingested in excess of this critical number have development stopped and have it began at a later time when adult numbers drop below a certain level. No specific mechanisms were postulated for this adult population control.

B. Environmentally conditioned larvae

In 1974, Armour and Bruce found that previously conditioned larvae (exposed to autumn conditions) of O. ostertagi spontaneously resumed development 23 weeks after the start of the conditioning treatment. It appeared that no arrested worms had developed up to that time. They equated this to an insect-like diapause, or cessation of growth for a prolonged period of time. Obviously these results were at variance with those of Michel, Lancaster and Hong (1975, 1976).

C. Depression in host resistance

Taylor and Michel (1953) discussed the significance of arrested larvae. They stated that inhibited larvae resumed development during

temporary depressions in the host's resistance. More recently Prichard, Donald and Hennessy (1974) showed with the use of corticosteroids that a depressed immune status of the host does not stimulate resumption of development of inhibited O. ostertagi larvae. The use of immunosuppressants in rabbits infected with O. cuniculi has produced somewhat conflicting results. Fox (1976) showed that regular injections of corticosteroids (9 fluoroprednisolone) from day 0-28 of infection resulted in egg counts and worm populations differing from controls which received no corticosteroids. In rabbits receiving injections, substantially more adult worms reached the late 5th stage. However, the egg count data showed that egg production was impaired in both treated and control animals. In a similar experiment Fox (1976) showed that rabbits injected with corticosteroids on day 20-26 of infection had significantly more adults than either the controls (no injections) or rabbits which were injected on day 0-6 of infection. Rabbits infected on days 20-26 also had egg counts similar to normal infections.

#### D. Lactation

Connan (1976) reported that during lactation, and sometimes during late pregnancy, the immune response of the host to gastrointestinal nematodes is partially suppressed. This phenomenon has been observed in the ewe, sow, heifer and rabbit. In the previous

paragraph it was mentioned that a suppressed immune status had no effect upon dormant O. ostertagi larvae. Brunsdon (1967) showed that the spring rise phenomenon in ewes depended upon the time of the year lambing occurred, and therefore, was independent of lactation.

#### E. Parturition

Crofton (1954) concluded that the rise in egg counts occurring in ewes in the spring was related to the time of lambing. He found the egg count rise to occur six to eight weeks after parturition. Brunsdon (1964) reported a similar rise in fecal egg counts six to eight weeks after lambing. Later, Brunsdon (1967) reported on experiments using two groups of ewes whose rise in fecal egg counts seemed to depend upon the time of the year in which lambing occurred. He failed to find a significant rise in egg counts in ewes lambed in the fall, whereas ewes from the same flock lambed in the spring showed a typical spring rise. Brunsdon (1967) postulated from these experiments that the physiological mechanism which triggers the rise in egg counts is closely associated with the reproductive cycle in normal lambing ewes, while in ewes lambing at other times this mechanism may not coincide.

#### F. Diet

Very little work has been done with effect of diet upon arrested larvae. Armour, Jennings and Urquhart (1969), in discussing

environmental changes during autumn, postulated that the host might be affected by alterations in the secretory rates of endocrine glands in response to various stimuli such as reduced daylight, low temperatures, falling plane of nutrition and other stresses. The host's diet was the subject of two of the experiments discussed in this paper.

#### Fecal Egg Counts

The number of eggs counted in a weighed amount of fecal material has been used routinely as an approximate indication of parasite burdens. When egg counts rise it is assumed that the parasite numbers are increasing. This assumption must be qualified by stating that under certain conditions (lactation) existing adult worms increase in their fecundity. The concept of increased worm fecundity is undoubtedly important at times, although the maturation of dormant larvae as a source for increased adult numbers and egg counts is considerably more important.

#### Obeliscoides cuniculi

Obeliscoides cuniculi, a trichostrongylid gastric nematode, was first described by Graybill (1923, 1924) from a natural infection in a domesticated rabbit. The life history was originally described by Alicata (1932). It has been shown that the domestic rabbit

(Oryctolagus), native cottontail and marsh rabbits (Sylvilagus spp.), the eastern woodchuck (Marmota monax) and guinea pigs can serve as suitable hosts. The use of the Obeliscoides rabbit system as a laboratory model which simulates bovine ostertagiosis has been postulated by Worley (1963), and Russell, Baker and Raizes (1966).

The prepatent period for O. cuniculi varies with the size of larval dose. Worley (1963), using larval doses between 100 and 10,000 infective larvae, showed an average prepatent period of 19 days. Russell, Baker and Raizes (1966) showed a prolonged prepatent period of up to 35 days when large larval doses (25,000 L<sub>3</sub>/Kg of body weight) were given. Egg production has been shown to be inversely related to increasing inoculation levels (Russell, Baker and Raizes, 1966). A similar finding was reported by Michel (1963) in calves infected with O. ostertagi.

Impaired larval development of O. cuniculi has been postulated to result from several different factors. Sollod (1968) showed that development is arrested at the early 4th stage when the worms have doubled in length after entering the host. Obeliscoides cuniculi has also been shown to suspend development when larvae are stored in a cold environment for a prolonged period of time (Fernando, Stockdale and Ashton, 1971; Stockdale, Fernando and Lee, 1970).

Russell, Baker and Raizes (1966) showed that the number of arrested larvae depended upon the size of larval dose. In rabbits inoculated with 2,500 and 25,000  $L_3$ /Kg of body weight, 6.0% and 70.9% of the total population, respectively, were arrested. Fox (1976) showed that both immunological status and degree of antigenic stimulation in rabbits affected the development of O. cuniculi such that large numbers of larvae were inhibited.

In wild rabbit populations, Gibbs et al. (1977) and Dorney (1963) have found that O. cuniculi infections vary seasonally. Spring and summer infections have been found to consist of predominately immature worms. This strongly suggests that O. cuniculi in nature uses arrested development as a mechanism to survive the winter. Both of these studies were conducted in the northern regions of the United States where winter conditions are unsuitable for the survival of free living larvae.

Fox (1978) stated that the effects of host nutrition, hormones and sex have not been studied for this host parasite system. The seasonal aspects of arrested development lead one to inquire whether seasonal changes in the host's diet contains specific nutrients which affect arrested larvae. Parturition by the rabbit also may stimulate arrested O. cuniculi larvae to resume development.

MATERIALS AND METHODS

Helminth Cultures

The strain of Obeliscoides cuniculi used in these experiments was originally isolated from a cottontail rabbit (Sylvilagus floridanus mearnsi) collected in Ohio in 1959. This strain has been maintained in laboratory rabbits by infecting regularly with 3,000-5,000 third stage larvae (L<sub>3</sub>). Passage of the strain occurred every 3-5 months. Feces were collected over wet wood shavings covered with cloth towels to avoid desiccation. Fecal pellets were softened by soaking in water for 1-2 hours, after which peat moss was mixed in with a wooden spoon (approx. 2/3 peat moss and 1/3 feces). This material was then stored in covered glass dishes at room temperature (approx. 25 C) for 10-12 days. Infective larvae were recovered from the cultures using the standard Baermann technique (Baermann, 1917). Baermannization was done through 2 layers of gauze supported by a 60 mesh screen in an 8-inch polyethylene funnel. The larvae were washed several times and used within 72 hours. During this short storage time the larvae were refrigerated at 4 C.

### Inoculations with Infective Larvae

The infected larvae were pooled prior to inoculation. An aliquot of this pool was taken and numbers of larvae were counted. Volumes of the suspension containing the appropriate number of larvae were then administered to the rabbits by oral inoculation. Intubation was accomplished with a size 6 Bard catheter (Bard woven venous cannula, C.R. Bard Inc., Murray Hill, N.J.) covered with a tight fitting piece of Tygon tubing (U.S. Stoneware, Akron, Ohio) as a stomach tube.

### Rabbits

Domestic rabbits (Oryctolagus cuniculus L.) were purchased from two local rabbit producers. Several breeds were used depending upon availability. French Lopps were used in one trial while cross-bred rabbits were used in the remaining experiments. The age of these rabbits ranged from 8 weeks to 17 weeks depending upon the experimental design. Each rabbit was caged individually in cages with expanded metal floors. Rabbits were usually allowed 10 days to acclimate to the laboratory animal room. The animal room was illuminated by overhead fluorescent lights which were controlled by a timeclock. In each 24 hour period the lights were on constantly for 18 hours. Antibiotics, other than that contained in the feed, were administered on two occasions for jaw abscesses. Rabbits were fed

ad libitum a medicated Peavey pelleted ration containing .02% furazolidone (Peavey Co., Minneapolis, Minnesota).

#### Fecal Examinations

Fecal egg counts were determined by using the modified Lane flotation procedure (Dewhirst and Hansen, 1961). Five grams of feces were macerated in 150 ml of water using an electric blender. Duplicate 15 ml aliquots were collected in 15 ml calibrated test tubes and sedimented at 1,500 g for 3 min. The supernate was removed and replaced with saturated NaCl solution (specific gravity 1.18). The sediment was resuspended by stirring and additional NaCl solution was added until a meniscus was formed at the top of the tube. A coverslip (22 mm sq., No. 2 thickness) was then placed on top of each tube after which they were centrifuged at 700 g for 2 min. The coverslips were then removed and placed on a microscope slide and examined under low magnification using a compound microscope. All eggs were counted and a correction factor (2) was used to arrive at the count of eggs per gram of feces (EPG).

#### Wheat Sprouts

Kernels of wheat were sprouted between layers of moistened paper towels. The sprouts were kept moist until an overall size of 2-4

inches was attained (approx. 2-3 weeks). After reaching this size the sprouts were fed to the rabbits. The sprouts were grown under fluorescent light which was illuminated continually for 18 hours during each 24 hour period. The variety of wheat used in this experiment was New Gaines, a soft wheat, high in starch and low in gluten.

#### Plant Growth Hormone

A plant growth hormone, gibberellic acid, was purchased as a lyophilized powder from Sigma Chemical Company, St. Louis, Missouri. Gibberellic acid was dissolved in 0.8% physiological saline solution (PSS) at various concentrations. There were minute particles in suspension at the highest concentration level used (50 mg/3 ml PSS). This hormone was administered to rabbits via subcutaneous injection just posterior to the ears, and just lateral to the dorsal midline. No foreign body reaction occurred during this study.

#### Breeding of Rabbits

Female cross-bred rabbits were inoculated with infective larvae at approximately 4 months of age. Once patency was confirmed, each doe was randomly placed in a cage with a male rabbit. Each pair was observed to see if mating occurred. Once the females were bred, they

were returned to their cage. Breeding of the rabbits required a total of 7 days. Just prior to parturition cotton was placed into each expectant female's cage to aid in nest building. Newborn rabbits were permitted to nurse "on demand" to stimulate lactation. Litters were caged with their mothers until they were weaned at 6-7 weeks of age.

RESULTS

Wheat Sprouts in Diet of Rabbits Infected  
with Obeliscoides cuniculi

The objective of this experiment was to study the effect of wheat sprouts in the diet of rabbits infected with Obeliscoides cuniculi. It was hoped that wheat sprouts introduced in the diet might, in the simplest of terms, elicit an early spring diet response similar to that observed in cattle or sheep which undergo the "spring rise" phenomenon. This experiment was modeled somewhat after the experiments of Negus (1966) and Hinkley (1966). In their work they showed that wheat sprouts or ether extracts of wheat sprouts placed into the diets of Microtus montanus had profound effects upon reproduction and fertility (Negus and Pinter, 1965). The current experiment was designed to detect any stimulatory effect exerted by 'green feed' in the diet of fertility or fecundity of the gastric nematode, O. cuniculi.

In male M. montanus, wheat sprouts placed into the diet stimulated gonadotropin secretion. It is known that host hormone levels affect nematode parasitism (Connan, 1966; Dunsmore, 1966; 1971). Work done in Maine with wild hare populations (Gibbs et al., 1977) has shown a seasonal increase in intensity of infection with O. cuniculi, the maximum egg counts coinciding exactly with the breeding season.

In this experiment, 16 male domestic rabbits (avg. age 8 weeks) were each infected with 750 3rd stage larvae ( $L_3$ ) of O. cuniculi. following a 30-day post-inoculation period, quantitative egg counts were started using the Lane flotation method. Egg counts were recorded periodically during the next 15 days. Following this period, two groups were formed consisting of 8 rabbits in each group. These groups were selected in order to equalize the egg counts between groups. Following the 45-day post-inoculation period, group I began receiving 15 grams of sprouted wheat daily. This was fed in conjunction with commercial pellets and water ad libitum. The wheat sprouts were eaten immediately upon placing them in the feeding pans. Group II was fed commercial pellets and water ad libitum. Egg counts were recorded periodically over the next 28 days.

Prior to the feeding of wheat sprouts, the egg counts between groups were very similar (Fig 1). After wheat sprout feeding began the egg production between groups remained very similar. Analysis of variance failed to show any significant difference in fecal egg counts at any time.

Effect of Gibberellic Acid upon  
Obeliscoides cuniculi Infections

The purpose of this experiment was to determine the effects of daily injections of gibberellic acid ( $GA_3$ ) upon larval and adult O.

















































