HOST RESISTANCE TO TICK INFESTATIONS
IN TWO BREEDS OF GOATS, USING THE TICK
RHIP ICEPHALUS APPENDICULATUS NEUMANN
(THE "BROWN EAR" TICK). //

BY

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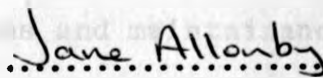NAIROBI.

1983

DECLARATION BY CANDIDATE

This Thesis is my original work
and has not been presented for a
degree in any other University.

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DECLARATION BY SUPERVISOR

This Thesis has been submitted for
examination with my approval as
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. . . . Jane Allonby . . . . 31 August 1983

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TABLE OF CONTENTS

CHAPTER   1

CHAPTER   2

CHAPTER   3

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENT

ABSTRACT

The objectives of this study were to observe and compare the levels of any innate resistance and development of any acquired resistance to Rhipicephalus appendiculatus ticks in two breeds of goats, viz. the Local East African (L.E.A.) meat goats and exotic Toggenburg (T.G.) milk goats.

Ticks were applied to feed on the animals' ears which were covered by cloth bags to prevent loss of ticks. Each day the distal ends of the ear bags were opened. Engorged ticks were collected, counted and weighed after each feeding period. The two groups of goats were alternately challenged with increasing burdens of both nymphal and adult ticks: 50 nymphs (NN), 25 adult females (AA), 100 NN, 50AA, 200NN, 100AA, 400NN, 150AA and 50NN (final test) from ticks maintained in culture. Each time different rabbits, naive to R. appendiculatus, were used as controls. They were infested at each level of challenge in order to monitor the viability of the tick culture. In all parameters measured there were no significant differences between the results from each tick test on the control rabbits indicating that the ticks were of uniform quality and the experimental results therefore were comparable.

Feeding success of ticks, as an index of goat resistance to infestation, was assessed and found to decrease with successive tick infestations. The number of nymphal and adult ticks able to engorge on the goats, and their engorgement weights, decreased significantly in most cases with successive tick challenges. The

duration of feeding by the attached ticks was significantly
(P<0.001) lengthened. The proportion of engorged nymphs that
succeeded in moulting to adults decreased, while the duration
of the development period increased.

The pre-oviposition period for engorged female ticks was
also significantly (P<0 .001) prolonged, and there was a significant
reduction in mean egg output and in the percentage of eggs that
hatched. Hence female ticks fed on resistant goats did experience
reduced fecundity, and fertility decreased significantly. The
net result was that there were far fewer larvae produced in the
next generation.

Responses in the two breeds of goats were significantly
different in some of the parameters. However, resistance was
observed to develop in both breeds. The L.E.A. showed greater
innate resistance perhaps due to the previous history of the
mothers (does) which had already encountered ticks in the field.
It is likely that some immunity against ticks was transferred to
their progeny.

The reduction of tick populations has important implications
from epidemiological and economic standpoints. Tick resistant goats
could be kept with cattle in order to reduce the size of the tick
populations experienced by the latter. This would be a feasible
method of biological tick control. The transmission of tick-borne
diseases may also be reduced since the ability of the ticks to
feed is impaired.

C H A P T E R   I

1:1   INTRODUCTION

It has long been known though not
documented, that Maasai herdsmen herd goats and
sheep in front of their prized cattle as they
pioneer any new area of grazing. Any ticks on
the ground are thereby "mopped up" by the less
valuable small stock, helping to reduce tick worry
and any subsequent disease to their valuable cattle.
However, the economic importance of goats and sheep
in their own right is increasing tremendously, par-
ticularly amongst the Kenyan small scale farmers.
Goats provide products such as meat, milk and hides
and are much cheaper to maintain in terms of feeding
and disease control than cattle and they can no
longer be ignored.

About 500 species of ixodid ticks have been
identified in the world today, many of which occur
in Africa (Hoogstraal, 1956). In Kenya, 73 species,
classified in 9 genera, have been recorded (Walker,
1970), of which about 10 species are economically
important. One of the most important tick species
in Kenya is Rhipicephalus appendiculatus Neumann as
it is found in most of the high potential livestock
areas.

R. appendiculatus   the "brown ear"
tick, is an ixodid tick feeding on three separate

hosts as larvae, nymphs and adults respectively.
They drop off the host after each blood meal, under-
going moulting and egg laying activities on the
ground. The larvae emerging from eggs laid on the
ground climb onto a passing host to feed for a few
days, engorge, then drop off to moult on
the ground. The emerging nymphs attach to a second
host and feed to full engorgement over a period of
between five and seven days. Fully fed, they drop to
the ground to moult through to adult male or female
ticks which attach onto a third host, feed and mate.
The female feeds to full engorgement and then drops
to the ground to lay her eggs.

R. appendiculatus is distributed in
Kenya from sea level to altitudes over 2100m, in
areas where there is adequate moisture, that is, areas
with 600mm. of rain or more per annum and a relative
humidity of over 85%. Over 2100m , the numbers of
R. appendiculatus infesting livestock declines
as this tick cannot withstand low temperatures or
frost found at extremely high altitudes. It is found
in the coastal strip as far inland as the Kurwa parts
of Taita hills; in the wetter parts of Eastern Province
in Machakos, Kitui, Embu and Meru districts; all
districts of Central Province; the wetter parts of
the Rift Valley Province such as Kajiado,
Elgeyo Marakwet, Tranzoia, Narok and Kericho districts;

most parts of Western Province; and all parts
of Nyanza Province especially Kisii district.(Walker, 1974).

The main hosts of R. appendiculatus
are cattle, goats /and sheep but horses, mules, donkeys
and dogs may also be attacked. However, cattle
appear to be the most important host of
R. appendiculatus , in Kenya. The tick prefers the
ears of the cattle, particularly along the edges
and the inside of the ear. These ticks may also
attach onto bases of horns, eyelids, cheeks and
on the basis of the tail switch.

R. appendiculatus , is an important
ectoparasite because it is a blood sucker, its
bite wounds can develop secondary infections, and
during feeding the tick can inject toxins as well
as a number of endoparasites into their hosts.
Toxins can cause irritation , annoyance and sometimes
paralysis to the host. R. appendiculatus
is the most efficient vector of Theileria parva
which causes East Coast Fever (E.C.F.), a disease
responsible for many cattle deaths in Kenya today.

As goats, sheep and cattle usually occur
in the same ecosystem, control of the ticks on
the cattle alone cannot eradicate the problem as
the life cycle of the R. appendiculatus .can
be perpetuated by the small stock. To help the

cattle industry and also to make the goat and sheep industry itself more efficient and productive broad front control methods are needed. Up to now two methods of tick control have been used in Kenya: regular dipping of livestock, and rotation of pasture lands.

In the past the dip chemicals used to kill the ticks were chlorinated hydrocarbons such as Dichloro diphenol trichloroethene (D.D.T), benzene hexachloride (B.H.C.) and toxaphene. They were used so extensively that ticks started to show resistance to these chemicals. Tick resistance to toxaphene is now widespread, especially in blue ticks and red-legged ticks; and more recently resistance has spread to brown ear ticks (Wellcome Eastern Africa Ltd., 1980). Instance of resistance to organophosphorous compounds is currently confined to blue ticks (Wellcome Eastern Africa Ltd., 1980), but there is every reason to suppose that tick resistance will eventually become widespread (Wellcome Eastern Africa Ltd., 1980). Carbamates are similar to organophosphorous compounds and ticks appear to be able to develop cross-resistance between the two groups; and some resistance has been reported to carbaryl especially blue tick strains (Wellcome Eastern Africa Ltd., 1980). Since acaricide resistance is increasing, and the search

for new safe dip chemicals is obviously a continuous
and expensive task, other methods of tick control
must be sought to help alleviate the problem.
Recently Wellcome Eastern Africa Ltd. have deve-
loped a new drug known as Clexon as prophylaxis
against East coast fever. Currently the drug is
expensive to make and hence not generally availa-
ble to the small farmer.

The breeding of tick resistant animals
could possibly be the answer. Already research
is in progress/to produce resistant cattle.   /which aims
Chiera and Newson (personal communication, 1982)
have shown that if ticks in the field have only
resistant cattle on which to feed, tick numbers
can be reduced to very low levels. Laboratory
studies have confirmed that $\underline{R}$. appendiculatus
ticks fed on resistant cattle are smaller at each
stage than those fed on susceptible hosts and much
fewer eggs are produced by the adult female ticks
(Chiera and Newson, personal communication, 1982).
Hence the smaller sized ticks, after passage
through resistant cattle could be a potent factor
in influencing the reduction of tick numbers in the
field.

The use of cattle which are resistant to
ticks offers a method of control which does not
require any extra managerial skills or continual

expense incurred where chemicals are the sole
method of control. The use of resistant cattle
and minimal use of chemical control therefore
seem to be desirable long term objective s.

It is obvious that there is now also a
need to develop resistant sheep and goats since
they all occur under the same farming situation.
In this research an indizenous meat goat, the
Local East African (L.E.A) goat 'was' investigated
to this end. It is this breed of goat which grazes
most commonly side by side with the Zebu cattle in
a local farming situation. In an attempt to
observe any natural resistance which may have bred
into this breed, it was decided to observe the
exotic Toggenburg breed of goats (T.G.) too as
Toggenburgs are milk goats used in many cross
breeding programmes in Kenya.

1:1:1   OBJECTIVES:

The objectives of this study were to observe
and compare the levels of any innate resistance and
the development of any acquired resistance to
R. appendiculatus   in two breeds of goats i.e.
indigenous Local East African meat goats and exotic
Toggenburg milk goats. R. appendiculatus   naive
rabbits were used at each experimental infestation as
controls to ensure that the ticks used from the culture

were stable and would give comparable results.
"Resistance indicators" monitored at each level
of tick challenge were as described below.

(a) The numbers of R. appendiculatus
nymphs and adult females which engorged
and were collected daily in the ear bags
of both breeds of goats were recorded at
each level of tick challenge i.e. at 50,
100, 200, 400 and 50 nymphs; and 25, 50,
100 and 150 adult female ticks. The
percentage engorgement of each tick
challenge was calculated by:

$$\frac{\text{Total number of engorged ticks } (X_1) \times 100}{\text{Total number of ticks applied } (X_0).}\%$$

where, X_0 stands for 50, 100, 200, 400 and 50
nymphs; 25, 50, 100 and 150 adult female
ticks.

X_1 = Total number of ticks engorged per
tick challenge. This was variable.

(b) The numbers of squashed or dead nymphs and
adult female R. appendiculatus   were
collected daily, and their percentage
calculated by:-

$$\frac{\text{Total number squashed or dead } (X_1) \times 100}{\text{Total number of ticks applied } (X_0)}\%$$

Where, X_1 = Total number of ticks squashed or dead

.X_0 = Total number of ticks applied at a

given challenge.

(c)   Individual weights at engorgement (in mg.) of

nymphs and adult female R. appendiculatus

were measured; means and standard deviations

were calculated as described below:-

Mean = $\dfrac{\text{Total weight of all individual tick observations}}{\text{Total number of ticks weighed.}}$

standard deviation (SD) = square root of variance

N.B.   Standard deviation was also directly obtained

from a desk calculator.

(d)   The length of feeding period on the host (in

days) were recorded for both nymphs and adult

female R. appendiculatus.

(e)   The number of days taken for the nymphs to

moult to adult R. appendiculatus   were

recorded/then the moulting percentage was
      and

calculated.

Moulting Percentage = $\dfrac{\text{Total number of ticks that moulted x 100}}{\text{Total number of engorged nymphs put to moult.}}$ %

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*   Casio fx-39 scientific calculator made in Japan

by Casio Computer Co. Ltd. was used to calculate

standard deviations directly.

(f) The number of days before adult females
      laid eggs were recorded and percentage
      calculated.

      Egg laying percentage

      $= \dfrac{\text{Total number of ticks that laid eggs x 100}}{\text{Total number of engorged females put to lay.}}$ %

(h) The length of time for eggs to hatch and the
      weights of the egg batches were recorded. The
      percentage of eggs hatching was calculated.

      Hatching $= \dfrac{\text{Total number of egg batches that hatched x 100}}{\text{Total number of egg batches laid.}}$ %

C H A P T E R   2

2:1   LITERATURE REVIEW

Acquired resistance by cattle to tick
infestations was first reported   (Johnston and
Bancroft,   1918). Since then many workers have
reported on the innate component in addition to the
acquired responsiveness of many hosts to tick
infection. Bos indicus cattle, or those with a
significant Bos indicus genetic background, were
considered to be more innately resistant to tick
infestation than cattle of Bos taurus genetic
composition (Kelley, 1943; Riek, 1962; Francis and
Little, 1964; Wharton, et al 1970; and Seifert, 1971). How-
ever, more recently Wagland (1975, 1978) reported
that B. taurus and B. indicus cattle not previously
infested with Boophilus microplus were equally suscep-
tible to infestation, a finding that brought contro-
versy on the validity of the concept of different
levels of innate resistance in each breed of hosts.
The development and use of cattle with defined
immunogenetic characteristics would greatly facilitate
these studies and allow for a more accurate assessment
of the concert of "innate resistance."

Acquired resistance to tick infestation by
bovines has been reported by many investigators.
These workers suggested an immunological basis for

acquired resistance, but the actual mechanisms still
remains to be described.

Trager (1939b) described acquired resistance
in guinea pigs, to infestations with Dermacentor
and other species of ticks. He suggested that immu-
nological mechanisms caused the reduced blood feeding
shown by larvae infesting resistant animals. Further
evidence to support this suggestion was provided by
Allen (1973), Bagnall (1975) and Wikel and Allen (1976a,
b, 1977).

In their experiments with Dermacentor species
larvae , Allen (1973) and Wikel (1976) and Wikel
and Allen (1976a, b, 1977) assayed the tick resistance
of guinea pigs by comparing the weights and percentage
of larvae which had engorged on normal and resistant
animals at the end of a standard 5 - day infestation
period. It was found that, generally, about 80% of
the larvae engorged on normal hosts but only 5-10% of
larvae from the same batch engorged on resistant animals.
In histological studies (Allen, 1973; Allen and Wikel,
1978) it was shown that a very marked infiltration of
basophil leucocytes occurred in the skin of challenged
resistant guinea-pigs, particularly high concentrations
of degranulated basophils were seen in epidermal
vesicles which developed beneath the attachment   sites
of larvae. Such vesicles were evident by day 2 of the
challenge infestation and reached macroscopic propor-

tions by day 4. It was suggested that such responses
resemble cutaneous basophil hypersensitivity reactions
of the guinea pig and that the degranulation of
basophils close to the mouthparts of the challenging
tick might have released mediators which disrupted
the normal behaviour of the tick (Allen and Humphreys, 1979).

A single exposure of guinea pigs to Ixodes
holocyclus larvae conferred immunity, the percentage
of larvae engorging fell from 40% on primary infestation
to only 4-5% on secondary infestation and the majority
of the larvae died on the host (Bagnall 1975a, and bb

It was hypothesised that immediate hypersen-
sitivity reactions were an important protective response
against blood feeding arthropods (Stebbings,
1974). Protection was achieved through avoidance
behaviour or by a direct deleterious effect on the
parasite. According to Snowball (1964), Bennett (1969)
and Koudstaal et al (1978) grooming was shown to be
important in limiting tick infestation.

Acquired high level resistance to Boophilus
microplus Canestrini is confined to certain strains
of cattle, being more evident in Bos indicus than in
Bos taurus. The ability to acquire resistance varies
markedly from animal to animal within and between breeds.
The larvae of Boophilus microplus were rejected

tions by day 4. It was suggested that such responses
resemble cutaneous basophil hypersensitivity reactions
of the guinea pig and that the degranulation of
basophils close to the mouthparts of the challenging
tick might have released mediators which disrupted
the normal behaviour of the tick (Allen and Humpreys, 1979).

A single exposure of guinea pigs to Ixodes
holocyclus larvae conferred immunity, the percentage
of larvae engorging fell from 40% on primary infestation
to only 4-2% on secondary infestation and the majority
of the larvae died on the host (Bagnall 1975a, and b).

It was hypothesized that immediate hypersen-
sitivity reactions were an important protective response
against blood feeding arthropods (Stebbings,
1974). Protection was achieved through avoidance
behaviour or by a direct deleterious effect on the
parasite. According to Snowball (1956), Bennett (1969)
and   Koudstaal et al (1978) grooming was shown to be
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Acquired high level resistance to Boophilus
microplus Canestrini is confined to certain strains
of cattle, being more evident in Bos indicus than in
Bos taurus. The ability to acquire resistance varies
markedly from animal to animal within and between breeds.
The larvae of Boophilus microplus were rejected

in the first 24 hours of feeding on the Bos taurus
(Riek, 1962; Roberts, 1968a; Wagland, 1975, 1979).
Kooudstaal, Kemp and Kerr (1978) reported that some
larvae of B. microplus also died when feeding on
resistant hosts. Wagland (1978) reported that the
engorged weights of female ticks on immune Bos indicus
cattle were reduced by 30%. Observed prolongation of
feeding time, reduced egg-out put and egg viability
in ticks fed on resistant Bos indicus has been repor-
ted by Hewetson (1971) and Wagland (1975).

Bowessidjaou et al (1977) reported that
repeated infestations of laboratory rabbits with
Ixodes ricinus Linnaeus produced a marked decrease
in percentages of ticks engorging, engorged weights,
female oviposition/ and the viability of eggs. There was an
increase in the feeding period. Similar studies
performed with Rhipicephalus appendiculatus
by Branagan (1974) suggested that host resistance
was acquired with repeated infestations. Köhler et al.
(1967) reported that the feeding time for both
R. appendiculatus and R. sanguineus were signifi-
cantly prolonged. Fujisaki (1978) reported that
immunity of rabbits to Haemaphysalis longicornis
was somewhat different in that the number of female
ticks engorging, the length of engorgement time and the
hatching rate of eggs were all unaltered though female
engorgement weights were reduced.

Willadsen (1980) reported that the manifes-
tation of tick resistance varied greatly depending
on the host and tick species concerned. The general
effects and features of acquired tick resistance
included a rejection of the parasite, interference
with feeding, prolongation of feeding time, reduction
in engorgement weights, inhibition of egg laying,
decreased viability of eggs and death of the parasite
on the host. Acquired tick resistance is accompanied
by a cutaneous hypersensitivity to antigens derived
from the salivary glands of the tick. A substantial
degree of resistance has been induced artificially
in guinea pigs, rabbits and cattle using such antigens
(Allen and Humpreys, 1979; Masiga, 1980). This
resistance could also be produced by intracutaneous
inoculation of an extract of larval ticks and be
passively transferred by intraperitoneal inoculation
of serum from guinea pigs hyperimmunized by repeated
infestations with nymphs of Dermacentor variabilis
(Trager, 1939a).

Specific antibodies induced by infestations
and nonspecific antibodies to B. microplus salivary
gland antigens were detected / along with an increase
in cattle
in gamma globulin after infestation (Roberts and Kerr,
1976). Willadsen et al (1978) found that high levels
of precipitating antibody to B. microplus correlated
with low levels of acquired resistance. Wikel and

Osburn (1982) reported that a very low level of
infestation
Dermacentor andersoni/was sufficient to stimulate a

precipitating antibody response by Bos taurus calves.

Significant evidence of a humoral factor in resistance

was provided by Roberts and Kerr (1976) when they

passively transferred resistance to B. microplus with

plasma. The plasma components responsible for this

reactivity were not characterized, an important subject

for future study. Reich and Zorzopulos (1980) found

that cattle previously infested with B. microplus

developed antibodies to larval phosphomonoesterase,

suggesting a target in the feeding tick.

Willadsen et al (1979) observed that the

amount of histamine in the skin of cattle resistant

to B. microplus correlated directly with the degree of

resistance and the intensity of immediate hypersensitivity

reactions. Masiga (1980) suggested that tick resistant

cattle immunized against target antigens from ticks can

be used successfully to control tick populations. It has

been shown by Bell et al (1979), and by Wikel (1980, 1981)

that tick resistance can provide protection against the

transmisssion of a tick-borne pathogen. Allen and Humpreys

(1979) demonstrated that antigens from the gut and other

internal organs of female D. andersoni ticks were effective

in inducing resistance to tick infestation. An antigen

mixture consisting of various tick internal organs -

salivary glands, muscle, gut and female reproductive

tissues has been suggested (Wikel, 1981).

Wikel and Osburn (submitted for publication) observed delayed skin reactions when cows and calves were experimentally given low level infestations of D. andersoni and were subsequently administered an intradermal injection of salivary gland antigens. Antigen-specific in vitro proliferation of peripheral blood lymphocytes from cows and calves given three or four low level D. andersoni infestations was stimulated by salivary gland antigen (Wikel and Osburn, submitted for publication. Ann. Trop. Med. Parasitol.).

Chiera and Newson (personal communication, 1982) found that when R. appendiculatus Neumann fed on susceptible cattle a large proportion succeeded in engorging and reached high average weights, but on subsequent feeds a few ticks engorged and their average weights decreased as the host developed resistance. Furthermore they showed that if ticks in the field had only resistant cattle to feed on, their numbers could be reduced to low levels and their size before feeding also reduced. Laboratory studies performed by (Chiera and Newson, personal communication, 1982) confirmed that ticks fed on resistant cattle were smaller at each stage than those that were fed on susceptible hosts and much fewer eggs were laid by the females. The duration of survival of unfed ticks stressed by dry conditions was related to their individual size. Hence they found out that the smaller sized ticks after passage through resistant cattle were a potent factor in influencing the reduction of tick numbers in the field.

Despite numerous studies, it is still not known what mechanisms are involved in the expression of acquired resistance by bovines to ixodid tick infestation. The exact importance of antibody, cell-mediated immunity, and soluble mediators must be determined, since suggestive evidence indicates that all these factors could be potentially involved in the expression of resistance.

Blood feeding arthropods are important vectors of pathogens, the majority of which are transmitted with the salivary secretions . In the case of saliva-borne agents, an intense immune response directed toward the blood feeding arthropod might create a local environment hostile to the development and spread of the pathogen. This would seem most likely for arthropods that obtain their blood meal over a prolonged period, thus providing a sustained antigenic stimulus (Mellanby, 1946).

Cattle resistant to infestation by Boophilus microplus were significantly less likely to develop babesiosis than animals susceptible to tick infestation (Francis and Little, 1964), and it was reported that animals resistant to tick infestation were significantly protected against the transmission of highly virulent tick-borne bacteria (Bell et al 1979,Wikel, 1980). In addition Wikel (personal communication) observed the presence of interferon at tick attachment sites on animals

resistant to infestation, a finding that might indicate
a potentially hostile environment for transmitted
viruses.

As yet work on tick resistance has not been
performed on goats and sheep, a sad omission   as these
small stock graze side by side with cattle in the
field situation. The author wished to rectify this
omission and the first ever studies were begun on
R. appendiculatus   resistance in goats.

CHAPTER 3

3:1   MATERIALS AND METHODS

3:1:1   EXPERIMENTAL ANIMALS

Two breeds of goats were used in this research study; indigenous Local East African meat goats and exotic Togggenburg milk goats.

3:1:2   LOCAL EAST AFRICAN GOATS

Pregnant Local East African goats (L.E.A.) were bought from small stock farmers in Kitui District of the Eastern Province of Kenya. These animals had not been dipped regularly and had been subjected virtually continuously to high tick challenge.

The pregnant goats were maintained in the Department of Zoology, Kenyatta University College. Ticks were removed by the application of an acaricide wash (Delnav D.F.F.) and the goats

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*   Delnav, D.F.F.   - An organosphorous compound. A highly concentrated diluent - free formulation (D.F.F.) made by Wellcome Kenya Ltd.

were dewormed with *Nemafax. The goats were maintained in covered, concrete - floored pens to ensure that no further ticks were picked up. At no time were the goats allowed to graze.

The kids born to these goats were reared tick free by continuing to maintain them in covered concreted floored pens until of an age suitable to be used in the sequence of experiments, which was approximately 5-6 months   when they weighed approximately 20 kilogrammes.

3:1:3 TOGGENBURG GOATS.

(T.G.)

The Toggenburg goats /, a very rare commodity in Kenya, had been reared tick free in a private well managed herd. Once trans- ferred to the Zoology Department, Kenyatta University College they were maintained in a similar fashion to the L.E.A. kids.

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* 'Nemafax' - is effective against all the important stomach and intestine roundworms in cattle, sheep and goats and lethal to worm eggs in the gut. Made in England, May and Baker Ltd. Dagenham·

3:1:4   FOOD RATIONS AND MAINTAINANCE OF GOATS.

The two breeds of R. appendiculatus
naive kids i.e. L.E.A. and T.G. were fed ad libitum
on ranch cubes, dairy meal, dairy cubes, wheat
bran, napier grass, hay and merclic salt blocks.
They were also provided with plenty of drinking
water. The goat and sheep building was thoroughly
cleaned twice weekly.

Precautions were taken that no ticks
could move in from the surrounding area. Buffer
zones were made around the goat and sheep building
by clearing the vegetation. A concrete trench
filled with water bordered one side of the
building.

3:1:5   NEWZEALAND WHITE RABBITS.

Six month old Newzealand white male
rabbits immunologically naive to R. appendiculatus
Neum. weighing about 2.0 kilogrammes were used as
controls. They were reared in the Department of
Zoology, Kenyatta University College (K.U.C.) and
maintained in rabbit cages measuring 60 by 60 cm^2.
The rabbits were fed on rabbit pellets, green
vegetables and carrots. They were also provided
with plenty of drinking water.

3:1:6   RHIPICEPHALUS APPENDICULATUS CULTURE

R. appendiculatus   culture was
established at the Department of Zoology, K.U.C.
to ensure a steady supply of the tick at
different stages of the   life cycle, i.e. eggs,
larvae, nymphs and adults. All ticks used were
disease free. The basic stock was supplied by
Dr. Robin Newson and Dr. Matt Cunningham, of
International Centre of Insect Physiology and
Ecology (ICIPE), Nairobi, Kenya; the ticks were
maintained in cotton wool and gauze plugged tubes,
larvae and nymphs in 50 x 12 mm and adults in
75 x 25 mm sized tubes. These tubes were kept   at
22°C in a desiccator over a saturated potassium
chloride solution (KCl) which gave a relative
humidity of 85%. R. appendiculatus   naive
rabbits described earlier were used as hosts to
feed the larvae, nymphs and adult stages of
R. appendiculatus .   A feeding batch was
conveniently confined to the ears of the host
rabbit by using ear bags so that the successfully
engorged instars would be collected easily and
efficiently.

The ear bag consisted   of a sleeve made
of white   fabric 8 cm. wide and 18 cm.
long. These were fixed over the ears of the

rabbits after first shaving away the hair at
the base of the ears with an electric shaver.
The bases of the ear bags were secured to the ears
by means of zinc oxide adhesive tape. The other end
which extended well beyond the ear tip was doubled
over and secured with an elastic band and elastoplast
adhesive, thereby making the sleeve into an 'ear bag'.
Collection of engorged ticks was quickly carried out
by removing the elastoplast and elastic band rolling
back the ear bag, and tapping gently allowing the
engorged ticks to be collected on an enamel tray held
beneath the open end. The process was then reversed
to close the ear bags. The two outward ends of ear
bags were secured to the top of a rabbit neck collar
to prevent the ear bags and the tick harvest from
becoming soiled in urine or drinking water, and
to prevent the rabbit using his rear legs to scratch
at his ears. The neck collar was made of leather
which was flexible enough not to cause any discomfort
to the rabbit. The collar was secured to the rabbit
by means of leather laces. As rabbit skin is very
subject to pressure necrosis, the securing laces were
not too thin or tied too tight.

Ticks were applied within cotton wool
and gauze plugged 50 x 12 mm tubes. These tubes
were placed within the ear bags, then unplugged

inside the ear bag to allow the ticks to climb out
and attach to the ear. By the following day all of
the ticks /attached to the ear and the tubes and
had
plugs were removed. In this way tubes of larvae,
nymphs or adults were applied, fed, collected,
allowed to moult, harden and reapplied on rabbits
for feeding.

Engorged larvae and nymphs were collected
from the culture system of rabbit host each day
for 3-4 days and were placed in a desiccator at
$22^{o}C$ with 85% relative humidity and left to moult
to nymphs or adults   (approximately
17 days). They were then left for 10 further days
to harden. Now they were ready to be used in the
experimental infections.

3:1:7   EXPERIMENTAL PROCEDURE

Five Local East African (L.E.A.) goats
and five Toggenburg (T.G.) goats were tethered
separately in covered compartments with concrete
floors in the goat and sheep building in the
Department of Zoology, K.U.C. Eighteen
R. appendiculatus   naive Newzealand white
rabbits were used in the investigation as controls.
At no time were the rabbits reinfected. Only
R. appendiculatus   naive rabbits were used at
each stage of the experiment. The animals were
identified by having tag numbers on their necks.

One L.E.A. goat was accidentally hurt and
it was excluded from the experiment so only
four L.E.A. goats continued to the end of the
experiment.

All the animals were carefully shaved
around the bases of their ears using an
electric shaving machine. Cloth bags were fixed
over the ears of the L.E.A. goats, T.G. goats
and control rabbits (C.R.) as described
earlier and secured to the bases of ears by
means of zinc oxide adhesive tape. The goats
were then placed in the experimental compartments
and the two rabbits restricted to rabbit cages
for about 3 days in order to get used to the
ear bags before applying the ticks on them. The
animals were given food and plenty of water as
described earlier.

3:1:8   METHODS OF COUNTING TICKS FOR INFESTATION

Counting of unfed nymphs and adults
was performed by placing the tube containing
the ticks into the middle of a clean enamel
dish 30 cm x 30 cm and removing the cotton wool -
gauze plug. The dish was smeared around the
edge with vaseline to help prevent the ticks
from escaping. The enamel dish was then placed
in a bigger dish containing some water, ensuring

that any ticks that could escape from the small tray would not be able to escape to the surrounding bench top. Using a damp paint brush (size No.6) the ticks were quickly separated, counted and put in tubes of 50 x 12 mm with strips of filter paper to increase the surface area available to the ticks. The tubes were sealed with cotton wool and gauze plugs. The gauze was used to prevent ticks from getting entangled in the cotton wool. An attempt was made to keep the sizes of the female adult ticks to be applied approximately uniform through the sequence of infestations.

3:1:9 FLOW CHART OF THE EXPERIMENTS PERFORMED TO OBSERVE THE
DEVELOPMENT OF ACQUIRED IMMUNITY.

DAY	TICKS APPLIED TO EACH GOAT	TICKS APPLIED TO EACH RABBIT CONTROL
0	50 NN	50 NN
14	50 AA	50 AA
31	100 NN	100 NN
45	100 AA	100 AA
100	200 NN	200 NN
115	200 AA	200 AA
134	400 NN	400 AA
154	300 AA	300 AA
191	50 NN	50 NN

All feeding ticks split equally between left and right

ears except feeds on day 14 which consisted of 13 males:

13 females per left ear and 12 males: 12 females per right

ear.

NN = Nymphs

AA = Equal numbers of males and females.

The 50 nymph test, the initial test was used as an index to ascertain the relative levels of any innate resistance.

A series of 50 x 12 mm tubes containing 25 R. appendiculatus nymphs and plugged with cotton wool gauze were made ready. Each tube containing the nymphs was placed in the left and right ears of all experimental goats and control rabbits. The distal

end of the ear bag extending well beyond the ear tip was doubled over and closed with an elastic band and elastoplast adhesive. The tubes were then unplugged inside the ear bags to allow the ticks to get out and attach to the ear. The empty tubes were removed 48 hours later. Each day (starting at day 0) the distal ends of the ear bags were opened by removing the adhesive tape and the elastic band. Each ear bag was then rolled back so that the nymphs that had engorged and fallen off the ear into the ear bag could be collected on a clean enamel tray and counted using a paint brush. The ear bags were resealed to await the next day's harvest. The number of dead and squashed nymphs were also counted and recorded. The individual nymphs collected each day from every animal were weighed individually and as a group using a Sartorius balance. The nymphs were then placed in a desiccator at 22°C and 85% relative humidity in the laboratory where they

were left to moult to adults and the time taken for
this to happen recorded. The same procedure was
repeated for 100, 200, 400 and 50 (final nymph test)
respectively.

In the case of adults R. appendiculatus
ticks the same procedure was followed as for the
nymphs. A sequence of experimental infections of
increasing numbers were performed alternating nymph
and adult stages. The first infestation of adults
consisted of 25 adult female and male
R. appendiculatus   ticks. The other challenge
infestations consisted of 50 male and females, 100
male and females and finally 150 male and females.
Any male ticks recovered were discarded. The time
taken for adult females to lay eggs was recorded. The
weights of egg batches per female tick were recorded
after 20 days. The eggs were then left to hatch and
length of time to hatch, and the hatching percentage
for each egg batch were also calculated.

It should be emphasized that the sequence of
experimental infections of increasing size was per-
formed alternating nymph and adult stages. After
all the engorged ticks had fallen off the experimental
animals, they were rested for one week to allow any
wounds due to tick feeding to heal before reinfestation.

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*   Sartorius precision Analytical Model 2472-
    Sartorius Werke GMBH, West Germany.

C H A P T E R 4

4:1   RESULTS

4:1:1   Nymphal infestations of rabbits.

In the initial experimental infestation of
the two R. appendiculatus   naive control
rabbits, 50 R. appendiculatus   nymphs were
applied.   Daily collection of engorged nymphs
started on day 3 when a mean of 4.0 ± 0.7 engorged
nymphs were collected in the ear bags (Table 1a).
A maximum number was collected on day 4 when
40 ± 1.4 were collected, followed by a gradual
reduction in the daily collection to day 6 when
2.0 ± 0.7 were collected (Figure 1a).   The mean engor-
ging period was 4.7 ± 0.2 days (Table 1b) and the
engorgement percentage was calculated to be 95.0% ±
1.4 (Table 1d).   The percentage of squashed ticks
was 2.0% ± 0.0 (Table 1d) and the percentage
collected dead was 3.0% ± 1.4 (Table 1e).   The mean
weight   of engorged nymphs (Table 1c) was
calculated to be 8.54 mg ± 0.03.   The moulting mean time
was 17.2 ± 0.1 days and the moulting percentage
was 100.0% ± 0.0 (Table 2a).

A similar pattern was observed at each
experimental infestation of nymphs on the rabbits.
In each case it must be remembered that fresh
R. appendiculatus   naive rabbits were used.

Table 1a: The mean number of R. appendiculatus nymphs at
engorgement which had been fed on L.E.A., T.G. goats
and control rabbits during successive feeds.

Number of days	Number of nymphs applied and engorged daily + S.D.				
	50	100	200	400	50
	TOGGENBURG GOATS.				
4	12+5.3	17+3.3	29+0.0	40+10.1	2+1.8
5	24+6.9	35+3.1	52+8.4	79+17.6	5+0.7
6	11+5.4	25+5.5	31+9.4	28+14.4	2+1.1
7	1+0.8	7+5.2	17+7.9	12+5.1	1+0.0
8		1+0.3	2+1.6	1+0.5	1+0.6
9					1+0.3
	LOCAL EAST AFRICAN GOATS				
4	9+2.9	16+3.5	21+6.3	35+17.1	1+0.6
5	12+2.1	22+2.6	33+10.6	27+0.5	2+0.8
6	24+2.1	33+5.3	55+7.9	59+13.9	5+1.4
7	2+0.0	6+4.7	9+7.7	7+1.5	1+0.0
8			2+0.8	3+1.8	
	CONTROL RABBITS				
3	4+0.7	13+4.2	13+1.4	34+5.0	7+3.5
4	40+1.4	54+5.7	150+5.7	246+7.8	30+2.8
5	3+0.7	14+5.7	15+2.1	60+2.1	18+2.8
6	2+0.7	12+5.7	9+5.0	28+17.7	4+2.1

Figure Ia: showing the mean number of nymphs R. appendiculatus ticks at engorgement which had been fed on L.E.A., T.G. goats and control rabbits during the first infestation

- 32. -
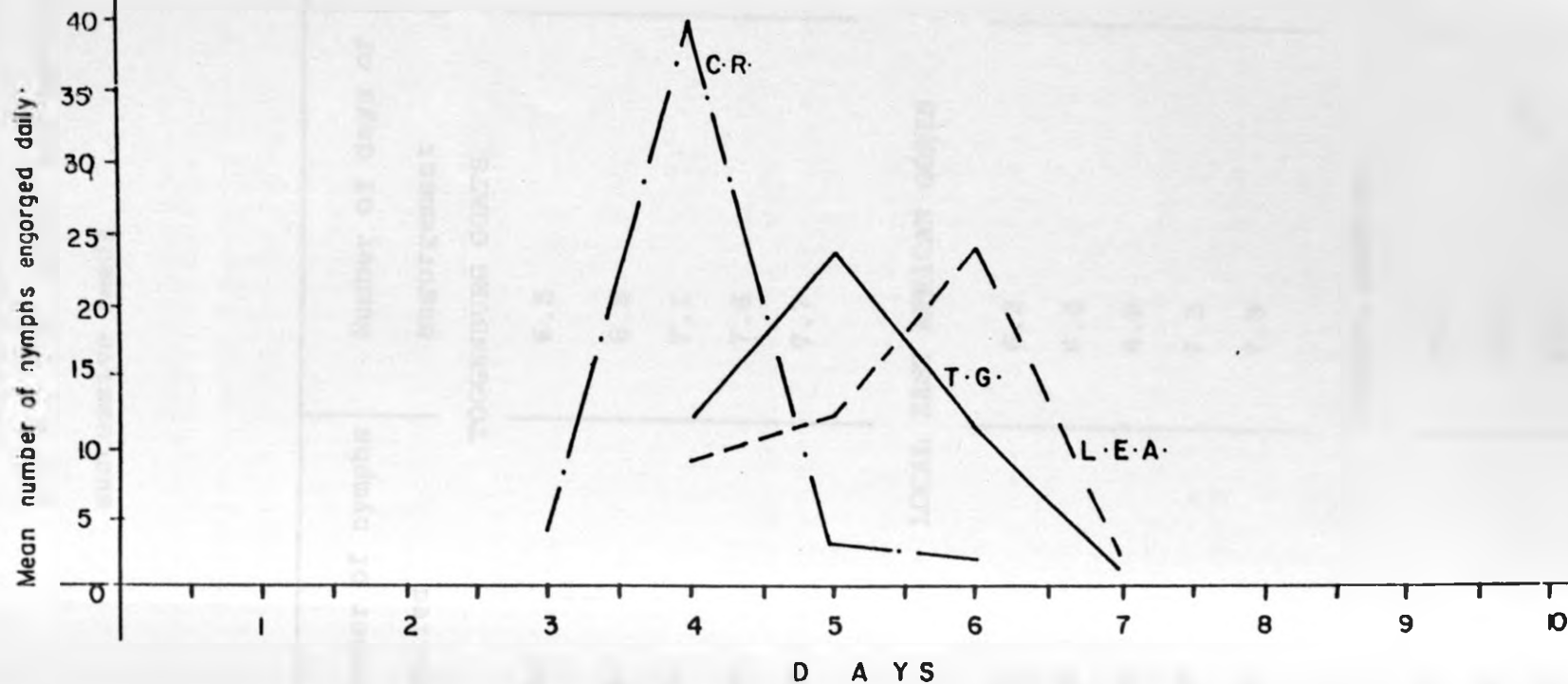
TABLE 1b.   The mean engorging period (in days) of nymphs
R. appendiculatus ticks which had been fed on
L.E.A., T.G. goats and control rabbits during
successive feeds.

Number of nymphs applied	Number of days of engorgement	± SD
TOGGENBURG GOATS.		
50	6.5	0.1
100	6.8	0.2
200	7.1	0.1
400	7.6	0.1
50	7.7	0.2
LOCAL EAST AFRICAN GOATS		
50	6.2	0.1
100	6.5	0.1
200	6.6	0.1
400	7.3	0.1
50	7.3	0.2
CONTROL RABBITS		
50	4.7	0.2
100	4.6	0.1
200	4.6	0.1
400	4.5	0.0
50	4.6	0.0

TABLE 1c. The mean weights (mg) of nymphs R. appendiculatus
ticks at engorgement which had been fed on L.E.A.,
T.G. goats and control rabbits during successive
feeds.

Number of nymphs applied	Engorgement wt. (mg)	± S.D.
TOGGENBURG GOATS		
50	9.44	0.03
100	9.23	0.06
200	5.71	0.03
400	2.38	0.05
50	1.36	0.08
LOCAL EAST AFRICAN GOATS		
50	8.49	0.06
100	7.54	0.10
200	4.29	0.05
400	1.31	0.07
50	0.61	0.04
CONTROL RABBITS		
50	8.54	0.03
100	8.49	0.01
200	8.48	0.00
400	8.46	0.00
50	8.51	0.06

TABLE 1d: The mean engorgement and squashed percentage of nymphs R. appendiculatus ticks which had been fed on L.E.A., T.G. goats and control rabbits during successive feeds.

Number of nymphs applied	Percentage engorgement	± S.D.	Percentage squashed	± S.D.
TOGGENBURG GOATS				
50	94.0	2.5	3.6	2.2
100	84.4	1.9	7.9	2.4
200	64.9	2.0	15.4	3.4
400	38.2	1.5	17.5	1.0
50	22.0	2.0	29.2	3.0
LOCAL EAST AFRICAN GOATS				
50	92.0	1.6	5.2	1.2
100	81.0	2.9	10.5	1.7
200	58.9	2.4	16.9	1.5
400	32.7	1.2	26.1	3.1
50	18.0	1.6	37.0	2.6
CONTROL RABBITS				
50	95.0	1.4	2.0	0.0
100	93.0	1.4	4.0	0.0
200	93.0	1.4	3.8	1.8
400	91.5	0.7	4.9	0.2
50	96.0	2.8	3.0	1.4

TABLE 1e: The mean dead percentage of nymphs
R. appendiculatus ticks which had been fed
on L.E.A., T.G. goats and control rabbits
during successive feeds.

Number of nymphs applied	Percentage dead	± S.D.
.TOGGENBURG GOATS.		
50	3.0	1.2
100	7.4	2.4
200	19.7	3.6
400	44.4	1.6
50	48.8	2.3
LOCAL EAST AFRICAN GOATS		
50	3.0	1.2
100	8.5	4.2
200	24.3	3.1
400	41.3	3.2
50	45.0	2.6
CONTROL RABBITS		
50	3.0	1.4
100	3.0	1.4
200	3.3	0.4
400	3.6	0.5
50	2.0	0.0

TABLE 2a: The mean moulting time (days) and moulting percentage of nymphs R. appendiculatus ticks which had been fed on L.E.A., T.G. goats and control rabbits during successive feeds.

Number of nymphs applied	Number of days taken to moult	± S.D.	Percentage moulting	± S.D.
TOGGENBURG GOATS				
50	17.3	0.1	100.0	0.0
100	18.3	0.1	93.9	2.0
200	20.0	0.4	47.6	4.7
400	22.7	0.1	23.5	1.9
50	23.3	0.2	18.3	1.7
LOCAL EAST AFRICAN GOATS				
50	18.3	0.1	99.5	1.1
100	19.4	0.4	87.6	2.5
200	22.6	0.1	33.9	4.9
400	23.2	0.1	18.2	1.1
50	24.3	0.1	11.2	1.0
CONTROL RABBITS				
50	17.2	0.1	100.0	0.0
100	17.2	0.3	100.0	0.0
200	17.2	0.1	99.7	0.4
400	17.2	0.1	99.9	1.1
50	17.3	0.1	100.0	0.0

Figure 3a: Mean engorgement weights (mg) of nymphal R appendiculatus fed on two breed of goats (L E A and T G) and control rabbits during successive infestations.
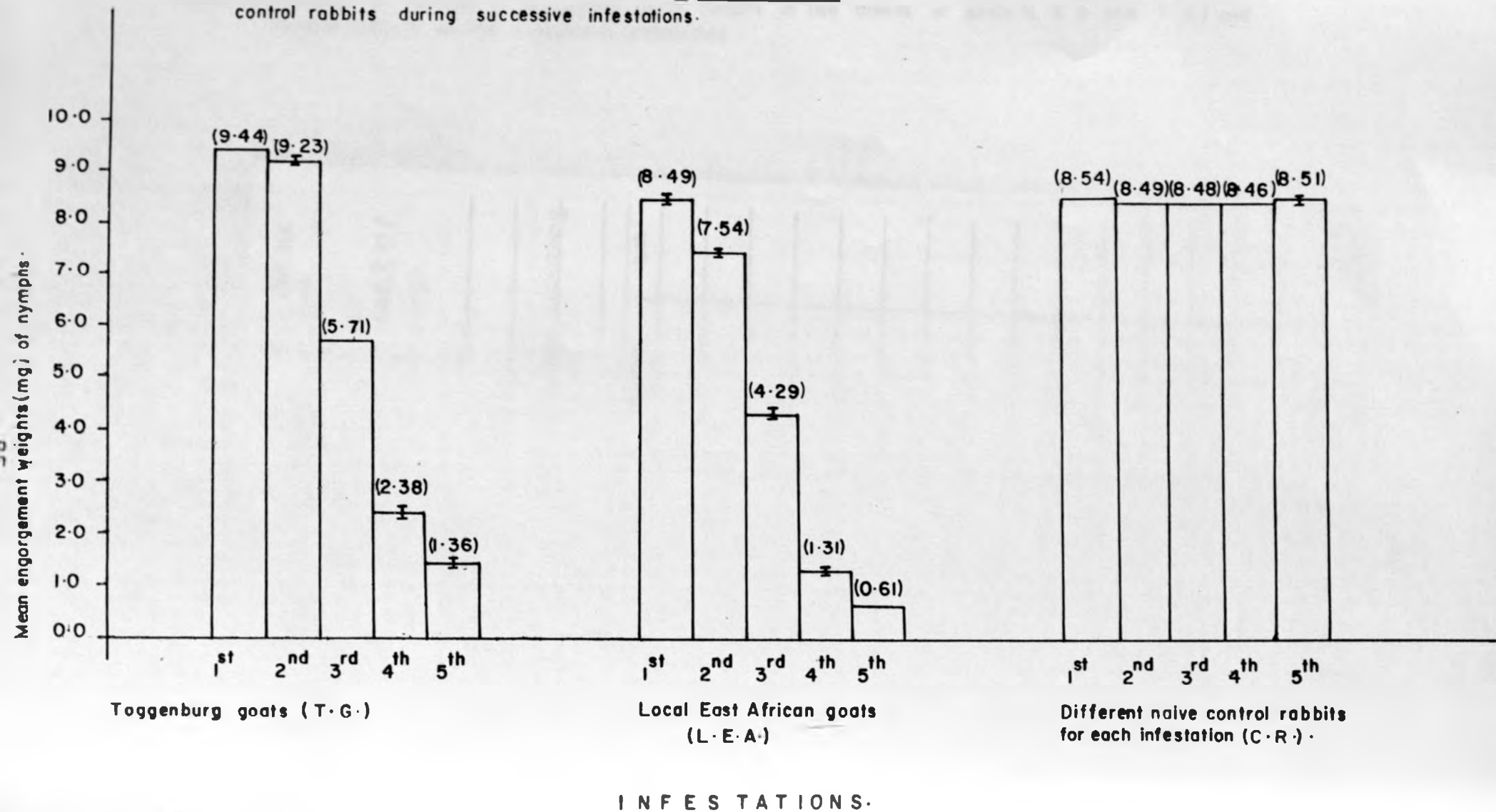
Figure 3b· Duration of feeding of R·appendiculatus nymphs in two breeds of goats (L·E·A· and T·G·) and control rabbits during successive infestations·

-39-
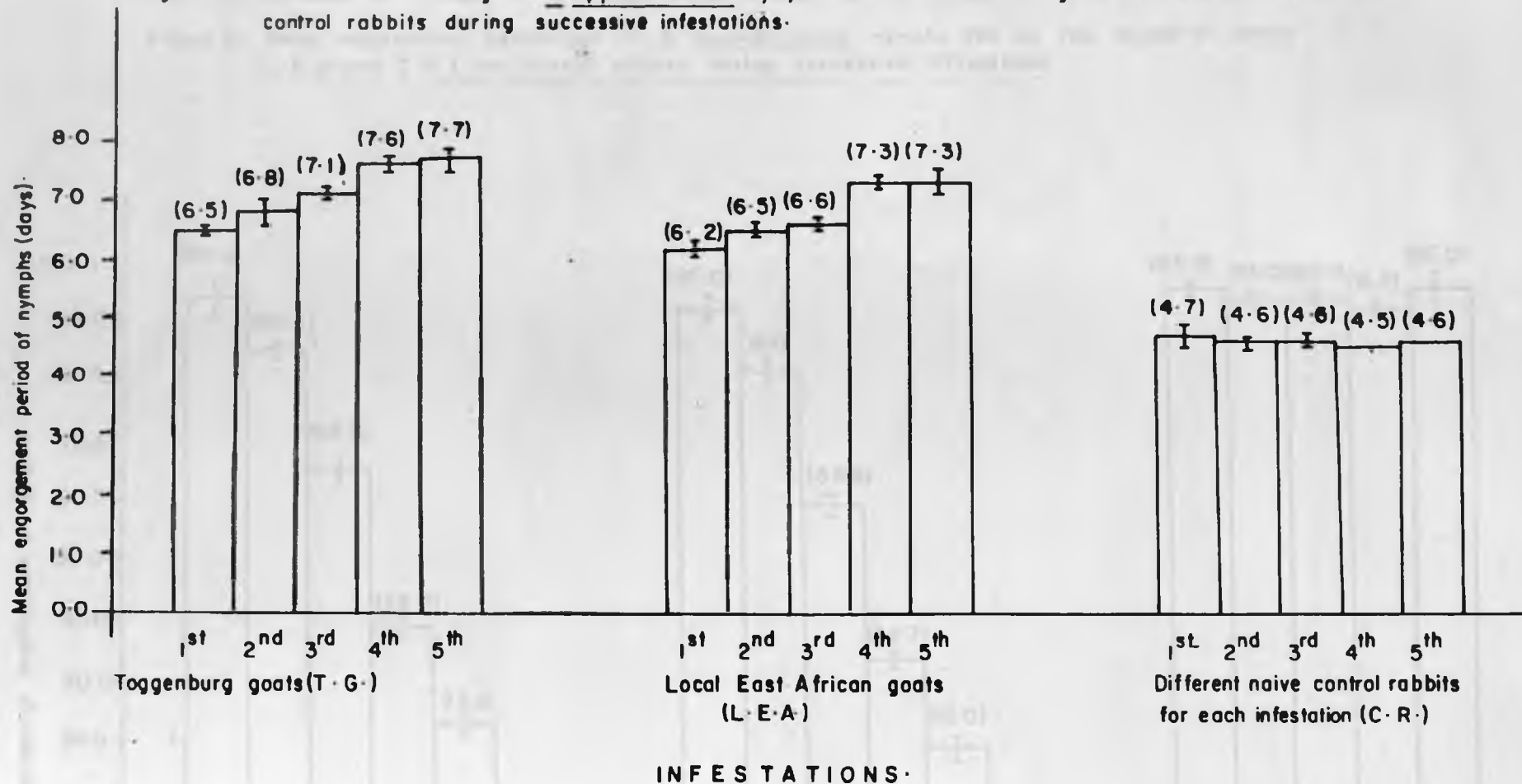
Figure 3c: Mean engorgement percentage of _R appendiculatus_ nymphs fed on two breeds of goats (L E A and T·G·) and control rabbits during successive infestations.

- 46 -
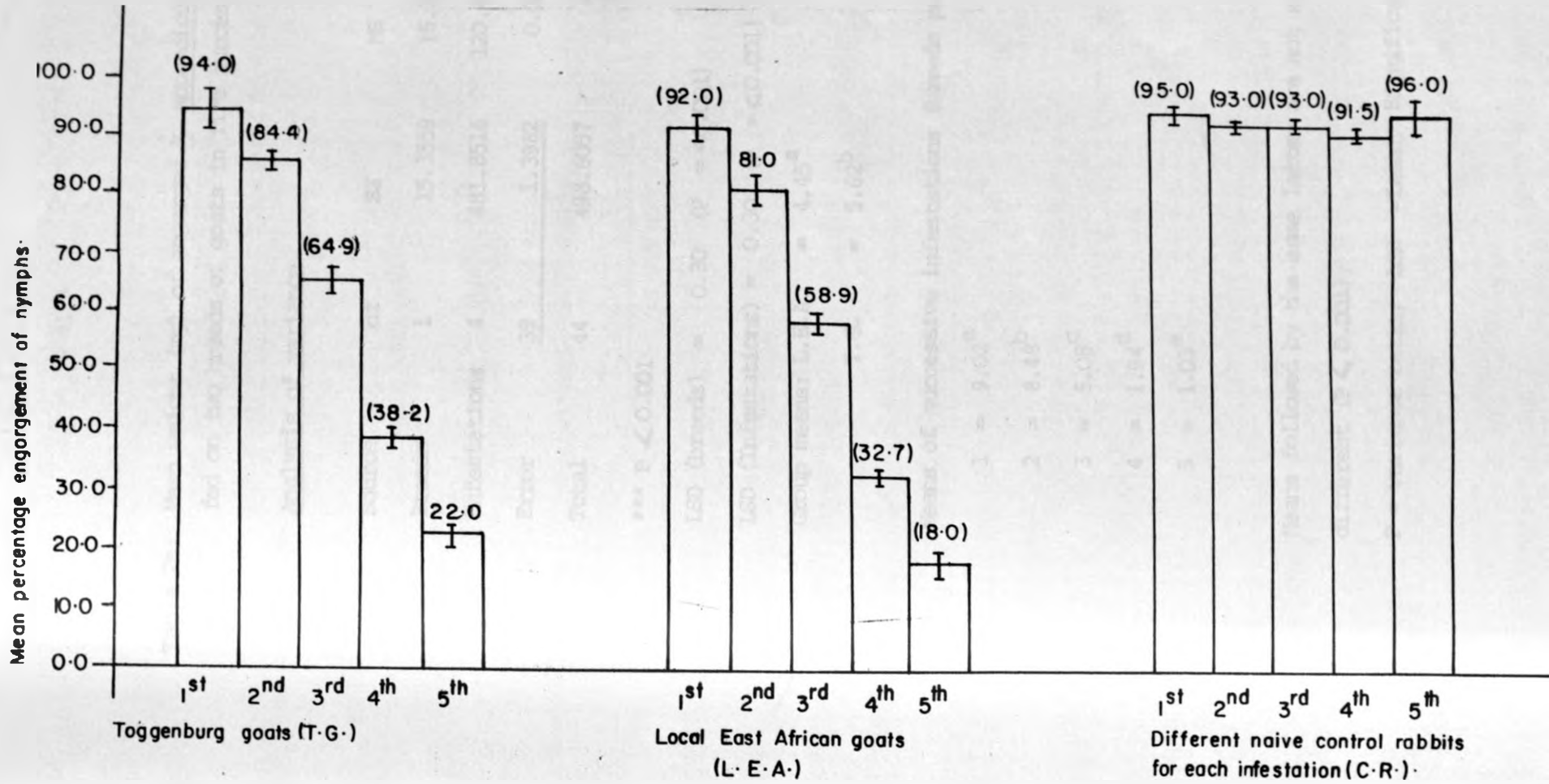
Table 2b: Mean weight (mg) of engorged R. appendiculatus nymphs
fed on two breeds of goats in five successive infestations.

Analysis of variance

Source	df	SS	MS	F
Breeds	1	15.3559	15.3559	428.3289***
Infestations	4	481.8516	120.4629	3360.1252***
Error	39	1.3982	0.0359	
Total	44	498.6057		

*** P $<$ 0.001

LSD (breeds) = 0.30 (P = $<$0.001)

LSD (Infestations) = 0.30 (P = $<$0.001)

Group means: L.E.A. = 4.45^a

T.G. = 5.62^b

Means of successive infestations (breeds pooled):

1 = 9.02^a

2 = 8.48^b

3 = 5.08^c

4 = 1.94^d

5 = 1.03^e

Means followed by the same letter are not significantly
different (P $<$ 0.001);

F = variance ratio; LSD = Least Significant difference.

Table 2c: Mean percentage that engorged (transformed to Arcsine)
of R. appendiculatus nymphs fed on two breeds of goats
in five successive infestations.

Analysis of variance

Source	df	SS	MS	F
Breeds	1	97.9704	97.9704	40.8309***
Infestations	4	14313.5982	3578.3996	1491.3599***
Error	39	93.5774	2.3994	
Total	44	14505.1460		

*** P $<$ 0.001

LSD (breeds)   = 2.4

LSD (infestations) = 2.6

Group means: L.E.A. = 49.6^a

T.G.   = 52.6^b

Means of successive infestations (breeds pooled):

1 = 75.0^a

2 = 65.8^b

3 = 52.1^c

4 = 36.7^d

5 = 26.7^e

Means followed by the same letter are not significantly
different (P $<$ 0.001); F = variance ratio, LSD = Least
significant difference.

Figure 4a. The rate of nymphal development of R·appendiculatus nymphs fed on the ....
and control rabbits during successive infestations.

- 143 -
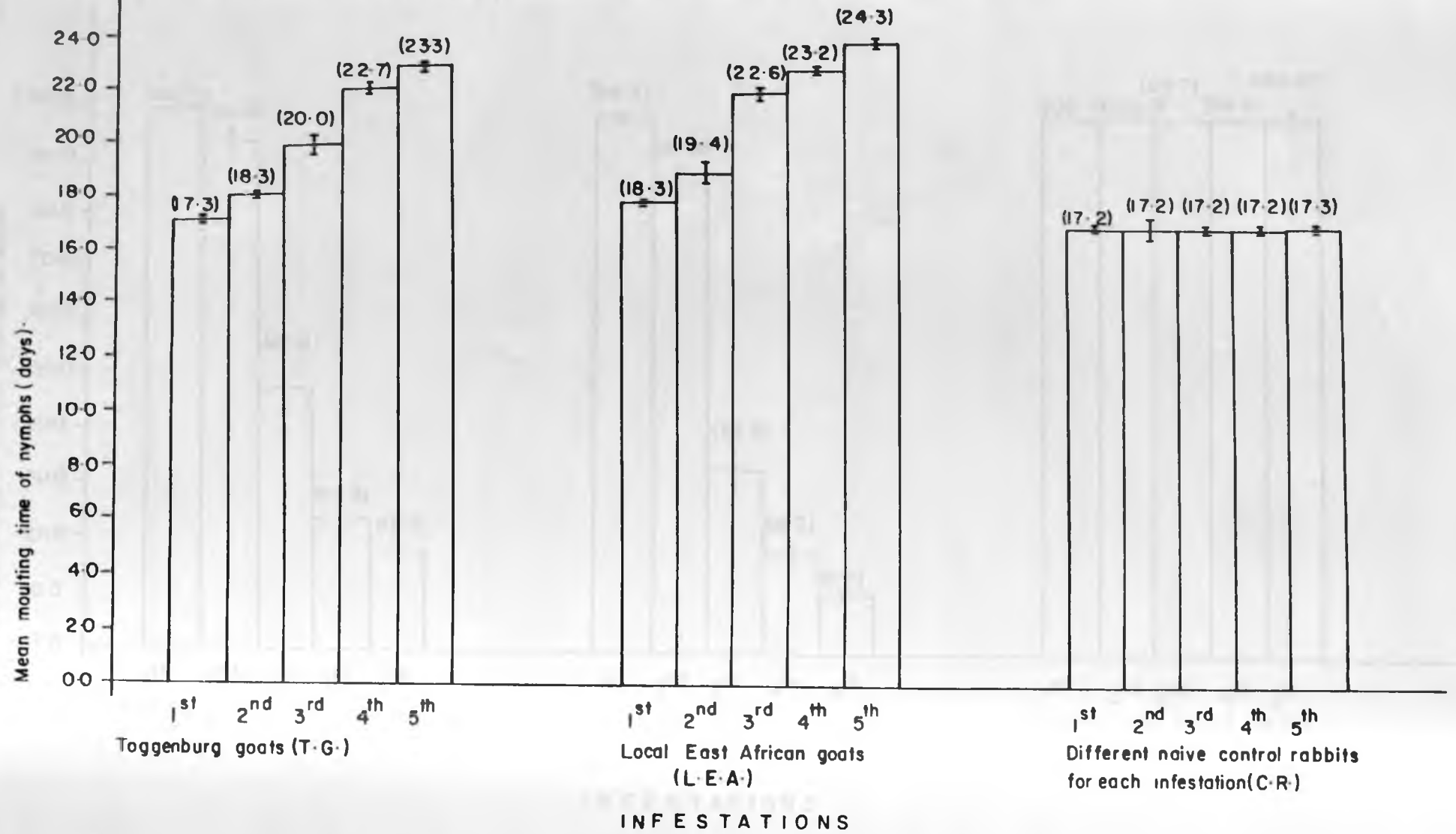
Figure 4b· Percentage moulting of R· appendiculatus nymphs fed on two breeds of goats (L·E·A· and T·G·) and control rabbits during infestations·

-44-

Mean percentage of nymphs moulting to adults·

100·0
90·0
80·0
70·0
60·0
50·0
40·0
30·0
20·0
10·0
0·0

(100·0) (93·9) (47·6) (23·5) (18·3)
1st 2nd 3rd 4th 5th
Toggenburg goats (T·G·)

(99·5) (87·6) (33·9) (18·2) (11·2)
1st 2nd 3rd 4th 5th
Local East African goats (L·E·A·)

(100·0)(100·0) (99·7) (99·9) (100·0)
1st 2nd 3rd 4th 5th
Different naive control rabbits for each infestation (C·R·)·

INFESTATIONS
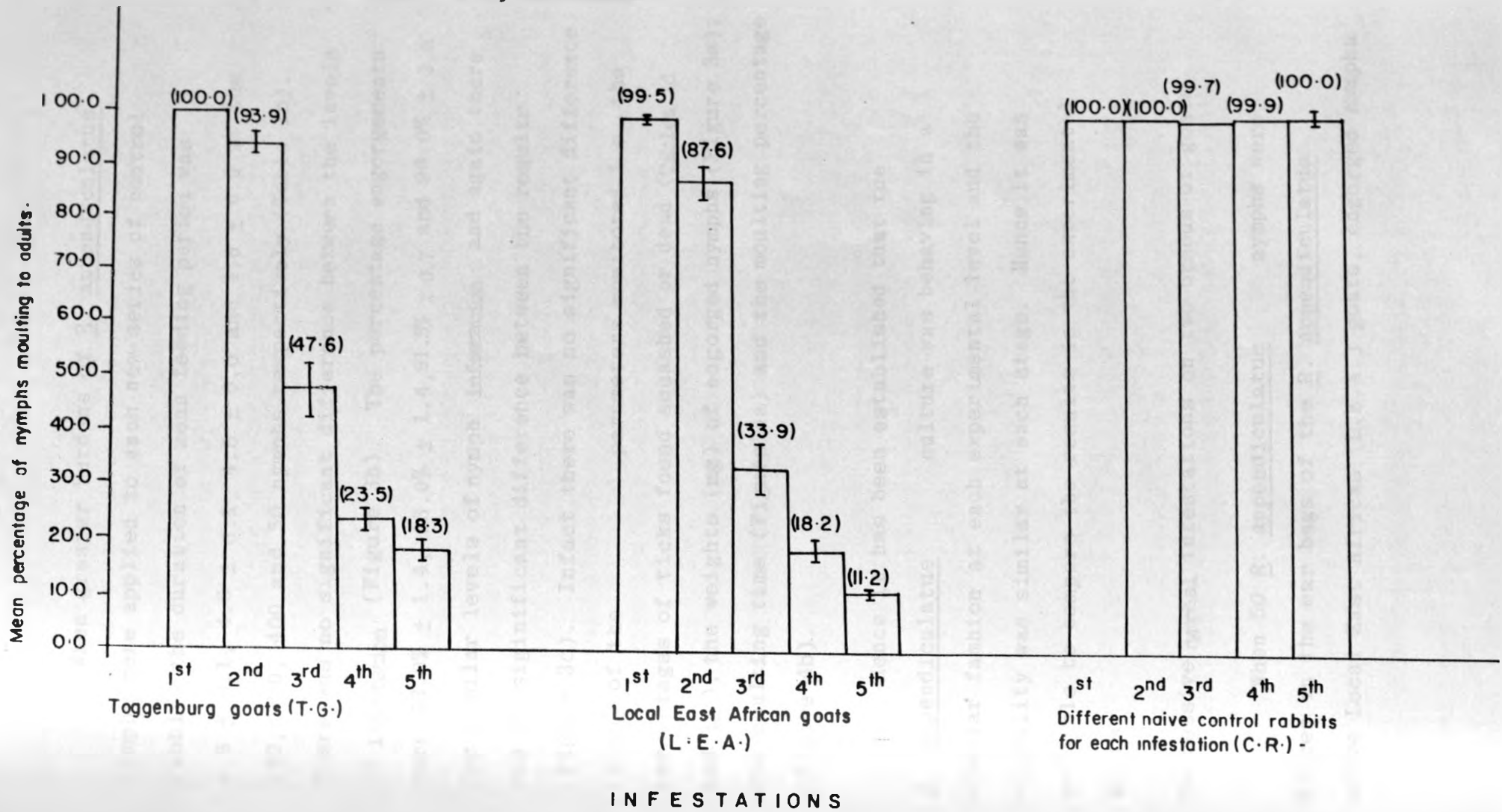
When greater burdens of R. appendiculatus nymphs were applied to each new series of control rabbits the duration of mean feeding period was 4.6 ± 0.1, 4.6 ± 0.1, 4.5 ± 0.0 and 4.6 ± 0.0 at the 100, 200, 400 and 50 nymphs respectively (Table 1b). There was no significant difference between the levels of infestation (Figure 3b). The percentage engorgements were 93.0% ± 1.4, 93.0% ± 1.4, 91.5% ± 0.7 and 96.0% ± 2.8 for similar levels of nymph infestation and again there was no siginificant difference between the results (Figure 3c). Infact there was no significant difference in any of the parameters monitored i.e. the percentages of ticks found squashed or dead (Tables 1d and 1e), the weights (mg) of engorged nymphs (Figure 3a); the moulting time (Figure 4a) and the moulting percentage (Figure 4b).

Hence it has been established that the R. appendiculatus culture was behaving in a similar fashion at each experimental level and the viability was similar at each stage. Hence it was possible to compare the results in the experimental goats.

4:1:2 Successive nymphal infestations on two breeds of goats.

When 50 R. appendiculatus nymphs were placed in the ear bags of the R. appendiculatus naive Local East African (L.E.A.) goats, engorged nymphs

started to drop off the ears on day 4 when a mean of
9.0 ± 2.9 nymphs (Table 1a) were collected. A maximum
collection occurred on day 6 when 24.0 ± 2.1 were
collected, followed by a sharp reduction in the number
of nymphs collected between days 6 and 7 (Figure 1a).
When the Toggenburg (T.G.) goats were infested with a
similar size of R. appendiculatus nymph burden
(50 nymphal), engorged nymphs began to be collected on day
4 i.e. the same day as for the L.E.A. goats when a
mean of 12.0 ± 5.3 nymphs were collected but the peak
collection was a day earlier than in the L.E.A. goats,
thereby followed a sharp reduction in engorged nymph
numbers between days 6 and 8 (Figure 1a).

The engorging period was 6.2 ± 0.1 days and
6.5 ± 0.1 in the ticks fed on L.E.A. and T.G. goats
respectively; the engorgement percentage was 92.0% ± 1.6
and 94.0% ± 2.5 respectively (Table 1d); the percentage
squashed was 5.0% ± 1.2 and 3.6% ± 2.2 respectively
(Table 1d); the percentage dead was 3.0% ± 1.2 and
3.0%± 1.2 respectively (Table 1e); the mean engorged
nymph weights were 8.49 mg ± 0.06 and 9.44 mg ± 0.03
respectively (Table 1c); the moulting time was 18.3 ± 0.1
days and 17.3 ± 0.1 days respectively (Table 2a) and
moulting percentage was 99.5% ± 1.1 and 100.0% ± 0.0
(Table 2a) respectively. There were significant
differences at (P < •001) in these resistant parameters
in the two breeds of goats, indicating that the levels
of innate immunity were quite different.

Table 2d: Mean duration (days) of feeding of _P. appendiculatus_ nymphs on two breeds of goats in five successive infestations.

Analysis of variance

Source	df	SS	MS	F
Breeds	1	1.4161	1.4161	94.8584***
Infestations	4	8.6147	2.1537	144.2670***
Breeds x Infestations	4	0.0747	0.0187	1.2526
Error	35	0.5225	0.0149	
Total	44	10.6280		

*** $P < 0.001$

LSD (breeds)   = 0.5 (P = $<$0.001)

LSD (infestations) = 0.5 (P = $<$0.001)

Group means: L.E.A. = 6.8^a

T.G.   = 7.1^a

Means of successive infestations (breeds pooled):

1 = 6.4^a

2 = 6.7^{ab} .

3 = 6.9^b

4 = 7.4^c

5 = 7.5^c

Means followed by the same letter are not significantly different ($P < 0.001$); F = variance ratio; LSD = Least significant difference

Table 2e: Mean percentage moulting (transformed to Arcsine) of
R. appendiculatus nymphs fed on two breeds of goats
in five successive tick infestations.

Analysis of variance

Source	df	SS	MS	F
Breeds	1	301.8443	301.8443	55,9982[***]
Infestations	4	30696.4158	7674.1040	1423.7013[***]
Error	39	210.2197	5.3902	
Total	44	31208.4798		

*** P $<$ 0.001

LSD (breeds)   = 2.5 (P = $<$0.001)

LSD (infestations) = 3.9 (P = $<$0.001)

Group means: L.E.A. = 47.5^a

T.G.   = 52.7^b

Means of successive infestations (breeds pooled)

1 = 89.1^a

2 = 73.0^b

3 = 40.0^c

4 = 27.3^d

5 = 22.7^e

Means followed by the same letter are not significantly
different (P $<$0.001); F = variance ratio; LSD = Least
significant difference.

As greater sized R. appendiculatus
nymph burdens were applied to the same animals signifi-
cant differences became evident in many of the parameters
monitored both between the result of each challenge
infestation and between the two breeds of goats.

The duration of feeding time (Figure 3b) was
increased significantly in both breeds of goats with
each successive infestation. In many cases they were
significantly different at (P $<$.001). Nymphs stayed
on the T.G. goats for a significantly longer period
than on the L.E.A. goats (Table 1b).

The mean engorgement weights (Figure 3a)
decreased gradually and in many cases significantly
at each successive infestation in both goat groups.
There was a tendency for the nymphs fed on the L.E.A.
goats to have a lower engorgement mean weights than
the T.G. goats (Table 1c). Table 2b indicates that the
two breeds of goats were significantly different at
(P $<$ .001) in the mean engorgement weights of fed nymphs.

The percentage of squashed and dead nymphs
increased significantly with successive infestations.
There was a tendency for ticks from the L.E.A. goats
to have higher percentages found squashed as compared
to those fed on T.G. goats, yet the result were vice
versa for the number of dead ticks.

The moulting time increased with successive nymph infestations in most cases significantly at (P<001) and the mean moulting percentage decreased with successive nymph infestations. There was significant difference at (P<001) between the two breeds of goats (Table 2e).

4.2 RESULTS

4:2:1 Female infestations on two R. appendiculatus naive control rabbits.

Table 3a indicates that when 25 adult female R. appendiculatus Neum.ticks were introduced to the ear bags of each R. appendiculatus naive control rabbits the mean numbers of engorged female adult R. appendiculatus ticks collected daily indicated that engorgement of females started on day 5 when a mean of 2.0 ± 0.7 were collected; increased to a maximum number collected on day 6 when a mean of 12.0 ± 2.1 were collected; followed by a gradual reduction upto day 9 when 1.0 ± 0.0 female adult was collected (Figure 2a). The mean feeding period was 9.3 ± 0.3 days (Table 3b). Table 3d shows that the mean engorgement percentage was 94.0% ± 2.8; the percentage squashed was 1.6% ± 0.0 (Table 3d); the mean percentage of ticks found dead was 4.0% ± 0.0 (Table 3e) and the mean engorgement weight was 388.40 mg ± 0.21 (Table 3c).

The mean pre-oviposition period in days was found to be 3.6 ± 0.0 (Table 4a); the percentage of

Table 3a: The mean number of female R. appendiculatus ticks at engorgement which had been fed on L.E.A., T.G. goats and control rabbits during successive feeds.

Number of days	Number of females applied and number engorged daily with their $\pm$ S.D.			
	25	50	100	150
	TOGGENBURG GOATS			
5	3 + 1.5	4 + 2.2	1 + 0.3	1 + 0.9
6	5 + 3.9	5 + 2.1	2 + 1.8	3 + 1.0
7	5 + 3.3	5 + 2.6	2 + 1.6	4 + 0.8
8	6 + 2.3	12 + 1.6	3 + 2.1	4 + 2.8
9	4 + 3.3	6 + 2.6	10 + 4.0	6 + 1.4
10		5 + 2.5	13 + 3.9	7 + 2.6
11		1 + 0.3	10 + 1.8	8 + 6.1
12			4 + 2.1	3 + 1.1
	LOCAL EAST AFRICAN GOATS			
5	3 + 2.4	1 + 0.6		
6	4 + 2.4	2 + 1.2	3 + 1.3	6 + 4.3
7	10 + 2.8	13 + 2.6	7 + 4.5	9 + 4.3
8	5 + 2.2	13 + 4.5	11 + 5.4	6 + 2.5
9	0	3 + 0.8	9 + 7.7	2 + 1.0
10		1 + 1.0	3 + 2.4	1 + 0.8
11				1 + 1.0
12				
13				
	CONTROL RABBITS			
5	2 + 0.7	4 + 1.4	9 + 0.0	11 + 2.2
6	12 + 2.1	21 + 1.4	26 + 1.4	28 + 2.0
7	8 + 1.4	11 + 1.7	45 + 2.1	45 + 1.2
8	2 + 0.7	4 + 1.4	10 + 2.1	3? + ?.?
9	1 + 0.0	1 + 2.4	5 + 2.1	17 + 5.7
10		2 + 0.7		7 + 4.0
11		1 + 0.7		

Figure 2a: showing the mean number of female R. appendiculatus ticks at which had been fed on L.E.A. , control rabbits during the first infestation.

- 52 -
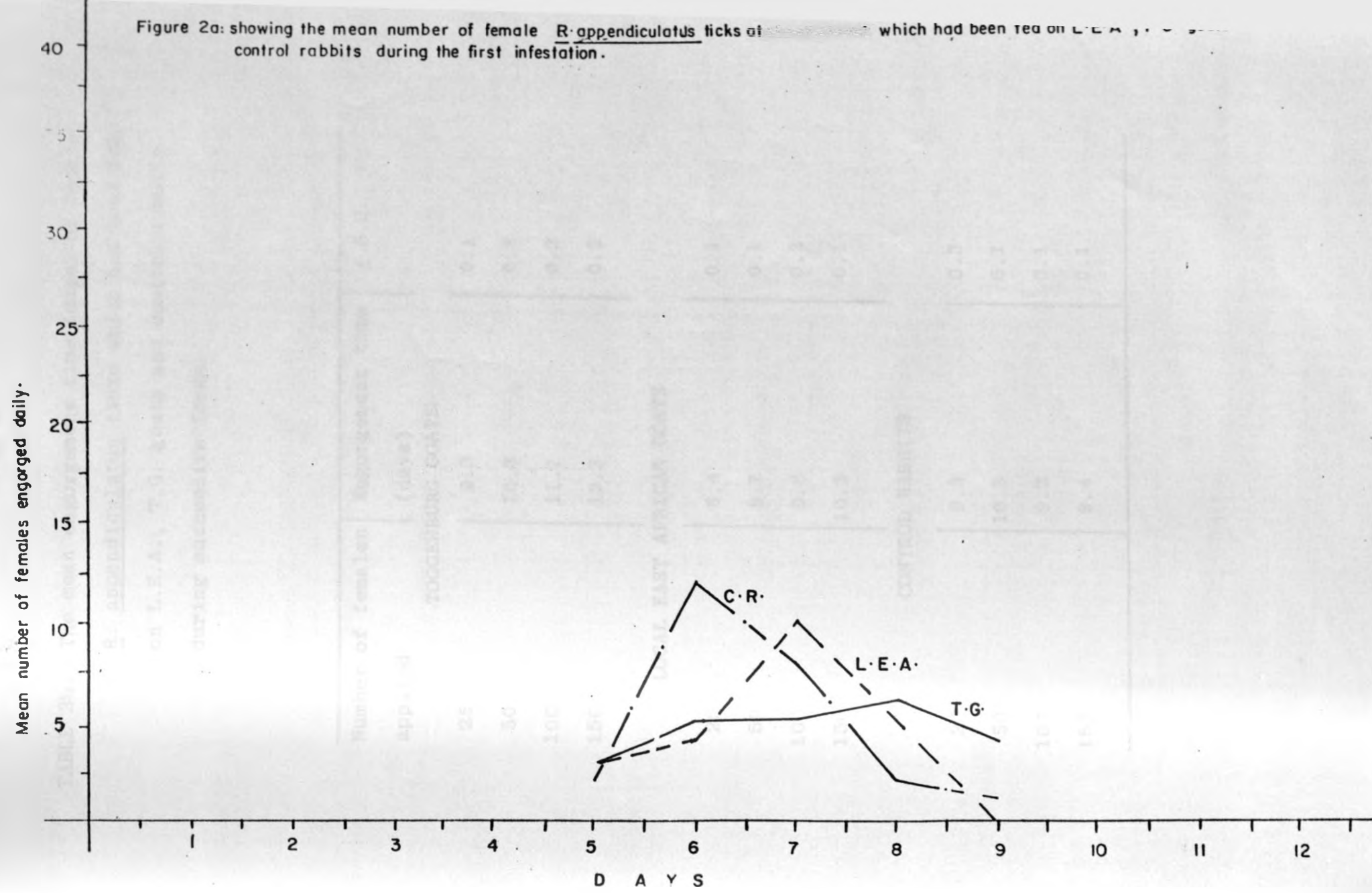
TABLE 3b: The mean engorgement time (days) of female
R. appendiculatus ticks which had been fed
on L.E.A., T.G. goats and control rabbits
during successive feeds.

Number of females applied	Engorgement time (days)	± S.D.
TOGGENBURG GOATS		
25	9.3	0.1
50	10.3	0.2
100	11.7	0.2
150	12.3	0.2
LOCAL EAST AFRICAN GOATS		
25	8.4	0.1
50	8.7	0.1
100	9.8	0.2
150	10.5	0.1
CONTROL RABBITS		
25	9.3	0.3
50	10.3	0.1
100	9.2	0.1
150	9.4	0.1

TABLE 3c: The mean weight (mg) of female

R. appendiculatus ticks at engorgement which

had been fed on L.E.A., T.G. goats and control

rabbits during successive feeds.

Number of females applied	Engorged weight (mg)	± S.D
TOGGENBURG GOATS.		
25	385.10	1.0
50	235.49	0.6
100	36.60	0.3
150	19.33	0.5
LOCAL EAST AFRICAN GOATS		
25	308.10	0.53
50	152.34	0.79
100	23.74	0.14
150	16.19	0.25
CONTROL RABBITS		
25	388.40	0.21
50	388.65	1.37
100	388.99	2.67
150	388.29	1.63

TABLE 3d: The mean engorgement and squashed percentage
of female R. appendiculatus ticks which had
been fed on L.E.A., T.G. goats and control
rabbits during successive feeds.

Number of females applied	Percentage engorgement	± S.D.	Percentage squashed	± S.D.
- TOGGENBURG GOATS-				
25	96.8	3.4	1.6	0.0
50	73.6	2.6	11.2	2.3
100	44.0	1.6	21.4	5.1
150	24.3	1.4	32.9	3.5
LOCAL EAST AFRICAN GOATS				
25	85.0	3.8	7.0	2.0
50	63.0	2.6	22.5	3.4
100	32.3	2.1	29.0	4.6
150	17.8	1.7	40.0	1.6
CONTROL RABBITS				
25	94.0	2.8	4.0	0.0
50	93.0	1.4	3.0	1.4
100	93.0	1.4	3.5	0.7
150	93.0	1.4	4.3	2.4

TABLE 3e: The mean dead percentage of female
R. appendiculatus ticks which had
been fed on L.E.A., T.G. goats and
control rabbits during successive feeds.

Number of females applied	Percentage dead	± S.D.
TOGGENBURG GOATS.		
25	4.0	0.0
50	15.2	1.8
100	34.6	4.4
150	42.8	2.7
LOCAL EAST AFRICAN GOATS		
25	8.0	3.3
40	14.5	1.9
100	38.8	3.0
150	42.2	1.4
CONTROL RABBITS		
25	4.0	0.0
50	4.0	0.0
100	3.5	2.1
150	2.7	0.9

TABLE 4a: The mean pre-oviposition time (days) of female R. appendiculatus ticks which had been fed on L.E.A., T.G. goats and control rabbits during successive feeds.

Number of females applied	Pre-oviposition time (days)	± S.D.
TOGGENBURG GOATS.		
25	7.4	0.1
50	7.5	0.1
100	7.6	0.1
150	7.7	0.1
LOCAL EAST AFRICAN GOATS		
25	7.6	0.1
50	7.7	0.1
100	7.9	0.1
150	8.5	1.0
CONTROL RABBITS		
25	3.6	0.0
50	3.7	0.1
100	3.7	0.1
150	3.7	0.1

TABLE 4b: The mean percentage of female
R. appendiculatus ovipositing of those
which engorged and egg batch weight (mg)
of females which had been fed on L.E.A.,
T.G. goats and control rabbits during
successive feeds.

Number of females applied	Percentage of females ovipositing	± S.D.	Egg batch weight (mg)	± S.D.
TOGGENBURG GOATS				
25	99.2	1.9	205.82	0.62
50	82.6	1.3	138.57	0.74
100	32.3	1.8	37.46	0.46
150	18.6	1.4	2.00	0.04
LOCAL EAST AFRICAN GOATS				
25	95.0	4.1	184.24	0.34
50	69.8	2.3	99.27	0.28
100	27.1	1.6	27.68	0.43
150	12.1	1.0	1.62	0.08
CONTROL RABBITS				
25	100.0	0.0	286.19	8.03
50	100.0	0.0	287.47	4.02
100	99.5	0.8	288.30	4.46
150	99.7	0.5	287.27	3.78

TABLE 4c: The mean percentage of eggs hatching and time taken for eggs to hatch (days) laid by female R. appendiculatus ticks which had been fed on L.E.A., T.G. goats and control rabbits during successive feeds.

Number of females applied	Percentage of egg hatching	± S.D.	Time taken for eggs to hatch (in days)	± S.D.
TOGGENBURG GOATS				
25	97.5	2.3	26.2	0.1
50	66.5	3.4	28.4	0.1
100	35.1	3.4	31.3	0.1
150	17.0	36.2	36.2	0.1
LOCAL EAST AFRICAN GOATS				
25	84.4	7.0	27.3	0.1
50	53.4	2.9	29.2	0.1
100	25.6	3.3	33.3	0.1
150	6.3	0.0	37.3	0.1
CONTROL RABBITS				
25	100.0	0.0	26.3	0.1
50	99.0	1.5	26.3	0.1
100	99.5	0.8	26.3	0.1
150	99.9	0.5	26.3	0.1

Figure 5a: Mean engorgement weights (mg) of female R. appendiculatus rea on two breeds of goats (L·E·A· and T·G·) and control rabbits during successive infestations.

Toggenburg goats (T·G·).

Local East African goats (L·E·A·)

Different naive control rabbits for each infestation·

INFESTATIONS·

Figure 5b. Mean engorgement percentage of female R appendiculatus fed on two breeds of goats (L·E·A and T·G·) and control rabbits during successive infestations.
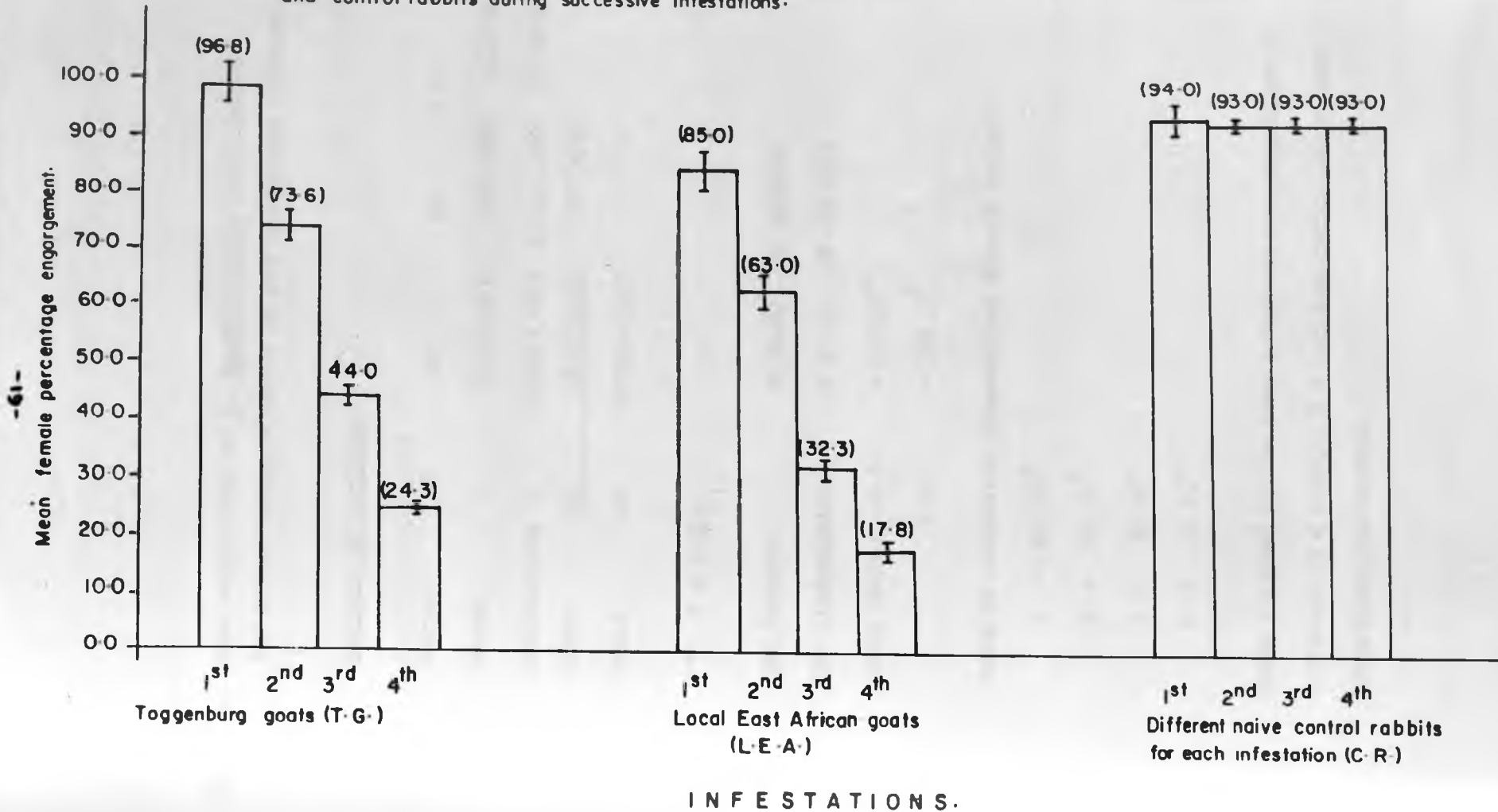
INFESTATIONS.

Table 5a: Mean weight (mg) of R. appendiculatus adult females
fed on two breeds of goats in four successive infestations.

Analysis of variance

Source	df	SS	MS	F
Breeds	1	17240.2900	17240.2900	45.6788 ***
Infestations	3	669043.6827	223014.5609	590.8850 ***
Error	31	11700.1630	377.4246	
Total	35	697984.1357		

*** P < 0.001

LSD (breeds)   = 33.53   (P =<0.001)

LSD (infestations)   = 33.33   (P =<0.001)

Group means: L.E.A.   = 125.09^a

             T.G.   = 169.13^b

Means of successive infestations (breeds pooled):

    1 = 350.88^a

    2 = 198.53^b

    3 = 30.88^c

    4 = 17.93^c

Means followed by the same letter are not significantly
different (P < 0.001); F = variance ratio; LSD = Least
significant difference.

Table 5b: Mean percentage that engorged (transformed to Arcsine) of R. appendiculatus females fed on two breeds of goats in four successive infestations.

Analysis of variance

Source	df	SS	MS	F
Breeds	1	598.9104	598.9104	44.0879[***]
Infestations	3	11963.0502	3987.6834	293.5474[***]
Error	31	421.1183	13.5845	
Total	35	12983.0789		

*** $P < 0.001$

LSD (breeds)   = 6.3

LSD (infestations) = 6.3

Group means: L.E.A. = 44.9[a]

T.G.   = 53.1[b]

Means of successive infestations (breeds pooled):

1 = 75.5[a]

2 = 56.2[b]

3 = 38.5[c]

4 = 27.5[d]

Means followed by the same letter are not significantly different ($P < 0.001$); F = variance ratio; LSD = Least significant difference;

Figure 5c: Duration of feeding of R. appendiculatus female in two ___ of goats (L.E.A and T.G.) and control rabbits during successive infestations.
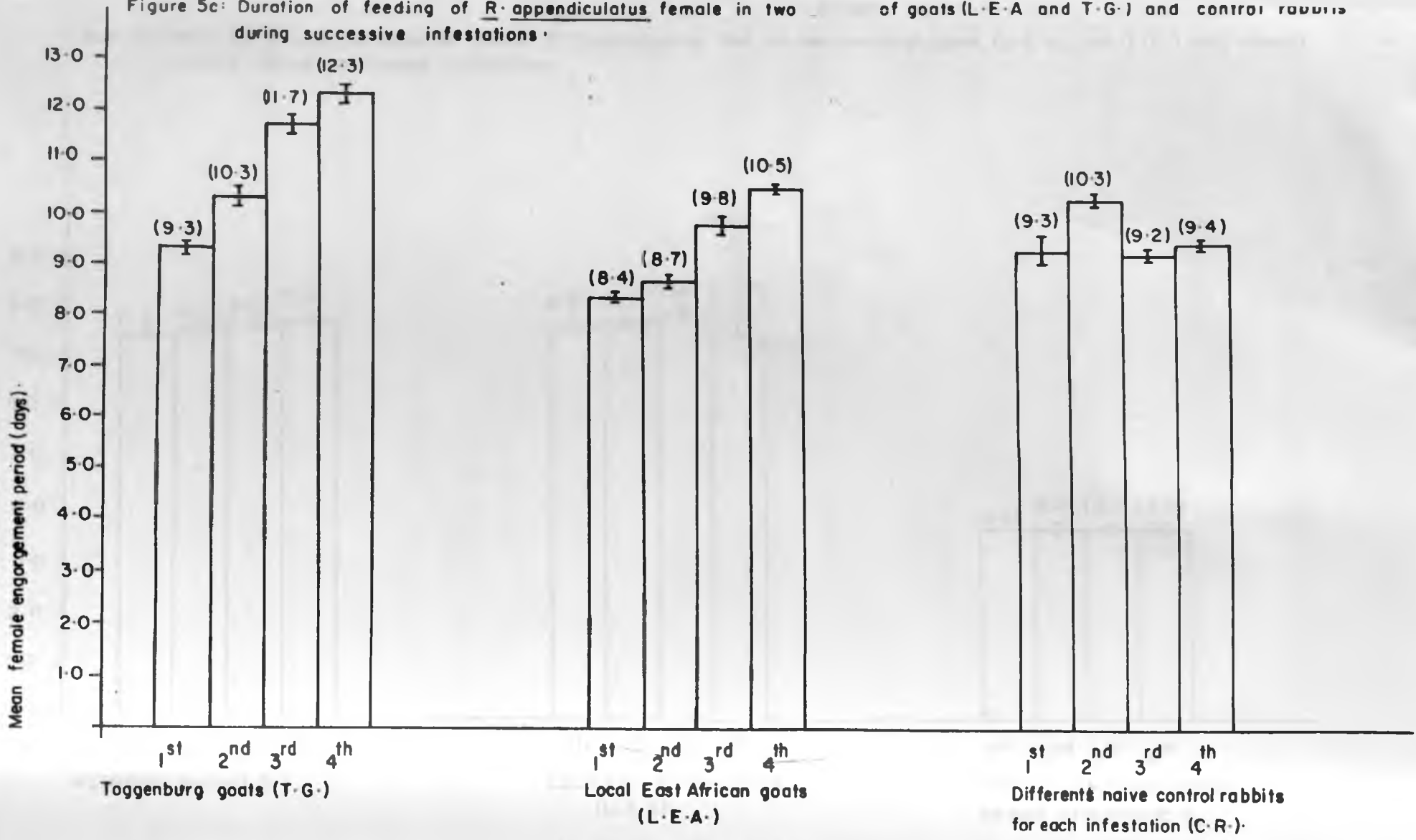
Figure 6a: Mean pre-oviposition period of female R. appendiculatus fed on two breeds of goats (L.E.A. and T.G.) and control rabbits during successive infestations.
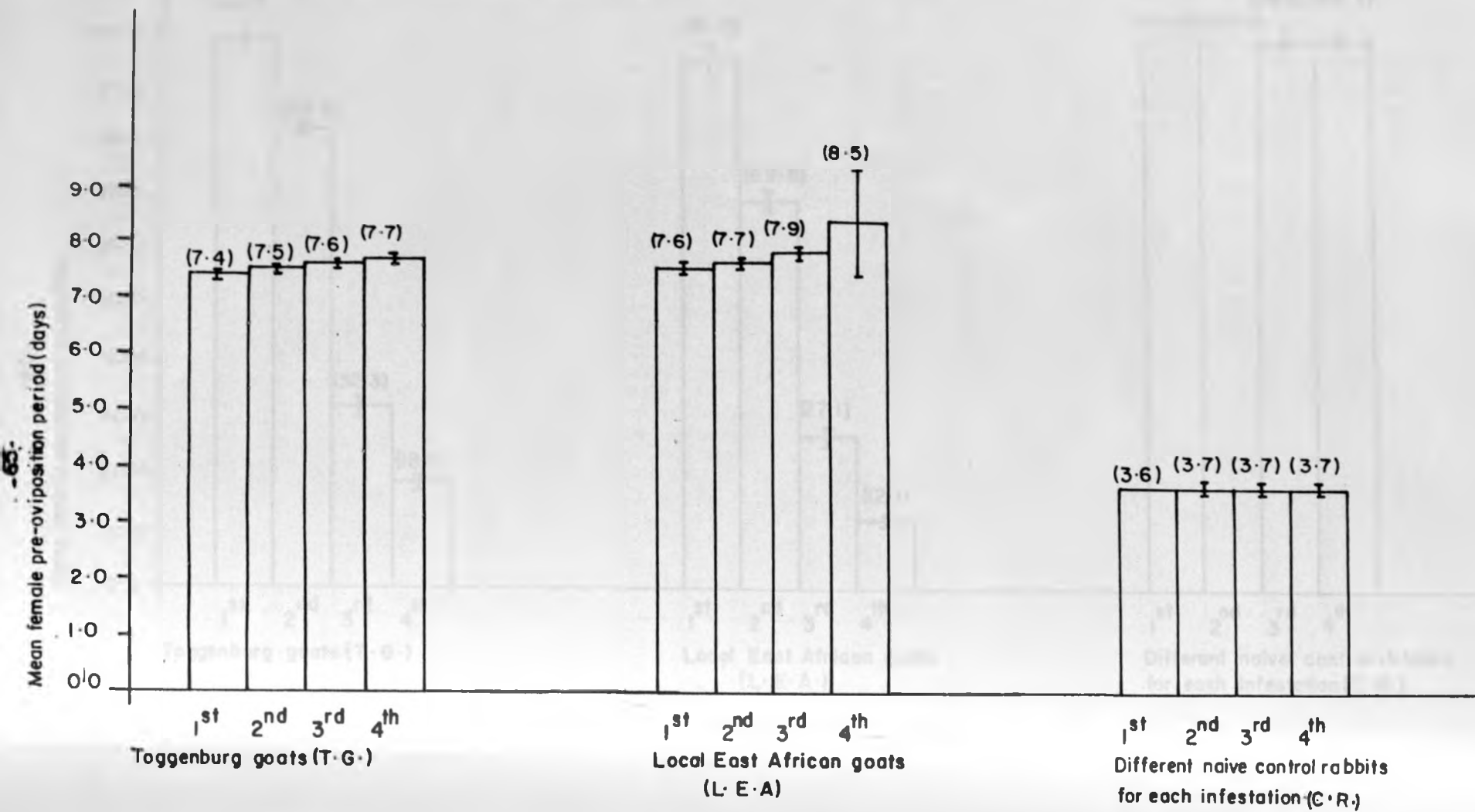
Figure 6b: Mean percentage oviposition of female R. appendiculatus fed on two breeds of goats (L·E·A· and T·G) and control rabbits during successive infestations.

-66-
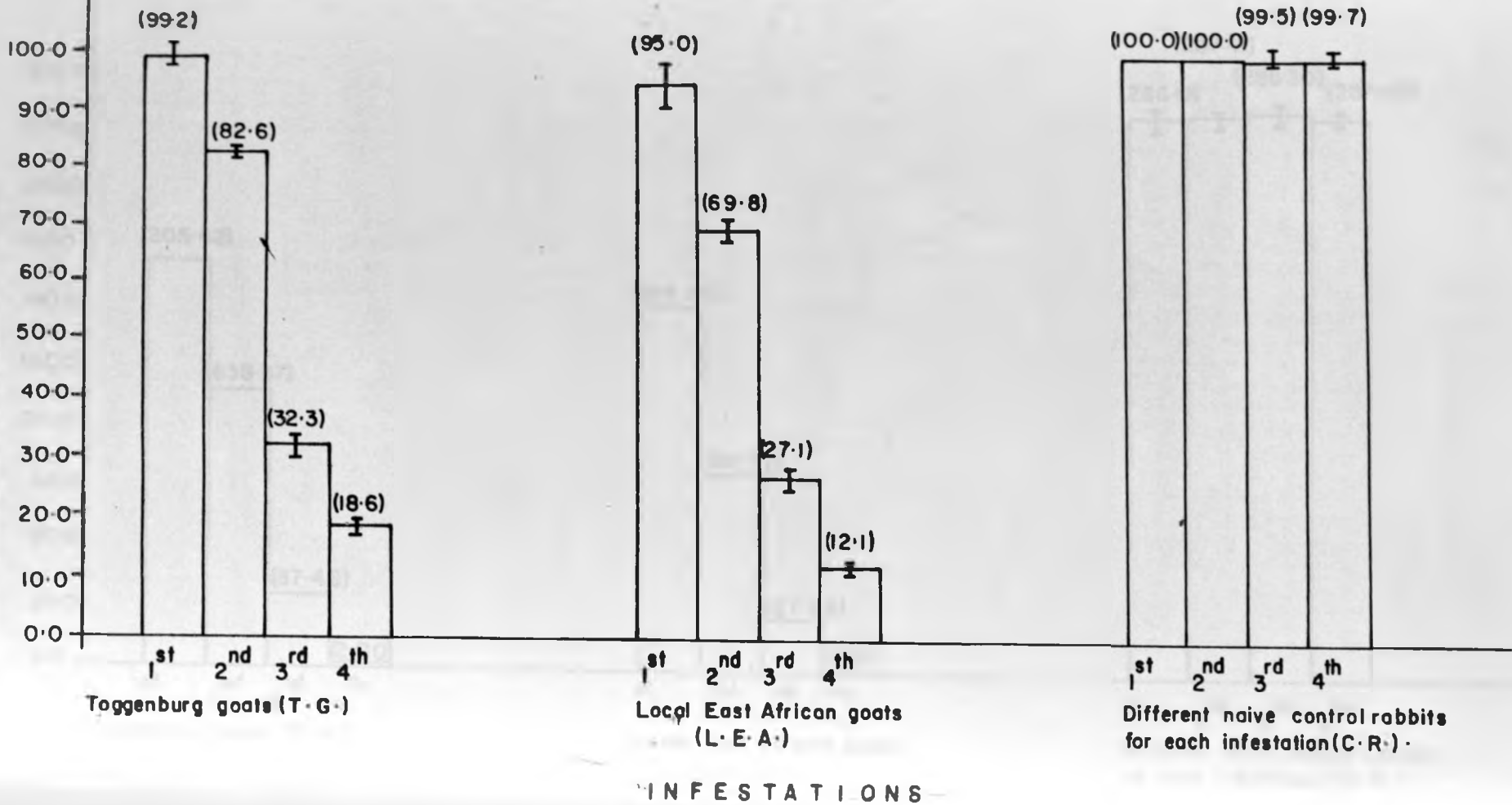
Figure 6c: Mean egg batch weight (mg) laid by female _R appendiculatus_ which fed on two breeds of goats (L·E·A and T·G·) and control rabbits during successive infestations.
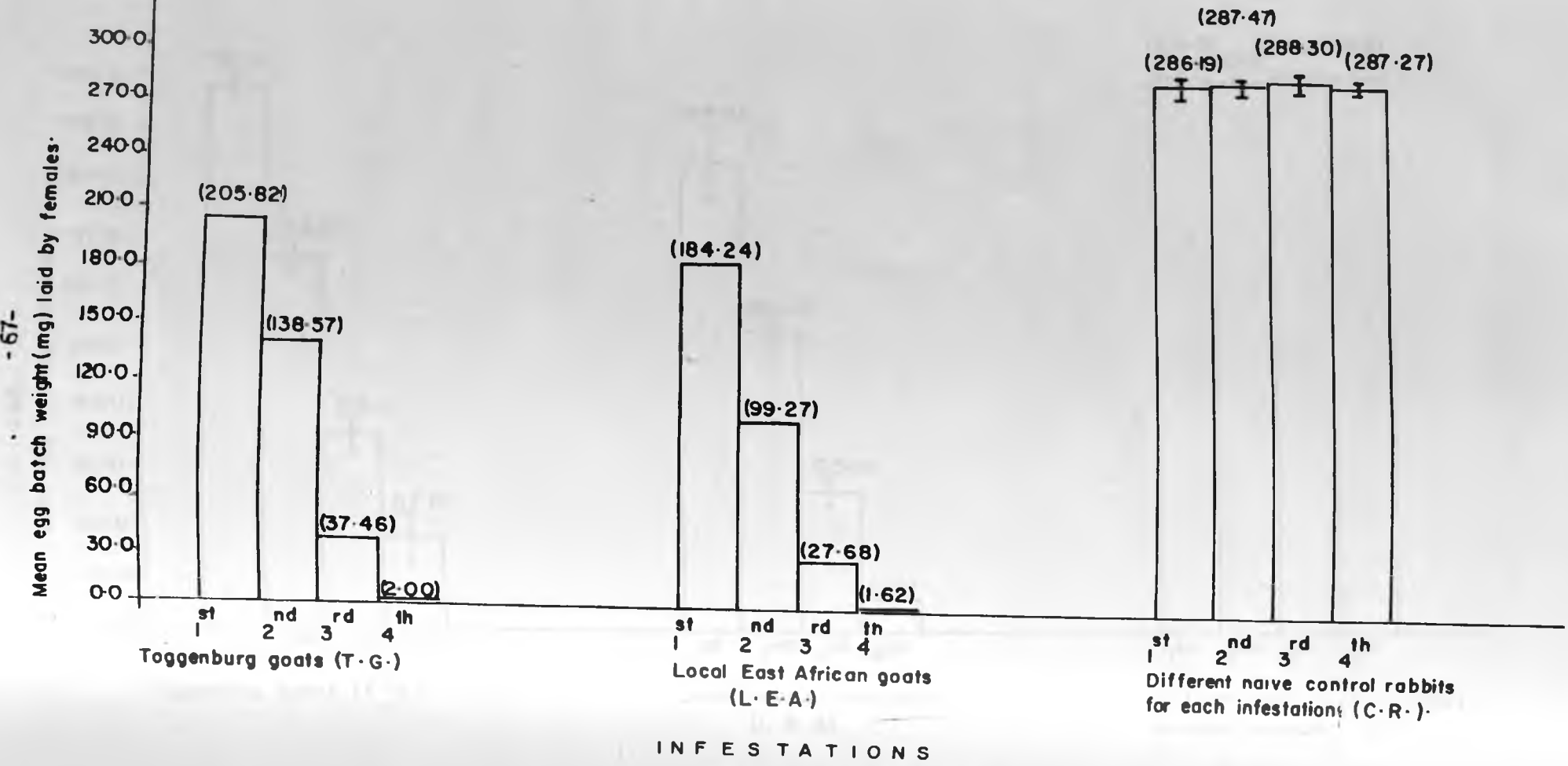
Figure 6d: Mean egg percentage hatchability of eggs laid by female R appendiculatus fed on two breeds of goats (L·E·A· and T·G·) and control rabbits during successive infestations.
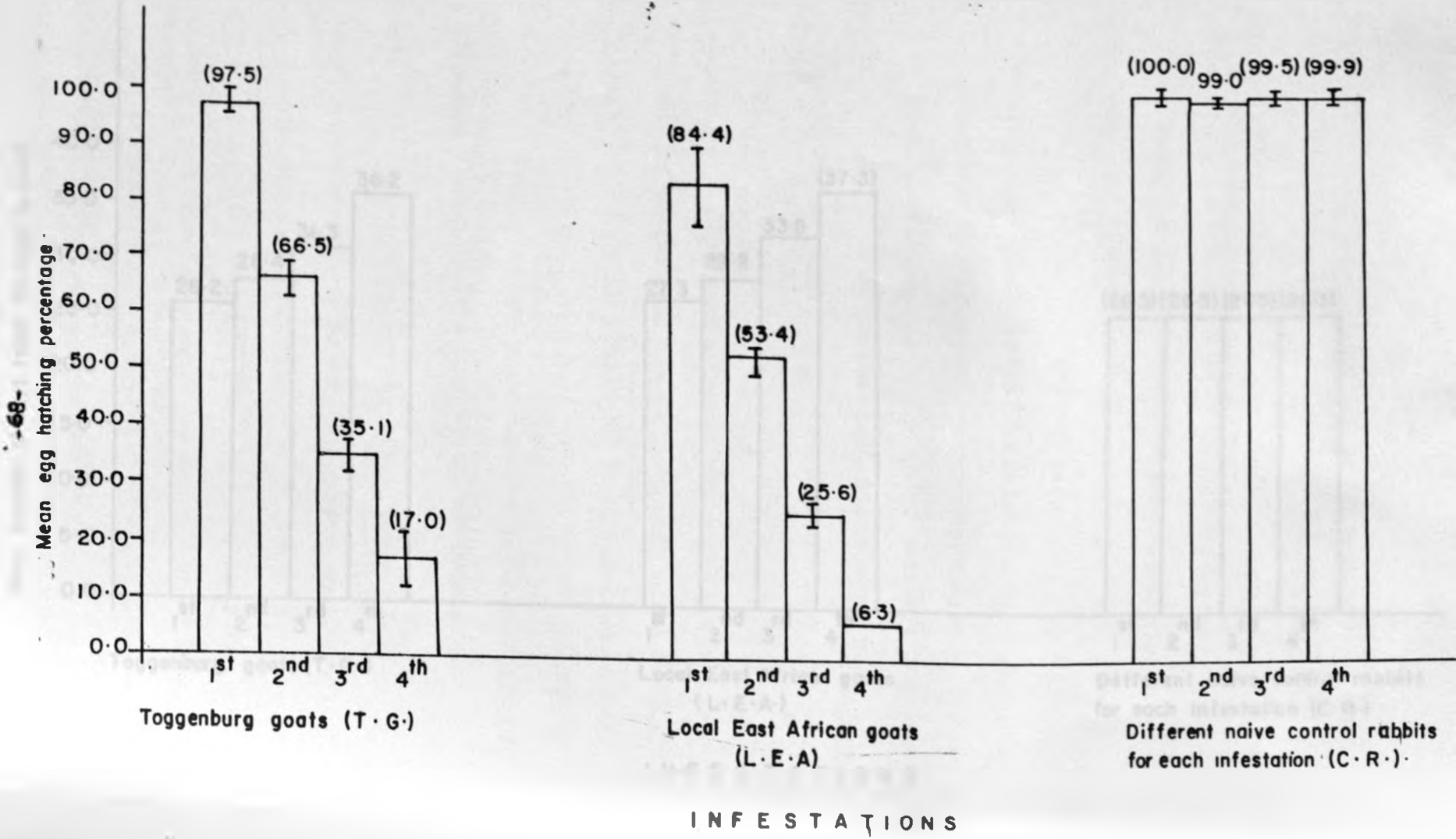
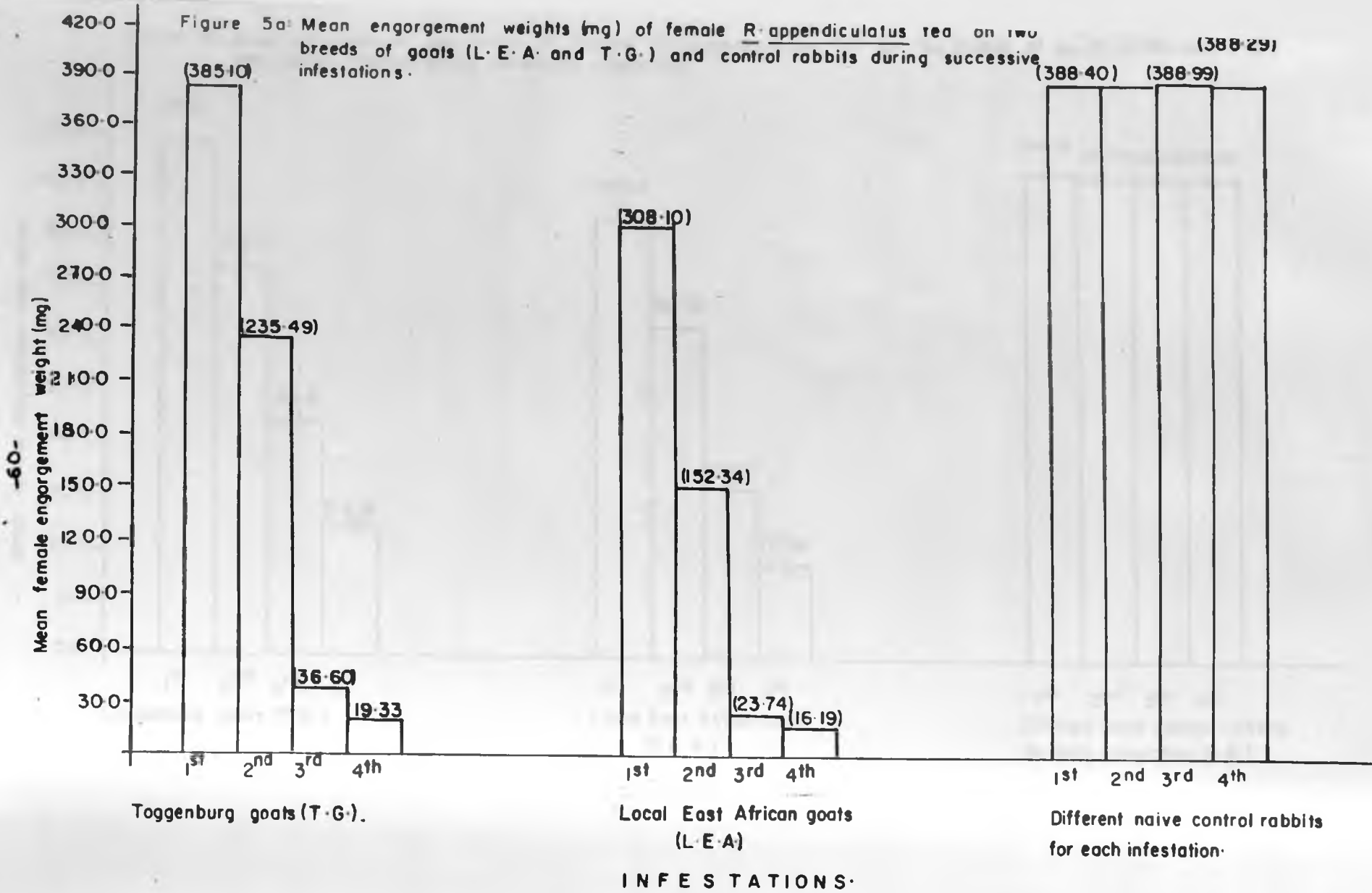Figure 6e: Mean incubation period of eggs laid by female R·appendiculatus fed on two breeds of goats (L·E·A and T·G·) and control rabbits during successive infestations.
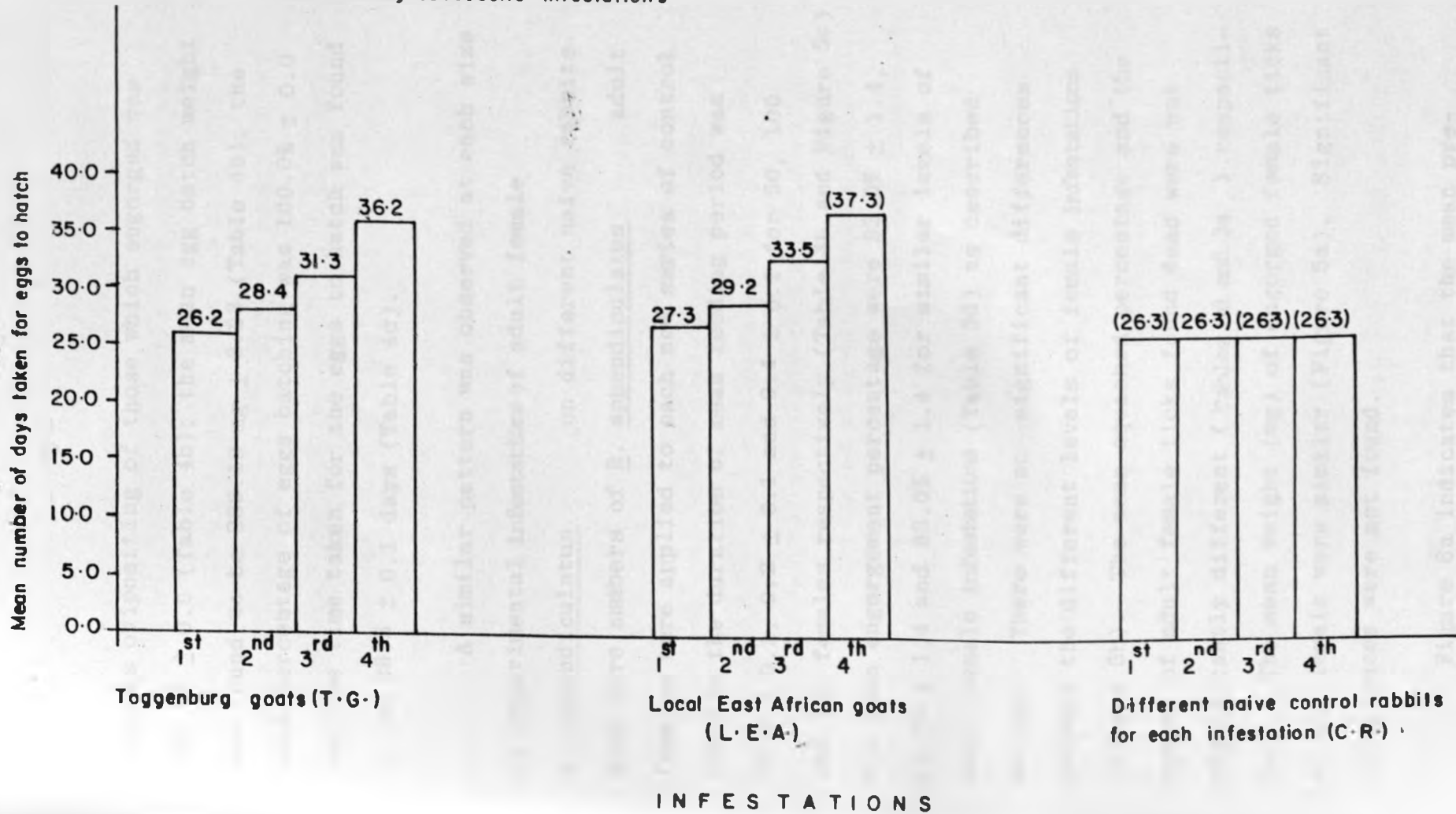
females ovipositing of those which engorged was
100.0% ± 0.0 (Table 4b); the mean egg batch weight
was found to be 286.19 mg ± 8.03 (Table 4b); the
mean percentage of eggs hatching was 100.0% ± 0.0
and the time taken for the eggs to hatch was found
to be 26.3 ± 0.1 days (Table 4c).

A similar pattern was observed at each size
of experimental infestations of adult female
R. appendiculatus on different naive rabbits.
When more numbers of R. appendiculatus adult
females were applied to each new series of control
rabbits the duration of mean feeding period was
10.3 ± 0.1, 9.2 ± 0.1 and 9.4 ± 0.1 for 50, 100
and 150 females respectively (Table 3b and Figure 5c).
The mean engorgement percentage were 93.0% ± 1.4,
93.0% ± 1.4 and 93.0% ± 1.4 for similar levels of
adult female infestations (Table 3d) as described
earlier. There were nc significant differences
between the different levels of female infestations
(Figure 5b). The mean squashed percentage and the
number of adult female ticks found dead were not
significantly different (Tables 3d and 3e) respecti-
vely. The mean weight (mg) of engorged female ticks
at all levels were similar (Figure 5a). Significant
differences were not found.

Figure 6a indicates that the mean pre-
oviposition period in days for the female ticks was
constant at all levels of infestations. The mean egg

egg batch weight (mg) was found to be similar
(n.s.) at all levels of female tick infestations
(Figure 6c). Table 4c shows that the mean egg
hatching percentages were 99.0% ± 1.5, 99.5% ± 0.8
and 99.9% ± 0.5 for similar levels of female infesta-
tions. There was no significant difference between
various levels of female infestations. The mean time
taken for eggs to hatch were not significantly
different i.e. 26.3 ± 0.1, 26.3 ± 0.1 and 26.3 ± 0.1
days for similar levels of female infestations (Table
4c).

It has been established therefore that the
R. appendiculatus   adults culture were behaving
in a similar fashion at each experimental level, the
viability was similar at each stage and it was
possible to compare the results in the experimental
goats.

4:2:2   Successive female R. appendiculatus   infestations
on the two breeds of goats.

When 25 adult female R. appendiculatus
were introduced to the ear bags of the Local East
African (L.E.A.) goats, engorged females started to
drop off the ears on day 5 when a mean of 3.0 ± 2.4
females (Table 3a) were collected. A maximum number
was collected on day 7 when 10 ± 2.8 females were
collected, followed by a sharp reduction upto day 8
when a mean of 5 ± 2.2 engorged female adults was
collected (Figure 2a). In the Toggenburg (T.G.) goats

Table 5c: Mean duration of feeding (days) of R. appendiculatus females fed on two breeds of goats in four successive infestations.

Analysis of variance:

Source	df	SS	MS	F
Breeds	1	21.2867	21.2867	304.5714***
Infestations	3	36.5164	12.1721	174.1591***
Error	31	2.1665	0.0699	
Total	35	59.9697		

*** P $<$ 0.001

LSD (breeds)	= 0.5	(P $=<$ 0.001)
LSD (infestations)	= 0.5	(P $=<$ 0.001)
Group means: L.E.A.	= 9.3^a	
T.G.	= 10.9^b	

Means of successive infestations (breeds pooled):

1 = 8.9^a

2 = 9.6^b

3 = 10.8^c

4 = 11.5^d

Means followed by the same letter are not significantly different (P $<$ 0.001); F = variance ratio; LSD = Least significant difference.

Table 5d: Mean pre-oviposition period (days) of R. appendiculatus
females fed on two breeds of goats in four successive
infestations.

Analysis of variance:

Source	df	SS	MS	F
Breeds	1	1.1520	1.1520	208.1032***
Infestations	3	2.0444	0.6815	123.1097***
Breeds x infestations	3	0.5886	0.1962	35.4426***
Error	28	0.1550	0.0055	
Total	35	3.9400		

*** P < 0.001

LSD (breeds) = 0.2 (P = <0.001)
LSD (infestations) = 0.2 (P = <0.001)
Group Means: L.E.A. = 7.9^a
            T.G. = 7.5^b

Means of successive infestations (breeds pooled):

1 = 7.5^a
2 = 7.5^a
3 = 7.7^b
4 = 8.1^c

Means followed by the same letter are not significantly
different (P < 0.001); F = variance ratio; LSD = Least
significant difference.

Table 5e: Mean percentage ovipositing (transformed to Arcsine) of
R. appendiculatus females fed on two breeds of goats in
four successive infestations.

Analysis of variance:

Source	df	SS	MS	F
Breeds	1	369.9290	369.9290	30.9454***
Infestations	3	20436.6603	6812.2201	569.8572***
Error	31	370.5820	11.9543	
Total	35	21177.1713		

*** $P < 0.001$

LSD (breeds)	= 6.0	$(P = < 0.001)$	
LSD (infestations)	= 5.9	$(P = < 0.001)$	
Group means: L.E.A.	= 46.8^a		
T.G.	= 53.3^b		

Means of successive infestations (breeds pooled)

$1 = 83.8^a$

$2 = 61.5^b$

$3 = 33.2^c$

$4 = 23.2^d$

Means followed by the same letter are not significantly
different $(P < 0.001)$; F = variance ratio; LSD = Least
significant difference.

Table 5f: Mean weight (mg) of egg batch laid by R. appendiculatus females fed on two breeds of goats in four successive infestations.

Analysis of variance

Source	df	SS	MS	F
Breeds	1	2803.4751	2803.4751	71.1514***
Infestations	3	209895.3655	69965.1218	1775.6944***
Error	31	1221.4482		
Total	35	213920.2888		

*** P < 0.001

LSD (breeds)   = 10.85 (P =<0.001)

LSD (infestations)   = 10.75 (P =<0.001)

Group means: L.E.A.   = 78.20^a

              T.G.   = 95.96^b

Means of successive infestations (breeds pooled)

    1 = 196.23^a

    2 = 121.10^b

    3 =   32.44^c

    4 =   1.82^d

Means followed by the same letter are not significantly different (P < 0.001); F = variance ratio; LSD = Least significant difference.

Table 5g: Mean percentage (transformed to Arcsine) of hatched
eggs laid by female R. appendiculatus fed on two breeds
of goats in four successive infestations.

Analysis of variance

Source	df	SS	MS	F
Breeds	1	1178.0358	1178.0358	28.8938 [***]
Infestations	3	17285.2044	5761.7348	141.3188 [***]
Error	31	1263.9068	40.7712	
Total	35	19727.1470		

*** P $<$ 0.001

LSD (breeds)   = 11.0 (P $=<$0.001)

LSD (infestations)   =   0.5 (P $=<$0.001)

Group means: L.E.A.   = 38.0[a]

.   T.G.   = 49.5[b]

Means of successive infestations (breeds pooled):

1 = 36.7[a]

2 = 32.2[b]

3 = 28.7[c]

4 = 26.7[d]

Means followed by the same letter are not significantly
different (P $<$0.001); F = variance ratio; LSD = Least
significant difference.

Table 5h: Mean pre-eclosion period (days) of eggs laid by female
R. appendiculatus fed on two breeds of goats in four
successive infestations.

Analysis of variance:

Source	df	SS	MS	F
Breeds	1	518.1870	518.1870	7843.4977***
Infestations	3	13.6208	4.5403	68.7239***
Error	31	2.0480	0.0661	
Total	35	533.8558		

*** P < 0.001

LSD (breeds) = 0.5 (P = <0.001)

LSD (infestations) = 23.3 (P = <0.001)

Group means: L.E.A. = 31.8^a

T.G. = 30.5^b

Means of successive infestations (breeds pooled):

1 = 76.0^a

2 = 51.2^b

3 = 33.7^{bc}

4 = 16.8^c

Means followed by the same letter are not significantly
different (P < 0.001); F = variance ratio; LSD = Least
significant difference.

experimental infestations with the same number of adult
female R. appendiculatus Neum.,engorged females
started to drop off the ears on the same day as for the
L.E.A. goats with a mean of 3.0 ± 1.5 engorged females
collected (Figure 2a). A maximum number was collected
on day 8 when 6.0 ± 2.3 engorged females were collected,
followed by a gradual reduction upto day 9 when
4.0 ± 3.3 engorged females were collected (Figure 2a).
The two breeds of goats showed significant differences
at (P < .001) in the feeding period at all levels of
adult female challenge (Table 5c).

There were significant differences at
(P < .001) between the two breeds of goats in the
engorgement percentage results (Table 5b) and the
mean engorgement percentage decreased significantly
with successive adult female infestations in the two
breeds of goats. Table 3d shows that the mean
squashed percentage of females was calculated to be
7.0% ± 2.0 and 1.6% ± 0.0 for 25 female ticks applied
on the L.E.A. and T.G. goats respectively. There
were significant differences between
percentage of adults found squashed from the two
breeds of goats (Table 3d). The mean percentage of
female R. appendiculatus squashed increased
significantly in most cases with successive infestations
(Table 3d). From Table 3d the L.E.A. goats had
higher percentage squashed compared with T.G. goats.

Table 3e indicates that the mean dead female per-
centage was found to be 8.0% ± 3.3 for the L.E.A.
goats for the 25 females applied, whereas for the
T.G. goats it was calculated to be 4.0% ± 0.0.
There were significant differences found between
the two breeds of goats. Table 3e
indicates that the mean percentage of dead females
increased with successive infestations. There were
significant differences when female infestations were
compared within each breed of goats.

The mean engorgement weight (mg) of
female ticks from both breeds of goats were
significantly different at $P < .001$ (Table 5a).
There were significant difference between successive
infestations within each breed of goat (Table 5a).
The mean pre-oviposition period was 7.6 ± 0.1 and
7.4 ± 0.1 for 25 female T.G. goats respectively.
The two breeds of goats were significantly different
at $(P < .001)$ when compared at the same levels of
infestation i.e. same burden of adult tick females
(Table 5d). Figure 6a indicates that the mean
pre-oviposition period was increasing with successive
infestations of adult ticks.   The L.E.A. goats
took significantly a longer time compared with T.G.
goats $(P < .001)$.

The mean percentage of females ovipositing
was 95.0% ± 4.1 and 99.2% ± 1.9 for 25 female ticks
applied on the L.E.A. and T.G. goats respectively
(Table 4b). Significant differences were found between
the two breeds of goats (Table 5e). There were
significant differences at (P $<$ .001) between
successive infestations within each breed of goat
(Table 5e). Figure 6b indicates that the mean percentage
of ovipositing females was decreasing with successive
female infestations. It was found to decrease more in
the L.E.A. compared with the T.G. goats. The mean egg
batch weight was calculated to be 184.24 mg ± 0.34 and
205.82 mg ± 0.62 for 25 female ticks applied on the
L.E.A. and T.G. goats respectively (Table 4b). Signifi-
cant differences were found between the two breeds of
goats (Table 5f). There were significant difference
between successive infestation= within each breed of goat
(Table 5f). Figure 6c indicates that the mean egg batch
weight (mg) was decreasing in both breeds of goats with
successive female tick infestations.

Table 4c shows that the mean percentage of eggs
hatching was 84.4% ± 7.0 and 97.5% ± 2.3 for 25 female
adult ticks applied on the L.E.A. and T.G. goats respec-
tively. Significant difference was found between the
two breeds of goats (Table 5g). There were significant
differences between successive infestations within each
breed of goats (Table 5g). The hatching percentage of

eggs laid decreased with successive female infestations
in both breeds of goats (Figure 6d). The mean time
taken for eggs to hatch was 27.3 ± 0.1 and 26.2 ± 0.1
days for 25 female adult ticks applied on the L.E.A.
and T.G. goats respectively (Table 4c). There were
significant differences between the two breeds of goats
(Table 5h). There were significant differences between
successive infestations within. each breed of goats
(Table 5h). Figure 6e indicates that the mean hatching
days were increasing with successive female infestations in
the two breeds of goats.

When the initial 50 nymphal test was compared with
the 50 final nymphal test, it was found to be highly
significant when various parameters of resistance were
compared. It was found that all the resistance parameters
were significantly different at (P < .001) when they
were compared within each breed of goats. However, there
was no significant difference found between the two breeds
of goats when the final 50 nymphal test was performed even
though some parameters were significantly different.

This showed that both breeds of goats were
resistant to tick infestation / with R. appendiculatus
almost at the same level of tick numbers.

CHAPTER 5

5:1   DISCUSSION AND CONCLUSIONS

Cattle can develop resistance to the feeding
activities of Amblyomma americanum and B. microplus adult
ticks after a single infestation. There are significant
reductions in the yield of engorged ticks, mean weight
and mean egg production during subsequent infestations
(Strother et al., 1974; Riek 1956, 1962). Similar decreases
in feeding success and fecundity in R. appendiculatus on
two breeds of goats are reported in the present study.
The goats acquired levels of resistance to R. appendiculatus
which increased with/successive tick infestation.   /each

The goats rejected ticks during the attachment
phase and significantly impaired the ability of ticks to
take in a normal blood meal and to produce their full
potential yield of eggs. This finding suggests a double
mechanism with one component directed against the tick
attaching phase and the other against the feeding phase.
My results demonstrate that it is possible for goats to
acquire resistance which interferes considerably with the
survival potential of R. appendiculatus. The resistance
phenomenon was characterized by a pronounced decrease in
the percentage of the number of ticks attaching and
engorging, increased mortality, prolonged time to drop
off the host and decreased engorgement weights. O'Kelley

and Spiers (1976) concluded that different breeds of the
host vary in their level of innate resistance to ticks as
I have also found in the present study.

The term "innate resistance" has been frequently
used and loosely used in the tick literature. If some
individuals of a species that is normally a host for a
particular parasite are found to be resistant to it before
initial exposure to that parasite, then they can reasonably
be described as "innately resistant" (Roberts, 1968b;
Riek, 1962). However, there has been a tendency to use
the term to describe interspecies variation, in particular
in the immunity of B. indicus and B. taurus cattle to
B. microplus (for example, O'Kelley and Spiers, 1976).

Three points can be made here. First, interspecies
variation is to be expected in the response of any parasite
to its hosts. Second, for the particular case mentioned,
there is now very good evidence that B. indicus cattle
before exposure to B. microplus are as susceptible to the
parasite as are B. taurus (Hewetson, 1971; Wagland, 1975,
1978). Third, the immunological response of an animal to
ticks can be affected by its previous history and earlier
parasitisms (Callow and Stewart, 1978). The present results
agree with what was found by Callow and Stewart (1978).
The L.E.A. goats tended to exhibit stronger resistance than
the T.G., possibly due to the historical background of the
L.E.A. goats which have always been reared on tick infested

pasturelands. This is similar to earlier findings of an
innate component in addition to the acquired responsiveness
of many hosts to tick infestation. Kelley (1943),
Riek (1962) and Francis and Little (1964) all considered
that cattle with a significant B. indicus genetic background
were more innately resistant than pure B. taurus cattle.

The ability to acquire resistance also varies
markedly from animal to animal within breeds. Although
host resistance to ticks might have an innate component
which varies with breed differences, a significant component
of tick resistance is acquired (Branagan, 1974; Allen
et al., 1977). In this study, acquired resistance was
expressed by lengthened feeding time and post embryonic
development (nymphs), reduced oviposition and deaths amongst
feeding ticks. These findings are in agreement with those
of Riek (1956), and more recently Schleger et al., (1976),
who hypothesized that bovine immunity to B. microplus must
be a mast-dependent cutaneous hypersensitivity, characterized
by cutaneous eosinophilia. This conclusion resulted from
several observations. First, serous exudates were observed
at tick attachment sites in sensitized cattle, within a few
hours of infestation, and were accompanied by intense
scratching. The observed pruritis probably resulted from
mast cell release of vasoactive mediators, such as histamine,
at the tick attachment sites as a result of cellular
degranulation following interaction of cell surface bound

anti-tick antibody with tick derived salivary substances.
Similar grooming behaviour in tick sensitized goats was
observed in this study at each challenge. Histamine and
other mediators derived from mast cells could contribute
to the inflammatory process by altering the permeability
of local blood vessels resulting in oedema that could make
the tick feeding environment less suitable and an accumulation
of effector cells that could act directly on ticks, or
release additional mediators that could adversely affect
physiological processes crucial to tick feeding, ovipositional
success and survival.

Bowessidjaou et al. (1977) working on laboratory
rabbits with the tick Ixodes ricinus and Hewetson (1971)
and Willadsen (1980) with B. microplus on cattle all found
an increase in the length of the feeding period, as was
also found in my study for R. appendiculatus in both breeds
of goats. The feeding periods of both nymphs and adults were
always significantly longer on the T.G. than on the L.E.A.
goats. In this case, it appeared that the T.G. goats were
acquiring R. appendiculatus resistance more quickly than the
L.E.A. goats. The tick attachment sites on the T.G. goats
were more inflamed than those on the L.E.A. goats suggesting
the presence of cellular infiltrates at the sites of tick
attachment.

The first 50 nymphal feed on the goats when they were

still tick naive, indicated significant differences
between the two breeds of goats in their levels of
resistance. The present findings agree with those of
Willadser, (1980) who demonstrated that the manifestation
of tick resistance varied greatly, depending on the species
of hosts and ticks concerned.

Bowessidjaou et al. (1977) reported that repeated
infestations on laboratory rabbits with I. ricinus produced
a marked decrease in the percentages of ticks engorging,
female engorged weights, oviposition, viability of eggs
and an increase in the length of the feeding period. The
present findings for both breeds of goats are in conformity
with these. The manifestation of resistance to R.
appendiculatus in goats was also similar to that of the
mouse, Apodemus sylvaticus to Ixodes trianguliceps larvae
(Randolph, 1979).

There was a constantly high tick engorgement
percentage at each tick feed on the naive control rabbits,
whereas the goats became increasingly resistant with each
repeated tick infestation. This demonstrated very clearly
the importance and possibility of using acquired resistance
to ticks by hosts for biological control purposes. If this
method could work in the field, it would have some advantage
over chemical methods. The development of tick resistance
to acaricides has become a matter of great concern in many

parts of the world and has stimulated interest in non-
chemical methods of tick control. The mechanisms of these
resistance responses by cattle have not been determined,
but the fact that some animals are innately resistant to
ticks whereas others must acquire resistance by tick
infestation, has been the basis for selective breeding of
cattle for resistance to ticks in Australia (O'Kelley and
Spiers, 1976; Utech et al., 1978). Similar work should be
done on goats.

Resistance to tick infestation leads to a reduction
in the number of ticks feeding to engorgement and in turn
leads to a reduction in tick density. Newson and Chiera
(Personal communication) have shown that if ticks in the
field have only resistant cattle on which to feed, tick
numbers can be reduced to very low levels. Laboratory
studies have confirmed that R. appendiculatus fed on
resistant cattle are smaller at each stage than those fed
on susceptible hosts and far fewer eggs are produced (Chiera,
Newson and Cunningham, Personal communication).

There is evidence that host resistance to tick
infestation can also alter the ability of pathogen bearing
ticks to transmit these pathogens between hosts. Mellanby
(1946) working on the human reaction to mosquito bites,
reported that an intense immune response directed towards
the blood feeding arthropod might create a local environment

hostile to the development and spread of the pathogen. This would seem to be even more likely for arthropods that obtain their blood meal over a long period, thus providing a sustained antigenic stimulus to the host. Similarly, Francis and Little (1964) demonstrated that cattle resistant to infestation by B. microplus were significantly less likely to develop babesiosis than animals naive to tick infestation. Bell et al. (1979) reported that rabbits resistant to tick infestation were also significantly protected against the transmission of highly virulent tick borne bacteria.

The percentage of ticks collected squashed or dead, increased progressively in both breeds of goats. Doube and Kemp (1975) showed that when cattle exposed to Ixodes holocyclus acquire immunity, the irritation caused by the feeding ticks results in their removal by grooming or in the death of the ticks in situ by physical damage. Loomis (1971) attempted to rear B. microplus on rabbits but was unsuccessful because immunity was rapidly acquired and some ticks died on the host. The increase in the number of squashed ticks was probably due to the increased irritation the ticks were causing to the goats, as they shook their heads increasingly at each tick challenge. The L.E.A. goats had a higher percentage squashed than those on the T.G. goats and the differences were significantly different. The inference could be that the L.E.A. goats

were irritated more than the T.G. goats. The numbers of
both nymphal and adult ticks collected dead also increased
over successive tick infestations. Similarly Kooudstaal,
Kemp and Kerr (1978) reported that the percentage of B.
microplus larvae that died when feeding increased at each
successive infestation. Bowessidjaou et al. (1977)
suggested that there was a toxic effect on female I. ricinus
which had been fed on an immune rabbit. They also noted
that antibody to I. ricinus salivary gland as measured by
indirect immunofluorescence, appeared towards the end of
the first infestation and reached high titres on a second
one, but did not increase thereafter, although the hosts
became progressively more immune. A similar explanation can
be advanced for the present study. There was probably some
antigenic reaction specific to the tick; that is, since the
goats were already sensitized with tick saliva, they produced
antibodies against it, retarding its feeding. Very few ticks
were found squashed or dead on the naive control rabbits
indicating perhaps that they were not very much irritated
by the ticks. Trager (1939a) noted that the first batch of
Dermacentor variabilis larvae placed on guinea pigs nearly
all reached full engorgement, whereas this number was greatly
reduced during subsequent infestations on the same animal.
These observations suggested that one infestation was enough
to induce effective resistance to subsequent infestations
with larvae. Goats acquired resistance to R. appendiculatus
infestation more slowly.

The mean engorged weights of nymphs and female
R. appendiculatus in both breeds of goats decreased
considerably with repeated tick infestation. Wagland
(1978) reported that the mean engorged weight of female
ticks fed on immune B. indicus cattle were reduced by 30%.
Similar effects have been observed in R. appendiculatus
(Branagan, 1974) and in the present study. This indicated
that with successive tick infestations, the goats were
mounting an increasingly effective immune response to the
ticks. Similar findings have been reported by Wishitemi
(Personal communication) who observed that the percentage
of R. appendiculatus engorging and engorgement weights on
Red Maasai sheep reduced significantly.

The time taken for the engorged nymphs to moult
into adults was significantly different for the two breeds
of goats although there was a progressive increase in
moulting time in both breeds with successive tick
infestations. However, there was no significant change in
the moulting time in ticks fed on the naive control rabbits.
Similar findings have been reported by Wishitemi (personal
communication) who noted that the duration of moulting of
nymphs increased with successive infestations of R.
appendiculatus. This shows that there was a significant
effect on nymphal development. The reduced moulting
percentage and prolonged moulting time could be solely due
to inadequate blood meal taken in and hence poor nutrition.

The other possible explanation is that the blood meal taken by the nymphs from the goats had a toxic effect. Hence with repeated tick infestations the goats were acquiring a higher concentration of this blood factor which interfered with the development of any nymphs that had fed on them. Brossard (1976) found that serum gamma globulin concentration was significantly increased following B. microplus infestation. Using indirect immunofluorescence, he showed the presence of specific and non-specific antibodies to the salivary gland of female ticks. Specific antibody appeared following initial infestation of cattle with ticks, reached high titres, which were maintained throughout infestation, and then declined over a period of months once infestation was finished. This blood factor suggested in this study was likely to be antibody mediated response. Fujisaki (1978) found that rabbits developed an IgG anti-body to Haemaphysalis longicornis. Tracey-Patte (1979) found that the activity of an enzyme which B. microplus secretes into the host's skin within 1h of attachment can be neutralised by a host previously exposed to the tick. This reaction could be antibody-dependent.

The incubation period of the eggs lengthened with successive tick infestations in both breeds of goats, with eggs from females fed on L.E.A. goats taking longer to hatch than those from T.G. goats. In both breeds of goats, tick reproductive potential was significantly reduced as

demonstrated by female engorgement and egg mass weight.
Egg viability was also reduced with successive tick
infestations. All these results are in conformity with
Bowessidjaou et al. (1977), who also demonstrated that
the viability of eggs laid by female I. ricinus fed on
resistant rabbits was reduced markedly. The pre-oviposition
period of engorged R. appendiculatus females on two breeds
of goats was prolonged significantly. This was probably
due to poor nutrition and some toxic effect obtained from
the blood meal. Wishitemi (personal communication) obtained
similar results for R. appendiculatus on sheep.

Despite numerous studies it is still not known
what exactly are the factors involved in the acquisition
of resistance by bovines or caprines to ixodid tick
infestations and further work is needed. The tick could
perhaps secrete an antigen into the host in order to
stimulate an immune reaction. Unless this is the case,
however, any antigen should be characterized not only by
its immunological reactivity but also by a biochemical
function, for example, as a feeding enzyme. Characterization
of tick antigens to which hosts react under natural
infestation in terms of their biochemical function has
been reported only for B. microplus. Of the three antigens
studied in this tick, one is a hydrolytic enzyme, a serine
esterase (Willadsen and Williams, 1976; Willadsen, 1976) and
another an inhibitor of proteolytic enzymes (Willadsen

and Riding, 1979). A similar approach should be followed for R. appendiculatus.

To date then, attempts to immunize artificially against ticks have been at best partially successful. This lack of success has been, if anything, a stimulus to proposals about how one might immunize against ticks and an inducement to enthusiastic speculation about the potential efficacy of immunization in tick control (Allen and Humphreys, 1979; Smith, 1979). There is currently no reason for such optimism, not only because success in laboratory experiments has so far been limited but also because none of the methods of immunization used is likely to be acceptable and economically sound in practice.

It is hoped that goats made resistant by artificial infestation could be used in the field to try and control the numbers of R. appendiculatus. This should lead to reduction in the transmission of tick borne diseases. The effect of reducing tick populations therefore, has important implications from epidemiological and economic standpoints.

In summary, resistance by two breeds of goats to R. appendiculatus ticks builds up over repeated tick infestations. The result is a fall in tick yield and impaired feeding, leading to reduced production of larvae. This resistance is an immune response associated with some

blood factors and probably some cellular reactions.
These findings need to be investigated further. The
mechanism by which goat immune response adversely affects
tick feeding, development and reproductive potential is
also not known, and deserves further study.

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APPENDIX

Formula for calculating standard deviation:

$$S = \sqrt{\left(\frac{\sum x^2 - \frac{(\sum x)^2}{N}}{N - 1} \right)}$$

Where,

$\sum x^2$ = Sum of the squares of all values of X taken singly.

$(\sum x)^2$ = The square of the sum of all values of X.

N = Number of observations.