Study 3.6
Patterns of Water Usage

3.6.1 Introduction

In the course of studies into the nesting behaviour of hens of three different strains in laying cages (Study 3.5) it was noted that a number of hens drank quite avidly during the 10 or 20 minutes immediately prior to oviposition. Most reports in the literature, for example, Lifschitz *et al.* (1967), Wood-Gush and Horne (1970), Woodard and Wilson (1970), Mongin and Sauveur (1974), Nys *et al.* (1976) and Savory (1978), have indicated that water intake of individually caged hens is reduced during a period of from one to three hours before oviposition. Water intake, in these studies, increased after oviposition. The observation that some hens in the studies conducted by this author drank considerably during the immediate pre-lay period seemed to be inconsistent with these reports. The following experiment was conducted in an effort to investigate the water intakes of hens used in the behavioural studies in greater detail.

3.6.2 Materials and Methods

At the completion of the behaviour studies described in Study 3.5, the 18 hens of Group A were moved back into their original individual cages from the pens in which they had been housed during the latter half of the behavioural study. These were six hens of each of the B x W, R x W and B x R strains. Unfortunately, one B x R and one R x W hen had been severely vent-pecked towards the end of Study 3.5 and did not lay on their return to the cages and so were omitted from these studies. The husbandry of these birds was the same as it had been in the original study when they had previously been housed in the same cages. However, the continuous water troughs at the back of the cage-sets were removed and replaced by individual metal water cans attached at the back of each cage. These water troughs were small enough and positioned in such a way that hens could only drink from their own troughs.

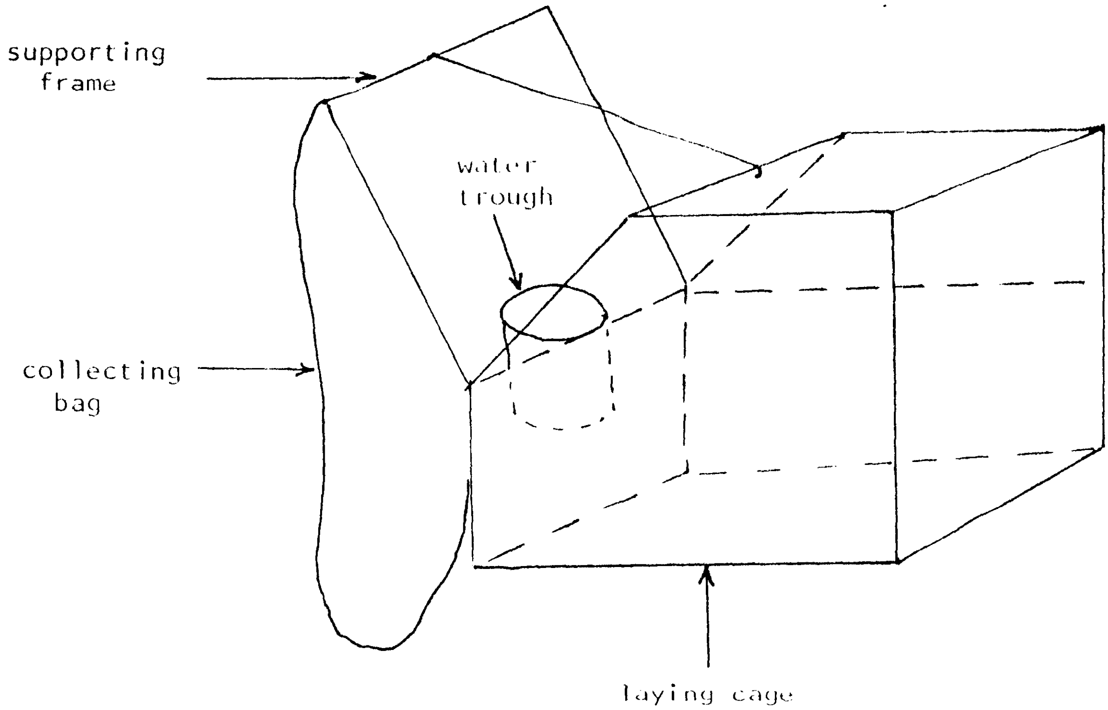
Hens were allowed three days to become accustomed to the cage conditions again and to the new water troughs before water usage was first measured. Starting at either 5.30 am, 6.30 am, 7.30 am and 8.30 am each morning, each water trough was removed, weighed and recorded and water added to a weight determined to be the weight of that trough plus 400 mls of water. The troughs were then returned to the cages from which they had been removed. This procedure was repeated at hourly intervals precisely throughout the day to 7.30 pm or 8.30 pm. Hens were closely observed during this period and when a hen laid the time of oviposition was recorded. At the same time the water trough was weighed and refilled.

Excluding the first measurement each day, which reflected water usage from the last recording on the previous night to that time, the hourly water usage of individual hens was determined from these measurements on eight days. All data were converted to millilitres used per minute to allow for comparisons between hourly figures and pre- and post-lay periods which were somewhere less than an hour due to the fact that ovipositions could occur at any time between the hourly recording intervals. Mean water usage per minute was then calculated for the five full hour periods and the extra pre-lay period (approximately 30 minutes) before, and the extra post-lay period (approximately 30 minutes) and five full hours after oviposition for each hen using each day's data. One hen, a B x W cross, consistently laid early each day and on no occasion could the full five hour pre-lay water usage data be obtained. This hen was excluded from the analyses. Five hens of each strain therefore contributed data for the analysis of water usage patterns.

These pre- and post-lay water usage data were analysed using the BMDP P2V programme for analysis of variance with repeated measures available on the DEC20 computer at the UNE.

Using data from any hens which did not lay on one or several days during the eight days over which recordings were taken, a daily pattern of water usage on non-laying days was established. In addition, the environmental temperature in the shed was recorded hourly at the same time that water usage measurements were taken to give the average daily temperature pattern. Two water troughs were also kept in the shed and weighed and topped up hourly to give an indication of daily evaporation patterns from the troughs.

During the early stages of this experiment it became obvious that a large amount of water was being wasted from the troughs each day. This appeared to be mainly a result of the drinking action of many of the hens, which involved a great deal of water splash (water not swallowed but thrown from the trough during the process of drinking), as the hens threw their heads back in drinking. The amount of splash seemed to vary throughout the day and appeared to be greatest in the hottest part of the day which coincided approximately with the time of day that many eggs were laid. In an effort to assess the extent of this water splash throughout the day, light weight plastic bags were attached by means of wire supporting frames to the back of each cage (see Figure 3.6.1). These bags completely surrounded the water trough, extending back 25 cm from the cage, and were elevated 20 cm above the level of the top of the water trough. This device managed to catch most of the forwards and upwards splash from the trough. No attempt was made to catch water flung backwards over the drinking hen or upwards and forwards over the top of the collecting device. However,



<u>Figure 3.6.1</u> Device used to collect water splashed from drinking troughs

water splash in these directions was observed to be minor.

Water splash was collected and recorded at hourly intervals throughout the three final days of the water usage experiment. Plastic bags were removed and replaced at the same times that water troughs were measured and the amount of water collected again determined by weight. For each hen, the amount of water splash collected during the five full hour periods and extra pre-lay period before, and the full five hour periods and extra post-lay period after oviposition were calculated and again converted to millilitres per minute.

To investigate the possibility that hens approaching oviposition may in fact be drinking in a different manner than at other times of day, the drinking activities of individual hens were observed in detail during the pre- and post-lay periods. It was thought that these may explain the conflict between the reports in the literature and the observations from these studies. Six hens, two of each strain, were videotaped at close range at the water trough before, during and after one oviposition in their home cage. From the videotape the numbers of 'drinking' movements of several different types were recorded for 10 minute periods both before and after laying. The types of drinking movements recorded were as follows:

1 = one complete drinking movement; the bill was dipped into the water and the head thrown back with swallowing occurring,

√ = one movement of the above type, but which was accompanied by a side to side head shake. This movement involved the splashing of water from the bill and head of the hen but appeared to result in at least some intake of water as evidenced by swallowing,

x = bill dipped into water but followed by head shaking and apparently no drinking; head not thrown back and no swallowing apparent,

0 = one pecking movement at the water with some swallowing,

\- = one pecking movement at the water with no apparent swallowing.

3.6.3 Results

The water usage patterns of each hen for the five full hour periods and pre-lay period before, and post-lay period and five full hour periods after lay are given in Figure 3.6.2. The average time at which oviposition took place on the days from which these data came is also given for each hen in this Figure, as is the graph of mean daily water usage rates for the hens on non-laying days.

From Figure 3.6.2 it can be seen that when averaged over all hens and the several occasions recorded for each hen, ovipositions occurred approximately mid-way through an hour period. Therefore, the average lengths of the pre- and post-lay periods recorded were approximately 30 minutes each. Mean values of water usage for time periods before and after lay are given in Table 3.6.1.

Table 3.6.1 Mean water usage (mls/min) during pre- and post-lay periods for BxW, RxW and BxR strains and for all strains

	Water Usage (mls/min)											
	Before Oviposition (hours)						After Oviposition (hours)					
Strain	-5½	-4½	-3½	-2½	-1½	-½	½	1½	2½	3½	4½	5½
B x W	0.36	0.32	0.38	0.29	0.26	0.79	0.44	0.52	0.64	0.70	0.68	0.77
R x W	2.88	1.20	1.12	0.84	0.75	0.71	1.68	1.22	1.49	1.41	1.41	1.38
B x R	1.04	0.87	0.83	0.71	0.58	0.43	0.99	0.64	0.74	0.86	0.75	0.80
All	1.27	0.75	0.72	0.57	0.51	0.65	1.00	0.77	0.94	0.97	0.93	0.98

Mean daily temperature and control evaporation rates are shown in Figure 3.6.3. Early afternoon temperatures rose as high as 32°C on some occasions. However, evaporation rates never exceeded 0.03 mls/min and this level of evaporation was considered insignificant in contributing to the water loss recorded from water troughs.

Figure 3.6.2 Water usage (mls/min) for B x W, R x W and B x R hens before and after oviposition

([hatched box] = full hour period;

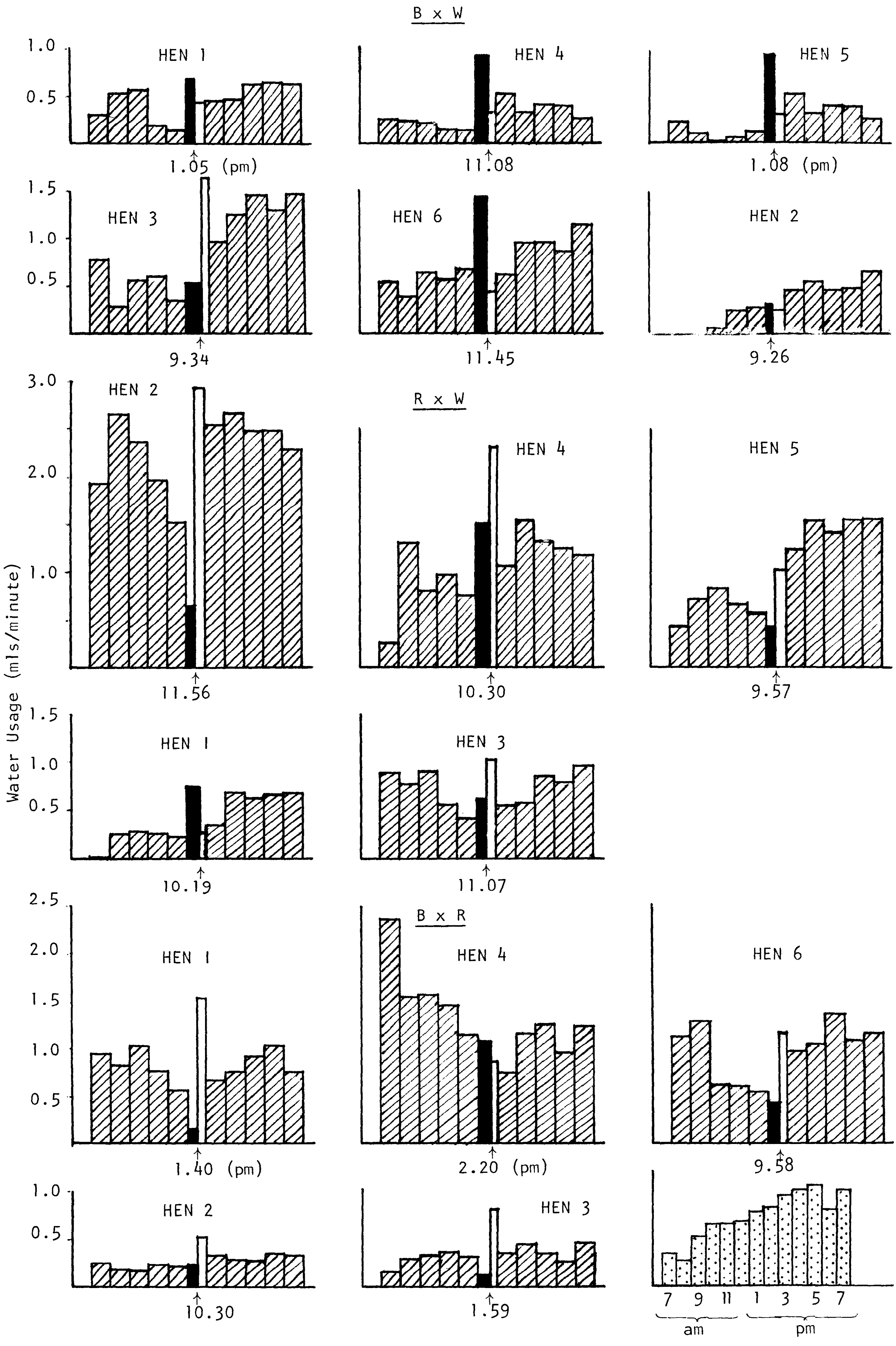
[solid black box] = immediate pre-lay period;

[open box] = immediate post-lay period;

width of bar represents duration of period;

↑ = time of oviposition;

[dotted box] = water usage on non-laying days)


B x W
HEN 1
HEN 4
HEN 5
1.05 (pm)
11.08
1.08 (pm)
HEN 3
HEN 6
HEN 2
9.34
11.45
9.26
HEN 2
R x W
HEN 4
HEN 5
11.56
10.30
9.57
HEN 1
HEN 3
10.19
11.07
B x R
HEN 1
HEN 4
HEN 6
1.40 (pm)
2.20 (pm)
9.58
HEN 2
HEN 3
10.30
1.59
7 9 11 1 3 5 7
am
pm
Water Usage (mls/minute)
1.0
0.5
1.5
3.0
2.0
2.5

The data recorded in Figure 3.6.2 indicate that not only the total water usage but the pattern of water usage about oviposition varied considerably between hens. This is reflected in the highly significant hen within strain effect revealed in the analysis of variance shown in Table 3.6.2.

Table 3.6.2 Analyses of variance of water usage before, after and both before and after oviposition for hens of BxW, RxW and BxR strains

	Water Usage During Pre-lay Time Periods				
Source	D.F.	S.S.	M.S.	F	Significance
Strain	2	2.685	1.342	1.01	N.S.
Error (a)	12	15.897	1.325		
Period	5	0.582	0.116	1.462	N.S.
Period x Strain	10	2.689	0.269	3.376	***
Error (b)	60	4.779	0.80		
Total	89	26.632			
	Water Usage During Post-Lay Time Periods				
Source	D.F.	S.S.	M.S.	F	Significance
Strain	2	7.899	3.949	2.13	N.S.
Error (a)	12	22.199	1.850		
Period	5	0.585	0.117	2.62	*
Period x Strain	10	0.328	0.033	0.74	N.S.
Error (b)	60	2.675	0.045		
Total	89	33.686			
	Water Usage During Pre- and Post-Lay Time Periods				
Source	D.F.	S.S.	M.S.	F	Significance
Strain	2	9.330	4.665	14.46	***
Hen Within Strain	12	34.225	2.852	8.84	***
Error (a)	12	3.872	0.323		
Before/After Lay (B/A)	1	3.254	3.254	52.39	***
B/A x Strain	2	1.254	0.627	10.09	***
Period Within B/A	10	1.167	0.117	1.88	-
Strain x Period w B/A	20	3.017	0.151	2.43	***
Error (b)	120	7.454	0.062		
Total	179	63.572			

N.S. = P > .10 ; - = .05< P < .10 ; * = .01< P < .05 ; *** = P < .001

Pre-lay water usage patterns were variable between hens. Overall, however, there was a tendency for water usage to decrease during the five full hours prior to oviposition. In the immediate pre-lay period, approximately 30 minutes preceding oviposition, water usage increased dramatically in the case of B x W birds in general, remained much the same for the R x W strain and decreased further in the case of the B x R strain. The significant period by strain interaction component of the pre-lay analysis of variance reflects these differences in water usage during the immediate pre-lay period.

Individual differences were also evident in the post-lay water usage patterns of hens. However, a tendency for water usage to increase during the first few hours after oviposition is apparent, and a significant effect of period was revealed by post-lay analysis of variance. The time at which an increase in water usage occurred varied, some hens increasing their water usage dramatically in the immediate post-lay period, for example, B x W hen 3, R x W hens 2, 3 and 4, and B x R hens 1 and 3. In other cases, for example B x W Hens 4, 5 and 6 and R x W hen 1, water usage increased gradually over the first one to three hours after oviposition. Hens which tended to display reduced water usage during the immediate pre-lay period tended also to be those for which dramatic increases in usage during the immediate post-lay period were detected. A slight drop in water usage seemed to occur in many cases between four and five hours after oviposition and this appeared to be followed by a further slight increase in water usage during the next hour period.

Overall analysis shows that the different strains differed in the extent to which they used water from the troughs but also, as previously pointed out, different hens within these strains used water to different extents. In general, B x W hens used least water and R x W hens most water during the study period. Significant differences also were found between water usage before as against after oviposition. In general, more water was used in the time periods after oviposition than before. This tendency was, however, influenced by strain, the effect being less obvious in the case of B x R hens. Within the before and after lay data, significant strain by time period effects were found. The most dramatic of these was the marked tendency for water usage of B x W hens to increase in the immediate pre-lay period, whereas it tended to be low in the case of B x R hens, and then drop in the immediate post-lay period and increase over the following few hours. In the B x Rs, water usage usually increased immediately after oviposition.

Water splash levels were, except in a few cases, quite low and rarely exceeded 0.1 mls/min. Mean water splash values corrected to two decimal places are given in Table 3.6.3.

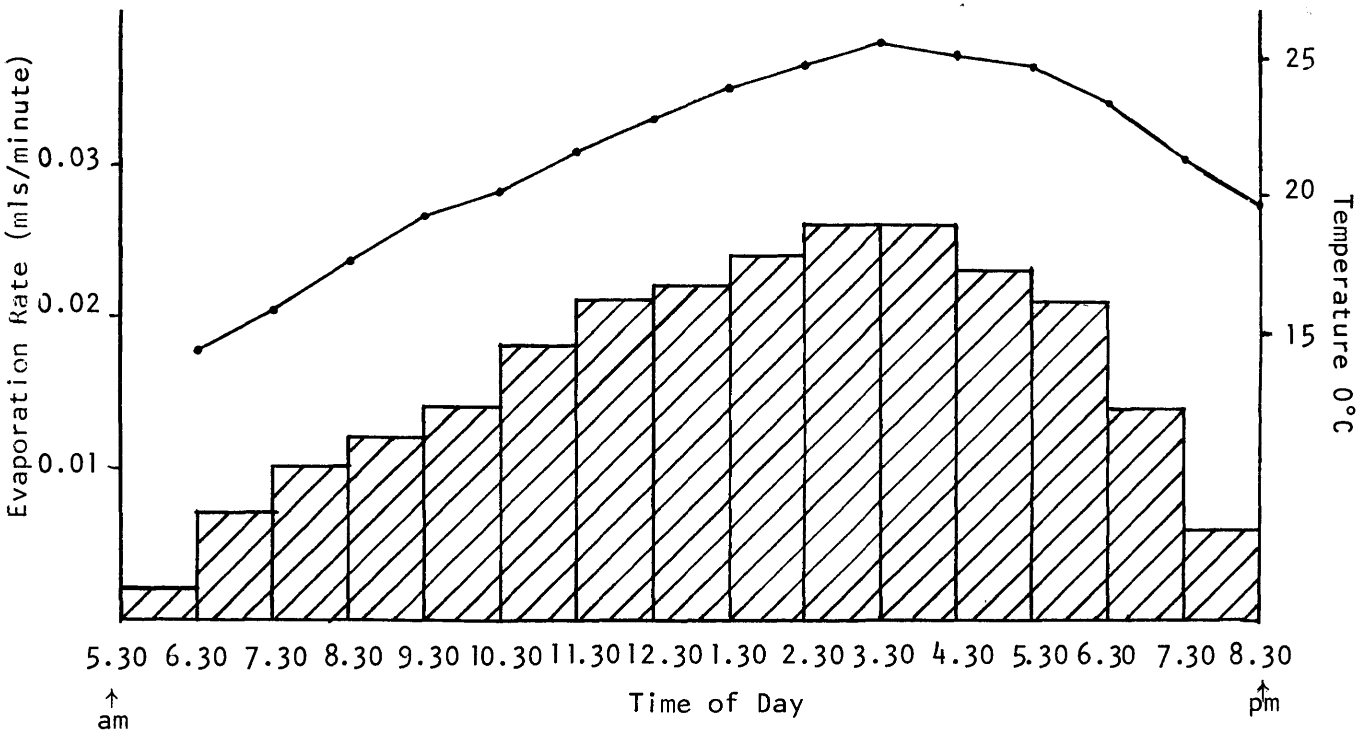


Figure 3.6.3 Average evaporation rates (▨) and temperatures (——) at hourly intervals throughout the period of study

Table 3.6.3 Mean water splash values (mls/min) for pre- and post-lay periods for each hen

		Water Splash (mls/min)											
		Before Oviposition (hours)						After Oviposition (hours)					
Strain	Hen	$-5\frac{1}{2}$	$-4\frac{1}{2}$	$-3\frac{1}{2}$	$-2\frac{1}{2}$	$-1\frac{1}{2}$	$-\frac{1}{2}$	$\frac{1}{2}$	$1\frac{1}{2}$	$2\frac{1}{2}$	$3\frac{1}{2}$	$4\frac{1}{2}$	$5\frac{1}{2}$
B x W	1		0.01	0.02	0.01	0.01	0.01	0.00	0.04	0.04	0.07	0.08	0.08
	2				0.01	0.05	0.01	0.05	0.05	0.04	0.06	0.06	0.07
	3			0.02	0.02	0.01	0.03	0.06	0.06	0.05	0.07	0.07	0.12
	4	0.01	0.04	0.01	0.02	0.02	0.04	0.02	0.05	0.03	0.06	0.04	0.10
	5	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00
	6		0.02	0.08	0.10	0.09	0.18	0.04	0.12	0.17	0.23	0.21	0.22
R x W	1		0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.02	0.01	0.01
	2	1.10	1.48	1.05	0.99	0.53	0.25	1.44	1.17	1.15	1.00	1.14	1.10
	3	0.07	0.04	0.05	0.03	0.05	0.04	0.02	0.02	0.03	0.10	0.06	0.08
	4				0.14	0.09	0.10	0.00	0.14	0.20	0.15	0.14	0.12
	5					0.23	0.22	0.01	0.03	0.05	0.09	0.12	0.10
B x R	1	0.33	0.16	0.08	0.05	0.04	0.00	0.08	0.04	0.01	0.02	0.02	
	2	0.05	0.01	0.02	0.03	0.04	0.03	0.02	0.01	0.02	0.01	0.01	0.02
	3	0.01	0.06	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.03
	4	0.97	0.60	0.43	0.39	0.31	0.03	0.08	0.24	0.12	0.13	0.09	
	6	0.05	0.07	0.03	0.05	0.03	0.27	0.06	0.06	0.06	0.06	0.05	0.07

Water usage and water splash data from the corresponding days for six of the hens, two of each strain, for which water splash levels were comparatively high, are graphed in Figure 3.6.4. This Figure shows that a large proportion of the water used by the two highest 'water users' of the hens, R x W hen 2 and B x R hen 2, was actually splashed from the troughs. This Figure also shows that the amount of water splashed in each of the time periods is roughly proportional to the amount of water used. A possible exception to this may occur in either the immediate pre- or post-lay periods with elevated water usage levels. In all but one of the illustrated cases, water splash during the high water use period either immediately before or after oviposition appears to account for less of the water used in that period than in any other.

The numbers of movements at the water trough recorded for each of two hens of each strain before and after one oviposition are shown in Figure 3.6.5. These results show a high proportion of complete drinking movements during the immediate pre- and post-lay periods. The occurrence of other types of drinking action seems to be quite variable and dependent on the individual bird.

Figure 3.6.4 Mean levels of water splash (■) and of water usage (▨ for full hour periods; □ for immediate pre- and post-lay periods) from corresponding days for a number of B x W, R x W and B x R hens before and after oviposition (↑)

Figure 3.6.5 Number of drinking movements performed in 10 minute periods before and after oviposition (↑):

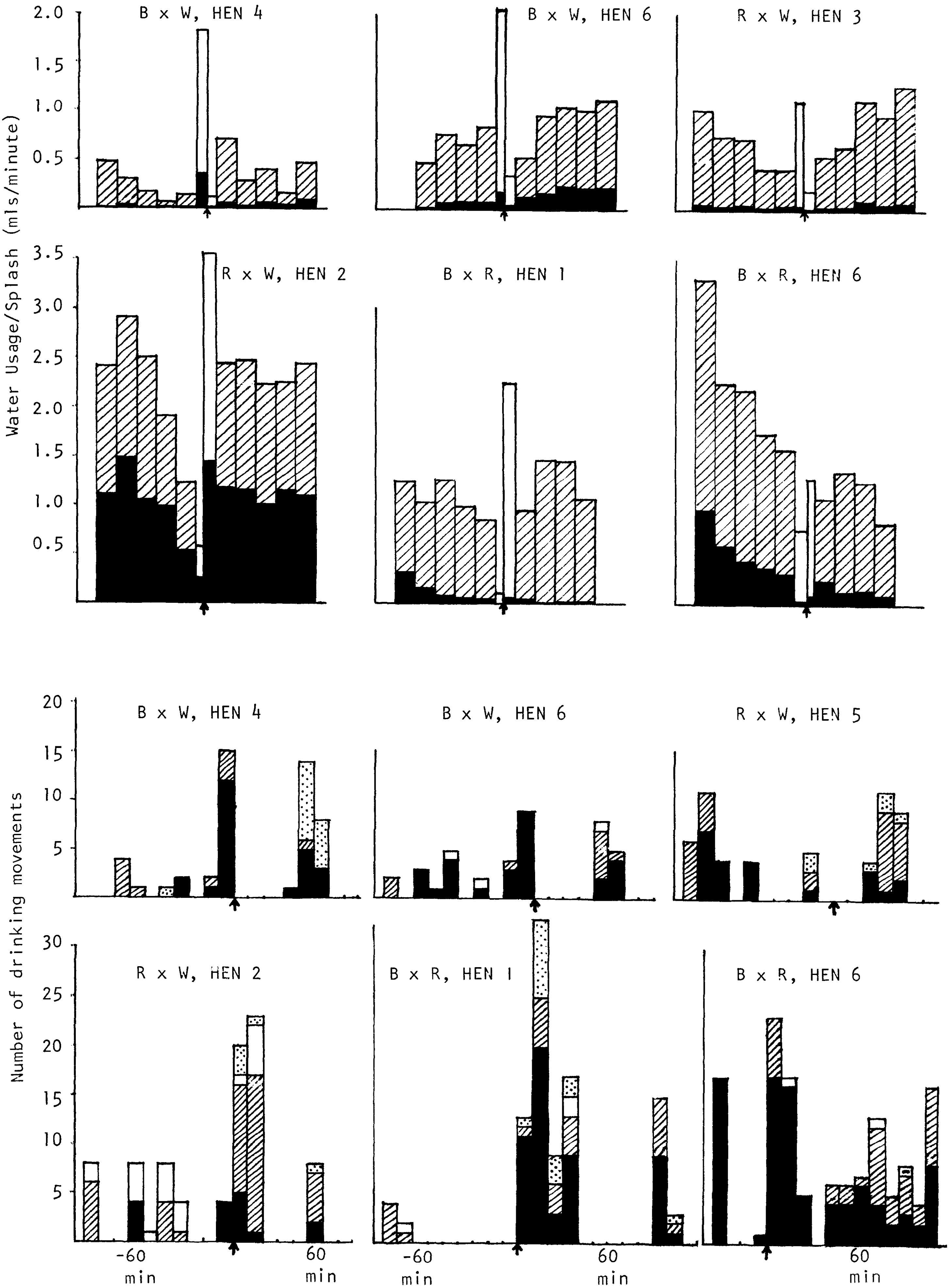
■ type 1 (complete drinking);

▨ type √ (complete drinking with head shake);

□ type X (head shake only);

⁘ type 0 (pecking with swallowing);

⊟ type - (pecking, no swallowing)



Water Usage/Splash (mls/minute)
B x W, HEN 4
B x W, HEN 6
R x W, HEN 3
R x W, HEN 2
B x R, HEN 1
B x R, HEN 6
2.0
1.5
1.0
0.5
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Number of drinking movements
B x W, HEN 4
B x W, HEN 6
R x W, HEN 5
R x W, HEN 2
B x R, HEN 1
B x R, HEN 6
20
15
10
5
30
25
20
15
10
5
-60 min
60 min
-60 min
60 min
60 min

3.6.4 Discussion

The results of the present experiment concur with those of other researchers, previously mentioned, in some respects. Assuming water usage patterns as determined in the present study can be roughly equated with water intake patterns, several parallels with their findings can be seen. Water usage of hens in this study generally dropped during the two or three full hour periods before oviposition or were very low during these periods, although strain effects were found in this respect. Lifschitz *et al.* (1967), Wood-Gush and Horne (1970), Woodard and Wilson (1970), Mongin and Sauveur (1974), Nys *et al.* (1976) and Savory (1978) also report a decline in water intake in the two to three hours preceding oviposition.

In the present study, mean water usage increased after oviposition, highest post-lay rates being found in the immediate post-lay period of approximately 30 minutes and during the periods three and five hours after this. An increase in drinking after lay is noted in all the abovementioned studies. Wood-Gush and Horne (1970) found that ingestion increased during the hour of laying and remained high for one to two hours afterwards. The water intake of hens in the study conducted by Mongin and Sauveur (1974) were highest just after oviposition and during the period corresponding with albumen plumping of the next egg in the sequence. The results presented by Lifschitz *et al.* (1967) also show that the dramatic increase in water intake at 0 hour, the hour in which the egg is laid, is followed by another higher peak seven hours after oviposition, although these workers do not draw attention to this peak.

Nys *et al.* (1976) found that water intake of hens effectively desynchronised as a result of being reared from hatching in continuous light reached a peak six to eight hours after oviposition. The persistence of this post-lay peak under continuous lighting conditions was taken as confirmation of the role of albumen plumping in the regulation of thirst. The increase in water usage in the present study during the fifth full hour after oviposition, which represents a period from about 4½ to 5½ hours after oviposition, may be attributable to increased water demands related to this process also. Unfortunately, water usage data from time periods beyond 5½ hours post-oviposition was limited, and so no information as to the extent or duration of this post-lay peak is available. Although records were taken of whether or not hens laid on the day following each recording of water usage patterns, occurrences of ovipositions at the end of a sequence (i.e. not followed by ovulation on the same day) were insufficient to allow comparisons of water usage patterns after oviposition on days on which albumen plumping either did or did not occur. To clarify whether or not the increase in water usage which seems to occur four to six hours after oviposition, is associated with albumen plumping, further studies would need to be conducted either using a larger number of hens or recording over more days both in a sequence and at the end of a sequence of ovipositions.

The increased water usage detected for a number of hens during the immediate pre-lay period is not recorded in the other reports in the literature. However, since the time of oviposition cannot be predicted and since water intake in most of these studies was determined by hourly water usage, the actual water intakes during the time periods immediately preceding or following oviposition cannot be accurately determined because most ovipositions are likely to occur some time between the hourly recordings. The method of determining water usage in the present study did, however, enable the extra recording at the point of oviposition since the experimenter was at hand at most times of the day and so could see when hens were about to lay.

Wood-Gush and Horne (1970) found that ingestion increased during the hour of laying but were unable to show at what point in that hour the increase occurred. Similarly, Lifschitz *et al.* (1967), Mongin and Sauveur (1974), Nys *et al.* (1976) and Savory (1978) recorded water intake automatically at hourly intervals and so, presumably, could not determine the exact level of water intake in the periods immediately before or after oviposition. Woodard and Wilson (1970), on the other hand, recorded frequencies of drinking in hens by means of a 'trip' mechanism attached to the lip of each hen's waterer which would be activated when the hen attempted to drink. This action would then be automatically recorded on an event recorder. The time of oviposition was also recorded and so frequency of drinking could be determined and arranged on a true hourly basis pre- and post-lay. They found that drinking decreased over several hours pre-oviposition and that frequency of drinking was lowest in the hour immediately preceding oviposition.

The results of the present study would therefore appear to be inconsistent with other reports of water intake during the immediate pre-lay period. The fact that hens are actually drinking during this phase and not merely pecking at or 'playing' with the water as some sort of displacement activity is borne out by the water splash and drinking behaviour data. Mongin and Sauveur (1974) produced evidence to suggest that the low levels of water intake just before and high levels just after oviposition found in their study were associated with the oviposition itself and not with the ovulation which generally follows it. They also suggest that the increase in drinking after oviposition may be a consequence of the release of arginine vasotocin. They cite reports in the literature which indicate a role of this hormone in stimulating oviposition. While there is some evidence that vasotocin is not necessary for the induction of oviposition and may in fact be related to ovulation instead (Sturkie and Lin, 1967), Mongin and Sauveur's suggestion seems quite reasonable.

A possible role of arginine vasotocin in the control of patterns of water usage associated with oviposition is indicated by a number of studies. Drinking and the release of vasopressin (the mammalian analogue of vasotocin) appear to be controlled by the same regulatory mechanism (Fitzsimons, 1976). Vasotocin also seems to be in some way connected with oviposition (see Chapter 2), although the potentiality of vasotocin to produce antidiuretic effects, and therefore probably thirst, is likely to be greater than its potentiality in influencing oviposition. In humans, plasma levels of vasotocin may be affected positively by exercise (Wade and Claybaugh, 1980; Geyssant *et al.*, 1981) and in humans and rats elevated vasopressin levels have been found to be associated with stress (Yates *et al.*, 1971; Devane and Porter, 1980; Hashimoto *et al.*, 1981). It is interesting, therefore, that the hens which tended to perform vigorous pacing or escape activities in the pre-laying period in the present studies also tended to be those which drank avidly in the immediate pre-lay period. The activity involved, and perhaps stress related to frustration, in nesting during this phase may have resulted in elevated vasopressin levels which, in turn, may have produced the observed drinking activity in the immediate pre-oviposition period in such hens. It is also possible that, although a delay in the time of oviposition may have occurred in the nesting of some hens, release of vasotocin may have taken place at the expected time of oviposition anyway, resulting in an increase in drinking activity, but that the effect on oviposition may have been blocked by some other factor.

However, the studies reported by Woodard and Wilson (1970), Wood-Gush and Horne (1970), Mongin and Sauveur (1974) and Nys *et al.* (1976) have all been conducted in controlled or near constant temperatures. The present experiment was conducted under a range of environmental temperatures during a hot Australian summer. Maximum daily temperatures in excess of 28°C were common and on several occasions hens showed symptoms of heat stress. In addition, oviposition in quail has been shown to be associated with a significant increase in body temperature which begins about one hour before oviposition and peaks at oviposition (Woodard and Wilson, 1970). Increased body temperature related to oviposition has also been reported for chickens (Winget *et al.*, 1965; Cain and Wilson, 1971; Van Kampen, 1976; Bobr and Sheldon, 1977). This increase has been attributed to the changes in locomotor activity of hens during the nesting phase (Van Kampen, 1976; Cain and Wilson, 1971) but also to hormonal changes associated with nesting and oviposition. Bobr and Sheldon (1977) found that the temperature peak which occurs at oviposition, like pre-laying behaviour, is dependent on the intact post-ovulatory follicle and suggested that the posterior pituitary hormones may play a role in the temperature increase associated with oviposition.

The increase in body temperature associated with oviposition, whether merely resulting from increased locomotor activity or hormonally induced, when coupled with the high environmental temperatures, may be responsible for the increased drinking activity of some hens in the period immediately prior to oviposition observed in the present study. These effects may be compounded by the preceding hours of reduced water intake and possible delays in oviposition resulting from stress induced by caging during the pre-laying phase, as indicated by elevated corticosterone levels associated with oviposition (Beuving and Vonder, 1977), or heat stress. It is interesting to note that hens may achieve evaporative cooling by splashing water over their combs and wattles (Wilson, 1949). Although splash was apparently no worse during immediate pre- or post-lay periods, the rather high rates of water usage and splash of certain birds, usually those particularly active individuals, may be related to water usage for the purposes of evaporative cooling by this strategy.

The significant strain by period effect found for water usage in the periods preceding oviposition may also be attributable to differing heat loads or heat tolerance. There appears to be no correlation between the type of water usage pattern shown by hens and the average time at which they laid, which could influence the environmental temperature at the time of oviposition. However, it has already been noted (Study 3.5) that the strains differed in the type of nesting pattern they displayed. The B x R strain exhibited a much less vigorous nesting pattern than did the B x W strain in particular. B x R hens spent more time sitting during the pre-laying phase in cages and pursued less vigorous forms of pacing and escape behaviour. The reduced locomotor activity of these hens may therefore have resulted in a lower heat output which may have enabled them to cope with the high environmental temperatures better than the B x W hens. As a result, these hens may not have needed to increase their water intake during the immediate pre-lay period. On the other hand, it is also possible that the mechanisms which control nesting behaviour and produce the different nesting forms displayed by the strains, may also control pre-lay drinking patterns and have produced the dissimilar behavioural types with regards to drinking. However, it is interesting to note that hens derived from a White Leghorn cross were more often of the 'pre-lay drinker' type, and yet many of the studies previously conducted which have indicated very low levels of drinking during the hour immediately preceding oviposition have been conducted on White Leghorn hens.

It is quite possible that the drinking itself, before ovipositon and the excessive drinking noted for certain hens, may have been psychogenic in origin. Polydipsia has been reported in caged Brown Leghorn hens by Lintern-Moore (1972) who suggested that the polydipsia that was apparent in her experimental birds may have been in response to 'boredom' in their restricted environments. A similar explanation could be offered for the apparently excessive drinking noted for some individuals and likewise the drinking response of some hens as they approached oviposition.

The general increase in water usage following oviposition was probably as compensation for the reduced levels prior to lay. Wood-Gush and Horne (1970) put the depressed intake levels they observed in the pre-lay period down to the fact that during that period hens are involved in other, more specifically nesting,activities. They also suggest that the increased ingestion observed after lay is probably the result of compensation for the self-imposed deprivation and increased energy output prior to oviposition. The results of the present study support this suggestion, in particular, the observation that hens which did exhibit a high level of water usage in the immediate pre-lay period tended not to show such dramatically elevated water usage in the post-lay period as was typical of hens which tended to abstain from drinking much prior to oviposition.

The observation that water usage tended to decline slightly after the initial and immedate post-lay peak probably reflects the fact that, after a substantial post-lay drink, most hens directed their attention to feeding, an activity from which they had largely abstained during the pre-laying phase. Most hens tended to eat quite voraciously during the first hour or so after oviposition. With time, however, their attention to feed waned somewhat and other activities such as drinking were more commonly observed.

The considerable differences observed between hens with respect to levels of water usage were probably more a reflection of the way that individuals drank than of actual water intake. This is indicated by the high levels of water splash recorded for hens with unusually high levels of water intake such as R x W hen 2. Actual water splashed would have been somewhat larger than the levels recorded, since only some proportion of the total water splashed could be collected by the collecting device used in this study. Individual differences in drinking technique were also indicated by the limited data obtained for this parameter. More reliable information on the water intake of hens could be obtained in further studies, if some other form of drinking facility, such as nipple drinkers, were used in an effort to minimise usage of water for purposes other than actual drinking.

Conclusions - Nesting Behaviour of Domestic Hens

The studies conducted and reported in this Chapter suggest that the form of the behaviour pattern associated with nesting may be influenced by a number of factors. While it may be true that the behaviour of the individual hen, at least in the case of a mature bird, will be stable in a constant environment (Gilbert and Wood-Gush, 1963; Wood-Gush and Gilbert, 1970b), the results of the present research suggest that the pattern may be influenced by social, environmental, genetic and age or experiential factors.

Results of Study 3.1 indicate that the nesting behavioural pattern displayed by broiler hens changes quantitatively as the individuals mature or have more nesting experiences. Hens paced and called less as they matured and this is suggested to be associated with a change from the emphasis on nest-seeking behaviour as hens developed attachments to particular nest sites. Activities related to nest attentiveness, such as nest building and material gathering behaviours, became more apparent over time as such site attachments developed. Material gathering movements to the back and sides of hens were not seen during the initial days of the laying phase, but tended to occur to a greater extent in later nestings.

Individual differences were found between birds in their tolerance of other hens in potential nesting sites and also in terms of the behavioural patterns displayed. The social rank of individual hens influenced the expression of some aspects of the behavioural sequence. Hens higher in the flock hierarchy tend to perform behaviours appropriate to nest attentiveness to a greater extent than their counterparts lower in the flock hierarchy. This may indicate a means by which usage of suitable nesting sites may be determined according to a priority system in situations in which potential sites are limited.

The behaviour of hens which selected floor sites in which to lay was similar to that shown by hens which selected provided nests. Hens nesting in floor sites tended to spend approximately the same amount of time in the site as their counterparts and also displayed conservatism in the selection of a site.

Most of the behaviour patterns associated with nesting in the floor pen situation, although often appearing purposeless or irrelevant when performed in that environment, can be seen to be adaptive behaviours for hens nesting in a natural habitat. Most of the behaviour patterns were also seen in the laying cage situation (Study 3.5) although many were seen at a lower frequency, in only rudimentary forms, or occurred as vacuum activities.

Pacing and nest calling activities were found to occur to a greater extent, or at high intensities, when hens were prevented access to established nest sites or were moved to a new pen (Study 3.2) or were housed in a cage as opposed to a pen environment (Study 3.5). The performance of activities related to nest attentiveness was generally inhibited in such circumstances. This provides further evidence that pacing and calling are component activities of the nest seeking drive associated with situations in which hens have not previously established a nest site, are forced to establish a new nest or are unable to establish a nest site because suitable sites are not readily found.

The results of these studies also suggest that at least part of the pacing component in some situations may be a stereotyped response indicative of frustration as the bird is thwarted in its attempts to use an established nest or is unable to find appropriate nest sites in its home environment.

The length of time that hens will spend in certain pre-laying behaviours and the overall length of the pre-laying phase, seems to be determined to some extent by the time of day that the oviposition eventually takes place (Study 3.3). This may represent an increasing attentiveness to the nest as the sequence or clutch progresses, a tendency which is noted for wild gallinaceous birds nesting in a natural habitat (see Chapter 2) which then go on to incubate their clutch.

Quantitative differences were also found in the extent to which hens of different breeds exhibited certain nesting activities, although the nesting sequences displayed were essentially similar (Study 3.4). Bantam hens tended to perform activities appropriate to nest attentiveness to a greater extent and activities appropriate to the search for a nest to a lesser extent than did broiler breeder hens and particularly White Leghorn hens. It is suggested that the bantam hens, which have retained a quite highly developed incubation and brooding tendency, may have also retained the ability to respond to the nest with appropriate attentive behaviours and with a particularly strong attachment to the specific nest site. White Leghorn hens, in which the tendency to perform behaviours associated with the incubation phase has been substantially reduced directly and indirectly through genetic selection, may have partly lost the ability to respond to an established nest during nest selection and to show appropriate attentiveness towards the nest.

The nesting behaviour of hens appeared to be unaffected by the presence of cockerels or by the behaviours exhibited by them (Study 3.4). It is suggested that cockerels do not have an essential role in the selection and establishment of a nest site in domestic fowl, at least in the floor pen situation.

The nesting drive and behaviours associated with it occupy a considerable portion of a hen's laying day (Study 3.3) and also have a considerable influence on the expression of other behaviours. The daily pattern of water usage was found to be markedly affected by the occurrence of nesting and oviposition (Study 3.6), although the pattern varied somewhat between individual hens.

While the type of nesting pattern exhibited by hens was determined by such factors as breed, age or experience, environment and even the time of day at which it occurred, the actual form of the behaviour pattern displayed and the eventual nest selected was very much an individual characteristic. Hens usually displayed a high degree of conservatism in the way that they approached the selection of a nest and also in the nest or nest site selected.

CHAPTER 4

CHARACTERISTICS OF NEST SELECTION AND NEST PREFERENCES

Introduction

A number of studies have been conducted in the past which have attempted to establish the basis of nest site selection, or at least some factors involved in it, and 'preference' of hens for particular factors. These are reviewed in Chapter 2. Unfortunately, there has been little consistency in the manner in which these studies have been conducted or in the factors that have been studied. Such studies have often proved to be difficult to design and interpret because of compications arising from reduced opportunity of individuals to approach preffered alternatives in a whole flock situation and because of possible interactions of the factor under investigation with other environmental or experiential factors (Hurnik *et al.*, 1973b). One finding common to most studies, however, is a large degree of variability between individual hens. This has tended to suggest that there may be no one nest type that is acceptable to all hens. Strain effects apparently further complicate matters.

The object of the present studies was to investigate the effect of a number of factors on selection of nest site in a number of strains and to assess the relative importance of these factors. Factors studied were selected to include some that would influence selection of nests in several ways, through influencing or 'releasing' approach to and investigation of potential nesting areas, releasing entry to specific nests and, finally, encouraging 'remaining' at the nest or sustained attention to the nest, possibly by releasing sitting in the nest.

As previously indicated in Chapter 1, this work was intended as the first step in a two stage investigation of nest site selection by hens. It was therefore intended that the present studies should be used to indicate the influences of a range of factors on nest selection, and the possible reasons for these, rather than to carry out more complete and detailed investigations into a narrow range of selection responses. Those factors to which hens responded markedly or which had potential in terms of utilization for improved nest design would be investigated more fully in the work following on after completion of this exploratory research described in this thesis.

Several techniques were used to study selection of alternative nest sites in this study. As two were common to many of the experiments to be reported in this Chapter, these will be described here.

Technique A. Alternative nest types available to groups of hens - random allocation of alternatives

This approach was used in most early studies of nest selection and involved the presentation of nest alternatives, generally still in the form of conventional nest-boxes, to groups of hens in some sort of deep litter floor pen situation. Usually, all nests were available in a bank of nests or a nest-set and the position of each alternative in the set altered at regular intervals, usually each day, by random allocation. Eggs were, in most cases, collected regularly throughout the day to minimise the influence of the presence of eggs in alternative nests on selection for other parameters.

This method of comparing selection of nests for or against certain parameters had several advantages. Large numbers of hens could be studied at any one time and therefore data could be quickly and easily obtained from a number of hens. Another advantage was that hens could be left to select nest sites in a familiar environment and did not need to be disturbed by handling or movement from the home flock situation for testing. It was also probable that hens would accept the nest-boxes offered and so preferences for particular alternatives could be readily established.

This technique also had a number of disadvantages. Possibly the most important of these was that selection of available nests could be influenced by other factors in the shed such as proximity to other shed facilities, the light source and so on, and could also be affected by position of alternatives in the nest-set, differing light intensities in different nests and what the hens could see from different nests. Although alternatives were reallocated randomly to avoid these possible effects, experience gained during the first occasion on which the alternatives were used by hens may have affected their sudsequent selections since it is known that hens rapidly form 'habits' in the use of particular nests. It is also possible that gross differences such as position of a nest in the set and differences in light intensities in different nests may mask the expression of preferences for some other factors which are those under investigation at the time.

Another problem associated with this technique is that hens could be influenced by the presence of, or more importantly, the selection of nests by, other hens. In the initial behavioural study conducted by the author, tendencies for different hens to either select or avoid nests which were already occupied were noted. The presence of other nesting flock-mates not only introduces the possibility of competition for nests, but may also influence selection through preferences for or against occupied nests. There is the additional difficulty, also, of identifying eggs laid by particular individuals, although many of these studies were accompanied by detailed observations, in which case identification of where individuals laid was not a problem.

Prior experience probably considerably influences selection of nest site. Prior experience with nesting in particular sites is likely to influence subsequent selection of nest site. It was therefore considered desirable, but was not always practical, to use hens which had no previous nesting experience for testing. However, hens in the group testing situation also had prior experience of the pen environment, which may have influenced their subsequent nest selection, and also may have been influenced by the selection of hens which came into production earlier than they did.

Although the group test technique proved particularly useful in the investigation of a number of factors for which particularly strong preferences for or against alternatives were exhibited, an improved technique for establishing selection of nest options was sought.

Technique B. Test-pen

In an effort to minimise the problems associated with studying nesting preferences in the flock home-pen situation, a test chamber was designed for investigation of nesting preferences. The intention was to provide a situation in which hens were allowed to select nests which were presented in a symmetrical enclosure in which factors other than those specifically under investigation could, to a large extent, be controlled.

A description of the test-pen used and the procedure for testing hens is given in the General Materials and Methods that follows. Nest options were provided in recesses in the pen in such a way that they were presented in a symmetrical pattern, all facing in towards the centre of the pen. Since the options were arranged about the pen in this way, interactions with other environmental factors were minimised. Light intensity in the pen and in each nest option offered could be controlled. In the absence of any light and sound proof rooms in existing facilities at UNE, in which testing would have ideally been conducted, test-pens were enclosed in light proof plastic 'tents' to overcome the complications created by external light sources.

One possible influence which could not be eliminated in most experiments was that the 'view' from each of the options included more of one particular option, that which was directly opposite the one occupied, than any other. This effect was minimised in some experiments by curtaining of the entrances of all nest options and random allocation of options to position in each pen used.

Another factor which could not be controlled was that of outside noise such as the sound of vehicles passing on a nearby road or sounds of other hens in the shed. However, all hens were tested in the sheds in which they were ordinarily housed, and test-pens and the enclosures in which they were housed were placed in sheds so that sounds of other occupants of the shed tended to come from all directions.

Except in cases in which the effect of another hen was of specific interest, hens were placed in test-pens alone to select a nest. In an attempt to overcome the possible complication of prior nesting experience on patterns of nest selection shown, hens were usually tested from their first nest selection.

Results of the initial study conducted in these pens (see Study 4.4.1) suggested that hens of a White Leghorn x Black Australorp strain (white feathered) were more acceptable subjects than heavier Black Australorp x New Hampshire strain (black feathered) for these studies. Palpation was easier and more reliable with the White Leghorn x Black Australorp hens and they did not react adversely to placement in the test-pen as did the other strain. These black feathered hens often tried frantically to escape once placed in the pen. Although this may have resulted from the fact that hens in this experiment did have the opportunity to lay in their home environments, and so may not have been a problem when tests were conducted on naive pullets, it was decided to use the less reactive white hens anyway, particularly since they laid well in the first few weeks of lay and so could quickly be run through the series of tests. The heavier black hens tended also to withhold their eggs when placed into pens. This tendency was not apparent in the case of the white hens. Laying cages were considered to be more acceptable home environments than were deep litter floor pens because hens from them were also less liable to act in an agitated manner when placed in the test-pen or to withhold their eggs during testing.

Despite these initial problems encountered during the pilot study, it was found that hens generally responded very well to the testing situation. In fact, as described in Study 4.4.1, many hens which had only experienced nesting in the test enclosure behaved in an agitated manner in their home cages while approaching oviposition. When they were moved into the test-pen they would immediately examine nest options, select sites and sit peacefully within the selected site until they had laid. Almost without exception, young laying hens took to the test-pens veryreadily, settling in to the selection of a nest quickly and rarely attempting to get out of the pen. Where problems did occur, they were invariably in one or both of two situations. Firstly, where hens had been housed in floor pens some hens reacted adversely to the test situation, attempting to escape from the test pen. Secondly, if hens had not laid their first few eggs in the test-pen but had instead laid a number, or even one or two eggs in their home environment prior to or during their period of testing, then they sometimes acted in a similar manner. However, this was generally only the case when hens were also housed in floor pens. With the possible exception of Study 4.5, cage housed hens exhibited very little, if any, agitation or nervousness in the test-pen even from their initial encounter with it.

One group of hens to react particularly strongly to the test situation were floor housed broiler hens, a number of which would delay laying for considerable lengths of time in the test situation. However, this was believed to have resulted form the fact that they had laid in their home environment on several occasions prior to, or during, testing. In general, however, it could be said that hens accepted the test situation very well and were not observably distressed by it or nervous in it. In all, unusual or agitated responses to the test situation were only recorded for approximately 6% of all the hens ever tested.

Hens were not put through a 'familiarisation' period in the test-pen before coming into lay. Firstly, initial casual investigations using a number of B x W hens nesting for the first time suggested that hens, at least cage reared ones, reacted very well when placed into the test-pen, displaying very little nervousness or apparent agitation. Slightly older cage reared hens likewise displayed little, if any, agitation and readily began examining nest options almost immediately upon being placed in a test-pen and readily selected, sat and laid in these. Although the 'first-time' nesters did tend to lay in mid-pen sites rather than in nest recesses, this could not be regarded as an indication of nervousness or disturbance in the test situation since it is well known from the experience of commercial poultrymen that some proportion of floor eggs, or eggs laid outside provided nests, is always expected at the onset of lay, regardless of how long hens have been housed in the laying shed. Secondly, time and the availability of test-pens throughout the study period were limited, and allowing hens a period of familiarisation in the test-pens prior to their first nest selection would have almost doubled the time required to complete each study.

Hens were tested, and their responses tabulated, from their first nest selections for several reasons. The types of factors that hens responded to during their initial selections, perhaps due to a certain nervousness in the unfamiliar nesting condition or to incompletely developed nest-seeking 'instinct', may not be those to which they will respond as experienced nesters. Since these early responses are extremely important with respect to floor-laying tendencies, most floor eggs being expected during the initial period of production, it was considered important that these be investigated. If we Knew what types of nests or factors hens are attracted to in this initial period of nesting we might be able to suggest types of nests which accomodate these tendencies or eliminate such factors from the floor environment, at least during early stages of lay. Although each individual hen's response may change over time, the fact that in commercial situations large numbers of hens are housed together and hens mature at different ages means that one hen's response or selection can influence another's through the presence of the egg which she deposits in certain sites. Therefore, nesting sites which would not be expected to be used by mature, experienced hens may continue to be used due to their altered attractiveness resulting from the presence of eggs in them and then the formation of attachments to previously used sites. Hence, although the initial responses of hens should theoretically only affect nest selection patterns for a short period of time, residual effects may be felt over the flock's entire laying period.

Since these initial responses of hens may be of considerable importance, total results for the first 15 nestings were collected for each hen in the test-pen situation. It has already been indicated that many hens go through a period of somewhat random egg-laying during the first few days to weeks after coming into lay and only after this period do they form attachments to particular nests (see Study 3.1). Changes in the type of nest selected throughout the first 15 nestings would therefore be expected. As a result, Chi-square analyses were applied to the total data. However, these results are not strictly independent, coming from repeated measurements on the same individuals which, at some point through the 15 nestings, would be expected to begin to show a certain degree of repeatability in their selection of a nest. As a result, the analyses must be interpreted with some caution. Effects were only considered significant where the probability level obtained was very low ($P < .001$). Analyses resulting in probability levels higher than this but lower than .05 were interpreted as indicative of trends only. This approach was adopted, rather than leaving the results unanalysed, because serial correlation must be extreme for probabilities to be greatly affected.

In addition, the most commonly recorded, or 'predominant', selection response in the last five days of testing ie. testings 11 to 15, was tabulated for each hen. It is suggested that this would provide a reasonable measure of the type of nest that the hen would respond to as an experienced nester familiar with the nesting environment and the nest options available. Unfortunately, only a limited number of hens were available at any one time for these studies and facilities for their housing and testing also limited, resulting in low numbers of hens per study. This gave rise to insufficiently large expected values to permit adequate Chi-square analysis.

In addition, in some cases where mature hens were studied over a number of nestings, total numbers of occasions that each nest option was selected were compared by Chi-square analysis. Although repeated measurements on individual hens may not have been in-ependent, due to the expected repeatability of nest site selection often displayed by hens, these analyses were performed to enable some recognition of those nest types that either initially attract hens or are of secondary importance to them. As indicated in Study 3.1, many hens tend to display attachment to more than one preferrred nest site, or have secondary preferences. Hens may also tend to form new nest attachments over time. Variaility in responsiveness to many stimuli from the nest, within as well as between hens, has been suggested by other research (see Chapter 2). Selection of a nest on the basis of many stimuli is therefore not an 'all-or-none' response situation, hens responding to a range of possible nest types relating to that factor. If only the most frequently observed responses were recorded and analysed for each hen, then the likely importance of these alternative selections would be overlooked. However, to back up findings based on overall numbers of hens which selected each possible nest option more often than any other (the predominant or typical response) was tabulated. Again because only a small number of hens could be studied, these results could not be analysed.

The technique used to collect data from mature hens was refined slightly in several later studies. Hens were tested over successive nestings until they had registered five consecutive nestings in the one nest option, at which point they were said to have made their 'final selection' or 'final choice'. The numbers of hens making each nest option their 'final selection' were tabulated but again could not be analysed because of insufficient numbers of hens. The numbers of selections of each option made up to and including those made during the 'final selection' were also tabulated. These were analysed by Chi-square analysis and, as elsewhere when non-independent data were collected, only highly significant ($P<.001$) results were taken as evidence of any relationship.

In the Chi-square analyses of test-pen data, numbers of selections of each possible nest option were compared and then the total number of these nest option selections compared with the total number of times that the mid-pen area, or areas outside nest recesses, were selected. This approach was adopted because initial studies (see Study 3.1) indicated that hens responded to proximity to some dimension of confinement, for example a wall, strongly. Even more marked was their orientation towards a corner formed between confinement in two dimensions. To illustrate this high degree of responsiveness, results from Study 3.1 show that approximately 82% of all eggs were laid in corners. By contrast, these corners occupied approximately 3.6% of the total floor area. This high level of responsiveness displayed by hens which were studied during their initial weeks of nesting suggested that hens should be quite responsive to the nest recesses in which the test factors were presented. Assuming that hens orient towards corners because of the dimensions of confinement provided and that hens actually examine all potential nest options, the selection of one particular nest recess should be based on the hen's response to and preference for the factor/s under study. Therefore, the number of responses to each of the nest options provided should indicate the relative 'attractive-ess' of each of the nest types provided.

Failure to respond to the stimuli provided by the nest recesses at all, as indicated by mid-pen nestings, should be regarded as something completely different, perhaps even as a basic lack of responsiveness. Therefore, the number of nest selections occurring outside them ('mid-pen) are compared and analysed indepently of the comparisons of nest option usage. Since mid-pen nestings can, perhaps, be roughly equated with at least some component of floor-laying in a flock situation, they may also be of considerable significance. If analysed in a five option comparison, along with results for each individual nest option, or if only 'typical' hen responses were recorded and analysed, these results and their significance would be lost because most hens only laid a few eggs mid-pen before adopting nest recess habits.

General Materials and Methods

(a) *Birds and Their Housing*

A number of breeds of hen were used in these studies which took place in either deep litter floor pens or test-pen situations.

a. White Leghorns, Old English Game bantams and broiler hens which had been observed and described in Studies 3.1 and 3.4 were used in several studies. The housing and management of all these breeds was the same in the studies of nest preferences described in this Chapter as they were in the earlier studies.

b. A commercial White Leghorn x Black Australorp (B x W) strain of layer hen was used extensively in the present studies. The birds were purchased from a commercial breeder between 10 and 16 weeks of age and had to that point been reared in deep litter floor pens. Upon purchase, they were moved directly into individual laying cages or into a deep litter floor pen in an isolation shed on the UNE campus. Laying cages were 43 cm x 46 cm x 21 cm in size with 1 in 5 sloped floors. The floor pen was 3 m x 2.98 m in dimension, and contained two tubular feeders, two waterers, no perches and, initially, no nest-boxes. A constant 16 hour daylength lighting regime was provided in the shed.

Birds were fed a commercial pullet grower ration up until 26 weeks of age, at which point they were transferred to a commercial layer ration. From 18 weeks of age the birds were placed on a time restriction feeding programme to delay the onset of sexual maturity and were brought into lay between 26 and 30 weeks of age. After each individual became reproductively active it was provided with feed and shell grit *ad libitum*.

Birds were either tested as 'naive' hens, in which case they were tested on the first occasion that they ever nested and laid and thereafter, usually for 15 consecutive nestings, or as 'mature' hens, with testing commencing after the birds had laid in their home environment for a variable period of time determined by the particular study being conducted.

Hens bought into the University and managed in the above way will subsequently be referred to as 'purchased' birds.

Other layer strainB x W pullets were reared from day old on the UNE's poultry unit, 'Laureldale', and will subsequently be referred to as 'Laureldale' birds. They were reared in deep litter floor pens under natural daylength and on a commercial pullet grower ration until transferred to deep litter pens or laying cages for the purposes of these studies. At onset of lay, at about 26 weeks of age, they were fed a commercial layer ration and shell grit *ad libitum*.

c. White Leghorn x New Hampshire (R x W) and Black Australorp x New Hampshire (B x R) birds, also commercial layer strains of hen, were used in one study and were reared as per the ' Laureldale' B x W birds. These birds were also used in a behavioural study (see Study 3.5) and details of their housing and management can be obtained from that Study.

d. Broiler breeder hens of a commercial Steggles* strain were used in several studies. They had been reared on deep litter in a commercial shed before being transferred to the UNE and placed in a 3 m x 3 m, deep litter floor pen in an isolation shed at 16 weeks of age. The pen contained one

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* Steggles Pty. Ltd., Hawthorne Street, Beresfield, N.S.W. 2322

tubular feeder, one waterer, no perches and a set of metal nest-boxes to be described later. The hens were restricted fed to 60% of *ad libitum* intake up to point of lay, which was at 27 weeks of age, and to 80% thereafter. They were fed on commercial pullet grower and layer rations during these periods. For the duration of their stay in the isolation shed, the birds received a constant 16 hour daylength pattern.

e. Several studies were conducted on groups of 'feral' fowl. The original 24 birds were feral fowl captured on North-West Island off the coast of Queensland, Australia, in May, 1980, and housed in six large deep litter pens in an isolation pen on a UNE rural property. Two or three hens were placed in with a cockerel in each of these pens and the birds were fed on a commercial crumble ration *ad libitum* and were housed under conditions of natural daylength. Little was known of the previous nesting history of these hens, but most appeared old enough to have laid previously. It was known that one hen had successfully incubated and brooded before, since she was captured with her brood of seven chicks. For a description of the original island fowl population from which these birds were taken, see McBride *et al.* (1969).

Other studies were conducted on a group of first generation offspring of these original feral fowl, which were incubator hatched and reared in deep litter floor pens under natural daylength conditions to point of lay. Thereafter they were housed in the deep litter floor pen described in Study 3.1 and under conditions of constant 16 hour daylength. These birds were fed a commercial crumble ration *ad libitum* throughout the rearing and laying phase.

All groups of hens used in these studies, except for the White Leghorns, were leg banded for the purposes of individual identification.

(b) *Testing Situation and Procedure*

Tests carried out using Technique A were conducted in a number of deep litter floor pens and a number of flocks which are specified for each individual study. Results of such studies conducted on flocks of hens in deep litter floor pens were analysed using analysis of variance or by Chi-square analysis as specified in each particular study. All analyses were performed on data obtained for the flock as a whole.

The design and arrangement of the test-pen used for studies conducted using Technique B is shown in Figure 4.1. The original pen constructed was made of 16, 45 cm wide x 75 cm high metal panels hinged together so that each panel could be moved independently of any other. Fixed hinges attached pairs

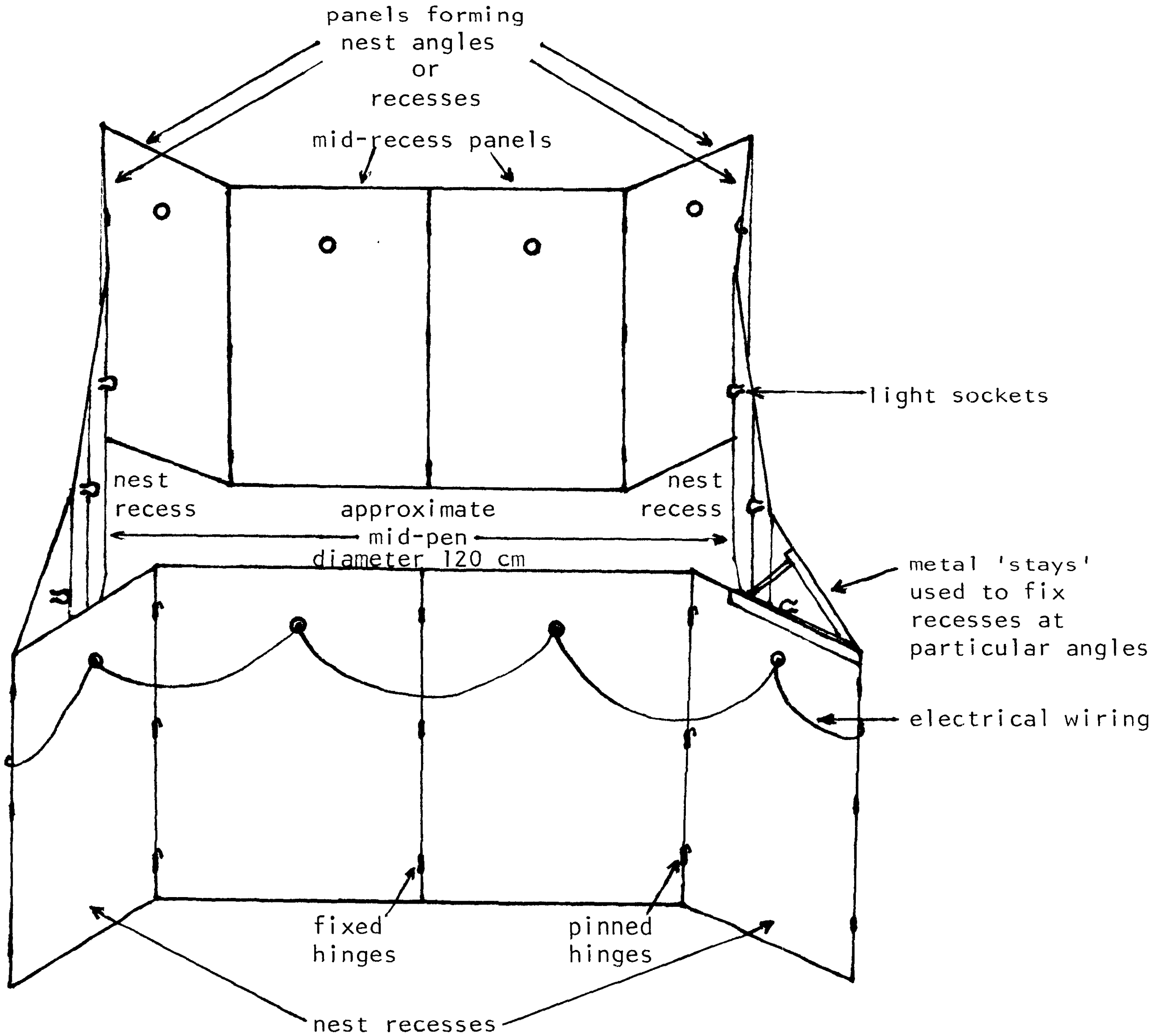


Figure 4.1 The design of the test-pen in which hens were tested showing four nest recesses, in which various options were presented, and four pairs of mid-recess panels

of panels together, and pairs of panels were connected by pinned hinges which allowed the entire pen to be dismantled into two-panel segments. Each panel was wired and fitted with a light socket so that each panel could be illuminated independently of any other. Sockets were situated 15 cm from the top of each panel, but panels could be inverted so that lighting came from near the base of the pen rather than the top. All joins between panels were covered on the outside of the pen with black tape so that hens could not see out of pens at any point in the perimeter of the pen.

The pen was covered over with wire mesh which was suspended several centimetres above the top of the pen and covered with hessian. An angle-iron frame supported an observation chair above the pen. The entire pen and frame area was enclosed in a large, black plastic 'tent' which did not permit light into the enclosure. In this way, a light-proof enclosure could be created in any of the existing isolation pens on the University campus. When the lights were turned on in test-pens, nothing could be seen of the area outside the pen from within the pen, at least by the human eye, because of reflection of light from the hessian covering and complete darkness in the area outside the pen. An observer looking in through the hessian to the lighted area inside the pen could watch the activities of hens in the pens quite easily. Hens could presumably see nothing of the observer, only the hessian covering the pen, but the observer could watch hens placed in the test-pen.

The pen could be positioned so that any number of nest 'recesses' up to a maximum of eight could be formed by moving panels to form angles out from the centre of the pen. Different options could be offered in these recesses or angles, as potential nesting sites.

Five additional test-pens were made which were all 12-panelled since studies mostly required only four nesting recesses. These pens were the same as the original, except that four panels in each were 16 cm wider and all panels were 15 cm higher than in the original, so that the actual possible size of a complete pen was slightly smaller than the original (29 cm smaller in diameter when four nest recesses were provided). Each of these four panels could be dismantled from the four fixed pairs of panels which were used as the nesting recesses. The width of all panels from which these nesting recesses were formed was the same for all pens being 45 cm. As a result, these panels could be removed from one pen and fixed to another to provide additional nesting recesses if required by certain experiments. Size of the nesting recesses could be fixed with metal 'stays' at the top of the pen. These specified the angles that would form the recess.

The most common set-up, in which four nest sites or recesses were provided, is represented diagrammatically in Figure 4.1. In this case, as in all set-ups, the recesses were all arranged in a symmetrical fashion about the pen, and all faced directly into the centre of the pen. Alterations were made to the area inside these recesses to form the required nest options. Lighting could be controlled so that the intensity in each option could be the same or different in nest alternatives as determined by the experiment. Light intensity was measured from the entrance of the nest recess using a photographic light meter.

In a number of experiments curtains were hung over all options, so that all nest sites were hidden behind black curtains and hens in any one nest could not see out into the pen. The arrangement of the options was allocated randomly in each pen used in every experiment and hens were placed in different pens for consecutive nestings and so the options that they could see most clearly from a particular nest option was likely to be different on consecutive testings.

All panels which formed nesting recesses were painted with matt grey paint, marginally darker than the colour of the other sheet metal panels in the pen, in an effort to attract as many hens as possible into the nest sites. Wood shavings were also spread on the cement floor in each pen and in the nest recesses unless otherwise stated.

The procedure followed for investigating nest selection was as follows. Hens were palpated each morning to ascertain which hens were going to lay that day. Hens were placed into the pen some time prior to the predicted time of oviposition or when nesting behaviours were displayed in the home environment. Hens which began to display nesting behaviours but did not have a hard-shelled egg in the uterus were also tested initially, as these were apparently internal layers. In later studies however, such hens which were suspected of internally laying on occasions prior to their first oviposition were not used as test subjects, being replaced by other hens not having this characteristic.

Hens were removed from their home environment and placed in the centre of a test-pen. Access to the pens for the purpose of getting hens in or removing them again was through a side panel which was unpinned during these procedures. Once a hen had been placed in the pen the access would be pinned shut again and the lights in the pen turned on.

Hens would remain in the test-pens for at least several minutes after they had laid, or finally settled in a nest site and risen to leave the site in the case of internal layers, at which point they were removed from the pen and returned to their home cage or pen. The activities of these hens as they approached oviposition in the pens was recorded, as well as entries into nest recesses and sites in which nest building was conducted. The site of eventual oviposition, or final nesting in the case of internal layers, was also recorded.

Except in cases in which the effect of another hen was of specific interest, hens were placed in test-pens alone to select a nest. In many of the experiments conducted in these pens, hens were tested for their first nest selection, which may or may not have coincided with their first oviposition, and for each subsequent oviposition or nesting thereafter for at least 15 days. Internal layers were identified by the behaviour patterns that they displayed, which were typical of nesting hens. Considerable trouble was taken to ensure that hens did not lay in their home environment during the test period when studies were conducted on these otherwise naive nesters. Hens were occasionally left in test-pens overnight so that ovipositions were not missed.

In some experiments water, but not food, was available in the centre of each pen. Hens were without food for the duration of their time in test-pens, which varied from 30 minutes up to four hours, depending on the individual and how close she was to oviposition when placed in the test-pen.

Except where otherwise stated, hens used in these experiments were only used in one experiment and so at the beginning of testing had not previously used the test-pens. Pullets were not allowed a period of familiarisation with the test-pen before testing commenced on the first day of nest selection except where otherwise indicated.

Results of studies using the test-pen technique were analysed by Chi-square analysis. In these analyses, the total frequency of selection of each nest option was compared and the number of mid-pen selections compared with nest option selections for each treatment applied. Analyses were performed on total data for all hens in each treatment and for all testings of each hen. Results for individual hens will not be given but are available from the Physiology Department, UNE.

All studies were conducted between July, 1979 and September, 1982. Recording was carried out by the author with the occasional help of two assistants. Observational data were collected only by the author, mostly directly, but sometimes from records taken on videotape.

I. Factors Related to Nest Entry and Sitting

4.1 The Presence of an Egg

Introduction

Results of studies conducted by other researchers have suggested that domestic hens show considerable interest in their own eggs (Wood-Gush, 1975a; Brantas, 1978) and will use nests in which real or artificial eggs are placed to a greater extent than nests which do not contain eggs (Turpin, 1918). Preliminary studies carried out by the author also indicated that hens showed a marked preference for nests containing an egg over empty nests. The following studies were conducted to provide further information on the nature of this relationship between the presence of eggs and selection of nest site.

Study 4.1.1
Daily Distribution of Eggs Between Identical Nests in a Set

In the course of earlier behavioural studies, it had been noted that flocks of hens with access to a bank of nest-boxes tended to lay a large number of eggs each day in two or three nest-boxes, while most of the other boxes were not used at all. The objective of this study was to investigate the daily distribution of eggs between different nest-boxes in a set to see if this tendency, referred to as 'clumping', was in fact occurring.

Materials and Methods

The 25 White Leghorn hens used in Study 3.4 were studied, with housing and management as per that study. The hens had been laying for five weeks at the commencement of recording. A nest-set consisting of two tiers of nests with seven nests per tier, as described in Study 3.1, was available to the hens.

Daily distributions of eggs between all nests in the pen were recorded over a period of 19 days to investigate the possibility that clumping of eggs in nests was occurring. Eggs were collected and recorded only once daily at 5.00 pm. Hens were observed continuously over three consecutive mornings during the period of study and notes made of the activities of hens at the nest-set.

Overall Chi-square analyses were performed on the daily data for both top and bottom level nests. Chi-square tests were not applied to each day's results independently because of the low expected values obtained. Similar analyses were also performed on top and bottom level data summed over all days to see if any particular nest-box(es) was used to a greater extent than any others. The number of times nest-boxes were found to contain 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 (the maximum recorded) eggs was recorded and a Poisson distribution model fitted to the data (Steel and Torrie, 1980, p. 528).

Results and Discussion

Daily numbers of eggs collected from each nest are shown in Table 4.1.1.

Table 4.1.1 Numbers of eggs laid in nests in bottom and top levels, Chi-square values and levels of significance for these

	Number of Eggs Laid																	
	Bottom Nests									Top Nests								
Day	A	B	C	D	E	F	G			A	B	C	D	E	F	G		
1	1	3	1	1	2	1	1			0	0	0	0	0	0	0		
2	7	3	0	0	3	2	0			0	0	0	0	0	0	0		
3	7	0	0	0	0	1	0			2	2	0	0	0	0	1		
4	1	0	1	0	3	0	3			0	0	0	0	0	1	3		
5	1	0	2	0	0	0	3			5	0	0	0	0	0	1		
6	2	0	0	0	1	1	3			0	0	0	1	0	4	1		
7	3	1	0	0	0	0	3			0	0	0	0	0	5	2		
8	1	0	2	0	1	2	3			0	0	0	0	0	3	1		
9	3	0	1	0	2	0	2			0	6	0	0	0	0	0		
10	5	2	2	0	0	0	1			2	5	0	0	0	0	0		
11	1	0	0	1	2	0	4			0	0	0	1	1	6	1		
12	2	1	0	0	2	3	3			0	0	1	0	4	0	0		
13	1	1	2	3	0	1	0			0	3	3	0	0	0	0		
14	0	0	0	7	7	0	0			0	0	0	0	3	3	1		
15	0	2	4	0	0	5	0			0	0	0	0	0	2	0		
16	1	0	4	0	2	5	0			0	1	1	0	3	0	0		
17	7	2	1	0	1	0	0			0	0	0	0	1	0	1		
18	0	1	0	10	0	6	0			0	0	0	0	2	0	0		
19	1	3	4	0	0	1	0			0	4	0	0	0	1	0		
	For all days: χ^2_{38}						=	153.2	***	For all days: χ^2_{38}						=	159.4	***
Total	44	19	24	23	25	28	26	14.2	*	9	21	5	2	14	23	11	30.0	***

When all data were collected it became apparent that clumping was in fact occurring. In other words, eggs were not being laid in different nests with equal frequency. Instead, several nests would be found to contain a relatively large proportion of the eggs and other nests none at all. The numbers of occasions on which nests were found to contain different numbers of eggs were found to differ significantly from a Poisson distribution ($.001 < P < .01$), and are shown in Figure 4.1.1. Nests containing no eggs at the end of each day occurred most frequently.

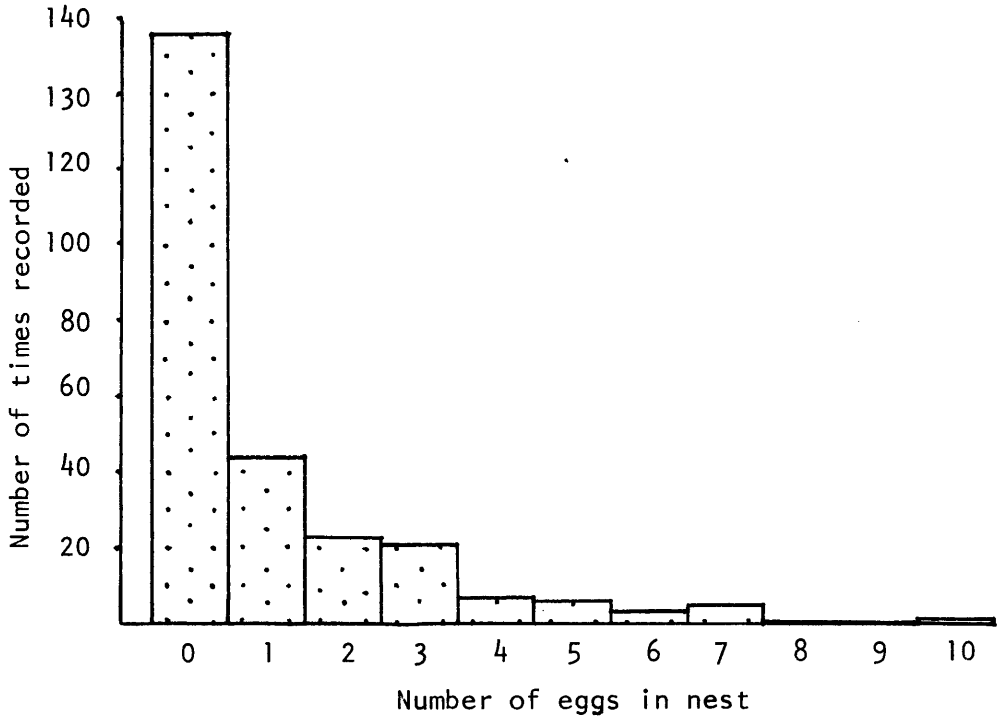


Figure 4.1.1 Numbers of occasions on which nests were found to contain different numbers of eggs

Preferences for particular nests were evident in both top and bottom level data, but this could not account for all the difference in distribution of eggs between nests throughout the experiment. *A posteriori* analysis of partitioned data indicated significant differences between the numbers of eggs laid in nest A, which was the nest furthest from the door of the pen, and the rest of the nests ($\chi^2_{A\ \mathrm{vs\ rest,\ 1df}} = 12.5^{***}$; $\chi^2_{BCDEFG,5df}$ N.S.) in bottom level nests, nest A having contained 23.3% of all eggs laid at that level. Similar partitioning of top level data indicated a significant tendency for higher nest usage of the second nests in from the ends of the set and lowest levels of nest usage in the case of two middle nests ($\chi^2_{BF\ \mathrm{vs}\ AEG\ \mathrm{vs}\ CD,2df} = 28.5^{***}$; $\chi^2_{BF}, \chi^2_{AEG}, \chi^2_{CD}$ all N.S.).

Study 4.1.2
The Effect of Size and Number of 'Nest-Eggs'

This study was conducted in order to determine what influence size, number and colour of nest-eggs had on selection of otherwise identical nest options and how different breeds of hen, having different egg characteristics, responded in this respect.

Materials and Methods

The pen and 25 White Leghorn hens described in Study 3.4 were again used in this study. In addition, the adjacent pen and 18 wheaten Old English Gamebantam hens, as also used and described in Study 3.4, were studied. Leghorns were 57 weeks old and bantams 52 weeks old at the commencement of this study. Pens and management were the same as described in Study 3.4 except that three nests in each nest level in both pens were blocked off.

In the remaining nests were placed one or two hard boiled eggs of either Leghorn or bantam origin. The white Leghorn eggs weighed, on average, 60g, and the pale buff bantam eggs 35g. In each nest level, one nest containing one bantam egg, one nest containing two bantam eggs, one nest containing one Leghorn egg, and the final nest containing two Leghorn eggs, were provided. The nest-boxes to which these treatments were applied were reallocated randomly at the end of each day.

Eggs were collected and recorded hourly so that eggs did not accumulate in nests and so interfere substantially with the results of the study. During periods of heavy nest usage, collections were made half hourly. Hard boiled nest-eggs were returned to the appropriate nests at each of these visits. The number of eggs that had been laid in each nest during that period was recorded, but for analysis only the first egg laid was counted. In other words, if more than one egg had been laid over the time period between collections, this was counted as only one. This was done to minimise any possible effect of eggs, other than those purposely provided in the nests, on selection.

Analysis of variance using the DEC20 computer NEVA programme for multivariate analysis of factorial experiments (Burr, 1980) was performed on the data recorded from 20 consecutive days with levels as replicates. Since there is the possibility of serial correlation in these results, the findings must be viewed with some caution. However, this should not be a serious problem in general particularly since the group of hens which provided the data each day was not neccessarily the same, although each day's results came from the same overall flock of hens.

A similar experiment was then applied in the same pens for ten days after the completion of this initial trial. This was exactly the same as the experiment just described, except that dark brown eggs laid by broiler breeder hens and averaging 60 g in weight were used as nest-eggs instead of bantam eggs.

Results and Discussion

Numbers of eggs recorded in nests with one or two Leghorn or bantam nest-eggs for both nest levels and both White Leghorns and bantams are shown in Figure 4.1.2. These are total figures for all 20 days of recording.

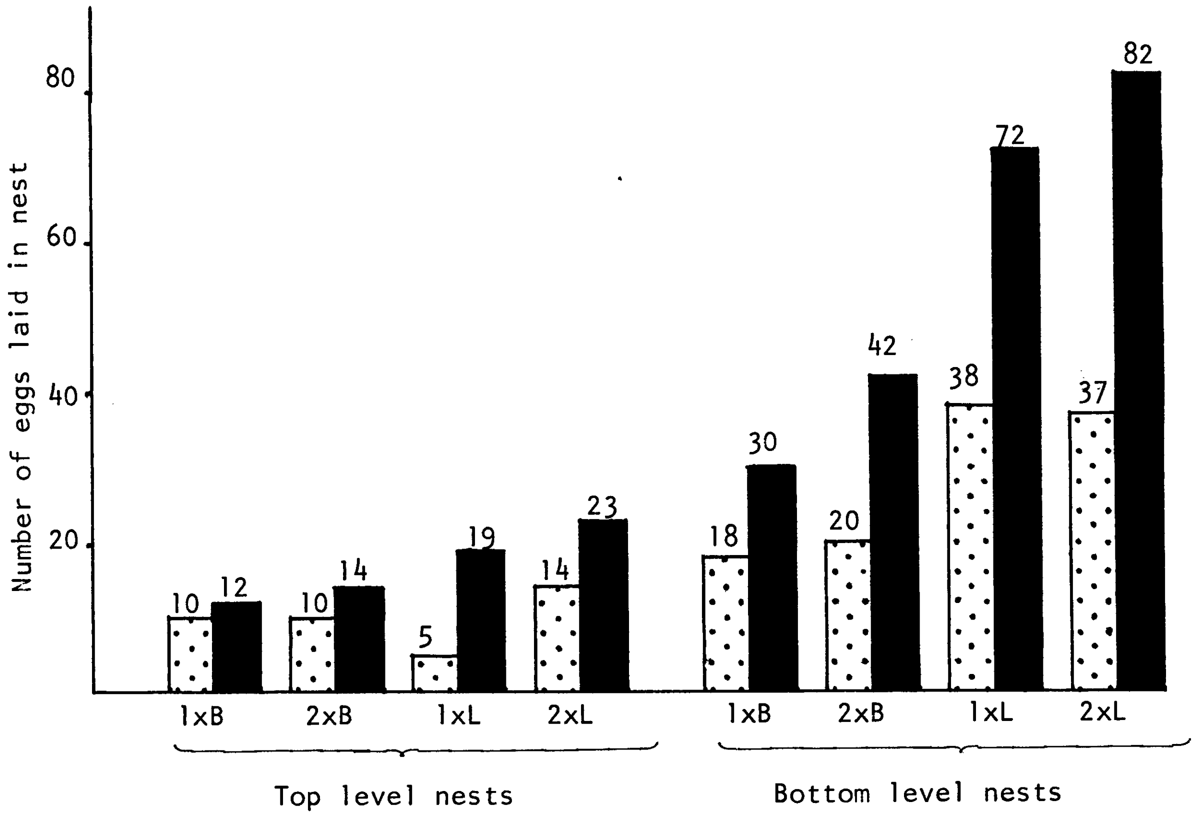


Figure 4.1.2 Numbers of eggs laid in nests containing one (1 x B) or two (2 x B) bantam nest-eggs, or one (1 x L) or two (2 x L) Leghorn nest-eggs by bantam ( ) and White Leghorn flocks ( )

Hens laid more eggs in nests in which the larger White Leghorn eggs were used as nest-eggs than in nests containing bantam nest-eggs ($0.01 < P < 0.05$). This was even the case in the bantam pen. Overall, 65% of all eggs were laid in nests with Leghorn nest-eggs and 35% in nests with bantam nest-eggs. Also, 45.7% and 54.3% of all eggs were laid in nests with one or two nest-eggs respectively, but no significant effect of egg number was found on analysis. No other significant effects were found.

Total numbers of eggs laid in nests with one or two brown or white nest-eggs for both breeds and for top and bottom nest levels are given in Table 4.1.2. Analysis indicated that greater numbers of eggs were laid in nests containing two rather than one nest-egg ($.001 < P < .01$).

Results of analyses of both experiments indicate that more eggs were laid in nests which contained two as opposed to one nest-egg, that contained the larger nest-eggs and that colour of the nest-eggs, at least within the range of that which is usually produced by the domestic hen, was not apparently associated with differences in nest usage exhibited by the two breeds studied.

Table 4.1.2 Total numbers of eggs laid in nests with one or two brown or white nest-eggs by White Leghorn and bantam flocks

	Number of Eggs Laid							
	Top Level				Bottom Level			
	Brown		White		Brown		White	
Breed	1 egg	2 eggs	1 egg	2 eggs	1 egg	2 eggs	1 egg	2 eggs
Leghorns	5	8	6	11	26	29	23	38
Bantams	6	11	4	8	9	17	8	16

Study 4.1.3

The Effect of 'Nest-Eggs' and Nesting Material

The results of Study 4.1.2 indicated that hens selected nests differentially on the basis of the type of nest-eggs they contained. The relative importance of nest-eggs and nesting material in determining preferences for nests was investigated in the following study.

Materials and Methods

The same White Leghorn and bantam hens and the same pens as in Study 4.1.2 were used in this investigation, except that seven hens were removed from the White Leghorn flock to even up the numbers of hens in both pens. Hens had been laying for approximately six months at the commencement of this experiment.

Nest alternatives provided in top and bottom nest levels in both pens were the presence or absence of wood shavings spread to a depth of 5 cm in the otherwise bare sheet metal nests, and the presence or absence of a hard boiled Leghorn nest-egg in the nest. Therefore, in each level hens were provided with one nest with both nesting material (wood shavings) and a nest-egg, one with nesting material and no nest-egg, one with no nesting material and a nest-egg, and one with no nesting material and no nest-egg. Treatments were reallocated to nest-boxes randomly each day, the necessary changes being made in the late afternoon.

Eggs were collected hourly, or more frequently when required, throughout the day. Records were again taken of the number of eggs laid in each nest, discarding all but the 'first' egg laid in each nest, as was done in the previous study. Recordings were taken for 22 consecutive days.

Analyses of variance were performed on the data, daily and overall, with nest levels as replicates. Although not considered a serious problem, the possibility of serial correlation was recognised and results interpretted accordingly.

Results and Discussion

It had initially been intended that the study would be conducted over a 40 day period, but a number of bantam hens became broody, sitting on nests containing nest-eggs and preventing access of other hens into these nests, so the study was terminated prematurely. Only the data obtained during the first 22 days of the study, before any of the bantams had begun to show broody behaviour, were retained.

The total numbers of eggs that were recorded from each nest type and for both breeds are shown in Figure 4.1.3.

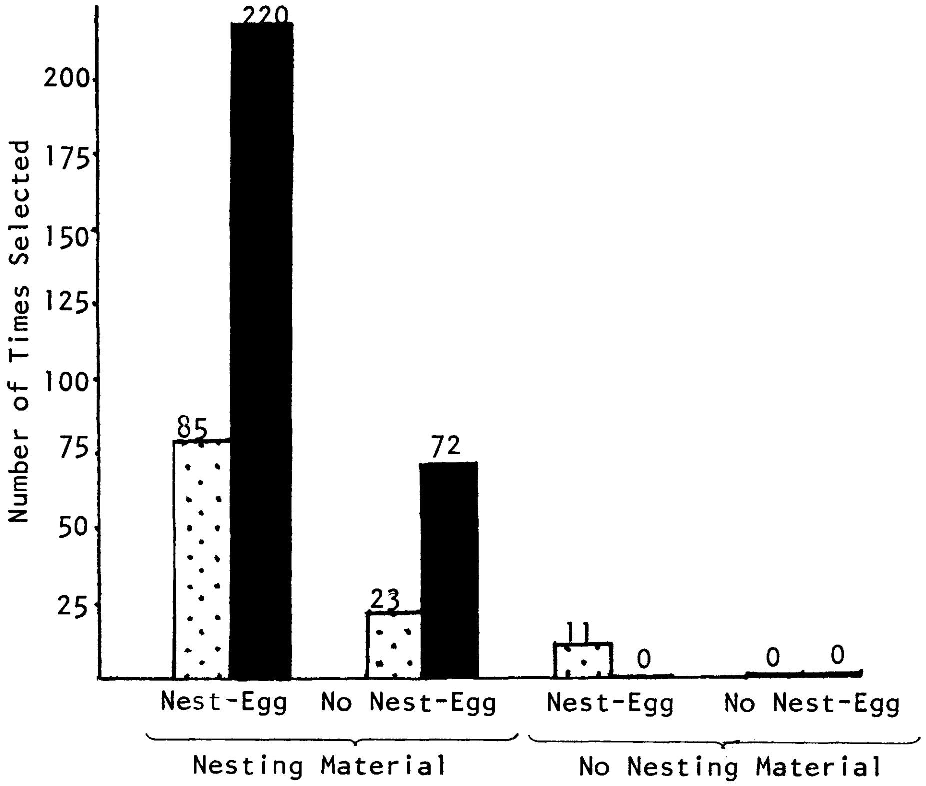


Figure 4.1.3 Numbers of eggs laid in nests containing either a nest-egg or no nest-egg and nesting material or no nesting material by bantam ( ) and White Leghorn flocks ( )

Analyses revealed highly significant differences between numbers of eggs laid in different nests on most days. Daily data for both breeds are presented in Appendix 4.1.3, along with the results of analyses performed on them. The results of analysis of data from the entire study period are shown in Table 4.1.3.

Table 4.1.3 Analysis of variance of numbers of eggs laid in nests with or without nesting material and with or without a nest-egg for Leghorn and bantam flocks

Source	D.F.	S.S.	M.S.	F	Significance
Breed (B)	1	85.03	85.03	15.02	-
Error (a)	2	11.32	5.66		
Egg (E)	1	138.75	138.75	29.33	**
Nesting Material (N)	1	429.89	429.89	90.88	***
E x N	1	112.50	112.50	23.78	**
B x E	1	15.98	15.98	3.38	N.S.
B x N	1	108.03	108.03	22.83	**
B x E x N	1	26.73	26.73	5.65	-
Error (b)	6	28.38	4.73		
Day (D)	21	9.55	0.45	0.61	N.S.
B x D	21	19.91	0.95	1.27	N.S.
E x D	21	15.68	0.75	1.00	N.S.
N x D	21	17.80	0.85	1.13	N.S.
E x N x D	21	15.68	0.75	1.00	N.S.
B x E x D	21	28.96	1.38	1.84	*
B x N x D	21	11.66	0.56	0.74	N.S.
B x E x N x D	21	21.46	1.02	1.36	N.S.
Error (c)	168	125.80	0.75		
Total	351	1223.11			

Nests which did not contain nesting material were rarely laid in. More eggs, however, were laid in nests containing nest-eggs than in those without nest-eggs. The presence or absence of a nest-egg did not have as great an influence on numbers of eggs laid in nests as that exerted by the presence of nesting material. Nests containing a combination of both nest-eggs and nesting material were most popular and nests containing neither nest-eggs nor nesting material extremely unpopular.

Nesting material appeared to be of comparatively less importance to bantams than it was to the Leghorns, which never laid in any nest which did not contain wood shavings. The efficacy of nest-eggs in attracting the different breeds into nests to lay differed slightly for different days.

Observation of hens using nests on two days in each of the two pens revealed that hens made many more entries into nests which contained nest-eggs than into nests without nest-eggs. During the periods that pens were observed, some 98 entries were recorded into nests with nest-eggs as compared with 46 entries into nests without nest-eggs. Hens appeared not to remain in nests which they had entered for as long if the nest did not contain an egg, but this was complicated by the fact that hens seemed to react more strongly in this respect to the presence or absence of nesting material.

Study 4.1.4
Responses of Hens of Different Age and Experience to Eggs

The object of this study was to investigate the response of pullets nesting for the first few times and of hens which had no previous experience of laying an egg, to the presence of an egg during the nesting phase. In order to do this, hens were required which would nest but not lay. Wood-Gush (1963) and Wood-Gush and Gilbert (1965) have described experiments in which hens were rendered chronic internal layers by ligation or by stitching up of the infundibulum. These birds apparently nest normally but do not lay. It was therefore decided to use hens rendered internal layers by similar surgical techniques for the purposes of the present study.

Materials and Methods

Hens used in these studies were White Leghorn x Black Australorp hens (B x W) purchased at 14 weeks of age and housed in individual laying cages.

At about 21 weeks of age, at which point four pullets were showing signs of approaching reproductive activity, 26 pullets were selected at random and were subjected to surgical intervention which was to render them internal layers. These birds will subsequently be referred to as surgically altered birds.

Surgery was performed under general anaesthesia using ether. The body cavity was opened on the left side, two ligatures tied on the oviduct and the infundibulum removed if identified.

Another group of 26 pullets was subjected to sham operations in which the body cavity was opened and the oviduct manipulated but not tied or removed.

All pullets were returned to their cages on the same day that the operations were performed and were injected with penicillin/streptomycin i.m. for three days after the operation. Five pullets died under anaesthetic but all others which survived surgery recovered quickly once returned to their home cages. One of the surgically altered pullets was subsequently found to lay normally and another was never observed to nest. However, surgery was successful in all other cases and hens were recorded to nest quite regularly, but not lay. Detection of nesting was reasonably simple as most hens tended to perform the pacing, calling and sometimes sitting activities typical of hens of the same genotype when approaching oviposition in cages, as described in Study 3.5.

Ten surgically altered birds and ten sham operated controls were assigned to treatment 1 which involved testing for responses to eggs during the first week of nesting. Another ten surgically altered birds and sham operated controls were designated to treatment 2 to be tested for responses to eggs about three months after they had first reached sexual maturity.

The testing procedure involved placing the hen to be studied in a test-pen, as described previously as Technique B. Four 60° nest recesses were provided in the pen set-up, all containing wood shavings but only one containing a nest-egg. The nest-egg provided was a fresh egg laid by a hen of the same strain. The activity patterns of hens in these pens were observed directly or videotaped and perused at a later date. The video camera was sited above the pen, but the field it covered included only about one quarter of the total pen area which encompassed the nest option which contained the nest-egg.

Records taken included the number of pecks at the nest-egg, complete egg rolling movements, movements of the hen over the egg or movement of the egg with the feet, and the nest eventually laid in. One egg rolling movement was recorded if the hen stretched its neck out towards the egg, placed its head over the egg and its bill behind the egg and pulled the neck back towards the body with or without the egg. Hens in treatment 1 were tested on five consecutive nesting occasions, starting from the first nesting they performed. Hens in treatment 2 were also tested on five consecutive nesting occasions. Hens were observed for about 30 minutes after laying and were then removed from the pen and returned to their home cages. In the case of surgically altered birds, subjects were removed from the pen if they had ceased nesting or sitting for 30 minutes.

Results and Discussion

Of the original ten surgically altered hens allocated to treatment 1, results from five had to be discarded. The reasons for this included uncertainty that the first nesting recorded was actually the first which had occurred, uncertainty in determining which nest was actually the final nest selected, and apparent 'missed' nestings suggested by the failure of the hen to recommence nesting behavioursor to search and sit in a nest once transferred to the test-pen. Incomplete recordings were also a problem with several control treatment 1 hens and suspected for several treatment 2 surgically altered hens. As a result, recordings were only continued for five of the ten hens in all groups.

The total numbers of pecks which were directed at the nest-egg, complete egg rolling movements, other manipulations of the egg (moving over the egg and moving the egg with the feet) before 'laying' and times that hens used the alternative containing the egg for final nesting are shown for all groups in Table 4.1.4.

Table 4.1.4 Numbers of pecks at the nest-egg, egg rollings and other manipulations of the nest-egg prior to oviposition and the number of occasions on which the nest containing a nest-egg was finally selected

Treatment (5 Hens, 5 Nestings)	Pecks	Egg Rolling	Other Manipulations	Times Selected
Surgically Altered at Onset of Lay	41	0	2	6
Controls at Onset of Lay	30	1	6	10
Surgically Altered 3 Months into Production	66	4	10	10
Controls 3 Months into Production	55	11	16	12

These results were not analysed for several reasons. They do, however, show that egg rolling was not seen in surgically altered pullets in the first five days that they nested, was rarely seen in normal laying pullets over the same period, and was less frequently seen in surgically altered hens than in mature laying hens of the same age. In addition, five hens of similar genotype and age, which had been housed in floor pens for the duration of their laying life, were tested in the same test situation on five occasions each. These hens had all previously used provided nest-boxes containing wood shavings for nesting purposes. These hens between them recorded 32 individual instances of complete egg rolling and selected the nest option which contained the nest-egg on 13 of the possible 25 occasions.

Pecking at the egg was observed in a number of cases in both control and surgically altered groups in treatment 1. Most of these were observed during the later days of testing. Very little interest was shown in the nest-egg by hens of either group during the first two or three days that the hens ever nested. In fact, little attention was paid to specific nest sites during these initial nestings. Hens during these early nestings would usually continue pacing about the pen up to the point of oviposition and then suddenly squat and 'lay', relax and then resume other activities. The site at which this occurred was often in none of the nest recesses but in the middle of the pen or along a pen wall.

Results of selection of final nest are limited and do not give much indication of whether any of the groups of hens respond to the presence of an egg in the nest in their nest selection more than any other.

The above observations are only made very tentatively, since the results may have been subject to considerable error and were not considered suitable for analysis.

General Discussion - The Presence of an Egg

Results presented for studies pertaining to selection of nests on the basis of whether other eggs are present suggest several possibilities. Study 4.1.1 results indicate that hens may prefer to lay in sites which have already been laid in, although it is possible in this study that the presence of other hens in the nest could also have been important. The results concur with those of Turpin (1918) who found that more eggs would be laid in nests in which one or a number of eggs were placed as nest-eggs.

The clumping of eggs in nests was apparent in Study 4.1.1, but the particular nest used for this varied day to day. This would tend to indicate that the decision of the first hen to lay on any particular day will determine the overall daily distribution of eggs about the nest-set. Since it is likely that there will be day to day variability in the individual hen that lays first, so being presented with a completely empty set of nests, and since each hen may base her selection of nest on different criteria in this situation, it is easy to understand how the distribution of eggs in a set would vary each day.

Attractiveness of nests was increased if two nest-eggs, rather than one, were included in the nest, at least in Study 3.1.3. Perhaps this relates to the fact that a hen may become increasingly attached to a site or nest the more times she has used it for successive eggs in a clutch, and so nests with more eggs are more attractive. However, Turpin (1918) found that the number of eggs that would be laid in nests with nest-eggs declined after the first egg. Analysis of his results by this author showed that significantly more eggs were laid in nests with one or two eggs than in nests with either three or four nest-eggs, which, in turn, were more popular than nests in which no nest-eggs were provided ($\chi^2_{2df} = 91.0^{***}$). However, as Turpin pointed out, since eggs were gathered only once every day the numbers of eggs collected would probably not represent the actual relative attractiveness of nests. It is possible that addition of eggs to these nests resulted in accumulations of eggs which the hen was unable to cover satisfactorily. If this were the case, hens may opt for smaller accumulations. Nests which initially contained small numbers of eggs could be added to by a greater number of eggs before this stage was reached.

It would be interesting to further investigate the selection of nests with differing numbers of eggs present. Of particular interest would be an analysis of nest entries and ovipositions in nests containing larger accumulations of eggs. It may well be that increasingly large accumulations of eggs are increasingly effective in releasing nest entry, but the stimulus provided by larger accumulations once the bird is on the nest may encourage her to stand and leave the nest.

It is well known that many types of birds respond to supernormal visual stimuli, particularly with respect to eggs (e.g. Tinbergen, 1951). Perhaps the responses noted in the present studies indicate that larger numbers of eggs act as supernormal stimuli releasing nest entry. It is possible that accumulations of eggs serve to release different component activities of nesting through two different cues, one visual, which may be involved in the nest entry response, and the other tactile, controlling sitting or 'remaining' in the nest.

Nests with very large accumulations of eggs may be avoided for a number of reasons. Wild gallinaceous birds tend to lay their eggs in clutches which generally do not exceed ten or a dozen at the most (Baker, 1930). Even those domestic strains which do show broodiness will rarely produce and sit on clutches much larger than this, if not interfered with by man. Large accumulations of eggs may be more conducive to egg breakage. In a large accumulation of eggs not all may be effectively covered by a hen at all times and this could result in significant reductions in incubation success since eggs are moved about during incubation and all may be subject to chilling. If nests with very large accumulations of eggs are avoided, it would be of interest to see whether this trend is as strong in those strains for which genetic selection against broodiness has been operative in recent decades as in strains which have not been similarly selected.

The apparent lack of response to numbers of eggs in Study 4.1.2 may be attributable to the fact that the response to the larger sized eggs may have masked any additional effect of egg number, particularly since size and number may together constitute 'amount' of eggs in the nest site.

Results of Studies 4.1.2 and 4.1.3 indicate that nest-eggs are as attractive to White Leghorns as they are to bantams. It would therefore seem that the Leghorns have not lost, through genetic selection, the ability to respond to the visual cues provided by the presence of eggs in the nest. Domestic strains may have even generalised in this respect to eggs which are not their own. The author is unaware of any reports of responses of jungle-fowl to own and alien eggs, but it is possible that hens may be able to distinguish between their own eggs and those of other hens, as birds of some other species are believed to do.

It is more likely, however, that Gallus hens identify their own nests by site. If this is the case, hens may not identify their eggs specifically. Failure to recognise own eggs from those of conspecifics or other egg-like objects has been noted for many other bird species. This has been shown to be the case for barn swallows, which were also shown to distinguish own nests by their siting (Grzybowski, 1979).

Failure of hens to recognise or to respond preferentially to eggs typical of their own strain is shown for both bantams, which laid more eggs in nests containing Leghorn eggs than bantam eggs, and also for White Leghorns, which laid in nests containing brown broiler eggs to the same extent as they laid in nests containing other White Leghorn eggs. Turpin (1918) found that Rhode Island Red hens showed no preference for nests containing either brown or white nest-eggs, which also indicates that colour of eggs outright, or similarity in colour to that laid by particular hens, is unimportant in determining response to the egg. If colour were to fall outside the usual range that might be expected for domestic hens, however, different responses may perhaps occur. It would also be interesting to test responses to eggs of greenish colouration like pheasant eggs.

Turpin (1918) also found that nests containing real or china nest-eggs were more attractive to nesting hens than nests which did not contain a nest-egg. However, real eggs were more attractive than the china eggs. In a further experiment, he found that real, wooden or plaster of paris nest-eggs were all effective in increasing usage of nests in which they were placed. Subsequent analysis of his results by this author showed that all three of these nest-eggs were equally effective in this respect and all produced nest usage figures considerably greater than those recorded for nests which did not contain nest-eggs ($\chi^2_{1df} = 16.7^{***}$). His results suggest that hens respond in much the same way to egg models which approximate the colour, size and shape or contours of real eggs as they do to real eggs. Hens apparently distinguished between real and model if the model egg was china. Possibly some cue other than the visual cue operated to influence preferences in this case and it is conceivable that heat retention qualities of eggs may be involved.

Failure to recognise own eggs may be a contributing factor in the previously discussed tendency for hens to use nests in which other hens have nested. It has already been noted that wild gallinaceous hens tend to share and adopt nests, and also lay some eggs in dump nests. However, such cases seem to be the exception rather than the rule. It may be that the comparatively 'promiscuous' nesting habits of hens within clutches may be a result of inability of hens to identify own nests in a rather less intricate environment in which cues for distinguishing specific nest sites are either limited or occur repeatedly elsewhere in the environment. As a result, hens may more easily make 'mistakes' in identifying previously used sites. Accumulations of eggs may be all the more effective in attracting hens to nests because of this difficulty in distinguishing sites.

Egg rolling is a behaviour commonly recorded for a wide range of bird species. Egg rolling and other manipulations of the egg are reported by Wood-Gush (1975a) both before and after oviposition in the case of individually penned Rhode Island Red hens. Results of Study 4.1.4 suggest that this behaviour will be shown by sexually mature hens even if they have had no previous experience of oviposition. There is some evidence to suggest, however, that hens which have some prior contact with eggs or with oviposition are more likely to perform the activity.

No analyses were attempted on these data for several reasons. Firstly, hens were placed into the test-pen when their behaviour in their home pen indicated strongly that they were in the nesting phase. Eventual nest selection occurred at extremely variable intervals following transfer to the test-pen and hens may have had more or less opportunity to express egg-related responses as a result. The age of the hens and their previous experience with egg laying may have influenced this opportunity by affecting the stage at which they first showed obvious signs of nesting. Also, it was apparent that some mistakes in the determination of where and when surgically altered hens finally nested were probably occurring, particularly when recordings were taken from video-tape, in which case the activity of hens in three quarters of the pen could not be followed. 'Missed' nestings were also a possibility in the case of several surgically altered hens.

Despite these, and other possible short-comings, the results seem to suggest that complete egg rolling responses may be more prevalent in older hens and in hens which have had some experience of egg laying than in pullets or hens with no laying experience. Although these hens had been maintained in laying cages all their laying life, some had undoubtedly had some contact with the eggs that they had laid. Contact of this sort has been documented for caged hens in Study 3.5.

Egg rolling was not commonly seen to be performed by hens in the first week during which they nested and laid. Similarly, pecking at the egg was most commonly observed for mature hens.

It is not known whether these pecks at the nest-egg were responses directly related to nesting or whether they were simply investigatory. However, it is known that hens peck at eggs in a nesting situation and that this often precedes egg rolling. This has been indicated in Study 3.1. It has also been suggested that this activity may serve to dislodge eggs and make them available for egg rolling. Incidences of pecks at eggs were slightly higher in the older hens, as were incidences of other responses to the nest-egg. Pecking at eggs, which may therefore be a form of egg manipulation or 'recognition', was, however, seen in these young hens to some extent, suggesting that hens may be capable of responding to eggs at this stage, but that complete egg rolling behaviour has either not developed or that it is suppressed in the novelty and perhaps 'confusion' of the first nesting situation.

Whatever the case, complete responses to eggs do not appear to occur as frequently in naive as in mature nesters, nor in birds which have had no experience of 'own' eggs as compared to others which have. It is interesting to note that Beer (1963) reports 'shifting', a behaviour apparently analogous to egg rolling, in juvenile Black-headed Gull females in the sexually maturing phase. He states that the behaviour was identical to that performed by gulls in full adult plumage and at the same stage in the pre-laying phase, despite the fact that they had presumably never seen eggs before. This lends further support to the suggestion that prior experience with eggs is not necessary for the expression of this behaviour pattern and may indicate a hormonal mechanism similar to that controlling pre-laying and nesting behaviours operant in the control and expression of egg-related behaviours. Wood-Gush (1975a) discusses the possible roles of prolactin and/or progesterone in the control of early incubation behaviour and therefore possibly egg rolling behaviour and also considers the implications of this in terms of egg production.

Further research into the effect of experience with egg laying and contact with eggs, and of maturity on the response to eggs would be of value. One simple but potentially useful means of doing this might be to follow the responses of hens to eggs in pens with nests from the onset of lay to sexual maturity to see at what point hens begin to respond or respond maximally to eggs and whether this relates in any way to expression of other nesting behaviours. Also of interest could be studies of the effect of hormones known to be associated with nesting and incubation on the expression of responses to eggs such as egg rolling.

4.2 The Presence of a Nesting Material

Introduction

A number of studies have indicated differences in nest usage associated with different nesting materials (Hansen *et al.*, 1948; Siegel and Howes, 1959; Daly *et al.*, 1964) in situations in which hens were free to select nest alternatives. In experiments comparing selection of nests differing in light intensity, height of nests and the presence or absence of litter in wooden trap-nests, Murphy (1969) found no significant differences in the frequency of selection of nests with or without nesting material (litter). However, evidence provided by studies of pre-laying behaviours of hens in cages with litter floors as opposed to wire floors (Wood-Gush, 1975b) suggests that the presence of a nesting material may influence or release sitting behaviour in the nesting phase.

In the light of these conflicting reports, it was decided to investigate the role of nesting material in nest selection further. It was intended that, firstly, gross differences in response to the presence or absence of a nesting material would be determined and then the influence of previous experience on preferences for different floor materials investigated.

Study 4.2.1
Responses to Nesting Material and Nest Curtains

This study was conducted to establish whether hens would differentiate in their selection of nest site on the basis of whether nests contained nesting material or not. The relative effectiveness of nest curtains, as a source of additional sense of enclosure or confinement to the nest, in influencing nest selection was also investigated.

Materials and Methods

Hens, 18 White Leghorns and 18 bantams, pens and management were the same as in Study 4.1.3. The present study was, however, conducted at an earlier stage than Study 4.1.3, and was commenced when the hens were in their fourth month of production.

Four nests in each nest level were available to the hens. The nest alternatives offered to the hens were the presence or absence of nesting material (wood shavings) and the presence or absence of a nest curtain over the front of the nest. The nest curtain treatment was offered in an attempt to provide an added dimension of confinement to the nests and to determine the relative attractiveness of the two parameters; nesting material and confinement. The nest curtains were made of hessian and hung from wire rods across the entire entrance of the particular individual nests to which they were fitted. Hens had to gain access to the nests via a split in the curtain about mid-way along the nest entrance or at the sides of the curtains.

Wood shavings were spread in the appropriate nests to a depth of about 5 cm and were topped up as required. In each nest level all four possible combinations of nesting material/no nesting material and curtains/no curtains were offered. Treatments were reallocated to nest-boxes randomly each day, the necessary alterations being made in the late afternoon. Eggs were collected and recorded at 9.00 am, 11.00 am and 1.00 pm daily. All eggs collected contributed to the data analysed. Recordings were taken on 40 consecutive days.

The daily results were subjected to analysis of variance, daily and overall, with levels as replicates.

In addition, hens were observed in the Leghorn flock from 7.00 am until 1.00 pm on days 1, 7 and 36 of the study, and bantams observed in the same way on days 2, 8 and 37. During these observation periods records were made of the numbers of nest entries into different nests and any other activities of interest at the nests or in them.

Results and Discussion

The most obvious trend found each day was for hens to lay significantly more eggs in nests containing wood shavings (99.2% of all selections) than in nests which did not contain a nesting material. This trend was significant for all but two days over which the study was conducted.

Total numbers of eggs laid in the four possible nest alternatives by both White Leghorns and bantams are shown in Table 4.2.1a.

Table 4.2.1a Numbers of eggs laid in nests with or without nesting material and with or without nest curtains by White Leghorn and bantam flocks

	Total Eggs Laid in Alternative:			
	Nesting Material		No Nesting Material	
Breed	Curtain	No Curtain	Curtain	No curtain
Leghorn	243	340	0	0
Bantam	149	125	7	0

Overall analysis of variance is shown in Table 4.2.1b.

Table 4.2.1b Analysis of variance of numbers of eggs laid in nests with or without nesting material and with or without nest curtains by Leghorn and bantam flocks

Source	D.F.	S.S.	M.S.	F	Significance
Breed (B)	1	142.51	142.51	14.55	-
Error (a)	2	19.58	9.79		
Curtains (C)	1	6.81	6.81	1.69	N.S.
Nesting Material (N)	1	1128.91	1128.91	280.84	***
CN	1	10.00	10.00	2.49	N.S.
BC	1	25.60	25.60	6.37	*
BN	1	156.03	156.03	38.81	***
BCN	1	20.31	20.31	5.05	-
Error (b)	6	24.12	4.02		
D	39	23.48	0.60	0.30	N.S.
DB	39	29.87	0.77	0.39	N.S.
DC	39	205.57	5.27	2.66	***
DN	39	29.87	0.76	0.38	N.S.
DCN	39	191.88	4.92	2.48	***
DBC	39	97.28	2.49	1.26	N.S.
DBN	39	47.85	1.23	0.62	N.S.
DBCN	39	113.07	2.90	1.46	*
Error (c)	312	619.30	1.98		
Total	640	4058.00			

Hens exhibited a highly significant tendency to lay in nests which contained nesting material. In fact, almost all eggs were laid in such nests except on one occasion on which one bantam hen laid an egg in a bare, sheet metal nest and other hens either got into the nest with her and laid, or entered and laid in the same nest when they observed eggs in it.

Overall, the numbers of eggs laid in curtained and uncurtained nests were not significantly different. However, breed differences were detected in the level of usage of curtained nests, bantams laying in curtained nests to a greater extent than Leghorns. Observation suggested that hens of both breeds initially experienced some difficulty using the curtained nests. Hens had no prior training with these curtains and initially appeared to be hesitant to use them. Leghorns experienced greater difficulty in getting into the nests than did bantams. The initial phase of entry, in which hens had to push in through the curtain while moving from the nest approach into the nest itself, appeared to be, literally, the stumbling block. Bantams seemed to adjust to this quickly, but throughout the study many hens continued to enter these curtained nests only with some hesitance.

The highly significant interactions found between breed and the presence or otherwise of nesting material, and between the two factors under study on different days, are mostly a reflection of the one occasion on which the bantams opted to lay in the bare, curtained nest once it had been entered and laid in by one flock member.

Use of nests with curtains changed significantly through the period of study. The percentage of total eggs laid by bantams and Leghorns in uncurtained nests in four consecutive ten day periods in the study are illustrated in Figure 4.2.1. As the study progressed, more and more hens were beginning to lay in curtained nests. By the end of the study, the numbers of eggs laid in curtained nests were significantly higher than the numbers in uncurtained nests on a daily analysis. Hens had possibly overcome their fear of the nest curtains, had learnt to use the curtains and were more confident of using them, or had become more responsive to the stimuli provided by them throughout the study.

Greatest differences in nest usage were consistently associated with presence or absence of a nesting material. However, it should be remembered that hens had previously only had access to nests containing nesting material. As a result, nests containing wood shavings may have been doubly attractive since they were not only of the inherently preferred type, but were also the type of nests hens had obtained previous nesting experience in. Nests with curtains, on the other hand, may also provide a more 'attractive' nesting environment, but the expression of this may be suppressed because curtains are unfamiliar to the hens which have experience with nesting in the opposite alternative.

Results of recordings of nest entry are given in Table 4.2.1c, pooled over both breeds.

Table 4.2.1c Numbers of nest entries recorded during observation periods at the beginning of the study, a week after it commenced and towards the end of the study

Days on Which Recorded	Number of Nest Entries: Nesting Material		No Nesting Material	
	Curtains	No Curtains	Curtains	No Curtains
1 and 2	19	28	22	33
7 and 8	24	54	6	12
36 and 37	39	49	8	9

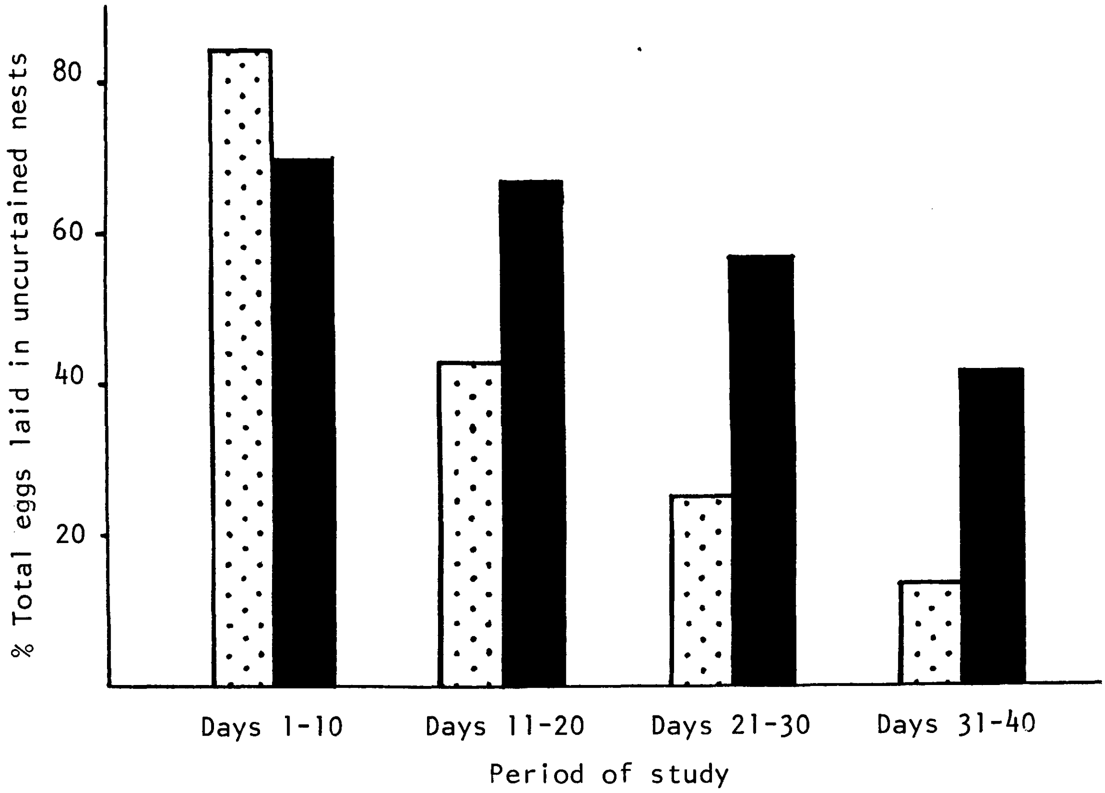


Figure 4.2.1 The percentage of total eggs laid in uncurtained nests in four time periods throughout the study by bantam ( ) and White Leghorn ( ) hens

The observational data show that nests both with or without nesting material were entered with about the same frequency during the first few days of the study. However, at the end of the first week, hens tended to mainly enter nests containing the nesting material. This trend was still apparent towards the end of the study. Additional notes made during observation indicate that initially, hens seemed to examine and enter both alternatives equally. Once in the nest, hens would often sit and scratch about until they eventually left the nest to examine and enter another, or until they laid. Hens which entered nests which did not contain wood shavings would sometimes sit and attempt to scratch about, but would usually stand and sit again several times before eventually standing for a short interval before leaving the nest. Hens never laid in these bare metal nests in the absence of nesting material in these early observations.

When observed again at the end of the first week of the study, a different pattern was noticed. Hens approached and examined most alternatives, seemingly spending less time outside bare nests, but tended only to enter those containing nesting material. This same pattern was again noted during the final observation period when even less attention was paid to the bare nests. All nests were examined from the approach outside the nests, but only nests containing the nesting material were usually entered.

The numbers of nest entries into curtained as opposed to uncurtained nests tended to reflect the forementioned tendency for hens to be somewhat hesitant to attempt entries into curtained nests and also for a proportion of entry attempts to be unsuccessful. Curtained nests were, however, frequently examined by hens which would stick their heads into the nest at the edges or slits in the curtains. Hesitancy in front of the curtained nests appeared to decline in subsequent observation periods and the number of nest entries seemed to have increased.

Study 4.2.2

Responses of Hens from Different Environments to Nest Floor Type

The object of this investigation was to follow the selection of different floor types for nesting by hens which had dissimilar rearing and initial nesting experiences.

Materials and Methods

The 24 hens used in this study were B x W strain birds purchased at 14 weeks of age. On purchase, half the birds were moved into individual laying cages and the other half into a deep litter floor pen in an isolation shed at the UNE campus (see General Materials and Methods). No nests were provided in this pen up until a week before testing began, at which point, three cardboard nests were placed together on the floor of the pen in one corner.

When the pullets began to show signs of sexual maturation, five of the individuals in each environment were transferred into the alternative environment (five pullets from cages to deep litter pen; five pullets from deep litter pen to cages). Within several days of transfer, most of these pullets laid their first eggs. They were allowed to lay in their respective environments until tested, about four months after they had first laid.

All testing was carried out in a test-pen situation as described in Technique B at the beginning of this Chapter. All recesses were formed by an angle of 60° in the two panels forming them. Therefore, the width of the entrance to the nest site was 45 cm. Lighting was provided on the mid-recess panels using 40 watt light globes. The light intensity in the nest options was the same.

Four nest options were made available to hens selecting nest sites in the test-pen. The first was a 1.5 cm square wire mesh floor which was fitted into a nest recess. Another was bare cement provided by the shed floor. The third option was a dark green short pile carpet and the final option was the same carpet on which was spread a covering of wood shavings to a maximum depth of one centimetre.

In addition, four hens that had not been transferred to the alternative environment at point of lay, two from cages and two from the pen, were tested in a similar situation. The nest options available to them were the same carpet and carpet + litter (wood shavings) options but the cement and wire options were replaced by carpet and carpet + litter options in which the type of carpet used was of a colour very closely approximating that of the wood shavings. This small study was conducted to ensure that hens in the main study were not simply responding to litter on the basis of colour.

Hens were tested in the following way. Hens in cages were palpated each morning to determine which individuals were to lay that day. Those which were to lay were placed into the test-pen when their behaviour indicated that oviposition was imminent. Hens in the deep litter floor pen were placed into the test-pen when they were found to have entered a nest in the home pen. Records were taken of where hens laid. In addition, notes were taken of the activities that hens performed once in the test-pen, although this was only done on a casual basis. Most nestings took place without the observer being present throughout the nesting period. Shortly after the hens had laid and left the site, they were returned to their respective home environments.

Hens of the main study were placed in the test-pen for at least five nestings. Options selected were recorded. Once the hen selected (laid in) the same option on five consecutive testings she was deemed to have made a 'final' choice and was no longer tested. Since the hens were mature layers it was assumed that they would form attachments to particular sites fairly quickly and so five nestings would be sufficient to establish preference.

Hens were not necessarily tested on all days that they laid since they already had considerable nesting experience outside the test-pen situation.

The numbers of hens from each treatment that selected each particular nest as a final choice were tabulated. These results could not be analysed as insufficient hens were available for study, giving rise to expected values insufficiently large to permit Chi-square analysis. The total number of times that different nest options were laid in, both before and in the final nest selection, was tabulated. Although results from individual hens may not necessarily have been independent, these results were considred worthy of Chi-square analysis since features of the nest which may initially attract hens in their first few nest selections in a novel environment may not necessarily be those which encourage hens to form attachments to sites. Selection responses would therefore change throughout the testing period and some indication of initial responses obtained.

Hens from the subsidiary study with different coloured carpets and litter options were tested on ten occasions and the total number of times hens selected each option tabulated.

Results and Discussion

The numbers of selections of nest options prior to final selection of a nest type and the numbers of hens settling in different nest options in each treatment are given in Table 4.2.2a.

Table 4.2.2a Number of times that hens laid in different nest options prior to 'final selection' of a particular nest type, and numbers of hens which finally selected each option

		Number of Times Selected Prior to 'Final Selection'				Number of Hens Making Option the 'Final Selection'			
Rearing	Laying	Wire	Cement	Carpet	Litter	Wire	Cement	Carpet	Litter
Floor Pen	Floor Pen	2	4	7	5	0	1	0	4
Cage	Floor Pen	0	4	9	0	0	1	0	4
Floor Pen	Cage	2	5	5	2	0	0	0	5
Cage	Cage	1	4	3	4	0	0	0	5

Eight of the ten hens made the nest option with wood shavings their final choice,laying in it for five consecutive testings. This was plainly the most popular nest type. Only two hens did not make this choice, both of these laying five eggs in a row in the cement floor option. Although both these hens were from floor pen environments, this does not provide sufficient evidence to suggest that selection of the final nest was affected by eitherrearing or laying environment. Hens with only previous experience of nesting in laying cages eventually selected only nest recesses which contained wood shavings on carpet to lay in repeatedly. It should be noted, however, that all hens had some previous experience with litter floors because they had been reared to 14 weeks of age in deep litter floor pens.

Analysis of data for all nest selections up to and including those made during the period of successive selection of final nest, indicate that hens laid more eggs in the nest option with wood shavings than in the cement or carpet options, which in turn were laid in more often than the wire-floored nest ($\chi^2_{\text{litter vs carpet/cement vs wire,2df}} = 14.4$***). No differences were found between groups with different rearing or laying experiences in this respect, nor were there any significant differences between housing treatments in the number of selections made before reaching the 'final selection' stage. In short, hens from all housing treatments responded in the same way in this testing situation.

One behavioural pattern noted during periods of observation of hens in the test-pen, was for two hens, both of which had been housed in cages both through the rearing and the laying phases, to sit or stand for considerable periods of time in the wire option after being moved to the test-pen and after laying. These hens repeated this procedure on many testing occasions although only one of these hens ever laid an egg in the same site and even then on only one occasion. The tendency to perform this activity seemed to diminish towards the end of the test period. Another observation was that a number of hens from the pen environment became extremely agitated when placed in the test-pen and often attempted to get out of the pen. The same hens were suspected of withholding their eggs and always remained in the test-pens considerably longer than predicted before eventually laying.

The numbers of eggs laid in the 'green/brown carpet' and 'carpet with wood shavings' nest options by each of four additional hens are shown in Table 4.2.2b.

Table 4.2.2b Numbers of eggs laid in different nests by four hens tested on four occasions each

Previous Experience	Number of Eggs Laid in Nest Option: Green Carpet		Brown Carpet	
	Carpet	Carpet + Litter	Carpet	Carpet + Litter
Reared and Laid in Pen - Hen 1	0	6	0	4
- Hen 2	2	3	0	5
Reared and Laid in Cage - Hen 1	0	6	1	3
- Hen 2	0	2	0	3

The results of testing with green and brown nest carpets indicate that hens still laid most of their eggs in nests containing wood shavings regardless of the colour of the carpet in the other nest options.

Observation of hens in the different nest types seemed to indicate that hens, which sat to nest in the nest options with carpet alone, would perform rotations and foot scraping activities in the nest and appeared to look about from their seated positions as if looking for some material to peck at or pick up. This would often be followed by the hen standing, as if to leave the nest, but sitting again. The procedure would be repeated a number of times, usually ending with the hen going elsewhere to nest and lay, often in the litter option.

Hens in nest options containing wood shavings would sit, rotate, perform foot scraping activities and peck at the litter, sometimes nest building to the chest, side or back. They did leave the nest from time to time, but appeared to remain in the nest to a greater extent than hens using carpeted nest options. Despite all their activities there was insufficient nesting material in these sites for a rim of any size to be built or a depression made.

Hens entered cement and wire nest options, particularly during the early testing sessions, but did not usually remain in them or sit, particularly in the wire option. The exception to this was in the case mentioned earlier in which two hens tended to spend much of their non-nesting time in the pen in the wire-floored nests.

It should be noted that the above observations were only impressions gained by the observer from incomplete and unanalysed data. Analysis of these sorts of observations in the given testing situation were not considered worthwhile, particularly because of the differences found between times of placement in the test-pen and times of oviposition for different individuals and different testing occasions. Nevertheless, these general observations were considered of particular interest and so have been included.

General Discussion - The Presence of a Nesting Material/Floor Type

The results of the present studies and those of Study 4.1.3, indicate that the presence of a nesting material may be very important in the eventual selection of a nest. However, it must be remembered that all hens used in these studies had had some previous experience with litter at some stage in their lives. However, even hens which had only had previous experience of nesting in laying cages showed marked preferences for nests containing wood shavings.

The possibility that this may have been due to preferences for colour is doubtful in the light of the findings when several hens were given the option of nests with carpets similar in colour to that of the wood shavings used as a nesting material.

The results of the present research are apparently in conflict with those of Murphy (1969) which indicated no preference for nests with or without litter. However, in that study, hens were only given the one opportunity to select a nest option, and once a nest had been entered the hen had no choice but to lay in it, since nests used were trap-nests. The hens had prior experience of nests containing variable amounts of litter, but presumably not of completely empty or litterless nests. It may well be that hens need to have entered and experienced a litterless nest before they can establish a preference against it, or for that matter, that they must enter a nest containing nesting material before they can show a preference for it. The stimulus qualities that nest material possesses for nesting may only be apparent once the hen has actually entered a nest containing material. Hens without experience of entry into a bare nest may therefore fail to identify a bare nest as less attractive on examination from outside the nest and so initially enter it.

Records of nest entry from Study 4.2.1 show that hens may, in fact, learn to identify nests with or without nesting material visually. Initially, hens seemed to examine and enter nests with or without nesting material equally. However, after some experience with the nests the same hens examined all nests but only entered those containing wood shavings, the preferred alternative. Thus, it seems probable that although the stimulus characteristics of nesting material which may release certain of the behaviours relevant to nesting may only be experienced whilst the bird is actually on the nest, hens may learn, through prior experience, only to enter the preferred alternative.

The recordings of nest entries at different stages through the study also suggest that nest examination may be functionally discrete from nest entry and may in fact involve discriminatory processes.

The results of the present research and also those presented by Wood-Gush (1975b) suggest that nesting material may have a role in releasing the sitting component of nesting. Wood-Gush found significant differences between the amounts of time hens would spend in sitting during the half hour before oviposition in cages with wire floors as opposed to metal trays containing litter on the floor. However, he was unable to differentiate between the factors, litter and slope, since wire floors were sloped and litter floors flat. Nevertheless, hens spent more of the pre-laying period sitting in cages with the litter floors.

Although not quantified, the observations of Study 4.2.2 tend to support the suggestion that nesting material stimulates sitting. Hens seem to be in some way disturbed in the situations where nesting occurred on nests without shavings, frequently rising and settling again. However, why hens entered and sat in these sites at all, when the apparently more acceptable nests containing shavings were at hand, is not understood. However, the tendency was for hens to develop their preferences for nests containing shavings as the study progressed, suggesting that hens may have been disturbed initially by the handling and unfamiliarity of the testing situation and that this may have affected their selection. Certainly, preferences for nesting material were found for all hens of both Leghorn and bantam breeds in Studies 4.1.3 and 4.2.1, and these were found to be particularly strong and, in fact, more important in determining nest selection than either the presence of eggs in nests or the existence of added dimensions of confinement.

The features of nesting material which provide the stimuli giving rise to preference are not determined by these studies. Possibly of importance is that the material may be manipulated to form a depression. Formation of such a depression may be important to the wild ancestors of the hen, in that the bowl created will hold the eggs together so that they will not roll away from the site to be lost or to attract predators during the accumulation phase. The ability of the depression to hold the eggs together would increase the likelihood of successful incubation. The actual capacity to maintain such a structure may not be important, since it has already been noted that hens in Study 4.2.2 selected nests containing material even though there was not enough present to form any sort of a depression.

The possibility that hens may tend to remain in a site that can be, or already is, 'moulded', is given credence by the findings of Bressler (1961). He reported that levels of floor-laying in pens with nests containing fibrous roll-away nest cushions which were moulded into a nest-cup shape were similar to floor-laying levels in pens with more conventional nests containing wood shavings. This does not mean that the nest-cushions would be as popular as the nests with shavings if the two options were presented together, but it does suggest that the acceptance of the nest-cushions was nevertheless quite good. Whether it was the shape of these nests or the presence of the fibrous material from which they were constructed that attracted hens can not be determined.

Wood-Gush (1975a) noted a tendency for hens to be less likely to add feathers to their first nest on a subsequent visit if their egg had been left in it. It is possible that both eggs and nesting material may provide hens with something to manipulate whilst on the nest, and it may be this characteristic which encourages hens to remain in a nest containing them.

Other stimuli from the nesting material that may be involved in the determination of nesting preferences may include the heat retention and humidity conditions provided by the material. However, it is not known whether the hens could detect or appreciate such differences.

The actual senses through which the stimuli from the nesting material are recorded are not known. If manipulability of a nesting material were the important feature, then it may be that several senses are involved as cues. The hen may respond to the presence of a loose, friable substrate visually, or may act on tactile cues. These could include the ability of material to be felt to move with the bird or to 'give way' as the hen scrapes out sideways with her feet in nest building. Further research is required to determine the stimulus qualities of nesting material which influence sitting and remaining in the nest.

These studies have only indicated the effectiveness of one type of nesting material in determining nest preferences. It is possible that different types of nesting material may not produce the same results. Hansen *et al.* (1948), Siegel and Howes (1959) and Daly *et al.* (1964) have all reported apparently differential attractiveness of a number of nesting materials, although similar studies (Baker, 1962) have also failed to show such differences. Studies of hen responses to different nesting materials in terms of times spent sitting and the selection of site of oviposition, could prove useful. They may provide clues as to the common characteristics of more preferred types and so to a better understanding of the stimuli that hens may be responding to. This could prove particularly useful in the design of more satisfactory flooring materials for situations in which the provision of litter in nests is impractical. Nest usage in pens provided with roll-away nests is often poor (Anon., 1964) and problems associated with poor usage of roll-away nests in get-away cages when sand-boxes are included in the environment have also been reported (Wegner, 1980). While floor slope may be involved, it is likely that these problems are related to the lack of a nesting material. Further automation of nesting systems may necessitate the investigation of stimuli presented by nesting materials more fully.

4.3 The Shape of the Nest

Introduction

As was indicated in Chapter 2, most gallinaceous birds nest in rounded, saucer-like depressions which they either form themselves or which occur naturally. The possible importance of a nesting material in providing the hen with something that can be moulded to form such a depression has already been discussed. However, in well concealed sites in undergrowth or in clumps of vegetation, nests may be seen to have a particular aspect, an entrance and even a shape in terms of its enclosure by surrounding confining structures. It was decided that several investigations would be undertaken to see if these features could be manipulated to establish individual or flock preferences for certain design features which could be incorporated in the construction of nest-boxes.

The first factor considered was the type of entrance to the nest. Subsequent studies looked at responses of hens to nests of different shape and to nests which either diverged or converged once entered. As far as possible, other factors were controlled so that influences such as space available in the nest and light intensity would not interfere in the results.

Study 4.3.1

Responses to Nest Entrance Shape

Since the nesting environment available in a natural habitat may offer a range of potential sites in, under or between various types of cover, it seems possible that hens may be able to distinguish nest types on the basis of their shape or characteristics of their entrances. The objective of this study was to determine if hens of a number of different breeds would exhibit preferences for nest options on the basis of the shape of the entrance.

Materials and Methods

Usage of nests which differed in the shape and design of the entrance to the nest was studied using three breeds of hen, these being broiler, first generation 'feral' and broiler breeder strains (see General Materials and Methods). All nest alternatives offered to the hens were internally of identical dimensions and were offered in a bank of six nest-boxes in the hens' pen. For each flock studied, entrance types were reallocated to positions in the set daily, or as specified, according to a Latin Square design, necessary alterations being made in the late afternoon. Eggs were collected at hourly intervals between 9.00 am and 4.00 pm each day to avoid the accumulation of eggs in the nests.

Nest-sets offering the alternative nest types were hung mid-way along the wall in the pen under study. The nest-set and the six alternatives offered are illustrated in Figure 4.3.1. Only the lower bank of nests was available to broiler and broiler breeder hens, whereas upper and lower banks were made available to 'feral' hens. Hens were given access to the nests by means of two wooden runged platform approaches. The entrance alternatives differed not only in shape, but also in the size of the gap available to hens to get into and out of the nests, and so in the amount of light that they admitted.

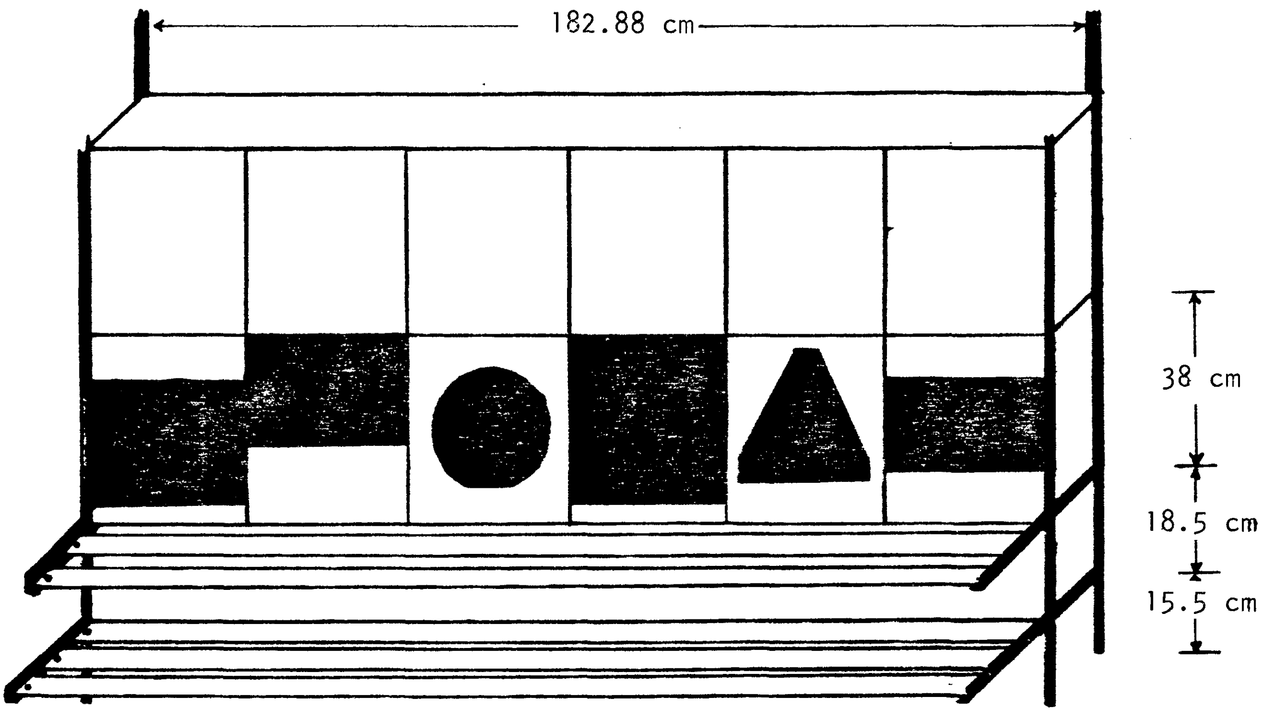


Figure 4.3.1 The nest-set and nest entrance alternatives provided for nest entrance studies

The alternatives offered, corresponding to the diagrammatic representation in Figure 4.3.1, from left to right were an entrance with a 3.5 cm lower lip and 8 cm upper lip, one having a 15 cm lower lip and no top section, one with a circular entrance with the entrance 10 cm from the bottom of the nest, an open nest with only a 3.5 cm lower lip to stop eggs rolling out of it, a triangular entrance, the bottom of which was 10 cm above the base of the floor and, lastly, an entrance with 10 cm and 8 cm bottom and top sections. As a result of the positioning of the entrance holes, alternatives also differed in the distance from the nest approach to the nest opening.

All nest fronts were constructed of sheet metal, like the rest of the nest-set, and were easily moved from one box to another, as they slid into slots which ran down the sides of each nest-box. Once in position the nest fronts fitted firmly onto the nests. Wood shavings were used as a nesting material and were topped up to a depth of 3 cm throughout the studies.

Studies on all groups of birds were conducted in their home floor pens (see General Materials and Methods). Bird densities were similar, being 2.16, 1.75 and 2.22 birds per sq. metre for broiler, 'ferals' and broiler breeders respectively. Hen to nest ratios in each pen were 6.17, 2.50 and 3.33 hens per nest for broilers, 'ferals' and broiler breeders respectively, although many of the broilers did not use the nests.

The first study that was conducted using these nests involved 37 broiler hens. The experiments were commenced when the hens were five months into lay.

Eggs laid in particular nest options were recorded on a daily basis. Two recording periods, henceforth called 'trials', each of six days as dictated by the Latin Square design, were conducted. Analyses of variance were conducted for each trial and overall.

Hens were then given the same nesting options during a further four day period. Hens were observed during this period from a position along the pen wall opposite to that on which the nest-set was hung. Numbers of nest entries were recorded, as well as the numbers of times that nest options were laid in. The distribution of nest entries and eggs laid between the nests was subjected to Chi-square analysis. The numbers of entries that were followed by oviposition in the same site were compared with the number of entries that did not result in oviposition for each nest option by Chi-square analysis also.

The second study, using 30 first generation 'feral' fowl, commenced shortly after the hens had begun to lay for the first time. Both top and bottom nest levels of the same nest-set were made available, unlike the studies using broiler and broiler breeder hens in which only the lower bank of nests was available to the hens. All six of the nest alternatives appeared in the upper level as well as in the bottom level of nests. Alternatives were allocated to positions in the set according to a Latin Square design, top and bottom levels treated as separate trials. Because hens in this flock were not laying particularly well the position of alternatives was only changed every third afternoon. Recording was continued for one complete trial period which, because options were only reallocated every third day, lasted for 18 days. Eggs were collected at 9.00 am, 11.00am and 1.00pm each day and totals for each three day session recorded.

Results for the entire 'feral' study period were subjected to analysis of variance. Chi-square analyses were also performed on the total and partitioned data to establish what alternatives were the most favoured.

The third study was conducted on 20 broiler breeder hens. These birds had been subjects of a study involving the test-pen technique early in their laying history (see Study 4.5.7), and were used in the present study when they had been laying for about two months.

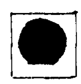
The same nest alternatives as offered to the other groups were made available to these hens, only the bottom level of nests being provided. Alternatives were again allocated to positions in the set by a Latin Square design, repositioning taking place daily. Recording was continued for six complete six day trials. Analyses of variance were performed on the data from each trial and overall. Chi-square analyses were also performed on the complete and partitioned data.

Results and Discussion

The numbers of eggs found in the different nest alternatives in the broiler study, summed over both trials, are given in Table 4.3.1a.

Table 4.3.1a Numbers of eggs laid in nests with different entrances by broiler hens

Nest Entrance Type:

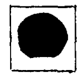
					
21	20	14	20	17	20

Numbers of Eggs Laid

Analysis of these results revealed no significant differences between the numbers of eggs laid in nests with alternative entrances or in different positions in the nest-set in either trial or overall. It had originally been intended that further trials with these hens would be conducted, but the results from the first two trials suggested that the study was not worth continuing.

In the subsequent period of observation, the numbers of nest entries as well as eggs laid in particular nests were recorded and these data are presented in Table 4.3.1b.

Table 4.3.1b Numbers of nest entries and eggs laid in nests with different entrances by broiler hens

	Nest Entrance Type:					
						
Number of Entries	35	30	18	23	20	22
Number of Eggs Laid	7	8	6	11	5	6

Analysis indicated that no significant differences existed between numbers of entries into, or between numbers of eggs laid in, nests with different entrances. The impression gained by the observer was that hens tended to enter the alternative with the 3.5 cm lip quite often but rarely laid in it, whereas they entered the 10 cm bottom/8 cm top less frequently but tended to remain and lay in it to a greater extent. *A posteriori* analysis of the numbers of occasions on which entry into nests resulted in oviposition as compared to occasions that it did not result in oviposition for the 3.5 cm, the 10 cm/8 cm and all other entrance types combined failed to reveal any significant differences. However, when the 10 cm/8 cm alternative was compared with all other alternatives in this way, some difference was detected ($\chi^2_{10cm/8cm \text{ vs rest}, 1df} = 4.7^*$). Although this result must be viewed with considerable caution as it is an *a posteriori* test, it may suggest that hens may have failed to register preferences because of the difficulty experienced in getting into certain alternatives. It had been observed that hens did experience some difficulties in entering several nests which provided smaller openings. Once in a nest, the rather large broiler hens occupied most of the nest although they could easily turn around in it.

Results of the studies on first generation 'feral' hens produced very different patterns of nest usage. These data are shown in Figure 4.3.1a. Results of analyses of variance of top level and bottom level data are presented in Appendix 4.3.1. Chi-square analysis of top and bottom data revealed that the trends in distribution of eggs between different nest types were consistent over levels.

These results indicate distinct nest preferences, by far the most frequently selected nest being the alternative with the high nest front. It was noted that the small feral hens would tend to settle in these nests and sit very 'close' and low in them. From outside, the observer could see very little of any hen sitting in this alternative and frequently overlooked hens within it. Whilst

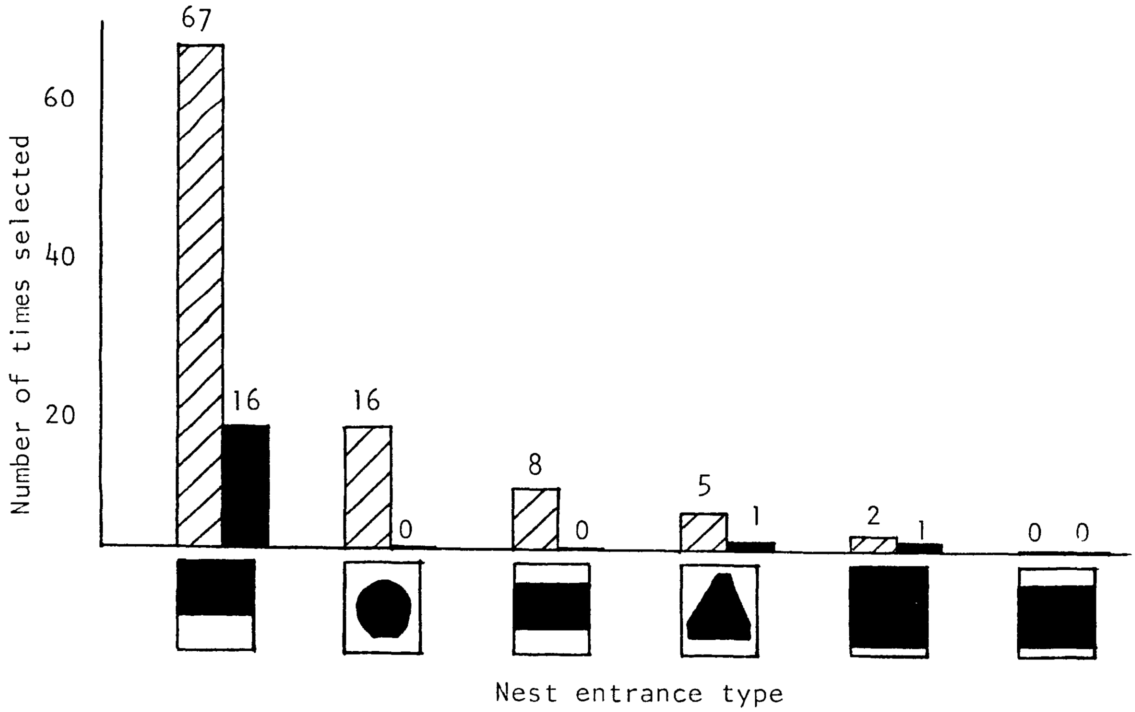


Figure 4.3.1a Number of times nests of different entrance type were selected by first generation 'feral' hens (lower level nests; top level nests)

sitting in this nest, hens would usually face out towards the nest entrance, although it is doubtful that they could see out into the pen because of the depth of the nest and elevation of the nest-set. Hens in other alternatives seemed to have no such preference for the direction that they faced whilst sitting in the nest. Other hens which moved along the platform approach outside the nests, examining potential nest sites, could easily see into the preferred deep nests and their interest in such nests tended to be greater than of other types of nests. Most nests appeared to be entered however. Results of a later study stimulated by these observations (see Study 4.4.4) suggest that the apparent preference for nest entrance types by ferals may have actually been a response to nest depth, and that the lack of response by broilers may have been due to their larger size which rendered all nests insufficiently deep to afford them sufficient cover or concealment.

The numbers of eggs laid in different nests in each of the trial periods with broiler breeder hens are shown in Table 4.3.1c. Total numbers of eggs laid in nest-boxes in different positions in the set are shown in Figure 4.3.1b. Analyses of these data reveal significant differences in the numbers of eggs in nests in different positions in the set (χ^2_{5df} = 155.2***) and also in the numbers of eggs laid in nests with different entrances (χ^2_{5df} = 42.3***). From Figure 4.3.1b it can be seen that the

Table 4.3.1c Numbers of eggs laid in nests with different entrances by broiler breeder hens and results of analyses of variance performed on the data

Time Period	No. of Eggs Laid in Different Nests						Variance Ratio (F) and Significance		
							Entrance	Position	Day
1	6	3	1	2	8	7	2.12-	4.89**	N.S.
2	7	1	3	0	9	8	3.06*	4.16**	N.S.
3	11	3	4	4	12	16	2.71*	4.98**	N.S.
4	4	1	6	10	5	7	N.S.	3.19*	N.S.
5	8	2	7	8	11	8	2.29-	19.65***	N.S.
6	12	3	7	5	14	9	4.52**	12.80***	N.S.

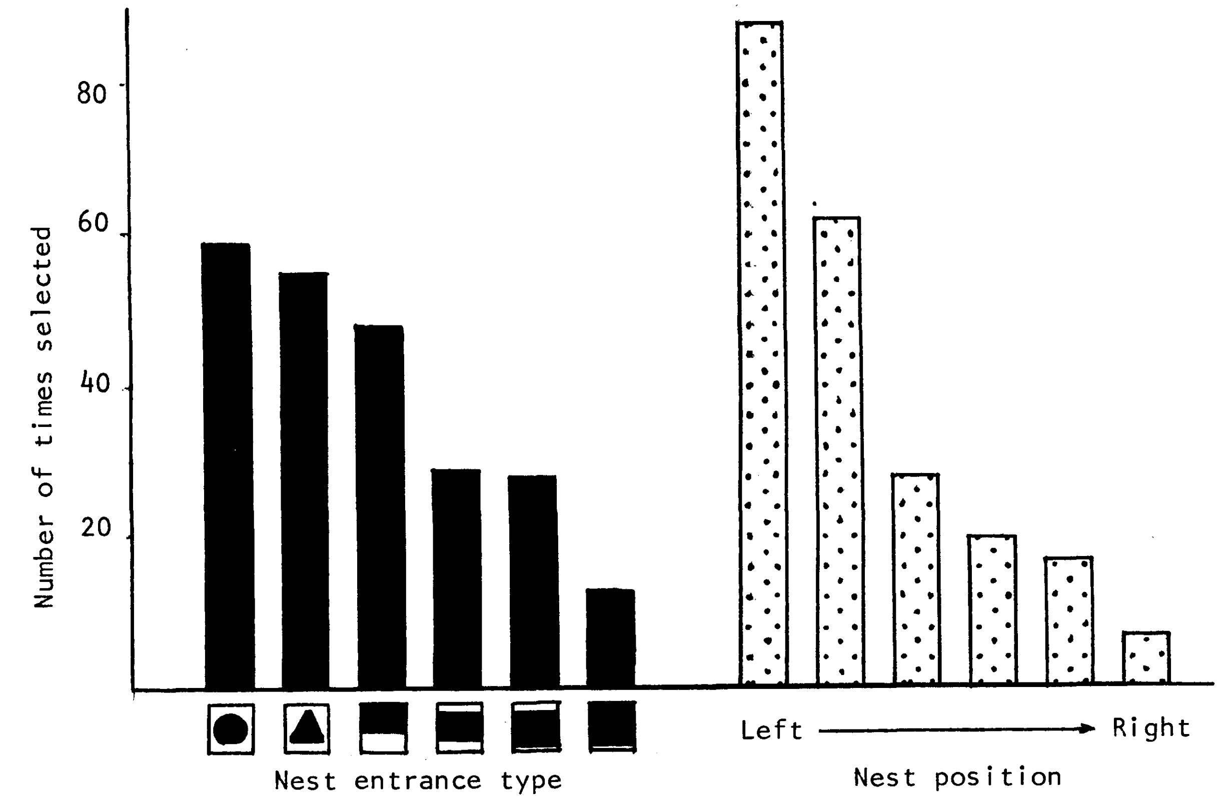


Figure 4.3.1b Number of times nests of different entrance type or different position in the nest-set were selected by broiler breeder hens

popularity of nests declined from left to right. The nests to the far right of the set were those closest to the door of the shed and the front work bay in the shed. No difference in light intensity was recorded for any one nest alternative in any of the positions in the set.

Upon partitioning of the total data relevant to each nest entrance type, it was found that the deep nest (15 cm base), the triangular and the circular types of nest entrances were used to the same extent. Hens used these alternatives for nesting to a greater extent than they did the two alternatives with upper 'lips', which in turn were used to a greater extent than the open nest with 3.5 cm lip ($\chi^2_{2df} = 40.6$***).

Light intensities in nests with different nest fronts varied somewhat. The darkest alternative was the 8 cm/10 cm nest option which recorded an intensity of 3 lux. Light intensity in the same nest-box with deep (15 cm), circular and triangular nest entrances was 4 lux, although some differences in intensity were found to exist in different areas of the nest, particularly in the case of the deep nest. Light intensities in the nest with 8 cm/3.5 cm and 3.5 cm lip entrances were 6 and 8 lux respectively.

Many hens in all three flocks did not use the provided nest-set at all, electing to lay on the floor. Positioning of popular floor-laying sites did not seem to be related in any way to popular nest positions in the nest-set.

Study 4.3.2

Responses to Nest Shape

This study was designed to investigate the possibility that hens may respond to the shape of the nests available.

Materials and Methods

Hens used in this study were the original feral fowl captured on North-West Island and housed in large deep litter pens in an isolation shed at the UNE (see General Materials and Methods). The study was commenced as the hens began to lay in their first spring (southern hemisphere) in captivity and was continued over four months.

Three nest types were made available to these hens. All were constructed so that they were of equal length (35 cm from the open end of the nest to the back of the nest) and offered the same area of opening at the entrance to the nest (vertical cross sectional area of nest - 529 sq. cm). Consequently, since the nest fronts were completely open, the volumes inside the nests were identical.

The three nest alternatives, termed cylindrical, cubical and triangular, are represented diagrammatically in Figure 4.3.2. All were made of sheet metal and litter was spread in the bottom of each to a depth of about 2.5 cm.

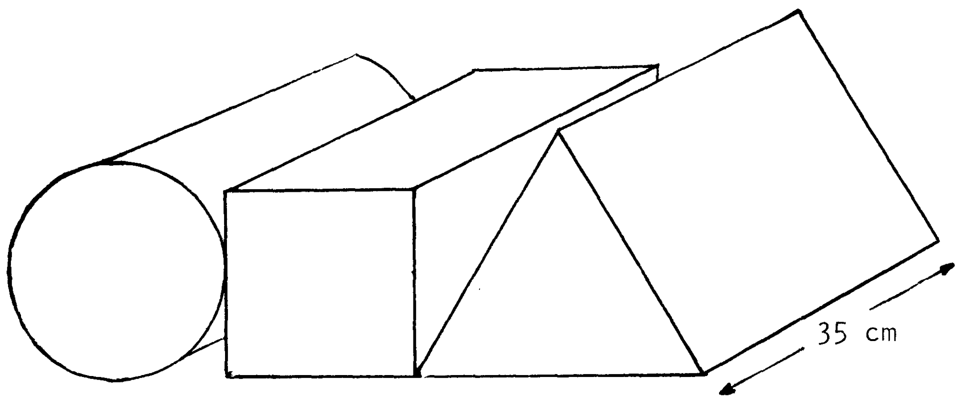


Figure 4.3.2 The three nest shapes offered to feral hens; left to right: cylindrical, cubical, triangular

All options were placed as a set in the middle of and against one end wall in each of five pens (named pens 1, 2, 3, 4 and 5). The positions of the three alternatives in the set were reallocated randomly each month. Therefore, four different combinations were present in each pen throughout the study period. Since the birds were housed in disease free conditions, and a long distance from the UNE, eggs were only collected and recorded every second day. Observation of the hens was only carried out on a casual basis on several days during the study period.

The numbers of eggs laid in different nests and on the floor during each month and for each pen were tabulated and analysed for trends in nest selection using Chi-square analyses.

Results and Discussion

Monthly nest usage data for each pen studied are shown in Table 4.3.2.

Marked differences were apparent in the number of eggs that would be laid in alternative nests in each pen during each month of recording. It was particularly interesting to note that the initial preference shown in most pens was for cylindrical nests in preference to any other. This tendency changed during subsequent months in all but pen 4. Preferences swung to cubical nests during the last two months in pen 2, from cubical nests to floor sites in pen 1 and from cylindrical nests towards floor sites in pens 3 and 5. Overall comparison

Table 4.3.2 Numbers of eggs laid in cylindrical, cubical and triangular nest alternatives and on the floor during each month period by 'feral' hens in each of five pens

Pen	Month	Number of Eggs Laid Cylindrical	Cubical	Triangular	Floor Sites
1	August	10	13	0	0
	September	3	27	0	7
	October	3	2	0	21
	November	0	0	0	16
	All Months	16	42	0	44
2	August	21	0	0	14
	September	27	7	0	14
	October	0	34	4	4
	November	0	17	0	1
	All Months	48	58	4	33
3	August	18	12	0	7
	September	17	3	0	8
	October	16	1	0	20
	November	8	0	0	8
	All Months	59	15	0	43
4	August	10	4	0	1
	September	24	8	0	0
	October	31	14	1	7
	November	20	0	0	1
	All Months	85	26	1	9
5	August	15	3	9	9
	September	40	0	0	4
	October	34	8	9	15
	November	4	1	7	39
	All Months	93	12	25	67
All Pens/Months		301	153	30	196

of the distribution of eggs between the three nest alternatives provided indicated that the differences between numbers of eggs laid in each were highly significant (χ^2_{2df} = 228.3***). Most eggs were laid in cylindrical nests, least in triangular nests, with numbers in cubical nests being intermediate. It should be noted that the original arrangement of nests in all pens was for the cylindrical nest to appear on the left hand side of the set, the cubical nest in the middle and the triangular nest to be on the other side of the set.

Analysis of the distribution of eggs between the three nest alternatives and floor sites in the different pens revealed that nest usage tendencies were not consistent for different pens (χ^2_{12df} = 225.6***). Of the three nest types, cubical nests were overall more popular in two pens, while cylindrical nests were most popular in the other three pens. Levels of floor-laying were markedly different in different pens also. Since only two or three hens were contributing data to the results of each pen, it may be possible to say that these differences between pens may be attributable to differences between individual hens.

Although observation of these hens was not possible throughout most of the study some general comments can be made. The behaviour of the hens when approaching oviposition was similar to that described for bantam hens in pens (see Study 3.4). The nesting phase was characterised by stealth and secrecy. Once settled on a nest these hens sat very tight and would allow the approach of the observer right to the entrance in some cases. Once disturbed on the nest, however, the hens would 'flush' from the nest and dash or even fly off in an extremely alarmed state. The behaviour of hens in the nest examination and nest entry phase gave no indication of how their preferences for nest alternatives were established nor of what stimuli they may have been responding to in their selection of a nest.

Study 4.3.3
Responses to Diverging/Converging Nest Types

This study was conducted in an attempt to evaluate whether hens will prefer to lay in nests which diverge from the entrance, or in other words appear to expand outwards in dimension towards the 'back' of the nest, or in nests which converge or get smaller from the entrance backwards.

Materials and Methods

Hens used in this study were 26 week old R x W strain birds which had been reared in deep litter floor pens at the 'Laureldale' poultry unit, UNE. For two weeks prior to the hens commencing production the 18 hens to be used in this study were moved into a deep litter floor pen, of 18.93 sq. metres area, in which they received a natural lighting pattern and food and water *ad libitum*. The two nest alternatives were provided at either end of a wooden nesting platform. This platform was 60 cm above the floor of the pen and 180 cm long. The 90 cm length of platform between the nests was blocked off with cardboard. The nests were accessible from wooden approach rungs running along the front of the nest platform. Hens seeking out a nest could walk along this approach and only come upon openings to a nest site at either end of the approach, these being the entrances to the two provided nest alternatives.

The nest alternatives offered to the hens were constructed of thick corrugated cardboard and were painted on the outside with a dark grey matt paint. The design of the nests is shown in Figure 4.3.3. Both nest types were

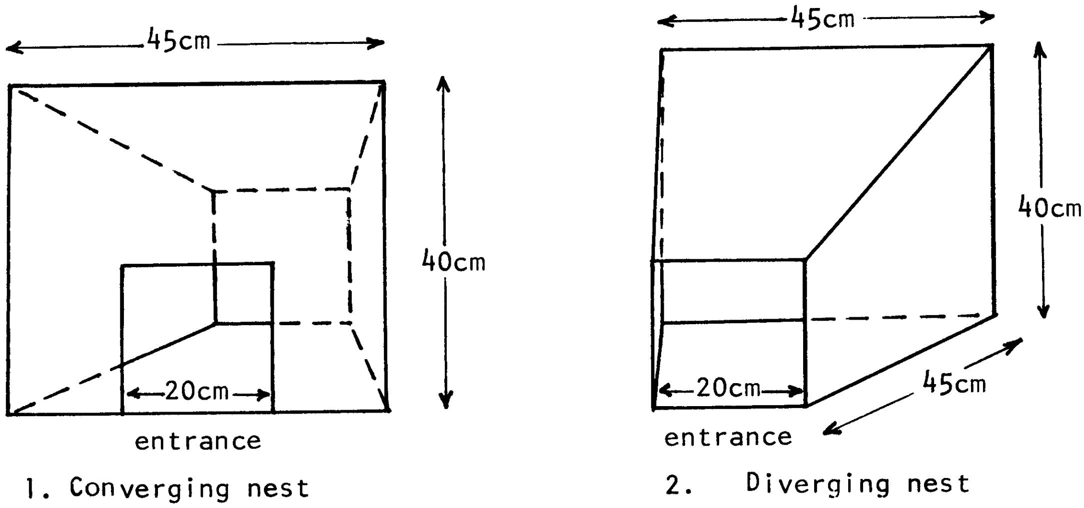


Figure 4.3.3 Converging and diverging nest types available to R x W hens

identical, except that the entrances occurred at different ends. In the converging nest, an opening the same size as that created by the entire front wall in the diverging alternative, was cut in the middle and the bottom of the front wall. Thus, hens approaching or entering either alternative were presented with the same sized aperture to look into or enter the nest. This precaution also meant that the amount of light that could be admitted into the nest was the same for both nest types. This was verified by measurement of light intensity in both nests.

Hens were allowed to lay in their home pen for several weeks to ensure that they were familiar with the nest approaches and nesting area before the two nest types were introduced into the pen. The two nests were alternated between opposite ends of the nesting platform each day. Nests were topped up with litter to a depth of 3 cm each day. After a two day familiarisation period the numbers of eggs that were laid in each nest type were collected and recorded daily. After eggs were collected the nests were swapped to opposite ends of the nesting platform.

The activities of hens at the nests were recorded during observation sessions conducted on the 10th, 11th and 13th days of the study. During these periods the nests that individual hens laid in were recorded. Hens were identified with coloured marking pens, on tail, back and wing areas and also by gross morphological characteristics, for the purposes of these studies.

Results and Discussion

The total numbers of eggs that were laid in the two nest alternatives and at either end of the nest unit, and the numbers of days that most eggs were found in either nest type or position are given in Table 4.3.3.

Table 4.3.3 Total numbers of eggs laid and numbers of days that most eggs were found in diverging and converging nests and in nests at either end of the nest unit

Number of Eggs Laid/ *Days Predominantly Selected*					
Nest Type			Nest Position		
Diverging	Converging	χ^2 Value	Left	Right	χ^2 Value
82	71	0.8 N.S.	75	78	0.1 N.S.
8	*6*	*0.3 N.S.*	*7*	*7*	*0.0 N.S.*

The hens did not lay in one nest type any more often than in the other, nor did they use either end of the nesting platform to a greater extent than the other overall. Observational records did, however, indicate some interesting trends. Of all the hens that were observed to lay during the observation periods, 11 were recorded to lay on all three days. Of these, four laid only in diverging nests, two laid only in converging nests and a further three laid all three times in either left nests only or right nests only. Two hens were found to lay in both diverging and converging nests and both left or right sites. It would therefore appear that although no overall preferences were established, individual preferences for either nest type or nest position existed.

General Discussion - The Shape of the Nest

The foregoing studies, although very limited, do serve to suggest several possibilities. Firstly, factors associated with the nest shape or entrance shape would not seem to be very effective in determining patterns of overall nest usage, at least within the limited range offered by the alternatives in these studies. It seems unlikely that nest entrance shape itself, for example, could be manipulated to affect nest usage patterns, particularly if one considers that position in a set of nests can have more wide ranging effects, as noted for broiler breeders in Study 4.3.1.

The lack of response to diverging or converging nests was interesting. One characteristics of nest sites is that they tend to be placed in junctions of some sort, for example in a corner or crevice created by walls or banks of vegetation. It is conceivable that hens could use a visual cue of such a 'closing in' illusion in selection of nests with these characteristics. A converging visual illusion might then be expected to create such conditions. Since it did not have such an effect in the present study, we can assume that the alternatives did not differ sufficiently to produce a differential response, that the alternatives did not converge or diverge to a sufficient extent to release such a response or that the nest-box provided sufficient stimulus value in that it already possesses plenty of corners and ends. Also, it may not matter whether the appropriate conditions are perceived as the hen looks into the nest or looks out from it. More likely, however, hens may simply not respond to such stimuli in the selection of a nest.

The observation that individual hens would differentiate between nest types and position in a set may relate to the fact that hens need to be able to identify their own nest specifically. It would not be adaptive for any hen which is going to incubate its own eggs to continue to search for, or continue responding to key stimuli for nesting in their environment beyond a certain point in their laying history. This could result in hens finding and nesting in new and 'better' nests each day and so the possibility of building up a clutch would diminish. This would possibly also result in huge and unmanageable clutches of eggs building up in sites that are particularly attractive to a number of hens, which is a condition infrequently seen in the wild except under conditions of very high population density (see Chapter 2). Hens may need to be able to respond to or 'recognise' some other characteristics that are specific to their own nest. In the somewhat barren environment provided by a floor pen, in which all the potential nesting sites are almost identical, factors like position and shape may serve as factors by which hens can identify the nests in which they have previously deposited eggs.

It is also possible that individual hens may respond to a wider range of factors associated with the nest than previously considered to be the case. This could function to ensure that hens in the one area do not all lay in the same nest or similar nest types. This could have disastrous effects on the susceptibility of the species to predation and also on the overall adaptability of the species. Whether responsiveness to minor factors such as position in a set, specific nest shape or other factors, for example colour (Hurnik, 1973a, 1973b), is an adaptation to avoid such problems or is merely an artifact is open to speculation.

The preference shown for cylindrical nests by feral hens may have come about for several reasons. Firstly, it is possible that this may have been some sort of positional effect, since all such nests were initially 'end' nests. However, triangular nests were also originally 'end' nests, admittedly at the other end of the set, and yet these nests were shunned. One fact that may have influenced the relative attractiveness of the cylindrical nests could have been the floor type that it provided. These nests had rounded bottoms, unlike the flat bases of the other two alternatives. Litter in these rounded bases tended to be held together by the inclining walls of the nest and may therefore have provided a suitable nest 'mould' for the hen. The rounded shape of the base itself may have served to keep hens sitting in the nest depression that it created. It would be worthwhile to repeat the trial using nests which all have similar flat or rounded tray bases fitted to see if this were the case.

The possibility that hens may, in fact, have been responding to the shape of the nest itself rather than to position or to floor type cannot be ruled out entirely. Doty (1979) reported a significantly greater use of provided conical shaped nests for nesting in wild duck when they were provided with open basket type, conical and cylindrical alternatives. It is possible that similar differences could exist for gallinaceous species, but the particular advantage that such nests would have is not known.

The tendency for feral hens to alter their nesting preferences through the season is of interest. It may have come about because of occupation of initially preferred types by brooding hens and a resulting migration of hens to other, unoccupied nest types. Certainly, a number of hens did become broody during the latter stages of the trial, but since these hens tended to follow nests which had eggs deposited in them, this is unlikely to be the complete explanation. Perhaps hens tended to abandon particular nests if they proved to be unsuccessful. Since all eggs were removed from the nests, all nesting attempts could be said to be unsuccessful. The eventual migration of hens from nest to floor sites in a number of pens may have been an avoidance of previously unsuccessful nest type. On the other hand, it is also possible that succession to other nest types normally occurs with these birds and functions as an anti-predator or hygienic device. Whatever the case, it is apparent that gallinaceous birds display a tendency to avoid previously used sites, except in adding to or completing current clutches.

Perhaps the most impressive response noted in these studies was for the first generation feral hens to nest almost entirely in one nest alternative in the nest entrance comparisons. The sitting behaviour of hens in these nest types suggested that the depth of the nest, and therefore the apparent concealment value of the site, may have been important. In the light of these observations, further experiments were designed to look at the responses of hens of different breeds, and of different sizes, to nest depth (see Study 4.4.4).