Studies On
The Energy Exchanges and Glycogen Levels
In
Laying and Non-Laying White Leg Horn Hens

A THESIS

Submitted to the Faculty of
Veterinary Science and Animal Husbandry
Magadh University
In Partial Fulfilment of the Requirements
for the Degree of
Master of Science (Animal Husbandry)

November, 1966

BY

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Bihar Veterinary College
Patna

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BIHAR VETERINARY COLLEGE
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P A T N A .

Dated the 30 November, 1966 .

This is to certify that the research work presented in this Thesis entitled " Studies on the Energy Exchangens and the Glycogen levels in laying and non-laying White-leg Horn hens" has been carried out by Shri N.S.Dravid , a candidate for the award of MASTER OF SCIENCE (A.H.) under my personal supervision and guidance and that it incorporates the results of his independent study .

(A. K. Ray)

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N.S. DRAVID

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STUDIES ON

THE ENERGY EXCHANGES AND GLYCOGEN LEVELS

IN

LAYING AND NON-LAYING WHITE-LEG HORN HENS

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INTRODUCTION

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CHAPTER-I

INTRODUCTION

GENERAL:-

Development of poultry has played an important role in solving the food problems of various thickly populated countries and one of the surest and quickest means of increasing the food production .

The total poultry population of India was 75 million in 1951, it increased to 97 million in 1956 and has further increased to 117 million according to the latest census report (1961) .

Eventhough the poultry population in India is 117 million, the number of birds per 100 persons is the lowest in the world .

TABLE NO.1

Table showing the distribution of number of chickens per 100 persons in India and other countries. Tulsaram (1965) Indian Livestock.

Sl. No.	Number of chiken per 100 persons	Country
1.	373	Canada
2.	286	U.S.A.
3.	179	U.K.
4.	540	Denmark
5.	552	Ireland
6.	150	European countries
7.	25	India .

Out of these 127 million birds only 45 millionn birds are hens with laying capacity of 60 eggs per bird per year while the world average is 130 eggs per bird per year . Even at this rate of production the poultry industry contributed to national income of Rs. 45/- crores in the form of eggs and meat and is expected to contribute of Rs. 100/- crores annually by the end of third five year plan .

The second poultry research conference, held at Poona in 1955, recommended that

1. As the egg production is the result of interaction between genotype and environment , the random sample laying test should be undertaken in different agroclimatic conditions .
2. Evolving Economic Poultry ration .

As the result of research work done on economic poultry rations, it is now possible to utilize industrial by-products in poultry feeds and thereby reduce the use of cereals in poultry rations .

A co-ordinated project for investing and evaluating the biological and biochemical values of poultry feeds should be taken up with a veiw of developing feed testing organisation in the country . Investigation should also be taken up quickly to obtain basic data and optimum requirements of energy, proteins minerals, and vitamins and antibiotic feed supplements under different conditions of productions .

In veiw of the above the study of energy matabolism is playing important role . The term metabolism in the brodest

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sense includes all the chemical reactions involved in growth, development, production, maintainance of the nutritive state of an individual . The problem of metabolism can roughly devided into two classes

1. Those that deal with the nature and extent of chemical reactions that occur in the body .
2. Those which deal with the changes in the environment in which these reactions occur and the influence of these environmental changes on the reactions .

Metabolism it-self can be divided into :-

1. Energy producing reactions which provide the energy for vital activities, these terminate in the oxidative combustion and ultimately be measured in terms of the oxygen consumed .
2. Operative reactions which contribute to these activities, the specific qualities and & direction which make them significant .

GENERAL OBJECTIVES :-

From the above mentioned points it was clear that a systematic study should be undertaken for the factors responsible for egg laying .Egg laying depends upon the metabolic activities. Metabolic activity is measured by energy output. Energy is required for work, for digestion, for assimilation of food-stuff and also to maintain body temperature. Since the energy expended to maintain body temperature is dependent on the temperature of environment . This factor could have profound influence on metabolic activity and thus on feed consumption and egg

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production in chickens .

The factors affecting the metabolic activities in laying and non-laying of same age, sex and breed can be summarised as follows :-

1. Environmental temperature, pressure and humidity .
2. Neuro-hormonal intrinsic factors
3. Feeding levels and composition of ration .
4. Quality of breeding stock
5. Management .

Gerhartzs (1914) reported that basal metabolism of laying hen is definately higher than that of non-laying hens

Dukes (1937) studied the energy metabolism in hen by Haldane's gravimetric method and observed that fasting metabolic rate during egg laying was greater in comparison with non-laying stage .

As the rate of metabolic activity is measured by oxygen consumption and carbondioxide and moisture production . It was decided to find out oxygen consumption and carbondioxide and moisture production in laying and non-laying birds .

After determination of oxygen consumption and carbon-dioxide production , the gaseous values are converted on N.T.P. basis and the respiratory quotient is determined. With the help of respiratory quotient and oxygen consumption the heat values are determined by referring a standard table prepared by Lusk (1924) .

As the liver is chief laboratory of body and also chief storage orgen for glycogen in the body which is playing

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the most important role in carbohydrate metabolism. The estimation of liver glycogen was undertaken in laying and non-laying stages. The objective underlined in determining the % of liver glycogen is to observe the rate of storage and deposition of glycogen also to study the various synthetic and destructive processes going on in the body during laying and non-laying stages. Side by side the feeding levels and composition of ration is also studied.

After liver, the next organ of storage of glycogen and seat of heat production is the muscles. The % glycogen of obliqus abdominal externus muscle is determined. As we know the % of muscle glycogen varies according to the nature of activity of that muscle. The energy required for muscle can be divided in to two parts.

1. Energy required for maintainee of the muscle.
2. Energy required to carry out the specific function or activity of that muscle. (Guyton 1965).

After determining the % of muscle glycogen ; the glucose utilisation and glycogen synthesis was studied with the help of Warburg's appearatus and by incubating the muscle at 40° C in the Kreb's Ringer Phosphate buffer (pH 7.4 and glucose level 157 mgm %). The idea behind this particular work is to study the energy utilisation and its synthetic and destructive use at tissue level.

This is a very simple attempt to know about the metabolic activities of laying and non-laying stages under Indian Climatic conditions.

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Before formulating the energy rations for laying and non-laying birds we must know their exact energy requirements, their rate of utilization and absorption of energy rich substances from intestinal tract; their rate of storage and deposition in the various parts of the body; their synthetic and destructive end products and their final expressions and achievements in the form of egg. If we can substitute energy rich agro-industrial by products, we will have to observe their mode of behaviour in the body whether they are really giving sufficient energy or not and simaltaneously their effect on egg production . By substituting these agro-industrial by-products we can save the humane consumable cereals and grains such as Maize, Gram, Oats, Millets etc. and that will give great food savings to the country .

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CHAPTER-II

RECENT STATE OF KNOWLEDGE REGARDING METABOLISM IN BIRDS.

CHAPTER - II

RECENT STATE OF KNOWLEDGE REGARDING METABOLISM IN BIRDS

The doctorine of the insesible perspiration as held by the hippocratic School was based upon the observation that the average adult does not gain in weight inspite of the fact that the total ingesta of food and fluid is in a definate excess of the total measurable excreta. (King 1924)

The history of metabolism is closely bound with that of respiration and the next epoch making work was that of Lavoisier (1780) who gave the name oxygen . He constructed a crude calorimeter by means of which heat eliminated was melted measured quantitatively through the amount of ice that was melted . Lavoisier convinced that the oxygen in the gaseous stage entered and unite with the carbon and hydrogen in the body resulted in the formation of carbon dioxide and water and in the production of heat .

The great value of Lavoisier's work however laid in his demonstrations of 3 foundamental principles of Basal metabolism :-

1. Exposure to cold increases the absorption of oxygen in the body .
2. Digestion of food increases the absorption of oxygen .
3. Physical exercise increases the absorption of oxygen .

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We know that an increase in oxygen absorption means an increase in heat production and increase in the total metabolism of b-ody . Lavoisier was under the false impression that oxygen decomposed some fluid in the lung causing the liberation of hydrogen and carbon with subsequent oxidation of these elements and their excretion as carbondioxide, water in the expired air . Scientists of later date abandoned a theory of oxidation in the lungs and favoured the blood as a site of these chemical changes The belief was strengthened by the discovery of gases in the blood by Magnus in 1837 . Later it become known that oxidation occured in the tissues and blood simply carried the gases to and from the lungs .

Under the radiating influence of Lavoisier in 1842 Liebig proposed the theory that carbohydrates and fats are united with the air in the body and portain is the source of urinary nitrogen.

The name of Carl Voit is associated with the great advances in the study of metabolism and gas exchanges Voit made the important discovery that muscular exercise did not increases the protein metabolism and from that his pupils . Lusk who studied the analysis of oxidation of mixtures of carbohydrates and fats (1924) Rubner (1883) who determined the fuel values of food stuffs ; and by means of respiratory exchange was the same as that obtained by the direct measurement of the heat given off by the body .

In 1883 , Rubner demonstrated the relationship between the surface area of the body and the heat production; thus

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providing a basis for the comparison of the metabolism of different individuals .

In 1894 calorimeter had advanced to the point at which it was possible to compare the results obtained through direct calorimetry and indirect calorimetry .

In 1915 Dubois devised the most satisfactory method for estimating the surface area of body and later published normal standards of heat productions for males and females . According to him surface area (A) in square centimeters = $Wt.^{0.425}$ X $Ht.^{0.75}$ X 71.84

Where | Wt = weight in kilogrames
| Ht = height in centimeters

In the animal calorimetry, the elaboration of this simple form of calorimeter has culminated in the construction of the Atwater,Rosa,Benedict Respiration calorimeter with this apparatus the heat of the body is determined indirectly from the respiratory exchanges and directly by a careful computation based on the observations of all the known means of heat loss (1905)

The principle of this apparatus was applied by Armsby in the elaboration of calorimeter for use with farm animals . Benedicts and associates (1905) also devised a respiration chambers for use with domestic animals . Respiration calorimeters have provided the means of aquiring most of the present day knowledge of energy metabolism . They have been extremely valuable in proving the accuracy of indirect calorimetry .

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He studied the effect of fasting on respiratory quotient; metabolic rate . 50 tests were undertaken on white plymouth rock hens, aging 10-13 months old average respiratory quotient is 0.74 by the end of first day fasting and 0.70 by the end of second day fasting .

He studied the basal metabolism in white plymouth rock hens, 18 to 22 months old, weighing 1.95 to 3.89 kg (average weight 2.51 - 0.03 kg) eliminated 2.53 - 0.03 calories per hour per kg .

TABLE NO.-2

Table showing the percentage change in metabolism and egg production in white plymouth rock laying hens (age 18 to 22 months old) reproduced from Journal of Nutrition 1937)

Hen No.	Date	Weight	Cal./hr./kg	Change in metabolism	Egg production in the test period
2321	11.2.31	2.31	2.41	- 7%	11
		2.50	2.51		
2440	11.9.31	3.11	1.91	- 17%	13
	2.10.31	3.17	2.31		
1532	11.12.31	2.10	2.24		
	1.8.32	2.46	2.08	- 3%	-
	2.11.32	2.44	2.85	-37%	-

The metabolism tests forming the basis of above data were made over a period of several months. Records of the egg production of the flock were kept during that time; since several of the hens laid fairly well in which their metabolic rates were determined .

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In the above article Dukes concluded that :-

1. The average B.M.R. of matuare hens after a fast of 24-30 hours duration was not far from 2.4 calories/hour/kg and 32.4 calories per square meter area per hour or 57.6 calories/kg/day and 778 calories/squaremeter/day . The average respiratory quotient is 0.73 .
2. The basal metabolism is lower in older hens .
3. The heat loss due to vaporization of moisture(basal) varied from 12 to 25 % of the total heat loss and averaged to 19 % .
4. Basal insensible loss and basal metabolism showed a positive correlation .
5. Egg production was accompnied by a small increase in the Basal metabolism .

C.F. Winchester in 1940 studied the lability of metabolic processes in laying hen . During a period of 1 month, frequent measurements were made of normal heat production, heart rate, respiratory quotient, body temperature and body weight of 4 laying new hampshire hens (age 2 years old). Variation in heat production were as large as 40 % of the maximum rate measured and variation in the heart rate were equally great . In general the curves of heat production and heart rate were tend to rise and fall . The results indicate that in making seasonal studies of metabolic processes in laying hens, either very frequent measurements of small number of hens should be taken or less frequent measurments of fairly large

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group of hens are necessary to minimise the influence of individual lability on group average .

C.F. Winchester studied the seasonal metabolic rhythms in domestic fowls in (1940) . His research is concerned with seasonal matabolic rhythms particularly in relation to egg production. Because of lability of heat production is relatively large in laying hens (Winchester 1939), group of 11 to 15 hens were used in this studies to minimize the influence of the lability of individual data on group averages .

1. Periodic measurments were made of fasting heat production,resting heat production,respiratory quotient and body temperature of new hampshire pullets which were hatched in February,March and April and began to lay in autumn and laid heavily in winter .

2. The curve of fasting heat production ascended as the egg production increased and reach its peak 2 months prior to achiev maximum egg production . Heat production began to decline several weeks before the decline in egg production. From which it appears that decline in metabolism with the latter decline in sex activity .

3. Although heat production appeared to be inversly related to environmental temperature, during the larger part of production year, the fact that low heat production coincide with low environmental temperature when egg production was also low indicates that heat production is not mearly function of environmental temperature but it is influenced by other factors such as velocities of energy exchanges in the body .

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4. The production cycle depends upon the sex rhythm and endocrine activity .

Barrot and Pringle in 1946 studied the energy and gaseous metabolism of the Rhod I s-land Red chickens from hatch to maturity as affected by temperature . He observed R.I.R. birds, 12 weeks old weighing 1030 gms consumed 1.1 to 0.98 millilitres of oxygen/hour/gram of live weight . R.I.R. birds having the age 23 weeks old, weighing 1960 grams consumed 1.c.c to 0.70 c.c. of oxygen/hour/gram of live weight .

TABLE NO 3

Table showing carbondioxide elimination of chickens at various environmental temperature (as per Borrot and Pringle, 1946) (reproduced from Journal of Nutrition 1946.)

Temperature °F	Average weight in grams 1030	1610	1960	2430
	C.C.of CO_2 / hour / gram of live weight .			
70	0.69	0.51	0.47	0.44
75	0.66	0.51	0.47	0.44
80	0.64	0.52	0.48	0.45
85.	0.64	0.52	0.48	0.46
90	0.63	0.53	0.49	-
95	0.64	0.53	0.51	-
100	0.66	0.54	-	-

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TABLE NO 4

Table showing the heat elimination of chickens at various temperature (Barrot and Pringle 1946) Journal of Nutrition 1946 .

Temperature	Average weight in grams			
oF	1030	1510	1960	2430
	Calories /hour/ kg of body weight .			
70	4.25	3.15	3.00	2.75
75	4.10	3.10	2.95	2.75
80	4.00	3.10	2.25	2.80
85	3.99	3.15	3.00	2.85
90	3.90	3.25	3.10	2.90
95	3.95	3.40	3.20	3.00

In the exhaustive treaties on Basal energy metabolism Brody States (Bioenergetics and growth Brody 1945)." We refer to minimum energy cost of the automatic body processes representing the excess of endothermic over exothermic reactions as the Basal metabolism . Energy used in circulation,excretion secretion,respiration accounts for 25 % of the cost; the balance being required for maintaining muscle tone and body temperature .

Basal metabolism has been measured for animals of many different sizes and form such data it is clear that

1. The basal heat production is affected by body weight
2. The metabolism of small animals is greater than that of large animals per unit of body weight .

Theoretical consideration suggest that basal metabolism

is related to the surface area of the body by which heat is eliminated out and if surface area might be factor then 2/3 power of body weight (i.e. $W^{2/3}$ or $W^{0.66}$) is a better index of surface than to the weight of first power (i.e. W^{1}). However we must note that exterior body surface is not a constant in living animals; nor can be measured satisfactorily, and hence the basal heat loss are dependent on the external body surface consequently we may conclude that the relation between surface area and basal metabolism is not direct expression of cause and effect. Rather we should consider that W^{n} is a measure of physiological effective body size or metabolic size and that value of exponent 'n' should be determined from the data in question.

The relation may be expressed mathematically as follows:

$$Y = a \times x^{b}$$

or

$$\log Y = \log a - b \log x$$

Brody states that the direct control of metabolic curve does not reside in the external surface but in neuro-endocrine system which tends to vary in size with surface area rather than the simple body weight. So it comes out that the size of neuro-endocrine components, the surface the heat dissipation and heat production all tend to vary in parallel. They may all said to vary with W^{n} and the value of n tends to be near 0.7 . It will be shown presently that the quantity of milk energy production and egg energy production likewise tends to vary with $W^{0.7}$ ha whith may be termed physiological

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weight in contrast to W^1 which is physical or gravitational weight it is suggested that $W^{0.7}$ be tentatively adopted as a reference base for basal energy metabolism, endogenous nitrogen metabolism, milk energy production and egg energy production and related processes .

In observing metabolic changes in growing chicken H.H. Kibler and S.Brody (1946) gave the calories eliminated/day in Rhod Island Red birds of different ages under fed and fasting conditions .

T A B L E N O 5

Table showing the metabolic changes in growing chickens reproduced from J. of Nutrition, Kibler and Brody(1946)
* Starred indicates 24 hours fasting birds .

RHOD ISLAND RED FEMALES

Age in months	No.of observation.	Body weight in grams	Calories/ day	Calories/sqm per day
1.	2.	3.	4.	5.
2-3	16	679	78.8	1001
2-3 *	16	631	59.4	794
3-4	14	1152	104	913
3-4 *	12	1048	83	778
4-5	6	1602	139	992
4-5 *	9	1479	109	834
5-6	9	1724	139	917
5-6 *	3	1319	109	872
6-7	6	1784	156	1008
6-7 *	4	1679	134	906

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1.	2.	3.	4.	5.
7-8	9	1925	149	913
7-8 *	9	1822	106	674
8-9	9	2123	176	1003
8-9 *	4	1864	112	705
9-10	7	2054	172	-
9-10 *	8	1944	131	-

In 1962, Huston, Cotton, Carmen studied the influence of high environmental temperature on the oxygen consumption of mature fowls . Individual oxygen consumptions records were taken for 5 adult male and 5 adult female white leg horn breed . It was observed that at 90° F W.L.H. males consumed 190 ml. of oxygen per hour per pound of body weight while in females it is 242 ml. of oxygen/hour/pound .

At variable temperature W.L.H. males consumed 259 ml/ hour pound while in females it is 236 ml. per hour per pound . The oxygen consumption for birds held at variable temperature was statistically higher than that of the birds held at high environmental temperature .

They used a vertical spirometer to obtain oxygen consumption and also duration of test period is 10 minutes .

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TABLE NO 5A

Table showing the comparison between 3 oxygen measurment trials on the same hens taken at intervals over a period of 2 months . Data by Huston,Cotton,Carman,Poultry Science, Vol.41 1962

Treatment and breed	Millilitres oxygen consumption/hour pound of body weight			
1. High temperature(90°F)	Trial 1	Trial 2	Trial 3	Average
a) White leg horn	203	253	266	241
b) New hampshire	171	156	174	167
c) White plymouth rock	133	197	217	182
2. Variable temperature				
a) White leg horn	342	200	288	277
b) New hampshire	159	182	215	185
c) White plymouth rock	267	247	205	239

In 1942 Golden and Long studied the absorption and deposition of glucose in the chicks by cori's technique . A rate of 400 mgms of glucose per hour per 100 grams of body weight is obtained. This rate appears to be constant over a 4 hour period and does not seem to be affected by moderate differences in concentration of administered glucose . After 4 hours of absorption the liver glycogen has risen to a level of 6 % ; while muscle glycogen rose to about 1300 mgm % . 12 % could be accounted for increased of liver glycogen but not more than 8 % was estimated for deposition in muscle .

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TABLE No 8

Table showing the percentage deposition of liver and muscle glycogen with the volume of concentration of administered glucose . Reproduced from American Jaurnal of Physiology, Golden and Long (1942)

Absorption time	No. of birds	Liver Glycogen mgm %	Muscle Glycogen mgm %	% Absorbed glucose as liver glycogen.	Volume of concentration of administered glucose .
1. Hour	9	1448 ±112	915±54	7.4 %	5 c.c. of 30 %
2 Hour	8	3494 ±296	1150±57	11.8 %	5 c.c. of 43 %
3 Hour	14	4397 ±176	1344±115	11.3 %	10 c.c. of 35 %
4 Hour	13	5362 ±223	1306±118	12.3 %	10 c.c. of 40 %

Golden and Long in 1942 studied the effects of small doses of insulin and cortical extract on liver and muscle glycogen of the fasted chicks . They found that small doses of insulin (i.e. 1 to 5 units/kg/day) depresses the % of liver and muscle glycogen .

Riddle and Opdyke (1942) studied the effect of large doses (80 units/kg) reported elevated blood glucose and liver glycogen in 24 hours after last injection .

Riddle and Coworkers (1937) reported that adrenal cortical extract (ACE) injected into normal, hypophysectomized , thyroidectomised, adrenalectomised doves and pigeons increase blood sugar level by 15 % seven hours after injection. Likewise in the fed or fasted chickens, ACE (8 to 11 c.c.) administered

over an 8 hour period increases significantly blood glucose and liver glycogen but has no significant effect on muscle glycogen.

Smalie and Henry (1933) studied the glycogen formation in the Barred plymouth rock chicks weghing 100 to 200 grams. The average value for liver glycogen is 0.126 % - 0.029 in males while in 15 females it is 0.136 % - 0.045 fasted for 24 hours.

Murray and Rosenberg (1953) reported that, no detectable glycogen remained in the livers of 6 weeks old new homshire chicks when they are fasted for 24 hours. When they were fed cracked yellow corn the liver glycogen concentration increased gradually. After 7 hours corn feeding the average liver glycogen % of 12 cockrels was 6.07 %. Other cockrels concerrently fasted for 24 hours and then fed low grade sugar showed a faster rate of glycogen deposition after 7 hours on low grade sugar, 12 cockrels had on and average liver glycogen concentration of 7.75 %.

Saxena,Jensen,Mc Ginnis (1962) observed the influence of Raw Soyabeans on liver and muscle glycogen content of chicks. The Chicks (3 weeks old) fed raw soya bean meal showed 596 mgm % while fed autoclared soya bean meal showed 2600 mgm % liver glycogen.

Nakatani and Gotoh (1961) studied effect of fasting on liver glycogen content of 10 W.L.H. cockrels, age 80 to 90 days old, of average body weight 1009 grams. The levels are determined before and after fasting for 18, 24, 48, 72 hours. Liver glycogen decreased rapidly to 394 - 57 mg per 100 gram of fresh material at 18 hours from the initial value of 2174 - 165 mgm.

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The minimum was 137 - 36 mgm at 24 hours and thereafter a rise to 228 - 47 mgm at 72 hours. Muscle glycogen was 539 - 38 mgm per 100 gram of fresh material at start and decreased slightly at 18 hours and reached minimum of 319 - 27 mgm at 24 hours. It then recovered to 501 - 46 at 48 hours .

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CHAPTER-III

ENERGY AND GASEOUS METABOLISM

CHAPTER - III

ENERGY AND GASEOUS METABOLISM OF THE LAYING AND NON-LAYING WHITE LEGHORN HENS.

INTRODUCTION

Metabolic activity is measured by energy out put . All the energy displayed by the animal body i.e. heat,mechanical , electrical energy comes from the oxidation of food-stuffs i.e. carbohydrates, proteins, fats . When the energy producing food stuffs are oxidised into the body, the latent or potential energy locked up in their molecules is released and serves to supply the animal with its energy requirements . Heat is produced by the oxidations in the active protoplasm of the body-muscles by the and glands, making up the greater parts of the active tissue , and are therefore principal seats of heat production. The liver because of its active metabolism and large size is next chief organ in the body responsible for heat production .

When carbohydrates, fats or proteins are oxidised or burned outside the body they yield heat energy or calories . The number of calories produced by the oxidation of 1 gram of pure carbohydrate is 4.1 and for 1 gram of fat 9.3 . This is also true when oxidised in the body because the end products are carbondioxide and water . One gram of protein when oxidised in the body yields approximately 4.1 calories but about 25 % more than this when oxidised outside the body (Rubner 1885). Thus proteins are not completely oxidised into the body and protein nitrogen in the form of urea and mainly uric-acid in the

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bird is excreted in the urine . This energy is therefore lost to the animal .

Other sources of energy are combustible materials in the feces and increase heat production which occurs when eating. Calorigenic effect of food is not fully known but it is believed to be due to extra-pesistalsis; secretion,excretion,necessiated by ingestion and metabolism of food (Lusk 1922)

Calorigenic effect of proteins is due to specific dynamic action which is related to deamination of amino acids . Oxidative deamination is accompnied by large heat incriment and transamination by small heat incriment.(Sadhu and Brody 1947) .

The total or gross energy of food is the amount of heat energy produced upon complete combustion of it . The amount of heat energy utilized by the animal, apart from the calorigenic heat represents the net energy of food (Armsby 1923)

By determining the amount of heat energy eliminated by the body, this is outgoing of energy and knowing the net income of energy it is possible to determine the gain or loss of energy by the body (Armsby 1923)

Heat production or metabolism can be determined by measuring the oxygen consumption and production of carbondioxide. Indirect calorimetry is based on the fact that normally the consumption of oxygen and production of carbon dioxide are closely correlated with heat production .

The increase rate of heat elimination in laying indicates the increase metabolic activity which depends upon the activity of thyroid gland .

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Skanes (1949) reported that metabolic rate may be considered to be an index of impact of thyroid hormone on the body cells. The serum protein bound iodine is an index of hormonal level proper . The excretion of iodine reflects the glands activity for iodine .

The thyroxine secretion level of laying pullets varies with the rate of production (Brooker and Sturkie 1950) . The rate for pullets laying two eggs in sequence averaged 10.85 micrograms of thyroxine per day, for pullets laying four eggs in sequence 13.75 micrograms per day .

Cruickshank (1930) reported that thyroid weights in fowls shows a marked seasonal variation. The weight being greatest from January to March (0.13 grams) and minimum from mid March to Jyly (0.085 grams) . Iodine content of the thyroids was found to vary with the weight of organ .

Turner, Irwin,Reineke (1945) who fed thyroprotein at 3 different levels to white leg horn hens during their second year of production and reported an increase in number of eggs laid. The three groups of birds received 5,10,20 grams of thyroprotein per 100 pounds of feed. The rates of egg production for the 3 groups of birds for the period were 38.1 , 40.6 and 30.7 % respectively as compared to 22.6 % . for the controls . Most of the increased production in these experiment occured during summer months when normally thyroid activity is at minimum .

The activity of the thyroid is governed by thyrotropic hormone secreated by pituitary . Therefore it also indirectly

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acts on the process of egg laying . Opel and Nalbandov (1961) reported growth of ovarian follicles in hypophysectomised laying hens by daily intramuscular injections of mammelian follicular stimulating hormone , pregnant more serum and chicken anterior pituitary powder . The mammelian gonadotropins caused a pronounced increased in the number of follicles entering the initial phase of yellow yolk depositiong but did not support growth of follicles to mature size . With timed sequence of ovulation inducing hormone substances up to 5 consequitive daily ovulations were obtained .

The number of ovulations was limited by the ability of follicles to attain ovulable size and was independent of the type of gonadotropins used to stimulate follicular growth .

The gonadotropins of chicken pituitaries differ qualitatively from mammelian hormones. Chicken Anterior piluitary contain thyroid stimulating hormone and adrenocortical tropic hormone and it is possible that these hormones play a role in Yolk deposition (Taber 1958)

b) <u>Factors affecting rate of metabolism in laying and non-laying stages:-</u>

In veiw of the abovecommon factors which are aggregately acted upon the rate of metabolism in laying and non-laying should be studied .

1. <u>F O O D</u>

Because of the calorigenic effect of food the composition of ration should be considered .

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TABLE NO 7

Table showing the composition of poultry mash fed during the experimental period for W.L.H. laying and non-laying hens. (period-March,April,May,June, 1966).

Ingredients	% incorporated in mash	Proteins %	Fats %	Corbohydrates %
Maize	50	11.11	4.39	82.56
Ground-nut-cake	25	40.00	6.00	40.00
Wheat bran	15	11.39	1.79	76.98
Fish meal	5	70.00	4.00	5.00
Bone meal	2	-	-	-
Minerals	3	-	-	-
Terramycin 5	100 Kg of feed mixed with 100 grams of Terramycin - 5			

Mallon,Hills,Dukes (1952) reported that chickens fed high energy diet have higher metabolic rate than a chicken fed low energy diet .

Here in this case the average feed consumption per day in laying birds is 4 ounces while in non.laying it is 3 ounces .

2. AGE AND GROWTH :-

The metabolism of the chickens of various ages has determined by a number of investigators . Here the experimental birds are 6 to 10 months old and the age of maturity under Indian condition is about 6 to 7 months .

3. EFFECTS OF ENVIRONMENTAL TEMPERATURE :-

In northern India it is extremely hot during May and June, 1966 and birds greately suffer from hot and dry winds. The experimental temperature range is from 33°C to 39°C.

4. INDIVIDUAL VARIATION AND ACTIVITY:-

Mieghton and Hutchinson (1940) reported the effect of different types of activity on metabolic rate in hens. He stated that

i. Metabolism in standing position is 40 to 50 % higher than in the sitting position.

ii. Streching of neck in any direction so that feathers are separated increases metabolism as much as 20 %.

iii. Crowing produces a momentary increase in metabolism.

iv. In rising from sitting to standing position metabolism may go up to 200 % but this increase is momentary.

v. When the birds goes to sleep with head underwing metabolism drops to 12 % and remains at this levels.

In order to minimize the changes in metabolism due to activity factor in the present experiment; the determinations were made for the period of 2 hours. Generally it is observed that the birds in the animal chamber have got tendency to sleep or sit comfortably. Only at the time of keeping the bird in the metabolic chamber they have got tendancy to struggle, but that struggle will lasts only for 5 to 10 minutes, up to recording the weight of chamber with bird; then afterwards bird will adipt to particular surroundings.

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For observing the different activities of the bird a glass window was provided to animal chamber , but some mischevious birds found pleasure to strike against the glass window with beack . Then the towel is covered from out side surrounding the window to minimize the particular activity .

5. SEASONAL METABOLIC CHANGES AND EGG PRODUCTION :-

Winchester (1940) made a detail one year study of metabolism of birds at different rates of laying . He concluded that heat production increases steadily 2 months earlier to reach peak egg yield .

Here the experimental work is carried out in 2 seasons i.e. spring and summer . Actually peak egg yield is getting in the month of February and March . The egg yield is appreciably low during summer months (May and June and July)

a) Principle of working

The amount of heat produced in the body can be calculated from the gaseous metabolism as determined by some of forms of respiration apparatus .

In the present experimentation Haldane's modification of Pattenkoffer method is adopted. The apparatus consist of animal chamber with an ingoing chain of absorber bottles, an outgoing chain provided with absorber bottles for moisture and carbondioxide and a succétion pump . Air entering the animal chamber is made free of water vapour and carbondoxide by passage through sulfuric acid and soda-lime or caustic alkali . The outgoing air contains vater vapour and carbondioxide derived from

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the animal . The carbondioxide is determined directly by the gain in weight of carbondioxide absorber bottle of the outgoing chain . The oxygen consumption,indirectly, by the gain in weight of the water and carbondioxide absorbers less the loss in weight of the animal and chamber i.e. insensible loss .

The oxygen determination is based on the long known principle that the insensible loss of an animal equals the weight of gaseous outgo minus the weight of gaseous income . Since it is assumed that carbondioxide and water vapour are the only gaseous excreta of significance and the gaseous income is oxygen . It is evident that

$$\text{Insensible loss} = (H_2O - CO_2) - O_2$$

$$O_2 = (H_2O - CO_2) - \text{Insensible loss}$$

d) Materials and methods:-

Throughout the experiment, white leg horn laying and non-laying birds were procured from Government Central Poultry Farm Patna belonging to Pen.No.5 . The birds are selected from Pen No.5 in order to minimize the genetic variation. Birds belonging to Pen No.5 , having the age of 6 to 10 months old, are healthy, active,alert,consuming the same ration mentioned above .

The laying birds are selected in a such manner that their laying stage is confirmed from Farm authorities, also the laying bird is kept under observation one day prior to metabolic trial. During the 24 hours observation the bird must lay down one egg as per the laying sequence . The laying stage is also confirmed after sacrificing the animal by observing the size and

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shape of the developing follicles and subsequent stages of developing ova .

The bird usually collected at 10 A.M. from the Farm and kept under observation in laboratory, during that period provided with plenty of fresh water and food (which has got same composition as Farm .) From 2 P.M. the feeding was stopped and fasting was continued through-out night . In early morning hours at 9.00 A.M. next day the bird was ready for metabolic trial. A uniform fasting of 20 to 24 hours was continued. During fasting adlib quantity of water is provided .

Reagents and procedure:-

Absorber bulbs, of concentrated sulphuric acid and 50 % potassium hydroxide are prepared .

1. Sulphuric acid bulbs :-

In a conical flask about 70 c.c. of pure concentrated sulphuric acid (Bengal chemical) is slowly poured. The flask is provided with rubber cork fitted with inlet and outlet glass tubes. The inlet glass tube is sufficiently long to dip into sulphuric acid. The outlet tube is sufficiently high about 6 to 7 cms from the level of sulphuric acid. The rubber cork is fitted to the flask with the application of vasseline. 3 such bulbs are prepared for each experiment . At the end of each experiment concentrated sulphuric acid is discarded and fresh bulbs are prepared .

2. Caustic alkali bulb :-

50 grams of potassium hydroxide (E.Merk) is dissolved in 100 c.c. distilled water . The solution is filtred and kept

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over night . Out of that solution 70 c.c. is transferred to conical flask having rubber cork inlet and outlet tubes just same as described above. The cork is properly fitted to flask by applying vasseline .

Here in the first few trial experiments 20 and 30 % potassium hydroxide solutions are used but these solutions are leaving their water content when they are connected with sulphuric acid bulbs and the results for carbondioxide production are going abnormally high .

Soda-lime (Indian-Anchor) has also been tried in few trial experiments but due to the presence of impurities in soda-lime , it could not absorb the total amount of carbondioxide .

So finally 50 % strength of potassium hydroxide is fixed for experimental procedure .

Two such bulbs are prepared for each experiment. At the end of each experiment the 50 % potassium hydroxide solution is discarded and fresh bulbs are prepared at each time .

An animal chamber is prepared locally having the diamensions of 9" x 12" x 13" made up of thinner tin sheet which is airlight, water proof provided with one side glass window to observe the activities of bird and also with a provision of air inlet and outlet . A chamber is cleaned properly prior to start the experimental work .

Prior to starting each experiment the absorber bulbs are numbered as a follows :-

No. 1 bulb	50 % potassium hydroxide solution
No. 2 bulb	Concentroted sulphuric acid .

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PHOTOGRAPH NO-1.

Photograph showing the experimental procedure for determination of geseous exchange Animal chamber with bird, outgoing absorber bulbs no.3 , no. 4 , no. 5 in front and ingoing chain of absorber bulb no.1 , no.2 in the back side all connected series with electrical sucction pump .

No. 3 bulb — Concentrated sulphuric acid

No. 4 bulb — 50 % potassium hydroxide solution.

No. 5 bulb — Concentrated sulphuric acid .

The initial weight of all the bulbs are recorded then the weight of empty thorougly cleaned animal chamber is recorded. Then the bird is kept inside the animal chamber and intial weight is recorded. The difference between empty chamber and chamber with animal will give weight of the animal .

The series of connections are made as follows:-

Bulb No.1 (50 % KOH) with its out let joined with rubber tubing with the inlet of sulphuric acid bulb No.2 . The out let of sulphuric acid bulb No.2 is attached with the respiratory inlet of animal chamber. The outlet of animal chamber is attached with the inlet of sulphuric acid bulb No.3 . The outlet of H_2SO_4 bulb No.3 is attached with the inlet of bulb No.4 (50 % KOH) and so on upto bulb No.5. The outlet of bulb No.5 is attached with electrical suction pump. An electrical vaccum pump is kept in order to evacuate expired gaseous waste products from animal chamber and it also helps to circulate air with giving maximum comfort to bird. The speed of the electrical vaccum pump is kept at minimum so that rate of bubling from bulb No.1 to No.5 is kept at slow speed and sufficient time will get to absorb the moisture and carbondioxide in the respective bulbs. The experiment was ran for the period of 2 hours and the temperature and pressure during that time are recorded . The final weights of all the bulbs, the chamber including and excluding the bird are recorded. The initial weight and final

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weight difference of bulb No.4 will give carbondioxide produced by the animal . The oxygen consumption is computed by finding out the insensible loss as already stated above .

No feed or water has been provided to the animal between 2 hours test period .

e) Consideration of Subjective and Objective errors in metabolic trial technique .

Subjective errors are due to:-

1. Abnormal respirations, insufficient rest, restlessness nervousness are likely to give high values .
2. Faulty recording of duration of activity .
3. Technical difficulties in measuring the metabolic cost of an activity .

Now a days in human beings a training is imparted to the persons undergoing such metabolic tests, but that is not possible in birds .

In order to minimize the errors, the following precautionary measures can be adopted :-

i. In order to get average value the duration of experiment is kept for 2 hours.

ii. A record is kept of activity during the trial .

Any restlessness or nervousness is avoided by giving utmost attention for the comfort of bird. The cause for particular activity should be removed off. In this experiment it was observed that birds found pleasure to hit on glass window with the beak or leg. The glass window is covered from outside with towels and they stopped this habbit .

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Objective errors are due to :-

1. Due to leakages:-

For checking air leakages in the whole circuit a test should be made by connecting all series and after starging suction pump the equal number of bubling should come out from all the bulbs from No.1 to 5 and if some leakage is there then the bubling is not uniform and equal . Each joint and fitting should be tested for leakage. Rubber tubing and joints should be lubricated and fixed properly with the application of vasseline and checked repeatedly .

2. The defects and faults of animal chamber :-

The faults can be corrected by taking the 2 or 3 successive records with the same bird. The respiratory inlet and outlet should be checked . The door of the animal chamber should be closed tightly with the help of screws .

3. Inlet air is coming with sulfuric acid fumes and causing respiratory distress in bird. The effort should be made to keep the outlet of sulphuric acid bulb sufficiently high and also adjusting the rate of suction so that bubling fumes could not reach up to inlet .

4. Clesning of animal chamber :-

After completing each experiment the animal chamber should be clesned properly in order to remove droppings,feathers dung etc. Otherwise few grams of dung stuked to the floor of animal chamber will spoil the next experiment. Usually the floor of animal chamber is first clesned with dry cotton and blotting paper and finally by duster. The chances of errors are thus

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minimized .

f) Basis of calculations :-

The gaseous values obtained by experimental data i.e. grams of oxygen consumed and carbondioxide produce/hour/kilogram of body weight are converted on normal temperature and pressure by using following equation :-

$$\frac{P_0V_0}{T_0} = \frac{P_1V_1}{T_1}$$ Combination of Boyle's and Charle's law

V_1 = To be found out(in litres/hr/kg)

(1 gram of gas at 273° Absolute temperature and 760 m.m. pressure occupies 22.4 litres volum) Avagadro's law .

Here P_0 - 760 m.m. pressure

V_0 = 22.4 grams of CO_2 or O_2/hr/kg

T_0 = 273° Absolute temperature value

T_1 = Experimental room pressure in m.m.

V_1 = To be found out in question .

T_1 = 273 - Experimental room temperature in centigrade .

After obtaining volumes of O_2 consumed and CO_2 produced respiratory quotient is computed by following equation

$$\text{Respiratory quotient} = \frac{\text{Volume of Carbondioxide produced}}{\text{Volume of oxygen consumed .}}$$

By obtaining the values of respiratory quotient and oxygen consumed per hour per kilogram of body weight the thermal values (i.e. calories eliminated/hour/kg of body weight) are computed by referring standard table prepared by Lusk (1924) .

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TABLE No 2

Table showing weight of the birds, oxygen consumption, carbondioxide production, room temperature, room pressure, respiratory quotient, calories eliminated per day per kg of body weight and total calories eliminated/day in white-leg Horn laying hens. fasted for 20 to 24 hours, age 6 to 10 months old.

Sl. No.	Weight of the animal in grams	Oxygen consumption per hour per kg of body weight.		CO_2 produced per hour per kg of body weight.		Room temperature ^0C	Room pressure in inches	Respiratory quotient	Calories/day/kg of live weight	Total calories eliminated on total live weight.
		O_2 in gms	O_2 in litre at N.T.P.	CO_2 in gms	CO_2 in litres at N.T.P.					
1.	1328	1.02	0.8195	0.993	0.735	35	29.3	0.892	93.35	124
2.	1915	1.02	0.8195	1.07	0.6254	35	29.4	0.763	93.05	177
3.	1796.2	1.11	0.895	1.086	0.6385	34	29.2	0.713	107.3	191
4.	1597.5	1.192	0.9562	1.12	0.6589	36	29.3	0.860	111	176
5.	1532.5	0.900	0.7526	1.02	0.5898	34	28	0.825	84.01	128.6
6.	1648	0.98	0.7873	0.874	0.571	34	29.3	0.818	90.90	149.3
7.	1328	1.28	1.076	1.23	0.752	34	28	0.700	118	155.6
8.	1397	1.00	0.8231	1.06	0.627	34	29	0.764	93.87	130
9.	1796	0.9474	0.6047	0.913	0.540	34	29.3	0.88	71.1	127

TABLE NO 2

Table showing weight of the birds, oxygen consumption, carbondioxide production, room temperature, room pressure, respiratory quotient, calories eliminated per day per kg of body weight and total calories eliminated/day in white-leg Horn non-laying hens. fasted for 20 to 24 hours, age 6 to 10 months old.

Sl. No.	Weight of the animal in grams	Oxygen consumption per hour per kg of body weight.		CO_2 produced per hour per kg of body weight.		Room temperature ^oC	Room pressure in inches	Respiratory quotient	Calories day/kg of live weight	Total calories eliminated on total live weight.
		O_2 in gms.	O_2 in litre N.T.P.	CO_2 in gms.	CO_2 in litres at N.T.P.					
1.	1108	0.9090	0.7695	0.8500	0.5257	36	28.0	0.748	87.07	96.33
2.	1308	0.9060	0.7827	0.8840	0.5530	36	28.0	0.706	88.53	115.71
3.	1278	0.9384	0.7160	0.7829	0.7700	36	29.2	-	-	-
4.	1494	1.0000	0.8420	0.9300	0.5719	39	29.2	0.700	94.00	140.44
5.	1525	0.9613	0.6437	0.8200	0.5244	35	28.0	0.810	74.00	112.77
6.	1663	0.9020	0.7370	0.9620	0.5586	32	29.5	0.759	82.00	136.00
7.	1061	0.8920	0.7060	0.8460	0.4870	33.0	29.6	0.700	76.00	80.24
8.	1394	0.8243	0.6913	0.7190	0.5516	33	28.0	0.790	77.00	107.00
9.	1228	0.8970	0.7133	0.8560	0.4930	33	29.5	0.700	78.00	95.763

TABLE NO 12.

Table showing oxygen consumption in laying litres/hour per kg of body weight in laying and non;laying W.L.H. birds (age 6 to 10 months old) fasting for 20 to 24 hours

Analysis of Data from table No. 8 & 9 .

Item	No. of observations	Mean	Standard Error	Difference between means
1. Oxygen consumption in				
a) Laying	9	0.8371 =	0.0404	0.1036 *
b) Non-laying	9	0.7335 =	0.0193	

Results-

* By running " t test " the difference between the means of laying and non-laying birds as regards to oxygen consumption is found to be significant. Calculated t 16 df at 5 % level = 2.16 while the tabulated value of t 16 df at 5 % level = 2.12

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TABLE NO 11

Table showing the calories eliminated per day per kg of body weight in laying and non-laying W.L.H. hens age 6 to 10 months old and fasted for 20 to 24 hours.

Analysis of Data from table No. 8 & 9.

Item	No. of observations	Mean	Standard Error	Difference between means
1. Calories eliminated in				
a) Laying	9	95.84 ±	4.78	13.76 *
b) Non-laying	8	82.08 ±	2.51	

RESULTS:-

* By running " t test " the difference between the means of laying and non-laying as regards the calories eliminated per day per kg of body weight is found to be significant. Calculated t 15 df = 2.476 at 5 % level as against the tabulated value of t 15 df = 2.13 at 5 % level.

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D I S C U S S I O N

From the table it is evident that oxygen consumption in laying group is 0.8371 - 0.0404 litres/hr/kg of body weight where as in non-laying group it is 0.7335 - 0.0193 litres/hr/kg of body weight. The carbondioxide value in laying group is 0.6267 litres/hr/kg of body weight in comparison to non-laying it is 0.559 litres/hr/kg of body weight. As the oxygen consumption and carbondioxide production are the representative values of the metabolic activities going on the body and metabolic activity is measured by energy out put . It can be concluded that in laying group, the average calories liberated per day per kg of body weight is significantly higher . The higher calorific value in laying group indicates the increased metabolic rate .

Metabolic rate is influenced by the activity of thyroid gland. Overactivity of gland elevates and under activity depresses metabolic rate removal of the thyroid depresses metabolic rate (Marvin Smith 1943) .

Winchester (1940) concluded that the seasonal rhythm of heat production is not simply effect of changes in environmental temperature, but that level of metabolism is influenced by egg production and other energy exchanges of the body.

Dukes (1937) reported that metabolic rate of high laying hens is slightly greater than that of poor laying hen.

Salter (1950) considered that the amount of circulating thyroxine (Protein bound iodine) as the best indication of thyroid activity .

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The thyroxine secretion rate of laying pullets varies with the rate of egg production (Brooker and Sturkie 1950). For pullets laying two egg sequences averaged 10.85 micrograms of D.L. thyroxine per day while for pullets laying four eggs in sequences 13.75 micrograms per day .

Turner (1948) studied the effects of season upon the thyroid secretion rate in 2 year old birds (white leg horn hens) in their second year of egg production. D.L. thyroxine secretion level ranging from 10.05 to 12 micrograms per day .

In relation with hypophysial control of ovulation, the gonadotrophic potency of the serum of immatuate males,immature females and non-laying hen is about the same and is higher than in laying hens and adult males (Bailey and Phillips 1952). One cubic centimeter of serum of non-laying hens contains 1 rat units.

Release of Luteinizing hormone (L.H.) from the chicken pituitary occurs six to eight hours before ovulation this was demonstrated by injecting pituitary extract and also by hypophysectomizing laying birds prior to ovulation (Hammond)

L.H. potencies in terms of ovulation units are 200, 25,17 units respectively for the pituiteries of males, non-laying and laying hens (Fraps 1943)

The ovulation inducing potercy of male pituitary is some 10-12 times that of pituitary from laying hens and about 8 times that of non-laying hens (Fraps 1943)

It might be concluded from the follicular responses to gonadotrophins that the ovulation is caused by the release of an ovulation inducing hormone (OIH) from the anterior pituitary

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body at a definate interval before actual ovulation. Evidence that this is so come from the results of hypophysectomy of regularly ovulating mature hens (Rothchild and Fraps 1949) . Release of OIH into the blood stream is believed to take place 6 to 8 hours before the ensuring ovulation, though evidence is not conclusive .

The over all effect of above 3 hormonal factors gives the clear idea regarding 13.76 extra calorific value per day per kg in laying group .

Values for O_2 consumption and for CO_2 production were computed on N.T.P. fasis for each experiment. The values for O_2 consumption and CO_2 production are closely in agreement with Barott and Pringle (1946)

He observed that R.I.H. birds weighing 1039 grams consumed 1.1 to 0.9 litres of oxygen, while birds weighing 1978 gms consumed 0.8 - 0.2 litres of oxygen/hr/kg of body weight while bird weighing 1030 gms produced 0.64 litres of carbon-dioxide and birds weighing 1610 gms produced 0.53 to 0.54 litres of CO_2/hr/kg at 95° F environmental temperature (Baroot and Pringle 1946) . He reported that the metabolic rate also increases at temperatures above that for minimal metabolic rate. This increase is due to extra effort entailed in panting to evaporate sufficient water from respiratory tract.

Baroot and Pringle worked on R.I.H. females fasted for 12 hours and he had not specifically mentioned whether his experimental R.I.R. females are in laying or non-laying stage .

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Huston Cotton carmon in 1962 observed the influence of high environmental temperature on oxygen consumption of mature fowl. He stated that O_2 consumption at 90° F is 219 millilitres per hour per pound in males where as in females it is 226 to 203 millilitres per hour per pound .

The above values are converted on kilogramme units and the comparison is made with the present experimental data .

The values for O_2 consumption (in the present experimental date) both for laying and non-laying are higher (i.e. 0.298 litres/hr/kg in laying and 0.194 litres/hr/kg in non-laying) than that of the values observed by Huston and Carmon. Of course they have not specifically mentioned the O_2 consumption for laying and non;laying W.L.H. female . Their technique is also different from present experimentation. They used a vertical spirometer to obtain O_2 consumption and also duration of test period is 10 minutes.

Berman and Shapiar (1965) studied a relation of fasting and resting metabolic rate in domestic fowl. They derived a fix formula for calculating oxygen uptake in resting and fasting state weighing the bird from 1 kg to 4 kg .

According to their formula O_2 uptake in litres/hr/kg

$$= 0.916 \text{ (body weight in Kg)}^{0.59}$$

(for resting).

O_2 uptake in litres/hr/kg $= 0.795$ (body weight in kg)$^{0.61}$

(for fasting)

The average values of weights for laying and non-laying are fitted into the fasting equation for calculating the O_2 uptake.

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The equation values for O_2 uptake/hr/kg is 1.0546 for laying while experimental value is 0.8371 - 0.0404 litres/hr/kg. The equation value for O_2 uptake 0.97 litres/hr/kg for non-laying while experimental value is 0.7335 - 0.0193 litres/hr/kg . The experimental lower values for both laying and non-laying can be attributed due to environmental temperature hread and age factors.

Kibler and Brody (1944) studied the growth and metabolism in R.I.R. chickens. According to their findings R.I.R. female birds aging 9 to 10 months weighing on and average 1944 grams fasting for 24 hours eliminated 131 calories per day. The data is converted on 1 kg basis and it was observed that 57.5 calories required per day per kg of ~~ats~~ live body weight .

Kibler and Brody calculated the heat production on the assumption that 1 litre of oxygen has a heat equivalent of 4.9 calories for the fed chicken and 4.7 calories for fasted chicken.

In the present experiment the average value for laying is 95.85 - 4.78 calories per day per kg and in non-laying it is ~~82.28x~~ 82.08 - 2.51 calories/day/kg body lot. The difference of 14.5 extra calories/day/kg (i.e. experimental W.L.H. non-laying female 82.08 calories per day per kg and Brody's R.I.R. female 67.5/day/kg can be attributed due to breed difference between W.L.H., and R.I.R. also due climatic and feeding variation. Also the values of respiratory quotient are estimated separately for each experiment. From the R.Q. values and O_2 uptake values in litres the respective calorific values are taken from standard table constructed by Lusk (1924)

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b) Theoretical consideration of metabolism and its application with the present experimental data :-

According to the law of Newton, Stephans and Boltzman ( ) the rate of cooling of body is proportional to its surface area. Now if the heat loss is proportional to surfaces, the heat production is also proportional to surfaces since in homeotherms heat loss is proportional to heat production .

The relation of heat production to linear size of surface area was first formulated by Rameaux and Sarrus . Metabolically effective body size and surface area :-

1. Surface area of living animal is not constant
2. Surface area as it relates to heat loss and changes with the environmental temperature but also by developing heat conserving and heat dessipation device (feathers, wool, fur etc.)
3. It is true that surface area varies with the 2/3 power of weight .

Now in the analysis of present data the question arose to what basis the comparison should adopt i.e. whether to express the result as a function of body surface of a live animal especially the birds are covered with the feathers. However it is very easy to obtain accurate weight .

As the metabolic activity depends upon the amount of active protoplasm in the living cells of the body and also it is closely associated with the neuro-endocrine system (Brody 1945)

In laying and non-laying white leg horn females the intrinsic neuro-hormonal factors are not clarified as yet .

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The calculations are undertaken as follows:

$$Y = a \cdot x^b$$

or expressing the above equation on exponential form

$$\text{Log } Y = \text{Log } a - b \log x$$

Where Y = Oxygen consumed in litres per hour

and x = Weight of the bird in kilogram.

'b' value is calculated by simple regression equation

$$b = \frac{XY - X \cdot Y/n}{X^2 - (X)^2/n}$$

b = 0.7861 for laying W.L.H. birds

b = 0.7150 for non-laying W.L.H. birds.

After calculating the b value by simple regression equation the X and $\bar{Y}$ values are converted on logarithmic number and power factor (b) is confirmed by using following equations:-

$$\text{Log } Y = N \log a - b \log X \ldots\ldots (1)$$

$$\text{Log } X \cdot \log Y = \log a \log X - b \log^2 x^2 \ldots\ldots (2)$$

By dividing the respective coefficients and substracting the equation (1) from (2) we can obtain b value.

A straight line regression equation is found out for laying and non-laying which was plotted on semilogarithmic graph.

$Y = 0.074 - 0.7861 x$ ..... For laying

$Y = 0.055 - 0.7150 x$ .....For non-laying.

$Y = .0740 - .7861 \times 1$

$= -.7121$

$Y = .0740 - .5722$

$= -1.4982$

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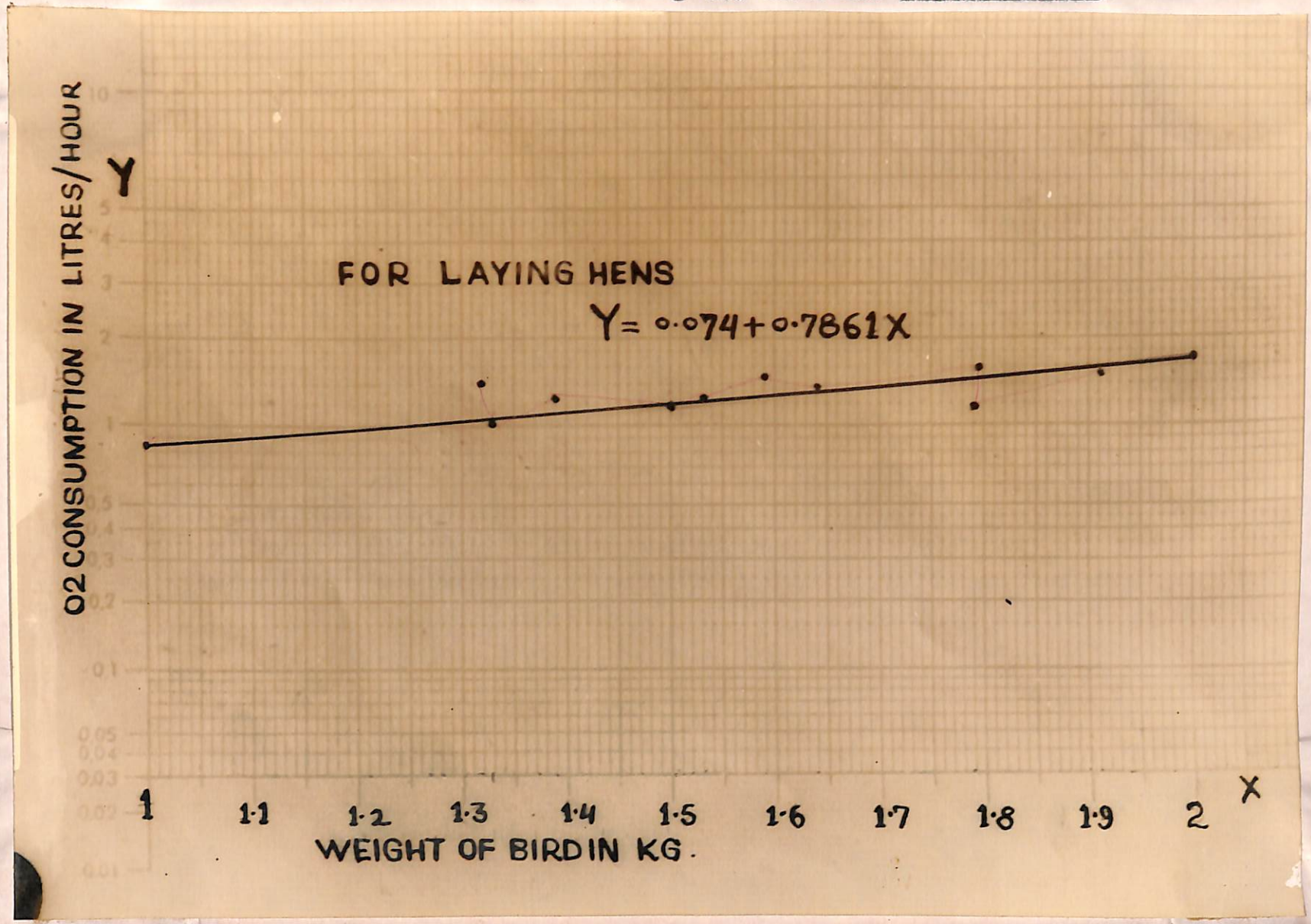
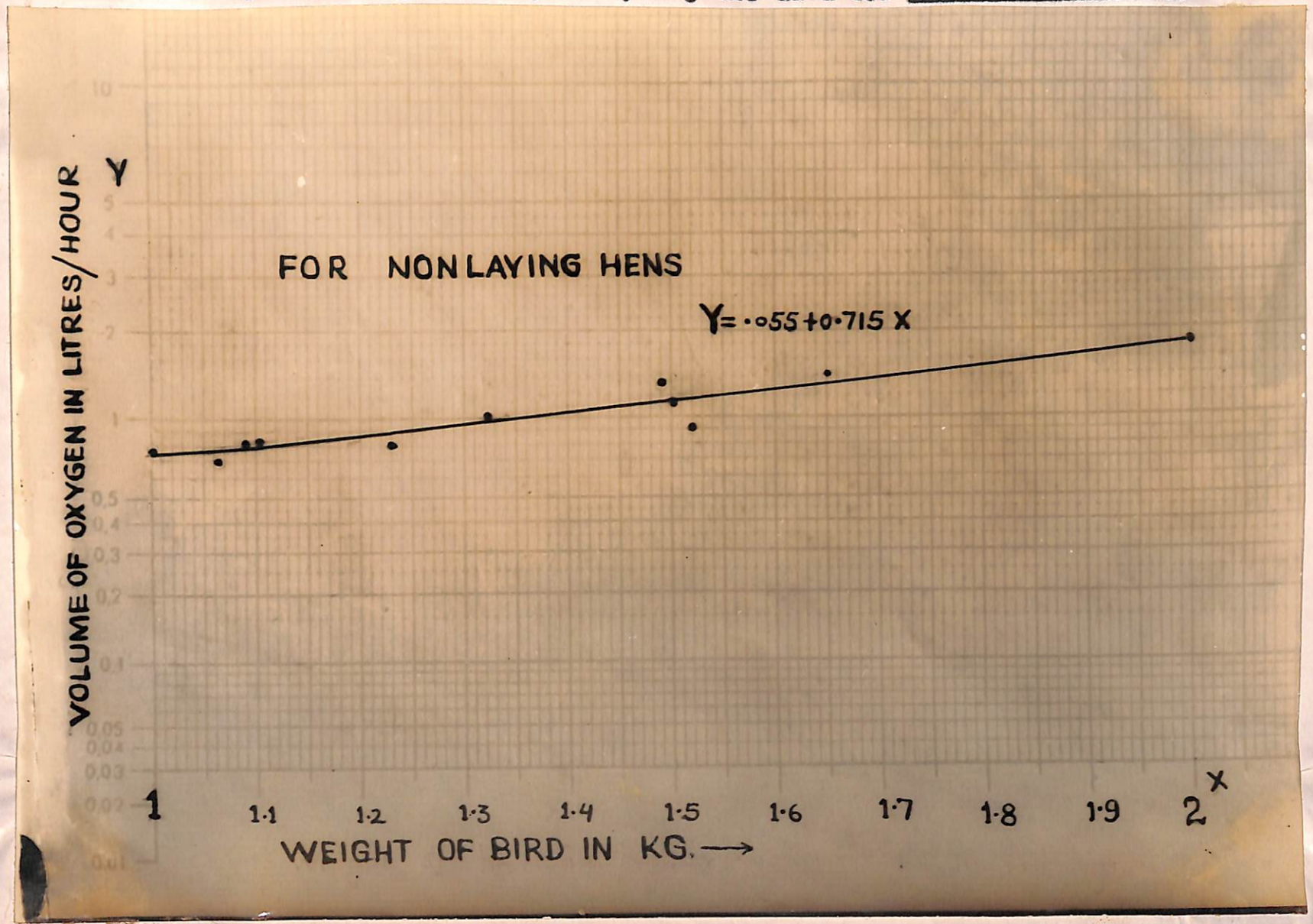
PHOTOGRAPH NO-2.

A semilogarithmic chart showing the weight of the bird in kilogram (on X axis) and Oxygen consumption in litres per hour per kilogram (on Y axis) a curve plotted by simple regression equation by analyzing the data for laying birds.



PHOTOGRAPH NO-2.

A semilogarithmic chart showing the weight of the bird in kilogram (on X axis) and Oxygen consumption in litres per hour per kilogram (on Y axis) a curve plotted by simple regression equation by analyzing the data for non-laying birds.



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CHAPTER-IV

LIVER GLYCOGEN

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LIVER GLYCOGEN.

a) Factors affecting the level of liver glycogen.

As cited in the previous chapter, the liver because of its active metabolism and large size is next chief organ in the body responsible for heat production. The present study is under taken to find out the quantitative variation and lability of liver glycogen in laying and non-laying stages .

To claude Bernard (1876) physiology owes the discovery of glycogen or animal starch and its function as form of stored energy. The molecular weight of glycogen is about 2000 . This peculiarity adapts its role as form in which carbohydrate may be stored in the cell when it is immediately available. Because of its large size it can not escape by diffusion through the cellular membrane and exerts an almost negligible osmotic pressure . By converting glucose to glycogen the cell is able to hold large amounts of energy .

Cori, Cori and Schmidt (1939) demonstrated the chemical reactions that take place as a blood glucose into liver glycogen. Glucose is first transported through the cell membrane of each liver cell and inside the cell it is synthesised under the influence of glucokinase into glucose phosphate then polymerised under the influence of phosphorylase into liver glycogen. The process is known as glycogenesis . The glycogenolysis is reverse process repeating in reverse order of chemical reaction and glycogen is converted into glucose .

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The glycogen is also formed from intermidiary process of carbohydrate metabolism, other organic compounds like glycerol odd chain fatty acids several amino acids (Cori and Cori 1929, Dakin 1913)

Deuel et all (1937) found that glycerol can be used by the liver to form glycogen .

Janney (1915) reported the glucose can be synthesised in the body from different proteins .

The rate of formation of glycogen varies with the glycogenic material upon which the organ has to work. The rate of glycogenesis can not be estimated merely from the quantities of glycogen found in the liver at a given interval after the administration of given amount of sugar, because the glycogen in liver is not determined by the rate of glycogenesis alone but by the relative rates of glycogenesis and glycogenolysis. When the sugars are given by mouth. The relative rates at which they absorbed is also important (Cori 1925) .

Murschhauser (1911) found the increase of liver glycogen causing after feeding 50 grams of glucose was rated as 100, the other sugars ranked as follows levulose 65,Sucrose 58, maltose 16, galactose 13, lactose 6 .

The rate of glycogen formation is not only dependent on the nature of glycogenic material but also condition of the animal when these materials ingested (Duel 1933)

The usual physiological conditions which deplete liver glycogen are starvation, exercise cold etc. Starvation alone may reduce the liver glycogen to less than 1 % but never cause its

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complete dissappearance when the exogenous supply is cut off. Tissue proteins are utilized to form glycogen (Macleod and Prendergast 1921).

The thyroid hormone accelarates energy production in the body and carbohydrate metabolism the relative rate of circulating thyroxine is definately high in laying than in non-laying(Brooker and Sturkie 1950).

Thyroxine accelarates the rate of absorption of sugars from gastrointestinal tract and also increases the rate of glucose utilization of cells (Guyton 1965).

The pituitary and insulin and other hormonal factors which are affecting the level of liver glycogen in laying and non-laying stages are not fully known. The relative levels of their secretions are also not clarified as yet.

b) Determination of liver glycogen.

For estimation of liver glycogen the method of Good, Krammer, Somogyi was followed (1933) which was based on the principle that glycogen content of the tissue is precipitated by alcohol from its homogenised condition in KOH solution and glycogen obtained is hydrolysed to glucose by N-Sulphuric acid and digested for 3 hours in boiling water-bath and then estimated as per the blood glucose estimation procedure followed by Hagedorn and Jensen method (1923).

Ferricyanide for quantitative sugar determination was introduced by Hagedorn and Jensen in 1923. If the glucose solution is heated in an alkaline solution for a definate time with known

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excess amount of potassium ferricyanide some of the ferricyanide i.e. $Fe(CN)_6^{3}$ is reduced to $Fe(CN)_6^{4}$. The reverse oxidative reaction taking place in the presence of oxygen in air is prevented by precipitation of ferrocyanide ion as a double potassium zinc sulphate in the presence of zinc sulphate . The excess of unchanged ferricyanide $Fe(CN)_6^{3}$ is then reduced by iodine solution in an acid medium to liberate iodine which is then titrated with standard thio-sulphate solution using starch as an indicator this back titration gives a measure of amount of ferricyanide originally reduced by the sugar of the solution. The chemical reaction in this process is as follows : -

$2 K_3 Fe(CN)_6 - 2 KI = 2 K_4 Fe(CN)_6 - I_2$ (Peters and Vanslyke 1958) .

Reagents : -

1. 30 % and 20 % KOH solution .
2. 96 % and 60 % alcohol .
3. N-Sulphuric acid .
4. 0.005 N-alkaline potassium ferricyanide .

1.65 grams of potassium ferricyanide and 10.6 grams of fused sodium carbonate are dissolved in water and made up to 1 litre . The solution was thus prepared and protected from sunlight .

5. Sulfate chloride solution .

The solution was prepared by dissolving 12.5 grams of zinc sulfate and 62.5 grams of sodium chloride in 250 of distilled water and filtred .

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6. Potassium iodide solution

15 grams of potassium iodide was dissolved in 100 c.c. of distilled water . The solution was kept in dark .

7. 0.005 N-Potassium iodate solution

This was prepared by accurately dissolving 0.1783 grams of water free potassium iodate (A.R.) in 1000 c.c. of distilled water . It was permanent solution for checking the strength of 0.005 N-sodium thio-sulphate solution .

8. 3 % Acetic acid solution

This was prepared by dissolving 3 c.c. of glacial acetic acid in 100 c.c. of distilled water .

9. 0.005 N-Sodium thio-sulphate solution

This was prepared by dissolving 0.7 gram of sodium thio-sulphate in 500 c.c. of distilled water this was approximate solution and its strength was checked by 0.005 N-KIO_3 solution which was exact in strength .

10. Starch solution

1 gram of soluble starch was dissolved in 100 c.c. of saturated solution of sodium chloride .

Chemicals of highest purity were used through out the experiment .

Analytical procedure :-

The liver sample for the analysis of liver glycogen was obtained from the sacrificed animal . Care was taken to take the liver sample within 2 to 3 minutes after the animal was sacrificed .

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200 to 250 mgm of liver tissue from the biggest lobe of the liver was blotted in the filter paper weighed accurately in torsion balance and dropped in 0.5 ml. of 30 % potassium hydroxide solution in 10 c.c. centrifuge tube. The centrifuge tube was corked with stopper in which a long tube was the inserted to act as a reflux condenser . After heating the centrifuge tube for 15 to 20 minutes which was shaken occassionally during the process of boiling, it was taken out and 8 c.c. of 96 % alcohol was poured into it and again placed in a water bath for a minute or two till bubbles. It was then taken out and kept at room temperature for half an hour . Afterwards it was centrifuged for 10 minutes at 3000 revolutions per minute; inverted and allow to drain on filter paper . After 10 minutes of drainage the mouth of the centrifuge tube was blotted with filter paper and precipitate in it was washed with 2 c.c. of 60 % alcohol and alcohol was again allowed to drain in the same way after centrifugation for 10 minutes .

For hydrolysis of glycogen, 3 c.c. of N-Sulphuric acid was added over the precipitate and then the centrifuge tube was corked with the same stopper containing the condenser and kept in boiling water bath for 2.5 to 3 hours, after which the contents of the centrifuge tube was transferred to 50 c.c. volumetric flask by washing the centrifuge tube repeatedly by distilled water . The solution so transferred to volumetric flask was neuterlised with 20 % potassium hydroxide solution using a drop of phenol-red as indicator and the volume was made up to mark .

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The sugar thus obtained by the hydrolysis of liver glycogen was estimated by Hagedorn and Jensen method for which 1 c.c. of the solution in volumetric flask was transferred in the test tube containing 2 c.c. alkaline potassium ferricynide solution and heated in a boiling water bath for 15 minutes; cooled under tap water and to it was added 3 c.c. of sulphate chloride solution and 0.5 c.c. of potassium iodide solution and 2 c.c. of 3 % acetic acid and then titrated with 0.005 N-sodium thio-sulfate solution using one drop of starch solution as an indicator.

Blank determination :-

A blank determination is performed with addition of all the reagents except the addition of sugar solution .

Standardization of thio-sulfate solution :-

A solution of 3 c.c. of sulfate-chloride solution and 0.5 c.c. of potassium iodide and 2. c.c. of 3 % acetic acid and exactly 2 c.c. of the standard potassium iodate solution is used. Thio-sulfate is added from the microburette untill most of the iodine has disappeared then one drop of starch was added and the solution was carefully titrated untill the red-blue color vanishes. The factor for the 0.005 N-thio-sulfate factor

$$\text{Factor} = \frac{2}{\text{c.c. of thio-sulfate}}$$

Precautions:-

The potassium ferricyanide solution must be added quantitatively from a calibrated micro pipette . The titration with sodium thio-sulfate was carefully performed using a calibrated micro-burette divided in intervals of 0.001 c.c.

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c)

Calculations for liver glycogen :-

The liver glycogen was calculated by running side by side blank experiment. The reading of the 0.005 N-Sodium thio-sulfate was substracted from blank and again multiplied by factor for correction . The corrected reading of 0.005 N-thio-sulphate will give the milligrams of glucose present in 1 c.c. of the sugar solution obtained by hydrolysis of glycogen by reffering the table prepared by Hagedorn and Jensen for blood sugar estimation(Peters Vanslyke 1958) and hence to get the amount of sugar in whole sample of liver tissue taken the above value was multiplied by 50 and hence the % of glycogen was calculated as milly grams of glucose per 100 gms of liver tissue .

d) Discussion :-

From the table number 13 and 14 it is evident that in the laying group the liver glycogen is 8200.9 - 170.5 mgm per 100 gms of wet liver tissue is found to be significantly higher than that of non-laying group (i.e. 6584.6 - 235.89 mgm per 100 gram of wet liver)

The rate of storage and release of glucose by the liver is influenced by the amount of glucose absorbed and by starvation and certain hormones (Sturkie 1954), glucose and glycogen may also formed in the liver from amino acids, lactic acid, pyruvic acid which are the products of muscle metabolism .

Certain fatty acids such as propionic acid and other containing odd number of carbon atoms and glycerol can also be converted into glucose and glycogen (Sturkie 1954)

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TABLE NO 13

Table showing the % of liver glycogen with its standard error in laying and non-laying W.L.H. birds (milligrams of glycogen per 100 gms of wet liver tissue). Birds fasted for 20 to 24 hours (Age 6 to 10 months old)

	mgm/100 grams of liver tissue .	mgm/100 grams of liver tissue .
Sl. No.	Laying birds .	Non-laying birds .
1.	9000	7160
2.	7190	6603
3.	8053	6500
4.	7890	6690
5.	8496	7575
6.	8580	5280
7.	8744	6424
8.	8392	6856
9.	7870	5434
10.	7794	7330
Average	8200.9 - 170.5	6584.6 - 235.89

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TABLE NO. 14

Table showing the % of liver glycogen (mgm of liver glycogen per 100 gms of liver tissue with its standard error mean and mean difference between laying and non-laying birds. Analysis of data from table No. 13 .

Item	No. of observation.	Mean	Standard error	Difference between the means.
% Liver Glycogen				
1. Laying	10	8200.9	170.50	1616.3 *
2. Non-laying	10	6584.6	235.89	

Results :-

* By running the t test the difference between the laying and non-laying birds as regards % of liver glycogen is found to be highly significant .

Calculated t 18 df at 1 % level

= 4.849 as against the tabulated t_{18} df at 1 % level

= 2.88

High liver glycogen content in laying stage can be attributed due to following reasons :-

1. During the experimental procedure it was found that the average feed consumption for laying group is 4 ounces per day while in non-laying it is 3 ounces per day .
2. It might concluded be that extra glycogen content in laying period indicates the extra reserved energy to cope up with the normal physiological act of egg laying which consist of ovulation, movement of ova through reproductive tract, formation of Yolk, albumen, deposition of shell membranes etc .
3. Ramanoff and Ramanoff (1949) studied the composition of egg it clearly indicates that egg contain 1.9 % carbohydrates and 32.6 % of fat .

It indicates that the percentage of carbohydrate in the egg is not appreciably high but % of fat is definately higher . The fat which is taking part in the formation of egg may be the resultant end product of carbohydrate metabolism in the liver .

4. The whole mechanism of egg laying indicates that it increases the muscle metabolism . The liver is a constant reservoir to store some additional energy in the form of glycogen which is showing significantly higher value in laying group .

The hormonal effect upon the % liver glycogen in laying and non-laying is not so clear .

Opdyke (1942) studied the effects of large doses of insulin on liver glycogen of fasted and non-fasted chickens.

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Large doses of insulin injected over a 1 to 4 days did not depress blood glucose or liver glycogen in the non-fasted chickens. Infact there was an increase blood glucose and liver glycogen in those birds receiving the larger doses suggesting again that the insulin stimulated the release of adrenal hormones which are responsible for rise. There is no evidence of increase of adrenal hormones in laying than that of non-laying .

After injecting 60 units/kg/day of insulin on third day liver glycogen was 6840 - 659 mgm % for non-fasted chicks whereas in control birds the level is 2550 - 195 . The weights of the birds are ranging from 250 to 400 grams (Opdyke 1942).

Riddle and Coworkers (1937) reported that adrenal cortical extracts injected in normal doves and pegeious increase blood sugar level by 15 % seven hours after injection.

Golden and Long (1942) reported the increase in blood glucose and liver-glycogen after administration of 8 to 11 c.c. of adrenal cortical extracts .

Rosenberg and Murray (1953) studied the glycogen deposition after feeding the cracked yellow corn in 6 weeks old new hampshire chicks. After 10 hours feed of cracked yellow corn, cockrels had on and average 7.75 % liver glycogen .

Golden and Long (1942) studied the rate of absorption and deposition of glucose from the intestinal tract in the chicks. He administered 5 c.c. of 30 % glucose and after 1 hour absorption time, liver glycogen was found 1448 - 112 mgm % . They observed 5862 - 223 mgm % liver glycogen after administration of 10 c.c. of 40 % glucose .

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Nakatani, Gotoh (1961) found 2174 - 165 mgm % liver glycogen in white leg horn cockrels aging 80 to 90 days having average body weight 1009 gms.

Wilson and Lewis (1929) pointed out that liver and muscle glycogen may vary according to the breed and environment.

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CHAPTER-V

MUSCLE GLYCOGEN

CHAPTER - V

MUSCLE GLYCOGEN .

a) Factors affecting the level of muscle glycogen :-

Muscles making up the greater part of the active tissue of the body and therefore principle seat of heat production. In view of the above an estimation of muscle glycogen is undertaken in laying and non-laying stages .

Muscle glycogen is chemically indistinguishable from liver glycogen (Young 1937) , but is much more stable in comparison with the liver glycogen . Glycogen in the muscle and other extra hepatic tissue is primarily related to the energy mechanism concerned with the metabolism and to carry out specific functions of these tissues. It is not a direct supply of glucose to the blood and other tissues.

Heat production is increased by muscular exercise in direct proportion to the amount of energy expended in the muscular activity .

Meyerhof (1919) and Hill (1924) found that muscle glycogen was broken down in muscular activity .

Mann (1925) found that only glucose and its polymers and glycogen could be utilized freely by the liverless animals. All tx other monosaccharides and other compounds and amino-acids must apparently transferred by the liver before they can be made into glycogen or oxidised in the tissues. The preparatory process consist of conversion to glucose, probably through intermidiary stage of glycogen(Mann 1925) .

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PHOTOGRAPH NO-4.

Photograph showing the site of collection of abdominal muscle for estimation of muscle glycogen content .

Variation of muscle glycogen during fasting and feeding condition was studied by Murray and Rosenberging (1963) . They reported that after 16 hours in fasted chicks, the muscle glycogen content was low i.e. 0.05 % while after feeding cracked yellow corn it was raised to 0.33 % .

b) Materials and methods :-

For the estimation of muscle glycogen the same analytical method is followed as in liver glycogen . Immediately after sacrificing the animal, a median longitudinal incison was given on the ventral surface of the animal just 2 inch below the sternum . Skin fascia and sub-cutanious fat was removed and about 250 to 300 mgms of " Obligus abdominal externus " muscle piece was weighed in torsion balance and immediately transferred to 0.5 c.c. of potassium hydroxide tube and the same analytical procedure was followed as per the liver glycogen up to the stage of N-sulphuric acid digestion . The contents are transferred to test tubes and then neuterlised with 20 % KOH using drop of phenol red as an indicator and then the final volume was made up to 10 c.c. by adding distilled water . The caculations were made as per the final volume .

c) Discussion :-

From the table No. 15 and 16 , it is evident that the muscle glycogen in laying group is 1275 - 41.99 mgms per 100 grams of muscle tissue while in non-laying it is 1213 - 63.89 . The quantita quantitative difference is non-significant .

The glycogen content of the muscle depends upon the type

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of muscular activity that is to be performed by that muscle . Murray and Rosenberg (1963) studied the glycogen content of breast muscle and lower thigh muscle . They have found that the breast muscle contains 0.33 % while in lower thigh muscle it is 0.23 % glycogen . It is clear that breast muscle and lower thigh muscle have got different type of activity . The type of activity depends upon the nature of work that is to be performed in the body .

In human beings it is observed that the total quantity of blood glucose is only 17 grams and the total quantities of stored glycogen averages 245 grams in the muscle and 108 grams in the liver (Guyton 1965) .

These are the entire stores of metabolically useful carbohydrates in the body and it can be calculated that at normal rates of metabolism all of these carbohydrate stores would be capable of supplying the energy value only for 12 hours(Guyton 1965).

Murray and Rosenberg (1963) found 0.05 % breast muscle glycogen in the chicks fasted for 16 hours while after 1 to 10 hours feeding it was raised up to 0.33 % .

In 60 white leg horn cockrels, aging 80 to 90 days old , of average body weight 1000 grams were studied before and after fasting for 18 , 24 , 48 hours , 10 birds at each time .Muscle glycogen was 539 - 38 mgms per 100 grams of fresh material at start . It decreased slightly after 18 hours and reached minimum of 319 - 27 mgms at 24 hours . It then recovered to 501 - 46 mgms at 48 hours (Nakatani and Gotoh 1961) .

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Golden and Long (1942) reported the % of muscle glycogen in the chicks weighing 250 to 400 grams the value at 0 hours fasting is 1093 - 34 mgms % while after 24 hours fasting it was lowered down to 773 - 20 mgms % .

From the above, it was clear that variability of % of muscle glycogen depends on : -

1. Fasting hours .
2. The type of muscular activity that is to be performed by the muscle .
3. The relative rate of glycolysis .
4. Rate of intermidiary metabolism .

The effect of moderate small doses of insulin (0.5 to 2 units/kg) has not got any effect on muscle glycogen (Golden and Long 1942) .

The effect of epinephrine on muscle glycogen of the fasted chicks was studied by Golden and Long in 1942 . They have reported that after injection of 0.5 mg/kg of epinephrine the muscle glycogen was 687 - 31 mgms % while in normal control birds it was 910 - 56 mgms % .

The different levels of hormones, their secretion, their power of activiting the muscle is not clarified in laying and non-laying stages as yet .

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TABLE NO 15

Table showing the % of muscle glycogen (in mgms per 100 grams of wet muscle) along with its standared error in laying and non-laying W.L.H. birds, fasted for 20 to 24 hours, age 6 to 10 months old .

	mgm/100 grams of muscle tissue .	mgm/100 grams of muscle tissue .
Sl. No.	Laying birds .	Non-laying birds .
1.	1383	1000
2.	1498	1104
3.	1333	1160
4.	1216	1104
5.	1159	981
6.	1046	1379
7.	1266	1457
8.	1364	1496
9.	1328	1239
10.	1158	-
Average	1275 - 41.99	1213 - 63.89

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TABLE NO 16

Table showing the % of muscle glycogen in laying and non-laying birds with number of observations , mean , standard error etc . Analysis of data from table number 15 .

Item	No. of observations.	Mean	Standard error.	Difference between the means .
% Muscle Glycogen				
1. Laying	10	1275.1	- 41.99	61.8 *
2. Non-laying	9	1213.3	- 63.86	

* Results - By running the " t test " the difference between the means of laying and non-laying birds as regards % of muscle glycogen is found to be non-significant .

Calculated t_{17} df at 5 % level = 0.1104 as against the tabulated value of t_{17} df at 5 % level = 2.11 .

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CHAPTER-VI

MUSCULAR ACTIVITY

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CHAPTER-VI

MUSCULAR ACTIVITY

Although in the investigation of muscle metabolism diaphragm tissue isalways studied because of its specialised function to carry out constant activity and it has been pointed out that with respect to oxidative metabolism diaphragm occupies the intermidiate position between constantly active heart muscle and intermittently active skeletal muscle (Peterson et all 1961)

As the diaphragm in the bird is rudimentary (Sisson and Grossman 1947) the obliqus obdominal externus muscle was preffered to study the muscular activity in laying and non-laying stages.

a) Synthesis of glycogen and glucose utilization :-

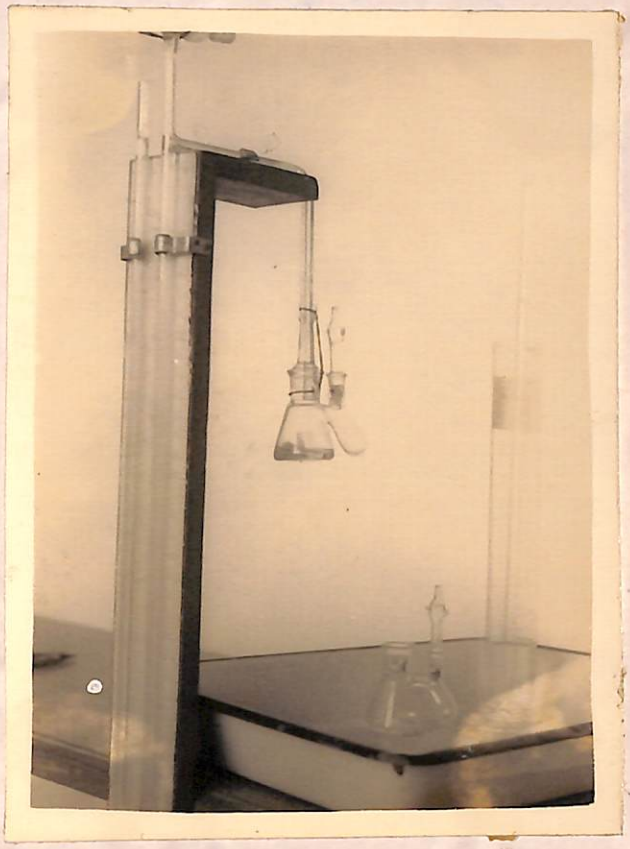
The bird was sacrificed by decapitation and obliqus abdominal externus muscle was removed by opening the skin and subcutanious fat and then immersed in ice-cold Kreb's Ringer Phosphate Solution buffer (pH 7.4) prepared according to Umbreit (1947).

Composition of Kreb's Ringer Phosphate Buffer Solution :-

1. 0.9 % Sodium chloride (0.154 M)
2. 1.15 % Potassium chloride (0.154 M)
3. 1.22 % Calcium chloride (0.11 M)
4. 2.11 % Potassium dihydrogen phosphate (0.154 M)
5. 3.82 % Magnessium sulphate 7 H_2O (0.154 M)
6. 1.32 % Sodium hydrogen carbonate (0.154 M)

PHOTOGRAPH NO-5.

A photograph showing the Warburg's manometer alongwith Warburg's flask contained 2.5 ml Kreb's Ringer Phosphate buffer solution (pH 7.4 glucose level 157 mg %) and a piece of abdominal muscle ready for incubation at 40° C .



7. 0.1 M Phosphate buffer pH 7.4 :- 17.8 grams of $Na_2HPO_4 2H_2O$ - 20 ml of Hcl dilute up to litre .

The above solutions are taken in the following proportion :-

100 parts of solution	1.
4 parts of solution	2.
3 parts of solution	3.
1 parts of solution	4.
1. parts of solution	5.
12 parts of solution	7.

Solution 6. is kept for adjusting pH at 7.4 . A proper pH is adjusted with the help of pH paper .

All the possible fat and connective tissues were separated and removed from the muscle piece . Each piece was blotted and weighed in torsion balance and then dropped in a Warburg's flask . Each Warburg flask contained 2.5 ml of Kreb's Ringer Phosphate buffer pH 7.4 with glucose (157 mgm/100 ml of buffer i.e. as per the blood glucose level of poultry) and 0.2 ml of 10 % (W/V) potassium hydroxide in central well .

Warburg flasks were attached to the manometers and gas phase was filled with air . The closed manometer was started for incubation at 40°C with 110 oscillations per minute . After 10 minutes of incubation muscle was removed from one flask quickly washed in distilled water and dropped in 30 % KOH for glycogen estimation . An aliquot of the buffer in the flask was taken for sugar estimation .

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Incubation at 40°C was continued in the other flask for 2 hours and then removed quickly, washed in distilled water and dropped in 30 % KOH solution and the procedure for its glycogen estimation was followed. An aliquot from the buffer in the second flask was also taken for glucose estimation .

Glycogen was estimated from the muscle by following the same principle as was undertaken for glycogen estimation of liver. Some modifications were however necessary for volume make up as the glycogen present in the muscle is comparitavely lesser in quantity than liver .

b) Discussion :-

From the table number 17 it is evident that mgm of glucose used per 100 mgm of wet muscle per hour is 0.1575 -0.0112 in laying which is significantly higher than in comparison to non-laying stage i.e. 0.1064 - 0.0009 .

From the table number 18 it is evident that mgms of glycogen synthesised per 100 mgm of wet muscle per hour is 0.1064 in non-laying while it is significantly lower in laying stage .

From table number 19 and 20 it can be concluded that glucose utilization of abdominal muscle is significantly higher in laying stage but as compared to that rela tive rate of glycogen formation is significantly lower .

It is definately clear that glucose is utilized by the tissue but it is not so effectively deposited in the form of glycogen . The relative rate of deposition of glycogen is significantly lower in laying . The glucose utilized is used to obtain immediate energy .

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Roherer (1924) was first to demonstrate in vitro that the oxygen consumption of minced tissue derived from animals which have been fed thyroid is greater than that of the tissues from normal animals .

The rate of utilization of glucose by muscle tissue depends upon the rate of oxidative processes in that particular tissue (Guyton 1965) . The oxidative processes of particular tissue depends upon the necessary energy required to carry out activity and to maintain normal metabolic reactions for survival of that tissue (Guyton 1965) thyroxine increases the rate of glucose utilization of cells (Guyton 1965) .

The relative rates of thyroxine secretion in pullets laying 2 eggs in sequences is 10.85 micrograms per day while pullets laying 4 eggs in sequences is 13.75 microgram per day . (Brorker and Sturkie 1950).

Shorr and Baker (1939) Stadie, Zapp, Lukens (1940) agree that the addition of insulin increases the consumption of oxygen and thereby utilization of glucose by minced pigion breast muscle .

Ray and Sadhu (1959) reported 0.163 mgm of glycogen synthesised per 100 mgm of wet diaphragm per hour by utilizing 0.318 mgms of glucose per hour per 100 mgm of wet diaphragm in rats .

Peterson,Beaty and Bocek (1963) reported that the addition of 1 milliunit/ml of medium increased the glucose uptake in skeletal muscle and diphragms from depancreatectomized

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rats . Insulin increased the glycogen levels (2 hours incubation) in diaphragm but not in muscle . The glucose uptake is 1.15 - 0.07 mg/gm/hr wet skeletal muscle while in diaphragm it is 2.1 - 0.11 .

So from the above it can be concluded that the glucose uptake and relative rate of glycogen formation and depostion is dependent on the several factors such as : -

1. Primarily the glucose uptake depends upon the nature of activity of the muscle .
2. The activity depends upon the rate of thyroxine secretion .
3. The glucose uptake and glycogen synthsis is also depends upon the insulin secretion level .

Stah, Pipes and Turner (1961) observed the thyroxine secretion rate (T.S.R.) in new Hampshire pullets at 26 to 28 weeks of age . The combined T.S.R. of the pullets ranged from 1.40 ug to 1.58 ug . The pullets showing rise in T.S.R. to a mean of 1.99 ug during egg production .

Huston and Carman (1962) observed the influence of high environmental temperature on thyroid size of domestic fowl. They observed in white leg horn females at 90° F the mean thyroid weight is 92.8 - 28.2 milligrams while at variable temperature it is 131.9 - 19.4 milligrams .

Nakajo and Imai (1961) reported that the weight of anterior pituitary during non-laying stage is 8.4 - 0.68 mg. while in laying stage 9.0 - 0.59 in white leg horn female birds.

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TABLE NO - 17

Table showing the milligrams of glucose utilized per hour per 100 milligrams of wet muscle in laying and non-laying birds. (Fasted for 20 to 24 hours, incubated at 40°C for 2 hours with buffer glucose level 157 milligrams %.)

	Glucose utilized in mgm/ 100/mgm wet muscle/hour	Glucose utilized in mgm/100 mgm wet muscle/hour.
Sl. No.	Laying birds	Non-laying birds
1.	0.150	0.095
2.	0.174	0.051
3.	0.180	0.103
4.	0.118	0.096
5.	0.120	0.132
6.	0.110	0.134
7.	0.145	0.152
8.	0.187	0.095
9.	0.221	0.100
10.	0.170	-
Average	0.1575 - 0.0112	0.1064 - 0.009

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TABLE NO 18

Table showing milligrams of glycogen synthesis by 100 milligrams of wet muscle per hour in W.L.H. laying and non-laying birds. (Fasted for 20 to 24 hours, incubating the muscle at 40° C in buffer glucose level 157 milligrams %).

	Milligrams of glycogen synthesised by 100 mgms of muscle/hour.	Milligrams of glycogen synthesised by 100 mgms of muscle/hour.
Sl. No.	Laying birds	Non-laying birds
1.	0.057	0.095
2.	0.060	0.051
3.	0.055	0.103
4.	0.020	0.096
5.	0.046	0.132
6.	0.030	0.152
7.	0.016	0.095
8.	0.031	0.134
9.	0.064	0.100
Average with standard error	0.0421 - 0.003	0.1064 - 0.010

TABLE NO. 12

Table showing milligrams of glucose used per hour per 100 mgm of wet muscle in laying and non-laying birds with Special reference to Standard Error, Mean number of observations. Analysis of data from table number 17.

Item	No. of observations.	Mean	Standard error	Difference between the means.
Milligrams of glucose used per hour per 100 mgm of wet muscle.				
1. Laying	10	0.1575	0.0112	0.0511 *
2. Non-laying	9	0.1064	0.009	

Results :-

* By running the t test the difference between the means of the mgms of glucose used per hour per 100 mgm of wet muscle in between laying and non-laying birds is found to be highly significant. Calculated t_{17} df at 1 % level = 225.37 as against the tabulated value of t_{17} df at 1 % level = 2.90.

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TABLE NO. 20

Table showing the glycogen synthesis per 100 milligrams of wet muscle per hour in laying and non-laying W.L.H.bird and its Standard Error, Mean etc. Analysis of data from table number 18 .

Item	No. of observations	Mean	Standard error	Difference between the means.
Milligrams of glycogen synthesised per 100 mgm of wet muscle per hour				
1. Laying	9	0.0421	0.003	Difference = 0.0643 *
2. Non-laying	9	0.1064	0.01	

Results :-

* By running the t test the difference between the means of non-laying and laying as regards the mgms of glycogen synthesised per 100 mgm of wet muscle per hour is found to be highly significant .

Calculated t_{16} df at 1 % level = 6.25

Tabulated value t_{16} df at 1 % level = 2.92 .

From the above, it can be concluded that as T.S.R. level is high in laying stage naturally glucose utilization is significantly high and also the glucose utilized is used for the increased oxidative rate to supply energy and therefore the glycogen formation rate is significantly lower in laying stage .

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CHAPTER-VII

SUMMARY AND CONCLUSIONS

CHAPTER NO - VII

SUMMARY AND CONCLUSIONS.

Obviously, before we can make any useful start in formulating energy rations we must have resonably clear working knowledge of what such energy rich rations must contain . This is also necessary for the precise understanding of physiological roles by which these substances are needed in metabolism both in laying and non-laying stages .

For many years investigaters are trying to correlate between energy rich nutrients in rations with that of metabolic studies. There are no finalizing figures that can be called energy requirements, because in feed we can substitute the proper energy rich substances but their fate is decided by rate of digestion, assimilation, utilization, destruction, decomposition etc. by the animal body .

The common factors affecting the level of metabolism is studied in laying and non-laying stages. The environmental temperature is playing profound influence on the level of metabolism, the experimental temperature range is from 32° to 39° C . At temperatures higher than normal body temperature, metabolism is probably also increased due to accelarating effect of heat on chemical reactions. Since heat is not only lost by radiation and conduction but also from vaporization of water from the skin and expired air. (Benedict,Benedict and DuBois 1925 . Landis,Long,Dunn, Jackson, Meyer 1926) .

In veiw of the above, a comparative study is being

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made for obtaining quantitative data regarding calories eliminated per day per kilogram is laying and non-laying stages. It was found that in 9 white leg horn laying hens the average value is 95.84 - 4.78 calories per day per kilogram of live weight while in 9 non-laying birds it is 82.08 - 2.51 . In lying group the heat elimination is significantly higher(at 1 % level of P) than in non-laying stage .

Initially for determination of oxygen consumption and CO_2 and moisture production Haldane's gravimetric method is used. It was found that oxygen consumption in laying group is 0.8371 - 0.0409 litres per hour per kilogram of body weight while in non-laying it is 0.7335 - 0.0193 . The oxygen consumption in laying group is significantly higher than that non-laying

As previously stated a significantly increased heat production and oxygen consumption in laying indicates the increased metabolic activity which is related to neuro-hormonal factors, thyroxine and ovulation inducing hormone .

The utilization of carbohydrate in the normal fed animal appears to be correlated with the quantities of glycogen in the liver . The usual physiological conditions which deplete the liver glycogen are starvation, exercise and cold .Depletion of the liver glycogen may also occur due to glycogenolysis proceeds so much more rapidly that it out springs glycogenesis. The glycogen of liver, therefore appears to be broken down whenever glucose is required by the tissues . The estimation of % liver glycogen is carried out in laying and non-laying stages.

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It was found that the average value of liver glycogen is 8200.9-170.5 mgm % while in non-laying it is 6584.6 - 235 mgm % . The average value is significantly higher in laying group . This extra glycogen content in laying indicates the extra reserved energy which is deposited by the utilization of carbohydrate from ration. It was observed that during experimental period laying birds consumed 4 ounces of feed per day while non-laying are consuming 3 ounces per day . The extra glycogen content may be related with the extra energy required for normal physiological act of egg laying and also the carbohydrates and fats in the formation of egg may be derived from end products of carbohydrate and fat metabolism going on in the liver .

Muscles making up the greater part of the active tissue and due to their activity they are the principle seat of heat production. During muscular activity, muscle glycogen is the chief source of supplying the energy and it is primarily derived from blood glucose. A variable amount of carbohydrate is always utilized by the tissues for the maintainance of the metabolic processes and of this carbohydrate a proportion is oxidised to CO_2 and H_2O for the provision of energy . The average value for muscle glycogen is 1275 - 41.99 mg % in laying while in non-laying it is 1213 - 63.89 mg % . The difference between laying and non-laying is statistically not significant . The conclusion may be drawn that in the same muscle the level of glycogen is not very much affected by laying or non-laying stage .

Up to this time, we have seen the carbohydrate reserves of muscle but from that we could not conclude the rate of

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oxidative processes by the tissue . Therefore the obdominal muscle (weighing 250 to 300 mg) is is selected and glucose utilization in terms glycogen synthesis is observed by incubating the muscle at 40°C in Warburg's flask contained 2.5 ml Kreb's Ringer phosphate buffer solution (pH 7.4 and glucose level 157 mg %) for 2 hours, and it was found that glucose utilization in laying stage is 0.1575 - 0.0112 mgm in laying while in non-laying it is 0.1064 - 0.0009 mgm per hour per 100 mg of weight muscle . The glucose utilization is significantly higher in laying stage. But regarding glycogen synthesis the results are statistically high in non-laying . From the above it may be concluded that the glucose uptake is significantly high in laying stage but as compare to that glycogen synthesis is relatively low, the glucose is effectively utilized to liberate energy and taking part in the oxidative processes of the tissue. Glucose uptake and oxidative processes of the tissues are related with thyroxine secretion rate . The thyroxine secretion levels in normal pullets is ranged from 1.40 to 1.58 ug while in egg laying T.S.R. is 1.99 ug(Staph, Pipes, Turner 1961).

The achievements of coordinated project is to give clear idea of total energy eliminated per day per kg, energy levels in the liver, energy levels at muscle and energy utilization and synthesis at tissue level in laying and non-laying stages. The data will be useful in formulating energy rations and during substitution of agro-industrial by products .

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