Ser. J Korea Vol VII 1967

Effects of Air Pressures on the Physiology of Silkworm, *Bombyx mori* L.

空氣壓力이 蠶體生理에 미치는 影響

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I. Introduction

It is certain that the physiology of insect has to do with the oxygen consumption which is inpependent of oxygen content in the surroundings. In respiratory metabolism, it is known that oxygen is indispensable together with carbon dioxide, and respiratory quotient is always expressed as ratio of oxygen to carbon dioxide.

Insects are apt to low oxygen tension through morphological and physiological self-regulation. Many studies are done on the effects of oxygen tension on eggs, larvae, pupae, and adults. In a certain insect, oxygen tension has an influence on respiratory processes, depending directly upon the rate of metabolism. Especially the higher the temperature, the higher the carbon dioxide tension, and the older the age of the insects are, the more the effects of the high oxygen tension increase.

It was been reported on silkworm by many authors that the effects of oxygen tension do not give rise to the variation of larval respiration, but the oxygen consumption in diapause eggs is chiefly dependent on them.

Furthermore, many investigators found that the pupal period under the conditions of gases containing more than 10 per cent of oxygen is sometimes the same as that under normal conditions.

There are not any reports concerning the effects of air pressure on the insect and silkworm physiology for a wholle life cycle.

In this paper it is designed to investigate more detailed effects of air pressure on silkworm physiology from egg to adult.

This work was carried out at Department of Sericulture, College of Agriculture, Seoul National University, and at Sericultural Station, Korea.

II. Review of literatrure

It was studied by many investigators whether or not the respiratory metabolism of eggs, larvae, pupae, and abults had been affected by oxygen tension.

Bodine[8] reported that both diapause and non-diapause eggs consume oxygen at a uniform rate in the oxygen tensions, ranging from 60 to 160 milimeter Hg. But below 60 milimeter Hg of oxygen tension, the oxygen consumption progressively decreases, reaching zero at 2 milimeter Hg. Gaarder[1] reported that in the pupae and nymphs of a certain insect its respirations take place at a constant rate, ranging from 7.36 to 3.96 miligram of oxygen per liter and 5 per cent to 97 per cent of oxygen. According to Hirstand[2], larvae of caddis fly are able to withstand under both high and low tensions, showing a constant rate of respiration. Also Fraenkel and Herford[6] observed the same results in the blowfly.

Kalmus[4] reported that lowering of the oxygen tension may prolong the time required for pupal development of Drosophila.

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In other reports the action of high tension of oxygen appears to depend upon the metabolic state of the organism at the time of exposure. Gubin and Smaragdova[6] reported in the bee that the greatest consumption of metabolic stores occured at respiratory ratio of 50, that is, the ratio at which mortality was lowest, and the sugar metabolism was greatest at 10.

In silkworm, the effects of oxygen and carbon dioxide had been reported by Nunome[7,8,9,12], Kamioka[10], Yamazaki[11], Nittono[16-19], Kobayashi[20-22], I'o[4], Nishizawa[18], Kawase[14], and Ueda[15].

According to Nunome[8], in silkworm, it has be en proved that Krogh's diffusion theory and the formula of Fick's diffusion were applicable to the respiration of silkworm, and that the rate of decrease of the air pressure was k/d(d=distance from stigma).

Besides those, in case of diapausing eggs, oxygen consumption was dependent on the oxygen tensions, ranging from 1 to 99 per cent, and was 3,4 fold of normal rate at 99 ger cent (Nittono[17]).

And in larvae, oxygen tensions did not influence the larval respirtion. Nittono[19] and kobayashi[20] reported that normal oxygen consumptions were maintained until the oxygen tension reached 10 per cent in the respiration of silkworm pupa.

Nunome[7] reported that air pressure took an effect on physiology of egg and larvae and very much on the injury in the larval respiratory metabolism. When narrow air pressnre was applied to eggs, the hatching ratio increased, but there was no influence on hatching time.

Recent studies, in respiratory metabolism, reported that oxygen tension influences somewhat on respiratory enzymes (cytochrome oxidase and terminal oxidase).

III, Materials and Methods

1. Date and places of the experiments.

In spring rearing season of 1967, these experiments were carried out at Department of Sericulture, College of Agriculture, S.N.U., and at Sericultural Experiment Station, Suwon, Korea.

2. Incubation, rearing, and storage for pupae

For hatching, the eggs were exposed to the constant temperature of 24°C and the moisture of 80 per cent for 12 days.

Rearing methods;

Instar	Younger Instar	Advanced Instar
Temperature °C	25	23
Humidity %	85	70
Feeding hours a day	7	5

Storage for pupae; The pupae are stored under natural conditions.

3. Varieties used.

Varieties used in this experiment are all recommended in Korea.

Experiment on eggs: Sulak, Soyang, Moran, Taedong, Suwon 101, Suwon 102, Sulak × Soyang, 101 × 102.

Experiment on larvae and pupae: F_1 hybrid, Sulak × Soyang.

4. Investigative method.

a. Sampling methods:

The larvae were sampled at 10 A.M. everyday and this was repeated 15 times. They were narcotized with ethyl ether for 10 minutes, and dissected immediately. Sampling was made on 10 larvae per a block after third instar. A larva was seperated into silkgland, digestive organ, and others, and they were kept in desicator at the temperature of 60°C.

b, Processes:

Protein was analyzed by micro kjeldahl method. Fat was analyzed by soxlet aparatus. Sugar was analyzed by Hane's iodometric titration method. Somogyi's method were used for omitted-protein. Ash was obtained by general method.

5. Treatment of pressure.

The pressure was from 1 to 10 pound/inch square with vacuum pump. Desicator was used in low pressure and autoclave in high.

IV, Result and Discussion.

1. Comparisons of air pressures among races at the stage of egg.

As hown in Table 1, Suwon 101, Suwon 102, and F1 hybrid of them were very low in hatching ratio and practical ratio, the facts show that they might be special characters among races. The characters concerned with long period of incubation. Generally, it is appearent that the period of incubation depends direcly upon temperature. Chinese racea are relatively better than Japanese in hatching ratio.

Table 1. Hatching ratio and practical hatching ratio.

Ratio(%) / Blocks / Races	Hatching ratio				Practical hatching ratio				
	Control	−1p	−2p	1p	Control	−1p	−2p	1p	(pound/in. sq)
Sulak	96.4	96.4	95.4	96.7	62.8	91.7	55.2	83.1	
Soyang	97.5	96.8	98.8	95.3	95.7	93.3	95.3	86.2	
Moran	98.1	99.5	98.9	96.4	86.7	63.3	82.5	81.7	
Taedong	95.1	97.0	89.2	98.2	54.5	74.4	38.8	80.[illegible]	
Suwon 101	74.3	95.7	71.6	90.0	15.4	55.1	13.6	40.4	
Suwon 102	70.7	88.0	84.1	84.1	59.1	75.1	64.7	77.1	
Sulak × Soyang	94.5	99.4	90.0	99.1	82.5	69.5	82.9	94.0	
101 × 102	80.9	91.1	80.0	92.2	51.3	74.5	55.9	77.5	
Total	707.5	769.9	707.9	752.0	508.0	626.9	488.9	620.0	
$\bar{x}$	88.4	96.2	88.5	94.0	63.5	79.6	61.1	77.5	

Table 2. Analysis of variances for hatching ratio.

Sources	d. f.	MS	F
Races	7	46.35	1.91
Trearments	3	437.31	18.02**
Errors	21	24.26	

** Significant atlevel.

Table 3. Analysis of variances for practical hatching ratio.

Sources	d. f.	MS	F
Races	7	259.63	2.30
Treatments	3	3376.06	29.96**
Errors	21	112.67	

** Significant at 1% level.

From the table 2 and 3, it can be seen that there are not any differences among varieties, but there are among pressures.

The hatching ratio is considerably increased owing to the little change of pressure.

2. Effects of various air pressures on silkworm eggs.

Temperature and humidity influence the hatching ratio, hatching ability and period of incubation in eggs. As shown in Talbe (4-6) and Figure (1-3) effects of various air pressures were different in hatching ratio, and practical ratio.

Table 4. Data of hatching ratio, practical ratio, and hatching power.

A. Hatching ratio (%)

Blocks / Reces	−10p	−5p	−3p	−2p	−1p	C	1p	2p	3p	5p	10p	Mixed p
sulak	86.7	88.3	87.5	96.4	98.3	97.1	97.2	98.1	96.7	92.9	91.4	93.2
soyang	87.4	90.1	90.4	94.2	93.7	96.5	96.9	97.1	94.6	93.8	92.6	94.1

B. practical ratio (%)

sulak	56.7	83.2	85.0	85.9	86.7	87.0	92.3	83.8	80.5	75.2	73.9	71.4
soyang	67.1	80.1	81.6	83.1	89.1	84.7	90.6	86.4	83.1	82.0	71.7	72.1

C. hatching power

sulak	883	845	739	556	487	571	518	562	673	731	751	792
soyang	746	613	607	581	559	643	574	581	618	637	663	771

Analysis of variance., for hatching ratio

Sources	d. f	M.S	F
Races	1	33.14	6.61
Treatments	11	46.10	3.64**
Replications	2	27.41	2.17
Error	57		

** Significant at 1% level.

Fig. 1. Hatching Ratio

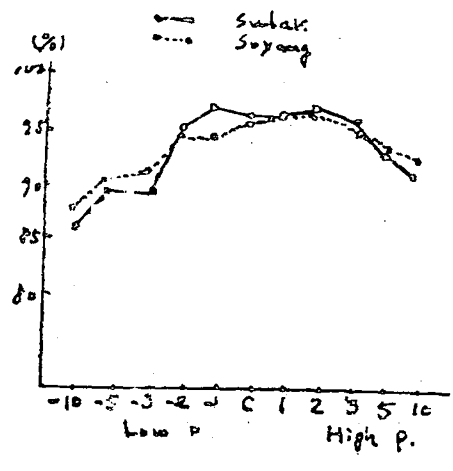


Fig. 2. Hatching power.

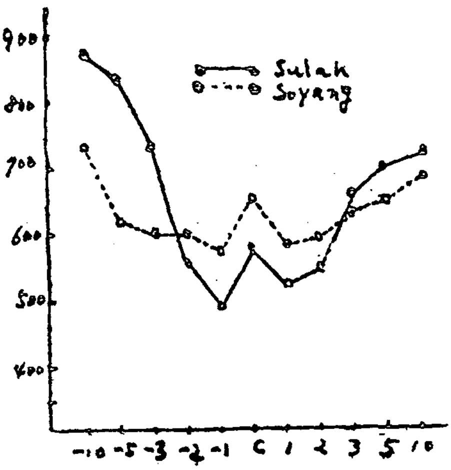


Table 5. Hatching power

Sources	d. f	MS	F
Races	1	2085.70	2.88
Treatments	11	2730.54	4.05**
Replications	•2	1613.43	2.58
Errors	57	673.85	

** Significant at 1% level.

Table 6. Analysis of variances for practical hatching ratio

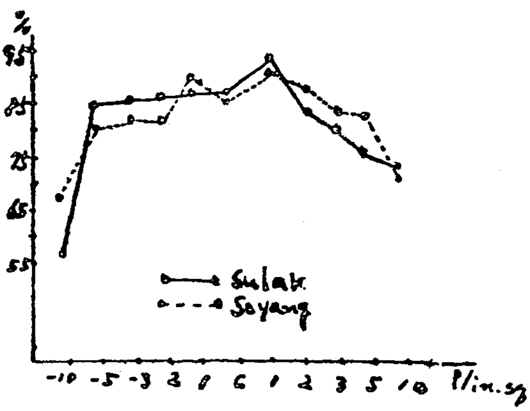
Sources	d. f	MS	F
Races	1	707.31	3.22
Treatments	11	912.47	4.14**
Replications	2	591.27	2.71
Errors	57	219.58	

** Significant at level.

It is very interesting to note that the hatching ratio in air pressure (high and low: pound per in.n square) is high.

When the low pressure was compared with high it is inclined that the former seems to be worse than the latter, this means that the decrease of pressures caused lack of oxygen contents. Therefore, the egg physiology depends not only upon temperature, humidity, and light, but also upon air pressure.

Fig. 3 Practical ratio



3. Rearing experiments under several kinds of pressure at larval stage.

Data on rearing experiments

A. Cocoon weight (g)   Using variety: Sulak × Soyang

Blocks / Replications	−10p	−5p	−3p	−2p	−1p	C	1p	2p	3p	5p	10p	mixed p
I	2.12	1.97	2.18	2.23	2.07	2.42	2.24	2.37	2.03	2.24	1.95	2.12
II	1.94	2.16	2.23	2.17	2.32	2.23	2.39	2.06	2.05	1.92	2.06	2.13
III	1.98	1.93	2.11	2.30	2.45	2.40	2.33	2.14	1.96	2.05	1.90	1.98
Total	5.94	6.06	6.42	6.60	6.84	7.05	6.96	6.57	6.24	6.21	5.91	6.22
$\bar{x}$	1.98	2.02	2.14	2.20	2.28	2.35	2.32	2.19	2.08	2.07	1.37	2.07

B. Cocoon layer ratio (%)

I	18.4	19.6	17.7	18.1	23.2	20.7	21.2	20.0	23.2	17.6	19.6	22.2
II	19.3	18.7	21.1	20.0	20.3	20.8	21.7	17.5	18.2	19.9	20.1	19.3
III	16.9	19.0	21.4	23.6	17.1	24.0	18.9	23.6	19.3	23.5	18.6	19.9
Tatal	54.6	57.3	60.2	61.7	61.6	65.5	61.8	61.1	60.7	61.0	58.3	61.4
$\bar{x}$	18.2	19.1	20.1	20.6	20.5	21.8	20.6	20.4	20.2	20.3	19.2	20.5

C. Fresh cocoon ratio

I	82	80	92	86	93	99	82	89	87	90	82	86
II	85	79	85	88	91	90	87	83	85	85	88	80
III	67	82	85	91	87	98	87	81	87	79	77	87
Total	234	241	262	265	271	287	267	263	259	254	247	253
$\bar{x}$	78	80	87	88	90	96	89	88	86	85	82	84

a. Cocoon weight and cocoon layer ratio.

Table 7. Analysis of variance for cocoon weight.

Sources	d. f	MS	F
Replications	2	70.36	1.34
Treatments	11	236.77	4.51**
Errors	18	52.52	

** Significant at 1% level

Table 8. Analysis of variance for cocoon layer ratio

Sources	d. f	MS	F
Replications	2	296.78	2.29
Treatments	11	441.93	3.42**
Errors	18	129.22	

Significant at 1% level.

Fig 4. Cocoon Weight

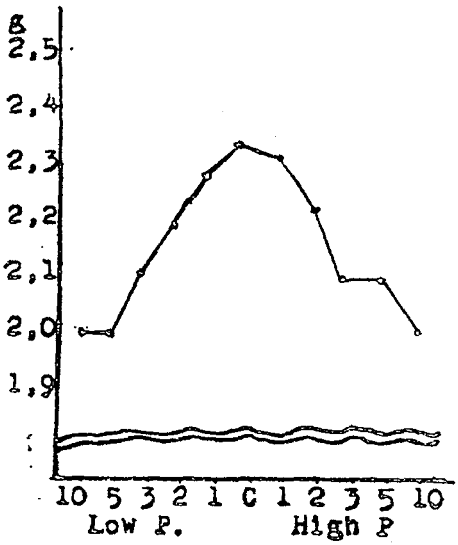
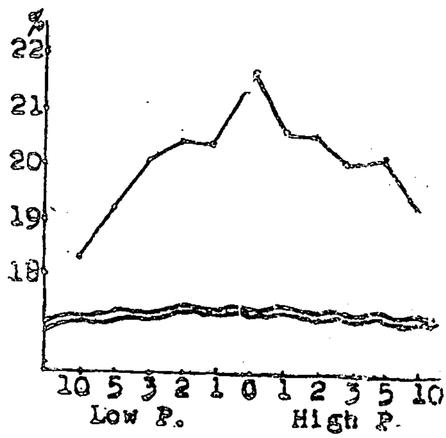


Fig 5. Cocoon Layer Ratio



As shown in Table(8-7) and Figure(4-5), high pressures did not affect, but low pressures remarkably did the cocoon weight and the cocoon layer ratio. The reason that there is no differences among theatments by the analysis of variance are considered because of the short period of treatment.

b. Fresh cocoon ratio.

Table 9. Analysis of variance for fresh cocoon

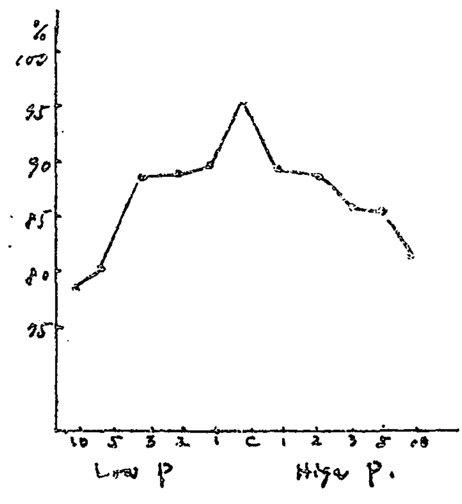
Sources	d. f	MS	F
Replications	2	27.62	1.07
Treatments	11	264.04	10.23**
Errors	18	25.81	

** Significant at 1% level.

As shown in Table 9 and Figure 6, it appears that there are remarkable differences among the average of the values obtained from the treated larvae. It means that the change of air prossure causes to the low vitality and abnormal nutrition metabolism.

It shows that cocoon quality of treated larvae is inferior to that of control. The effect of high pressure was not appeared clearly.

Fig 6. Fresh Cocoon ratio



4. Quantitative analysis of treated larvae with pressure.

Water, protein, fat, carbohydrate, etc. are present in larvae. They are supposed to cause variation in the chemical compositions of the silkworm, for the differences of air pressure affect the growth of larvae.

Table 10. Quantilative analysis of silkworm.

	Control			High Pressure			Low Pressure		
	Body.	Digesti.	Silkgland	Body.	Dige.	silkgland	Body.	Digestive.	silkgland
protein (%)	47.63	44.88	75.38	49.37	50.44	81.75	44.34	47.44	86.49
Fats (%)	29.64	12.91	8.82	34.62	12.39	8.92	25.83	12.44	7.62
Sugar (%)	5.66	5.73	5.80	7.10	7.25	7.31	4.83	5.66	4.35
Ash (%)	4.90	14.40	4.24	3.83	13.89	4.77	5.41	14.20	3.82

Fig. 7-1. Amount of Fat

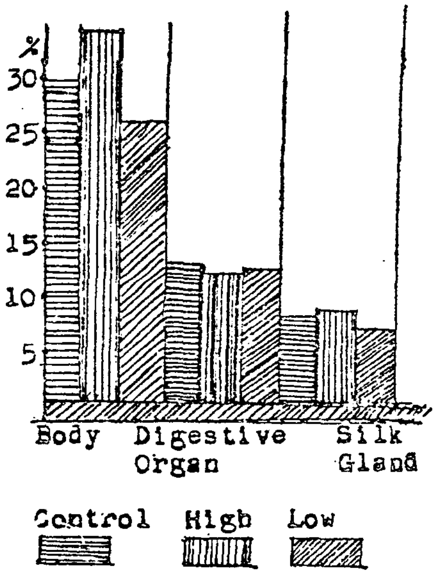
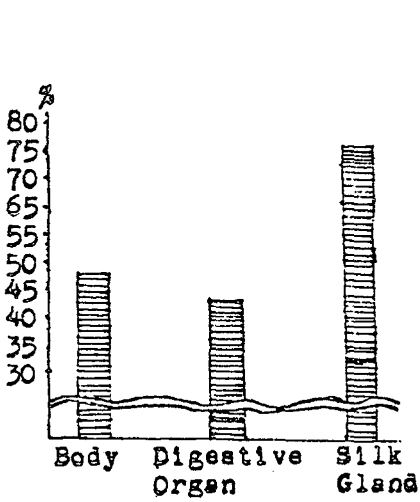


Fig. 7-2. Amount of protein



In Table 10 and Figure 7, it appears that fat contents show remarkable differencs among the tissues. Especially, silkgland contains little amount of fat. It is evident that the small amount of fat in silkworm body transfers to silkglands.

And it is of important means for silk formation that there are more protein content in the silkgland than in the other tissues. On the other hand, in sugar and ash there were no differences among treatments. This might be due to the fact the air pressure uniformly affected the nutrition metabolism.

5. Silkworm pupae and air pressure.

Pupation and emergence play an important role of silkworm physiology. These phenomena are dependent almost entirely upon temperature, oxygen, carbondioxide, etc.

Various air pressure were treated to pupae, obtaining the interesting results.

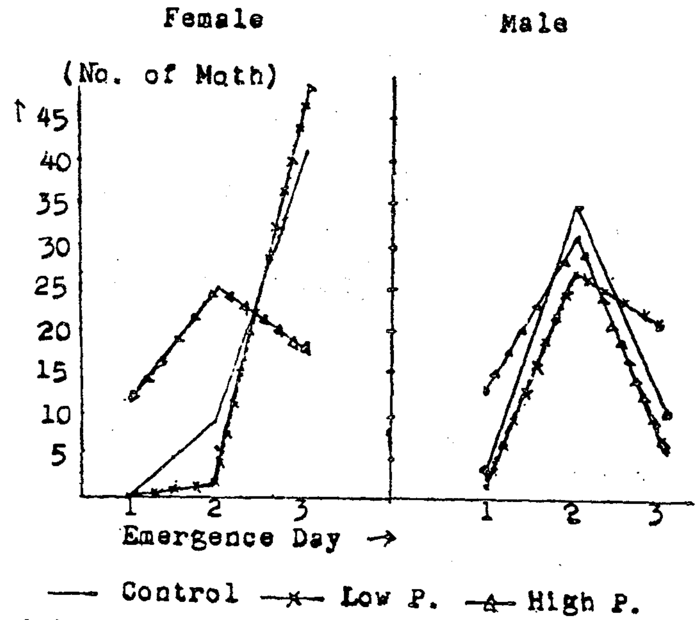
As shown in Table 11 and Figure 8, the results suggest that air pressures affected the duration of pupa. It was delayed more by pressure than by atmospheric pressure, while it was shortened by high pressure.

These results are similar to those of Kalmus[4], that the development of pupae in Drosophila was prolonged in low oxygen tension.

Table 11. Pressure and emergence

	Control		High(1 pound/in. sq.)		Low(1 pound in. sq.)	
	female	male	female	male	female	male
1st day	0	4	7	13	0	2
2nd day	9	35	25	31	2	27
3rd day	41	11	18	6	48	21

Fig. 8. Pressure and period of emergence



The writer and others may assert that time of emergence is regulated by air pressure, and there should be respiratory metabolism advantage to pupa, laying ability.

V. Conclusions.

This work was carried out to investigate the effects of air pressures (low and high pressures: 1 to 10 pound per inch square) on the physiology of silkworm, *Bombyx mori* L.

The results may be summarized as follows:

1. Among silkworm varieties, the effects of air pressures on the eggs were of no differences at all.
2. Narrow air pressures ranging from -1 to 1 pound per inch square resulted in high hatching ratio, and the short hatching period, but it was closely associated with the time of treatment.
3. The effects of air pressure, ranging from 1 to 10 pound per inch square on the weight of cocoon and cocoon layer ratio observed.
4. In silkworm larvae, the air pressure affected respiratory metabolism and larval vitality. Especially low pressures extremly injured the larvae.
5. It was meaningful for the silk formation that the protein content of silkgland was more or less increased.
6. Air pressure did not influence the fat content of silkgland.

7. Air pressure affected the emergence period of pupae, low pressures prolonged its period, but high pressures shortened.

8. The silkworm physiology depends not only upon temperature, humidity, and light but also upon air pressure.

摘　　要

本 實驗은 空氣壓力이 蠶體生理에 미치는 영향을 알고자 遂行되었으며 要約하면 다음과 같다.

1. 蠶卵 孵化에 對하여 미치는 空氣壓力의 影響은 蠶品種間에는 差가 없다.
2. 大氣壓에서 差가 적은 高壓(1 Pound/inch sguare) 또는 低壓(1 Pound/inch sguare)은 孵化를 좋게하나, 壓力이 클 수록 處理時間이 길수록 孵化에 나쁜 影響을 미친다.
3. 壓力은 繭重, 繭層比率, 上蔟比率에 影響을 미치며 大氣壓에서 差가 클 수록 處理時間이 길 수록 惡影響을 미친다.
4. 壓力은 蠶兒體力을 弱化시키며 低壓은 特히 해롭다.
5. 蠶兒組織(蠶體, 絹絲腺, 消化器)間에 있어서 脂肪成分에 현저한 差異가 있으며, 蠶體組織에서 絹絲腺으로 移動하는 脂肪成分은 그 量이 적다.
6. 繭形成에 있어서 各成分(炭水化物, 脂肪, 蛋白質, 灰分)은 서로 密接한 關係가 있다.
7. 壓力은 化蛾日에 影響을 미치며 低壓은 化蛾時間을 延長, 高壓은 短縮시킨다. 따라서 雌雄間化蛾日 調節을 壓力에 依해할 수 있다.
8. 蠶體生理에 미치는 環境要因은 溫度, 濕度, 光線뿐만 아니고 壓力도 큰 影響을 미친다.

Acknowledgement.

The author is most indebted to professors, Mun Hyup Kim, Nak Jung Kim, and Dr. Byong Hee Choe, for their advices and guidances throughout the progress of this work.

His sincere thanks goes also to Dr. Sang Ki Hahn and professor Kwang Eui Park for reading through the original manuscript.

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