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Effect of Acetic Acid Ester and KUMGANG Medicine Stone in Silkworm Rearing with Artificial Diet and Mulberry

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ABSTRACT

Raising silkworms by using artificial feed is not affected by the seasons and can realize the industrialization of silkworm breeding. The study has been conducted the research for using acetic acid as the material of organic acid in fresh mulberry and KUMGANG medicine stone in which various mineral salts are many contained as the material of inorganic salts in artificial feed. When acetic acid esterfied is added to artificial diet as rate of 3.3%, the setae dispersion within 48h was above 98% and the incentive was 100%. Antiseptic effect on artificial feed has been maintained for 90h since falling the feed. Acetic acid has raised the feeding habit on the 3rd instar larvae as the rate of 118.5% to128.4% and the body weight as the rate of 104.5%. KUMGANG medicine stone of the rate of 1% on artificial feed has promoted the growth and development of young silkworm. In the autumn with bad condition on rearing, KUMGANG medicine stone has been decreased the disease occurrence in larvae and raised the quality and yield of cocoon.

Keywords : Bombyx Mori L, Artificial feed Rearing, Organic acid, KUMGANG medicine stone.

I. INTRODUCTION

Raising silkworms using artificial feed can promote the growth of silkworms while not being affected by the seasons and realize the industrialization of silkworm breeding. Recently, there has been an active research project to raise silkworms by using artificial feed. There is also active research on the effects of organic acids on the growth and sanitary conditions of mulberry silkworms. Using a comparative protein approach, the effects of different diets on the growth and development of cultured silkworms at the protein level were analyzed [1].PSG revealed posterior silk gland as the most important factor responsible for synthesis and secretion of silk-mediated fibrin protein in silkworms, and analyzed several factors secreted by PSG [2].Carried on the comparison with the method of rearing with artificial diet method and rearing silkworm in bulk, analyzed the factors to artificial diet [3].A proteome study was conducted to characterize the protein profile of silkworm chrysalis and how to optimize the breeding strategy [4].The specific comparison of the contents of amino acids, carbohydrates and lipids in the feces of mulberry leaf-bred silkworm and artificial forage was made, raising a new improvement method for artificial forage [5].The factors affecting the feeding time of silkworm larvae, Bombyx mori, were investigated, and continuous observation showed that feeding was at regular intervals throughout the outbreak [6]. In vivo uptake of titanium dioxide (TiO2) nanoparticles by silkworms was studied to induce the direct production of modified silk [7]. The performance of

bivoltine Bombyx mori larvae cultured from different host plant varieties was specifically analyzed according to different contents [8]. The feedstock efficacy of silkworm Bombyx mori for V instar larvae fed by kanva-2 mulberry leaves with Riboflavin (Morus alba) and b-complex vitamins treated with Riboflavin (0.5%), Pantothenic acid (0.5%), Pyridoxal phosphate (0.5%) and Biotin (0.5%),Growth and economic variables were specifically studied [9].In this paper, based on the specific research analysis of the prior literatures, the effects of organic acids on the growth and sanitary conditions of mulberry silkworms were studied on how to esterify one of the organic acids in raw mulberry leaves and put it into artificial feeding. In addition, a young silkworm with a high requirement for inorganic salts, rich in mineral ingredients, has been used as the main inorganic source of artificial feeding.

II. MATERIALS AND METHODS

2.1 Materials

The composition of artificial feed was 40g of Mulberry Leaf powder, 33g of soy bean care, 5g of Maize powder into α-state, 5g of sugar, 5g of yeast, 5g of citric acid, 2g of Vitamin C, 5g of agar, 230ml of H2O and 30ml of antiseptic liquid.

The acetic acid ester is the primary liquid that glacial acetic acid, alcohol and sulfuric acid of the density with 90% are blended to composition of 4:4:1 and used as antiseptic liquid diluted to 11%.

KUMGANG medicine stone (YONSAN county, north of HWANGHA province.) is plain grey powder with 100 mesh of the particle size and the Mulberry silkworm variety was No.201 and 401 as normal one.

2.2 Methods

①Acetic acid ethylester by ratio of 11% as antiseptic liquid was put to amounts of 10ml(1.1%), 20ml(2.2%), 30ml(3.3%) per 100g of dry weight in artificial feed. In test were measured the incentive ratio which the larvae has dispersed for artificial diet after hatching, the setae dispersion within 48h, the eating habit and the body weight of the 3rd instar larvae.

Then, the antiseptic effect of acetic acid ethylester was investigated in contrasting occurrence quantity of *Aspergillus oryzae* and *Asp. flabus* on artificial diet with the contrast plot for 4days. In contrast test, propionic acid as antiseptic acid was put in rotio of 1.5%.

Eighther acetic acid ester of various densities was feeded on the 5th instar larva in autumn which mulbery leave has stiff and bad, then the effect of it on survivality of larava and cocoon quality was aasaied.

②KUMGANG medicine stone was added to artificial feed as amount of 1% and 3%. The setae dispersion within 48h from hatching time, the period of every instar larvae, the growth coefficient(contrasting with mulberry silkworm growth indicated as 1.0) and the every instar molted ratio, etc. were investigated.

Test for use of KUMGANG medicine stone has done in autumn with bad rearing condition.

For the purpose of disinfection and staining of silk worm egg surface, we got ready for the turbid liquid which KUMGANG stone powder was solved as quantity of 3% in alcohol by70%, and then a day ago silkworm egg hatch, it was put in the liquid for 3 minutes.

After hatching, the liquid was diluted as 3 times and fed to young lavae by one time a day with mulberry.

The survey indexes were arranged as the period and the molted ratio of every instar, the survivality of old silkworm, the yield and the quality of cocoon.

III. RESULTS

3.1 Effect of acetic acid ester to the growth of larva, the anticeptic property and the cocoon quality on silkworm rearing with artificial diet and mulberry.

1) Effect of acetic acid ethylester to the growth, the eating habit of silkworm and the antiseptic property on artificial feed.

Acetic acid ethylester has shown the incentive, and made the setae dispersion ratio and the growth of young silkworm larvae better and shown some antiseptic effect against artificial feed. (Table1)

Table1.The added density of acetic acid ethylester to the growth of silkworm and antiseptic effect against artificial feed.

Index / Test plot	Incentive %	Setae dispersion ratio %	Newly Molted ratio/ slept ratio %
Contrast plot	100	95	92/94
Acetic acid ester			
0%	0	78	68/75
0.55%	95	85	83/83
1.1%	96	92	90/93
2.2%	100	98	92/95
3.3%	100	98	92/96
4.4%	100	98	90/96

Contrast plot; Propionic acid adding. Variety; No 401.
Septic area; on 90h after newly hatching

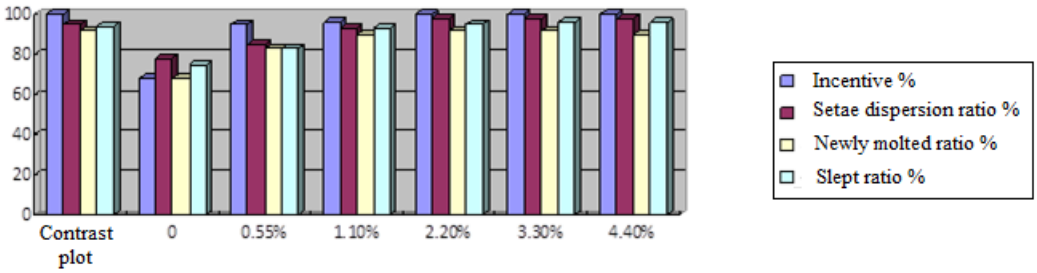

Figure1. Growth of silkworm by densities of ester against artificial feed

As shown in Table 1, the incentive are shown apparently on the rate of 2.2% and the setae dispersion ratio showed as many more than 98% on the rate of 3.3%.

The slept ratio and molted ratio of 1st instar larvae was similar to the contrast plot as 95% and 92% on rate of 2.2%.

The antiseptic effect of acetic acid ester appeared on the density of 0.55% and then the fungi flourishing on the artificial diet were inhibited on the density of above 3.3% for some 4 day (some 90h) since feeding on.

Table 2.The antiseptic effect of acetic acid ethylester against artificial feed.

Index / Test plot	Septic diet area %	% of it		Septic degree
		% *Asp. Flabus*	% *Asp. Oryzae*	
Contrast plot	0	0	0	0
Acetic acid ester 0%				
0.55%	100	60	40	+++
1.1%	16	10	6	+
2.2%	10	2	8	_
3.3%	0.5	0	0.5	...
4.4%	0	0	0	0
	0	0	0	0

Contrast plot; Propionic acid adding. Septic area; on 90h after newly hatching

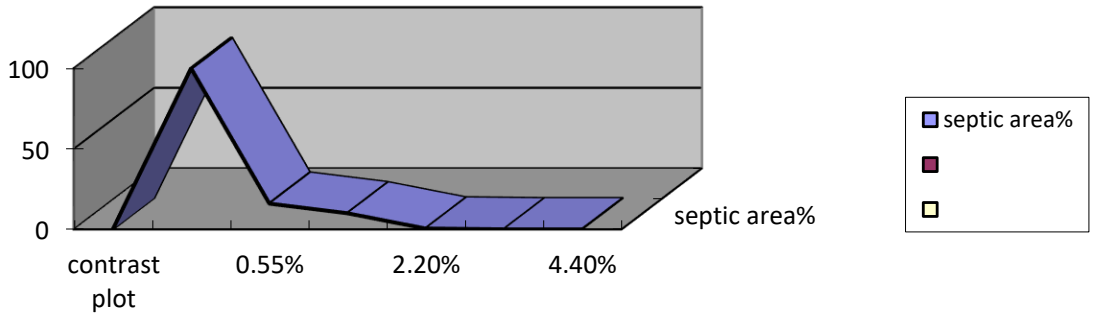

Figure 2. Antiseptic effect by densities of ester against artificial feed

The antiseptic effect of acetic acid ethylester against artificial feed inhibition action of each components for fungi. It is provided with acetic acid and alchol, secondarly supplied with sulpharic acid catalytic action for ester (Table 3).

Table 3. The antiseptic effect of each components consisting acetic acid ester

Index / Test plot	Septic diet area %	% of it		Septic degree
		% *Asp. Flabus*	% *Asp. Oryzae*	
Contrast plot	0	0	0	0
Acetic acid	10	10	0	_
Alchol	100	0	10	+++
Sulpharic acid	99.5	99.5	0	++_
Aceticacid+ Alchol	0	0	0	0
Aceticacid+Sulpharic acid	10	10	0	_
Alchol+Sulpharic acid	100	50	50	+

Diet area; 40×60cm^2, temperature 29°C, humidity 85% . On 72h after feeding

As the shown the table, the inhibition effect for fungi is the highest for mixture acetic acid and alchol, then ethyl alchol inhibited A*sp. Oryzae* but not *Asp. flabus*.

Acetic acid ester has raised the eating habit and the growth of the 3rd instar larva more than the mixture of acetic acid and alchol having the similar anticeptic action (Table 4).

Table 4. Effect of acetic acid ester to feeding habit and body weight of 3rd instar

Index / Test plot	2nd day of 3rd instar				3rd day of 3rd instar					
	Larva number off diet 1	Dead larva Number 2	Dropings per head 3	Contrast % 4	1	2	3	4	Body weight per head,	4
Acetic acid+ alcohol	2	0	10.8	100.0	0	2	23.6	100.0	116.7	100.0
Acetic acid ester	0	0	12.8	118.5	0	0	30.3	128.4	122.0	104.5

Variety; No 201. N=30heads larvae body weight; mg

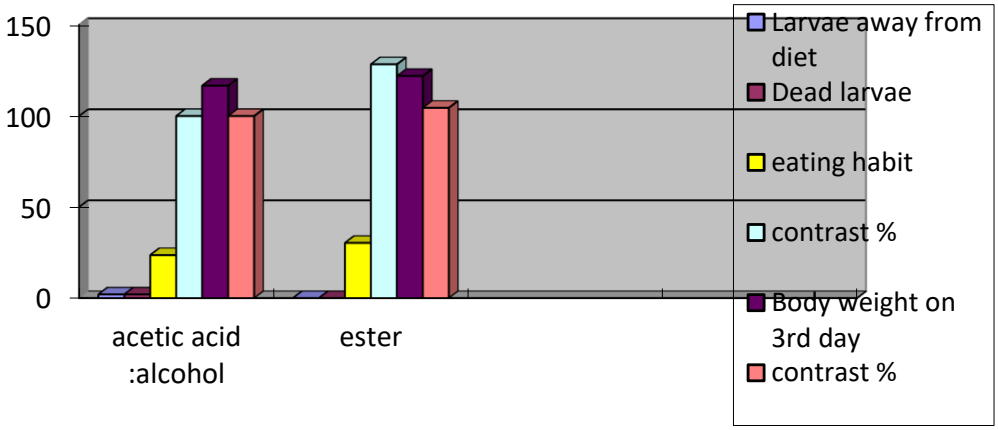

Figure 3. Eating habit and growth of silkworm on the feed esterized

As shown in the table 4, acetic acid ester has raised the feeding habit as ratio of 118.5% to 128.4%, and the body weight as ratio of 104.5% many more than the mixture of acetic acid and alcohol.

This means that when acetic acid is esterized with alcohol put to artificial feed it has raised the setae dispersion and eating habit and made growth of silkworm better.

2)The effect of acetic acid ester to survivality of 5th instar larva and cocoon quality in autumn.

When feeding acetic acid ester on 5rd instar larva in autumn having a few low quality of mulbery leaf, the larva period was shorten and the survivality and the cocoon quality was raised(Table 5).

Table 5. Effect of acetic acid ester on the period and the survivality of 5rd instar larva

Index / Test unit	5rd instar perriod date.hour	Survivality, % 1 2 3	$\sum_{Ti}$	Mean %
Mulbery contrast	9.10	88 90 92		90.0
Water contrast	9.00	90 91 92	273	91.0
Aceti cacid				
0.15%	8.18	89 92 92	273	91.0
0.3%	8.12	91 92 93	276	92.0
0.45%	8.06	93 94 94	280	93.3**
0.6%	8.09	89 91 93	273	91.0
0.75%	9.04	86 88 87	261	86.0**

Variety; No 201. N=200×3. $t_{0.05}$= 2.365 < $t_{H2O,0.45\%}$=5.11, $t_{0.01}$= 3.499 < $t_{H2O,0.18\%}$=4.27

As the shown the table, acetic acid ester has the 5th inster period more shorten as 8 hours for the rate of 0.4% th an water contrast. Then, the survivality of the 5th instar larva was painly raised as the rate of 93.3% on feeding e ster by the rate 0.45%, and it was proved on the significant level of 5% and 1% as the result of dicentralization f or investigation values. Also when adding an dillute solution of acetic acid ester on the 5th instar larva, the weig ht of cocoon and cocoon shell was both raised in autumn(Table-5).

Table 6. Effect on feeding of acetic acid ester to the 5th instar larva for the quality of cocoon in autumn.

Index / Test unit	The cocoon weight of per body g	%	The Cocoon shell weight of per body g	%	The cocoon shell percentage %
Mulbery contrast			0.30		21.0
Water contrast	1.43		0.30	100.0	20.8
Acetic acid ester	1.44	100			
0.15%			0.29	96.7	20.3
0.3%	1.43	100	0.33	110.0	20.6
0.45%	1.60	111.1	0.35	116.7	21.6
0.6%	1.62	112.5	0.34	113.3	20.8
0.75%	1.63	113.2	0.33	110.0	2.04
	1.62	112.5			

Variety; No 201. On feeding one time a day during 5th instar.

As the table, on the ester dencity 0.6%, the cocoon weight and cocoon shell weight was more higher as each 1. 63g and 0.34g than 1.44g and 0.3g of water contrast. When feeding acetic acid ester of the rate of 0.45% to the 5th instar larva in autumn, the survivality of larva has been raised more 2~3%, cocoon shell weight more 116.7 % than ones· of water contrast.

Thus, when feeding acetic acid ester of a low density with mulbery of a few organic acid in autumn, the surviva lity of larva and cocoon quality were both higher.

3.2 The effect of KUMGANG medicine stone on silkworm rearing by artificial diet and Mulberry.

1) The effect of KUMKANG medicine stone to growth and development of young silkworm by artificial feed.

KUMGANG medicine stone as inorganic salts of artificial feed has raise set-ae dispersion ratio and made the gro wth of newly hatched larvae better.

When adding KNMGANG medicine stone to artificial feed by ratio of 1% and 3%, the setae dispersion ratio on 48h after feeding was more higher as 93% and 95% than 90% of contrast plot. (Table 7)

Table 7. The Effect of KUMGANG medicine stone to young silkworm rearing by artificial feed

Test plot	Head number	1st instar			2nd instar			3rd instar		
		Setae dispersion ratio,%	Body size	Period	Molted ratio,%	Body size	Period	1	2	3
Contrast plot	1000	90	0.9	5.00	30	0.75	3.01	36	0.8	4.07
Kumgang 3%	1000	93	0.95	5.00	83	0.90	3.01	86	0.9	4.07
Kumgang 1%	1000	95	0.95	5.00	85	0.95	3.01	88	0.87	4.07

Body size; surveying on 3rd day of every instar.

The body size on the 3rd day in 1st instar larvae was remarkably larger as 0.95 than 0.90 of contrast plot and the tendency was further parent on the 2nd and 3rd instar larva.

Especially, since the 2nd instar larvae, the first molting ratio of KUMGANG test plot was remarkably as larger as 85% and 83% than 30% of the contrast plot and the tendency was similar to the 3rd instar larva.

This means that in artificial feed rearing KUMGANG medicine stone gives a good effect to raise the larvas growth and development as the setae dispersion, the body size and the 2nd~3rd hatching ratio.

2) The effect to the growth and the disease occurrence of silkworm in autumn by treatment and feeding of KUMGANG medicine stone.

In autumn with bad silkworm rearing, since the 3rd instar, the period of larvae was shorted and the development equilibrium was large and the disease occurrence ratio too was low by feeding of KUMGANG medicine stone .(Table 8)

Table 8. The effect of KUMGANG medicine stone on growth and disease occurrence of silkworm in autumn rearing

The test plot	Moth number	3rd instar		4th instar			5th instar		
		Period	Molted ratio %	1st day disease occurrence %	4th day disease occurrence %	Period	Disease occurrence %	Survivality %	Period
Contrast	5	4.15	30	7	90.0	7.06	15.0	25.3	8.00
Water	5	4.15	50	10	60.0	6.00	5.0	45.7	8.00
KUMGA-NG	5	4.03	90	0	5.0	5.0	2.0	93.1	8.00

*The period of 1st~2nd instar; 8days and 20hours.

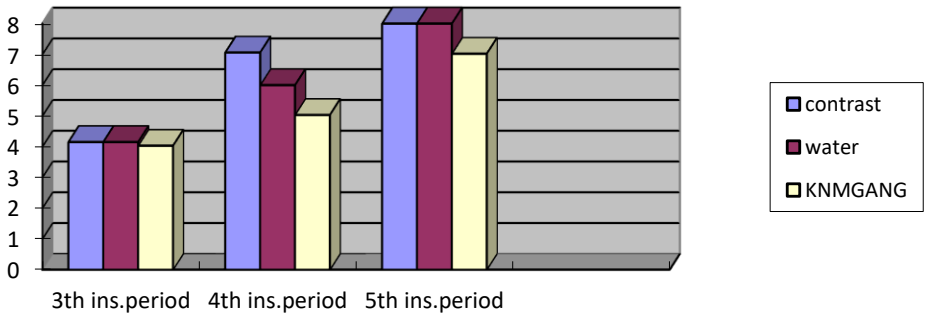

Figure 4. Adding of KUMGANGE medicine stone and period of the 3~5 instar larvae

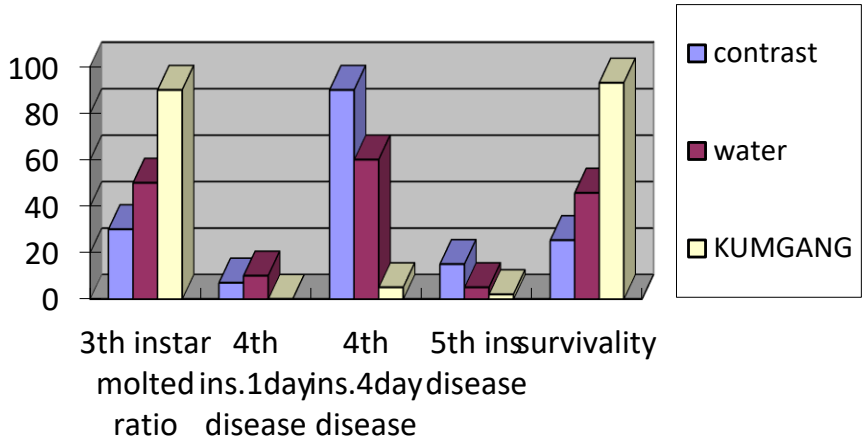

Figure 5. Adding of KUMGANG medicine stone and the 3rd instar molted ratio, disease occurrence in 4~5th instar larvae and survival ratio.

As shown in the table 8, in KUMGANG medicine stone test, there were none disease silkworms in 4th instar, but in the contrast plots of water and mulberry the disease silkworms have been occurring with 10% and 7% on first day of the 4th instar and there were suddenly many disease silkworms occurred with the ratio of 60% and 90% for the 4th instar.

The tendency either appeared in too 5th instar period, which the disease occurrence ratio of KUMGANG stone test plot was some of 2% but in contrast plots of mulberry and water were as many as 15% and 5%.

The 4th instar period has been shorted as 1 to 2 days and the 5th instar period shorted as one day than the contrast plots.

3) The effect of treatment and feeding by KUMGANG medicine stone on the yield and quality of cocoon.

In autumn silkworm rearing, the treatment on silkworm egg surface before hatching and the feeding to young silkworm with KUMGANG medicine stone solution have raised the yield and quality of cocoon. (Table 9)

As shown table 9, in KNMGANG medicine stone plot the disease occurrence ratio in period making cocoon was very lower as 6.9% than 74.7% or 54.3% of Mulberry plot or water plot, and the cocoon yield ratio was more larger as some two times as 93.1% than 45.7% of water plot.

In the cocoon quality of KUMGANG plot, the average weigh per head of female and male on cocoon and cocoon shell as apart of 1.85g and 0.36g were some larger as ratio of 107.5% and 112.5% than 1,72g and 0.32g of water plot, and the cocoon shell ratio too were rather larger as 19.5% than 18.9% of mulberry plot.

Table 9. The effect of KUMGANG medicine stone on the yield and quality of cocoon

Test plot	Cocoon harvest ratio %	Contrast %	Disease cocoon Ratio %	Per cocoon		Per cocoon shell		Cocoon shell ratio %
				Weight g	Contrast %	Weight g	Contrast %	
Contrast plot	25.3	0.55	74.7	1.75	101.7	0.33	103.1	18.9
Water	45.7	1.00	54.3	1.72	100.0	0.32	100.0	18.6
KNMGAN-G	93.1	2.04	6.90	1.85	107.6	0.36	112.5	19.5

*Contrast number and % were counted with water plot as normal.

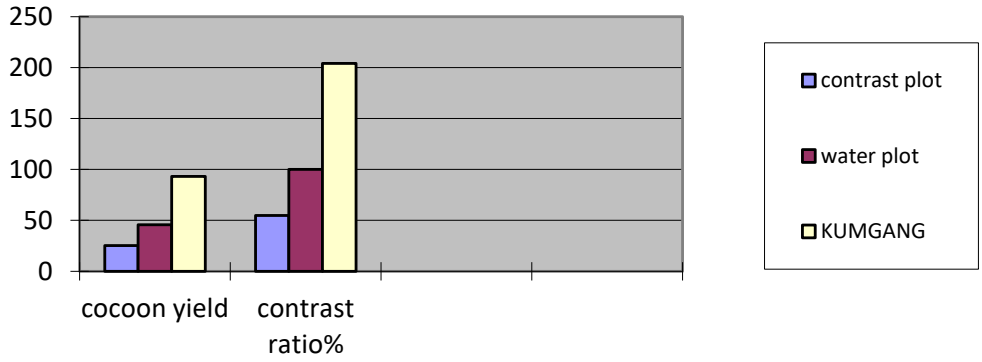

Figure 6. Yield of cocoon by adding of KUMGANG medicine stone

As thus, when feeding to young silkworm KUMGANG medicine stone as the nutrient source of inorganic salts, the silkworm growth has been promoted and the disease occurrence ratio was low, and the cocoon yield and quality both were improved. Thereby, when advancing the utilization method of KUMGANG medicine stone, we relive the effect will be large more than.

IV. DISCUSSION

Acetic acid ester raised the incentive and the setae dispersion of young silk worm and promoted the eating habit and growth of the 3rd instar larvae in artificial feed rearing, it is because of that the action of organic acids including acetic acid in fresh mulbery was raised. The property of acetic acid ester has appeared to raised the survivality of larva and the cocoon quality in autumn which the quality of mulbery leave falls to low, it is shown. Also, it inhibited the occurrence of fungi flourishing on artificial diet as similar to the contrast plot added propionic acid. It is shown that the definite antiseptic action of acetic acid ester is by the mixture of acetic acid and alchol. When using KUMGANG medicine stone as the inorganic salts material, it can raised the setae dispersion and every instar molted ratio and promoted the growth of larvae. Either, before and after of hatching in autumn rearing when feeding KUMGANG medicine stone to mulberry silk worm larvae, it can raise the survivality of larvae and improve the yield and quality of cocoon.

V. CONCLUSION

Based on the effect of organic acid on mulberry silkworm growth and sanitary condition of silkworm rearing, it was studied how to add to artificial diet by esterifying acetic acid as one of organic acid in fresh mulberry. Acetic acid ethylester has promoted the silkworm growth including the setae dispersion ratio when put as amounts of 30ml by Acetic acid ester 11% per 100g of dry weight in artificial diet. Then, it's antiseptic effect is maintained for 90hs after giving artificial feed. The feeding habit of the 3rd instar larva was large as 118.5~128.4% and the body weight was large as 104.5% with acetic acid ester. When feeding acetic acid ester of the rate of 0.45% to the 5th instar larva in autumn, the survivality of larva has been raised more 2~3%, cocoon weight per body more 113.2% and cocoon shell weight more 116.7% than ones of water contrast unit. KUMGANG medicine stone has promoted the silkworm growth and raised the molted ratio of silkworm in rearing with artificial feed remarkably. Before hatching the treatment of egg and the feeding to larva by KUMGANG medicine stone alcohol liquid have elevated the 3rd instar molted ratio and make the period of 4th to 5th instar larva shorted as 1~2days and have the occurrence ratio to very lower range in autumn silk worm rearing.

Either, the treatment and feeding by KUMGANG medicine stone in dilute alcohol solution have raised the cocoon yield as twice, the cocoon weight per head 107.6% and the cocoon shell weight as 112.5%.

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