

# STUDIES ON THE UTILIZATION OF FEEDSTUFFS AND PRODUCTIVE PERFORMANCES BY SOME FARM ANIMALS

## I. A Study on The In Vitro Digestibility of Cellulose by CheJu Korean Cattle, and Sheep

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### INTRODUCTION

The potency of cellulose utilization by the rumen micro population could be more significantly important than both quality and quantity of feedstuffs for digestion of low quality roughage or cellulose by ruminant animals. However, these two factors are closely interrelated each other. Pure cellulose was used in this experiment to eliminate the possible influences on the utilization of cellulose in the feedstuffs, thus seasonal variations of cellulolytic potencies in CheJu Korean cattle, sheep and dairy cattle could be measured accurately.

As Barnett and Reid (1961) mentioned, a simple incubation of rumen liquor technique was contributable to predict seasonal acclimatization of rumen micropopulation to low quality natural grass grown in CheJu Island.

The simple incubation of rumen liquor technique modified by the author was adopted for the experiment 1, 2 and 3.

#### Digestibility Filter Paper

Low digestibility of pure cellulose or filter paper could be due to its attack by a minor component of the flora which gradually increases in numbers because of the availability of its particular food, or it could be due to the plenty of existing food particle available for Ruminococcus and Butyrivibrio strains which produce a soluble

cellulase and *Bacteriodes succinogenes*. King(1956) reported that rumen fluid did not ordinarily contain much free cellulase, although soluble cellulose derivatives were attacked. Much of the cellulase was presumably attached to the cellulose. Hungate(1966) reported that filter paper(Whatman No.1) was not attacked to an appreciable extent by many bacterial strains which will attack the same filter paper after it was pebble-milled in water. Phillipson(1953) stated that under normal circumstances several factors can depress the digestion of cellulose but none can increase it. It is necessary to investigate what factor would depress cellulose digestibility.

### **Cellulose Determination**

According to Stolk(1956), relative values referred to filter paper No. 292 as 100 for the estimation of cellulose by different techniques show that 99.76%, 96.55%, 98.36% and 100.94% by the method of Norman and Jenkins(1933), Crampton and Maynard(1938), Viles and Silvean(1948), and Weende(A.O.A.C. crude fiber) methods respectively. In this experiment 98.5% in whatman No.1 filter paper and 98.8% in Toyo filter paper No.2 of cellulose were estimated by the Crampton and Maynard method modified Barnett (1957).

### **Carbohydrates Digestion**

Phillipson and McAnally(1942) reported that sugars, such as glucose, fructose and sucrose, were readily broken down in the rumen, and lactose, maltose galactose were much less efficiently utilized. Starch and cellulose were attacked only slowly. Stewart et al. (1958) found that molasses decreased acetic and corn meal increased propionic acid production. Chun-Akan (1963) found that molasses decreased pH of the rumen content and increased lactic and propionic acid production. But it did not cause the evolution of large quantities of acetic acid. Waldo and Schultz(1956) found that grain tends to increase the level of lactic acid in the rumen over hay alone. Large quantities of acetic acid are produced when fiber is digested(vanSoest 1963). EI-Shazly et al, (1952) reported that cellulose digestibility was lowered in the presence of starch or starchy feedstuff.

## Utilization of Nitrogenous Substances

Intensive studies on the rumen protein and non-protein nitrogen metabolism have been done in the presence of carbohydrates in the ration. Belasco(1956) found that the utilization of urea was greater with starch than with cellulose. In this study, starch yielded more acetic, butyric and valeric acids and less propionic acid than cellulose. High dextrose inhibited cellulose digestion while 1:1 ratios of starch:cellulose increased cellulose digestion(Belasco et al. 1956, Stewart et al. 1968). It has been reported that the wasteful effect of the bacterial deamination of protein can be considerably reduced when diets with a high content of readily fermentable carbohydrate are given to ruminants(Annison 1954, Fontenot et al. 1955, Lewis 1957, Shazley et al. 1952, Oh et al. 1969, Chicco et al. 1972). McDonad(1952) and Lewis and McDonald(1958) indicated that the best utilization of protein supplement is probably obtained when a carbohydrate is also present that can be fermented at a comparable rate. Lewis(1960) reported that the presence of casein stimulates the formation of volatile fatty acid from starch. He found that similar nitrogen-containing compounds are less effective than casein. Salsbury and Haenlein(1962) reported that certain simple mixture of amino acids increased cellulose digestion in vitro, particularly when used with the B-vitamin mixture. Price et al. (1972) investigated that as level of urea increased in the urea treatment diets, weight gains, feed consumptions, water intakes and terminal plasma urea levels increased linearly.

## Effect of Protein and Carbohydrates on Cellulose Utilization

A few studies on the effect of protein and starch or sugars on cellulose utilization have been reported. Head(1953), Borroughs et al. (1950) and Lewis and McDonald(1958) generally agree that adding starch depresses cellulose digestion and added protein has no influence on cellulose digestion. Salsbury et al. (1963) have found that a ration of cellulose, corn starch and urea is capable of maintaining the cellulose-digesting ability of rumen ingesta. Loosli and McDonald(1969) have reported that molasses-urea can be beneficial as a supplement of low quality forage diets. However, the interaction between carbohydrates and protein substances on the digestibility of cellulose has not been studied extensively.

## EXPERIMENTAL

### Animals

From Korean native cattle in Jeju Island (Jeju Korean Cattle), sheep (American Merino) at the experimental farm, Jeju Nation College the rumen liquor was collected.

### Materials

Ground filter paper (Whatman No.1 and Toyo filter paper No.2) was used for pure cellulose, urea as source of nitrogen, and dextrose for carbohydrates source were used in the experiment 1, 2, and 3.

### In Vitro Techniques and Method of Cellulose Analysis

The inoculum used in this experiment was collected through eight layers of cheese clothes into a thermoflask. A 0.2 gram of ground filterpaper were measured into a 50 ml. capacity-plastic flask which was equipped with the bunsen gas release system (Lee 1971). To these flask 20 ml. of previously prepared rumen inoculum was added. Each flask was incubated in a water bath maintained temperature at  $39 \pm 1^\circ\text{C}$ . After the end of each fermentation, samples were filtered through Toyo filter paper No.2, and dried them followed by cellulose analysis according to the method of Crampton and Maynard modified by Barnett(1957).

## RESULTS AND DISCUSSIONS

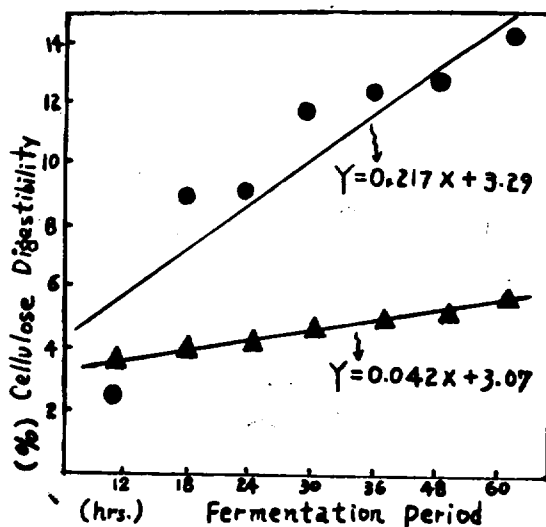
### Experiment 1. EFFECTS OF FERMENTATION PERIODS ON INVITRO CELLULOSE DIGESTIBILITY.

The fermentation periods did effect on cellulose digestibilities with significant ( $P < 0$ )

.05) regression coefficient,  $t=0.217$ , for Jeju Korean cattle and non significant regression coefficient,  $t=0.042$ , for sheep. Striking discrepancy was investigated between the degrees of cellulose digestion coefficients. In the Jeju Korean cattle, the range from the 12 hours-fermentation period to the 60 hours-fermentation period was 12% differences, while the range for sheep was 2.2%

**Table 1. Cellulose Digestibility at Various Time of Fermentation by JeJu Korean Cattle and Sheep.**

| Fermentation Periods.       | Animals | Cattle          | Sheep           |
|-----------------------------|---------|-----------------|-----------------|
| 12 hours.                   |         | 2.5%            | 3.6%            |
| 18                          |         | 9.2             | 3.8             |
| 24                          |         | 9.7             | 4.2             |
| 30                          |         | 12.1            | 4.5             |
| 36                          |         | 13.1            | 4.6             |
| 48                          |         | 13.8            | 4.8             |
| 60                          |         | 14.5            | 5.8             |
| Regression Coefficient,     |         | (b)=0.217*      | (b)=0.042N. S.  |
| M. S. Deviation Regression, |         | Sy. $x^2=5.88$  | Sy. $x^2=24.66$ |
| Regression Equation,        |         | $Y=0.217X+3.29$ | $Y=0.042X+3.07$ |



**Fig. 1. In Vitro Cellulose Digestibility at Various Fermentation Periods using a Simple Incubation of Rumen Liquor Technique.**

○.....Cattle,      ▲.....Sheep



the photo shows the technique of collection of rumen liquid from a sheep using vacuum power.

There was no individual variation for both of cattle and sheep. The reaction of cellulose digestibility was followed after 1st order reaction, 2nd order reaction and zero order reaction. The question was arose what factors would be involved in the cause of such low cellulose digestibilities for sheep. Protein and carbohydrates in the inoculum used might be the main factors to depress fermentation as earlier workers reported(Hungate 1966. Blaxter 1957). The following experiments 2 and 3 were conducted to investigate what levels of nitrogen and carbohydrates would effect on the digestibility of cellulose.

### **Experiment 2. EFFECTS OF NITROGEN AND CARBOHYDRATE ON CELLULOSE DIGESTIBILITY BY JEJU KOREAN CATTLE.**

The urea and dextrose effected significantly ( $P < 0.01$ ) on cellulose digestibility. It means the addition of urea and dextrose depressed cellulose digestibility at the level of 50% sample dry matter as protein equivalent, 100%, and 150% for the cattle. And the 0.5 ml., 1.0ml. and 1.5 ml. of 10% dextrose solution in 20 ml. of rumen liquid did depress the digestibility of cellulose. There was high significant effect ( $< 0.01$ ) in the interaction the urea and dextrose treatments. There also were high significant linear, quadratic and cubic effects ( $< 0.01$ ) in the urea treatments, and a linear effect in the carbohydrates treatment ( $P < 0.01$ ).

Individual comparison by means of Duncans new multiple range test indicated that there was no significant difference between the urea 0.0—dextrose 0.5 ml. treatment and the control group. As the level of dextrose were increased the digestibilities were decreased ( $P < 0.01$ ).

**Table 2. The Urea and Dextrose levels effected on Cellulose Digestibility in Jeju Korean Cattle.**

| Urea<br>% !           | Replication     |      |      | Mean | Individual Comperison |   |
|-----------------------|-----------------|------|------|------|-----------------------|---|
|                       | Dextrose<br>ml. | 1    | 2    |      |                       | 3 |
| 0                     | 0.0             | 14.3 | 14.2 | 15.1 | 14.53                 | a |
|                       | 0.5             | 12.1 | 16.3 | 13.3 | 13.90                 | a |
|                       | 1.0             | 10.4 | 7.2  | 9.9  | 9.16                  | b |
|                       | 1.5             | 11.8 | 6.4  | 7.3  | 8.50                  | b |
| 50                    | 0.0             | 4.3  | 4.2  | 5.4  | 4.63                  | c |
|                       | 0.5             | 3.9  | 4.9  | 4.7  | 4.50                  | c |
|                       | 1.0             | 3.9  | 4.0  | 4.4  | 4.10                  | c |
|                       | 1.5             | 3.4  | 3.4  | 4.0  | 3.60                  | c |
| 100                   | 0.0             | 3.8  | 4.1  | 4.9  | 4.26                  | c |
|                       | 0.5             | 3.7  | 3.8  | 4.5  | 4.00                  | c |
|                       | 1.0             | 3.5  | 3.2  | 4.2  | 3.63                  | c |
|                       | 1.5             | 3.2  | 3.1  | 3.7  | 3.33                  | c |
| 150                   | 0.0             | 3.5  | 3.5  | 4.3  | 3.76                  | c |
|                       | 0.5             | 3.4  | 3.1  | 4.0  | 3.50                  | c |
|                       | 1.0             | 3.1  | 3.1  | 3.7  | 3.30                  | c |
|                       | 1.5             | 2.6  | 3.1  | 3.5  | 3.10                  | c |
| Mean                  |                 | 5.68 | 5.48 | 6.05 |                       |   |
| Individual Comparison |                 | a    | a    | a    |                       |   |

! : % Sample dry matter as protein equivalent basis.

Individual comparison by Duncans new multiple range test indicates that there was no significant difference between similar symbols.

**Statistical Analysis in 4×4 Factorial Experiment.**

| Factors               | d. f. | S. S.  | M. S.  | F         |
|-----------------------|-------|--------|--------|-----------|
| Total                 | 47    | 669.29 | —      |           |
| Replications          | 2     | 2.72   | 1.36   |           |
| Treatment             | (15)  | 631.68 | 42.11  | 33.58 **  |
| Nitrogen(N)           | 3     | 539.29 | 179.76 | 143.35 ** |
| Carbohydrates(C)      | 3     | 40.38  | 13.45  | 10.73 **  |
| N. C. Interaction(NC) | 9     | 52.02  | 5.78   | 4.61 **   |
| Error                 | 30    | 37.61  | 1.254  |           |

## Regression in 4×4 Factorial Experiment.

| Factors           | d. f. | S. S     | M. S.     |
|-------------------|-------|----------|-----------|
| Nitrogen (N)      | (3)   | (539.29) |           |
| R. Linear         | 1     |          | 366.89 ** |
| N. Quadratic      | 1     |          | 143.86 ** |
| N. Cubic          | 1     |          | 28.64 **  |
| Carbohydrates (C) | (3)   | ( 40 38) |           |
| C. Linear         | 1     |          | 37.68 **  |
| C. Quadratic      | 1     |          | 0.03      |
| C. Cubic          |       |          | 2.67      |

In the experiment 2, the levels of urea were found to be high concentration comparing that of In Vitro trial of earlier workers. It was found that up to the 150% of sample dry matter level the micro population had a cellulase activity.

### Experiment 3. EFFECTS OF UREA AND DEXTROSE ON CELLULOSE DIGESTIBILITY BY AMERICAN MERINO SHEEP.

The concentration of urea in the experiment 3 was lowered than that of the experiment 2. The results of the 36 hours fermentation period effected on cellulose digestion are shown in the table 3,

Table 3. Effects of Urea and Dextrose on Cellulose Digestibility by Sheep.

| Urea %                 | Dextrose |        |        |        | Mean | Indiv. Comp. |
|------------------------|----------|--------|--------|--------|------|--------------|
|                        | 0.0ml.   | 0.5ml. | 1.0ml. | 1.5ml. |      |              |
| 00 *                   | 4.6 **   | 3.8    | 3.1    | 1.8    | 3.3  | a            |
| 25                     | 10.4     | 9.3    | 6.6    | 5.7    | 8.0  | b            |
| 50                     | 5.2      | 4.9    | 4.2    | 2.6    | 4.3  | a            |
| 75                     | 4.7      | 4.5    | 3.9    | 3.5    | 4.2  | a            |
| Mean                   | 6.2      | 5.6    | 4.5    | 3.5    |      |              |
| Individual***<br>Comp. | a        | ab     | ab     | b      |      |              |

\* % of sample D.M. as protein equivalent.

\*\* Mean value of 3 replications of fermentation flasks.

\*\*\* According to the Duncans new multiple range test.



The 25% urea-treatment plot was found significant increase of cellulose digestibility ( $P < 0.01$ ). This result has brought a fundamental support for further study in the field of establishing the feeding standard for ruminant animals and for the measurement of what chemical component of feedstuffs could be effectively utilized.

In the 60 hours of fermentation period plot, there was only 5.8% of the cellulose digestibility contrasting to 10.4% of cellulose digestibility which was twice as much as control plot. The 25% urea treatment-0.5% dextrose plot also showed significant increases. It was also interesting that the 0.15 ml. and the 0.1 ml. dextrose without urea treatment plots showed strikingly lower digestibility. These results were coincident with those results done by Hatch et al. (1972), Chicco et al. (1972), and Shultz et al. (1970) in their InVivo trials. Consequently the question arose from the experiment 1 was partially solved in the experiment 3 as these series of studies had intended to investigate at the start.

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### SUMMARY

A simple incubation of rumen liquor InVivo technique was adopted to determine the cellulose digestibility by the CheJu Native Korean cattle and sheep in the experiments 1, 2 and 3.

In the experiment 1, the fermentation periods increased cellulose digestibilities with regression coefficient of 0.017 ( $P < 0.05$ ) by CheJu Korean cattle, while slightly increased regression coefficient of 0.040 (N.S) was found in sheep. This discrepancy was solved by doing the experiments 2 and 3.

Addition of urea and dextrose caused significant decrease ( $P < 0.01$ ) in cellulose di-

gestibility. There were significant interactions ( $P < 0.01$ ) between the two additives. There were linear, quadratic and cubic effects ( $P < 0.01$ ) in the urea treatments, and linear effect in carbohydrates ( $P < 0.01$ ) by the CheJu Korean cattle in the experiment 2. The 0.25% of urea treatment plot in the sheep experiment 3, a high significant increase of cellulose digestibility was found showing twice as much as the control plot.

#### LITERATURE CITED

- Annison, E. F. 1954. Some Observations on Volatile Fatty Acids in the Sheep's Rumen. *Biochem. J.* 57: 400.
- Belasco, I. J. 1956. The Role of Carbohydrates in Urea Utilization, Cellulose Digestion and Fatty Acid Formation. *J. Anim. Sci.*, 15: 496.
- Bently, O. G., R. R. Johnson, S. Vanecko and C. H. Hunt. 1954. Studies on factors needed by rumen microorganisms for cellulose digestion *In vitro*. *J. Anim. Sci.* 13: 581.
- Blaxter, K. L. 1967. *The Energy Metabolism of Ruminants*. Hutchinson Scientific and Technical Press.
- Borroughs, W. Gall, L. S., Gerlaugh, P., and Bethke, R. M. 1960. The Influence of Casein upon Roughage Digestion in Cattle with Rumen Bacteriological Studies. *J. Anim. Sci.*, 9: 214.
- Chicco, C. F., T. A. Shultz, E. Shultz, A. A. Carnevali and C. B. Ammerman, 1972. Molasses-Urea for Restricted Forage fed Steers in The Tropics. *J. Anim. Sci.*, 35: 859.
- Crampton, E. W. and Maynard, L. A. 1938. The Relation of Cellulose and Lignin Content to the Nutritive Value of Animal Feeds. *J. Nutrition*, 15: 383.
- Chun-Akana, R. K. Y. 1963. *The Effect of Molasses on the Organic Acids in The Rumen*, of Animal Science, Univ. of Hawaii.
- Fontenot, J. P., Gallup, W. D., and Nelson, A. B. 1955. Effect of Added Carbohydrate on the Utilization by Steers of Nitrogen in Wintering Rations. *J. Anim. Sci.*, 14: 807.
- Hatch, C. F. and W. M. Beeion. 1972. Effect of Different Levels of Cane Molasses on Nitrogen and Energy Utilization in Urea Rations for Steers. *J. Anim. Sci.*, 35: 854.
- Head, M. J. 1953. The Effect of Quality and Quantity of Carbohydrate and Protein in the Ration of the Sheep on the Digestibility of Cellulose and Other Constituents of the Ration, with a Note on the Effect of Adding Vitamins of the B-Complex on the Digestibility and Retention of the Nutrients of Hay Ration. *J. Agr. Sci.*, 43: 281.
- Hungate, R. E. 1966. *The Rumen and Its Academic Press*.
- Kamstra, L. D., Moxon, A. L. and Bentley, O. G. 1958. The Effect of Stage of Maturity and Lignification the Digestion of Cellulose in Folage Plants by Rumen Microorganisms *in vitro*. *J. Anim. Sci.*, 17: 199.
- Lee, M. H. 1971. Comparison Studies of *In Vitro* Fermentation Methods on Organic Matter Digestibility. *Korean J. Anim. Sci.*, 13: 312.

- Lewis, D. and McDonald, I.W. 1958. The Inter-Relationships of Individual Proteins and Carbohydrates during Fermentation in the Rumen of the Sheep. I. The Fermentation of Casein in the Presence of Starch or Other Carbohydrate Materials. *J. Agr. Sci.*, 51 : 108.
- Lewis, D. 1962. The Inter-Relationships of Individual Proteins and Carbohydrates During Fermentation in the Rumen of the Sheep. *J. Agr. Sci.*, 58 : 73.
- Loosli, J.K. and I.W. McDonald. 1969. Non-Protein Nitrogen in the Nutrition of Ruminants. *F. A. O. Agr. Studies N 75*. Rome.
- McDonald, I.W. 1952. The Role of Ammonia in Ruminant Digestion of Protein. *Biochem. J.*, 51 : 86.
- McCl. H. K., W.M. Longhurst and M. B. Jones. 1969. Relation of Nitrogen Intake to Rumen Microbial Activity and Consumption of Low Quality Roughage by Sheep. *J. Anim. Sci.*, 28 : 272
- Phillipson, A. T. and McAnally, R. A. 1942. Studies on the Fate of Carbohydrates in the Rumen of Sheep. *J. Exp. Bio.*, 19 : 199.
- Price, W. D., J. A. Brown, E. E. Menvielle and W. H. Smith. 1972. Effect of High Levels of Urea in Purified Diets for Lambs: Growth and Metabolism. *J. Anim. Sci.*, 35 : 848.
- Salsbury, R. L., Hoefler, J. A., 1961. Effect of Feeding Certain Defined Nutrients on Cellulose Digestion and Volatile Fatty Acids Concentration of the Rumen. *J. Anim. Sci.*, 44 : 1122.
- Salsbury, R. L. and Haenlein, G. F. W. 1962. Some Nutrients Required for Cellulose Digestion by Rumen Microorganism. *J. Anim. Sci.*, 21 : 1012.
- Salsbury, R. L. and Haenlein, G. F. W. 1963. Influence of Nitrogen Source on Cellulose Digestion In Vitro. *J. Anim. Sci.*, 22 : 846.
- Shazley, K. E. 1952. Degradation of Protein in the Rumen of the Sheep. I. Some Volatile Fatty Acids, Including Branched-Chain Isomers, Found In Vitro. *Biochem. J.*, 51 : 640.
- Shazly, K., Dehority, B. A., and Johnson, R. R. 1961. Effect of Starch on the Digestion of Cellulose In Vitro and In Vivo by Rumen Microorganisms. *J. Anim. Sci.*, 20 : 268.
- Shultz, T. A., Elena Shultz and C. F. Chicco. 1972. Pressure Cooked Urea-Cassa Meal for Lambs Consuming Low Quality Hay. *J. Anim. Sci.*, 35 : 865.
- Snedecor, G. W. 1962. *Statistical Methods*. 5th ed. The Iowa State College Press, Ames, Iowa.
- Stewart, W. E. and Schultz, L. H. 1958. In Vitro Volatile Fatty Acid Production from Various Feeds by Bovine Rumen Microorganism. *J. Anim. Sci.*, 17 : 737.
- Van Soest, P. J. 1963. Ruminant Fat Metabolism with Particular Reference to Factors Affecting Low Milk Fat Efficiency. A Review. *J. Dairy Sci.*, 46 : 204.

〈要 約〉

## 飼料의 家畜에 依한 利用성과 産肉能力에 關한 研究

### (1) InVItro 纖維素消化利用에 關한 研究

李 弼 煥

濟州韓牛와 緬羊의 Cellulose 消化率을 測定하기 爲하여 第1, 第2 및 第3 試驗에서 第1 胃液의 單純醱酵法을 채택하였다.

第1 試驗 結果에 의하면 濟州韓牛는 醱酵時間을 달리함에 따라서 回歸係數  $b=0.217(P<0.05)$  로써 增加 現象을 보였으나 緬羊은  $b=0.042$  로써 有意的 增加現象은 보이지 않았는데, 이러한 不一致現象이 第2, 第3 試驗에서 研明되었다.

濟州韓牛의 第2 試驗에서, 尿素와 Dextrose 添加로 因하여 Cellulose 消化率의 현저한 감소 ( $P<0.01$ )를 보였으며, 尿素와 Dextrose間에 交互作用이 현저하게 나타났다( $P<0.01$ ). 또한 尿素處理에서 第1次, 第2次 및 第3次 回歸關係 ( $P<0.01$ )를 나타냈으며, 炭水化物區에서는 有意的 回歸直線 ( $P<0.01$ )關係가 있음을 發見하였다.

緬羊試驗에서 0.25% 尿素處理區는 高度의 有意的 纖維素 消化率을 보였고 對照區보다 2 倍의 消化率을 나타냈다.