JOINT RESEARCH REPORT

By Takeo Hasegawa [1), 2)] , Toshio Nagaishi [3)]

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- 2) Kyoto Prefectural University of Medicine , Graduate School of Medical Science
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THETA LACTO

Symbiotic effect of young Shiitake mushroom powder and Formula-1 (Lactobacillus Plantarum S-1) on bioactivity

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INDEX

What is Formula-1 lactic acid?

Common name	:	Formula-1（vegetable Lactobacillus）
Scientific name	:	Lactobacillus Plantarum S-1
Discovered by	:	Louis Pasteur Center for Medical Research
Source	:	Pickles（Japanese name: Suguki-zuke）
Features	:	Acid resistant. Reachable to intestine. Proof of activation of tumor necrosis factor related apoptosis inducing ligand: TRAIL

Suguki-zuke.
Traditional pickles in Kyoto

~Related paper~

Horinaka M, Yoshida T, Kishi A, Akatani K, Yasuda I, Kokura S, Wakada M, Sakai T. "Lactobacillus strains induce TRAIL production and facilitate natural killer activity against cancer cells" , FEBS Letters(587),P577-582, 2010

WHAT IS SHIITAKE MUSHROOM?

Habitat

- Shiitake Mushroom grows on dead broad-leaved tree as like chinquapin tree.

Shiitake mushroom

Nutritional Contents

- Shiitake contains plenty of 5'-guanylic acid, glutamic acid, β-glucan, and many vitamins.

- 5'-guanylic acid and glutamic acid are both Umami (Japanese for flavor) component and these two acids produce various symbiotic effects. Shiitake provides rich taste, and in Japan, it's very common to use the extract soup from dried shiitake as a stock/hidden flavor.

- B-glucan is one of the main components in mushroom.
- B-glucan already shows some good effects on human body, such as enhancing the immunity system, cancer control, etc.

CULTIVATION METHODS OF SHIITAKE MUSHROOM

1) **Log cultivation :** Cultivated on dried sawtooth oak (Quercus acutissima) or Japanese oak (Quercus serrata). It is very close to nature but mass production is difficult.

2) **Fungus bed cultivation :** Cultivated on artificial medium(*). Mass production is very easy and almost all of mushroom is of high quality and homogeneous.

3) **Hydroponics cultivation :** Cultivated on artificial medium immersed in water. Unorthodox method in recent years.

(*) artificial medium : made of mixed wood tip, rice bran, and nutrients.

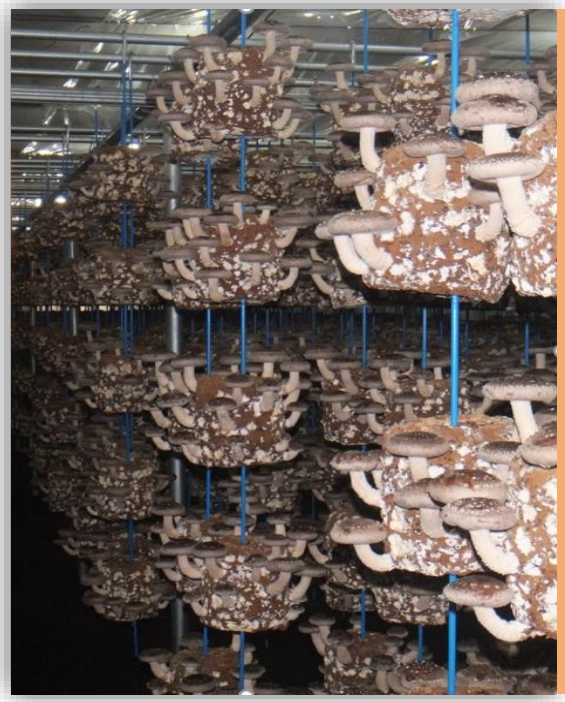
We cultivate our mushroom by Fungus Bed Cultivation method.

Images of each methods

Log cultivation

Fungus bed cultivation on shelf

Hydroponics cultivation



Our method

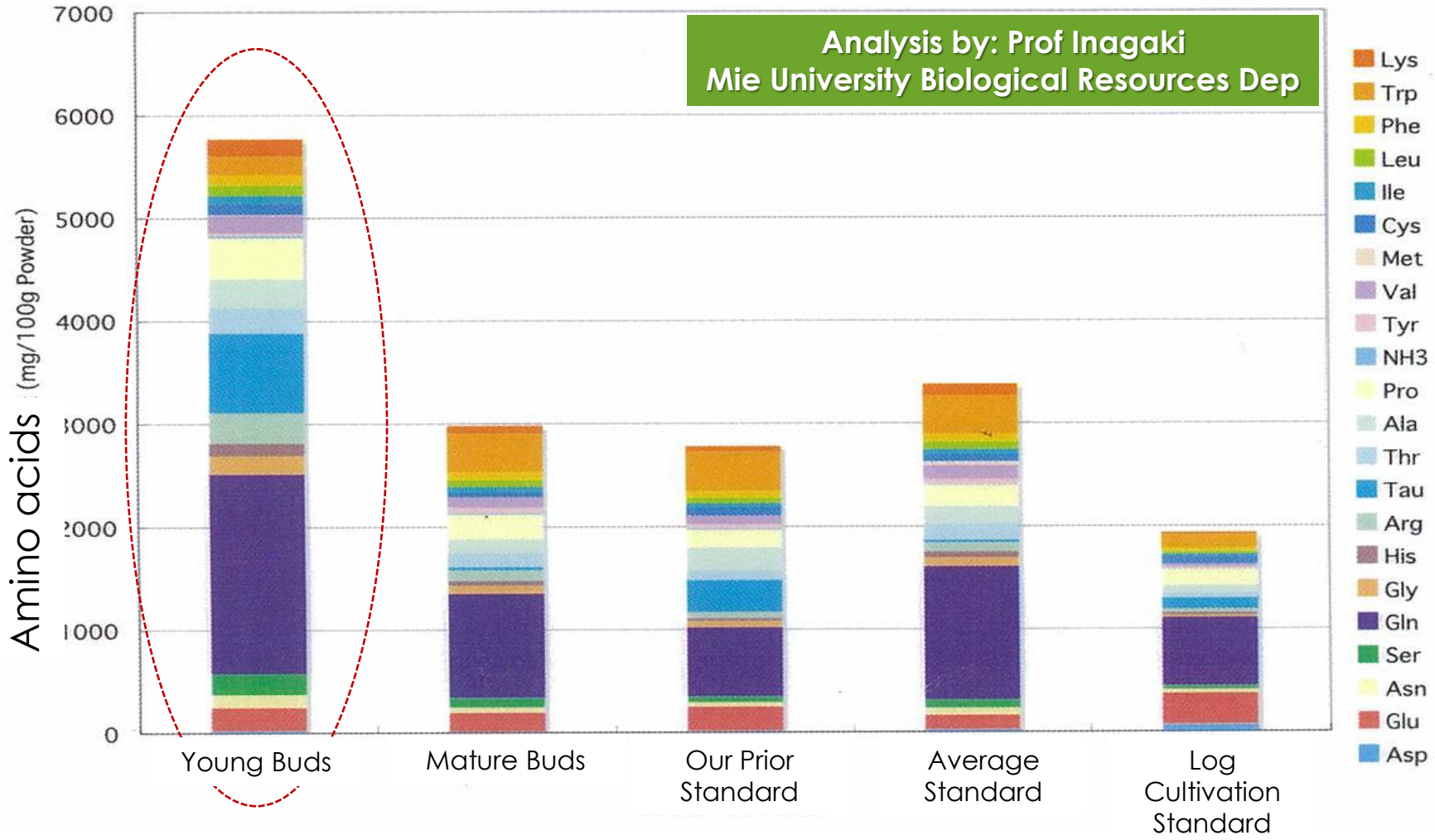
Hanged fungus bed cultivation (Hanged method)

Merits: Easy to maintain hygiene & grow environment.

High work efficiency.

Picture of young and mature Shiitake

Accelerating amino acid content of nipped mushroom sprouts



Graph: Comparison of Amino Acids

The amount of each Free Amino Acids

Table: The amount of amino acids in each sample (mg/ 100g powder)

Amino acids	young	mature	prior	others	Log
Asp	22.64	9.15	14.67	23.21	65.77
Glu	224.84	183.93	236.29	140.14	303.17
Asn	128.16	56.72	43.49	70.83	43.75
Ser	204.67	90.57	54.96	78.51	30.82
Gln	1933.38	1014.71	672.25	1299.96	664.59
Gly	178.92	73.20	58.45	78.24	25.12
His	120.85	44.95	29.60	60.48	22.08
Arg	301.60	110.20	61.26	88.82	37.79
Tau	766.56	28.26	307.68	24.92	105.85
Thr	251.45	132.95	94.40	157.46	45.24
Ala	277.82	138.83	223.85	173.83	77.00
Pro	401.06	238.84	172.11	202.30	155.13
NH3	18.61	13.08	10.65	10.67	3.23
Tyr	29.87	52.82	41.12	49.63	17.31
Val	166.40	97.64	77.24	128.72	21.08
Met	7.71	4.27	5.28	42.85	6.76
Cys	107.13	42.50	75.94	62.36	78.32
Ile	81.79	56.68	46.47	53.48	21.92
Leu	102.60	72.55	60.07	76.00	25.00
Phe	94.54	73.74	56.73	74.52	29.58
Trp	182.74	371.63	380.68	370.73	128.31
Lys	165.18	76.76	54.76	110.50	26.32

Properties of amino acids present in THETA LACTO

- **Asn** (asparagine) : collagen production
- **Ser** (serine) : enhancement of enzyme activity
- **Gln** (glutamine) : nutrition for muscle cells, source of energy
- **Gly** (glycine) : glycogen genic amino acid, natural sweetener
- **His** (histidine) : essential amino acid, source of histamine
- **Arg** (arginine) : essential amino acid, essential for growth, immune activity, cell replication, collagen production & fatigue recovery
- **Tau** (tau protein) : supporting the structure of nerve cell
- **Thr** (taurine) : essential amino acid, homeostasis, liver function
- **Ala** (alanine) : hemoglobin, raw material for chlorophyll
- **Pro** (proline) : collagen repair, prevention of over-ageing of skin
- **Val** (valine) : essential amino acid, metabolism, tissue repair
- **Cys** (cysteine) : antioxidant, radiation protection, immunity
- **Leu** (leucine) : growth of infants, nitrogen equilibrium for adults, maintenance of muscles

The mechanism of Fomula-1 and Shiitake mushroom on bioactivity

Lactobacillus

- From animal → Killed by stomach acid → Decomposition Products → Enhance immune system
- From vegetable e.g. Fomula-1 → Reachable to intestine → Improving intestinal environment → Enhances immune system. Inhibiting increase in blood sugar level

Shiitake mushroom →
- ・Low in carbohydrate
- ・plenty of vitamins and minerals

→ Provides bioactive substances Enhances immunity system

Bioactive substances in Fomula-1 and Shiitake

Fomula-1

Ribonucleic acid	Antiaging, recovery from fatigue
oligosaccharide	Enhancing immunity system
Peptide	Antitumor effect

Synergy

Shiitake

Ergosterol	Precursor of vitamin D
Lanthionine	Prevent Thrombosis and promote blood circulation
D-Eritadenine	Lowers blood pressure and cholesterol
Lentinan	Anti-tumor effect

Material and Method

Mouse : C57BL/6, male, 7 weeks old (n=12, each group)
Tumor cells : Cancer cells ($5*10^5$ /0.05mL) from lung
Lactobacillus : Fomula-1
Ingredient of shiitake bait : 50% shiitake powder , 30% rat bait, 10% soy flour, 5% cheese, and 5% rice flour
Normal bait : 100% rat bait

Measurement item

Weight, amount of food intake, tumor volume

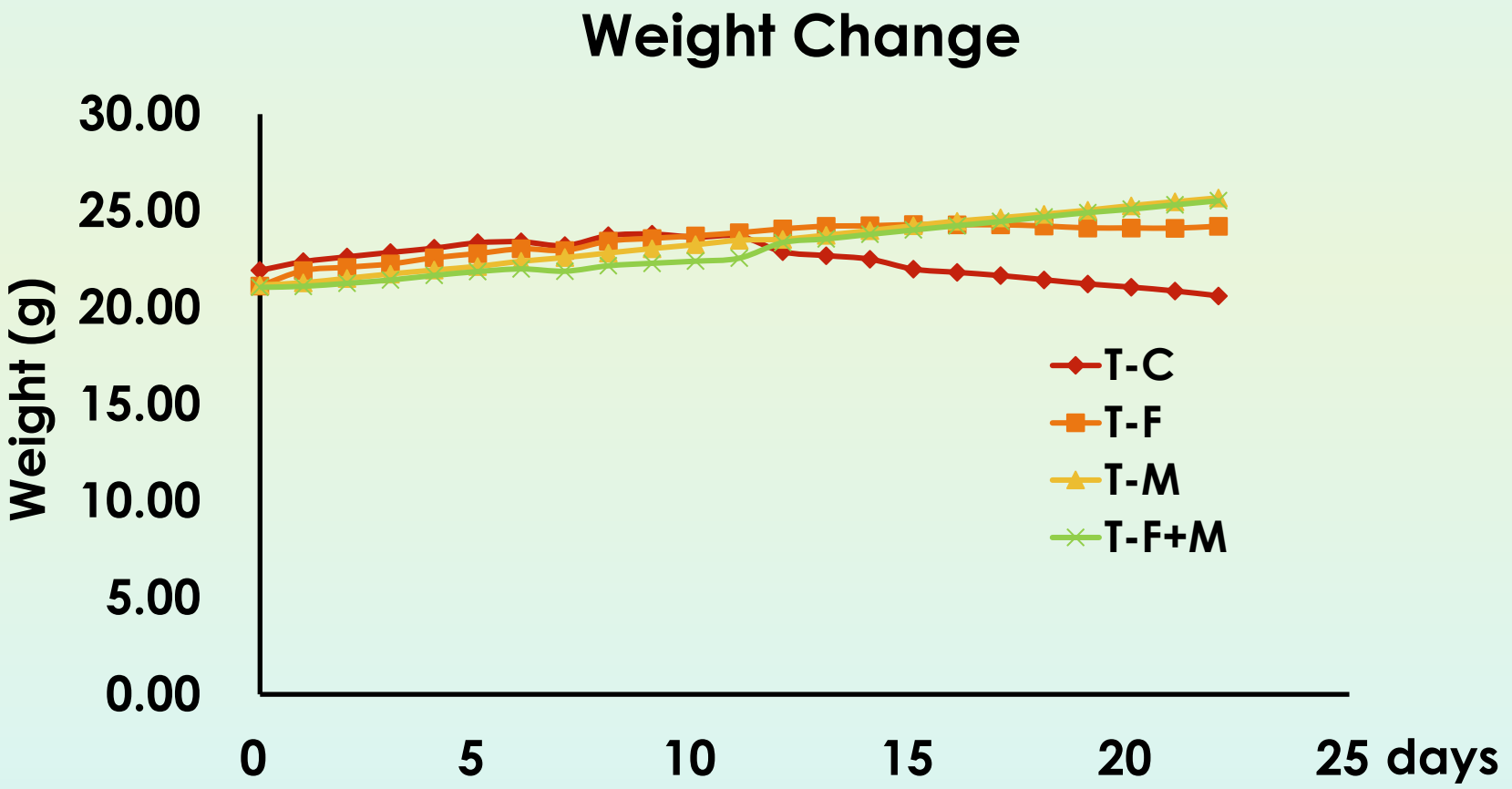
Method

Transplant cancer cells to thigh and divide into four groups.

1) T-C : Control group. Normal bait, not administered with Fomula-1
2) T-F : Fomula-1 group. Normal bait, administered with Fomula-1
3) T-M : Shiitake bait group. Shiitake bait, not administered with Fomula-1
4) T-F+M : Combination group. Shiitake bait, administered with Fomula-1

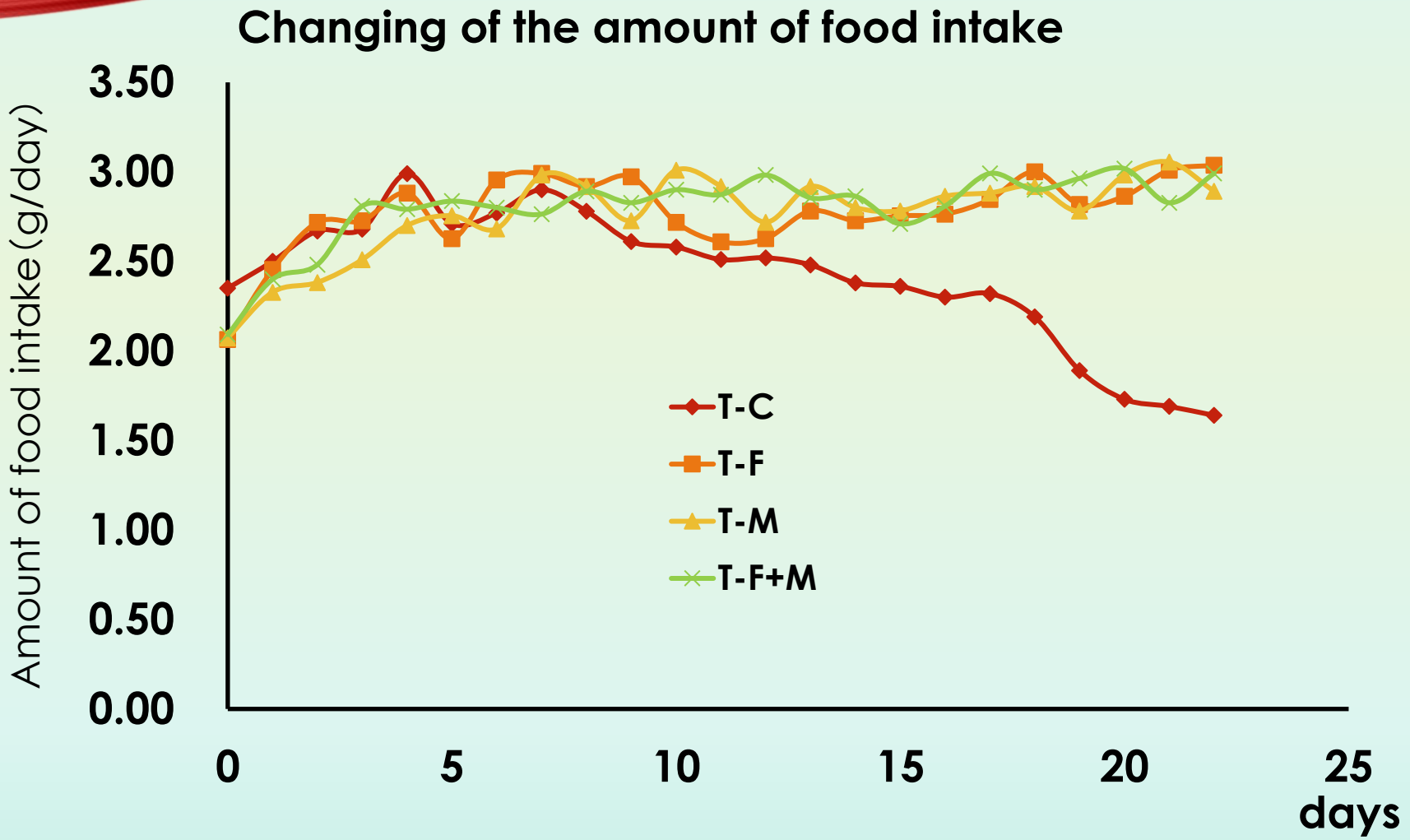
Orally administer 0.3ml Fomula-1 everyday.
Mouse are allowed to eat freely. Monitoring period: 30days

Results



The control group only lose its weight in the end.

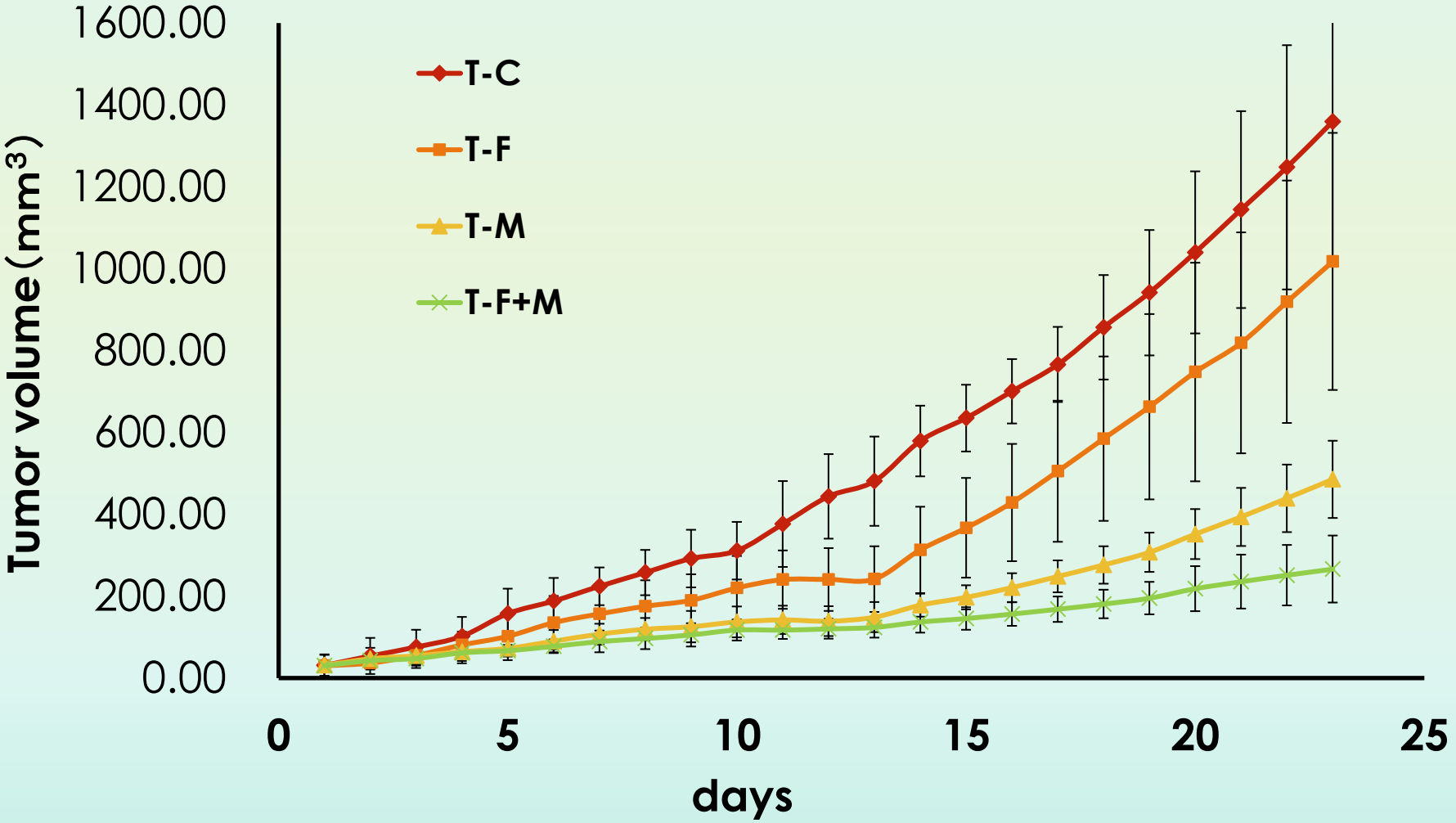
Results



The control group only lose the amount of food intake as the days goes by.

Results

Changing of tumor volume



The combination of Fomula-1 and Shiitake shows the best antitumor effect. Shiitake group shows better antitumor effect than Fomula-1 group.

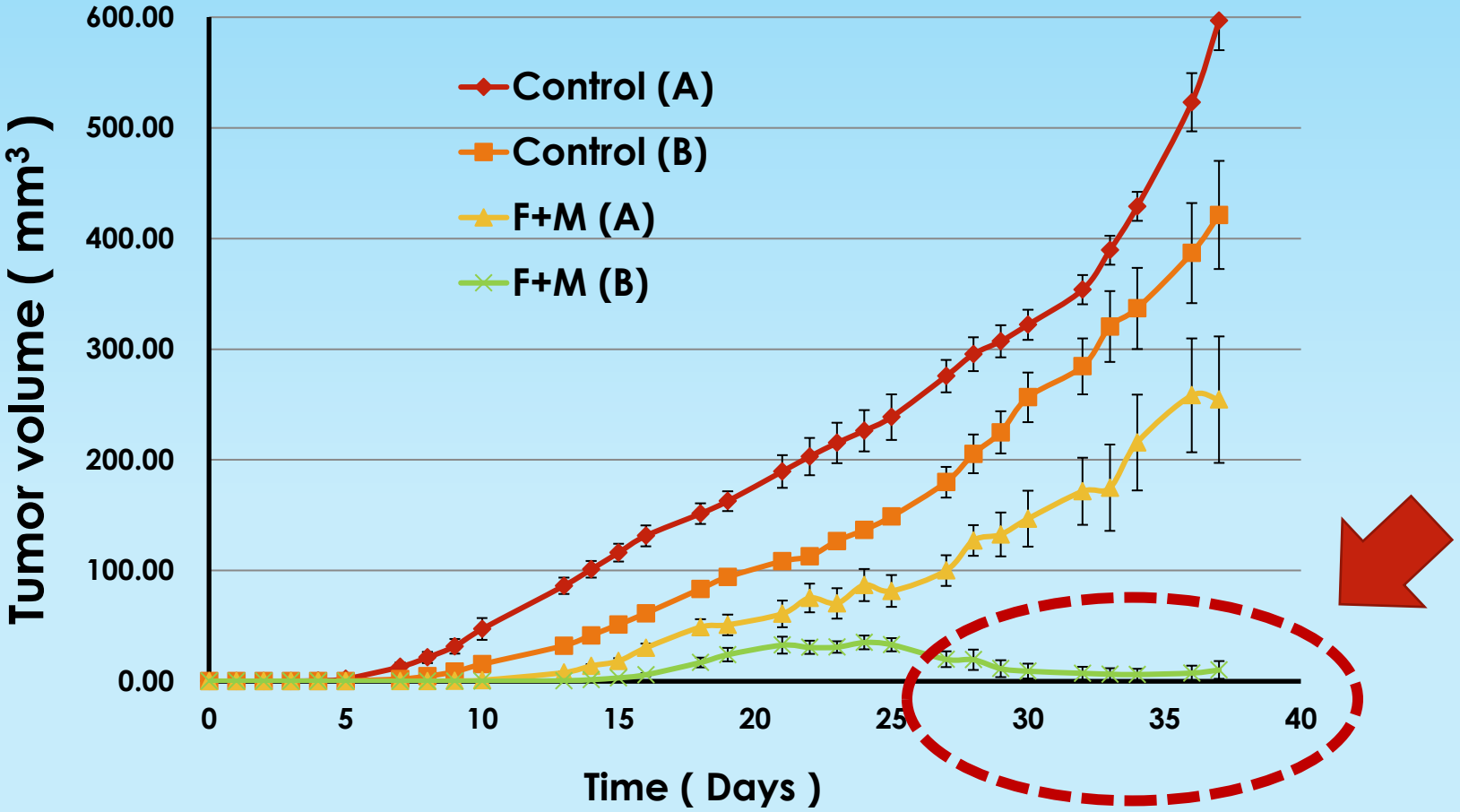
Results The antitumor effect at early stage

	days to 100mm^3	growth ratio
T-C	6.25	1.00
T-F	9.38	1.50
T-M	15.31	2.45
T-F+M	19.53	3.12

Slows down the tumor growth by 2/3

Additional Experiment

Anti-tumor effect on early stage tumor



A = 3.0x10⁵ Tumor Cell Transplantation Group
(average administration amount)

B = 1.5x10⁵ Tumor Cell Transplantation Group
(Minimum administration amount)

F+M
F1 Lactic Acid + Shiitake Powder
(THETA LACTO)

the combination of Fomula-1 and Shiitake powder shows significant reduction of early stage tumor

Conclusion

Food intake

T-C group lost the amount of food intake as time went by.

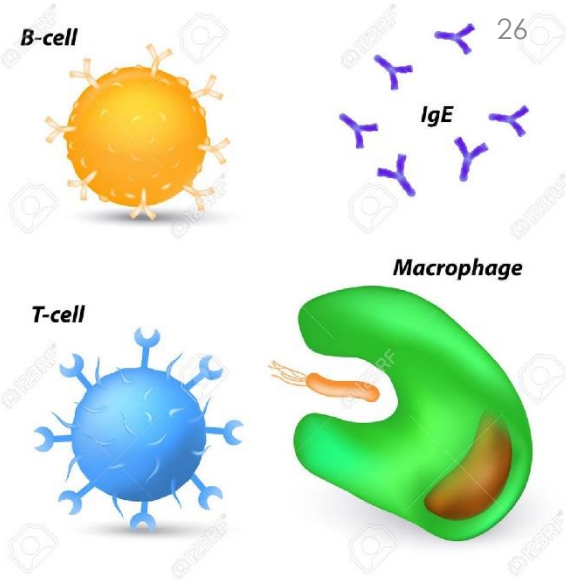
Weight

Only the T-C group lost its weight by the end of the experiment.

Antitumor effect

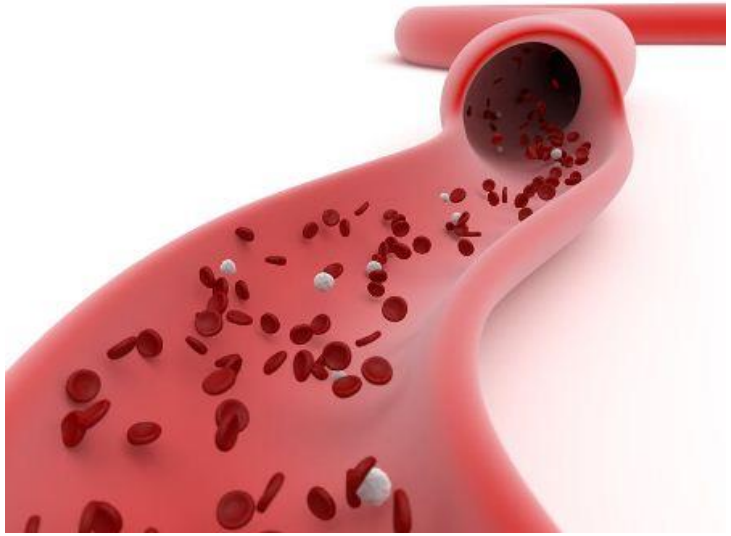
Comparing to T-C group, every other group shows anti-tumor effect.
Especially, T-F+M group shows significant antitumor effect.

Moreover, in early stage, combination of Fomula-1 and Shiitake could even reduce the tumor volume.



Second Experiment

Effects on blood sugar level and immunity



Material and Method

Material

Mouse :	C57BL/6, male, 7 weeks old (n=12, each group)
Lactobacillus :	Fomula-1
Ingredient of shiitake bait :	50% shiitake powder , 30% rat bait, 10% soy flour, 5% cheese, and 5% rice flour.
Normal bait :	100% rat bait

Measurement item

Weight, amount of food intake, Cytokine activity (important material for immune system), blood sugar level.

Method

Mouse are divided into four groups and fed different baits.

1) NT-C :	Control group. Normal bait, not administered with Fomula-1
2) NT-F :	Fomula-1 group. Normal bait, administered with Fomula-1
3) NT-M :	Shiitake bait group. Shiitake bait, not administered with Fomula-1
4) NT-F+M :	Synergy group. Administered with Shiitake bait and Fomula-1

Orally administer 0.3ml Fomula-1 everyday.
Mouse can eat the bait freely. Monitoring period - 30days

Function of Measured Cytokine

IL-2 : Stimulates lymphocytes and boosts immunity

IL-4 : Stimulates immune cells and differentiate into B-cells (Antibody producing cells)

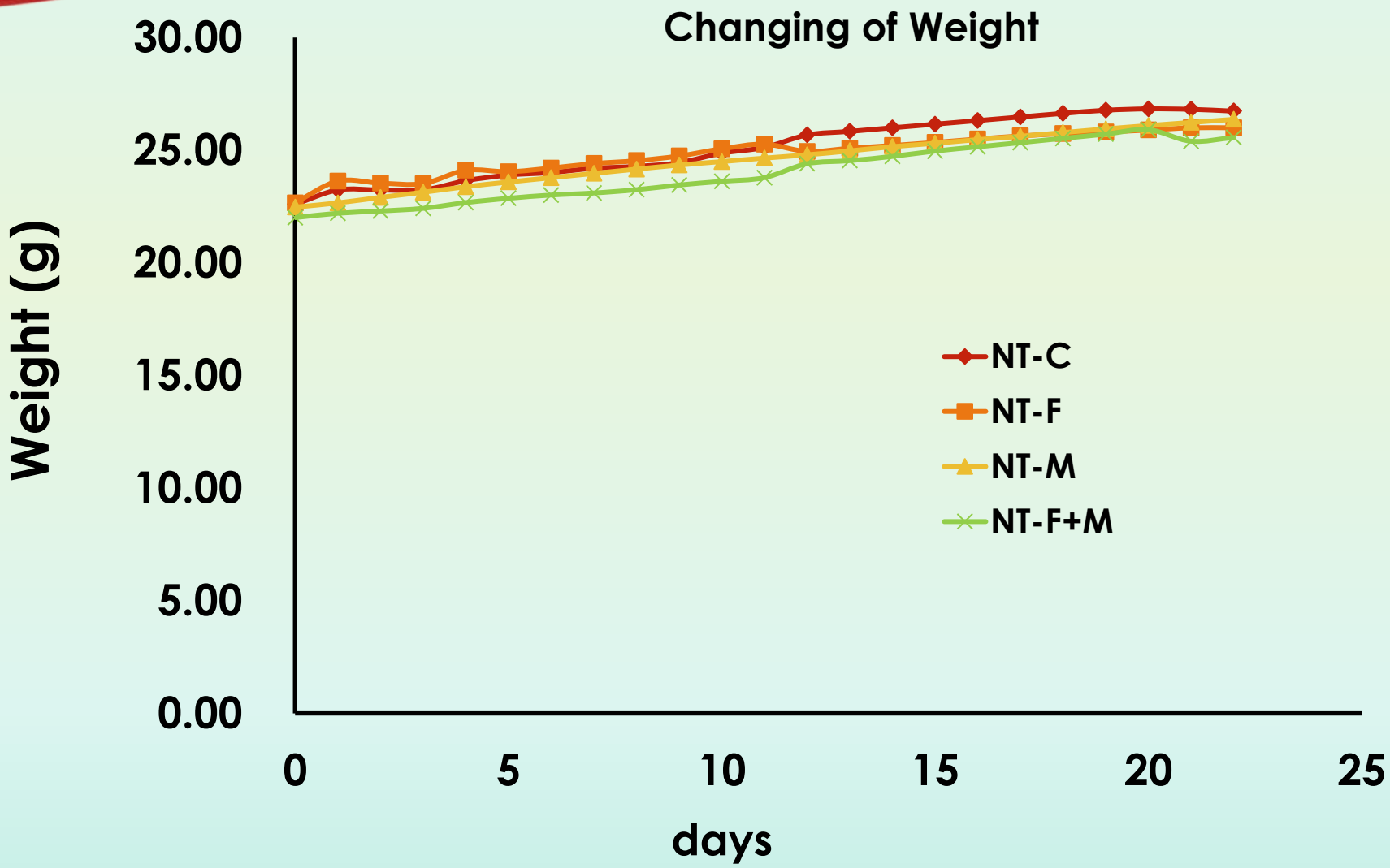
IL-10 : Reduces overreaction of immune system and inflammation

IL-12 : Activate NK (natural killer) cells and boosts immunity

IFN-γ : Shows Antiviral Activity.

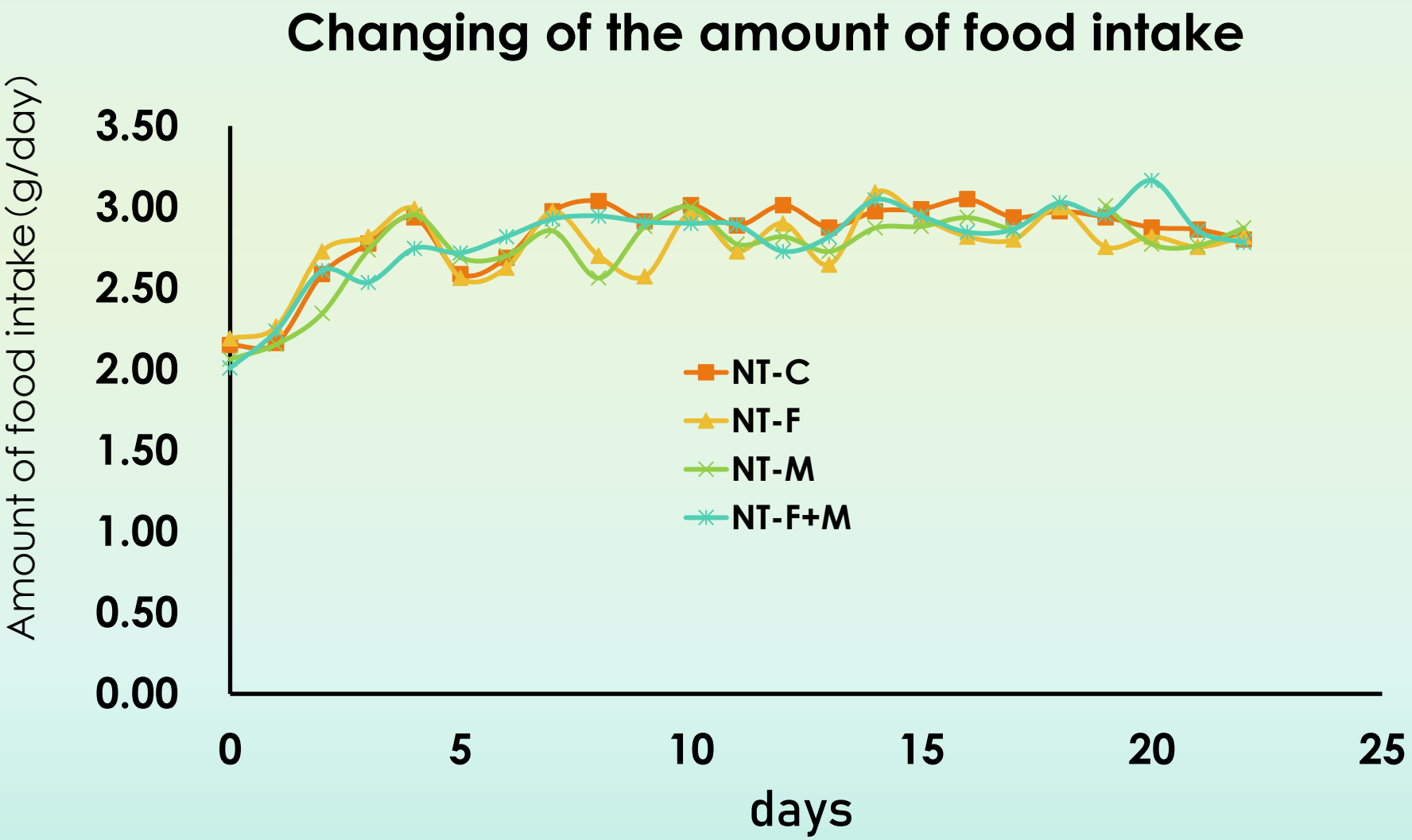
Activated IL-2 and IFN-γ stimulates NK cells and enhance immune system against pathogens and tumor, such as cancer cells.

Results



No significant differences.

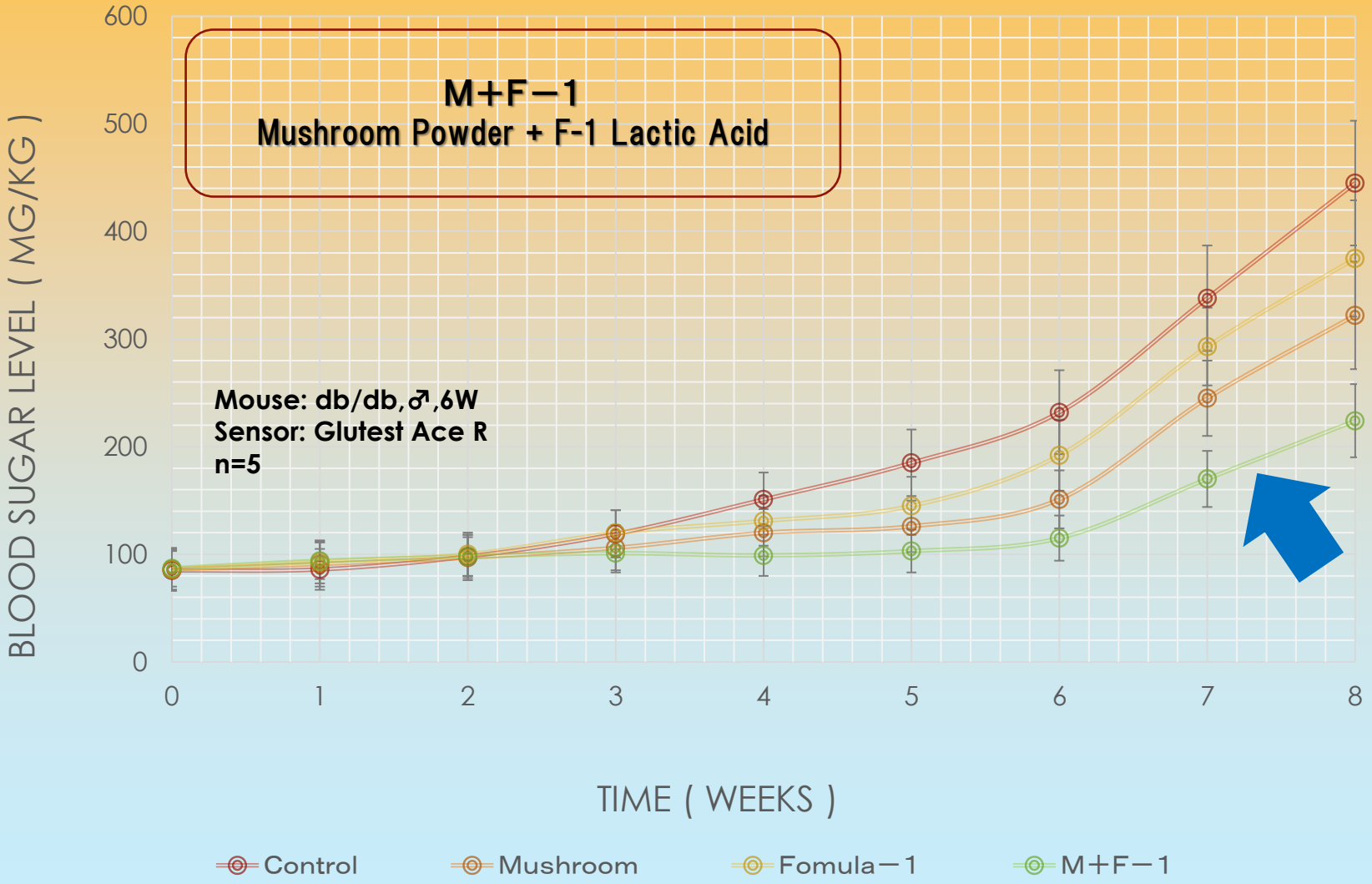
Results



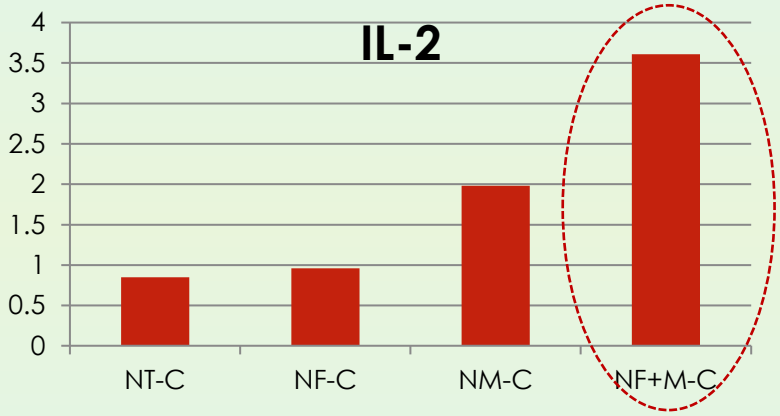
No significant differences

RESULTS

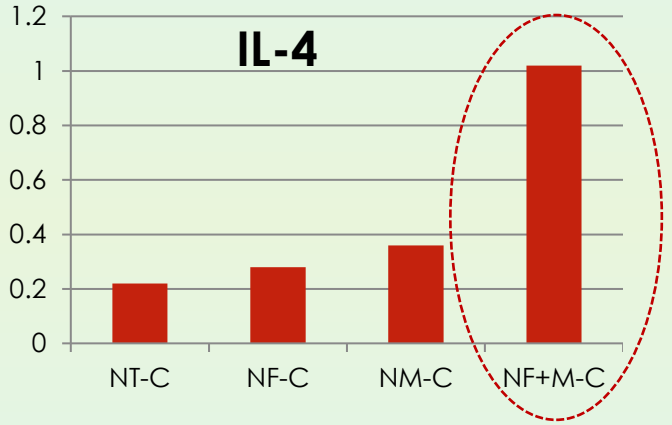
Effect on blood sugar level (mouse)



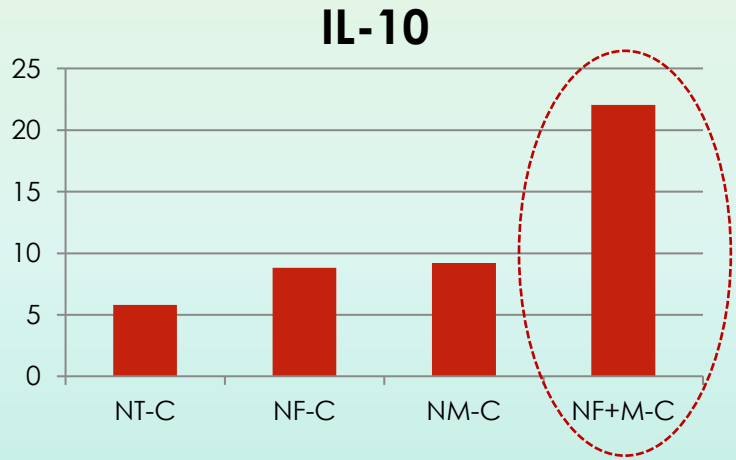
Cytokine（Immune System Activation Factor）



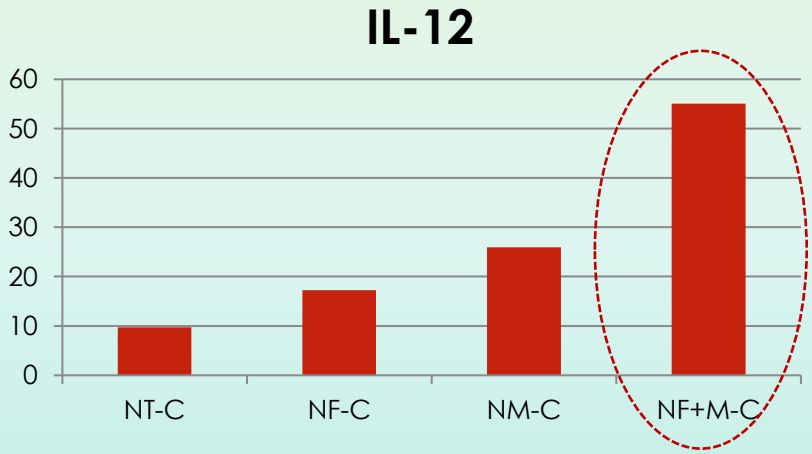
IL－2：Activation of lymphocytes



IL－4: Macrophage activation.

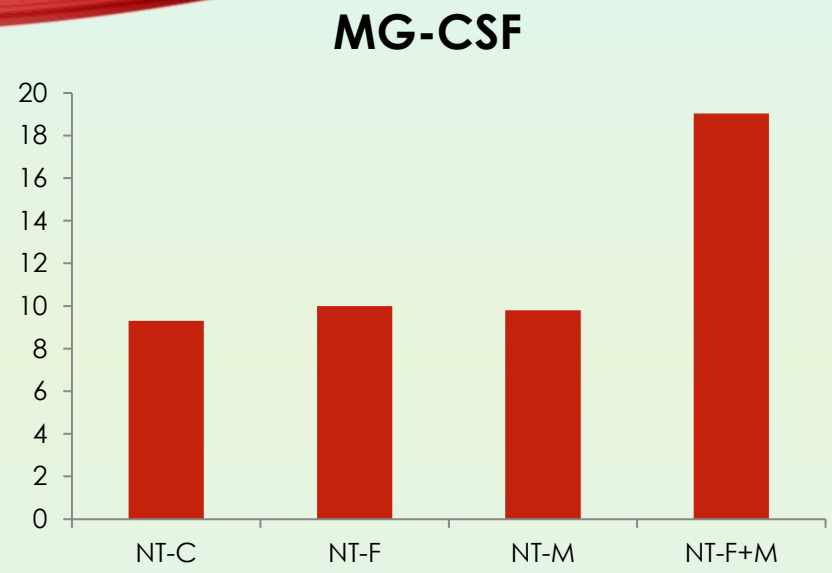


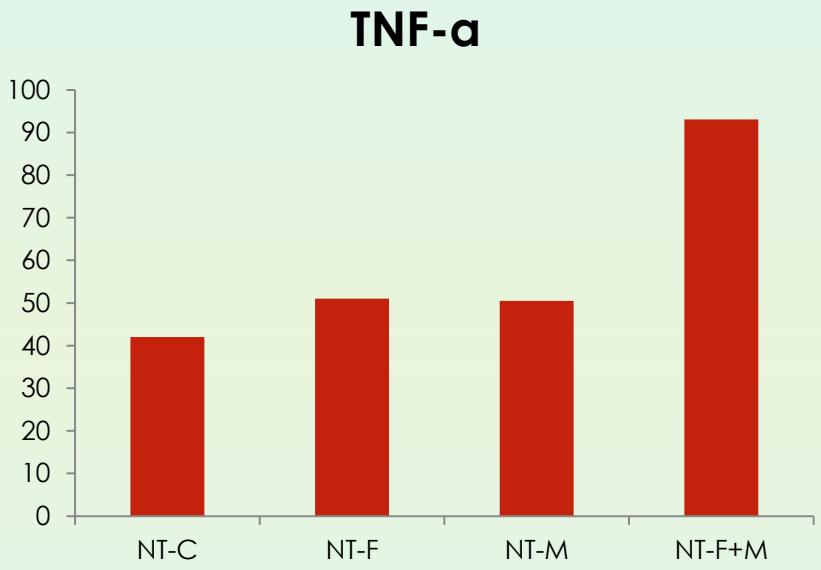
IL－10 ：Control of Th1 (T helper) cells and maintenance of QOL(quality of life).

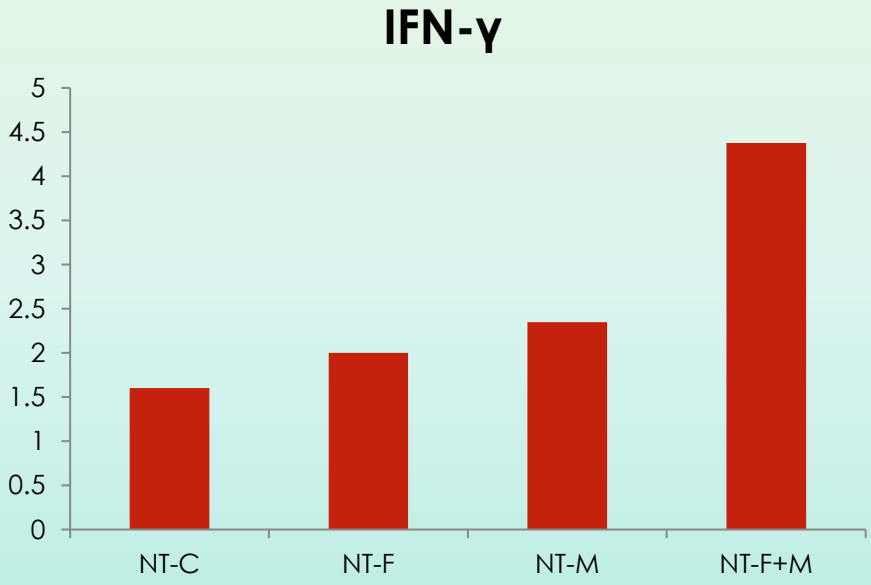


IL－12 ：Activation of NK (natural killer) cells and suppression of the cancer cells.

Results: activity of cytokine

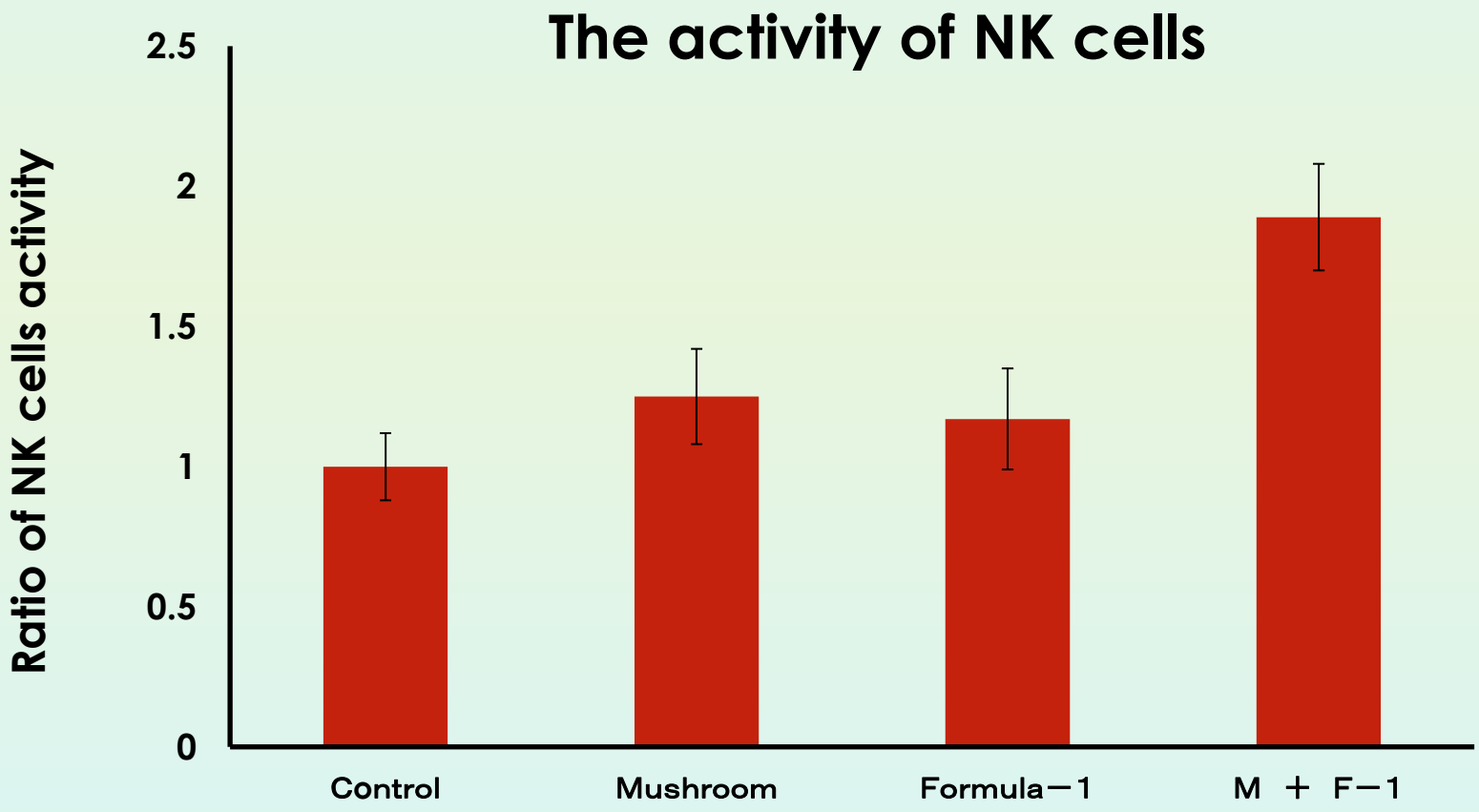






NT-F+M group shows the most activated cytokine in every cytokine

Results



M+F group shows the most activity of NK cells. This is one big reason that M+F group has significant anti-tumor effect.

CONCLUSION

Food intake & Weight

There were no significant differences among the groups.
This means that shiitake tastes good for mouse.

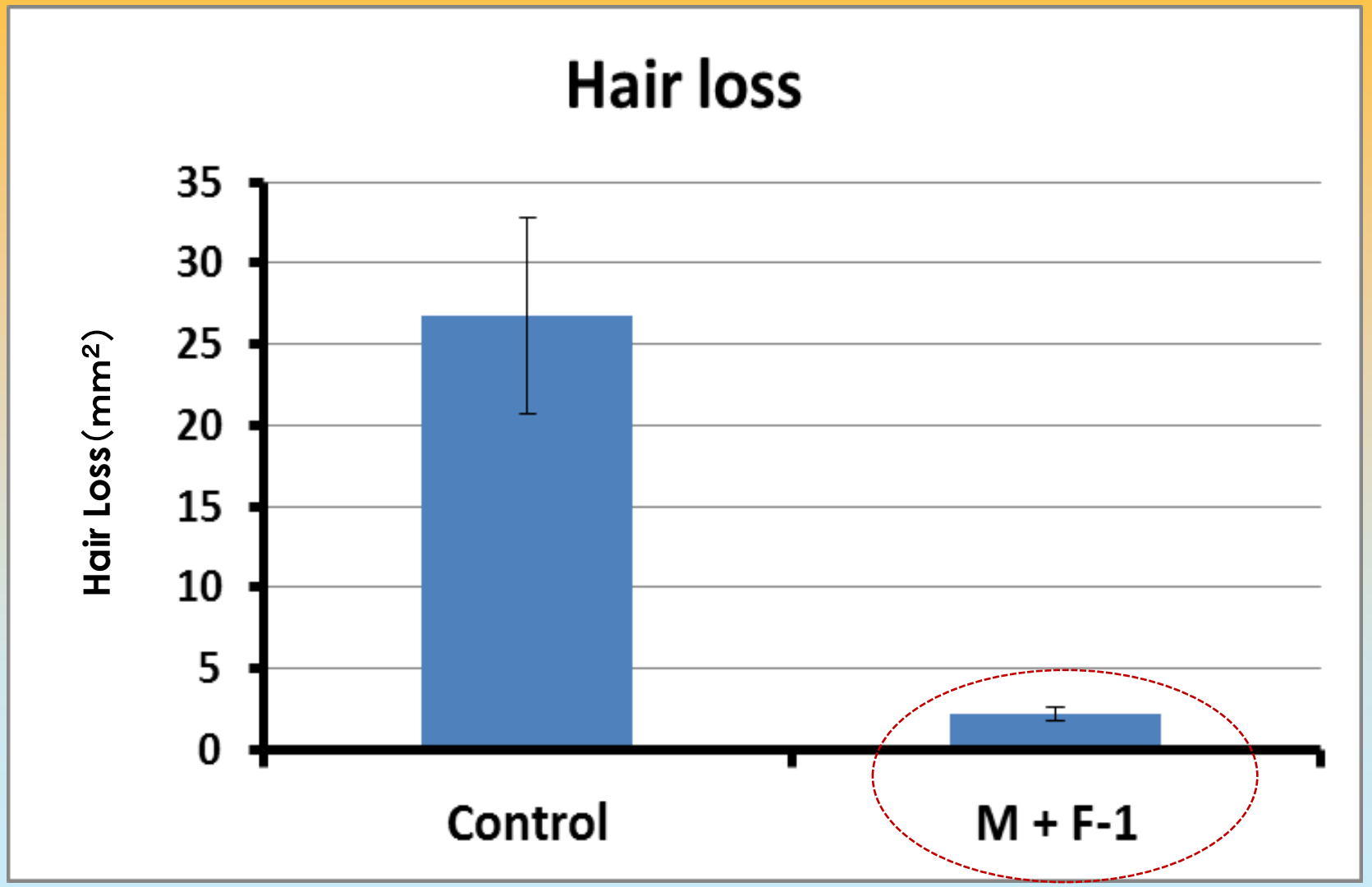
Effect on immunity

The Cytokine was activated the most in NT-F+M group.
This is the reason why T-F+M group showed the significant antitumor effects.

Effect on blood sugar level

NT-F+M group suppressed the rise in blood sugar levels.
Therefore the combination of Fomula-1 and Shiitake powder can be the inhibitor for blood sugar elevation.

Side effects: reducing hair loss



Document filed for patent

Experiment on the physiological effect of shiitake mushroom powder and F-1 lactic acid

1) LOUIS PASTEUR CENTER FOR MEDICAL RESEARCH: Kyoto
2) KYOTO PREFECTURAL UNIVERSITY OF MEDICINE: Gastroenterology department

【Experiment method & materials】

Mouse lung metastatic tumor cells (LLC cells: 1.5 x 105 / 0.05 mL, 3 x 105 / 0.05 mL) were transplanted subcutaneously into the thighs of C57 BL / 6 mice. The 4 groups were the untreated control group, the group treated with the plant lactic acid bacteria (Formula-1: F-1) alone, the group treated with shiitake mushroom bud powder (Mushroom: M) alone, and the group of F - 1 + M combined group. Their serum and lung tissues were collected from 10 days after starting the experiment and cytokines (IL-2, IL-4, IL-10, IL-12, IFN-γ) were examined. NK (natural killer) cell activity of the lymphocytes taken from the spleen was also examined after the treatment. 1 × 107 / 0.3 mL of lactic acid bacteria were orally administered daily. The rats were allowed free access to 1.3 to 1.5 g / day of solid dry shiitake mushroom powder.
Feed intake, body weight and tumor diameter were measured daily.

【Results】

No significant body weight change was observed between all experiment groups. The activation of cytokines (IL-2, IL-4, IL-10, IL-12, IFN-γ) was observed to be 1.3 to 2.5 times more even in the single treatment groups, but in the combination group between 4 ~ 8 times more synergistic activity was observed. The activity of NK (natural killer) cells was observed to be 1.2 to 1.3 times more in the single group and 1.5 to 2.2 times more in the combination group. In terms of antitumor effect, the number of days taken for the tumor to be 25 mm3 in volume was 8.1 days for the 3 × 105 / 0.05 mL transplantation control group, 11.4 days for the 1.5 × 105 / 0.05 mL tumor transplantation control group, 15.5 days for the 3 × 105 / transplanted mouse combination group, 19.2 days for the 1.5 × 105 / 0.05 mL transplanted mouse combination group.
If The tumor growth ratio of 3 × 105 / 0.05 mL tumor transplant control group was normalized to 1.00, 1.5 × 105 / 0.05 mL tumor control group was 1.34, 3 × 10 5 / 0.05 mL tumor-implanted combination group was 1.89, 1.5 × 105 / 0.05 mL tumor-implanted combination group was 2.34. Therefore a synergistic antitumor effect by the combination of lactic acid bacteria and shiitake mushroom powder was observed.

【Consideration】

There are many naturally extracted substances with physiological effects, but there are few reports that synergistic effects occur with the combined use of multiple substances. In this experiment, immunity competent cytokines were synergistically activated by the combined use of suspension-cultivated, dried shiitake mushroom powder and plant based F-1 lactic acid bacteria, and thus NK cell activity and antitumor effect were observed. IL-2 and IL-4 activate lymphocytes and NK (natural killer) cells, IL-10 is involved in maintaining QOL of the ill and the elderly, and IL-12 is involved in the progression of malignant tumors. These results indicate that Theta Lacto is effective in maintaining health and maintaining QOL by suppressing the carcinogenesis and activating the NK cells and immunocompetent cells. Therefore, clinical application is a real prospect.

Summary Focus on the Taste and nutrients

Shiitake mushroom tastes good, has low calories, has plenty of Umami ingredients, and rich in amino acids.

- Rich taste
- High nutritional value
- Healthy and easy to eat

Jerky
Name : *Matsutaro Jerky*

Features

- Low calorie
- Lots of Umami ingredients to stimulate the appetite
- Matsutaro Jerky lasts for 60 days

Summary - Focus on the Efficacy

The combination of Fomula-1 and Shiitake powder has **a lot of positive effects** on the human body.

- Antitumor Effects
- Suppression of blood sugar levels
- Enhance the immunity system

Expected Efficacy

- Inhibitor for the blood sugar elevation.
- Antitumor Effects.
- Enhancement of the immune system.

Supplement

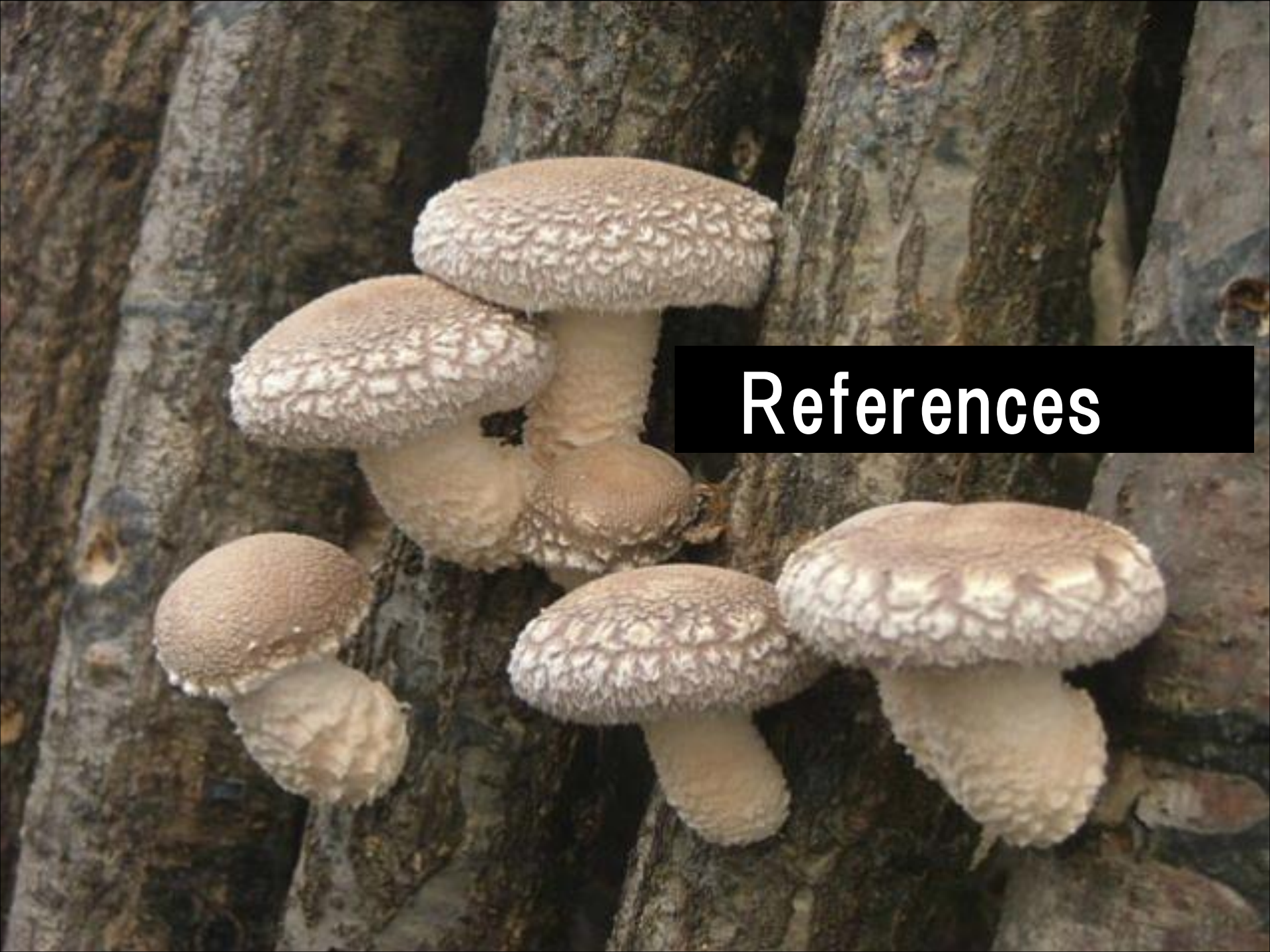
For human :*Super theta lacto*

For animal :*Theta lacto*

Current Spec & Future Plan

Development	Specifications
Fungal Bed	・Germination rate：60%±10% ・Harvest conditions：Temp 20°C ~±3° C / Humidity 75%~±2% ・Germination period：110 days ±15 days ※Room Temp 17~25° C ・Number of Germinations：3 times ※ Trial testing with coffee extract sand changing of the nutritional content and balance
Shiitake culture・cultivation & powdering	・Cultivation period：110 days ~ 120 days (max) ・Nurturing period：sprouting within 2 days ~ 3 days ・Harvesting sprout size：less than 5〜10mm in diameter ・Drying method : 1 hr sundry & low temp plasma dry (Eco-Fujin) ・Drying temp：48° C ・Drying time ：15 ~ 20 hours ・Testing for its effectiveness using different strands

Development	Specifications
Outsourced development: clinical human trial	・Test for Immune regulating effect (persons of over 50 years of age with normal health / 1 g per day) →Proof of the activation of helper T cells & killer T cells
	・Comparison of the dendritic cell count between control A/B →Immune function improvement, intestinal immune activation due to the increase in the number of peripheral blood dendritic cells

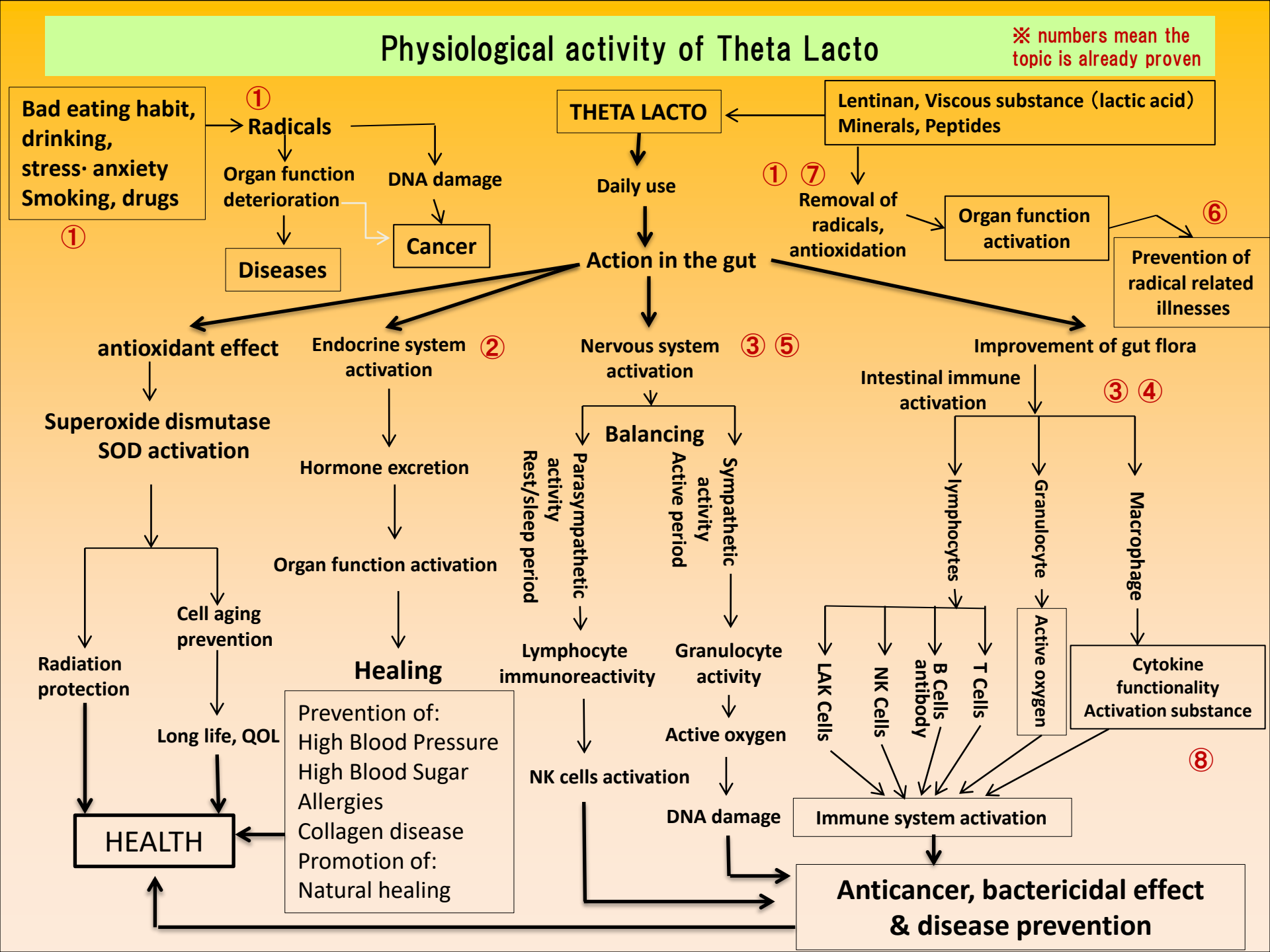

References

Plant – based lactobacillus (F-1)

Popular name	:	Formula-1（plant-based lactobacillus）
Scientific name	:	Lactobacillus plantarum S-1
Discovery	:	Louis Pasteur Center for Medical Research
Origin	:	Tsukemono (pickled & fermented Japanese turnip)
Characteristics	:	Strong resistance to gastric acid, works in the intestinal tract, long history of cultivation (Kyoto – Japan), nontoxic, antitumor factor（Tumor necrosis factor related apoptosis inducing ligand: TRAIL) Proven anti-tumor effect by activation

Related papers

Authors	:	Horinaka M, Yoshida T, Kishi A, Akatani K, Yasuda I, Kokura S, Wakada M, Sakai T.
Journal	:	FEBS Letters
Thesis	:	Lactobacillus strains induce TRAIL production and facilitate natural killer activity against cancer cells.
Pages	:	587、pp577－582
Published	:	2010


Physiological activity of Theta Lacto
※ numbers mean the topic is already proven
Bad eating habit, drinking, stress· anxiety Smoking, drugs
①
①
Radicals
Organ function deterioration
DNA damage
Diseases
Cancer
THETA LACTO
Lentinan, Viscous substance（lactic acid） Minerals, Peptides
Daily use
① ⑦
Removal of radicals, antioxidation
Organ function activation
⑥
Prevention of radical related illnesses
Action in the gut
antioxidant effect
Endocrine system activation ②
Nervous system activation ③ ⑤
Improvement of gut flora
Superoxide dismutase SOD activation
Hormone excretion
Organ function activation
Balancing
Parasympathetic activity Rest/sleep period
Sympathetic activity Active period
Intestinal immune activation
③ ④
Lymphocytes
Granulocyte
Macrophage
Radiation protection
Cell aging prevention
Long life, QOL
Healing
Lymphocyte immunoreactivity
Granulocyte activity
LAK Cells
NK Cells
B Cells antibody
T Cells
Active oxygen
Cytokine functionality Activation substance
⑧
Prevention of:
High Blood Pressure
High Blood Sugar
Allergies
Collagen disease
Promotion of:
Natural healing
NK cells activation
Active oxygen
DNA damage
Immune system activation
HEALTH
Anticancer, bactericidal effect & disease prevention

Literature References

【References】
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