Studies on Oral Care Supplements

(T-immune stick, Gangchi tntn) for Eliminating

Periodontitis, Plaque, Calculus and Malodor

Applicant	Prochall Korea Inc.
Period of study	03/03/2014~30/04/2014
Research institute	Industry-Academy Cooperation Foundation of Jeju University
Chief of research	Prof. Yoon, Young Min

Contents

1. Introduction

1) Title

Studies on canine oral care supplements (T-immune stick and Gangchi tntn) for eliminating periodontitis, plaque, calculus and malodor

2) Client: Prochall Korea Inc.

3) Research institute of test: Industry-Academy Cooperation Foundation of Jeju National University

4) Chief of research: Prof. Yoon, Young Min, Jeju National University

2. Materials

1) Manufacturer: Prochall Korea Inc.

2) Product: T-immune stick and Gangchi tntn

3) Date of manufactured: 02/05/2014

4) Registration number

T-immune stick: 99MOX0001

Gangchi tntn: 99MOX0023

5) Formulation: Components

(1) T-immune stick (per 100g)

Acetic acid 50mg

Eugenol 50mg

Ingredient: Vital wheat gluten, glycerine, Whey Protein Concentrates, cellulose, monosodium phosphate, Sodium hexametaphosphate, alpha-amylase, Xylanase, Protease, Acetic acid, Eugenol), Oregano oil

(2) Gangchi tntn (Per 100g)

Acetic acid 20 mg

Crude saponin 20 mg

Ingredient: Vital wheat gluten, Glycerine, Yeast extract, Cellulose, Xanthan gum, Fructooligosaccharide, Yucca extract, Parsley powder, Acetic acid, Beta-sitosterol

6) Dosage form

(1) **Dosage form**: Stick of solid matter

(2) **Description:**

T-immune stick; 20g of (8mm x 100mm)

Gangchi tntn; 4g of solid hexagonal stick beige colored (13mm x 30mm)

3. Period of examination

03/03/2014~30/04/2014 (2months)

4. Objectives of experiment

This studies were carried out to know the efficacies of oral care supplement (T-immune stick and Gangchi tntn) for eliminating periodontitis, plaque, calculus and malodor in companion dogs showing symptoms of gingivitis, plaque, calculus and malodor.

5. Experimental animals

The 20 companion dogs more than 5 years old showing symptoms of gingivitis, plaque, calculus and malodor.

6. Study design and schedule of assessment

1) Experimental animals

The 20 companion dogs (female, 12; male, 8; average age, 8±2.5 year; body weight, 5±3.2kg) of showing pre-periodontitis symptom of gingivitis, plaque, calculus and malodor were allotted randomly each 10 dogs in experiment of T-immune stick and Gangchi tntn, respectively.

2) Method

(1) **Feeding of T-immune stick**

All dogs were fed with adult dog feed (Royal Canin Adult, small dog under 10kg). The dog of test group (5 dogs), however, were supplied with T-immune stick (10g is relevant to 5 cm of T-immune stick) after finishing the feeding. To the below 6kg B.W. dogs, 10g of T-immune stick were supplied. And, 20g of T-immune stick were supplied to the 6~20kg B.W. dogs. All dogs were measured the eliminating efficacies of plaque, calculus and malodor weekly, and investigated the blood chemistry at 2 and 4 weeks after feeding of oral supplements.

(2) Feeding of Gangchi tntn

All dogs were fed with adult dog feed (Royal Canin Adult, small dog under 10kg). The dog of test group (5 dogs), however, were supplied 4g of Gangchi tntn (4g is relevant to 3 cm of Gangchi tntn) per 2kg B.W. as recommended dose after finishing the feeding. All dogs were measured the eliminating efficacies of gingivitis, plaque and malodor weekly, and investigated the blood chemistry at 2 and 4 weeks after feeding of oral supplements.

(3) Evaluation of Gingivitis

The condition of gingivitis was valuated using the Löe&Silness gingival index (GI) (Table 1) and scored the gingivitis through buccal gingival sulcus of each tooth.

① Tooth scored: Maxillary I3, C, P2, P3, P4, M1,

                Mandible C, P2, P3, P4, M1

② Valuation criteria

Table 1. Gingival Index.

Score	Criteria
1	**Mild inflammation** i.e. slightly redness and swelling but no bleeding, or delayed bleeding on gentle probing of the gingival sulcus
2	**Moderate inflammation** i.e. the gingiva is red, swollen and bleeds on gentle probing of the sulcus
3	**Severe inflammation** i.e. the gingiva is red or reddish-blue, the gingival margin is swollen, tendency to spontaneous hemorrhage or profuse hemorrhage on probing and/or ulcerations along the gingival margin

③ Calculation score: Averaged score of gingival condition

(4) Evaluation of plaque

The condition of plaque was valuated using Quigley and Hein (Turesky) index (PI) method (Table 2). The plaque of corona and gingiva of each tooth were confirmed and scored after applying plaque staining solution (Red cote, 1.5% D&C red No. 28, John O. Butler Company) and washing the buccal face of teeth.

① Tooth scored: Maxillary I3, C, P2, P3, P4, M1,

      Mandible C, P2, P3, P4, M1

② Valuation criteria

Table 2. Plaque Index

Score	Criteria	
	Coverage	Thickness
0	No observable plaque	
1	1 - 24% coverage	Light=pink to light red
2	25 - 49% coverage	Medium=red
3	50 - 74% coverage	Heavy=dark red
4	75 - 100% coverage	

③ Calculation of score: Half score was accounted for measurement through the coverage x thickness method and the other half score was added by measurement of gingiva and crown, and total score was measured by summing up each score of teeth. Total averaged score was measured by averaging the total score.

(5) Evaluation of calculus

The condition of calculus was valuated using the Schiff method (Table 3). After uncover the tooth by lifting the lips and drying the tooth in the air, the buccal face were divided vertically by the mesial, buccal and distal areas. The extent of calculus condition of each area were scored.

① Tooth scored: Maxillary I3, C, P2, P3, P4, M1,

               Mandible C, P2, P3, P4, M1

② Valuation criteria

Table 3. Calculus Index

| Score | Criteria |
	Coverage
0	No observable calculus
1	1 - 24% coverage
2	25 - 49% coverage
3	50 - 74% coverage
4	75 - 100% coverage

③ Calculation of score: Scored each tooth by summing up the score of each area. Total score was measured by averaging score of calculus.

(6) Evaluation of Stain index

Evaluation of staining was measured by the Schemehom method (Table

4). After dividing the tooth vertically by the mesial, buccal and distal areas, the score was measured by the staining based on plaque coverage area.

① Tooth scored: Maxillary I3, C, P2, P3, P4, M1,

   Mandible C, P2, P3, P4, M1

② Valuation criteria

Table 4. Stain Index.

Score	Criteria	
	Coverage	Intensity
0	No observable stain	
1	1 - 24% coverage	Light yellow or tan
2	25 - 49% coverage	Medium brown
3	50 - 74% coverage	Dark brown to black
4	75 - 100% coverage	

③ Calculation of score: Scoring each area of tooth by coverage x intensity method and summing up the scores. Total score was measured by averaging the scores.

T-immune stick group	Test group (2g/kg, q 1week for 4 weeks)		
	Control group		
Gangchi tntn group	Test group (2g/kg, q 1week for 4 weeks)		
	Control group		

Estimation for oral hygiene	↓	↓	↓	↓	↓
	Pre	1 wk	2 wks	3 wks	4 wks

Figure 1. Summary of the experimental groups and estimation. Estimation was carried out by observation of gingival index, plaque index, calculus index, stain index, blood chemistry.

(7) Evaluation of Malodor

Malodor was measured by instrument (Halimeter, Interscan Corporation, USA) that inhale the air in oral cavity for 30 seconds measuring volatile sulfur compound (VSC). Normal; 0~99ppb, subjectively recognized as unpleasant odor; 100~199ppb, objectively recognized as malodor; 200ppb, however, it is not used in dogs that inhalation from dogs' oral cavity and trachea because of its anatomical differences with human. In this study, measured odor of collected air from oral cavity before feeding (V0)the oral supplement and compared with that of every week for 1 month (V1, V2, V3, V4). Measuring by triplicate, odor values were averaged and improvement effect was presented by decreased percentage (%) of malodor.

Decreased percentage of malodor (%) = ((Vx-V0) x100)/V0

V0; malodor of first day, Vx; malodor of 1, 2, 3 and 4 week after treatment

① Place the probe to measure the odor after relaxation.

② Collect odor of oral cavity using suction pump for 30 seconds in collecting balloon.

③ Connect the collecting balloon to Halimeter probe.

④ Measure the collected odor maintaining pressure of the balloon.

Figure 2. Method of dog oral malodor estimation.

(8) Analysis of blood chemistry

To evaluate of T-immune stick and Gangchi tntn, change of blood and serum conditions were measured. Blood sample was collected in anticoagulant tube (EDTA-2K, Heparin) through jugular vein using at 0, 2 and 4 weeks after treatment. Complete blood count (CBC) was measured using Pochi-v to analyze the red blood cell index (RBC, Hct, Hb), white blood cells, platelet and total protein. For serochemical analysis, centrifuged the collected blood which had treated with heparin to get plasma, and measured ALT, ALP, total bilirubin, albumin, globulin, BUN, creatinine, phosphorus, amylase, electrolyte (Na, K, Ca) using VetScan instrument for screening the oral supplements' effect of organs (liver, kidney and pancreas).

3) Statistical analysis

All measured value in this study was presented by average±standard deviation. During experiment, the differences between treatment group and control group were confirmed statically using SPSS program (*ver* 19.0, Chicago, IL, USA) in 5% of significance.

7. Results

For dogs suffering periodontal disease (gingivitis, calculus and malodor), recover and improvement results after feeding of T-immune stick and Gangchi tntn for 4 weeks were shown below.

1) Improvement effects in gingivitis

The eliminating effects of gingivitis and plaque was good after supplying of tntn was good. The gingivitis index showed decreased percentages at 1, 2, 3 and 4 week after feeding the oral supplements as follows ; 20.5(82.0%), 16.5(66.0%), 14.5(58.0%) and 12.5(50.0%), respectively, compared with 25.5(100%) measured at the first day of experiment. This result showed that Gangchi tntn leads 50% of improvement in gingivitis 4 weeks after treatment (Figure 3, 4).

Right side before feeding　After 2 weeks　　　　After 4 weeks

Left side before feeding　　After 2 weeks　　　　After 4 weeks

Figure 3. The gingivitis estimation of bilateral teeth according to feeding the Gangchi tntn.

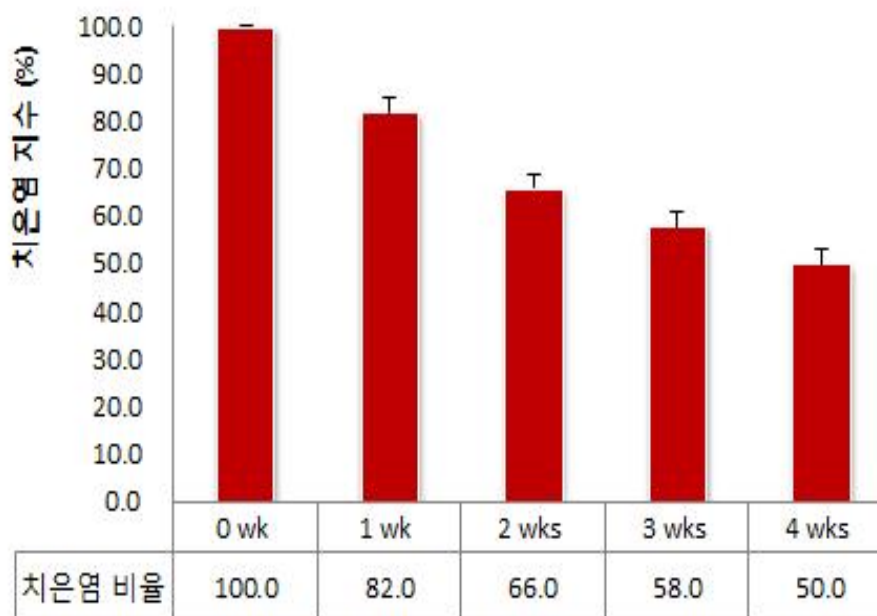|	0 wk	1 wk	2 wks	3 wks	4 wks
치은염 비율	100.0	82.0	66.0	58.0	50.0

Figure 4. Mean gingival index score at the pre-feeding and every week for 4 weeks by feeding Gangchi tntn.

2) Improvement effects in plaque

Evaluating the improvement of plaque at every week using Erythrosin B staining after feeding the T-immune stick and Gangchi tntn was shown in Figure 5. The plaque index showed decreased percentage as follows; 23.5(83.9%), 20.0 (71.4%), 15.5(55.4%) and 11.5(41.1%) at 1, 2, 3 and 4 week, respectively, after feeding the T-immune stick compared with 28.5(100%) measured at the first day of experiment. (Figure 6). Also Gangchi tntn reduced plaque index as follows; 25.5(91.1%), 22.0(78.6%), 19.0 (67.9%) and 15.5(55.4%) after 1, 2, 3 and 4 weeks, respectively, compared with 29(100%) measured at the first day of experiment (Figure 7). This result presented that T-immune stick and Gangchi tntn improved the plaque up to 58.9% and 44.6%, respectively, during 4 weeks.

A) T-immune stick feeding group

Right side before feeding   After 2 weeks   After 4 weeks

Left side before feeding   After 2 weeks   After 4 weeks

B) Gangchi tntn feeding group

Right side before feeding   After 2 weeks   After 4 weeks

Left side before feeding   After 2 weeks   After 4 weeks

Figure 5. The dental plaque Erythrosin B staining estimation of bilateral teeth according to feeding the T-immune stick(A) and Gangchi tntn(B).
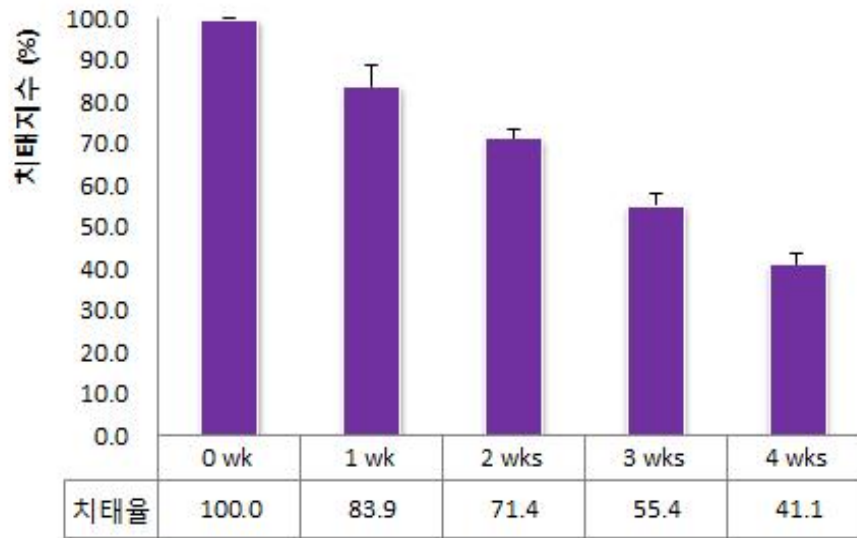
Figure 6. Mean dental plaque index score at the pre-feeding and every week for 4 weeks by feeding T-immune stick.
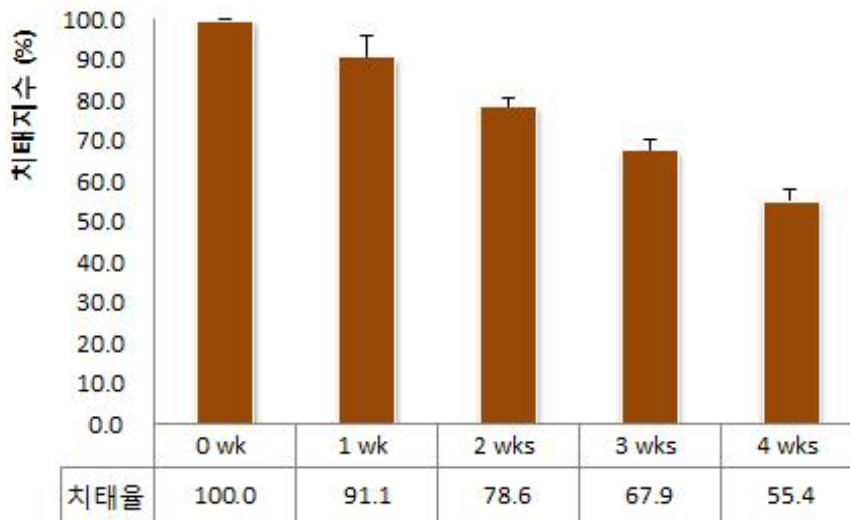

Figure 7. Mean dental plaque index score at the pre-feeding and every week for 4 weeks by feeding Gangchi tntn.

3) Improvement effects in calculus

The elimination of calculus in dogs suffering calculus at every week after feeding T-immune stick was evaluated. The calculus index were decreased continuously during all testing periods ; 24.0(80.0%), 20.0(66.6%), 16.0(53.3%) and 11.0(36.5%) at 1, 2, 3 and 4 week after feeding T-immune stick, respectively, compared with 30.0(100%) measured at the first day of experiment (Figure 8, 9). This result presented that T-immune stick improved the calculus up to 63.5% during 4 weeks.

Right side before feeding   After 2 weeks   After 4 weeks

Left side before feeding   After 2 weeks   After 4 weeks

Figure 8. The dental calculus estimation of bilateral teeth according to feeding the T-immune stick.

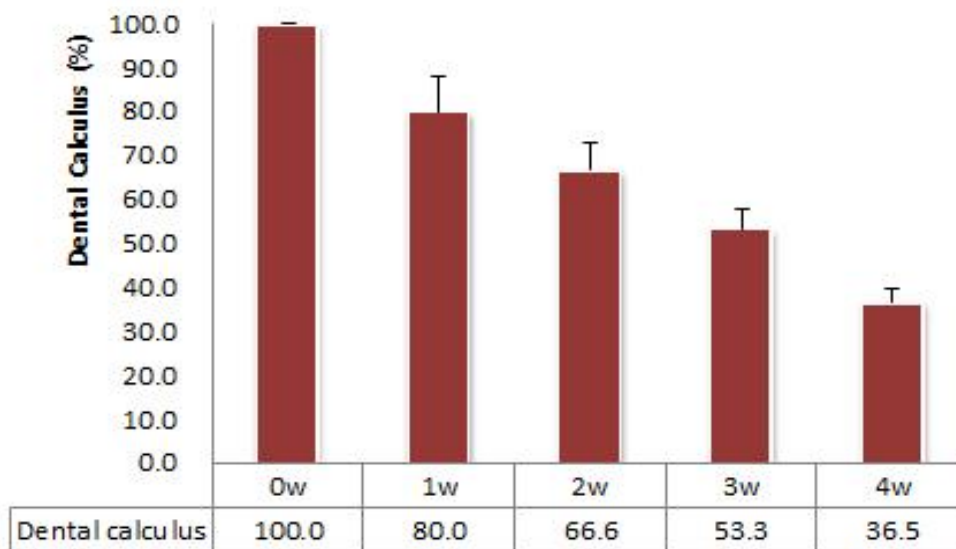| Dental calculus	0w	1w	2w	3w	4w
	100.0	80.0	66.6	53.3	36.5

Figure 9. Mean dental plaque index score at the pre-feeding and every week for 4 weeks by feeding T-immune stick.

4) Improvement effects in malodor

In evaluating the elimination of malodor in dogs at every week after feeding T-immune stick, the malodor index were decreased as follows; 34.0(83.0%), 28.5(69.6%), 24.5(59.8%) and 21.5(52.4%) at 1, 2, 3 and 4 week after feeding T-immune stick, respectively, compared with 41.0(100%) measured at the first day of experiment (Figure 10). This result presented that T-immune stick improved the calculus up to 63.5% during 4 weeks. Also Gangchi tntn reduced malodor index as follows; 38.5(79.0%), 32.5(66.7%), 28.5(58.5%) and 27.0(55.5%) after 1, 2, 3 and 4 weeks, respectively, compared with 48.5(100%) measured at the first day of experiment (Figure 11). This result presented that T-immune stick and Gangchi tntn improved the malodor up to 41.6% and 44.5%, respectively, during 4 weeks.
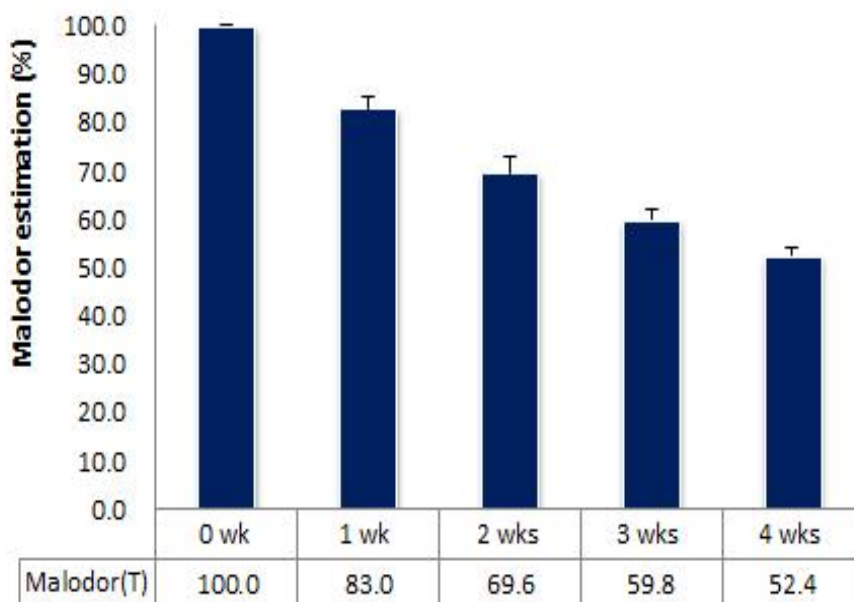
	0 wk	1 wk	2 wks	3 wks	4 wks
Malodor(T)	100.0	83.0	69.6	59.8	52.4

Figure 10. Malodor estimation at the pre-feeding and every week for 4 weeks by feeding T-immune stick.

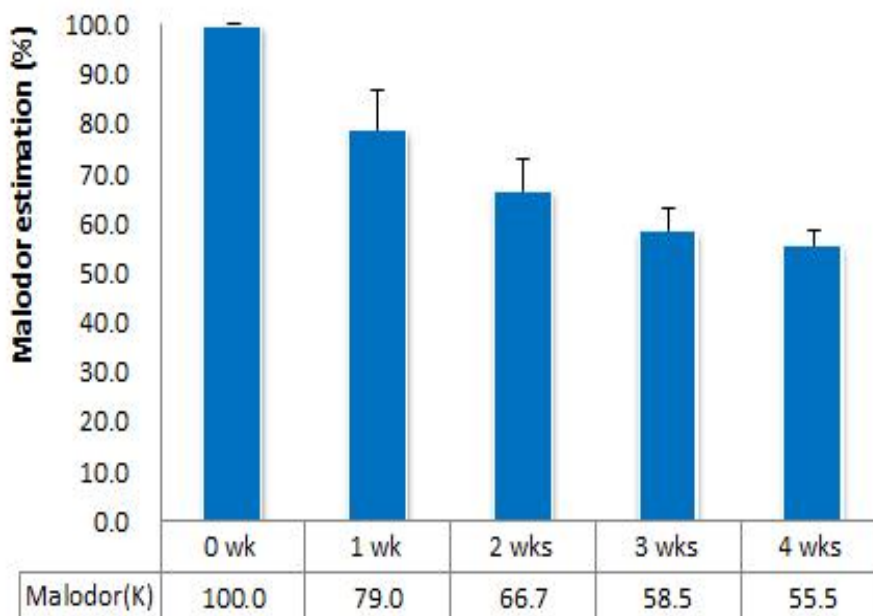|	0 wk	1 wk	2 wks	3 wks	4 wks
Malodor(K)	100.0	79.0	66.7	58.5	55.5

Figure 11. Malodor estimation at the pre-feeding and every week for 4 weeks by feeding Gangchi tntn.

5) Changes of blood chemistry

In analysis of blood and serum chemistry, there were no significant changes of that between 2 and 4 weeks after feeding T-immune stick (Table 5) and Gangchi tntn (Table 6), and showed normal range of red blood cells, electrolyte, enzymes of kidney, liver and pancreas, but there was a decreasing trend of white blood cells.

Table 5. Blood and serum chemistry on the 0, 2 and 4 weeks after feeding T-immune stick

Parameter	Week			Reference
	0	2	4	
WBC(10^3/µl)	14.5±3.95	13.0±2.08	10.6±0.33	6~17
RBC(10^6/µl)	7.0±1.30	6.9±1.22	7.5±0.89	5.5~8.5
Homoglobin(g/dl)	15.8±1.54	15.1±1.64	16.0±0.83	12~18
Hematocrit(%)	44.6±4.48	44.2±2.80	44.5±0.58	37~55
Platelet(10^3/µl)	438.0±148.11	394.3±55.27	370.0±49.67	200~500
Albumin(g/dl)	3.8±0.39	3.5±0.48	3.7±0.21	2.5~4,4
ALP(IU/L)	82.0±52.02	46.3±4.35	54.8±9.00	20~150
ALT(IU/L)	44.0±16.67	37.8±12.12	36.8±12.87	10~118
Amylase(IU/L)	595.0±184.77	663.8±118.84	645.0±88.13	200~1200
T-Bilirubin(mg/dl)	0.4±0.08	0.4±0.08	0.4±0.10	0.1~0.6
BUN(mg/dl)	16.5±4.12	13.3±2.75	16.5±1.73	7~25
Calcium(mg/dl)	10.6±0.60	10.5±0.67	10.0±0.29	8.6~11.8
Phosphrus(mg/dl)	3.6±0.64	3.7±0.28	3.9±0.30	2.9~6.6
Creatinine(mg/dl)	0.7±0.30	0.7±0.34	0.7±0.12	0.3~1.4
Glucose(mg/dl)	103.3±17.91	100.8±6.60	114.3±7.41	60~110
Na(mEq/L)	145.5±1.73	144.8±6.70	143.3±3.40	138~160
K(mEq/L)	4.6±0.56	4.7±0.39	5.0±0.10	3.7~5.8
TP(g/dl)	7.8±0.45	7.6±0.10	7.7±0.45	5.4~8.2
Globulin(g/dl)	4.0±0.51	4.1±0.56	4.0±0.43	2.3~5.2

Table 6. Blood and serum chemistry on the 0, 2 and 4 weeks after feeding Gangchi tntn.

Parameter	Week			Reference
	0	2	4	
WBC(10^3/μl)	13.5±4.56	11.4±1.79	9.4±0.85	6~17
RBC(10^6/μl)	6.8±0.84	6.7±1.56	7.3±1.63	5.5~8.5
Homoglobin(g/dl)	15.4±1.75	15.0±3.54	14.6±3.39	12~18
Hematocrit(%)	43.9±5.80	44.1±4.64	43.2±4.38	37~55
Platelet(10^3/μl)	315.3±60.00	340.8±97.78	396.5±67.42	200~500
Albumin(g/dl)	3.9±0.08	3.8±0.62	3.8±0.62	2.5~4,4
ALP(IU/L)	180.0±48.64	155.8±75.95	108.8±46.53	20~150
ALT(IU/L)	56.0±25.23	44.3±17.00	39.3±8.85	10~118
Amylase(IU/L)	716.5±329.02	777.5±310.39	750.0±279.25	200~1200
T-Bilirubin(mg/dl)	0.3±0.01	0.3±0.05	0.3±0.02	0.1~0.6
BUN(mg/dl)	13.3±1.26	14.3±2.06	13.8±1.50	7~25
Calcium(mg/dl)	10.4±0.47	10.5±0.64	10.2±0.49	8.6~11.8
Phosphrus(mg/dl)	3.1±1.00	3.1±0.94	3.1±0.87	2.9~6.6
Creatinine(mg/dl)	0.6±0.24	0.6±0.14	0.5±0.18	0.3~1.4
Glucose(mg/dl)	101.0±14.31	99.8±7.04	93.8±10.56	60~110
Na(mEq/L)	147.3±3.30	143.0±6.53	144.0±6.63	138~160
K(mEq/L)	4.4±0.26	4.5±0.10	4.5±0.10	3.7~5.8
TP(g/dl)	7.0±0.17	7.3±0.56	7.2±0.61	5.4~8.2
Globulin(g/dl)	3.3±0.48	3.5±1.16	3.4±1.22	2.3~5.2

8. Conclusion and Discussions

It is known that feeding dogs human food makes worsen plaque, calculus and malodor albeit pet food makes the symptoms slowly than human food. The periodontal diseases lead dogs to a cardiac disorder, malfunction of liver and kidney. It is important to care teeth with tooth brushing, dog chews and oral supplement after feeding to prevent the plaque, calculus and malodor and to maintain healthy life for dogs.

The effect of T-immune stick revealed that malodor and plaque were clearly improved despite of short period of experiment. It is supposed that malodor and plaque could be improved obviously if the supplements were provided for long term. Also we found that Gangchi tntn lead improvement of gingivitis in dogs suffering symptoms of that and it was consistent with evaluation index which had significant result. It is supposed that gingivitis could be improved more clearly if the supplement were provided for long term.

In the process of measuring odor from oral cavity, it was unsuccessful for dogs to exhale directly into Halimeter which requires constant air flow from oral cavity. We have designed new tool to collect the odor from oral cavity for 30 seconds and maintained constant pressure on it to measure averaged values of the malodor from dog's breath through triplicate trials. The upper limit of odor to be malodor is 200ppm in human, however, the value of experimental dogs' malodor was about 40~50ppm, which is thought to possibly be responsible for the influx of outer air. In this reason, we established the odor measured at the first day of experiment as 100% and measured decreased percentage (%) of malodor at 1, 2, 3 and 4 weeks after feeding the supplements. The improvement was revealed by either subjective evaluations of researcher or breeder, and objective measurement clearly confirmed its effectiveness.

It was observed that level of white blood cells was increased in analysis of blood and serum chemistry, however, the level was decreased by feeding T-immune stick and Gangchi tntn, which was thought to be responsible for

relieved inflammation by improvement of calculus and gingivitis. The analysis of serum chemistry presented normal range of enzymes in both of treatment and control groups, which suppose that the T-immune stick and Gangchi tntn have not affected the function of liver, kidney, pancreas and balance of electrolyte.

Overall, we have found that T-immune stick has improvement effect on plaque, calculus and malodor, and Gangchi tntn on plaque, malodor and gingivitis. This study concludes that T-immune stick and Gangchi tntn are highly recommended for oral care of pet animals.

9. References

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