Attachment 7: Effect of preventing Mastitis in Dairy Cows (10 pages)

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English Translation of Research Report of Takeyasu Bacteria on Mastitis in Cows

Efficacy of a Dietary Supplementation of Microbial Product to Prevent Mastitis during the Early Lactation Period in Dairy Cows

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Summary

Efficacy of dietary supplementation of the microbial product (Takeyasu-Bacteria) to prevent mastitis in the early lactation was evaluated using 10 multiparous dairy cows. Experiment of feeding with usual feed supplemented with or without 70 g/head/day of the Takeyasu-Bacteria (treatment or control) was performed from 30 days before estimated date of delivery through 90 days after delivery.

The following results were obtained:

1) Mastitis occurred in 4 out of 5 cows in control group, and 0 cows in treatment group, and the incidence rate of mastitis in treatment group was significantly ($P<0.05$) lower than that in control.
2) Although milk yield and milk compositions were not influenced by dietary supplementation with the microbial product, liner score of somatic cell count (LS) was decreased significantly ($P<0.05$) for the treatment(0.50 ± 1.00) than for the control (3.61 ± 2.12) at 2 month after the delivery. Additionally LS was tended ($P<0.10$) to be lower for the treatment than that for the control at 1 month after the delivery.
3) Blood parameters were not differed by dietary supplementation of the Takeyasu-Bacteria.

From these results, we consider that supplementation of the Takeyasu-Bacteria from dry period through early lactation period could prevent mastitis during high risk period of postpartum.

1. Introduction

Mastitis in cows has significant damages to dairy farmers. Its prevention has been recognized more important than the treatment in recent days. Mastitis incidence has a tendency to increase immediately before/after dry cow period, and before/after the calving. It is considered that the mastitis before/after calving occurs due to reduction of immunity function[2, 11]. On the other hand, it is reported that probiotics like *Lactobacillus* has immune-enhancing activity and antibacterial activity[3, 4, 5, 8, 10]. Therefore, preventive effect of Takeyasu-Bacteria, which has demonstrated immune-enhancing activity in *in-vitro* study, is evaluated against

mastitis in early lactating stage (calving to peak milk yield) in this study.

2. Materials and Methods

(1) Takeyasu-Bacteria

Takeyasu-Bacteria contain the mixture of three kinds of useful bacteria (*Lactobacillus, Yeast and Bacillus pumilus)*. The product is formulated with wheat bran as diluent.

(2) Test Animals

Ten Holstein multiparous cows at Awaji Agri. Tech. Center were used in September 2007 to January 2008, by the order of delivery; i.e., 5 cows in control group and 5 cows in test group. The 5 test group cows were administered with Takeyasu-Bacteria at 70 g/day/cow from 30 days before the expected calving to 90 days after the calving.

(3) Management of Animals

Test animals were kept in individual Comfort-Type Tie Stoll of 3.3 x 4.4 m. Milking was done at 8:00 a.m. and 17:00 daily. They were fed at 9:00 a.m. and 16:00 daily. Before the calving, hay was fed as a major feed and then shifted to complete formula feed by taking 1 week after the calving. Feed consumption was adjusted at the level of J-NCR twice a day (Feeding Volume ratio was Morning : Afternoon = 4 : 6).

(4) Measurements

i) Mastitis Status

At the milking time, issues on udder and milk were observed, and clinical mastitis was evaluated by CMT test (PL test, Zenoaq, Tokyo).

The cost for the mastitis treatment and loss due to milk discarding were calculated, excluding colostrums period for 5 days.

ii) Milk Yield

Milk production volume was measured by Milk Meter of the milker. Components of the milk were measured once a month for the 1 to 3 month period; i.e., general components of milk and somatic cell count, which were adjusted by the ratio of milk production volume in the morning and the afternoon. Somatic cell count was converted to Linear Score[1)] for statistical analysis.

iii) Blood Analysis

Blood sample was collected at 4 hours after the morning feeding on 30 days before the expected calving, and 30, 60 and 90 days after calving, and then

was used for analysis of hematocrit value, blood glucose, total protein, total cholesterol, BUN, AST, and GGT.

iv) Statistical Analysis

The mastitis incidence was analyzed by Fisher's exact test. Milk production volume, milk components, blood analysis results were analyzed by Student T-test. As one cow in the Takeyasu-Bacteria group had self-injury on udders one day after the calving, it was excluded in the analysis.

3. Results

(1) Mastitis Incidence

During the 90 days after the calving, 4 out of 5 cows showed mastitis in the control group, while there was no incidence in the Takeyasu-Bacteria group, which demonstrates the significant difference ($P< 0.05$).

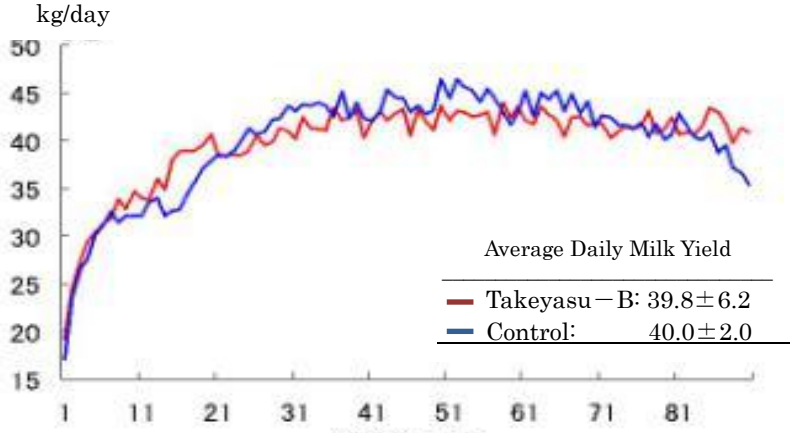
Incidence of Mastitis

	Normal	Mastitis	Total
Takeyasu-B group	4	0	4
Control	1	4	5

There was significant difference between the Takeyasu-Bacteria group and the Control by 2x2 Fisher's exact test ($P<0.05$).

(2) Milk Yield

Milk production volume in the control group and the Takeyasu-Bacteria group was 40.0±2.0 kg/day and 39.8±6.2 kg/day, respectively, with no significant difference. (Table 1)



Milk Yield

Somatice Linear Score was 3.60±2.19 in the control and 0.50±1.00 in the Takeyasu-Bacteria at the 2 months after the calving, which shows a significant difference (P<0.05). At the 3 months after the calving, the Takeyasu-Bacteria group shows a tendency of the reduction, but not significant (P< 0.10). (Table 1)

Table 1 . Production parameters for cows supplemented with or without a direct-fed microbial product

Item	Test [1] (n=4)	Control (n=5)	P-value [2]
Milk yield (kg/day)	39.8 ±6.2	40.0 ±2.0	NS
Milk composition			
Fat (%)			
1 M [3]	3.91 ±0.66	3.49 ±0.45	NS
2 M	4.06 ±0.79	3.52 ±0.25	NS
3 M	4.19 ±0.50	3.73 ±0.75	NS
Protein (%)			
1 M	3.03 ±0.10	3.02 ±0.10	NS
2 M	3.04 ±0.08	3.08 ±0.13	NS
3 M	3.10 ±0.10	3.38 ±0.67	NS
Solids Not-Fat (SNF) content (%)			
1 M	8.69 ±0.30	8.50 ±0.17	NS
2 M	8.68 ±0.17	8.60 ±0.28	NS
3 M	8.75 ±0.22	8.69 ±0.26	NS
Lactose (%)			
1 M	4.66 ±0.19	4.48 ±0.11	NS
2 M	4.65 ±0.11	4.52 ±0.17	NS
3 M	4.65 ±0.15	4.31 ±0.51	NS
Linear score (LS) [4]			
1 M	0.25 ±0.50	2.60 ±2.41	NS
2 M	0.50 ±1.00	3.60 ±2.19	P<0.05
3 M	0.75 ±0.50	4.60 ±3.85	P<0.10

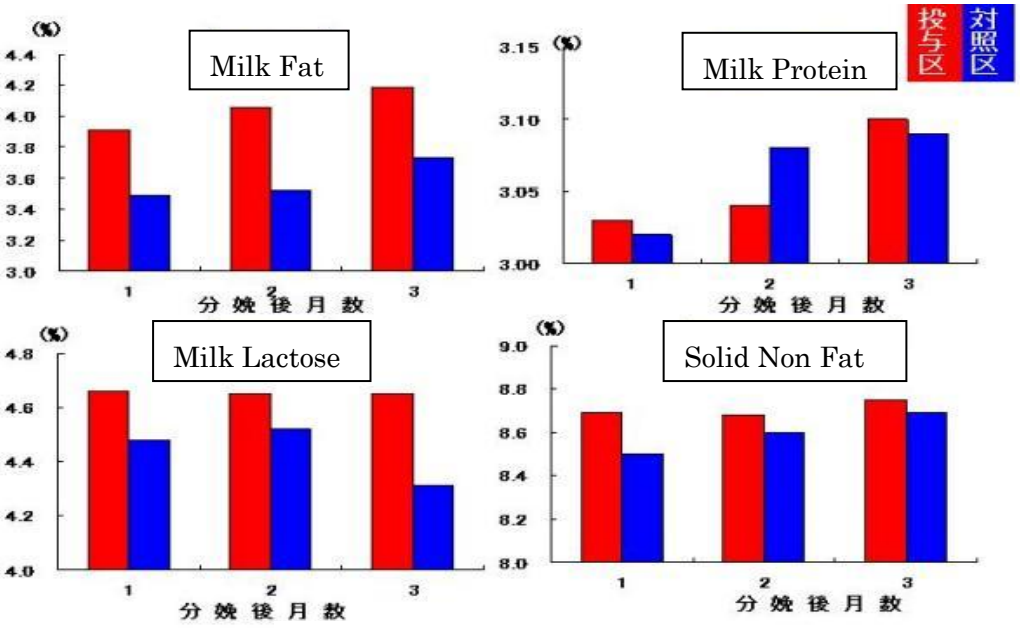
Mean ± SD

1) The microbial product was added 70 g/day/head.

2) NS:not significant.

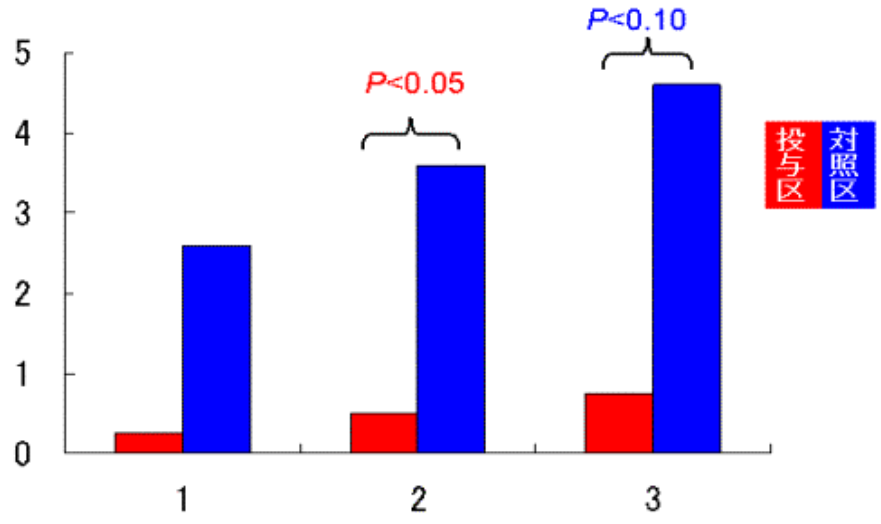
3) At month after parturition.

4) LS = Log_2(somatic cell count/10^5) +3 .



Milk Components

Note: Horizontal line shows months after the delivery. Red bar shows Takeyasu-Bacteria and blue bar shows Control.



Linear Score of Somatic Cells

Unit: $\log_2$(somatic cell No./100,000)+3

Note: Horizontal line shows months after the delivery. Red bar shows Takeyasu-Bacteria and blue bar shows Control.

(3) Blood Components

There was no significant difference between 2 groups on the parameters of blood analysis, at the 4-different sampling time. (Table 2)

Table 2. Blood parameters for cows supplemented with or without a direct-fed microbial product

Item	Test [1] (n=4)	Control (n=5)	P-value [2]
Glucose (Glu, mg/ dℓ)			
Preparturient	59.5 ±5.2	62.8 ±2.6	NS
1 M [4]	66.5 ±2.9	67.0 ±3.4	NS
2 M	68.3 ±4.3	67.5 ±5.1	NS
3 M	67.5 ±5.3	70.2 ±9.3	NS
Total cholesterol (TCho, mg/ dℓ)			
Preparturient	90.5 ± 9.3	92.8 ±52.1	NS
1 M	188.0 ±41.3	190.8 ±51.9	NS
2 M	226.0 ±43.6	248.2 ±59.4	NS
3 M	262.5 ±41.9	253.4 ±42.9	NS
Hematocrit (Ht, %)			
Preparturient	27.3 ±1.3	28.3 ±2.1	NS
1 M	26.5 ±2.1	25.3 ±2.5	NS
2 M	28.0 ±2.4	26.2 ±1.9	NS
3 M	28.3 ±2.1	7.4 ±1.5	NS
Total protein (TP, g/ dℓ)			
Preparturient	7.08 ±0.24	6.83 ±0.53	NS
1 M	7.83 ±0.67	7.68 ±0.48	NS
2 M	7.88 ±0.63	7.93 ±0.27	NS
3 M	8.03 ±0.50	7.94 ±0.41	NS
Albumin (Alb, g/ dℓ)			
Preparturient	3.53 ±0.17	3.50 ±0.08	NS
1 M	3.65 ±0.31	3.65 ±0.10	NS
2 M	3.70 ±0.18	3.62 ±0.19	NS
3 M	3.68 ±0.33	3.64 ±0.17	NS
Blood urea nitrogen (BUN, mg/ dℓ)			
Preparturient	4.10 ±0.89	7.53 ±3.92	NS
1 M	13.25 ±1.94	11.85 ±2.87	NS
2 M	12.70 ±3.38	13.42 ±1.27	NS
3 M	13.68 ±2.51	11.90 ±1.83	NS
Calcium (Ca, mg/ dℓ)			
Preparturient	10.63 ±0.22	10.35 ±0.39	NS
1 M	10.30 ±1.07	10.15 ±0.29	NS
2 M	10.50 ±0.94	8.97 ±1.81	NS
3 M	10.43 ±0.82	9.60 ±0.63	NS
Inorganic phosphorus (iP, mg/ dℓ)			
Preparturient	4.45 ±1.11	5.05 ±0.87	NS
1 M	4.25 ±1.25	4.27 ±1.13	NS
2 M	3.40 ±0.91	5.37 ±2.55	NS
3 M	3.38 ±1.11	4.40 ±1.37	NS
Aspartate aminotransferase (AST, IU/ ℓ)			
Preparturient	48.3 ± 5.9	46.3 ± 2.1	NS
1 M	75.0 ±12.2	71.5 ±12.7	NS
2 M	71.5 ± 9.3	71.5 ± 8.8	NS
3 M	90.0 ±16.8	74.0 ± 7.9	NS
γ -Glutamyl transpeptidase (GGT, IU/ ℓ)			
Preparturient	25.5 ±11.3	35.3 ± 3.0	NS
1 M	28.3 ±11.4	33.5 ±11.3	NS
2 M	33.3 ± 9.1	38.8 ± 6.5	NS
3 M	38.0 ± 8.8	42.0 ± 7.3	NS

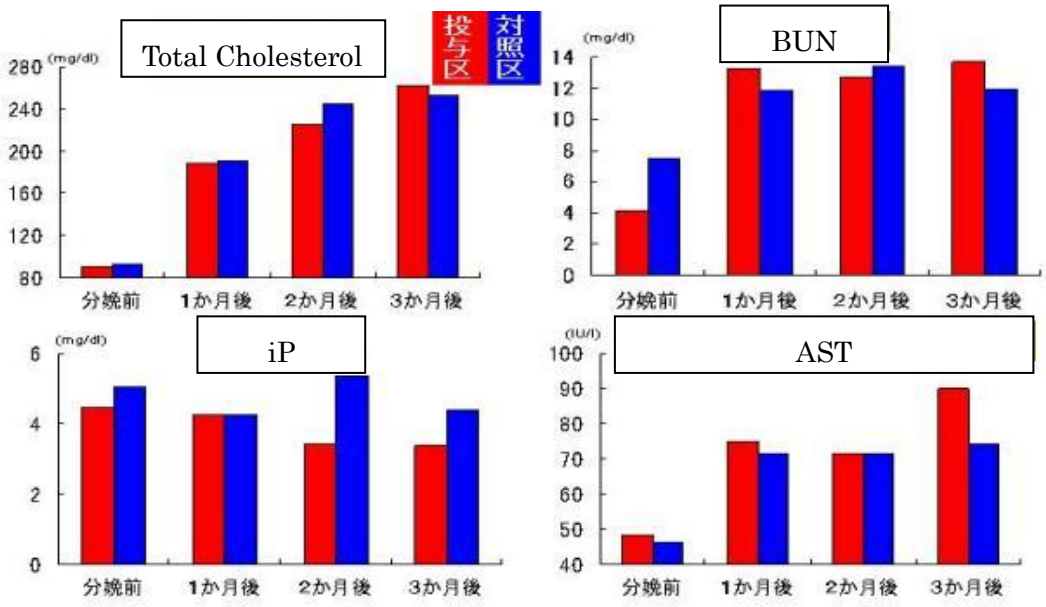
Mean ± SD

1) The microbial product was added 70 g/day/head.

2) NS: not significant.

3) 30 days befor estimated date of delivery. Just anterior to start supplement of the microbial product.

4) At month after parturition.



Blood Components

Note: Horizontal line shows timing of "before calving, 1 month later, 2 months later and 3 months later" from left to right.
Red bar shows Takeyasu-Bacteria and blue bar shows Control.

(4) Economical Loss by Mastitis

The loss by the mastitis together with the mastitis incidence was shown on Table 3. During the test period, Cow No. 99 had mastitis 4 times, Cow No. 115 with 2 times and other mastitis cows with 1 time. It occurred at immediately after the calving to 20 days later, and 60 days after the calving or later.

The milk loss by withholding the milk for the mastitis treatment and the required withdrawal periods was for 6 to 11 days in most of cases. Cow No. 99 at the 4th time mastitis had 35 milk-loss days due to the very acute mastitis with gram-negative bacteria.

Each loss by the mastitis incidence costed JPY 18,759 to 78,621. By excluding the longest loss time of Cow No. 99, the average loss by one mastitis incidence was JPY 28,059.

Table3. Economic loss by incidence of clinical mastitis in control group cows

Cow No.	Incidence No.	Day in milk	Treatment (days)	Milk disposal days[1]	Milk disposal volume(kg)[2]	Economic loss of milk[3] (yen)
#99	1	1	3	7	234.6	22,881
	2	17	3	6	193.6	18,961
	3	73	7	11	396.3	38,643
	4	88	14	35	813.0	78,621
#115	1	1	8	11	371.4	35,480
	2	87	6	9	340.0	33,293
#89	1	6	3	6	190.6	18,589
#81	1	60	3	6	295.6	28,567

1) Including days of treatment.
2) The range of unit value was 95.06-98.52 yen/kg
3) Except for 5 days after delivery in milk.

4. Consideration

In this study, the mastitis occurred in 4 out of 5 cows in the control group immediately after the calving to 20 days after the calving, and at the 60 days after the calving or later. As the period of "immediately after the calving" is reported as the lowest immunity activity period[11], it is considered as the high risk period against mastitis until the immunity recovers. Though the cows have peak milk production in 4 to 5 weeks after the calving, their dry matter intake becomes the highest in 8 to 10 weeks after the calving[2]. This creates a period of the most negative energy balance nutrititionally[11] at 60 days after the calving or later. As it is reported that immunity function decreases along with poor nutirtuion[9], it is considered that the high risk of mastitis at 60 days after the calving or later is created by the immunity reduction by the nutritional deficiency.

On the other hand, there was no incidence of mastitis in the Takeyasu-Bacteria group which shows the significant difference from the Control group. Therefore, the continuous administration of Takeyasu-Bacteria during the dry cow period to early lactation period has preventive effect against mastitis, when there is a high incidence of mastitis.

Probiotics administration in this period has demonstrated an increase of dry matter consumption, milk yield and milk protein, and an improvement of energy metabolism measured by blood components like free fatty acid and ketone body[7]. Since the results in this study did not show significant difference on milk yield, milk components and blood components, it is considered that the mastitis preventive effect by the Takeyasu-Bacteria is not related to the indirect effect by an improvement of the nutritional metabolism.

Takeyasu-Bacteria contain mainly *lactobacillus* and *yeast,* and its efficacy of immune-enhancing activity has been shown by in-vitro study (unpublished data). These bacteria mixture has been utilized in livestock industry, and an adjustment of intestinal function and immune-enhancing efficacy have been known[1, 5, 10]. There are some *lacutobacillus* to produce bacteriocin with antimicrobial activity[3, 8]. Therefore, it is considered that either or both of immune-enhancing activity and antimicrobial activity are related to the mastitis preventive efficacy by the Takeyasu-Bacteria.

In this study, the loss by the mastitis in the control group was JPY 28,059 per incidence

in average. The actual loss should be added with the treatment cost. The cost for Takeyasu-Bacetria is about JPY 7,000 per cow during the whole test period. As the Takeyasu-Bacteria group has shown the reduction of somatic cell count in milk, which can have positive impact on the milk price (though there was no incidence of mastitis in the group), it is considered that Takeyasu-Bacteria has good economical effect in cows.

Further studies should be conducted to increase the test animals and to study on the mode of action in future.

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