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Pharmacokinetics of a sustained-release bovine somatotropin in lactating cows

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Abstract : Bovine somatotropin is known to improve the growth rate and lactation in cattle. In this study, we examined the concentration-time profiles of a sustained-release formulation of bovine somatotropin (BST) and insulin-like growth factor-1 (IGF-1) in plasma and milk in cows. In addition, the possible effect of co-administrated vitamin ADE complex on the pharmacokinetic parameters of BST and IGF-1 was evaluated.

1. Plasma BST and IGF-1 levels reached the peak at 12~24 and 48 hours after the administration of BST, and plasma half-lives ranged 100 to 137 and 201 to 310 hours, respectively. To 8th day after administration, BST and IGF-1 levels in milk were not significantly different from the control levels.

2. Plasma BST levels showed cyclic pattern with high concentrations in early stage after each injection and following gradual declining during repeated administrations at 2 week intervals, while plasma IGF-1 levels in treated animals did not show such a cyclic pattern, but remained higher than the control levels.

3. Milk BST and IGF-1 levels during repeated treatments were not significantly different from the control levels.

4. Co-administration of vitamin ADE complex yielded slightly increased AUC of plasma BST for high dose group, but such effect was not evident in the IGF-1 levels. Co-administration of ADE complex tended to increase plasma BST levels and decrease the elimination half-life of IGF-1.

5. These results suggest that the BST formulation tested is one of the ideal sustained-release formulation for long term use in dairy industry. As for the co-administration of vitamin ADE complex, the benefit of co-administration with BST is needed to be further evaluated.

Key words : bovine somatotropin, insulin-like growth factor-1, pharmacokinetics, cow, vitamin ADE.

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Introduction

Somatotropin (growth hormone) is known to improve the rate of body growth and lactation in cattle. It's action largely depends on interaction between growth hormone and somatomedins such as insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-Ⅱ (IGF-2)[1]. The effects of bovine somatotropin (BST) have been well established in a number of tissues such as the mammary gland, muscle, pancreas and kidney[2]. In ruminants, the mechanisms inducing galactopoiesis are complex and involve a multiplicity of events in the whole animal[3]. Nevertheless, observations on goats have suggested that the effects of BST on the mammary gland itself are mediated in part by IGF-1 and the availability of IGF-1 to mammary tissue is an important component of the overall galactopoietic response to growth hormone[4]. The benefit and direct impact of BST on dairy industry is expected enormous[2].

With the advent of biotechnology, it has been possible that large quantity of somatotropin can be produced from the cloned bacteria with the gene coding BST. In fact, pharmaceutical companies have been actively involved in mass production of recombinant BST since 1982[5]. In 1990s, the slow-release forms of BST were also introduced to be used for dairy cows because BST in usual formulation should be administered at least once a day to produce its clinical effect because of its short biological half-life[6,7]. The positive effect of daily BST on milk production have been shown in many studies[8~14]. Numerous studies indicate that BST is not only promising drug for improving the productive efficiency of dairy cow but also considered safe to human[15,16]. No withdrawal period is required unlike most of other drugs used for cows. Hammond *et al*[15] and Juskevich and Guyer[16] well reviewed the scientific base on such conclusion as followings: 1) BST and other non-primate somatotropins are not active in human due to differences in amino acid sequences from human somatotropin, 2) BST is not orally active as degraded like other dietary proteins, 3) Residual BST in milk is very low and comparable to endogenous BST levels, 4) Residual IGF-1 in milk and meat is only marginally increased by BST treatment and biologically significant levels of intact IGF-1 would not be asborbed from the milk.

Therefore, BST seems to be one of the very promising drugs in livestock industry in the future in coping with the problems of low protein diet in the under-developed countries of the world.

The purpose of present work was to evaluate the concentration-time profiles of SR-BST, a sustained release form of BST manufactured by LG Chemical Ltd. and to determine its pharmacokinetic parameters and the changes of BST and IGF-1 levels in plasma and milk during repeated administration over 6-week period of lactation. In addition, the possible effect of co-administrated vitamin A, D and E on the pharmacokinetics of SR-BST was also evaulated.

Materials and Methods

Treatment groups and management : Lactating, multiparous Holstein cows (600 kg) were housed in a free stall barn and milked twice daily approximately 05 : 00 and 15 : 00 hour. Twenty cows were randomly assigned to each of 5 treatment groups; control, low dose group which is treated with 250 mg sustained-release BST (SR-BST), low dose+vitamin group treated with 250 mg SR-BST with vitamin ADE complex, high dose group treated with 500 mg SR-BST, and high dose+vitamin group treated with 500 mg SR-BST with vitamin ADE complex, respectively. The amounts of vitamin A, D and E in a dose of vitamin complex were 750, 2.81 and 693 ng, respectively. SR-BST was injected subcutaneously to the neck area on day 0, 14 and 28.

Sampling : Milk samples were collected at the days of -2, -1 day and 1, 2, 3, 4, 6 and 8 day following each administration. Blood samples were taken via venipuncture from jugular vein on -2, -1, 0 day, 0.25, 0.5, 1, 2, 3, 6, 12 hours, 1, 2, 3, 4, 6, 8, 10, 13, 15, 21, 28, 29, 35, 42, 44, 47, 50 days relative to the first adminstration of SR-BST. Plasma samples were obtained by centrifugation of whole blood samples for 10 min at 3,000 rpm. Collected milk and plasma samples were stored at -20℃ until analyzed. Treatment of SR-BST was administered just after taking samples on day 0.

Hormone measurements: BST and IGF-1 were determined by radioimmunoassay. Plasma BST and IGF-1 were measured by a similar methods developed for porcine somatotropin[17]. Briefly, plasma samples directly used for BST radioimmunoassays, but for IGF-1 assay the samples were first extracted with acid-ethanol by the method of Daughaday *et al*[18]. BST and IGF-1 in milk was determined by a modified method of Choi *et al*[17]. Monkey anti-BST and rabbit anti-IGF-1 were used for binding to BST and IGF-1, at the dilution of 1 : 50 and 1 : 400, respectively. ^{125}I-labeled BST and IGF-1 were prepared by the iodogen and chloramine T methods, respectively. Stock solutions of standard BST (100 μg/ml) and IGF-1 (100 μg/ml) were serially diluted with assay buffer in the range of 0.02~100 ng/ml. Assay sensitivity estimated from zero dose response of standard curve, were 2.755, 0.0635 ng/ml for BST and IGF-1, respectively.

Pharmacokinetic analysis : Pharmacokinetic parameters were obtained by a nonlinear regression methods from the averaged concentration-time data. The following equation is used for the calculation of parameters of one compartmental model.

$$y = a^{*}\alpha/(\alpha-\beta)^{*}(e^{-\beta^{*}t}-e^{-\alpha^{*}t})+y_{o} \quad \cdots\cdots\cdots\cdots\cdots\cdots (1)$$

Where 'a' is a constant representing F*Dose/Vd, and F, Dose and Vd are bioavailable, amount of drug administered and volume of distribution of the drug, respectively. Parameter 'a' is an intial absorption rate constant and 'β', elimination rate constant. Parameters 'y and y_o' are measured and background plasma levels of BST or IGF-1, respectively.

Pharmacokinetic parameters were calculated using standard equations[19,20]. The area under the plasma concentration-time curve and area under the monent curve (AUMC) were calculated using the linear trapezoidal rules-extrapolation method[21], Mathematically, this can be described by

$$AUC = \int_{0}^{t} Cpdt \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (2)$$

$$AUMC = \int_{0}^{t} t^{*}Cpdt \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (3)$$

Cp : concentration of plasma BST or IGF-1, t : time in hour

Mean residual time is identical to the turnover time of the endogenous substance. In this study, MRT was obtained by dividing AUMC with AUC. The half-life ($t_{1/2}$) was obtained from the relation, $t_{1/2} = 0.693/\beta$. β was obtained by curve fitting of the equation (1) to the concentration-time data.

Statistical analysis : The trial evaluated the effect of exogenous BST administration on BST and IGF-1 levels in plasma and milk. The response variables were milk BST, milk IGF-1, plasma BST and plasma IGF-1 levels. Levels of statistical significance were assessed using unpaired student *t*-test. Significant differences were judged as $p < 0.05$.

Drugs and hormones : The sustained-release formation of bovine somatotropin (SR-BST, Boostin® manufactured by LG chemical Ltd.), monkey anti-BST antibody (provided by Dr. AF Parlow Harbor UCLA Medical center, USA), and rabbit anti-IGF-1 antibody (provided by Dr. P Owens, CSIRO Division of Nutrition, Australia) were provided by Biotech Research Institute Ⅱ, LG Chemical Ltd. (Taejon, Korea). All other reagents were from Sigma (USA).

Results

The results of this work indicate that 1) plasma half-life of BST and IGF-1 were 4 and 11 days after single treatment of a sustained-release form of BST, SR-BST (250 mg), 2) plasma BST and IGF-1 levels were elevated and the BST levels showed a dose-dependent and cyclic pattern in response to repeated administrations, 3) BST and IGF-1 levels in milk during repeated treatments were not significantly different from the control levels, 4) co-administration of vitamin ADE complex tended to increase plasma BST levels, to decrease elimination half-life of IGF-1.

Fig 1A shows the plasma concentration-time profiles of BST after administration of SR-BST 250 and 500 mg/cow. After the administration, plasma BST levels were increased rapidly and reach the peak at 20 hours, then decreased slowly to control level at day 6. The concentration-time profiles from two dose groups showed little or very weak, if any, dose dependence. The solid and dotted lines were drawn by the best-fit parameters obtained from fitteing the measured data to the equation (1) as described in Materials and Methods. Resulting rate constants for initial absorption and

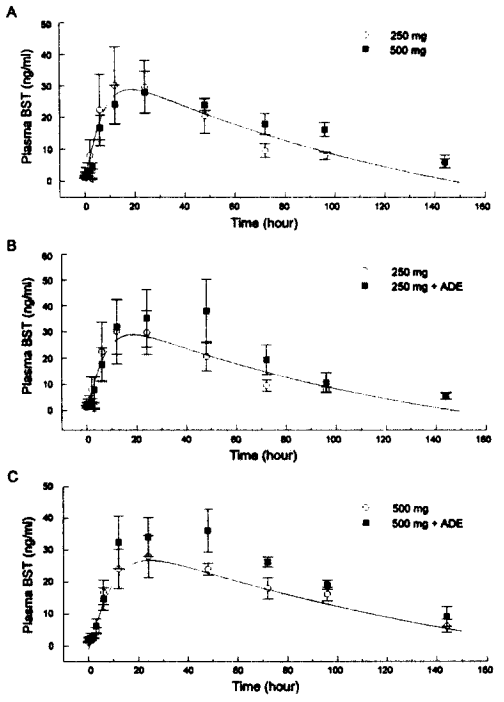

Fig 1. Plasma concentration-time profile of BST after first administration of Boostin, a sustained-release form of BST. Boostin was administered subcutaneously at 250, 500 mg/cow with or without vitamine ADE complex. Solid and dotted lines are drawn by the best-fit parameters obtained fitting the data to equation described in Materials and Methods. Symbols and bars are means of 4 measurements and sem, respectively. A plasma BST concentration-time profile at the doses of 250 and 500 mg/cow. B and C. Plasma BST concentration-time profiles at the doses of 250 and 500 mg/cow with and without vitamin ADE complex.

elimination were 0.18 ± 0.066 and 0.007 ± 0.0018 (h^{-1}) for low dose group and 0.133 ± 0.023, 0.005 ± 0.0007 ($t_{1/2}$ = 137 hours) for high dose group, respectively. The peak BST levels (ca. 30 ng/ml) are half of those of Zhao *et al*[6] (ca. 60 ng/ml) at the dose of 350 mg/cow, which is in the dose range of the present work.

Figs 1B and 1C illustrate the effect of vitamin ADE complex co-administered on the pharmacokinetics of BST. Co-administration of vitamin ADE complex appeared to increase the plasma BST concentration-time profiles without changing the elimination half-lives of BST (see also AUC values in Table 1). Table 1 is a summary of pharmacokinetic parameters obtained by the ways described in Materials and Methods from the mean concentration-time profiles of treatment groups. The elimination half-lives were 4.2 and 5.5 days for low and high dose groups, respectively.

IGF-1 is the major mediator of the effect of growth hormones, especially for lactation[4]. Therefore, we also studied the changes in IGF-1 level after treatment of SR-BST. The mean plasma IGF-1 concentration-time profiles were illustrated in Fig 2. IGF-1 levels changed in a similar pattern to that of plasma BST. However, the time to peak level was about

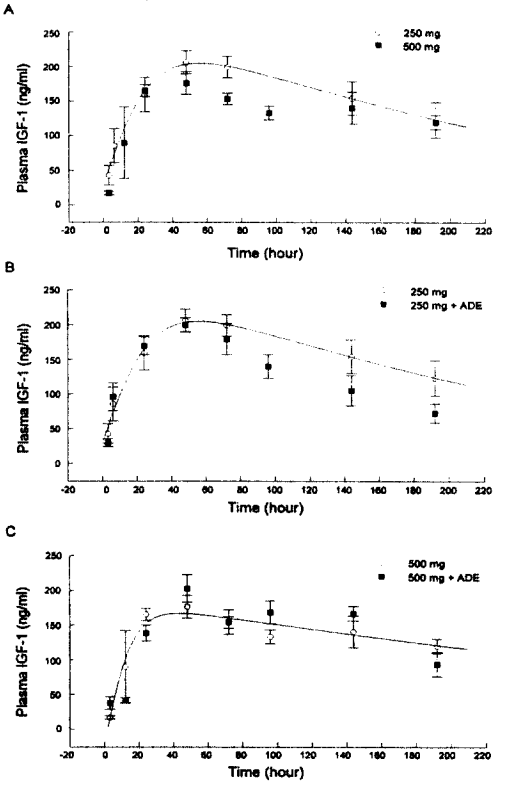

Fig 2. Plasma concentration-time profile of IGF-1 after first administration of Boostin, a sustained-release form of BST. Boostin was subcutaneously administered at 250, 500 mg/cow with or without vitamine ADE complex. Solid and dotted lines are drawn by the best-fit parameters obtained fitting the data to equation described in Materials and Methods. Symbols and bars are means of 4 measurements and sem, respectively. A plasma IGF-1 concentration-time profile at the doses of 250 and 500 mg/cow. B and C. Plasma IGF-1 concentration-time profiles at the doses of 250 and 500 mg/cow with and without vitamin ADE complex.

twice longer (48 hours) than that of BST. Like the case of plasma BST (Fig 1A), there were no difference in the concentration-time profiles of IGF-1 between two dose groups (250, 500 mg/cow). The pattern of changes, initial increase and later decrease of IGF-1, is also similar to those of BST but, 2~4 times slower than those of BST. In contrast to BST, the decay rate of IGF-1 was much slower than that of BST. IGF-1 concentration was far above the control level even at the 8th day after administration.

Co-administration of BST with vitamin ADE complex

Table 1. Pharmacokinetic parameters in plasma BST after administration of SR-BST

	250 mg	250 mg+ADE	500 mg	500 mg+ADE
α (h^{-1})	0.18±0.066	0.10±0.023	0.133±0.021	0.10±0.017
t_{max} (h)	12	48	24	48
C_{max} (ng/ml)	30.2±12.36	38.0±12.37	28.0±6.56	36.1±6.78
β (h^{-1})	0.007±0.0018	0.007±0.0009	0.005±0.0007	0.005±0.0007
$t_{1/2}$ (h)	100.0	105	136.7	132.3
y_o (ng/ml)	-0.36±0.041	-0.39±0.019	-0.39±0.016	-0.37±0.015
x^2	20.4	11.6	3.1	3.1
AUC (ng*h/ml)	1816	2605	2342	3040
AUMC (ng*h^2/ml)	85966	130054	136090	177577
MRT (h)	47.3	49.9	58.1	58.4

α, initial absorption rate constant; t_{max}, time to peak plasma concentration; C_{max}; peak plasma concentration; y_o, correction factor; β, elimentation rate constant; AUC, area under the concentration-time curve; AUMC, area under the first moment of the concentration-time curve; MRT, mean residual time; x^2: parameter for evaluation of goodness of the fit. C_{max} and t_{max} were obtained from real values at the averaged concentration-time data.

Table 2. Pharmacokinetic parameters in plasma IGF-1 after administration of SR-BST

	250 mg	250 mg+ADE	500 mg	500 mg+ADE
α (h^{-1})	0.06±0.013	0.08±0.029	0.077±0.025	0.04±0.029
t_{max} (h)	48	48	48	48
C_{max} (ng/ml)	206±16.3	200±10.5	177±16.5	203±19.8
β (h^{-1})	0.003±0.0007	0.003±0.0011	0.003±0.0006	0.002±0.0021
$t_{1/2}$ (h)	273.9	200.9	309.6	279.4
y_o (ng/ml)	-0.29±0.029	-0.34±0.056	-0.44±0.059	-0.36±0.087
x^2	92.3	236.0	174.5	960.9
AUC (ng*h/ml)	31080	25553	31975	27995
AUMC (ng*h^2/ml)	2916420	2176820	3749710	2733550
MRT (h)	93.8	85.2	117.3	97.6

α, initial absorption rate constant; t_{max}, time to peak plasma concentration; C_{max}; peak plasma concentration; y_o, correction factor; β, elimentation rate constant; AUC, area under the concentration-time curve; AUMC, area under the first moment of the concentration-time curve; MRT, mean residual time; x^2: parameter for evaluation of goodness of the fit. C_{max} and t_{max} were obtained from peak values at the averaged concentration-time data.

did not change the peak plasma concentration of IGF-1, but decreased the elimination half-lives of IGF-1 to 73 and 90% in low and high dose groups, respectively (Table 2, Fig 2B and 2C).

SR-BST formulation was designed for repeated administration to dairy cow. Fig 3 illustrates the mean concentration time profiles for plasma BST and IGF-1 during 3 repeated administrations of SR-BST (250 and 500 mg) with or without vitamin ADE at 2 week intervals. The plasma BST concentrations showed a cyclic pattern with high concentrations in early stage after each injection and following gradual declining. About one week after administration plasma BST concentrations returned to base levels. In contrast to plasma BST, plasma IGF-1 levels did not show clear cyclic pattern and remained higher than the control levels in the treated groups during the repeated treatments (Fig 3).

To estimate the degree of dose-dependence in plasma BST and IGF-1 between two dose groups (250, 500 mg/cow) during repeated administrations, we compared the AUC value of different groups shown in Table 3. For plasma BST, a tendency of dose-dependence was indicated but not for plasma IGF-1. In addition, co-administration of vitamin ADE yielded slightly increased AUC of plasma BST for both dose groups, but such effect was not evident in the IGF-1 levels. Similar trend was consistently seen in single treatment groups (see also Figs 1 and 2, Tables 1 and 2).

Fig 4 shows the concentration-time profiles of BST and IGF-1 in milk for 8 days after single administration. Par-

Table 3. AUC of the BST and IGF-1 plasma concentration-time curves during the repeated treatment

Mean±SEM (ng*day/ml, n = 4)

	BST	IGF-1
Control	97.5±28.79	3064±340
250 mg	302.8±109.54	6892±922**
250 mg+vit ADE	451.1±149.37	5698±314**
500 mg	499.5±121.59*	5882±249**
500 mg+vit ADE	613.4±110.73**	5926±844*

*p < 0.05, **p < 0.01 compared to control, respectively. AUC of plasma BST and IGF-1 obtained from concentration-time data during the repeated ad-ministration.

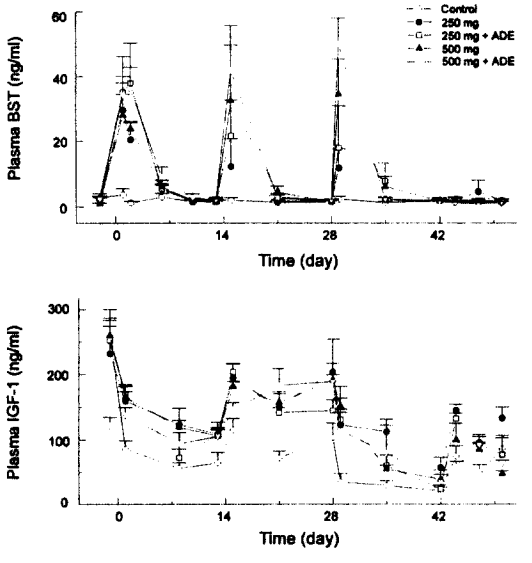

Fig 3. Plasma concentration-time profile of PST (upper) and IGF-1 (lower) at the doses of 250 and 500 mg/cow with and without vitamin ADE complex. The letters over the error bars, "a, b, c, and d" indicate that the values of respective treatment groups are significantly different from the control. Symbols and bars are mean of 4 experiments and sem, respectively. n = 4.

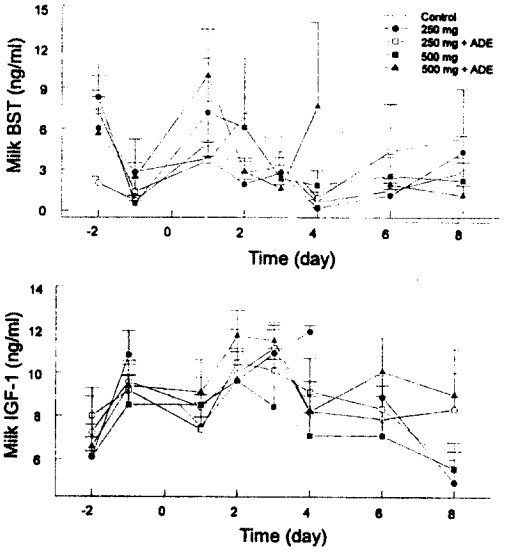

Fig 4. Milk concentration-time profile of BST (upper) and IGF-1 (lower) after single administration of Boostin. Boostin was administered subcutaneously at 250, 500 mg/cow with or without vitamin ADE complex. Symbols and bars are mean of 4 measurements and standard error of means, respectively.

ticularly, it is obvious that milk BST and IGF-1 levels in BST treated groups were not significantly different from the control. In addition, milk concentration-time profiles of BST and IGF-1 showed little correlation with the dose of BST or the co-administration of vitamin ADE.

Discussions

In this work we have shown that the sustained release form of bovine somatotropin(SR-BST, 250 and 500 mg/cow) increased plasma BST concentration for about 1 week and plasma IGF-1 concentration for more than 8 days while maintaining milk BST and IGF-1 levels at the control levels.

Limited reports were available for the pharmacokinetic parameters and concentration-time profiles of BST and IGF-1 during repeated administration. An extensive work of Zhao *et al*[6] is very similar to this work. They measured the BST and IGF-1 levels in plasma and milk during the treatment period of slow-release preparation of BST for 40 weeks and showed that the sustained injectable BST formulation (350 mg/head) elevated BST and IGF-1 levels in plasma and milk with cyclic pattern. The lowest level at the end of each dosing interval of 2 weeks was well over the control level during the repeated administrations. Perhaps, this is the major difference between the results of Zhao *et al*[6] and this work. Our data suggest that there is little accumulation in BST of IGF-1 levels in plasma and milk at the end of each administration cycle. One possible reason of such difference could arise from the differences in the release rate of two formulations. For example, the peak levels of plasma BST (30 ng/ml) was lower than that in the work of Zhao *et al*[6] where the peak level was 60 ng/ml at the dose of 350 mg/cow. The partial contribution of administered BST to peak plasma concentration was 0.12 and 0.17 ng/ml for SR-BST and for that used in Zhao *et al*[6], respectively. The release rate of BST from SR-BST is 1.4 times slower than that of Zhao *et al*[6].

It is well known that the major effect of BST is mediated by IGF-1 which is formed by the action of BST. The plasma BST levels appeared dose-dependent (Fig 1A and Table 1). However, such dose-dependence is not evident in the plasma IGF-1 levels (Fig 2A and Table 2). In other words, the IGF-1 levels in high dose group is not different from that of low dose group. The data suggest that the formation of IGF-1 by BST could be saturable at the dose of 250 mg/cows.

When vitamin ADE complex was co-administered with BST, the AUC values of BST after single and during repeated treatments (Figs 1 and 3, Table 3) were significantly higher in co-administered groups, indicating a positive effect on elevating plasma BST in BST-treated cows. It will be interesting to see whether the administration of only vitamin ADE complex can increase plasma BST level. If not, there could be a positive interaction between administered vitamin complex and BST for the absorption of BST. In contrast, the elimination half-lives of IGF-1 decreased to 73 and 90% of those of low and high dose groups after single treatment (Fig 2, Table 3). IGF-1 and vitamin ADE complex were not totally unrelated (Seeman *et al*, 1995), but little is available on the relations of plasma IGF-1 levels and vitamin ADE complex. Our data suggest that vitamin ADE complex or one of them may be involved in the processes of removal or metabolism of IGF-1 from blood. The overall effects of the co-administration of BST with vitamin ADE complex seems to be rather complicated and the benefit of co-administration of vitamin ADE complex should be carefully assessed based on the possible interactions between of vitamin ADE complex on BST and IGF-1.

In conclusion, the experimental results suggest that the BST formulation examined in this study is one of the ideal sustained-release formulation of BST for long term use in dairy industry. In addition, the benefit of co-administrating vitamin ADE complex is needed to be further examined.

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