ORIGINAL ARTICLE

# Influence of transgenic corn (CBH 351, named Starlink) on health condition of dairy cows and transfer of Cry9C protein and *cry9C* gene to milk, blood, liver and muscle

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## ABSTRACT

The influence of transgenic event CBH 351 (Starlink)-derived hybrid corn (SL) on the health condition, physiological function and lactational performance of dairy cows as well as the transfer of the *cry9C* gene and Cry9C protein present in SL to milk, blood, liver and muscles was examined, and compared with a diet containing non-transgenic (isogenic) control corn (non-SL). After adapting to a diet containing non-SL for 2 weeks, four Holstein cows were assigned to each of the non-SL and SL groups and were fed diets containing non-SL or SL, respectively, for 5 weeks. There were no significant influences on the physiological condition, milk yield or serum biochemical and hematological values after feeding with SL. There was also no influence on pH value, cell density of protozoa, or volatile fatty acid concentration and composition of rumen fluids. In addition, no significant differences were observed on histopathological examination of the major organs and tissues between the SL and non-SL groups. Moreover, the *cry9C* gene and Cry9C protein were not detected by the polymerase chain reaction method and ELISA in the milk, blood, liver and muscles of the cows at the end of the experiment.

**KEYWORDS:** lactating dairy cows, Starlink corn, transfer of *cry9C* and Cry9C.

## INTRODUCTION

Attempts to make genetically modified organisms (GMO) were started in the 1970s and a 'delayed softening tomato' was approved by the United States Food and Drug Administration (USFDA) in 1991. At the present time, 53 varieties of GMO crops and vegetables including corn, rapeseed, cotton, soybean, beet, potato, squash and chicory have been developed (USFDA 2002). Among them, 16 varieties of genetically modified corn (GM corn) that carry resistance to chemical herbicides and insects have been approved by the USFDA and the planted area of GM corn increased to 34% of the total planted area in the USA in the year 2002 (USDA 2002).

In Japan, more than 70% of feedstuffs are imported from foreign countries. In particular, more than 95% of imported feed corn comes from the USA (MAFF 2002). Under these circumstances, it is inevitable that GM corn will be used as feed in Japan. Therefore, after confirming their safety, some GM corn varieties have been approved for use as feedstuffs by the Ministry of Agriculture, Forestry and Fisheries of Japan.

The event CBH 351 corn, trade name Starlink (SL), is an insect-resistant corn which was developed in France by AgrEvo (now Bayer CropScience, Kansas City, MO, USA). It carries a novel gene from a naturally occurring soil bacterium, *Bacillus thuringiensis* ssp. *tolworthi*, that encodes for a form of the Bt endotoxin known as Cry9C protein (Schnepf *et al*. 1998).

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Because Cry9C protein is not digested completely *in vitro* and can be an allergen for humans, it has been approved only for use as a feedstuff and for alcohol production in the USA by the USFDA.

In contrast, SL is not approved for either food or feedstuff use in Japan and the discovery of SL in food and feedstuffs in Japan caused social anxiety relating to the safety of SL (MAFF 2000).

Bayer CropScience presented various data concerning the safety of SL to the USFDA. However, these data did not contain any results of feeding tests or the possibility of transferring the *cry9C* gene and Cry9C protein to animal tissues (USEPA 1998). Therefore, it is very important to confirm the effects of SL on the health of animals and the possibility of transferring the *cry9C* gene and Cry9C protein to animal products. The authors have already confirmed that SL does not affect the health of broiler chicks and that the *cry9C* gene and Cry9C protein is not transferred to the meat, liver or blood of broilers given free access to a diet containing 70% SL for 7 weeks (Yonemochi *et al*. 2002).

Following on from this work, the present study was conducted to confirm the effect of SL on the performance and physiological function of lactating dairy cows and the possibility of transferring the *cry9C* gene and Cry9C protein derived from SL to the milk, blood, liver and muscles of dairy cows fed a diet with 35% SL for 5 weeks.

## MATERIALS AND METHODS

### Corn grain

The two corn varieties, non-SL and SL, used in the present study were supplied by Bayer CropScience. In the non-SL, neither the *cry9*C gene nor the Cry9C protein were detected by the polymerase chain reaction method (PCR) or ELISA, respectively. The *cry9C* gene was carried by 89.3% of the SL grains.

There were no differences in chemical composition between non-SL and SL, as shown in Table 1.

**Table 1** Proximate analysis of SL and non-SL corn (%)

| | SL | Non-SL |
|---|---|---|
| Moisture | 10.6 | 10.4 |
| Crude protein | 7.7 | 7.8 |
| Ether extract | 4.4 | 4.3 |
| Nitrogen free extract | 74.3 | 74.6 |
| Crude fiber | 1.6 | 1.5 |
| Crude ash | 1.4 | 1.4 |

SL, Event CBH 351 corn (trade name Starlink); Non-SL, Non-transgenic (isogenic) control corn.

Before formulating the experimental diets, both SL and non-SL were ground with a hummer mill through a 1 mm screen.

### Diet

The compositions of the experimental diets are shown in Table 2. Each experimental diet contained 35% of either non-SL or SL. The cows were fed the experimental diet twice a day (at 09.00 hours and 17.00 hours), to provide 110% of the total digestible nutrients requirement for maintenance and the production of milk according to the Japanese Feeding Standard for Dairy Cattle (MAFF 1999) (Table 3).

### Experimental design

The experiment was performed between November 3 and December 22, 2000 in our Research Center. The characteristics of the dairy cows are shown in Table 3. During the 2-week pre-experimental period, eight Holstein cows were supplied the non-SL diet to adapt to the experimental environment, and then they were allotted to two groups, non-SL and SL dietary groups, each containing four cows. Each cow was tethered by a neck chain in a stall furnished with a $1.3 \times 1.8$ m rubber mat. An empty stall was left between stalls to prevent cross contamination. The SL group was separated by a vinyl sheet curtain from the non-SL group. Tap water was given ad libitum.

**Table 2** Ingredients and chemical compositions of the experimental diet

| | (%) |
|---|---|
| Ingredients | |
| Corn (SL or non-SL) | 35.0 |
| Soybean meal | 8.75 |
| Wheat bran | 2.7 |
| Soybean hull | 2.5 |
| Calcium carbonate | 0.5 |
| Dicalcium phosphate | 0.25 |
| Sodium chloride | 0.2 |
| Trace mineral premix† | 0.05 |
| Vitamin ADE premix‡ | 0.05 |
| Timothy hay | 35.0 |
| Alfalfa hay cube | 15.0 |
| Calculated analysis§ | |
| Neutral detergent fiber | 31.5 |
| Digestible crude protein | 12.8 |
| Total digestible nutrients | 66.1 |

†Contained (g/kg of premix): manganese 50, iron 50, copper 10, zinc 60, iodine 1. ‡Contained (IU or mg/kg of premix): vitamin A 10 000 IU, vitamin D3 2000 IU, DL-α-tocopherol acetate 10 mg. §Based on MAFF 1995. SL, Event CBH 351 corn (trade name Starlink); Non-SL, Non-transgenic (isogenic) control corn.

**Table 3** Characteristics of the cows and feed intake

| Group | Cow | Age | No. of parturitions | Stage of lactation (days after parturition) | Body weight (kg) | | Feed intake (kg/day) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Start | End | Concentrate | Timothy hay | Alfalfa hay cube |
| non-SL | | | | | | | | | |
| | A | 4 | 2 | 151 | 536 | 544 | 11.7 | 8.2 | 3.5 |
| | B | 4 | 2 | 27 | 615 | 616 | 9.1 | 6.3 | 2.7 |
| | C | 5 | 3 | 298 | 608 | 615 | 8.6 | 6.0 | 2.6 |
| | D | 3 | 1 | 209 | 689 | 696 | 11.8 | 8.3 | 3.5 |
| SL | | | | | | | | | |
| | E | 7 | 4 | 62 | 766 | 789 | 10.7 | 7.5 | 3.2 |
| | F | 3 | 1 | 293 | 546 | 567 | 10.2 | 7.1 | 3.0 |
| | G | 6 | 4 | 255 | 700 | 702 | 8.8 | 6.1 | 2.6 |
| | H | 2 | 1 | 169 | 627 | 647 | 10.0 | 7.0 | 3.0 |

SL, Event CBH 351 corn (trade name Starlink); Non-SL, Non-transgenic (isogenic) control corn.

## Health condition

The body weight of each cow was measured at the beginning and end of the experiment. The health status was diagnosed by observing the appearance of the animal's body and feces at each milking time.

## Milk production

The cows were milked at 09.00 hours and 17.00 hours by a bucket milker. The milk yield per day was calculated from the total amount of milk produced during one week or the entire experimental period.

## Serum biochemical, hematological and histopathological examination

Serum biochemical examination: Blood was collected from the jugular vein at the beginning and end of the experiment and the sera were separated. Lactate dehydrogenase (LDH), glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), neutral lipid (NL), total bilirubin (T-Bili), total cholesterol (T-Chol), total protein (T-P), albumin (Alb) and glucose (Glu) were determined by an autoanalyzer (model JCB MB8, Nihon Denshi, Tokyo, Japan). Globulin (Glob) and the Alb/Glob (A/G) ratio were calculated on the basis of T-P and Alb concentrations.

Hematological examination: Blood samples were collected from the jugular vein. Hemoglobin (Hb; azido-methemoglobin method), hematocrit (Ht; capillary method), red blood cells (RBC; Coulter Counter, model ZF; Coulter Electronics, Miami, FLO, USA), and white blood cells (WBC; indirect method) were determined.

Histopathological examination: At the end of the experiment, the cows were humanely slaughtered after anesthetizing with Rompun 2% solution (Bayer AG, Leverkusen, Germany) and then bled through the jugular vein. Tissue samples from the cerebrum, cerebellum, lung, heart, liver, kidney, pancreas, rumen, duodenum and jejunoileum were collected. After the specimens were fixed, thin sections were cut, stained with hemotoxylin and eosin, and examined under a light microscope.

## Analysis of rumen fluid

The rumen fluid was collected orally through a stomach tube at 13.00 hours at the beginning and end of the experiment. Its composition, pH value, protozoa counts and total volatile fatty acid (VFA) concentration were examined. The VFA were analyzed by gas chromatography, using a glass column packed with Chromosorb W (80/100 mesh) coated with 5% Thermo 1000 and 0.5% $H_3PO_4$ (Wako Pure Chemical Industries, Tokyo, Japan). The protozoa were counted according to the method of Imai and Katsuno (1977).

## Detection of *cry9C* gene and Cry9C protein in blood and tissue

At the end of the experiment, the blood, muscles and liver were collected individually.

Detection of the *cry9C* gene by PCR: Deoxyribonucleic acid (DNA) was isolated from the blood, liver and meat according to the procedures described in our previous report (Yonemochi *et al*. 2002). Deoxyribonucleic acid (DNA) was isolated from milk according to the procedures of Lipkin *et al*. (1993), except that the milk somatic cells were lysed by incubation in

boiling water for 10 min. Gel electrophoresis, PCR amplification, and the detection of PCR products on gels were performed by the methods described in our previous report (Yonemochi *et al*. 2002).

Detection of Cry9C protein by ELISA: The blood, liver and meat were treated according to the procedures described in our previous report (Yonemochi *et al*. 2002). Milk was treated in the same way as blood. The determination of Cry9C protein in the samples was as described in our previous report (Yonemochi *et al*. 2002).

## Statistical analysis

Data on milk yield were analyzed by a one-way analysis of variance (Yoshida 1983). Data on serum biological values, hematological values and rumen fermentation parameters were analyzed by a two-way analysis of variance. Then they were presented in a way in which the main effect of the group (SL group *vs* non-SL group) and sampling periods (the first day of the experiment *vs* the last day of the experiment) were shown, and the group by sampling periods interaction was included in the model. Significant differences were tested at a level of $P < 0.05$.

# RESULTS

## Health condition and milk production

There were no refusals of feed by any of the cows during the experimental period and no abnormalities in the health of the cows were observed.

Changes in milk yield during the experimental period are presented in Table 4. No significant differences in milk yield were found between the SL and non-SL groups during any week.

## Serum biochemical values

The serum biochemical values are presented in Table 5. There were no significant differences in LDH, GOT, ALP, T-Chol, T-P and the A/G ratio between the non-SL and SL groups and between the first and last days of the experiment. Also there were no differences in Glu and Alb between non-SL and SL groups. Glucose was higher and Alb was lower on the first day than on the last day. In contrast, GPT and BUN of the SL group were lower than those of the non-SL group, and GPT on the first day was higher than on the last day. However, these variations ranked within the normal values for a lactating dairy cow (Takahashi &

**Table 4** Change of milk yields (kg/head/day)

| Group | Week | | | | | Average |
|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 5th | |
| non-SL | 25.8 ± 6.4† | 25.0 ± 2.7 | 24.6 ± 3.1 | 23.9 ± 3.0 | 23.4 ± 2.5 | 24.5 ± 3.4 |
| SL | 25.0 ± 3.3 | 28.0 ± 6.0 | 27.2 ± 5.9 | 27.0 ± 5.4 | 26.0 ± 4.0 | 26.6 ± 4.4 |

†Values are means ± SD ($n = 4$). SL, Event CBH 351 corn (trade name Starlink); Non-SL, Non-transgenic (isogenic) control corn.

**Table 5** Serum biochemical values for cows at the start and end of the experiment

| Sampling periods Groups | First day of experiment | | Last day of experiment | | Probabilities‡ | | |
|---|---|---|---|---|---|---|---|
| | non-SL | SL | non-SL | SL | Group | Sampling periods | Interaction |
| LDH (IU/L) | 1633 ± 150† | 1643 ± 289 | 1752 ± 411 | 1712 ± 223 | NS | NS | NS |
| GOT (IU/L) | 60.3 ± 9.5 | 51.0 ± 10.4 | 57.8 ± 10.2 | 45.8 ± 11.9 | NS | NS | NS |
| GPT (IU/L) | 22.5 ± 3.3 | 15.0 ± 3.9 | 16.0 ± 2.7 | 13.0 ± 3.8 | * | * | NS |
| BUN (mg/dL) | 17.3 ± 2.8 | 13.5 ± 3.8 | 15.0 ± 1.2 | 11.8 ± 1.3 | * | NS | NS |
| NL (mg/dL) | <25 | <25 | <25 | <25 | –§ | – | – |
| T-Bili (mg/dL) | <0.30 | <0.30 | <0.30 | <0.30 | – | – | – |
| ALP (IU/L) | 186 ± 199 | 96 ± 43 | 184 ± 86 | 86 ± 27 | NS | NS | NS |
| T-Chol (g/dL) | 159 ± 30 | 164 ± 33 | 141 ± 33 | 150 ± 33 | NS | NS | NS |
| Glu (mg/dL) | 82.5 ± 10.9 | 79.8 ± 5.0 | 69.8 ± 4.9 | 76.5 ± 3.9 | NS | * | NS |
| T-P (g/dL) | 7.5 ± 0.7 | 7.5 ± 0.5 | 7.5 ± 0.4 | 7.5 ± 0.4 | NS | NS | NS |
| Alb (g/dL) | 3.7 ± 0.4 | 3.8 ± 0.2 | 4.0 ± 0.2 | 4.0 ± 0.1 | NS | * | NS |
| A/G | 1.05 ± 0.28 | 1.04 ± 0.16 | 1.22 ± 0.41 | 1.19 ± 0.18 | NS | NS | NS |

†Values are mean ± SD ($n = 4$). ‡NS, Not significant ($P > 0.05$); *, Significant ($P \leq 0.05$); §not applicable. SL, Event CBH 351 corn (trade name Starlink); Non-SL, Non-transgenic (isogenic) control corn. LDH, lactate dehydrogenase; GOT, glutamic-oxalacetic transaminase; GPT, glutamic-pyruvic transaminase; BUN, blood urea nitrogen; NL, neutral lipid; T-Bili, total bilirubin; ALP, alkaline phosphatase; T-Chol, total cholesterol; Glu, glucose; T-P, total protein; Alb, albumin; A/G, albumin/globulin.

**Table 6** Hematological values for cows at the start and end of the experiment

| Sampling periods Groups | First day of experiment | | Last day of experiment | | Probabilities‡ | | |
|---|---|---|---|---|---|---|---|
| | non-SL | SL | non-SL | SL | Group | Sampling periods | Interaction |
| RBC ($10^6$/μL) | 7.01 ± 0.39† | 7.36 ± 1.16 | 8.33 ± 0.46 | 8.05 ± 0.83 | NS | * | NS |
| WBC ($10^3$/μL) | 9.6 ± 1.6 | 8.4 ± 1.1 | 7.9 ± 1.8 | 6.8 ± 0.6 | NS | * | NS |
| Hb (g/dL) | 10.2 ± 1.1 | 10.8 ± 1.1 | 10.1 ± 0.7 | 10.0 ± 0.5 | NS | NS | NS |
| Ht (%) | 32.9 ± 1.7 | 34.8 ± 2.2 | 35.9 ± 1.6 | 37.5 ± 2.5 | NS | * | NS |

†Values are mean ± SD ($n = 4$). ‡NS, Not significant ($P > 0.05$); *, Significant ($P \leq 0.05$). RBC, red blood cells; WBC, white blood cells; Hb, hemoglobin; Ht, hematocrit.

**Table 7** Characteristics of rumen fluid from cows at the start and end of the experiment

| Sampling periods Groups | First day of experiment | | Last day of experiment | | Probabilities‡ | | |
|---|---|---|---|---|---|---|---|
| | non-SL | SL | non-SL | SL | Group | Sampling periods | Interaction |
| pH | 7.1 ± 0.2† | 7.2 ± 0.1 | 7.2 ± 0.3 | 7.3 ± 0.3 | NS | NS | NS |
| Protozoa count (105/mL) | 2.98 ± 1.60 | 3.63 ± 1.38 | 1.94 ± 0.29 | 3.02 ± 1.26 | NS | NS | NS |
| Total VFA (mmol/L) | 209.9 ± 26.4 | 208.1 ± 20.5 | 200.6 ± 58.6 | 202.1 ± 51.7 | NS | NS | NS |
| VFA composition (mol%) | | | | | | | |
| Acetic acid | 57.4 ± 0.8 | 56.9 ± 2.2 | 55.8 ± 1.2 | 55.8 ± 0.9 | NS | NS | NS |
| Propionic acid | 18.3 ± 1.6 | 18.0 ± 2.0 | 20.1 ± 1.7 | 18.1 ± 2.1 | NS | NS | NS |
| Butyric acid | 16.4 ± 0.6 | 17.9 ± 2.3 | 15.9 ± 1.1 | 17.9 ± 1.9 | NS | NS | NS |
| Others | 7.9 ± 1.1 | 7.4 ± 0.6 | 8.3 ± 1.4 | 8.2 ± 0.6 | NS | NS | NS |
| A/G | 3.15 ± 0.31 | 3.21 ± 0.47 | 2.80 ± 0.28 | 3.12 ± 0.41 | NS | NS | NS |

†Values are mean ± SD ($n = 4$). ‡NS, Not significant ($P > 0.05$). VFA, volatile fatty acids; A/G, albumin/globulin.

Itagaki 1975; Nakamura 1976; Sonota 1978), and there were no significant differences in the interaction between the groups and the sampling periods.

## Hematological values

The hematological values for both groups are presented in Table 6. There were no significant differences in Hb between the non-SL and SL groups on the first or last days of the experiment. Although there were no significant differences in RBC, WBC and Ht between non-SL and SL groups, RBC and Ht on the first day were significantly lower than on the last day. White blood cells on the first day was significantly higher than on the last day. These variations were also, however, within the normal values for a lactating dairy cow (Takahashi & Itagaki 1975; Nakamura 1976; Sonota 1978), and there were no significant differences in interaction between the groups and the sampling periods.

## Characteristics of rumen fluid

The results of the rumen fluid examination are presented in Table 7. There were no significant differences in the pH value, protozoa count, total VFA concentration and its composition between the non-SL and SL groups on the first and last days of the experiment. The interaction relationship between the groups and sampling periods were not significant.

## Necropsy finding and histopathological observation

Necropsy revealed several abnormalities. In the non-SL group, a liver abscess was found in Cow C. In the SL group, a lipoma around the colon was found in Cow E, a simple polyp on the rumen and hydronephrosis were found in Cow G, and a simple polyp was found on the rumen of Cow H. No specific abnormality was observed in any of the cows.

The results of the histopathological examination are presented in Table 8. No significant lesions were found in the cerebrum, cerebellum, heart, pancreas, duodenum or jejunoileum of any of the cows. Many of the lesions described in Table 8 are often found in specimens from conventional cows. Furthermore, these lesions were all fairly minor and not considered to have any effect on their physiological function.

**Table 8** Results of histopathological observation†

| Organ | non-SL | | SL | |
|---|---|---|---|---|
| | Cow | Findings | Cow | Findings |
| Lung | | | G | Deposit of hemosiderin on alveolus wall (migration of leukocyte on the part of bronchiole, minor). |
| Liver | C | Left lobe: bleeding in lobe (mild). Scattered leukocyte infiltration inside Glisson's capsule (middle degree) | H | Right lobe: scattered lymph-like cell infiltration between lobes (minor). |
| | D | Right lobe: scattered leukocyte infitration inside lobe and Glisson's capsule (minor). | | |
| Kidney | C | Left kidney: infiltration of lymph-like cell in cortex (mild). | E | Right kidney: scattered minor fattening of Bowman's capsule (mild). |
| | D | Left kidney: fattening of Bowman's capsule, minor infiltration of lymph-like cell in surrounding (minor). Cortex: nest-like infiltration of lymph-like cell which across vertical linear, necrosis of uriniferous tubule (middle degree). | G | Left kidney: infitration of lymph-like cell in cortex, fattening of Bowman's capusule (minor). Deposit of hemosiderin in urinary tubular cell (mild). Expansion of part of collecting tubule (minor). |
| Rumen | | | G, H | Fibroepithelioma (simple polyp) from mucous exists. |

†Only the regions and cows that showed abnormalities are presented. SL, Event CBH 351 corn (trade name Starlink); Non-SL, Non-transgenic (isogenic) control corn.

## Probability of transfer of *cry9C* gene and Cry9C protein to blood, liver, muscle and milk

On the final day of the experiment, neither the *cry9C* gene, nor the Cry9C protein, were detected in the milk, blood, liver or muscles of the cows in either the non-SL or SL groups, by PCR and ELISA, respectively (data not shown).

In all cases, DNA strands of 315-bp specific for the bovine growth hormone region were amplified by PCR from DNA obtained from the milk, blood, liver and muscles in both groups, while DNA strands of 379-bp specific for the *cry9C* region were not amplified. The agarose gel electrophoresis patterns of PCR products amplified from milk DNA are shown in Fig. 1. The same results were obtained for samples from the blood, liver and muscles (data not shown).

These results indicate that the *cry9C* gene and Cry9C protein contained in SL corn do not transfer to the milk, blood, liver and muscles.

## DISCUSSION

In the present study, the health condition of dairy cows was not affected when fed for 5 weeks on a diet with 35% SL corn. Furthermore, there were no differences in serum biochemical and hematological values, and feeding SL did not affect histpathological examination, because there were also no significant differences in the interaction between groups and sampling periods. The observed total VFA concentration was relatively high in both groups compared with the average, but these observed values ranked within normal values (Akay & Jackson 2001).

Although there has been no report on the effect of SL on the lactating performance of dairy cows, Folmer *et al*. (2000) and Donkin *et al*. (2000) reported the effect of other GM corn varieties (Bt-11, Folmer *et al*. 2000, and Roundup Ready, Donkin *et al*. 2000) on the lactating performance of dairy cows. These studies were conducted to elucidate the milk yield and composition using a Latin squire design (Folmer *et al*. 2000) or double reverse feeding (Donkin *et al*. 2000) which are commonly used for feeding experiments with dairy cows.

In contrast, the present study was conducted to elucidate whether or not the *cry9c* gene and Cry9C protein were transported into milk, blood, liver and muscle. In the present study, parallel comparative feeding was employed instead of reverse feeding, because transfer of the *cry9C* gene and Cry9C protein into the tissues had to be determined at the end of experiment.

There were no differences in milk yield between the SL and non-SL groups in the present study. This result was in agreement with those of Folmer *et al*. (2000) and Donkin *et al*. (2000). Moreover, in the present

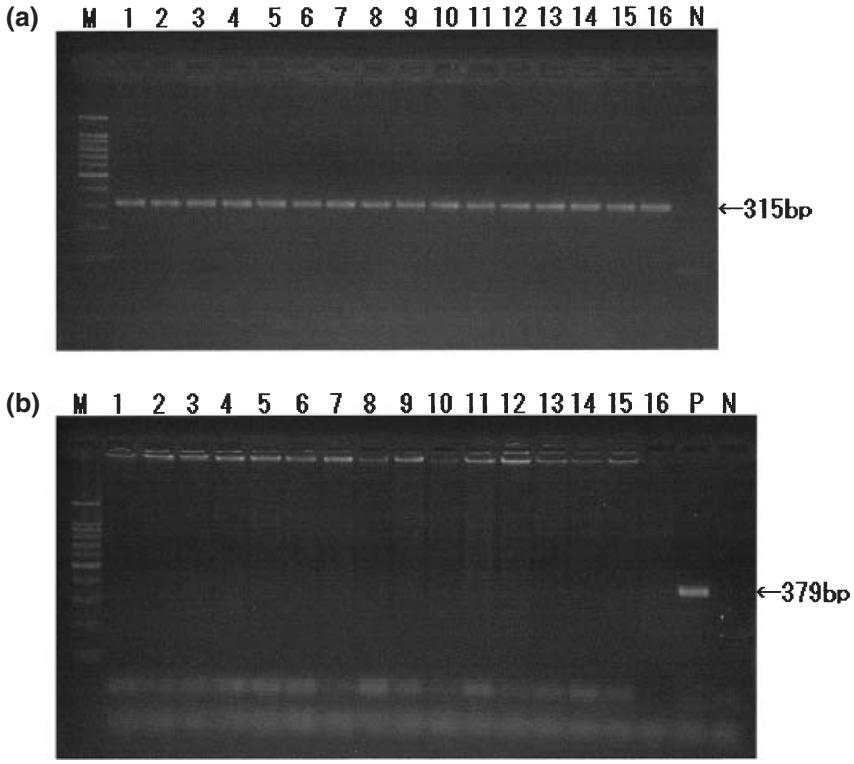


**Fig. 1** Detection of growth hormone gene and *cry9C* gene in milk of dairy cows by PCR. PCR product (315 bp) specific for growth hormone gene (A), and PCR product (379 bp) specific for *cry9C* gene region in SL (B). Two samples of milk for each cow were loaded onto the gel. M, 100 bp Ladder; Lanes 1–8, milk of cows from the non-SL group; lanes 9–16, milk of cows from the SL group; N, negative control (no template); P, positive control (PCR product amplified from Starlink corn).

study, the *cry9c* gene and Cry9C protein were not detected by PCR and ELISA in milk, blood, liver and muscle.

In conclusion, the results obtained in the present study indicate that feeding SL does not influence the health condition or physiological function in lactating dairy cows, compared with those fed non-SL. The *cry9c* gene and Cry9C are not transferred to milk, blood, liver and muscle of dairy cows.

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