INTRODUCTION

Soybean meal (SBM) is the most commonly used protein source in animal diet because of its excellent protein with amino acid, excellent nutrient availability and favorable palatability (Jeong and Kim, 2015; Wang et al., 2014). SBM is the most commonly used supplemental plant proteins for dairy cows (Mjoun et al., 2010; Awawdeh et al., 2007). A variety of anti nutritional factors (ANF) have limited its application in the diets for young animals (Wang et al., 2014; Rojas and Stein, 2013; Kim et al., 2010a). Also ANF of SBM damages the animal intestinal wall, increase diarrhea and reduce the absorption rate of vitamins and proteins (Song et al., 2010). A fermentation process could reduce the ANF in SBM which increased the nutrients digestibility in animals (Roh et al., 2015; Upadhaya and Kim, 2015). Furthermore, a variety of essential nutrients, such as vitamins, are also produced during the process (Kim et al., 2012). Previous studies have reported that fermented soybean meal (FSBM) improved growth performance and reduced diarrheal incidence in Escherichia coli challenged piglets (Hung et al., 2008; Feng et al., 2007). However, data on the effects of FSBM on growth performance and health parameter in sucking calves are not readily available.

This experiment was aimed to study the effects of soybean meal (SBM) fermented with Bacillus subtilis on per-
formance, nutrient digestibility and blood urea nitrogen concentration in sucking Holstein calves. We hypothesized that calves fed with FSBM diet can improve growth performance via improving nutrient retention during the sucking period.

MATERIALS AND METHODS

PREPARATION OF FERMENTED SOYBEAN MEAL
The bacteria were obtained from the Jilin Agricultural University, as *Bacillus subtilis* (Patent No:201510205215.1). The solid-state fermentation culture medium (containing 2.64kg soybean, 0.37kg wheat bran, 0.09kg sucrose and 2.48L distilled water) was mixed with probiotics (0.31L *Bacillus subtilis*), incubated at 37°C for 24h.

ANIMALS AND FEEDING
A total of 20 newborn Holstein calves (BW: 45.7±5.5 kg) were randomly allotted to four groups, every group has five calves. Each calf was in a single cage and a mechanical ventilation system. The calf was fed milk using plastic bottles (2L capacity) and fed diet three times a day. Liger-EDTA (0.4g) was provided in 1L milk and TiO₂ (0.4g) was provided in 100g diet at 20-30d for nutrient digestibility test.

DATE RECORDING AND SAMPLE COLLECTION
The feed intake was recorded daily. Body weight was measured on every phase of evaluation and blood was collected in 5-mL coagulation-promoting tubes (BD, USA) from the jugular vein at initial day, 20 day and 45 day. Serum was collected from coagulation blood samples after centrifugation at 1,600 × g at 4°C for 15 min and stored at -80°C until subsequent assays were conducted. Feces sample was collected from 23d to 30d, 10% sulfuric acid were added in feces and then keep it into the -20°C for further analysis.

GROWTH PERFORMANCE AND FEED EFFICACY
Growth performance results as average daily gain (ADG) and feed efficiency were calculated at phase I (1-20d), phase II (21-45d), phase III (1-45d).

CHEMICAL ANALYSIS
All of feed and fecal sample were analyzed for dry matter (DM) by an oven at 105°C for 5 h. Percentages of crude protein and crude fat were measured following the method AOAC (2004). Serum urea nitrogen (UREA) in whole blood was determined using fully automatic blood biochemical analyzer.

DIARRHEA SCORING
Diarrhea scoring was done followed the method by Kim et al. (2012). In brief, fecal scoring composed of fecal fluidity, consistency, and odor was conducted daily at 0800 h as follows: for fecal fluidity, normal=1, soft = 2, runny = 3, and watery = 4; for fecal consistency, normal = 1, foamy = 2, mucouslike = 3, sticky = 4, and constipated = 5; for fecal odor, normal = 1, slightly offensive = 2, and highly offensive= 3.

RESULTS

CHLORIDE COMPOSITION OF DIETS
Diets were formulated to meet or exceed the nutrient requirements by the NRC (2001) for calves. Nutrient and feed ingredients composition of the basal diet are shown in Table 1.

BODY WEIGHT GAIN
As shown in Table 2. Average daily gain (ADG) of calves fed FSBM was significantly (P<0.01) improved in 6L milk at phase I (1-20d), and was increased (P<0.01) in 4L milk at phase II (20 to 45d). But the interaction between fed milk and diet was not significant (P=0.071). Compared to calves fed with SBM, FSBM significantly increased ADG in fed 6L milk and diet at phase III (1-45d), but the interaction between fed milk and diet was no significant.

FEED EFFICIENCY
As shown in Table 3. Feed efficiency of calves fed FSBM were significantly (P<0.01) decreased in 6L milk during phase I (1-20d), but at Phase II (20 to 45d) feed efficiency was significant decreased in calves fed with 4L milk and diet (P<0.01; P<0.05). However, the interaction between fed milk and diet was no significant (P=0.186). At phase III (1-45d), FSBM significantly increased feed efficiency in calves fed with 4L milk and diet (P<0.05). But the interaction between fed milk and diet was no significant.

NUTRIENT DIGESTIBILITY ANALYSIS
The effects of dietary treatment on nutrient digestibility were shown in Table 4. Compared with SBM, FSBM could significantly increase the digestibility of CP in calves fed with 6 L milk and diet (P<0.05; P<0.01).The digestibility of EE was improved in calves fed with 6 L milk and diet (P<0.05; P< 0.01) and the interaction between milk and diet was significant (P< 0.05).

SERUM UREA-NITROGEN CONCENTRATION
The effects of the different feeding conditions on urea-nitrogen concentration in serum of calves were shown in Table 5. At 20d, urea concentration in serum was decreased in calves fed FSBM compared with SBM (diet effect, P<0.05)
Table 1: Nutrition and ingredients composition (%) of the soybean meal (SBM) and fermented soybean meal (FSBM) in experimental diets

| Items                                      | SBM  | FSBM |
|-------------------------------------------|------|------|
| Ingredient, %                             |      |      |
| Corn                                       | 49   | 50   |
| SBM (42% crude protein)                   | 26   | -    |
| FSBM (44% crude protein)                  | -    | 25   |
| Cottonseed meal                           | 5    | 5    |
| Distiller’s dried grains with solubles    | 11.8 | 11.8 |
| Wheat bran                                 | 5    | 5    |
| Calcium hydrogen phosphate                 | 0.5  | 0.5  |
| Sodium chloride                            | 0.4  | 0.4  |
| Mountain flour                             | 0.8  | 0.8  |
| Sodium bicarbonate                         | 1    | 1    |
| Premix (vitamins and trace minerals)      | 0.5  | 0.5  |

Analyzed chemical composition, %

| Items                                      | SBM  | FSBM |
|-------------------------------------------|------|------|
| Metabolic energy, kcal/g                  | 13.46| 13.79|
| Crude protein                             | 21.45| 21.14|
| Calcium                                   | 0.55 | 0.47 |
| Phosphorus                                | 0.57 | 0.41 |
| Sodium                                    | 0.38 | 0.37 |
| Chlorine                                  | 0.32 | 0.31 |

1 Premix supplied the following nutrients per kilogram of mixed feed: vitamin A, 1200 KIU; vitamin D₃, 400 KIU; vitamin E, 12000 IU; CuSO₄·5H₂O, 4000 mg; ZnSO₄, 10000 mg; MnSO₄, 8000 mg; CoCl₂, 60 mg; KI, 160 mg; Na₂SeO₃, 140 mg and FeSO₄, 2000 mg.

Table 2: Effect of experimental diets on average daily weight (g) gain in calves

| Treatment | SBM | FSBM | M | B | M×B |
|-----------|-----|------|--|---|-----|
| Phase I (1–20d) |     |      |   |   |     |
| 6L milk   | 290±80 | 360±80 | ** |   |   |
| 4L milk   | 100±40 | 110±30 |     |   |   |
| Phase II (21–45d) |     |      |   |   |     |
| 6L milk   | 600±140 | 650±30 |   |   |   |
| 4L milk   | 500±110 | 710±30 |   |   |   |
| Phase III (1–45d) |       |       |   |   |     |
| 6L milk   | 430±40 | 475±40 | ** | ** |     |
| 4L milk   | 320±70 | 380±50 |     |   |   |

1 6L milk=feeding 6L milk each calf daily; 4L milk=feeding 4L milk each calf daily.
2 SBM=soybean meal; FSBM=fermented soybean meal.
3 M=milk effect; B=diet effect; M×B=the interaction between milk and diet effect. **P<0.01.

Table 3: Effect of feed efficiency in calves

| Treatment | SBM   | FSBM   | M | B | M×B |
|-----------|-------|--------|--|---|-----|
| Phase I (1–20d) |     |        |   |   |     |
| 6L milk   | 2.64±0.86 | 2.11±0.65 | ** |   |   |
| 4L milk   | 5.83±1.55 | 5.39±0.96 |     |   |   |

1 6L milk=feeding 6L milk each calf daily; 4L milk=feeding 4L milk each calf daily.
2 SBM=soybean meal; FSBM=fermented soybean meal.
3 M=milk effect; B=diet effect; M×B=the interaction between milk and diet effect. **P<0.01.
| Items       | Treatment | Significance (P-value) |
|------------|-----------|-----------------------|
|            | SBM       | FSBM                  | M   | B | M×B |
| CP (%)     |           |                       |     |   |     |
| 6L milk    | 67.24±2.82| 80.02±4.90            | *   | **| 0.067 |
| 4L milk    | 67.56±2.49| 72.09±4.39            |     |   |     |
| EE (%)     |           |                       |     |   |     |
| 6L milk    | 81.73±3.88| 91.01±1.71            | *   | **|     |
| 4L milk    | 82.33±1.77| 83.07±3.01            |     |   |     |

Table 4: Nutrient digestibility in calves

Similarly, at day 45, urea-nitrogen concentration was decreased in calves fed with FSBM compared with SBM (milk and diet effect, P<0.05).

**DISCUSSIONS**

In this study, soybean meal was replaced 25% by fermented soybean meal in calves diet, could significantly improve
growth performance, feed efficiency, crude protein, crude fat digestibility, and could decrease serum urea concentration as well as diarrhea score on drinking 6L milk and 4L milk daily. Milk and diet was the main factor could improve ADG, feed efficiency and nutrient digestibility in calves. This study ensured the higher average daily body weight gain in calves fed with fermented soybean meal (FSBM). The present study hypothesized that the growth-promoting effects of calves may be due the improvement of nutrition retention especially crude protein (CP) and ether extract (EE) in calves fed with FSBM. A similar growth promoting effects of fermented soybean meal in calves were noted by Kim et al. (2012) who observed the higher body weight gain and higher dry matter intakes, when compared with those fed non fermented soybean meal (SBM) in calves at 4 wk of age. In addition, growth–promoting effect was also found by Wang et al. (2014) who reported that pigs fed soybean meal fermented with *Streptococcus thermophilus* and *Saccharomyces cerevisiae* had a greater average daily body weight gain (ADG) and improved gain/feed ratio than the control diets. Furthermore, Kim et al. (2014) showed soybean fermented with *Aspergillus oryzae* increased growth performance of nursery pigs. Improved growth performance in broilers fed with fermented soybean meal was also reported by Chun et al. (2014).

Our results showed an increase in nutrient utilization particularly protein and energy in calves fed with fermented soybean was also related with improve feed efficiency in FSBM groups. Feng et al. (2007a) reported that fermentation may reduce the anti nutritional factors like raffinose and stachyose in soybean meal which lead improvement of nutrition retention in calves. We also hypothesized that fermentation may increase the availability of nutrients in experimental fed group. It is certain that some of the anti-nutritional factors were eliminated through microbial fermentation, and nutrients were supposed to be more digested. Fermentation of soybean meal using *Bacillus* spp. has increased digestibility of soy proteins as well (Kiers et al., 2003).

Serum urea nitrogen is a microbial product that is known to have a negative health status in birds and animals (Mafuz et al., 2020). In this report, calves fed with FSBM showed lower serum urea concentration than unfermented one which ensured the sound health status in experimental calves which was similar with previous report (Feng et al., 2007b).

In our study, the diarrhea score of newborn calves were reduced at the whole period, which was similar with Kim et al. (2010b) who reported that the incidence and severity of diarrhea of calves were significantly lower in the FSBM group compared to those in the SBM group at 21 days and 42 days of age. In addition, Kim et al. (2012) noted that calves fed the FSBM had significantly lower fecal scores than those fed the SBM during both pre and post weaning periods.

**CONCLUSION**

Calves fed with fermented soybean meal (FSBM) diet could improve growth performance via improved nutrient retention, decrease serum urea concentration and lowered the incidence of diarrhea. Further experiment can be conducted to evaluate the health status of calves fed with FSBM in commercial level.

**ACKNOWLEDGEMENTS**

The work was supported by National Key R&D Program of China (No. 2018YFD0500600).

**CONFLICT OF INTEREST**

The authors claim that they have no competing interests.

**AUTHORS CONTRIBUTION**

All authors contributed equally in the planning of the study, drafting the manuscript. All of them approved the final version of the article.

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