Kafkas Univ Vet Fak Derg
23 (3): 473-479, 2017
DOI: 10.9775/kvfd.2016.17111

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Effects of Flavonoids from Mulberry Leaves and *Candida tropicalis* on Performance and Nutrient Digestibility in Calves

Le-Ying ZHANG [1] Pei-Bin QU [1,2] Yan TU [2] Chun-Tao YANG [2] Qi-Yu DIAO [2]

[1] College of Agriculture, Hebei University of Engineering, Handan 056021, CHINA
[2] Key Laboratory of Feed Biotechnology, Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, CHINA

Article Code: KVFD-2016-17111 Received: 17.11.2016 Accepted: 12.01.2017 Published Online: 13.01.2017

Citation of This Article

Zhang LY, Qu PB, Tu Y, Yang CT, Diao QY: Effects of flavonoids from mulberry leaves and *Candida tropicalis* on performance and nutrient digestibility in calves. *Kafkas Univ Vet Fak Derg,* 23 (3): 473-479, 2017. DOI: 10.9775/kvfd.2016.17111

Abstract

Flavonoids from mulberry leave (FML) are natural flavonoids, and *Candida tropicalis* (CT) is yeast like microbe. In this study, forty-eight male calves were selected with born weight (40.5±0.7 kg) and 20±2 days age, divided into 4 groups randomly. FML was supplemented in a dairy calf starter at 2 g/d per calf before weaning, or 4 g/d per calf after weaning (FML group), while CT was added in a dairy calf starter at 1 g/d per calf (CT group). Our results showed that FML could be used to enhance body weight (BW) of calves through enhancing apparent digestibility of ether extract (EE) of the diet, and increased the levels of serum growth hormone (GH) and insulin-like growth factors 1 (IGF-1) in calves after the age of 56 days. CT enhanced the BW of calves before weaning through increasing the apparent digestibility of neutral detergent fiber (NDF) of the diet, and elevated the apparent digestibility of EE of the diet of calves after weaning through increasing the level of serum IGF-1 in calves. Furthermore, the mixture of FML and CT plays a synergistic role in enhancing growth, improving feed intake and nutriment digestibility. In conclusion, FML and CT could be used as additives to increase growth and nutrient digestibility in calves.

Keywords: *Calves, Flavonoids from mulberry leave, Candida tropicalis, Nutriment digestibility, Hormone level*

Dut Yaprağından Elde Edilen Flavonoidler İle *Candida tropicalis*'in Buzağılarda Performans ve Besin Sindirilebilirliği Üzerine Etkileri

Özet

Dut yaprağından elde edilen flavonoidler (FML) doğal flavonoidler olup *Candida tropicalis* (CT) de mantar benzeri mikroplardır. Bu çalışmada, doğumda 40.5±0.7 kg ağırlığa sahip 20±2 günlük kırk sekiz erkek buzağı kullanılarak rastgele 4 gruba ayrıldı. FML sütten kesme öncesi her bir buzağı için 2 g/d olarak buzağı başlangıç yemi içerisinde veya her bir buzağı için 4 g/d olarak sütten kesme sonrası (FML grup), CT ise her bir buzağı için 1 g/d olarak buzağı başlangıç yemi içerisinde (CT grup) verildi. Çalışma sonucunda FML'nin diyetin eter ekstraktının (EE) sindirilebilirliğini ve serum büyüme hormonu (GH) ve insülin benzeri büyüme faktörü 1 (IGF-1)'in seviyelerini belirgin bir şekilde arttırmak yoluyla 56 günlükten sonrasında buzağıların vücut ağırlığını (BW) geliştirmek amacıyla kullanılabileceği tespit edilmiştir. CT; buzağıların vücut ağırlığını sütten kesme öncesinde diyetteki nötr deterjan fiberin sindirilebilirliğini belirgin ölçüde geliştirdi ve serum IGF-1'in seviyesini yükseltmek suretiyle sütten kesme sonrasında diyetteki eter ekstraktının sindirilebilirliğini belirgin ölçüde artırdı. FML ve CT karışımı büyümeyi geliştirme, gıda tüketimini ve besin sindirilebilirliğini iyileştirmekte sinerjistik bir rol oynadı. Sonuç olarak, FML ve CT büyüme ve besin sindirilebilirliğini artırmak amacıyla buzağılarda bir katkı olarak kullanılabilir.

Anahtar sözcükler: *Buzağı, Dut Yaprağı Flavonoidleri, Candida tropicalis, Besin sindirilebilirliği, Hormon seviyesi*

INTRODUCTION

It is well documented that there are a number of potential risks for human health in using antibiotics in food-producing animals, including drug residues in meat products, increasing bacterial resistance and environmental contamination [1]. Flavonoids are found in berries, tea, cocoa, soybeans, grains, and plant leaves, are a class of organic polyphenolic compounds [2]. It is through various mechanisms including protection against oxidative stress, and preservation of epithelial barrier function and immunomodulatory properties that flavonoids are used in acute or chronic intestinal inflammation [3]. Dietary flavonoids (quercetin and morin) have marked effects on the fatty

İletişim (Correspondence)
+86 10 82106055
diaoqiyu@caas.cn

acid composition of blood plasma, liver, or breast muscle lipids in vitamin E-deficient chicks [4]. Condensed tannins (a polyflavonoid) can mitigate methane emission by feeding *Leucaena leucocephala* in sheep [5]. It is effective in improving rumen fermentation and reducing the incidence of rumen acidosis through supplementation of natural flavonoids extract from bitter orange and grapefruit [6]. Sainfoin which contains rich condensed tannins can increase beneficial fatty acids and reduce skatole content in lamb meat [7]. Flavonoids from mulberry leaves (FML) have positive effects on the hypoglycaemic, antihypercholesterolemic and anti-oxidative potential in rats [8].

Probiotics are live microorganisms including bacteria and yeasts, which can improve intestinal health and immune response, prevent acute and antibiotic-associated diarrhea [9]. It is reported that GSY10 is the most promising oleaginous yeast for microbial lipid production from molasses, and it can be used as feed supplement for microbial lipid production in dairy cattle [10]. The yeast culture extract can activate natural killer (NK) cells and B lymphocytes *in vitro*, which plays a role in the anti-inflammatory effects [11]. Yeast-based immunogen (EpiCor) possesses conspicuous anti-inflammatory activity, and can directly induce activation of chemotactic awareness of lymphocyte subsets *in vitro* [12]. After experimental challenged with *Salmonella*, pre-weaned dairy cows fed with *Saccharomyces cerevisiae* fermentation products have fewer bouts of diarrhea and fever, more beneficial microbe in rumens and higher weight gain comparing with no-fed group [13]. *Lactic acid bacteria* or *Bacillus* species generally target the lower intestine to stabilize the gut microbiota, which decreases the risk of pathogen colonization in young ruminants [14]. As a fungal organism, *Candida tropicalis* (CT) can grow as yeast morphology [15]. It is known that the somatotropic axis primarily consists of growth hormone (GH), insulin-like growth factors (IGF), as well as their associated carrier proteins and receptors, which plays a key role in the control of the protein anabolism, fat deposition, and growth rate in animals [16]. It was hypothesized that supplementation of FML and CT in starter of calves might improve consumption of nutriment, accelerate animal growth, and feed intake. Thereby, the aim of current study was to determine the effects of supplementation FML and CT in the starter on growth, performance and the concentrations of GH and IGF-1 in plasma during the first 80 days of age in calves.

MATERIALS and METHODS

Materials

The extract of FML was purchased from Xi'an Feida Biotechnology Co. Ltd., and there were 50 mg of FML per g of extract, which was analyzed by the manufacturer. CT was from Beijing Vano Biological Engineering Co., Ltd., and the concentration of live bacteria was 5×10^9 CFU/g, which was provided by the manufacturer. The milk replacer is provided by Beijing Jingzhun Animal Nutrition Center, and the starter is from Beijing Sanyuan Luhe Feed Factory. The basal diet consists of milk replacer and starter with no antibiotics and microbial preparation. The ingredients and nutrient levels of basal diet are shown in *Table 1*.

Animals and Experimental Design

Holstein male calves were managed on the first farm of Western Suburbs, Beijing Sanyuan Luhe Cow Breeding Center. The experimental protocol was approved by the Chinese Academy of Agricultural Sciences Animal Ethical Committee, and humane animal care and handling procedures were followed throughout the experiment. Forty-eight male calves with 20±2 days old, and 40.5±0.7 kg birth weight were selected from cows with natural childbirth and between 3 and 5 years old. The calves were fed adequate colostrum during the first 3 days and then fed milk replacer until 80 days of age. Calves randomly were divided into 4 groups (n=12) based on parity and birth weight. The control group (Ctrl group) was fed with basal diet, while the other three groups were added with FML (FML group, 2 g/d per calf before weaning, or 4 g/d per calf after weaning), with CT (CT group, 1 g/d per calf) and with the above two additives (FML + CT group, FML

Table 1. *Nutrient composition and levels of basal diet (air-dry basis) %*

Items	Starter	Milk Replacer
Corn	20.0	-
Extrude corn	22.9	
Soybean meal	20.0	
Extruded soybean	18.0	
Whey powder	5.00	
Wheat bran	10.0	
$CaHPO_4$	0.800	
Limestone	1.80	
Salt	0.500	
Premix*	1.00	
Total	100	
Nutrient levels		
DM (Dry matter)	85.4	95.4
OM (Organic matter)	92.2	94.9
CP (Crude protein)	19.1	24.3
EE (Ether extract)	2.21	12.9
NDF (Neutral detergent fiber)	18.6	4.02
ADF (Acid detergent fiber)	10.7	2.11
Ca	1.09	1.07
P	0.473	0.482
GE (Gross energy) MJ/kg	15.5	19.9

** Premix supplemented with VA 15.000 IU, VD 5.000 IU, VE 50 mg, Fe 90 mg, Cu 12.5 mg, Mn 30 mg, Zn 90 mg, Se 0.3 mg, I 1.0 mg and Co 0.5 mg for per kg starter*

2 g/d + CT 1 g/d per calf before weaning, or FML 4 g/d + CT 1 g/d per calf after weaning). The experiment began at 21 days old and lasting 60 days. At the age of 55 days old, calves were weaned. The diet for calves contained milk replacer and starter. Milk replacer was offered daily at 10% of body weight (BW) (adjusted weekly) and starter was offered *ad libitum* throughout the 60 days trial period. Each calf was housed in an individual hutch during the whole experiment period except the metabolic study period during which each calf was raised in an individual metabolic cage. There are two metabolic trials in the whole experiment period. One trial began at the age of 43 days lasting 5 days (preweaning), the other began at the age of 60 days lasting 5 days too (postweaning). BW was measured at 21, 28, 42, 56 and 80 days old during the whole trial period, and starter intake was recorded daily.

Sample Collection and Analysis

Every three calves that were from the correspond group, and reached the average BW were selected for the metabolic trial. Urinary and fecal excretions of every calf (4 groups in all, one group having 3 calves) were entirely collected daily for analyzing the apparent digestibility of dry matter (DM), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF). The mixed sample was from 10% of total amount of feces, and 10 g mixed fecal sample was treated with 10 mL of 10% dilute hydrochloric acid for nitrogen fixation. Then a 500-g feces sample was taken, dried at 103°C for 48 h, ground in a Cyclotec 1093 mill (Tecator, Sweden). The digestion rate was calculated as previously described [17]. The blood samples (jugular venipuncture) were collected before the morning feeding at 28, 42, 56 and 80 days old, respectively. Plasma samples were stored at -19°C after centrifugation (3000×g, for 15 min, at 25°C) for analysis of GH and IGF-1 by radioimmunoassay (RIA) as previously described [18]. IGF-1 antibody (sc-1422, Santa Cruz Biotech, CA) was used to analyze the IGF-1 concentration, and the GH concentration was determined using an antibody (sc-10365, Santa Cruz Biotech, CA).

Statistical Analysis

The experimental design was a randomized complete block design. Continuous variables were analyzed by ANOVA using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, 2003). The model included fixed effect and the random effect. Treatments, days (as a repeated effect), and their interaction were as the fixed effects, and the calf was as the random effects. Restricted Maximum Likelihood was used to estimate least square mean values. Where treatment effects were significant the means were analyzed using Tukey's procedure for multiple comparisons. The initial BWs were modeled as a Covariate to further control the experimental error. Differences were considered statistically significant at the 95% confidence level ($P<0.05$).

RESULTS

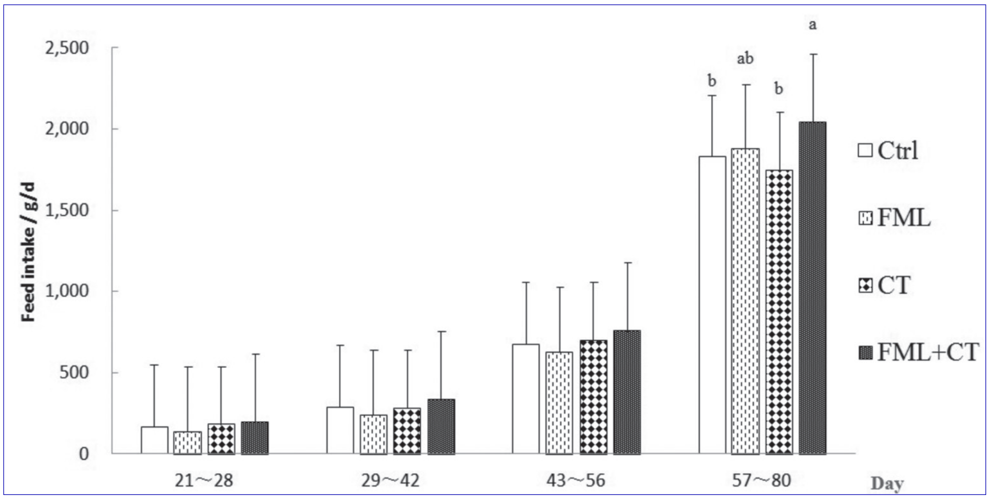
The Effect of FML on Performance and Plasma Hormone Level

It was shown in *Fig. 2* that the BW of FML group was significantly higher ($P<0.05$) than that of Ctrl group after weaning, but there was no significant effect of FML on BW before weaning. It was observed in *Fig. 1* that there was no significant effect of FML on starter intake throughout the experiment in calves ($P<0.05$). Meanwhile significant changes ($P<0.05$) were observed between FML and Ctrl group in the levels of plasma GH and IGF-1 at the age of 80 days *(Table 3)*, and the digestibility of EE in FML group was significantly higher ($P<0.05$) than that in Ctrl group after weaning *(Table 2)*.

The Effect of CT on Performance and Plasma Hormone Level

The diet supplementation with CT did not affect starter intake ($P>0.05$) throughout the experiment in calves *(Fig. 1)*, but the BW *(Fig. 2)* of calves of CT group was significantly higher than that of Ctrl group only at the age of 56 days ($P<0.05$), while the *apparent digestibility* of NDF

Fig 1. Effect of flavonoids from mulberry leaves and *Candida tropicalis* on feed intake. Ctrl, no additive; FML, FML 2 g/d per calf before weaning, or FML 4 g/d per calf after weaning; CT, *Candida tropicalis* (1 g/d per calf); FML+CT, FML (FML 2 g/d per calf before weaning, or FML 4 g/d per calf after weaning) + *Candida tropicalis* (1 g/d per calf). Within the same row with different superscripts indicated significant differences ($P<0.05$)



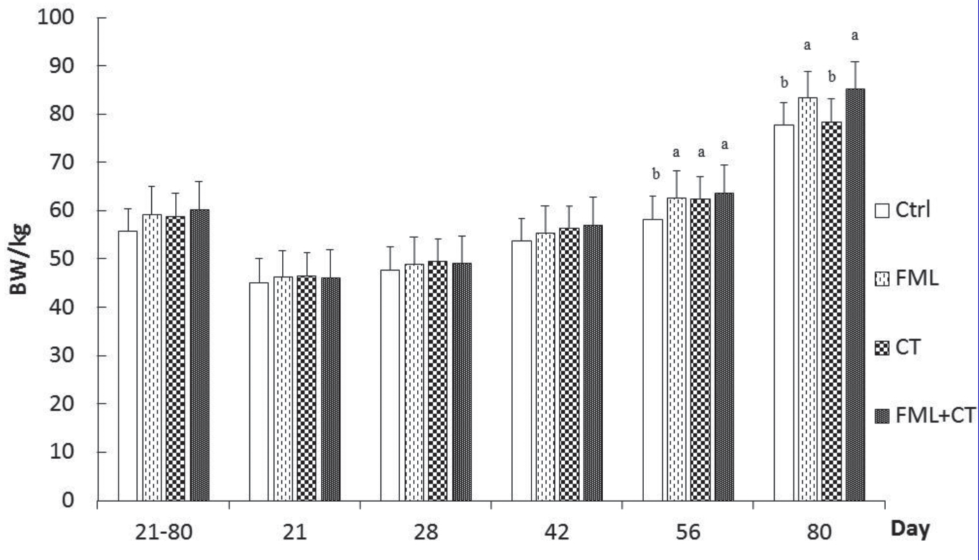


Fig 2. Effect of flavonoids from mulberry leaves and *Candida tropicalis* on body weight. Ctrl, no additive; FML, FML 2 g/d per calf before weaning, or FML 4 g/d per calf after weaning; CT, *Candida tropicalis* (1 g/d per calf); FML + CT, FML (FML 2 g/d per calf before weaning, or FML 4 g/d per calf after weaning) + *Candida tropicalis* (1 g/d per calf). Within the same row with different superscripts indicated significant differences (*P*<0.05)

Table 2. *Effect of flavonoids from mulberry leaves and Candida tropicalis on nutrient digestibility*

Items	Treatment				SEM	*P*-value
	Ctrl	FML	CT	FML+CT		
Pre-weaning male calves						
Dry matter	79.3	79.2	79.0	80.1	1.09	0.976
Organic matter	81.3	80.5	80.6	81.5	1.07	0.883
Ether extract	79.3	75.8	79.1	69.5	2.55	0.905
Neutral detergent fiber	19.2^{b}	21.3^{b}	25.7^{a}	19.8^{b}	0.983	0.163
Acid detergent fiber	27.4	29.6	27.7	29. 4	1.62	0.0994
Post-weaning male calves						
Dry matter	79.3	84.7	81.6	84.9	1.52	0.342
Organic matter	81.3	86.4	82.2	86.9	1.34	0.328
Ether extract	33.7^{b}	53.5^{a}	46.3^{a}	62.7^{a}	3.16	0.0317
Neutral detergent fiber	49.3^{b}	57.5^{ab}	48.3^{b}	60.7^{a}	2.03	0.0287
Acid detergent fiber	63.7^{b}	66.3^{ab}	65.4^{b}	72.5^{a}	1.36	0.0322

Ctrl, no additive; FML, FML 2 g/d per calf before weaning, or FML 4 g/d per calf after weaning; CT, Candida tropicalis (1 g/d per calf); FML+CT, FML (FML 2 g/d per calf before weaning, or FML 4 g/d per calf after weaning) + Candida tropicalis (1 g/d per calf). Within the same row with different superscripts indicated significant differences (P<0.05)

of CT group was significantly higher than that of Ctrl group before weaning (*P*<0.05). The digestibility of EE in CT group was significantly higher (*P*<0.05) than that in Ctrl group after weaning *(Table 2)*, while the level of plasma IGF-1 in CT group was obviously higher (*P*<0.05) than that in Ctrl group post weaning *(Table 3)*.

The Effect of FML + CT on Performance and Plasma Hormone Level

The results of current study *(Fig. 1, Fig. 2)* indicated that BW and starter feed in FML + CT group were significantly higher (*P*<0.05) than that in Ctrl group at the age of 57-80 days. There was no significant change in BW between FML + CT and Ctrl group at the age of 21-42 days (*P*>0.05). The levels of plasma GH and IGF-1 in FML + CT group were significantly higher (*P*<0.05) than that in Ctrl group at the age of 80 days *(Table 3)*. The diet supplementation with FML+CT simultaneously enhanced digestibility of EE, NDF and *ADF* (*P*<0.05) significantly compared with that no supplementation after weaning in calves *(Table 2)*.

DISCUSSION

The use of antibiotics for growth promotion has been totally banned in many countries, owing to drug residues in meat products and increasing bacterial resistance by use and misuse of in-feed antibiotics in food-producing animals [19], so utilization of phytogenic compounds in feed for food animal production has good potential [20]. It is fed flavonoids extracted from propolis that calves have

Table 3. Effect of flavonoids from mulberry leaves and Candida tropicalis on GH and IGF-1

Items	Treatment				SEM	P-value		
	Ctrl	FML	CT	FML+CT		Treatment	age	Treatment × Age
GH (ng/mL)								
28-80	3.21	3.31	3.33	3.39	0.0526	0.613	0.00157	0.169
28	3.28	2.95	3.02	3.09	0.0618	0.645		
42	3.23	3.18	3.25	3.16	0.0578	0.463	-	-
56	3.25	3.43	3.67	3.71	0.293	0.0681		
80	3.23^{b}	3.72^{a}	3.31^{ab}	3.75^{a}	0.181	0.0357		
IGF-1 (ng/mL)								
28-80	162	225	211	221	9.35	0.0452	0.0132	0.246
28	173	171	206	189	13.2	0.383		
42	167	195	194	158	13.5	0.336		
56	159	215	209	214	19. 5	0.0543	-	-
80	177^{b}	295^{a}	277^{a}	294^{a}	20. 60	0.00219		

Ctrl, no additive; FML, FML 2 g/d per calf before weaning, or FML 4 g/d per calf after weaning; CT, Candida tropicalis (1 g/d per calf); FML+CT, FML (FML 2 g/d per calf before weaning, or FML 4 g/d per calf after weaning) + Candida tropicalis (1 g/d per calf). Within the same row with different superscripts indicated significant differences (P<0.05)

higher BW than those fed no flavonoids until 120 days of age [21]. Results of our present study revealed that calves supplement with FML in the diet have higher BW than those fed no flavonoids after the age of 56 days, without significant difference in feed intake comparing to those fed no flavonoids, which is consistent with the previous reports. Flavonoids have beneficial effects on urinary tract infections, cognitive function and age-related cognitive decline, cancer and cardiovascular disease in human [22], and flavonoids and their metabolites modulated the expression and activity of several metabolic key enzymes, and are involved in regulation of lipid and carbohydrate metabolism [23]. Therefore, the higher BW in FML group may be due to its beneficial effects on several metabolic key enzymes.

Our results showed that the *apparent digestibility* of ADF and NDF was not affected by FML. It was also reported that feeding quebracho tannin extract, a diverse group of polymeric flavonoids, had no effect on ADF and NDF digestibility in Angus heifers [24], which was consistent with our results. However, there was significant effect on the EE of the diet of calves by supplementation with FML comparing Ctrl group after weaning in this study. It was reported that condensed tannins altered ruminal biohydrogenation process of unsaturated fatty acids [25], and the greater digestibility of EE in the FML group than that in Ctrl group may be due to the antioxidant capacities of FML after weaning.

Our results also indicated that there were higher levels of serum GH and IGF-1 in FML group comparing with that in Ctrl group at the age of 80 days. The binding of Genistein to estrogen receptors in the hypothalamus influences the production of GH and growth factors (GF), which lead to increasing the uterine weight, uterine wall thickness and ovarian weight in Sprague Dawley rats [26]. It is reported that flavanone 8-prenylnaringenin, as a phytoestrogen, increases serum GH, but decreases serum IGF-1 levels in rats [27], and IGF-1 has negative effect on GH gene expression in somatotroph cell line [28]. However, GH can strongly stimulate production of IGF-1 *in vivo*. Many tissues and cells can produce IGF-1, and IGF-1 is mainly secreted by the liver under the control of GH, meanwhile have effects on growth and development mediated partly by the effects of GH [29]. Our results may suggest that FML promotes the growth and development of calves by increasing the levels of serum GH and IGF-1. Therefore, FML enhanced apparent digestibility of EE of the diet, and increased the levels of serum GH and IGF-1 in calves, which led to the increased BW after the age of 56 days by supplemented with FML.

It is reported that yeast culture can enhance crude protein and cell wall digestibility, ruminal molar proportion of propionate and plasma glucose concentration in Baluchi lambs [30], and Jersey calves feed live yeast product have greater final BW at 63 days than calves fed none [31]. Our results indicated that there was significant effect on BW of calves supplemented with CT comparing with that no CT only at the age of 56 days, with no significantly difference in the feed intake during total experimental stage. Meanwhile the apparent digestibility of NDF of CT group was significantly higher than that of Ctrl group before weaning. Lesmeister *et al.*[32] reported that Holstein calves fed 2% yeast culture had greater BW at 42 days of age than calves with receiving no yeast. There are improvements in grain intake, BW gain, and blood parameters of calves when fed live yeast only during the pre-weaning period [33]. The calves fed *Saccharomyces cerevisiae* have greater BW during the pre-weaning period, because yeast can improve growth and activity of fiber-degrading bacteria and fungi, stabilize rumen pH, prevent lactate accumulation, improve ruminal microbial colonization, and set up fermentative processes [34], which is consistent with our study. Our study suggested that CT enhanced the BW of calves before weaning through manipulating rumen fermentation and increasing the apparent digestibility of NDF of the diet.

Furthermore, the results of present study showed that the apparent digestibility of EE in CT group was significantly higher than that in Ctrl group after weaning, and CT have significant effect on the level of serum IGF-1 comparing with Ctrl group in post-weaning male calves. It is supplemented with *Lactobacillus plantarum* that serum IGF-1 can return to pre-challenge values by day 13 post-challenge orally with *Salmonella* in pigs [35]. It is treated with *Lactobacillus rhamnosus* that Zebrafish exhibits a high gene expression level for IGF-1 comparing to untreated group at 6 days post fertilization [36]. Therefore, CT enhanced the apparent digestibility of EE of the diet in calves after weaning through increasing the level of IGF-1 and manipulating rumen fermentation.

Results of the current experiment showed that mixture of FML and CT plays a synergistic role in enhancing growth, improving feed intake and nutriment *digestibility*. It achieved the best effect among four treatments through simultaneously supplementation the two additives (FML + CT) to the starter of calves. BW, starter feed and the levels of plasma GH and IGF-1 were observed significantly higher ($P<0.05$) in FML + CT group compared with Ctrl group at the age of 80 days. Furthermore, the *digestibility of* EE, NDF and ADF in FML + CT group was significantly higher ($P<0.05$) than that in Ctrl group after weaning.

In conclusion, FML could be used to enhance BW of calves through enhancing apparent digestibility of EE of the diet, and increased the level of serum GH and IGF-1 in calves after the age of 56 days. CT enhanced the BW of calves before weaning through increasing the apparent digestibility of NDF of the diet, and elevated the apparent digestibility of EE of the diet of calves after weaning through increasing the level of serum IGF-1 in calves. Furthermore, the mixture of FML and CT plays a synergistic role in enhancing growth, improving feed intake and nutriment *digestibility*.

Acknowledgments

This work was supported by National Dairy Industry Technology System-Beijing Innovation Team, National Science and Technology Support Program, China (2012BAD12B06), and Science and Technology R&D Project of Handan City, China (1522101042-2).

REFERENCES

1. Redding LE, Cubas-Delgado F, Sammel MD, Smith G, Galligan DT, Levy MZ, Hennessy S: The use of antibiotics on small dairy farms in rural Peru. *Prev Vet Med,* 113, 88-95, 2014. DOI: 10.1016/j.prevetmed.2013.10.012

2. Bell L, Lamport DJ, Butler LT, Williams CM: A review of the cognitive effects observed in humans following acute supplementation with flavonoids, and their associated mechanisms of action. *Nutrients,* 7, 10290-10306, 2015. DOI: 10.3390/nu7125538

3. Vezza T, Rodríguez-Nogales A, Algieri F, Utrilla MP, Rodriguez-Cabezas ME, Galvez J: Flavonoids in inflammatory bowel disease: A review. *Nutrients,* 8, 211, 2016. DOI: 10.3390/nu8040211

4. Jenkins KJ, Atwal AS: Flavonoids increase tissue essential fatty acids in vitamin E-deficient chicks. *J Nutr Biochem,* 6, 97-103, 1995. DOI: 10.1016/0955-2863(94)00018-H

5. Soltan YA, Morsy AS, Sallam SM, Lucas RC, Louvandini H, Kreuzer M, Abdalla AL: Contribution of condensed tannins and mimosine to the methane mitigation caused by feeding *Leucaena leucocephala*. *Arch Anim Nutr,* 67, 169-184, 2013. *Arch Anim Nutr,* 2013. DOI: 10.1080/1745039X.2013.801139

6. Balcells J, Aris A, Serrano A, Seradj AR, Crespo J, Devant M: Effects of an extract of plant flavonoids (Bioflavex) on rumen fermentation and performance in heifers fed high-concentrate diets. *J Anim Sci,* 90, 4975-4984, 2012. DOI: 10.2527/jas.2011-4955

7. Girard M, Dohme-Meier F, Silacci P, Ampuero Kragten S, Kreuzer M, Bee G: Forage legumes rich in condensed tannins may increase n-3 fatty acid levels and sensory quality of lamb meat. *J Sci Food Agric,* 96,1923-1933. 2016. DOI: 10.1002/jsfa.7298

8. El-Sayyad HI, El-Sherbiny MA, Sobh MA, Abou-El-Naga AM, Ibrahim MA, Mousa SA: Protective effects of *Morus alba* leaves extract on ocular functions of pups from diabetic and hypercholesterolemic mother rats. *Int J Biol Sci,* 7, 715-728, 2011. DOI: 10.7150/ijbs.7.715

9. Wong A, Ngu DY, Dan LA, Ooi A, Lim RL: Detection of antibiotic resistance in probiotics of dietary supplements. *Nutr J,* 14, 95, 2015. DOI: 10.1186/s12937-015-0084-2

10. Paserakung A, Pattarajinda V, Vichitphan K, Froetschel MA: Selection and identification of oleaginous yeast isolated from soil, animal feed and ruminal fluid for use as feed supplement in dairy cattle. *Lett Appl Microbiol,* 61, 325-332, 2015. DOI: 10.1111/lam.12475

11. Jensen GS, Patterson KM, Yoon I. Yeast culture has anti-inflammatory effects and specifically activates NK cells. *Comp Immunol Microbiol Infect Dis,* 31, 487-500, 2008. DOI: 10.1016/j.cimid.2007.08.005

12. Jensen GS, Hart AN, Schauss AG: An antiinflammatory immunogen from yeast culture induces activation and alters chemokine receptor expression on human natural killer cells and B lymphocytes *in vitro*. *Nutr Res,* 27, 327-335, 2007. DOI: 10.1016/j.nutres.2007.04.008

13. Brewer MT, Anderson KL, Yoon I, Scott MF, Carlson SA: Amelioration of salmonellosis in pre-weaned dairy calves fed *Saccharomyces cerevisiae* fermentation products in feed and milk replacer. *Vet Microbiol,* 172, 248-255, 2014. DOI: 10.1016/j.vetmic.2014.05.026

14. Uyeno Y, Shigemori S, Shimosato T: Effect of probiotics/prebiotics on cattle health and productivity. *Microbes Environ,* 30, 126-132, 2015. DOI: 10.1264/jsme2.ME14176

15. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J: *Candida glabrata, Candida parapsilosis* and *Candida tropicalis*: Biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiol Rev,* 36, 288-305, 2012. DOI: 10.1111/j.1574-6976.2011.00278.x

16. Renaville R, Hammadi M, Portetelle D: Role of the somatotropic axis in the mammalian metabolism. *Domest Anim Endocrinol,* 23, 351-360, 2002. DOI: 10.1016/S0739-7240(02)00170-4

17. Kammes KL, Allen MS: Nutrient demand interacts with grass particle length to affect digestion responses and chewing activity in dairy cows. *J Dairy Sci,* 95, 807-823, 2012. DOI: 10.3168/jds.2011-4588

18. Mejía M, Gonzalez-Iglesias A, Díaz-Torga GS, Villafañe P, Formía N, Libertun C, Becú-Villalobos D, Lacau-Mengido IM: Effects of continuous ivermectin treatment from birth to puberty on growth and reproduction in dairy heifers. *J Anim Sci,* 77, 1329-1334, 1999. DOI: 10.2527/1999.7761329x

19. Casewell M, Friis C, Marco E, McMullin P, Phillips I: The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J Antimicrob Chemother,* 52, 159-161, 2003. DOI: 10.1093/jac/dkg313

20. Yang C, Chowdhury MA, Huo Y, Gong J: Phytogenic compounds as alternatives to in-feed antibiotics: Potentials and challenges in application. *Pathogens* 4, 137-56, 2015. DOI: 10.3390/pathogens4010137

21. Yaghoubi SM, Ghorbani GR, Rahmani HR, Nikkhah A: Growth, weaning performance and blood indicators of humoral immunity in Holstein calves fed supplemental flavonoids. *J Anim Physiol Anim Nutr (Berl),* 92, 456-462, 2008. DOI: 10.1111/j.1439-0396.2007.00734.x

22. Rodriguez-Mateos A, Vauzour D, Krueger CG, Shanmuganayagam D, Reed J, Calani L, Mena P, Del Rio D, Crozier A: Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: An update. *Arch Toxicol,* 88, 1803-1853, 2014. DOI: 10.1007/s00204-014-1330-7

23. Kobayashi Y, Miyazawa M, Kamei A, Abe K, Kojima T: Ameliorative effects of mulberry *(Morus alba* L.) leaves on hyperlipidemia in rats fed a high-fat diet: Induction of fatty acid oxidation, inhibition of lipogenesis, and suppression of oxidative stress. *Biosci Biotechnol Biochem,* 74, 2385-2395, 2010. DOI: 10.1271/bbb.100392

24. Beauchemin KA, McGinn SM, Martinez TF, McAllister TA: Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. *J Anim Sci,* 85, 1990-1996, 2007. DOI: 10.2527/jas.2006-686

25. Benchaar C, Chouinard PY: Short communication: Assessment of the potential of cinnamaldehyde, condensed tannins, and saponins to modify milk fatty acid composition of dairy cows. *J Dairy Sci,* 92, 3392-3396. 2009. DOI: 10.3168/jds.2009-2111

26. Zin SR, Omar SZ, Khan NL, Musameh NI, Das S, Kassim NM: Effects of the phytoestrogen genistein on the development of the reproductive system of Sprague Dawley rats. *Clinics (Sao Paulo),* 68, 253-262, 2013. DOI: 10.6061/clinics/2013(02)OA21

27. Böttner M, Christoffel J, Wuttke W: Effects of long-term treatment with 8-prenylnaringenin and oral estradiol on the GH-IGF-1 axis and lipid metabolism in rats. *J Endocrinol,* 198, 395-401, 2008. DOI: 10.1677/JOE08-0127

28. Romero CJ, Pine-Twaddell E, Sima DI, Miller RS, He L, Wondisford F, Radovick S: Insulin-like growth factor 1 mediates negative feed-back to somatotroph GH expression via POU1F1/CREB binding protein interactions. *Mol Cell Biol,* 32, 4258-4269, 2012. DOI: 10.1128/MCB.00171-12

29. Zhou Y, Capuco AV, Jiang H: Involvement of connective tissue growth factor (CTGF) in insulin-like growth factor-I (IGF1) stimulation of proliferation of a bovine mammary epithelial cell line. *Domest Anim Endocrinol,* 35, 180-189, 2008. DOI: 10.1016/j.domaniend.2008.05.003

30. Malekkhahi M, Tahmasbi AM, Naserian AA, Danesh Mesgaran M, Kleen JL, Parand AA: Effects of essential oils, yeast culture and malate on rumen fermentation, blood metabolites, growth performance and nutrient digestibility of Baluchi lambs fed high-concentrate diets. *J Anim Physiol Anim Nutr (Berl),* 99, 221-229, 2015. DOI: 10.1111/jpn.12230

31. Hill SR, Hopkins BA, Davidson S, Bolt SM, Diaz DE, Brownie C, Brown T, Huntington GB, Whitlow LW: The addition of cottonseed hulls to the starter and supplementation of live yeast or mannanoligosaccharide in the milk for young calves. *J Dairy Sci,* 92, 790-798, 2009. DOI: 10.3168/jds.2008-1320

32. Lesmeister KE, Heinrichs AJ, Gabler MT: Effects of supplemental yeast *(Saccharomyces cerevisiae)* culture on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. *J Dairy Sci,* 87, 1832-1839, 2004. DOI: 10.3168/jds.S0022-0302(04)73340-8

33. Galvão KN, Santos JE, Coscioni A, Villaseñor M, Sischo WM, Berge AC: Effect of feeding live yeast products to calves with failure of passive transfer on performance and patterns of antibiotic resistance in fecal *Escherichia coli*. *Reprod Nutr Dev,* 45, 427-440, 2005. DOI: 10.1051/rnd:2005040

34. Fonty G, Chaucheyras-Durand F: Effects and modes of action of live yeasts in the rumen. *Biologia,* 61, 741-750, 2006. DOI: 10.2478/s11756-006-0151-4

35. Gebru E, Lee JS, Son JC, Yang SY, Shin SA, Kim B, Kim MK, Park SC: Effect of probiotic-, bacteriophage-, or organic acid-supplemented feeds or fermented soybean meal on the growth performance, acute-phase response, and bacterial shedding of grower pigs challenged with *Salmonella enterica* serotype Typhimurium. *J Anim Sci,* 88, 3880-3886, 2010. DOI: 10.2527/jas.2010-2939

36. Avella MA, Place A, Du SJ, Williams E, Silvi S, Zohar Y, Carnevali O: *Lactobacillus rhamnosus* accelerates zebrafish backbone calcification and gonadal differentiation through effects on the GnRH and IGF systems. *PlosOne,* 7, e45572, 2012. DOI: 10.1371/journal.pone.0045572