Title

Performance of preweaned Holstein calves given 2 phytogenic feed supplements in their starter grain

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Performance of prewean Holstein calves given 2 phytogenic feed supplements in their starter grain

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ABSTRACT

Objective: Determine if prewean steer and heifer calves given two phytogenic starter supplements containing caraway, licorice, oak bark and vanilla which is soluble in milk (**PM**) or phytogenic supplement containing caraway, licorice, oak bark and vanilla (**PC**) or no phytogenic supplement, control (**C**), at a commercial calf ranch increases starter intake, weight gain or rumen development.

Materials and Methods: Holstein calves (124) were randomly assigned to 1 of 3 treatments, C, PM or PC, at 2 d of age. Supplements PM or PC were added to individual feed buckets at each feeding at the rate of 0.25g / kg starter at AM and PM feedings. Calves were weighed at enrollment and at weaning, and blood samples were collected from a subset of 13 calves per treatment to assess rumen development. Weekly average DMI, milk intake, Glu and BHB were analyzed using the Mixed procedure of SAS (v. 9.4)

Results and Discussion: Weekly average DMI ($P < 0.01$), BHB ($P < 0.01$) and Glu ($P < 0.01$) were different by week but not by treatment. Product PC group was numerically greater in total DMI, gain, ADG and had faster rumen development indicated by overall higher BHB values but were not different among treatments.

Implications and Applications: Addition of PM or PC to starter feed did not increase DMI, rumen development or body weight gain. But, the dose may have been too small or not fed long enough to elicit a response during the pre wean period.

KEYWORDS: bioactive compound, rumen development, calf BHB, calf ultrasound

INTRODUCTION

Phytogenic feed additives are bioactive compounds that are derived from secondary metabolism of plants. Examples sources of phytogenic compounds are spices, roots, peels, tree bark including essential oils from thyme, clove, licorice, caraway, oregano, cinnamon, vanilla, eucalyptus, oak bark and celery. Their activities have been associated with antimicrobial, antifungal, antiprotozoal, antiviral, antioxidative, anti-inflammatory functions resulting in improved health, rumen function, digestibility, palatability, and increased growth, intake, and reductions in methane production (Yang et al., 2015; Oh et al., 2017; Kholif et al., 2020). Because of these activities, interest in the use of phytogenic additives, alone or in combination, as growth promotants, stimulators of intake, rumen modifiers and replacements for antibiotics in dairy calf milk replacers or starter rations has increased.

Phytogenic additives have exhibited antimicrobial activity by changing bacterial, protozoal or yeast cell morphology through damaging cell walls, disrupting cell membranes and causing cell contents to leak (Calsamiglia et al., 2007; Oh and Hristov, 2016; Oh et al., 2017). In ruminants, phytogenic additives have shifted the microbial population and proportions of volatile fatty acids towards greater propionate and less methane production in the rumen (Neubauer et al., 2018). In the lower intestine, they can prevent oxidative stress and inflammation which is associated with changes in diet at weaning and exposure to gut pathogens to preserve gut mucosal activity, absorption and maintain feed intake and growth (Oh, et al., 2017). Phytogenic additives can increase palatability and therefore intake and growth because of their odors and flavors. Their antioxidant content can preserve feed quality and prevent undesirable odors (Yang et al., 2015;

Kholif et al., 2020). They have also been shown to interact with gut receptors to stimulate digestive secretions thereby increasing digestibility (Oh et al., 2013).

Despite the wide variety of activities attributed to phytogenic feed additives, results from feeding studies in cattle and calves have been mixed. Possible reasons why they may not be beneficial are that their activity is dependent on concentration and high concentrations may negatively affect palatability (Kholif et al., 2020). Other feed components or feed processing may interact with volatile phytogenic components and cause evaporation or alter phytogenic activity. Animals at different ages, health status, and production levels may already be functioning optimally and not impacted by phytogenic feed components (Yang et al., 2015). While phytogenic feed additives may have beneficial effects in vitro, their efficacy needs to be explored in vivo in commercial dairy settings. The objective of this study is to determine if pre wean steer and heifer calves given two phytogenic starter supplements, phytogenic supplement containing caraway, licorice, oak bark and vanilla which is soluble in milk (**PM**) or phytogenic supplement containing caraway, licorice, oak bark and vanilla (**PC**) or no phytogenic supplement, control (**C**), at a commercial calf ranch increases starter intake or weight gain, reduces diarrhea, or affects blood markers of metabolism and rumen development.

MATERIALS AND METHODS

All procedures involving animals were approved by the Animal Care and Use Committee of the University of California, Davis.

Animals and Experimental Design

One hundred and twenty four Holstein calves at 2 d of age were enrolled over three days in a completely randomized repeated measures design on a commercial calf ranch in Tulare, CA. All calves were from the same source. At enrollment, calves were weighed and loaded into individual wooden hutches with shade. Each calf was randomly assigned to one of three treatments: Control , PM or PC supplements fed at the rate of 0.25 g/kg starter as per manufacturer instructions (Biomin America Inc., Overland Park, KS). Even though the same phytogenic compounds were in PM and PC, they were both included in the study as different treatments to determine if PM results were different from PC when fed instead of mixed in milk.

Hutches were marked with a grease paint stick indicating color associated with treatment. Starter and water buckets were strategically placed to prevent calves from eating out of another feed bucket. Of the 124 calves enrolled, 120 calves (44 heifers and 76 steers) finished the trial with 33 bulls and 7 heifers for Control, 24 bulls and 16 heifers for PM and 20 bulls and 20 heifers for PC. Two calves died of pneumonia, 1 calf from bloat and 1 calf from mycoplasma pneumonia.

Feed and Milk Intake

Calves were fed a pelleted starter mix twice daily (Table 1) and had ad libitum access to water. Both starter and water buckets were cleaned daily and replaced if contaminated with feces.

Starter feed samples were collected daily and 100 g of each daily feed sample were pooled by week and sent to Analab (Fulton, Il) for analyses of DM, ADF, NDF, CP, fat, ash, and lignin using wet chemistry analyses (American Association for Analytical Chemists reference methods 935.29, 973.18, 2002.04, 990.03, 920.39, 942.05, 973.18, respectively), starch using NIRS (based on predictive equations developed at Analab), and mineral analyses (Ca, P, Mg, K, S, Na, Cl, Fe, Cu, Mn, Zn) using Inductively Coupled Plasma-Mass Spectrophotometry (American Association for Analytical Chemists reference methods 985.01 for Ca, P, Mg, K, Na, Fe, Cu, Mn and Zn, 923.01 for S, and 915.01 for Cl). Residual feed and feed intake were weighed each morning feeding and additional starter was weighed and added and hand mixed if needed at the afternoon feeding. Supplement PM or PC, depending on treatment assignment, were added to individual starter feed buckets at each feeding (am and pm) at the rate of 0.25g / kg starter added into each feed bucket and hand mixed into the starter grain.

Calves were fed approximately 1.9 L of pasteurized dairy waste milk twice a day for the first 2 wk of the trial, then a mixture of milk replacer and dairy milk twice a day for the next 2 wk and then only milk replacer for the rest of the pre wean period. On days in which temperatures were over 90° C, calves were also given electrolytes in their water buckets. Milk samples were collected daily from the morning feeding and were analyzed by Tulare County Dairy Herd Improvement Association to determine consistency in milk composition using a Foss NIR Milk Analyzer. At 8 wk of age, calves were only fed milk in the morning and then were weaned at 9 wk of age. Calves remained in the hutches for another week at which time they were weighed, ultrasounded, moved into group pens and treatments were stopped.

131 ***Measurements***

132 Fecal consistencies were observed daily on all calves in the morning using a fecal score (**FS**)
133 scale of 1 to 3 adapted from the University of Wisconsin's calf health scoring chart
134 (https://www.vetmed.wisc.edu/fapm/svm-dairy-apps/calf-health-scorer-chs). A score of 1 was
135 normal and formed feces, 2 as semi-formed or pasty and 3 as loose and watery feces. Diagnosis
136 and treatment of sick calves was carried out according to the farm protocol by farm staff. Calves
137 were evaluated and treated once daily by farm staff for signs of disease. Medications were also
138 recorded daily in the morning, approximately 1 hr after feeding.

139

140 Calves were weighed individually at enrollment just prior to entering the hutch and then when
141 they exited the hutch at the end of the trial using a single scale pulled by a tractor. Muscle
142 diameter growth was estimated in the front and hind limb at 1 and 9 wk using ultrasound
143 according to Davis and Rossow (2019).

144

145 Blood was collected from a subset of 13 calves per treatment via the jugular vein immediately
146 after the morning feeding. Weekly whole blood samples from these calves were analyzed for
147 BHB and glucose to estimate rumen development once a week using a Precision Xtra® blood
148 meter (Abbott Laboratories US, Abbott Park, IL) . At three time points, 1, 6 and 9 wk, whole
149 blood samples were also analyzed using a blood analyzer (VetScan® VS2, Abaxis Global
150 Diagnostics, CA) that measured albumin, alkaline phosphatase, aspartate aminotransferase, total
151 calcium, gamma-glutamyl transferase, total protein, globulins BUN, creatinine kinase,
152 phosphorus and magnesium.

153

154 ***Statistics***

155 For all analyses, calf was the experimental unit of interest and independent variables were
156 removed from the model if $P > 0.1$. The dependent variables initial and final body weight, gain,
157 average daily gain, gain to feed ratio, gain in ultrasound muscle diameter in the front and hind
158 leg, total DMI of starter, total milk intake and total medication events were analyzed using the
159 Mixed procedure of SAS (v. 9.4). The model used for statistical analyses of treatment effects on
160 production variables were : $y_{ijkl} = \mu + treatment_i + gender_j + group_k + treatment_i*gender_j +$
161 $trreatment_i*group_k + calf_i(treatment_i) + E_{ijkl}$, where y_{ijkl} = the dependent variable (observed
162 production variable). The μ was the overall mean of the dependent variables, fixed effects
163 treatment i (i = C, PM or PC), gender j (j = steer or heifer), enrollment group k (k = 1, 2 or 3 d),
164 interaction of treatment i with gender j, interaction of treatment i with enrollment group k, the
165 random effect of calf nested with in treatment i and random error E_{ijkl} .

166

167 Repeated measures data for calf nested within treatment by week, sampled at wk 1 to 10,
168 included the dependent variables weekly DMI, weekly milk intake per day, weekly fecal score,
169 weekly blood glucose, weekly BHB, and by period, samples at 1 wk, 6 wk and 9 wk, blood
170 albumin, alkaline phosphatase, aspartate aminotransferase, total Ca, gamma-glutamyl
171 transferase, total protein, globulins, BUN, creatinine kinase, P and Mg. These data were
172 analyzed using the Mixed Procedure of SAS (v. 9.4) and model: $y_{ijk} = \mu + treatment_i + gender_j +$
173 $week_k$ or $period_k + treatment_i*gender_j +$ $treatment_i*$ $week_k$ or $period_k + E_{ijk}$, where Y_{ijk} = the
174 dependent variable. The μ was the overall mean of the dependent variable, fixed effects
175 treatment i (i = C, PM or PC), gender j (j = steer or heifer), week k (k = 1 to 10) or period k (k =

1, 6 or 9 wk), interaction of treatment i and gender j, interaction of treatment i and week k or period k, repeated measure for each calf nested within treatment by week k and E_{ijk} as random error.

RESULTS AND DISCUSSION

Milk and Ration Composition

Milk composition had small variation during the 9 wk milk feeding period of the study indicating that milk replacers were well mixed and were similar to waste milk composition (Table 1). Starter nutrient composition was also consistent amongst all weeks of the trial. Overall protein fat ratio of the milk was 30 : 21 with total solids of 14.6 % and crude protein content of the starter was 28% which is above current Dairy Calf and Heifer Association adequate standards to reach target growth rates (DCHA, "Gold Standards", 2016).

Production Performance

Average levels of total proteins in blood of calves at 2 d of age was not different among treatments (Table 2). Total proteins were measured on a random third of the calves per treatment and are representative of passive transfer from colostrum. Average total protein levels over the entire trial were also above the minimum levels for calves (Table 3; Wilm et al., 2018), LSM fecal scores in the first wk of the trial were not different between treatments (Figure 1) and total medication events per calf were not different between treatments. For example, an injection of Baytril® would count as 1 medication event and if given over 3 d would count as three medication events. Lack of differences between these three health parameters indicate that there was no difference in immune status for all calves at the beginning of the study.

198

199 There were no differences in LSM initial bodyweights indicating that all treatments began with
200 similar initial bodyweights (Table 2). Production performance was not affected by gender or
201 treatment so results presented in Table 2 do not include gender effects. Final bodyweight, gain,
202 ADG, total DMI and gain in muscle size using ultrasound were numerically higher but not
203 different in calves given PM and PC. This implies that the number of calves per treatment may
204 have been too small to detect a difference or that the treatments PM and PC were not given long
205 enough or at high enough dosages to impact intake, palatability or performance. Because the
206 supplements were given in the starter feed relative to level of intake, very little of the
207 supplements were consumed in the first 5 wk of the study (Figure 2). Therefore the calves were
208 only exposed to PM and PC for the last 5 wk. This suggests that future studies should include a
209 post wean feeding period to fully explore the relationship between PM and PC starter intake.

210

211 ***Intake and Growth***

212 Phytogenic compounds have been associated with increased intakes and growth rates through
213 their ability to enhance palatability and growth. Brand et al. (2019) supplemented a similar
214 phytogenic compound at 0.05 and 0.1 g/kg TMR to crossbred yearling steers. Similar to this
215 study, DMI, gain, and G:F did not differ among treatments. But, ADG tended to increase with
216 increases in PC dose and longissimus muscle area was greatest at the 0.1 g/kg TMR PC dose.
217 The maximum doses per steer were comparable to the average doses fed to calves in this study, if
218 milk and starter intake were considered together. Although PC and PM had numerically higher
219 ADG and muscle size gain in the hind leg, the number of calves per treatment may not have been
220 high enough to detect these differences. But, pre wean calves are not ruminants and so if the

primary action of PC in the steers was to shift microbial fermentation and stabilize rumen microbial populations, then the mode of action and results in pre wean calves would be different.

Akbarian-Tefaghi et al. (2018) compared a similar phytogenic compound fed at 3 g/kg starter to supplementation of 3 other herbal plants, monensin and a control treatment (no supplementation) in pre wean Holstein calves. Similar to this study, ADG and G:F were not different amongst any treatment in the pre wean period (3 to 56 d of age) but, DMI tended to be higher with the phytogenic compound treatment. These calves were fed much higher levels of phytogenic compound than the current study which may account for the tendency for higher DMI, however, the higher DMI did not result in increased growth. Therefore feeding a higher level of PC may increase interest in the starter feed by increasing palatability but, did not have a growth promotant effect. The DMI were also approximately 0.2 kg/d higher but, ADG was similar to the current study, 0.62 - 0.70 kg/d. Considering that the calves were weaned 1 wk earlier than the current study, DMI and therefore dose of phytogenic compund was much higher than the current study. These results imply that levels of PM and PC may have to be even higher to impact pre wean calf intake and growth.

In general, gain, ADG, G:F and DMI (Table 3) in this study are similar to results in other pre wean Holstein calf feeding studies. In Teere et al. (2016), ADG was 0.57 - 0.62 kg/d, G:F was 0.57 - 0.62 and total DMI to 7 wk was 1.04 - 1.09 kg/d with calves at similar starting weights weaned at 6 wk. In Fouladgar et al. (2016), ADG was 0.5 - 0.56 kg/d, G:F was 0.51 - 0.56 and total DMI to 10 wk was 1.04 - 1.14 kg/d with calves at similar starting weights weaned at 7 wk. Although calves were not fully weaned until 9 wk in this study, milk offering was reduced at 8

wk so, on average ADG, G:F and total DMI were similar between these studies indicating that intake and growth was not unusual for pre wean Holstein calves.

Rumen Development

Phytogenic compounds have been associated with increasing DMI, changing microbial profiles and shifting VFA production thereby aiding rumen development. However in this study, changes in weekly DMI, milk intake, blood glucose and BHB were different by week but not by treatment (Table 3, Figure 2). There was an interaction of treatment by period for BHB at 63 d of age in which C BHB was higher than PM or PC. Akbarian-Tefaghi et al. (2018) conducted a similar study but used a higher dose and observed an increase in blood BHB and glucose at 70 d and a tendency for increased glucose at 35 d of age. Since calves were weaned earlier at 56 instead of 63 d of age, calves had a longer exposure and higher dose of phytogenic compounds. Therefore PC supplementation at a higher dose may influence rumen development more during the post wean period rather than the pre wean period.

Weekly PM increased with blood BHB levels indicating that more starter intake stimulated rumen development (Figure 2). Other studies have reported the same relationship (Quigley et al., 1991;Suarez et al., 2006; Overvest et al., 2016) and have validated the Precision Xtra® meter (Abbott Diabetes Care, Abingdon, UK) to detect pre wean BHB blood concentrations and as an indicator of rumen VFA production (Deelen et al., 2016). As weekly blood BHB increased during the pre wean period, weekly blood glucose decreased despite increased starter intake and consistent levels of milk intake. Other studies have also observed the decrease in blood glucose

(Klotz and Heitmann, 2007; Nemati et al., 2015; Fouladgar et al., 2016). Quigley et al.(1991). Akbarian-Tefaghi et al. (2018) reported that milk intake and level of concentrate can influence or delay a decrease in blood glucose. Because there were no differences in calf starter or milk composition among treatments other than the small doses of PC and PM, the increase in BHB and corresponding decrease in blood glucose reflects rumen development. Most adult non ruminants maintain a blood glucose concentration around 120 mg/dl and adult ruminants around 60 - 80 mg/dl. As pre wean calves age and switch to a functional rumen, VFA become the major energy sources (Quigley et al., 1991), microbes in the rumen begin to metabolize dietary sources of glucose, the liver switches to gluconeogenesis and blood glucose levels drop.

Fecal Scores

Because of their anti-inflammatory and anti-bacterial properties, it would be expected that phytogenic supplements would contribute to less diarrhea resulting in lower fecal scores. There were no differences in fecal scores among treatments except in week 3, PC fecal scores were lower than C or PM (Figure 1; $P < 0.01$). Therefore PC may aid stabilization of lower gut bacteria and function in pre wean calves, but it may take more exposure to the PC supplement earlier in the study to have an effect.

Fecal scores were different by week (Table 3) and showed that calves were having more digestive difficulties in the first 3 wk of the trial (Figure 1). Fecal score LSM for all three treatments were above 1 in the first 3 wk and then were approximately 1 for the rest of the study. Akbarian-Tefaghi et al. (2018) did not observe a difference in total fecal scores in pre wean or

post wean periods with phytogenic compound treatments but, they also used a 1-5 scale so it was difficult to compare results with this study. Azevedo et al. (2016) also observed that the diarrhea occurs in the first 3 wk of age and decreases over time.

Blood parameters indicating health

Phytogenic supplements are associated with improved health due to their anti inflammatory and anti oxidant properties. However in this study, blood parameters representing immune status and overall health were not different by treatment but were different by period (Table 3). Differences by period reflect changes associated with growth and development. There was an interaction between treatment and period for BHB reflecting that during the 8th wk, PC had the highest BHB and the following week C had the highest BHB. There were tendencies for C alkaline phosphatase and PM total proteins to be higher than other treatments but both were still within normal ranges for calves (Knowles et al., 2000; Fouladgar et al., 2016).

In this study, a relatively high level of CP in the starter grain and milk were fed but were not reflected in increased BUN levels as would typically be expected. But, the calves were changing from non ruminants to ruminants and so the relationship between CP and BUN may vary during the pre wean period. Dry matter intake was correlated with both aspartate aminotransferase activity ($R^2 = 0.51$) and BUN ($R^2 = 0.51$), however, there was no effect of treatment. Akbarian-Tefaghi et al. (2018) observed an increase in BUN at 70 d of age and a tendency for increased BUN at 35 d of age when a higher dose of phytogenic compounds were fed (3 g/kg PM). In this study, C was numerically higher at 63 d of age than PC or PM in both BUN and BHB (Figure 3).

310 Urea is equally distributed around body pools such as MUN, BUN, and salivary urea nitrogen
311 (SUN). Since SUN increases with DMI and is associated with rumen development, it may be
312 possible to use a saliva swab to collect and detect urea N to monitor rumen development in a less
313 invasive manner than collecting blood for BHB.

APPLICATIONS

Phytogenic feed additives have been reported to enhance palatability, support immune function, modulate microbial populations, and stimulate rumen development thereby increasing DMI and growth. However, their action is generally dose dependent. The dose of 0.25 g/kg of PC or PM added to starter feed and fed to pre wean Holstein calves in this study was probably not high enough or fed long enough to affect growth or rumen development.

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420 **Table 1.** Ingredients and nutrient composition of starter feed ration (10 samples) and milk
421 samples (47 samples)

Composition	Average	SD
Starter ingredients, % DM		
Steam flaked corn	21	
Steam flaked barley	15	
Soybean oil	3.0	
Vitamin-mineral, protein premix[1]	61	
Starter nutrient composition, % DM (unless noted)		
DM	89	0.88
CP	28	1.6
ADF	9.4	0.45
NDF	20	1.4
Starch	22	2.2
Fat	4.3	0.53
Ash	8.8	0.49
Na	0.28	0.031
Mg	0.36	0.018
P	0.70	0.020
S	0.31	0.0079
K	1.4	0.065
Ca	1.1	0.12
Cl	0.25	0.028

Mn, ppm	103	10
Fe, ppm	176	30
Cu, ppm	20.0	2.2
Zn, ppm	112	16
Milk nutrient composition, % As Fed		
Fat	3.12	0.24
Protein	4.42	0.43
Lactose	5.84	0.46
Solids not fat	11.5	0.68

[1] Corn gluten feed, Maize distillers dried grains, Soybean hulls, Cane Molasses, Calcium carbonate, Yeast culture, Cooked corn flour, Sodium sesquicarbonate, Salt, Dicalcium phosphate, Monocalcium phosphate, Propionic acid, DL Methionine hydroxy analog, Niacin, Folic acid, Biotin, Pyridoxine hydrochloride, Riboflavin, Thiamine mononitrate, Calcium pantothenate, Sodium selenite, Mineral oil, Vitamin B12, Vitamin E, Zinc sulfate, Manganous oxide, Vitamin A, Vitamin D, Copper sulfate, Cobalt carbonate, Manganese sulfate, Ferrous carbonate, Zinc oxide, Ethylenediamine Dihydriodide, Calcium iodate, Lasalocid (Cargill Animal Nutrition, Stockton CA)

Table 2. Least square means for gain and health of calves fed starter with and without supplements.

Parameter	Treatment[1]			SEM	*P*-Value
	Control	PM	PC		
	n = 40	n = 40	n = 40		
Initial body weight, kg	39.4	39.4	40.5	1.7	0.75
Final body weight, kg	83.9	84.9	86.0	2.1	0.74
Gain, kg	43.6	44.6	45.7	2.7	0.74
Average daily gain, kg/d	0.632	0.647	0.663	0.039	0.74
G:F[2], kg/kg	0.88	0.82	0.83	0.060	0.76
Gain in front leg muscle area[3], cm^2	1.72	1.87	1.74	0.14	0.79
Gain in hind leg muscle area[3], cm^2	1.77	2.09	1.98	0.40	0.72
Total DMI, kg	56.5	59.6	58.5	5.0	0.81
Total milk intake, L	103	103	102	0.41	0.30
Total blood proteins[4], g/dl	5.65	5.50	5.71	0.22	0.60
Total medication events, /calf	2.23	2.42	2.61	0.67	0.85

[1] Treatments are Control, no phytogenic supplement; PM, phytogenic supplement containing caraway, licorice, oak bark and vanilla soluble in milk; PC, phytogenic supplement containing caraway, licorice, oak bark and vanilla.

[2] G:F ratio was calculated as kg of BW gain/ kg of total DMI.

[3] Measured using ultrasound

[4] Measured at 2 d of age in a subset of 13 calves per treatment using a VetScan VS2, Abaxis Global Diagnostics, CA

441 **Table 3.** Least square means for blood parameters of calves fed starter with and without
442 supplements

Variables	Trreatments[1]				*P*-Value		
	Control	PM	PC	SEM	Trt	Period[2]	Trt x Period
DMI[3], kg/d	0.981	1.02	1.04	0.038	0.28	0.01	0.99
Milk intake, L/d	3.75	3.75	3.74	0.011	0.18	0.01	0.94
Fecal score	1.13	1.14	1.13	0.015	0.66	0.01	0.20
Glucose, mg/dl	109	112	108	3.2	0.46	0.01	0.99
BHB, mmol/L	0.14	0.13	0.15	0.010	0.50	0.01	0.04
Albumin, g/dl	3.43	3.44	3.36	0.060	0.37	0.01	0.33
Alkaline Phosphatase, U/L	230	191	200	16	0.06	0.01	0.87
Aspartate Aminotransferase, U/L	56.4	57.7	58.2	2.9	0.82	0.01	0.25
Total Ca, mg/dl	11.4	11.6	11.3	0.18	0.31	0.01	0.67
Gamma-glutamyl transferase, U/L	52.8	81.9	67.2	16	0.20	0.01	0.23
Total protein, g/dl	6.13	6.29	5.99	0.13	0.07	0.01	0.31
Globulins, g/dl	2.71	2.84	2.64	0.10	0.14	0.01	0.68
BUN, mg/dl	10.7	9.61	10.1	0.58	0.21	0.01	0.12
Creatinine Kinase, U/L	159	170	187	34	0.74	0.01	0.33
P, mg/dl	9.26	9.09	9.18	0.21	0.74	0.01	0.85
Mg, mg/dl	2.25	2.17	2.24	0.043	0.11	0.01	0.67

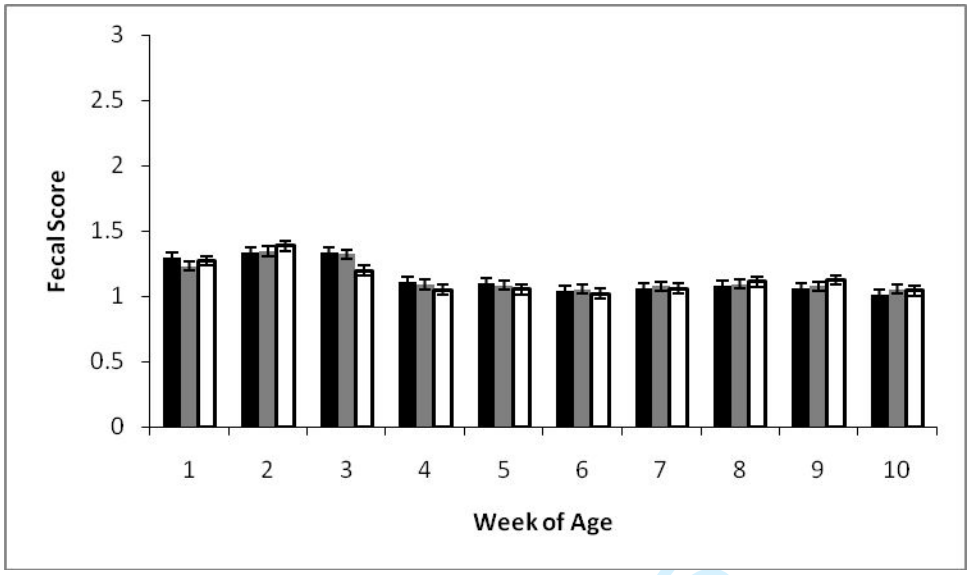
443 [1] Treatments are Control, no phytogenic supplment; PM, phytogenic supplement containing
444 caraway, licorice, oak bark and vanilla soluble in milk; PC, phytogenic supplement containing
445 caraway, licorice, oak bark and vanilla.

[2] Period represents weekly samples for DMI, Milk intake, fecal score, Glucose, BHB; Period represents samples collected at 1, 6 and 9 wk for the rest of the variables.

[3]Number of calves per treatment for DMI, milk intake and fecal score were 40. Number of calves for all other variables were 13 per treatment.

451 **Figure 1.** Weekly least square mean and standard errors of fecal scores by treatment. Black bars
452 represent Control, grey bars represent PM (phytogenic supplement containing caraway, licorice,
453 oak bark, vanilla with components soluble in milk) and blank bars represent PC (phytogenic
454 supplement containing caraway, licorice, oak bark, vanilla) treatments. Number of calves per
455 treatment were 40.

456



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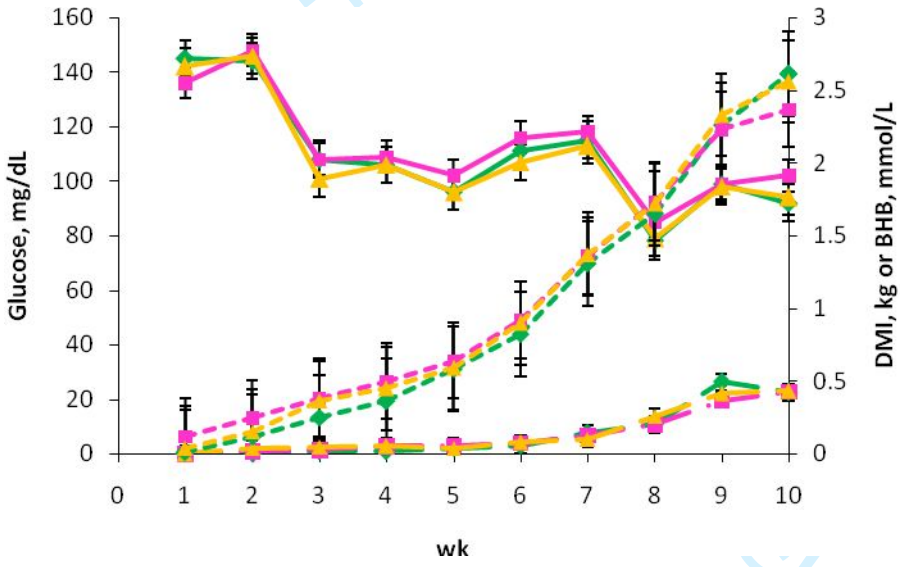
459 **Figure 2.**Weekly least square mean and standard errors of starter DMI and blood glucose and
460 BHB by treatment. Solid lines represent blood glucose (-), dashed lines are DMI (---) and dotted
461 lines are BHB (•••); diamonds are Control (◆), squares are PM (■;phytogenic supplement
462 containing caraway, licorice, oak bark, vanilla with components soluble in milk) and triangles
463 are PC (▲;phytogenic supplement containing caraway, licorice, oak bark, vanilla). Number of
464 calves per treatment were 13.

465



466

468 **Figure 3.** Weekly least square mean and standard errors of blood BHB and BUN by treatment.
469 Dotted lines represent BHB (•••) and dashed lines are BUN (---); diamonds are Control (◆),
470 squares are PM (■ ; phytogenic supplement containing caraway, licorice, oak bark, vanilla
471 components soluble in milk) and triangles are PC (▲; phytogenic supplement containing
472 caraway, licorice, oak bark, vanilla). Number of calves per treatments were 13.

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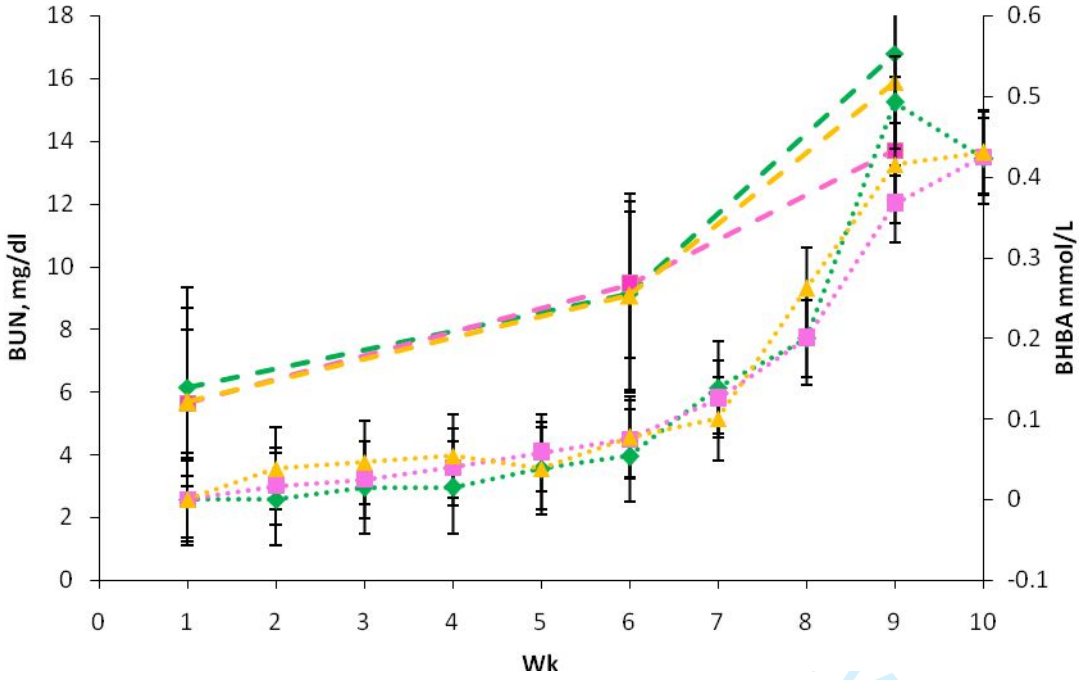

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