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Effects of Probiotic and Chromium-Methionine on Thyroid Hormones, Total Protein, Zinc, and Weight Gain in Dairy Holstein Calves During the Weaning Period

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Abstract

BACKGROUND: Many additives have been recommended to calf feed but the effects of each supplement have been under investigation especially during weaning stress.

OBJECTIVES: The effects of dietary supplementation of probiotic (Protexin) and chromium-methionine chelate (Cr-Met) on triiodothyronine (T3), thyroxine (T4), total protein, albumin, zinc, and growth body-weight gain in dairy calves during weaning period was investigated.

METHODS: A total of 28 dairy Holstein calves were randomly allocated to one of the four experimental treatments (n=7). The study commenced 21 days before weaning (average 70 days).The recommended dosage for both chromium-methionine chelate (Cr-Met) and probiotic (Protexin) is 2 gr/calf daily. Blood samples were collected from the jugular vein, 21 days before weaning and 3, 7 and 21 days after weaning. Serum consentrations of T3, T4, total protein, albumin, and zinc were measured. Bodyweight (BW) was measured 7 days before, at weaning and 7 days after weaning.

RESULTS: No effect of different diets nor interaction was found for body weight gain, total protein, and albumin. No effect of different diets was observed for T4 although the interaction was observed between groups and time. An effect of different diets and time was found for T3 and the mean T3 concentration was lowest in group Protexin + Cr-Met. The mean Zinc concentration was at its highest level 21 days after weaning, while different diets did not affect the Zinc concentrations.

CONCLUSIONS: There is no benefit associated with the separate or combined feeding of Protexin and Cr-Met on calves' growth performance according to weight gain, total protein, and thyroid hormones.

KEYWORDS: Chromium-methionine chelate (Cr-Met), Dairy Holstein calves, Probiotic, Thyroid hormones, Weaning period

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Introduction

Weaning of dairy calves is bound with a concurrent subjection of calves to a range of environmental stressors. Behavioral and physiological responses to weaning play a significant role in the well-being of calves. The development of weaning protocols aimed at precluding such stressors must be supported based on the scientific knowledge of the morphological, physiological and psychological mechanisms. Despite increasing the reproductive and productive performance of the herd, sudden weaning is a source of stress for the calves (Ungerfeld et al., 2011). Usually, weaning involves changes in the social and physical environment, which in turn causes stress for calves (Enríquez et al., 2011). Different weaning methods have been proposed to avoid the impact of weaning on behavior, performance and well-being. Around weaning time animals are susceptible to different pathogens. Antibiotics have been used to overcome such issues. However, the use of antibiotics in animal husbandry has certain limitations due to the antibiotic resistance to microorganisms. To replace calf feeds with antibiotics, many additives have been proposed, one of which is probiotics (Economou and Gousia, 2015). Probiotics are microbial food supplements which improve host interstitial microbial balance (Musa et al., 2009) and are alternatives to antibiotics in reducing diarrhea and improving immunity in calves (Heinrichs et al., 2009). Markowiak and Śliżewska (2018) reported that probiotics can enhance the weight gain of calves and piglets. Potential probiotic strains are normal inhabitants of a gut and have the ability to adhere to and colonize the epithelial cells of the gut (Musa et al., 2009). Probiotics dietary supplementation can significantly ameliorate feed intake and conversion rate, and daily weight gain in sheep, goat, cattle, pig, and horses (Casey et al., 2007; Chiofalo et al., 2004; Gobesso et al., 2018; Adriani et al., 2016; Torres-Rodriguez et al., 2007). Probiotics can affect animal health by competing against pathogens for colonization sites and nutritional sources and the production of toxics or stimulation of the immune system (Rai et al., 2013). These supplements have been associated with improvement in the immune system, and the stimulation of non-specific immune responses (Rai et al., 2013). The probiotics that increase immunoglobulin levels have a more positive influence on growth performance and the ability to resist disease (Roselli et al., 2017). A major convenience of such supplements is a proliferative effect on beneficial intestinal bacteria. Some gut microflora have been described to have positive effects on the whole body, including improved weight gain and immune function and decreased presence of pathogens (Liao and Nyachoti 2017). There are numerous growth promoter substances supplemented with animal feed improve animal production and potentially reduce the cost of animal management. These substances contain antibiotic growth promoters, such as flavomycin, probiotics, acidifiers, enzymes, herbal products, beta-agonists, microflora enhancers and immune-modulators. Probiotic preparations have shown encouraging results in different animal production areas. Generally, probiotics can be added to feed or water as mono or mixed cultures of live microorganisms (Todorov et al., 2008). Chromium (Cr) is an essential trace mineral participating in the metabolism of carbohydrates, lipids, proteins and nucleic acids (Li et al., 2013). The role of Cr has been researched for potential use as feed additives in animal production. Chromium deficiency in farm animals grows during physiological and environmental stress (Ohh and Lee 2005). Bailey (2014) indicated that chromium enrolls an important act in glucose metabolism and immunomodulation, which can, conclusively, affect animal health and performance. Previous studies revealed that proliferative activities in peripheral blood cells from chelated chromium supplemented calves were higher than those from calves fed inorganic chromium (CrCl3); also, they noticed the effect of chelated chromium on the non-specific immune response regarding neutrophil phagocytosis in dairy cows and chromium was able to enhance the humoral immune response. IL-2, TNF-α and INF-γ concentrations in the blood of periparturient cows with chelated chromium supplemented diet were significantly decreased (Al-Saiady et al., 2004). The objective of this study was to determine the beneficial effects of dietary supplementation of probiotic (Protexin) and chromium-methionine chelate (Cr-Met) on triiodothyronine (T3), thyroxine (T4), total protein, albumin, zinc, and growth bodyweight gain in dairy Holstein calves after and before weaning.

Materials and Methods

Animal ethics

All animal experiments were approved by the State Committee on Animal Ethics, Shiraz University, Shiraz, Iran (IACUC no: 4687/63). We further followed the recommendations of the European Council Directive (86/609/EC) of November 24, 1986, regarding the standards of protecting animals used for experimental purposes.

Animal management

In this work, 28 dairy Holstein calves with an initial body weight of 93.46 kg ±11.82 (mean±SD) were selected in a completely random design (n=7) and placed in one of the four experimental groups (from 21 days before weaning to 21 days after). The experimental groups (G) were comprised of (G1) control (G2) Protexin (G3) Cr-Met (G4), and Protexin + Cr-Met. All experimental animals were weaned on an average 70 days of life according to the farm policy. This experiment was carried out in Fars province of Iran from May to September 2016. The animals were kept in an individual pen and fed starter ration formulated according to NRC (2001) requirements and water ad libitum. The type of probiotic used in our study is a commercial multistrain symbiotic called Protexin (Probiotics International Ltd., South Petherton, UK). It contains the following strains of probiotics and prebiotics: *Lactobacillus plantarum, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus salivarius ssp. thermophilus, Enterococcus faecium, Aspergillus oryzae,* and *Candida pintolopesii*. We used a recommended dose of the product, which is 2 gr/calf daily. Chromium-methionine chelate (Cr-Met) was obtained from ZINPRO company and we used a recommended dose of the product (2 gr/calf daily). Fresh water was readily available at all times. The study started 21 days before weaning (average 70 days). All the calves received supplements in milk, before weaning, and 15 ml was added to water and drainage by syringe in their mouth to ensure that they have received supplements daily for 3 weeks after weaning.

Diet

Fed starter chemical analysis and feedstuff composition are presented in Table 1.

Table 1. Chemical analysis for treatment diets and feedstuff composition for farm mixed starter

Chemical Analysis		Ingredients	
Dry matter (%)	87.5	Cracked corn	25
Total Digestible Nutrients (%DM)	81	Soy Bean Meal	35
Crude protein (% DM)	23.2	Corn gluten meal	5
Neutral petergent Fiber (%DM)	19.5	Barley grain cracked	26
Acid detergent Fiber (%DM)	8.4	Molasses	3
Ash (%DM)	6.5	*Vitamin/mineral mix	3

* Each kg of vitamin premix provided (mg/kg), iron 30.0; manganese, 150.0; cobalt 3; iodine, 0.0; selenium, 0.5;vitamin A, 25,000 IU/kg; vitamin D, 5,000 IU/kg; vitamin E, 200 IU /kg.

Blood sampling

Blood samples were taken from the jugular vein from all calves at the same time in the day into plain vacutainer tubes, 21 days before weaning and 3, 7 and 21 days after weaning. The collected blood samples were quickly kept in an ice pack and sent to the laboratory. Samples were rapidly centrifuged at 3,000 rpm for 15 min. Sera were harvested and aliquots were stored at -20 °C until metabolites analysis.

Measurement of the biochemical parameters

Serum T3 was measured using a competitive enzyme immunoassay kit (Padtan Elm Co., Tehran, Iran). The intra- and inter-assay CVs of the assays were 12.6% and 13.2%, respectively. The sensitivity of the test was 0.3 nmol/L. Serum T4 was specified using a competitive enzyme immunoassay kit (Monobind Inc., Lake Forest, CA, USA). The intra- and inter-assay CVs of the assays were 3.0% and 3.7%, respectively. The sensitivity of the test was 5 nmol/L. Serum was analyzed for total protein by Biuret method (Commercial kit; Pars Azmoon, Tehran, Iran) and for albumin by the bromocresol green method (Commercial kit; Pars Azmoon, Tehran, Iran). Zinc serum was analyzed by atomic absorption spectrophotometry (Shimadzo AA-670, Kyoto, Japan).

Statistical analyses

Data were expressed as the mean ± deviation (SD). A repeated measures linear mixed model was used to compare the mean concentrations of different serological factors within similar weeks among the four different experimental groups. Statistical analysis was achieved using SPSS 16 (SPSS Inc., Chicago, Illinois). The level of statistical significance was set at P-value<0.05.

Results

Weight gain

The effect of treatment group and time on the weight gain is shown in Table 2. The effect of time was observed on the mean of weights ($P<0.05$), yet no effect of different diets and no interaction between different diets and time was found ($P>0.05$).

Average weight gain was at its lowest seven days before weaning (93.4± [88.7-98.1(CL)] kg) and at its highest seven days after weaning (132.8± [126-139.7(CL)] kg).

Total protein and albumin

The effect of different diets and time on the concentration of Total protein and albumin are shown in Figures 1 and 2, respectively. No effect of different diets and time was found (*P*>0.05) for total protein and albumin and no interaction between different diets and time was observed (*P*>0.05).

Table 2. Mean (± SEM) weight (kg) 7 days before weaning, at weaning and 7 days after weaning n=7 each group, * P-value<0.05

day Groups	-7	0	7
Control	97.57±0.6	109.28±0.8	137.14±0.5
Probiotic	94.14±0.5	106.42±0.6	133.57±0.6
Probiotic +Cr-Met	93.42±0.6	102.85±0.6	98.57±0.8
Cr-Met	88.71±0.7	98.57±0.4	129.71±0.8

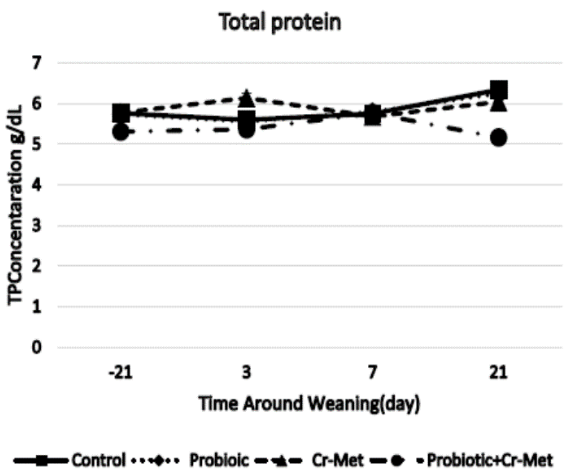

Figure 1. Mean (± SEM) serum concentrations of total protein (gr/dL) 21 days before weaning and 3, 7 and 21 days after weaning n=7 each group, * P-value<0.05 (normal value 5-6.5 gr/dL; Melvin, 1982; Constable et al., 2017).

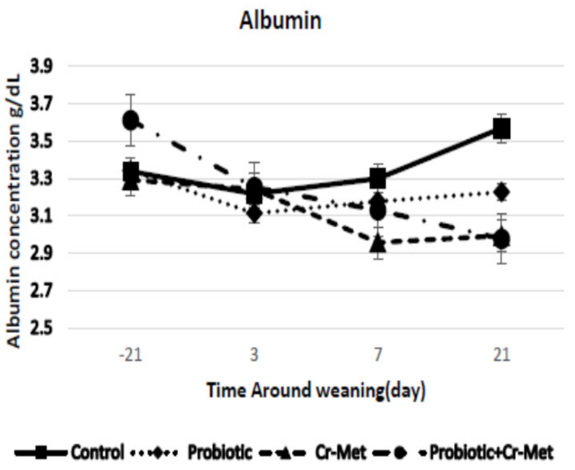

Figure 2. Mean (± SEM) serum concentrations of albumin (gr/dL) 21 days before weaning and 3, 7 and 21 days after weaning (n=7) in each group, * P-value<0.05 (normal value 4.6-5.3 gr/L; Bouda and Jagos, 1984; Constable et al., 2017).

Thyroid hormones

The effect of treatment groups and time on the concentration of thyroid hormones is shown in Figures 3 and 4, respectively. An effect of different diets and time on the mean T3 concentration was observed (*P*<0.05) but no interaction between group and time on the mean T3 concentration was observed (*P*>0.05).

The mean T3 concentration was at its lowest in Protexin + (Cr-Met) (2.7± [2.5-3(CL)] μg/L) group, the group where the mean T3 concentration was lower than the control and Protexin groups (*P*<0.05).

The mean T3 concentration was at its maximum 21 days before weaning (3.6± [3.4-3.7(CL)] μg/L). The effect of time was observed on the mean T4 concentration (*P*<0.05) and an interaction between different diets and time on the mean T4 concentration was observed (*P*<0.05).

The mean T4 concentration was at its highest 21 days before weaning (11.1± [102.12(CL)] μg/L).

Zinc

The effect of treatment groups and time on the concentration of Zinc is shown in Figure 6. The effect of time was observed on the mean zinc concentration (*P*<0.05) (see Figure 5) and an interaction was seen between different diets and time (*P*<0.05). The mean zinc concentration was highest, 21 days after weaning (0.25± [0.22-0.28(CL)] mg/L), at which point, it was higher compared with 3 and 7 days after weaning (*P*<0.05).

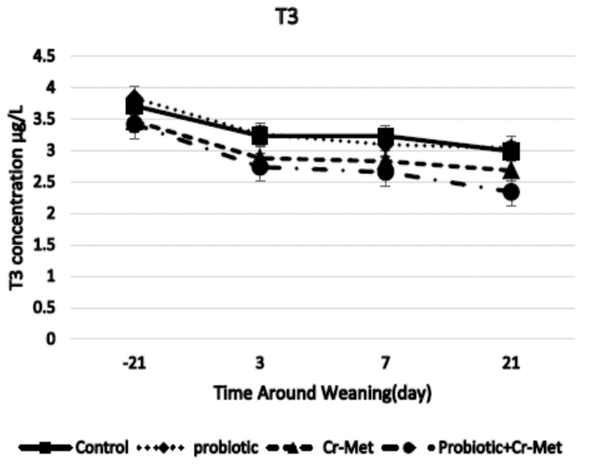

Figure 3. Mean (± SEM) serum concentrations of triiodothyronine (T3) (µg/L) 21 days before weaning and 3, 7 and 21 days after weaning (n=7) in each group, * P-value<0.05.

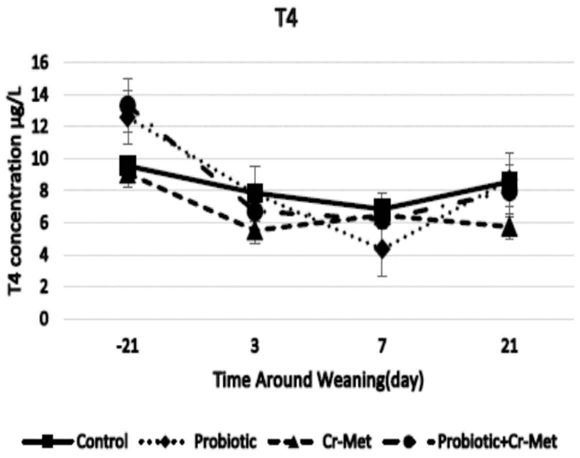

Figure 4. Mean (± SEM) serum concentrations of thyroxine (T4) (µg/L) 21 days before weaning and 3, 7 and 21 days after weaning (n=7) in each group, * P-value<0.05.

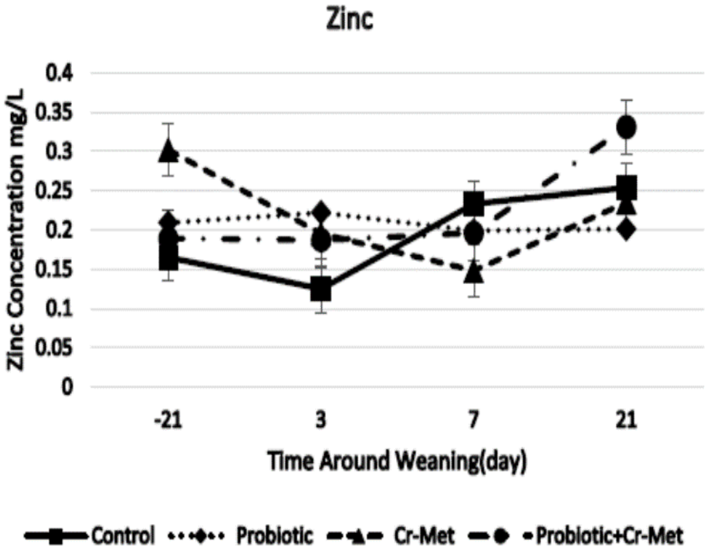

Figure 5. Mean (± SEM) serum concentrations of zinc (mg/L) 21 days before weaning and 3, 7 and 21 days after weaning (n=7) in each group, * P-value<0.05. Normal value of Zn 24-27 µmol/L (Bouda and Jagos, 1984).

Discussion

The results of the current study showed that there was no effect of different diets on average weight gain in calves fed chromium methionine (Cr-Met) and probiotics or both. Kegley et al. (2000) showed that beef steers fed chromium methionine chelate did not show any additional improvement on growth performances and feed efficiency compared with steers that were not supplemented with dietary chromium; on the other hand, chromium supplementation enhanced a glucose clearance rate and increased a serum insulin concentration of growing steers following intravenous insulin and glucose injection. In another study, Korean native steers supplemented with chromium methionine showed improvement in feed efficiency during growth periods; however, no difference was discovered in daily gain and dry matter intake although serum insulin concentration was the highest in chromium supplemented steers (Lee et al., 2016). This means that chromium methionine chelate has the potential to induce a positive effect on nutrient utilization in a relatively well-nourished animal without any adverse effect on growth performances. Lashkari et al. (2018) described that the daily gain in stressed feeder calves fed chelated chromium supplemented diet, was increased in the feedlot due to a notably reduced morbidity compared to calves with no chromium supplementation. But, another chelated chromium supplementation for stressed steers was not able to improve the growth performance and feed competency during growth, finishing and overall periods (De Oliveira et al., 2016). Therefore, it is still uncertain how a chelated chromium supplementation for growing cattle and calves does react under stressful states like transportation and weaning. The study by Roodposhti and Dabiri (2012) revealed that the average daily weight gain was greater in calves fed probiotics, prebiotics, and synbiotics at weeks 6, 7 and 8. Lesmeister et al. (2004) reported improvements in average daily weight gain (ADG) when a 2% supplemental probiotic was added to a calf diet. Probiotic bacteria also increased weight gain and feed efficiency in calves (Angelakis, 2017). The results of our study showed that experimental animals did not have zinc deficiency, T3 and T4 were in the range of the reference values. López et al. (2018) reported that T3 and T4 normal values in calves under 3 months are 1.50 ± 0.48 (ng.ml-1) and 8.21 ± 2.10 (µg.dl-1), respectively. Paulazo et al. (2019) indicated that experimentally induced zinc deficiency may decrease circulating TSH and T4 levels and Zinc may alter the binding of thyroid hormones. Concentrations of thyroid hormones are also influenced by such factors as fat- or starch-enriched diet (Greco et al., 2015). Pattanaik et al. (2001) observed reduced levels of circulatory thyroid hormones in sheep and goats when fed with protein-restricted diets. Accordingly, because the animals of the present study did not have a protein deficiency, their thyroid hormones were in a normal range. Another study by Barman et al. (2019) showed a trend towards reduced serum thyroid hormones in calves fed lower dietary protein levels, further pointing to a positive relationship between protein nutrition and thyroid status. Moreover, with the advancement of age, the T3 level in serum also increased. Since the animals were in the active growth phase, the thyroid gland must have been activated to secrete more T3 and T4, boosting their levels in the blood (Hajimohammadi et al. 2015; Constable et al. 2017). We observed that the total protein and albumin were in reference values and there was no effect of different diets and time on mean TP and albumin concentration. Total protein and albumin follow the availability of protein and their concentration reduced in the face of protein deficiency (Sangpuii et al., 2019). Albumin has a practically short half-life and can reflect protein deficiency problems over a duration of one or two months. We suggest that experimental animals do not have protein deficiency issues.

In conclusion, separate and mix feeding of dairy Holstein calves with chromium methionine and probiotic has no detectable effects on growth performance. Total protein and albumin were in the reference range, indicating that experimental animals did not have protein deficiency and also thyroid hormone and zinc were in a normal range.

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Conflict of Interest

The authors declared that there is no conflict of interest.

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مجله طب دامی ایران، ۱۳۹۹، دوره ۱۴، شماره ۲، ۱۸۸-۱۷۷

اثرات پروبیوتیک و کروم-متیونین بر هورمون‌های تیروئیدی، روی، پروتئین تام و وزن‌گیری گوساله‌های شیری هولشتاین در دوره از شیرگیری

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چکیده

زمینه‌مطالعه:

هدف: اثر مکمل خوراکی پروبیوتیک (پروتکسین) و کروم – متیونین چلاته بر روی هورمون‌های تیروئیدیT۳ و T۴، پروتئین تام، آلبومین، روی و وزن‌گیری در طول دوره ی از شیرگیری مورد بررسی قرار گرفت.

روش کار: تعداد ۲۱ راس گوساله هولشتاین شیرخوار به صورت تصادفی در ۴ گروه آزمایش شدند. این تحقیق ۲۱ روز قبل از شیرگیری آغاز شد (سن میانگین گوساله ها ۷۰ روزگی). دوز پیشنهادی برای هر دو مکمل پروبیوتیک (پروتکسین) و کروم – متیونین به میزان ۲ گرم در روز جهت هر راس گوساله بنا به توصیه شرکت سازنده در نظر گرفته شد. اولین نمونه خون در زمان شروع تحقیق در ۲۱ روز قبل از شیرگیری از ورید وداج گرفته شد و در روز های ۳، ۷ و ۲۱ روز پس از شیرگیری مجددا خونگیری انجام شد و غلظت سرمی هورمون های T۳ و T۴، پروتئین تام، آلبومین و روی اندازه‌گیری شد. وزن گوساله ها در ۷ روز قبل ازشیرگیری، روز از شیرگیری و ۷ روز پس ازشیرگیری اندازه گیری شد.

نتایج: هیچ تفاوتی در بین گروه های مختلف تغذیه ای مورد آزمایش و اثر متقابل گروه و بازه ی زمانی بر روی افزایش وزن روزانه، پروتئین تام و آلبومین مشاهده نشد. همچنین تفاوت معناداری در هورمون T۴بین گروه های مختلف تغذیه ای مشاهده نشد ولی اثر متقابل بین گروه و بازه ی زمانی مشاهده شد. تاثیر گروه‌های مختلف تغذیه‌ای و بازه ی زمانی بر روی هورمون T۳ مشاهده شد و میانگین غلظت هورمون T۳ در گروه دریافت کننده ی هر ۲ مکمل پروتکسین و کروم – متیونین در پایین ترین سطح ممکن بود. میانگین غلظت روی در ۲۱ روز بعد از ازشیرگیری در تمامی گروه ها در بالاترین میزان بود در صورتی که میانگین غلظت سرمی روی درگروه های مختلف تغذیه ای تفاوت معناداری نداشت.

نتیجه گیری نهایی: هیچ تاثیر بسزایی در مصرف به صورت جدا و یا ترکیبی هر دو مکمل پروبیوتیک (پروتکسین) و کروم – متیونین بر روی افزایش وزن، پروتئین تام و هورمون‌های تیروئیدی مشاهده نشد.

واژه‌های کلیدی:

پروبیوتیک، کروم-متیونین چلاته، دوره از شیرگیری، هورمون های تیروئیدی، گوساله شیری هولشتاین .

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