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Interaction Between Crude Protein Level and Rumen Protected Amino Acids in Starter Diet on Performance of Dairy Calves

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Abstract

BACKGROUND: A limited number of studies have investigated the inclusion of amino acids in calf starter.

OBJECTIVES: This study was aimed to evaluate different levels of crude protein in starter and supplementing some protected amino acids on efficiency, health status and blood metabolites of dairy calves and to compare it with unprotected amino acids.

METHODS: Forty-eight 3-day-old Holstein calves were randomly allocated to four treatments in a completely randomized design. Dietary treatments were (1) 18% Crude Protein starter without Amino Acid, (2) 18% Crude Protein with 0.0340% protected lysine and 0.016% protected methionine, (3) 18% Crude Protein starter with 0.215% un-protected lysine and 0.012% un-protected methionine, (4) 22% Crude Protein without Amino Acid. All calves received the same amount of whole milk, weaned on day 56; the study was finished on day 70.

RESULTS: Calves in treatment 4 had the greatest starter intake, weaning weight and final weight (P<0.05) but their feed efficiency was not different among treatments. There were no significant differences in Immunoglobulin G, total protein and Lysine and Methionine concentration in blood (P>0.05). Health score (eye, ear, nose and respiratory score), was not different among treatments but the fecal score revealed a significant difference (P<0.05). Starter diet with 22% Crude Protein resulted in higher serum urea concentration (P< 0.05)

CONCLUSIONS: Results of the present study indicate that, the performance of calves received protected or un-protected amino acid in starter was not different. Also, higher Crude Protein levels in diet did not lead to a better gain to feed ratio.

KEYWORDS: Dairy calves, Lysine, Methionine, Rumen protected, Starter

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Introduction

Reducing feed costs of the dairy calves is one of the critical issues that dairy farmers are faced with. Usually, protein accounts for the highest amount of the ration cost, while it is regarded as an important part of balancing diets for livestock. The amount of protein is very important in the diet of pre-ruminant calves, as reduced animal production, welfare, and polluted environment are the consequences of too low or too high protein concentrations. A concern of overfeeding protein is the amount of N that enters the environment; therefore, researchers have conducted research to reduce the amount of N lost to the environment while optimizing N usage within the cow (Erickson and Kalsheue, 2020). However, there are not enough studies on the most suitable amount of crude protein in the solid feed of pre-ruminant calves. As well, although the NRC (2001) has recommended the requirements of non-synthesizable AA (Amino Acid) for various animal species, their diets remain suboptimal regarding growth, development, reproduction, lactation and health (Guoyao et al., 2014). The NRC model indicates that 18% CP (DM basis) in starter should be adequate for calves fed with enhanced milk replacer, and that energy supply rather than CP supply would be a limiting factor for growth. However, data needed regarding the determination of the necessity to an increased CP or metabolizable protein concentration in the starter is inconclusive (Stamey et al., 2012).

In dairy cows, the advantage of improving the balance of absorbable AA is the increased efficiency of the use of absorbed AA for milk protein production. It has been demonstrated that improved lysine and methionine nutrition reduced the amount of dietary CP needed to achieve similar yields of milk protein (Socha, 2005). Individual infusion of lysine and methionine post-ruminally, has revealed that these two amino acids are the first and second limiting amino acids in dairy cows. The most effective way to increase the availability of methionine post rumen is the use of rumen-protected methionine (Rulquin, 2006). Awawdeh (2016), using rumen-protected methionine plus rumen-protected lysine reported improved milk yield and protein contents of dairy cows and the results were better than supplying rumen-protected methionine alone.

In dairy calves, the provision of adequate nutrients from liquid and solid feeds and maintaining average daily gain above 0.5 kg/d can enhance the first lactation performance of heifers when combined with proper post-weaning practices (Gelsinger et al., 2016). Increased Average Daily Gain (ADG) resulted from increasing amounts of CP and ratios of CP to energy in the feeding of calves only fed with milk has been reported in the study by Chapman et al. (2017). Hill et al. (2006b and 2007c) showed that in calves fed with starter, feeding a 26% CP and 17% fat milk replacer supported more ADG than a milk replacer containing 20% CP and 20% fat. Feeding a 20% CP milk replacer at 0.45 kg/d, supplemented by lysine and methionine improved ADG in all whey CP formulas. A 17% improvement was observed in ADG resulted from adding lysine and methionine, maximized with 2.34% lysine. The linear and quadratic ADG response to added methionine was equal to 13 and 7%, respectively, with a plateau appearing at 0.72% methionine. There was no ADG or efficiency response to added threonine (Hill et al., 2007b). Stamey et al. (2012) studied the growth performance of Holstein dairy calves that received different amounts of CP in their solid feed. In terms of solid feed intake and growth performance of calves, especially around weaning, moderate advantages were observed in starter with 25.5% CP (DM basis) compared to a starter containing 19.6% CP (Stamey, et al., 2012).

Besides, some studies have reported the effects of amino acids on immunity. Much non-essential AA and essential AA participate in cell signaling, gene expression, and metabolic regulation. Thus, functions of AA beyond protein synthesis must be considered in dietary formulations to improve the efficiency of nutrient use, growth, development, reproduction, lactation, and well-being in animals (Guoyao, et al., 2014). Amino acids might be a tool to transform animals from a pro- to an anti-inflammatory phenotype through the down-regulation of several genes related to immunity system (TLR-4, NF-κB, TNFa, IL-1β, IL-2, IL-6, IL-8, CCL-5 and CXCL-16) whose expression increases during inflammation (Tsiplakou, et al., 2018). In a study done by Montano et al. (2016) on feedlot calves, it was found that methionine and lysine were closely co-limiting amino acids.

Greater heifer growth rates can theoretically lead to an earlier breeding event and reduce the time that the heifer spends in a non-productive state (Roche et al., 2015).

In this respect, one of the strategies that can be used is to increase the metabolizable protein (MP) availability, which can be understood as an amino acids mixture that is absorbed in the small gut, and that can be used for muscle and mammary gland development, among other functions (Cervieri et al., 2001). Once the requirements of the RDP have been met, supplying rumen undegradable protein (RUP) could improve the supply of metabolizable protein and decrease the portion of nitrogen compounds that is recycled to the rumen thereby increasing the availability of nitrogen for anabolic purposes (Rufino et al., 2016).

In a study done by Silva (2017) in prepubertal and pubertal heifers, it was concluded that Microbial crude protein (CPmic) synthesis, microbial efficiency, and the efficient use of N for microbial synthesis decrease as the supplied RUP increases. The intestinal digestibility of CP is negatively affected by RUP levels, which is due to the reduction in the CPmic flow, which probably has greater intestinal digestibility than the protein of feedstuffs. The urinary N excretion decrease according to the RUP supply increase, which is due to the decrease in ruminal N losses as ammonia and the increase in N recycling.

Although categorizing dietary protein into RDP and RUP has not been extensively used for dairy calves, there are studies where adding protein as RUP has produced positive growth response, no response or a negative response (Kazemi-Bonchenari, 2016). In a study done by Kazemi-Bonchenari et al. (2016), the concentration of alanine aminotransferase was also lower ($P<0.05$) for calves fed high-fat starter compared to those fed low-fat starter on day 65, and these levels tended to increase with the addition of RUP. In conclusion, no effects were attributable to feeding a high-RUP starter.

Studies that evaluate whether reducing CP level in the starter and adding AA would improve dairy calves' performance or not, were not available in reviewed articles. Thus, the present study was aimed to assess the effect of crude protein level in the starter and adding rumen- protected and unprotected lysine and methionine on the growth performance, feed efficiency and well-being of calves as well as some blood metabolites.

Materials and Methods

Animals, management, and experimental design

The study was carried out in a commercial dairy farm (Goldasht Nemouneh) from December 2017 until March 2018, in Isfahan, Isfahan province, Iran.

Forty-eight 3-day-old Holstein calves (38.56 ± 1.75 kg of BW; mean ± SD) were weighted and moved to individual pens (1.2 × 2.5 meter) with a sand bed, replaced every 24 to 48 h as needed. They had been fed with four liters of colostrum within 6 h of birth and colostrum feeding continued for the first 3 days of their lives. Blood samples were taken from the jugular vein by venipuncture at 24 h after the first colostrum intake and were sent to the laboratory for analysis. Serum total protein was determined as an indicator of passive transfer of immunity. Neonatal calves with the serum level of protein greater than 6 gr/dl remained in the study (Mojahedi et al., 2018). Calves were bucket-fed twice a day with pasteurized whole milk; all calves were weaned at day 56 of the experiment.

Calves (n=12, six males and six females) were randomly allocated to four treatments in a completely randomized design. Treatments were: (1) 18% CP starter without AA (Amino Acid), (2) 18% CP with 0.0340% protected lysine and 0.016% protected methionine, (3) 18% CP starter with 0.215% un-protected lysine and 0.012% un-protected methionine, (4) 22% CP without AA.

Amino acids were added to experimental diets at the feed factory of the dairy farm and mixed thoroughly with other ingredients. The characteristics and amount of used amino acids were as shown below:

(1) Rumen-protected methionine, SmartamineM brand name, manufactured by Adisseo Co. with 74% purity at 0.016% of the diet. (2) DL Methionine, Rhodiment brand name, manufactured by Adisseo Co. with 99% purity at 0.012% of the diet. (3) Rumen protected lysine, LysiPearl brand name, manufactured by Kemin Co. with 50% purity at 0.340% of the diet. (4) L-Lysine, ADM, manufactured by Archer Daniels Midland Company with 78.8% purity at 0.215% of the diet.

Components of the experimental diets and their nutrients are shown in Table 1. Before the experiment initiation, basal diet (diet one) was made in the feed plant of the dairy farm, then a sample was taken and sent to the specialized laboratory to determine the current level of methionine and lysine in the diet using Pico-Tag, HPLC method (White et al., 1986) an additional level of methionine and lysine (20% more) were included in the diets according to the laboratory results.

Table 1. Ingredients, energy and nutrient composition of experimental diets fed to dairy calves for 70 days

Item	Experimental Diets			
	18CP	18CP+PAA	18CP+uPAA	22CP
Corn grain, %	63.15	62.794	62.923	51.300
Soybean meal, %	23.9	23.9	23.9	35.95
Soybean seed, %	5	5	5	5
Fat Supplement[1], %	2.95	2.95	2.95	2.75
Di- Calcium phosphate, %	2	2	2	2
Salt, %	0.5	0.5	0.5	0.5

Item	Experimental Diets			
	18CP	18CP+PAA	18CP+uPAA	22CP
Sodium bicarbonate, %	1	1	1	1
Mineral Supplement[2], %	0.75	0.75	0.75	0.75
Vitamin Supplement[3], %	0.75	0.75	0.75	0.75
Added Methionine[4,5], %	0	0.016	0.012	0
Added Lysine[6,7], %	0	0.340	0.215	0
Chemical Composition of the Diets				
Crude Protein (%), lab result	18.35 ±0.84	18.35 ±0.84	18.35 ±0.84	21.85 ±0.79
CP (%), AminoCow software*	18.46	18.43	18.44	22.63
Met, gr/kg	2.56	2.64	2.62	2.61
Lys, gr/kg	9.14	9.14	9.14	9.69
Lys: Met ratio	3.57	3.46	3.48	3.71
RDP, %	61.36	61.35	61.35	63.92
RUP, %	38.64	38.65	38.65	36.08
Microbial Protein (gr)	121.86	121.93	121.90	121.19
Total absorbable protein (gr)	161.99	162.01	161.99	170.82
ME (Mcal/kg)	3.18	3.18	3.18	3.19
NEg (Mcal/kg)	1.49	1.49	1.49	1.49
NEm (Mcal/kg)	1.96	1.96	1.96	2.1

AA= Amino acid; RDP= rumen degradable protein; RUP= rumen un-degradable protein; ME = metabolizable energy; NEG= net energy for growth; NEM= net energy for maintenance

1 Contained stearic acid, 5% of DM; palmitic acid, 30% of DM; oleic acid, 11% of DM; linoleic acid, 50% of DM; and linolenic acid, 4% of DM.

2 Contained (mg/kg) Co, 100; Cu, 4,290; I, 200; Mn, 10,000; Zn, 20,000; Mg, 67,500; and Ca, 240,000.

3 Contained (IU/kg) vitamin A, 1,300,000; vitamin D, 360,000; and vitamin E, 12,000.

4 Rumen protected methionine, SmartamineM brand name, manufactured by Adisseo co. with 74% purity.

5 DL Methionine, Rhodiment brand name, manufactured by Adisseo Co. with 99% purity.

6 Rumen protected lysine, LysiPearl brand name, manufactured by Kemin Co. with 50% purity.

7 L-Lysine, ADM, manufactured by Archer Daniels Midland Company with 78.8% purity.

* Despite adding amino acids to rations in protected or non-protected form, the CP percentage of diets are nearly similar to each other because the percentage of corn is reduced to maintain the total amount of ingredients constant.

Measurements, sampling, and analyses

The equal amount of milk was fed to all calves. Starter intake was measured every morning. Calves were weighed by 7-day intervals using an electronic balance. Average daily weight gain and feed efficiency ratio (kg of BW gain/kg of total dry matter intake) were computed for pre-weaning, post-weaning, and the overall experimental period. Blood samples were obtained from the jugular vein by venipuncture at day 30 and 70 of the study into an evacuated tube containing clot activator 3 h after morning feeding, and they were immediately placed on ice. Tubes were centrifuged at 3,000 × g for 15 min to separate the serum, reserved at

−20 °C for further analysis. Serum creatinine and urea were specified by colorimetric and enzymatic assays (Pars Azmoon Co., Tehran, Iran). Serum IgG was determined according to the immunoturbidimetric method using a commercial kit (Pars Azmoon Co., Tehran, Iran). Total protein was measured photometrically based on the Biuret method using a commercial kit (Pars Azmoon Co., Tehran, Iran). Feed samples were taken weekly from all diets, and were oven-dried and kept for subsequent analysis for CP. Skeletal growth including the length of body, body girth, height of withers, height of hip and the width of the hip of the calves were recorded at the start, weaning (day 56) and at the end of the study (day 70) according to the manner characterized in the study by Khan et al. (2007). As calves were fed with whole pasteurized milk produced in a dairy farm, two bulk milk samples from two consecutive days were collected bi-weekly and kept at 4 °C in tubes containing potassium dichromate as a preservative until analysis by Milkoscan. A total solid concentration of milk samples was used for the calculation of total whole milk DMI (Dry Matter Intake). Spot urinary samples were taken directly by stimulating the urinary canal on the last day of the experiment. These samples were immediately frozen at −15 °C for subsequent analysis.

Statistical analysis

The study was conducted based on a completely randomized design. Data on body weight, starter consumption, ADG, and Gain-to feed ratio (G: F) were analyzed using the MIXED procedure in SAS software (version 9.4, SAS Institute Inc., Cary, NC) along with Repeated Measures for pre-weaning (from day 1 to 56 of the study), post-weaning (from day 57 to 70 of the study), and overall period (from day 1 to 70). Initial values of body measurements were considered as a covariate for the analysis of body measurements. Significance was declared at $P<0.05$ and trends were considered when $0.05 < P < 0.10$. Means analysis was conducted using LSD for the probability. The statistical model was as below:

$$Yij=\mu+ Si+ Ti+ \beta(Xi-X) + eij$$

in which Yij is the dependent variable; μ is the overall mean; Si is the sex of calf; Ti is the effect of treatment; and eij is the residual error.

The comparison among treatments was done using 3 independent contrasts. The first contrast compares the level of protein (18% versus 22%) in starter diet; second contrast compares using AA in treatments against non-using AA diets and the third contrast compares protected AA versus unprotected one.

Results

Feed intake and growth

Starter intake, ADG, and feed efficiency data are reported in Table 2. Calves fed starter diet containing 22% CP without supplemented AA had the greatest amount of feed intake, total and pre-weaning ADG, weaning and final weight ($P<0.05$). However, feed efficiency was not different among treatments ($P>0.05$), neither during the whole experiment nor before and after weaning. Also, starter intake before weaning and ADG after weaning was not different among treatments ($P>0.05$). The contrasts show the difference between two treatments (CP18% versus 22%) as calves fed with 22% CP in starter diet had higher feed intake, ADG pre-weaning and during whole experiment and body weight ($P<0.05$). However, there was not any difference between using or not using AA and also between PAA or unprotected one in starter intake, ADG, gain to feed ratio and body weight.

Table 2. . Effects of crude protein level and adding rumen protected lysine and methionine or non-protected one on intake, ADG, feed efficiency, and BW of dairy calves

Item	Experimental Diets				SEM	P-Value	P-Value		
	18CP	18CP+PAA	18CP+uPAA	22CP			18CP vs 22CP	18CP vs 18CP+AA	PAA vs uPAA
Average daily milk DMI, kg/d	0.575	0.575	0.575	0.575	-	-	-	-	-
Starter intake, kg/d									
Pre-weaning	0.405	0.429	0.394	0.550	0.0461	0.109	0.018	0.904	0.596
Post-weaning	1.637^{b}	1.464^{b}	1.531^{b}	1.941^{a}	0.0880	0.006	0.001	0.213	0.598
Overall	0.651^{b}	0.636^{b}	0.622^{b}	0.828^{a}	0.0500	0.032	0.003	0.718	0.837
ADG, kg/ d									
Pre-weaning	0.579^{b}	0.580^{b}	0.596^{b}	0.708^{a}	0.0311	0.029	0.003	0.818	0.729
Post-weaning	0.613	0.671	0.783	0.796	0.0842	0.370	0.315	0.281	0.353
Overall	0.584^{b}	0.599^{b}	0.633^{ab}	0.725^{a}	0.0305	0.017	0.003	0.430	0.422
Gain- Feed ratio[1]									
Pre-weaning	0.619	0.595	0.639	0.668	0.0203	0.124	0.053	0.936	0.137
Post-weaning	0.426	0.566	0.571	0.459	0.0711	0.382	0.478	0.104	0.961
Overall	0.562	0.579	0.620	0.606	0.0194	0.147	0.429	0.116	0.138
Body-weight (kg)									
Initial (d 3)	38.570	38.570	38.570	38.570	-	-	-	-	-
Weaning (d 56)	71.430^{b}	71.501^{b}	72.376^{b}	78.658^{a}	1.8840	0.029	0.001	0.818	0.729
Final (d 70)	79.888^{b}	80.936^{b}	83.359^{ab}	89.757^{a}	2.1541	0.017	0.003	0.430	0.422
CP intake, gr/d[2]	119.551^{b}	116.613^{b}	114.052^{b}	180.577^{a}	10.0523	0.001>	0.001>	0.729	0.854
Lysine intake, gr/d[2]	5.955^{b}	5.814^{b}	5.683^{b}	8.022^{a}	0.4765	0.005	0.001>	0.721	0.842
Methionine intake, gr/d[2]	1.668^{b}	1.681^{b}	1.629^{b}	2.162^{a}	0.1306	0.031	0.003	0.937	0.782

AA=amino acid
1 milk consumption is included
2 only from starter
a,b Values within a row with different superscripts differ significantly at $P<0.05$.

Skeletal growth

Table 3 shows the data related to skeletal growth. Body length at weaning and the end of the experiment showed significant differences between treatments as calves received 18% CP with unprotected AA had the highest value ($P<0.05$). As other skeletal growth factors did not reveal the significant difference, it cannot be concluded whether CP level or using protected AA affect skeletal growth. The contrasts show that only protected AA versus unprotected one, resulted in a difference in body length ($P<0.001$).

Immunity measurement

Immunity measurements of calves including IgG and total protein concentration in serum are presented in Table 4. IgG and total protein in serum did not show any significant differences between treatment neither at day 30 nor at day 70 ($P>0.05$). Also there was not any difference between two levels of CP in the starter diet, using AA or not using it and the kind of AA ($P>0.05$).

Table 3. Effects of crude protein level and adding rumen protected lysine and methionine or non-protected one on skeletal growth of dairy calves

Item	Experimental Diets				SEM	P-Value	P-Value		
	18CP	18CP+PAA	18CP+uPAA	22CP			18CP vs 22CP	18CP vs 18CP+AA	PAA vs uPAA
Body length, cm									
Birth	45.3	45.1	45.3	44.5	0.920	0.923	0.523	0.931	0.843
Weaning	54.5^a	51.8^b	55.2^a	54.9^a	0.652	0.003	0.245	0.254	0.001>
Final	55.5^a	52.9^b	56.3^a	55.8^a	0.652	0.003	0.243	0.230	0.001>
Heart Girth, cm									
Birth	79.4	77.5	79.4	78.6	0.770	0.233	0.843	0.332	0.071
Weaning	91.8	91.8	91.8	91.3	0.780	0.065	0.368	0.293	0.057
Final	102.2	99.6	102.8	103.6	1.160	0.123	0.157	0.476	0.056
Withers height, cm									
Birth	74.3	74.1	76.0	75.3	0.703	0.068	0.638	0.240	0.059
Weaning	82.2	81.5	83.0	83.5	0.588	0.084	0.071	0.963	0.056
Final	89.8	88.6	89.3	90.9	0.806	0.296	0.097	0.379	0.525
Hip height, cm									
Birth	78.3	77.8	79.1	78.6	0.657	0.501	0.818	0.822	0.137
Weaning	90.6	89.7	89.7	91.4	0.833	0.489	0.184	0.380	0.977
Final	93.2	91.7	911.9	93.5	0.800	0.355	0.224	0.163	0.899
Hip width, cm									
Birth	17.3	17.5	17.6	17.5	0.242	0.056	0.053	0.429	0.735
Weaning	19.8	19.8	19.8	19.6	0.311	0.978	0.669	0.950	0.985
Final	20.8	20.7	20.7	20.6	0.301	0.958	0.659	0.830	0.965

AA= amino acid

a,b Values within a row with different superscripts are significantly different ($P < 0.05$)

Table 4. Effects of crude protein level and adding rumen-protected lysine and methionine or non-protected one on immunity measurement of dairy calves

Item	Experimental Diets				SEM	P-Value	P-Value		
	18CP	18CP+PAA	18CP+uPAA	22CP			18CP vs 22CP	18CP vs 18CP+AA	PAA vs uPAA
IgG concentration, mg/dl, d 30	143.460	206.980	160.870	135.960	54.4581	0.838	0.576	0.553	0.561
IgG concentration, mg/dl, d 70	26.869	205.640	77.327	17.255	57.8880	0.190	0.408	0.093	0.992
Total Protein, gr/dl, d 30	6.660	6.658	6.288	6.509	0.1405	0.196	0.849	0.245	0.069
Total Protein, gr/dl, d 70	6.501	6.536	6.568	6.670	0.1703	0.914	0.529	0.805	0.893

AA= amino acid; IgG =immunoglobulin G; d=day
a, b Values within a row with different superscripts are significantly different (P<0.05)

Health score

The results of health scores are presented in Table 5. Health scores except for fecal score, was not different between treatments. Calves received 18% CP with unprotected AA had the lowest (better) fecal score (P<0.05). Contrasts between treatment groups showed that using AA versus not using it and protected AA versus unprotected one resulted in a lower fecal score (P<0.05).

Urea and creatinine of serum

Results of serum urea and creatinine are presented in Table 6. There were no differences in serum creatinine and urea between treatments on day 30 of the experiment. However, the dietary effect on urea was significant at day 70 (P<0.05). Contrasts show that calves that received 18% CP, had less serum urea at day 70 (P<0.05), and calves fed with 22% CP in starter diet had less serum creatinine at day 30 (P<0.05).

Table 5. Effects of crude protein level and adding rumen-protected lysine and methionine or non-protected one on health score of dairy calves

Item	Experimental Diets				SEM	P-Value	P-Value		
	18CP	18CP+PAA	18CP+uPAA	22CP			18CP vs 22CP	18CP vs 18CP+AA	PAA vs uPAA
Eye Score	0.188	0.182	0.149	0.155	0.0380	0.861	0.705	0.636	0.543
Ear Score	0.212	0.226	0.179	0.21	0.0451	0.898	0.946	0.858	0.458
Nose Score	0.241	0.211	0.167	0.188	0.0432	0.644	0.738	0.320	0.466
Respiratory Score	0.138	0.096	0.155	0.223	0.0365	0.154	0.054	0.770	0.251
Fecal Score	0.492^{a}	0.530^{a}	0.174^{b}	0.421^{a}	0.0584	0.0001	0.759	0.048	0.001>

a, b Values within a row with different superscripts are significantly different (P < 0.05)

Serum amino acids level

The amount of lysine and methionine amino acids in serum are presented in Table 6. There was not any significant difference among treatments (P>0.05). Also, there was not any difference between the level of CP, using AA versus not using it, or the kind of AA (P>0.05).

Table 6. Effects of crude protein level and adding rumen-protected lysine and methionine or non-protected one on serum parameters of dairy calves

Item	Experimental Diets				SEM	P-Value	P-value		
	18CP	18CP+PAA	18CP+uPAA	22CP			18CP vs 22CP	18CP vs 18CP+AA	PAA vs uPAA
Urea, d 30 mg/dl	18.775	19.770	14.487	22.977	2.2661	0.367	0.369	0.292	0.541
Creatinine, d 30 mg/dl	1.403	1.368	1.369	1.064	0.1210	0.123	0.058	0.333	0.949
Urea, d 70 mg/dl	21.739[b]	20.376[b]	23.380[b]	31.644[a]	2.1315	0.009	0.001	0.958	0.328
Creatinine, d 70 mg/dl	1.209	1.096	1.140	1.181	0.0900	0.834	0.920	0.641	0.923
Lysine, µmol/l	90.407	105.800	92.877	117.230	8.2264	0.125	0.052	0.378	0.282
Methionine, µmol/l	22.586	26.552	23.859	27.985	2.0000	0.240	0.143	0.285	0.348

AA= amino acid; IgG =immunoglobulin G; d=day

a, b Values within a row with different superscripts are significantly different (P<0.05)

Discussion

In the study done by Lee et al. (2012), cows received lower CP in their diet and had lower feed intake too, which is in agreement with the present study. Also, in their study, adding protected lysine and methionine led to the same amount of feed intake that is again in line with the present study as there was no difference between treatments with the addition of AA, either protected or unprotected. So, maybe using AA in any form does not have any effect on calves feed consumption. In the study done by Tamura et al. (2019) no differences were found in milk yield, milk composition or cow health between cows received protected methionine and those who did not receive. Also, in the present study, there was no significant difference in performance (gain to feed ratio) of dairy calves.

In another study conducted by Socha et al. (2005) on cows in a transition period, there was no difference in feed intake with 2 levels of CP (16 and 18.5%) and basal diet and diet supplemented with rumen- protected lysine and methionine. While in the present study, during the whole experiment, higher CP in the starter led to higher feed intake which is probably due to higher total absorbable protein in diets with higher CP level.

In a study done by Kazemi-Bonchenari et al. (2016) on pre-weaned calves, there was no interaction between RUP and fat, nor was there any effect of high RUP on growth characteristics that are in line with the present study. It has been found that greater RUP content improved feed efficiency in dairy calves by reducing the intake but not increasing the gain (Kazemi-Bon-chenari et al., 2015). In the present study, no beneficial effects of high RUP content were observed related to ADG or feed efficiency which was in agreement with Swartz et al. (1991). Because the rumen is not fully functional in pre-weaned calves, providing RUP with protected amino acids may have had little effect on the amounts of AA composition of protein in the small intestine. More research with other sources of RUP might be able to determine if delivery to the small intestine of a RUP with different AA compositions may increase calf performance. Incomplete development of rumen function as well as an immature resident microbial population in pre-weaned calves, which may result in similar RUP contents of feedstuffs (Holtshausen and Crywagen, 2000), may explain why there are no conclusive benefits to formulating starters with higher RUP sources in the present study.

Variation in the starter intake by calves is the cause of more than half of the variation in weight gain in the same period. But in the present study, although calves that consumed a higher amount of starter had higher ADG, there was not a difference in feed efficiency between treatments. So, neither CP level nor the kind of AA affected these parameters. Although 18% of CP provided more microbial protein, 22% CP level provided more absorbable protein which neutralized their effect on each other, and led to a lack of feed efficiency between treatments.

The observed differences between skeletal growth factors were the result of low repeat per treatment or management. In a study done by Stamey et al. (2012), the kind of starter (two-level of CP; enriched and conventional) did not affect on skeletal growth which is in agreement with the present study. But in a study done by Tahmasebi et al. (2014), the protein source of the starter affected heart girth size that is not in line with this study. In a study done by Margerison, et al. (2013), at weaning, calves fed whole milk plus amino acids and plant carbohydrates had greater mean BW gain, a lower number of days to target BW, and a greater mean hip-width gain compared with calves fed with whole milk, which is not in agreement with present study as there were no differences between treatments that received amino acids and treatments that did not. But in their study, the mean gain in hip height did not differ among treatments, which is line with our study.

Moallem et al. (2004) found that increasing RUP in post-weaning heifers could be used to accelerate simultaneous increases in skeletal growth rates, whereas Kazemi-Bonchenari et al. (2015) observed no effect of RUP level on body measurements in pre-weaning calves. Bethard et al. (1997) indicated that in post-weaning heifers, the RUP level had no effect on hip height but that dietary energy greatly increased it. Generally, it appears that in the pre-weaning starter, the RUP level does not influence the body measurements of calves.

Total serum protein is correlated with IgG, and total protein above 5.2 gr/dl indicates the good inactive immunity transfer in a healthy calf (Wilm, 2018). As contrasts in Table 4 show, neither CP level nor the kind of AA had any effect on IgG concentration and total serum protein, indicating that dietary treatments did not have any effect on the immunity of calves. In the study conducted by Vailati-Riboni et al. (2017), using methionine pre-calving led to a higher immune response which is not in agreement with the present study. In a study done by Senevirathne et al. (2017), the level of CP did not affect diarrhea frequency which is in agreement with the present study as a fecal score of calves that consumed 22% CP was not different with calves that consumed 18% CP. The results of the study by Brscic et al.

(2014) showed that cows that received more CP/d, had fewer days of treatment for respiratory disease. While in the present study, the respiratory score was not different among treatments (*P*>0.05) that is in agreement with a study done by Ghassemi et al. (2013) that showed that different level of starter intake (and consequently different amount of CP), had no significant effect on respiratory score and days of drug administration for pneumonia and diarrhea. Besides, in a study done by Silva et al. (2018), supplementation of milk replacer with lysine and methionine and the association with glutamate and glutamine did not affect performance, fecal scores or metabolism of calves.

So it appears that neither CP level nor the kind of AA has any effect on the immunity and health situation of calves. That maybe because their basal diet was not deficient in essential amino acids.

In a study done by Klemesrud et al. (1998) adding rumen-protected lysine and methionine to the diet of fattening calves, did not have any effect on protein utilization which is in agreement with present study as there was not any difference between rumen-protected and unprotected ones.

Blood urea nitrogen concentration has a positive linear relationship with dietary CP intake, its ruminal degradability, and resultant ruminal ammonia concentration in cattle (Lohakare et al., 2006). More protein intake because of greater solid feed consumption and its ruminal degradation has probably resulted in greater concentrations of BUN in calves fed diet with higher CP. Greater concentration of BUN is also an index of renal dysfunction (Khan et al., 2007b); however, in this study the blood creatinine concentration in all calves was in the safe range and did not differ between treatments.

In a study done by Lee et al. (2012b), supplementing diets that were low in concentration of microbial protein with rumen-protected methionine and lysine, did not increase the serum level of lysine or methionine which is in agreement with the present study. In another study done by Rulquin et al. (2006), serum methionine concentration increased 110 and 65 percent after adding methioninand Smartamine respectively. In the study done by Tsukano et al. (2017), results showed that increases in plasma total amino acid (TAA) and branched-chain amino acid (BCAA) concentrations in diarrheic calves with severe acidemia were the result of an acceleration in proteolysis, similar to that in humans. In a study done by Tsukano and Suzuki (2019), concentrations of plasma essential amino acids, non-essential amino acids, branched-chain amino acids, glucogenic amino acids, and ketogenic amino acids in diarrheic calves with hypoaminoacidemia were significantly lower than those in healthy calves. No significant differences were observed between diarrheic calves with normoaminoacidemia and healthy calves when looking at these parameters that are in line with the resent study, because the concentration of serum lysine and methionine in calves with a low or high fecal score, was not different as they all were normoaminoacidemia. Lack of increase in methionine may indicate that diets were not deficient in energy or amino acids as a shortage of energy or other amino acids probably responds to methionine supplementation.

In conclusion, the results of the present study showed that higher CP levels in calf starter and adding amino acids to starter diet, either in protected form or unprotected one, led to some significant differences in ADG and body weight but did not result in overall calf performance regarding feed efficiency. In addition, there were not any differences between treatments in health status considering the amount of serum IgG and protein and health scores. Overall, it could be concluded that using amino acids in calf starter does not result in any significant differences.

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Conflict of Interest

The authors declared that there is no conflict of interest.

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اثر متقابل بین سطح پروتئین خام و اسیدآمینه های محافظت شده در شکمبه در استارتر بر عملکرد گوساله های شیرخوار

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چکیده

زمینه مطالعه: مطالعات محدودی در مورد اثر افزودن اسیدهای آمینه در استارتر گوساله ها وجود دارد.

هدف: هدف مطالعه حاضر، ارزیابی سطوح مختلف پروتئین خام در استارتر و نیز مقایسه اثر افزودن برخی اسیدهای آمینه پوشش دار روی بازده، سلامتی و متابولیت های خونی گوساله های شیرخوار و مقایسه آن با نوع معمولی بود.

روش کار: ۴۸ گوساله هلشتاین ۳ روزه به طور تصادفی به ۴ تیمار در قالب طرح کاملا تصادفی اختصاص یافتند. جیره های آزمایشی شامل (۱) ۱۸درصد پروتئین خام در استارتر بدون اسیدآمینه، (۲) ۱۸درصد پروتئین خام با ۰/۰۳۴ درصد لیزین و ۰/۰۱۶ درصد متیونین پوشش دار بیشتر، (۳) ۱۸درصد پروتئین خام با ۰/۲۱۵ درصد لیزین و ۰/۰۲ درصد متیونین معمولی و (۴) ۲۲ درصد پروتئین خام بدون اسیدآمینه بود. همه گوساله ها مقدار یکسان شیر کامل دریافت کردند، در ۵۶ روزگی شیرگیری شده و مطالعه در ۷۰ روزگی خاتمه یافت.

نتایج: گوساله ها در تیمار ۴ بالاترین مصرف استارتر ، وزن شیرگیری و وزن نهایی را داشتند ($P<۰/۰۵$) ولی بازده مصرف خوراک آنها با سایر تیمارها متفاوت نبود. تفاوت معنی داری در میزان ایمونوگلوبولین جی ،پروتئین تام و مقدار لیزین و متیونین سرم خون وجود نداشت ($P>۰/۰۵$). نمره سلامتی، به غیر از نمره مدفوع، بین تیمارها متفاوت نبود. استارتر با ۲۲ درصد پروتئین خام منجر به مقدار اوره سرمی بالاتر شد ($P<۰/۰۵$).

نتیجه گیری نهایی: نتایج مطالعه حاضر نشان داد که تفاوتی در عملکرد گوساله هایی که اسید آمینه محافظت شده یا معمولی در استارتر دریافت کردند، وجود ندارد. همچنین سطح پروتئین خام بالاتر در استارتر منجر به بهبود نسبت افزایش وزن به مصرف خوراک نشد.

واژه‌های کلیدی:

گوساله شیرخوار، لیزین، متیونین، محافظت شده در شکمبه، استارتر.

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