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Abstract

The objective of this research was to determine the effect of different starters consisted of dried whey and soy proteins with low anti-nutritive factors (ANFs) + amino acids (lysine and methionine) on body measurements, biochemical parameters, and feed consumption of early-weaned female Holstein calves. Based on the starter, calves were divided into four groups: i) control (control starter), ii) E1 (starter with added dry whey), iii) E2 (starter with added soy proteins with low ANFs + lysine and methionine), and iv) E3 (starter with added dry whey and soy proteins with low ANFs + lysine and methionine). Results indicate that different starters based on whey powder and soy proteins with low ANFs + lysine and methionine significantly affected the consumption of milk replacer and starter, the total consumption of dry matter, then withers in height, and body length (among body traits) as well as NEFA (among blood biochemical parameters) of female Holstein calves. Finally, starters based on dried whey and soy proteins with low ANFs + lysine and methionine could be recommended to optimize the growth and feed consumption.

Key words: calves; starter; consumption; body traits; biochemical traits

Introduction

During the process of rearing dairy calves, the use of adequate feeding technology can improve efficiency and economy of dairy cattle farming. According to Tozer and Heinrichs (2001), initial phase of rearing with liquid feed is the most expensive phase in the dairy business, representing approximately a fifth of a dairy farm's total cost. Drackley (2008) emphasized the importance of physical and metabolic development of reticulorumen since it provides an easier transition from pre-ruminant to mature ruminant state. Furthermore, Davis-Rincker et al. (2011) concluded that fast development of the digestive system is important for good immunological status of an animal, since it influences a long-term animal performance.

The consumption of solid feed in calf starter can affect ruminal epithelial development by altering microbial fermentation and products (Suarez et al., 2007; Khan et al., 2007a). This practice could increase butyrate production in the rumen and volatile fatty acid (VFA) which is responsible for development of epithelial tissue in reticulorumen (Bergman, 1990; Hill et al., 2006). The pre-weaned calf relies on lactose and fat as the primary energy sources, while supplementation of VFA is crucial for weaned ruminants. Baldwin and Connor (2017) pointed out the importance of the development of the pre-weaned calves for the absorption of VFA from the diet.

Morrill and Dayton (1974) concluded that concentrate with up to 10 % of whey powder can increase feed intake in young calves. Inclusion of lactose in the feeding process can increase the ruminal butyrate concentration (DeFrain et al., 2006; Oba et al., 2015), and can also increase the dry matter intake in mature cows (DeFrain et al., 2004). Seagusa et al. (2017) indicated that lactose inclusion up to 10 % of dry matter in calf starters did not affect the dry matter intake as well as growth performance of calves. On the other hand, they find out that higher lactose content and hay intake might be associated with decreased severity of sub-acute ruminal acidosis (SARA). Furthermore, according to Movahedi et al. (2016), growth performance of dairy calves can differ depending on forage sources that were used. According to those authors, the use of starter plus dried sugar beet pulp flakes in comparison with the other forages, can be a valuable source of forage for dairy calves during the transition from liquid to solid feed. Lallés et al. (1996) noted that soya bean meal (SBM) is often used as plant protein source in calf starters, but SBM contains anti-nutritive factors (ANFs) and allergens such as trypsin inhibitors, glycinin, ß-conglycinin, and oligosaccharides. Tukur et al. (1993) reported that ß-conglycinin is very resistant to digestion of the young calf.

Body measurements could be used for growth monitoring of female dairy cattle, primarily for estimation of body weight (Heinrichs et al., 1992.) and for determination of nutritional requirements (NRC, 2001). Some research (Morrill and Dayton, 1974; DeFrain et al., 2004; DeFrain et al., 2006; Oba et al., 2015; Seagusa et al. 2017) indicated that different types of starters influence the performances of calves.

The objective of this study was to evaluate the effect of the different feeding groups (including dried whey and soy proteins with low ANFs + lysine and methionine in the diet) on starter consumption, body measurements and blood biochemical parameters of early weaned female Holstein calves. It was hypothesized that whey and soy proteins with low ANFs would ensure dairy calves the easiest transition from milk replacer to solid feed by easier accessible nutrients.

Material and methods

The research was performed on a commercial dairy cattle farm in East Croatia. Twenty Holstein female calves with an average birth weight of 40.03±2.66 kg were selected and randomly allocated into 4 groups (5 calves per group). The experiment lasted until an average calf's age of 91 days. All calves had the same feeding plan during the experiment. They were fed with 4 L of colostrum within 2 h after birth. After administration of colostrum by drench, calves were fed first three days with non-pasteurized whole milk from transition cows (3 L twice a day). After that, they were fed with a solution of milk replacer (143 g/L of water) according to the feeding scheme presented in Table 1. From the 4th day of life, calves had *ad libitum* access to water and solid feed.

Based on given starters, calves were allocated into 4 groups as presented in the Table 2. Ingredients and nutrient composition of fed starters are shown in Table 3.

Furthermore, calf premix was provided (per kg of the starter) as follows: Vitamin A 10.000 IU, Vitamin D 2.000 IU, Vitamin E 40 mg, Vitamin B1 1 mg, Vitamin B2 2 mg, Vitamin B5 10 mg, Vitamin B6 10 mg, Vitamin B12 0,013 mg, nicotinic acid 30 mg, Fe 50 mg, Cu 12,5 mg, Mn 40 mg, Zn 41 mg, Se 0,3 mg, I 0,75 mg, and choline chloride 150 mg.

After weaning, each group of calves was fed with the same starter they ate before weaning till an average of 63 days of life. Then, all calves in the experiment were eating the same total mix ration (TMR).

The measurements of the traits analysed in this research were performed two times, at 50 days and at 91 days of age. Body measures were obtained using Lydtin's rod (wither height, hip height, body length, chest depth, and chest width) and measuring tape (hip-width, heart girth, and cannon bone circumference). For biochemical analysis, blood samples were taken and blood plasma was separated. Blood plasma samples were analysed on a biochemical analyser OLYMPUS AU 400 and the following parameters were determined: gamma-glutamyltransferase (GGT), glucose, urea, total protein, albumin, iron, phosphorus, calcium, non-esterified fatty acids (NEFA), ß-hydroxybutyric acid (BHBA) and insulin-like growth factor 1 (IGF-1). Finally, the consumption of the milk replacer solution (difference between the amount of offered and unconsumed) and the starter was monitored every day.

Analysed traits (body measurements, biochemical parameters, and consumption) in different feeding group (C,

Table 1. Milk replacer feeding scheme of calves

Period in days	Frequency, per day
4th-35th	2 x 3 L
36th-42nd	2 x 2 L
43rd-49th	1 x 2 L

Table 2. Starter feeding scheme by different trial groups

Group	Feeding
C (control)	Control starter
E1 (experimental 1)	Starter with added whey powder
E2 (experimental 2)	Starter with added soy proteins with low ANFs + lysine and methionine
E3 (experimental 3)	Starter with added d whey powder and soy proteins with low ANFs + lysine and methionine

Table 3. Ingredients and nutrient composition of the starters (air dry matter basis)

Ingredients, % / Group	C	E1	E2	E3
Wheat bran	25	25	25	25
Toasted full fat soy	10	10	10	10
Limestone	1	1	1	1
Mono-calcium phosphate	1.1	1.1	1.1	1.1
Mannan oligosaccharide (commercial Bio-Mos)	0.2	0.2	0.2	0.2
Aroma vanilla	0.05	0.05	0.05	0.05
Pellet binder	0.1	0.1	0.1	0.1
Calf premix[1]	0.25	0.25	0.25	0.25
Dry sugar beet pulp	3	3	3	3
Molasses	3	3	3	3
Dextrose	1	1	1	1
Salt	0.5	0.4	0.5	0.4
Corn	37.7	32	42.4	36.5
Soya meal	14.1	12.9	-	-
Rapeseed meal	3	3	-	-
Nucleotide (commercial NuPro)	-	-	3	1
Soya protein concentrate	-	-	7.3	7.9
DL-methionine	-	-	0.5	0.5
L-lysine HCl	-	-	1.6	1.6
Whey powder	-	7	-	7.4
Nutrient composition				
Dry matter (%)	88.15	88.64	88.91	89.34
Crude protein (%)	18.03	18.01	18.01	18.03
Crude fats (%)	4.00	3.85	3.84	3.74
Starch (%)	31.18	27.32	34.24	31.04
Lactose (%)	-	5.55	-	5.87
Carbohydrates total (%)	5.80	11.22	5.27	10.08
Lysine (%)	0.91	0.93	2.11	2.13
Methionine (%)	0.28	0.28	0.74	0.74
Ash (%)	6.16	6.65	6.00	6.34
ME (MJ/kg)	11.60	11.58	11.77	11.78

E1, E2, end E3) were tested separately for each measurement (I., and II.) using least square means in GLM procedure in SAS (SAS Institute Inc., 2000). Following statistical model was used:

$$y_{ijk} = \mu + b_1 a_i + b_2 a_i^2 + T_j + e_{ijk}$$

Where:

y_{ijk} = estimated trait (body measurements, biochemical parameters and consumption);

μ = intercept;

b_1, b_2 = regression coefficients;

a_i = age at measurement;

T_j = fixed effect of feeding group j (j = C/E1/E2/E3);

e_{ijk} = residual.

Scheffe's multiple comparisons in PROC GLM procedure in SAS (SAS Institute Inc., 2000) were used to test the differences in analysed traits (body measurements, biochemical parameters, and consumption) due to different feeding groups (C, E1, E2, end E3).

Results and discussion

Morrill and Dayton (1973) observed that starter consummation tended to increase when 10 % of whey was included. They also noticed that the highest inclusion of whey in calf starter depressed consumption. Feng et al. (2007) presented results regarding the influence of fermented soybean meal in comparison to soybean meal on the growth of piglets. They found that fermented soybean meal had a positive influence on piglet's growth, due to reduced ANFs and the greater concentrations of essential amino acids (AA) Following these findings, the influence of soy proteins with low ANFs was investigated on calves in this study. Statistical analyses showed that the experimental treatments did not differ (P>0.05) in body traits of calves at first measurement (Table 4). The withers height at the second measurement was highest (P<0.05) for the animals in the E1 group. Furthermore, a statistically significant differences (P<0.05) in wither heights were determined between animals in the E2 group and the E1 and E3 group (90.90: 92.25; 90.94 cm). Regarding body length (Table 4), animals in the E3 group had shorter (P<0.05) body length comparing to animals in groups C and E1 (90.07: 90.42; 90.40 cm), and longer (P<0.05) from animals in E2 group (90.07: 83.51 cm).

Calves from this research had a longer body, in comparison with body length from the research of the Mirghaffari et al. (2012). Although measured body weights of experimental groups were numerically different, the differences were not significant (P>0.05). Body weight of the calves from research by Pezhveh et al. (2014) was higher compared with calves measured in this research (52 average days of life), but lower in older calves (70 average days of life). The study tested effects of partially replacing corn with 2 forms of wheat on skeletal growth, blood metab-

Table 4. Least-squares means (LSMs) of body measurements of calves from different feeding groups at 50 (I. measurement) and 91 (II. measurement) day of age

Trait	I. measurement				II. measurement			
	C	E1	E2	E3	C	E1	E2	E3
Body weight, kg	59.87	62.75	61.79	62.39	93.33	97.20	87.26	99.32
Withers height, cm	84.30	83.71	83.47	84.72	86.70[A]	92.25[B]	90.90[AB]	90.94[B]
Hip height, cm	88.93	87.02	89.13	89.11	94.82[AB]	97.72[B]	93.68[A]	96.59[AB]
Body length, cm	80.19	77.09	76.15	77.57	90.42	90.40	83.51	90.07
Chest depth, cm	33.63	32.19	33.90	33.59	39.54	38.97	38.58	39.80
Chest width, cm	16.51	16.84	16.07	16.18	19.37	20.47	20.03	19.42
Heart girth, cm	93.63	93.82	93.83	95.43	108.87	108.73	107.92	109.28
Cannon bone circumference, cm	11.51	11.40	11.23	11.02	12.43	12.04	12.13	12.00
Hip width, cm	24.76	24.41	24.96	24.43	28.29	28.27	27.21	28.02

**Values within the same row within each measurement (I./II.) marked with different letter differ statistically (P<0.05)*

Table 5. Least-squares means (LSMs) of blood biochemical parameters of calves from different feeding groups at 50 (I. measurement) and 91 (II. measurement) day of age

Trait	I. measurement				II. measurement			
	C	E1	E2	E3	C	E1	E2	E3
GGT (U/L)	20.52	27.26	25.85	25.64	24.43	29.93	19.30	26.74
glucose (mmol/L)	4.57	5.76	5.00	6.06	6.06	6.06	6.17	5.58
urea (mmol/L)	3.50	3.83	4.24	5.69	3.15	5.10	4.97	3.00
TP (g/L)	61.42	73.78	68.31	78.03	86.12	83.67	76.30	68.73
albumin (g/L)	32.41	35.19	31.11	37.56	36.00	36.02	34.98	31.45
Fe (μmoL/L)	29.51	28.47	15.73	29.56	38.49	31.89	32.65	25.49
P (mmol/L)	2.61	2.69	2.63	3.62	2.88	3.22	3.04	2.68
Ca (mmol/L)	2.65	2.94	2.74	3.03	3.11	2.91	3.00	2.62
NEFA (mmol/L)	0.18	0.08	0.14	0.09	0.19[A]	0.08[B]	0.10[B]	0.09[B]
BHBA (mmol/L)	0.12	0.12	0.11	0.15	0.20	0.25	0.25	0.25
IGF-1 (ng/mL)	23.15	28.23	35.05[A]	41.33	21.09	28.15	27.16	24.10

***Values within the same row within each measurement (I./II.) marked with different letter differ statistically (P<0.05); GGT - gamma-glutamyl-transferase; TP - total protein; Fe - iron; P - phosphorus; Ca - calcium; NEFA - non-esterified fatty acids; BHBA - β-hydroxybutyric acid; IGF-1 - insulin-like growth factor 1*

olites, and feed efficiency of dairy calves until weaning within 52 days and after weaning till 70 days of age. Regarding the wither heights, hip height and body length of calves from the research by Pezhveh et al. (2014) were lower than those from calves in the present research, which could be explained by the difference in calves' age. Stamey et al. (2012) analysed both male and female Holstein calves and determined higher values of body weight, wither height, body length, and heart girth than were the values in this research. Similarly, Khan et al. (2007b) determined slightly higher values of body traits compared to this research.

In order to get a good insight into the metabolic and health status of certain animals, it is very useful to use the haematological and biochemical parameters of blood analysis. The values of different blood parameters in calves depend on age, due to colostrum intake at the beginning of life and transition from liquid to dry diet, in the pre-weaning and weaning period. Furthermore, in growing calves, the feeding and the rearing system had an important effect on the values of different blood parameters (Klinkon and Ježek, 2012). The effect of feeding becomes even more apparent after the fifth week of age when the consummation of dry food (hay, starter) increases.

From all biochemical parameters, the significant (P<0.05) differences were found only for NEFA in the second measurement, all experimental groups differentiated from control (Table 5). NEFA value was lower in the experimental group compared to the control group (0.08; 0.10; 0.09: 0.19), which is a sign that energy is not obtained from adipose tissue. The values of GGT in I. measurement (about 7th week of calves age) suggest that calves were healthy according to Ježek (2007), but in the measurement II. (about 12th week of calves age) values were slightly higher. Bouda and Jagoš (1984) noted that the reference value for GGT in plasma of calves with 2 months of age is 40.8 ± 15.6 U/L and for calves 3 months of age is 31.8 ± 6.6 U/L. The increased plasma level of GGT is usually associated with liver damage (Ježek, 2007), but in our research, the level of GGT was into reference value. When comparing results from this research with results of Ježek (2007), the values of urea, calcium, and phosphorus were similar,

Table 6. Least-squares means (LSMs) of milk replacer and starter consumptions of calves from different feeding groups at 50 (I. measurement) and 91 (II. measurement) day of age

Trait	C group	E1 group	E2 group	E3 group
Consumption of milk replacer, L/day	5.00^A	5.18^B	5.08^A	5.21^B
Consumption of milk replacer, g/day	625.27^A	648.04^B	634.54^A	651.06^B
Consumption of milk replacer DM, g/day	601.51^A	623.42^B	610.43^A	626.32^B
Consumption of starter, g/day	332.07^A	411.31^B	438.88^B	532.71^C
Consumption of starter DM, g/day	292.97^A	364.53^B	390.22^B	475.90^C
Total consumption DM, g/day	828.51^A	930.35^B	938.79^B	1043.38^C

**DM - dry matter; Values within the same row marked with different letter differ statistically (P<0.0001)*

but the values of iron and albumin were higher for both measurements. Albumin and total protein determined in Holstein calves in the research by Mirghaffari et al. (2012) were slightly or much lower compared to values obtained in this research, depending on the average age of calves (about 8th and 12th week), respectively. In both measurements, BHBA and glucose had lower values compared to calves from the research by Mirghaffari et al. (2012).

The consumption of milk replacer (as is in grams and litres, and on DM basis in grams) were statistically different (P<0.0001) between animals from E1 and E3 groups and those in groups C and E2 (Table 6). Regarding the consumption of starter (as is and on DM basis) and total consumption DM (g), animals in the control group consumed less in comparison to animals in experimental groups (E1, E2, E3). Furthermore, animals from the groups E1 and E2 had a lower starter consumption compared to animals in group E3 (P<0.0001). Stamey et al. (2012) concluded that calves should consume at least 1 kg of starter DM daily before weaning in order to ensure continued post-weaning growth. They found out that a starter with 25.5 % of crude protein provides the best starter intake (particularly around weaning) and growth for dairy calves, compared to a conventional starter with 19.6 % of crude protein. Holstein calves in the research of Mirghaffari et al. (2012) ate between 731 and 945 grams of the total dry matter, depending on the different group in the starter differentiated in the form of wheat, but slightly lower compared to the results of this research. Stamey et al. (2012) determined a higher dry matter intake of milk replacer and starter with crude proteins in comparison to the conventional starter in Holstein calves, and comparable with this research. Their calves had a higher intake of milk replacer but lower of the starter. Higher starter intake results in faster rumen development. Greater body weight, daily gain, glucose, albumin, and BHBA concentrations and higher insulin activity seem to be the results of a better rumen development in calves from experimental groups.

Conclusions

According to the obtained results, it could be concluded that different starters based on the whey powder and soy proteins with low ANFs + lysine and methionine, significantly affect the consumption of milk replacer and starter, and total consumption of dry matter, withers height, and body length (among body traits) and NEFA (among biochemical parameters) on the Holstein calves. Finally, in order to optimize the growth and feed consumption, the starters based on whey powder and soy proteins with low ANFs + lysine and methionine could be recommended, but this requires further research related to the effect on other performance traits of calves.

Utjecaj različitih startera na performanse i fiziološke osobine rano odbijene ženske teladi Holstein pasmine

Sažetak

Cilj istraživanja bio je utvrditi utjecaj različitih startera, koji sadrže sirutku u prahu i izvore bjelančevina s niskim udjelom antinutritivnih tvari (ANF) te metionin i lizin na: tjelesne mjere, biokemijske parametre te konzumaciju hrane rano odbijene ženske teladi holstein pasmine. Sukladno starteru, telad je podijeljena u četiri skupine; kontrolna (kontrolni starter), E1 (starter sa suhom sirutkom), E2 (starter sa izvorima proteina s niskim ANF-om + metionin i lizin) i E3 (starter sa suhom sirutkom i izvorima proteina s niskim ANF-om + metionin i lizin). Rezultati provedenog istraživanja ukazuju da različiti starteri koji su sadržavali sirutku i izvore bjelančevina s niskim ANF-om te metioninom i lizinom značajno utječu na konzumaciju mliječne zamjenice i startera te na ukupnu konzumaciju suhe tvari, visinu grebena, duljinu tijela te koncentracije NEFA u plazmi ženske teladi holstein pasmine. U konačnici, u cilju optimizacije porasta te konzumacije hrane teladi, može se preporučiti uporaba startera koji sadrže sirutku u prahu, izvore bjelančevina s niskim udjelom antinutritivnih tvari te metionina i lizina, ali to zahtjeva daljnja istraživanja o utjecaju na ostale performanse teladi.

Ključne riječi: telad; starter; konzumacija; svojstva tjelesnih mjera; biokemijska svojstva

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