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THE FEED VALUE OF A BY–PRODUCT OF THREONINE PRODUCTION BY FERMENTATION IN CATTLE FEEDING

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Abstract: Effects of mother liquor (a by–product of threonine production by fermentation) as a potential feed component for cattle were evaluated in an experiment with Holstein–Friesian steers. Experimental animals were surgically equipped with rumen cannula, and two were equipped with duodenum cannula in order to facilitate sample collection. The experiment consisted of a control and three consecutive experimental phases, each lasting 5 days and separated by 10–day pre–feeding periods. Composition of daily ration of control and experimental phases was identical except for the following supplementations in the different experimental phases: 1.0 kg mother liquor, 1.5 kg mother liquor, and 1.5 kg mother liquor with 1.0 kg molasses, respectively. The experimental supplements increased bacterial activity in the rumen resulting in a significant ($P<0.05$) increase in total volatile fatty acid production and an improved energy supply. Furthermore, supplementations increased the ammonia (NH_3) content of rumen fluid and thus improved N supply for ruminal bacteria. Combined mother liquor and molasses supplementation significantly ($P<0.05$) increased microbial protein ratio in duodenal chyme and improved the amino acid supply of the animals.
Keywords: Cattle, Threonine, Molasses, By–product, Rumen fermentation

1. INTRODUCTION

The production of farm animals – as a result of conscious breeding, the improvement of feeding and housing technology, as well as the preventive veterinary work – has been increasing to the extent that the essential amino acid requirement of some animal species (pigs, poultry species, dairy cows) cannot be covered by the amino acid content of the feed. This makes the increased use of industrially produced amino acids, especially lysine, methionine, threonine and tryptophan necessary. The amount of industrially produced amino acids has increased steadily in recent years due to the multipurpose use (food industry, pharmaceutical industry, chemical industry, animal nutrition). According to [2], at the beginning of the century 2.46 million tons of five important amino acids were produced (1.5 million tons of lysine; 600,000 tons of methionine; 230,000 tons of threonine; 80,000 tons of phenylalanine and 50,000 tons of tryptophan), most of which is presumed to have been used as feed. According to other sources [8], the amount of amino acid used for feeding has reached 4.5 million tons in 2017, and is expected to reach 6.2 million tons by 2022.

Aforementioned amino acids are produced by microbial fermentation with the exception of methionine, since only L–optical isomers of amino acids are produced by fermentation. Methionine is produced synthetically, in which process racematum (mixed D and L optical form) is produced, which is not an issue in the case of methionine, as cattle can effectively convert the D form of methionine into L optical isomer. During threonine fermentation two by–products arise that are suitable for feeding farm animals. One is the biomass that is formed when having completed the fermentation, the threonine producing bacteria are inactivated by heat treatment and removed from the fermentation broth by ultra– or diafiltration. After drying the biomass (based on its favorable chemical composition), it can be used for feeding monogastric animals, especially pigs. The threonine is recovered from the remaining fermentation broth by crystallization. The residue is the mother liquor, the composition of which is shown in Table 1. The mother liquor is rich in crude protein: 75.87% of its dry matter is made up of crude protein. 37.06% of the crude protein derives from ammonium sulphate, urea, and 62.94% from free amino acids. The high threonine content may create amino acid imbalance in monogastric animals but this does not occur in cattle, because the rumen microbes break down the excess threonine or use it for other purposes, e.g. for microbial protein synthesis.

Several by–products of amino acid production by fermentation contain valuable nutrients (e.g. amino acids) and can therefore be considered for animal feeding. These by–products are often characterized by high water content and are generated in vast amounts; however, their potential roles and effects on animal feeding are less studied. In vivo feeding experiments facilitate the understanding of the effects of these potentially novel feedstuffs (by–products) on ruminal microbial fermentation and animal production.

Our studies on the feed value of mother liquor started with basic physiological experiments, testing the effect of mother liquor feeding on the microbial processes (degradation and synthesis) in the rumen. The impetus was to determine the impact of mother liquor supplement on

— the activity of rumen microbes (degradation and synthesis processes)

— the synthesis of volatile fatty acids in the rumen
— the ammonia (NH_3) content of the rumen fluid
— the pH of the rumen fluid
— the amount of microbial protein synthesized in the rumen.

2. MATERIAL AND METHODS

— Animal experiments:

The batch experiment was performed in four Holstein–Friesian steers weighing 600–650 kg. For rumen fluid and chyme sampling, all steers were cannulated surgically to the rumen, and two animals of them to the duodenum, as well. The experiment consisted of a control and three consecutive experimental phases. All phases consisted of a pre–feeding and a sampling period, the length of which were 10 and 5 days, respectively. The composition and nutrient content of the feed rations are given in Table 2. In the experimental phases the animals received different amounts of supplements (phase 1: 1.0 kg mother liquor; phase 2: 1.5 kg mother liquor; phase 3: 1.5 kg mother liquor + 1.0 kg molasses) in addition to the feed described in Table 2. The animals were fed twice a day (at 7 am and at 3 pm), receiving 50–50% of the daily ration. Rumen fluid samples were collected from the animals by means of rumen cannula, four times a day (before morning feeding and 1, 2, and 3 hours after feeding) on each day of the experimental period. Furthermore, chyme samples were taken every 3 hours after the morning feeding (10 am, 1 and 4 pm) through the duodenal cannula for subsequent chemical testing. Throughout the study, animal handling and sampling were conducted in accordance with the standards recommended by Directive 2010/63/EU.

— Chemical tests:

Chemical tests were performed to monitor the processes in the rumen and duodenum. Dry matter, crude protein, crude fat, crude fiber, crude ash, Ca and P content of the diets as well as urea of the mother liquor were determined according to the Codex Pabularis Hungaricus II [6]. The ammonium sulphate content of the mother liquor was determined by the Kjeldahl method using Kjeltec 2200 apparatus. The amino acid content of the mother liquor and the DAPA (diaminopimelic acid) content of the chyme samples taken from the duodenum were determined by column chromatography using INGOS AAA 400 chromatograph. Column packing was LG ANB ion exchange resin.

Microbial activity of the rumen was examined by nitrite reduction test [19]. The method expresses the microbial activity of the rumen fluid by the lengths of time the rumen microbes require to reduce a given amount of KNO_2.

Volatile fatty acids were analyzed on a Biotronic 2000 type HPLC apparatus with column type Aminex Bio–Rad HPX 87H, size 300 mm x 7.8 mm. Separation was carried out at 45°C, eluting with 0.005 M H_2SO_4, pump flow 0.85ml/min, pressure 77kg/cm^2. The volatile fatty acid mixture of Supelco 10 was used as standard.

The pH of the rumen fluid was determined by an electrical pH meter of type OP–211/1 (Radelkis) and the NH_3 content was measured using the ammonia–sensitive electrode type OP–264–2 (Radelkis).

Table 1. Chemical composition of the mother liquor

Nutrient	g/kg mother liquor
Dry matter	405.35
Crude protein	307.56
Crude fat	4.00
Crude ash	27.40
N–free extracts	66.39
Crude protein	307.56
Free amino acid	251.56 (193.56 g crude protein)
arginine	7.62
alanine	4.64
aspartic acid	2.56
glutamic acid	43.14
histidine	1.25
isoleucine	8.27
leucine	4.09
lysine	1.77
methionine	5.81
phenylalanine	4.23
serine	3.76
threonine	141.46
valine	1.72
glycine	10.37
proline	1.25
cysteine	7.99
Tyrosin	1.63
Non–protein nitrogen	
Ammonium sulphate	72.54 (96.06 g crude protein)
Urea	6.15 (17.94 g crude protein)

Table 2. Composition and nutrient content of the daily feed ration

Feed	Daily dose
Maize silage	10.0 kg
Grass straw	2.0 kg
Mixed fodder[a]	3.0 kg
Nutrient content of daily ration	
Dry matter[b]	7.92 kg
Crude protein[b]	848.81 g
Crude fiber[b]	1626.09 g
NE_m[b]	48.58 MJ
NE_g[b]	29.55 MJ
MFE[b]	631.47 g
MFN[b]	533.05 g
Ca[b]	41.12 g
P[b]	29.00 g

[a] – composition: maize 88.2%, extracted sunflower meal 4.0%, feed limestone 5.6%, 1.2% monocalcium phosphate, bovine vitamin and microelement premix 0.5% (produced: Vitafort co., Hungary), salt 0.5%

[b] – calculated on the basis of the results of the chemical analysis of the components

For the determination of microbial protein of the total crude protein content of chyme we used the method of Csapó et al. (2008). The method determines the proportion of microbial protein using the chyme's DAPA or D–aspartic acid or D–glutamic acid and crude protein content. Csapó et al. (2008) determined the dry matter, crude protein, DAPA, D–glutamic acid and D–aspartic acid content of a mixture of 17 rumen bacterial strains; the test results on dry matter were as follows: crude protein 49.5%, DAPA 0.300%, D–aspartic acid 0.366%, D–glutamic acid 0.499%. The crude protein of the tested rumen bacteria mixture contained 0.606% DAPA, 0.739% D–aspartic acid, and 0.998% D–glutamic acid. Using these data, multiplication factors were calculated to determine the microbial protein proportion of the chyme's crude protein content. The multiplication factors for the three amino acid markers were as follows: DAPA: 100/0.606 = 165; D–asp.: 100/0.739 = 135; D–glut.: 100/0.998 = 100.

We calculated the crude proteins' microbial protein fraction of duodenal chyme on the basis of the DAPA content. DAPA is an amino acid that occurs naturally only in peptidoglycans involved in the construction of the cell wall of bacteria, including rumen bacteria. Therefore, it is an excellent marker in determining the microbial protein proportion of chyme. We were calculating with the factor determined for the DAPA (165) by Csapó et al. (2008).

— Statistical analyses:

The statistical analyses were performed on IBM SPSS Statistics for Windows v.20.0. Normality was determined by the Kolmagorov–Smirnov test. In case of normal distribution the Test of Homogeneity of Variances, as well as the Bonferroni and Games–Howell tests, whereas in the case of non–normal distribution, the Kruskal–Wallis and Mann–Whitney tests were applied.

3. RESULTS AND DISCUSSION

— Microbial activity:

Rumen microbial activity was evaluated by nitrite reduction tests (results presented in Table 3). Energy and N supply of microbes can be assessed based on changes in microbial activity. The time required for the reduction of potassium nitrite (KNO_2) added in various volumes (0.2, 0.5, and 0.7 ml, respectively) was between 3.55 and 5.45 min. In the control group, there were no significant ($P>0.05$) differences between reduction times of samples. In the control group, there were no significant ($P>0.05$) differences between reduction times of samples taken before and 3 hours after the feeding. Nevertheless, supplementations with mother liquor and molasses combined mother liquor significantly ($P<0.05$) decreased nitrite reduction time; in conclusion, microbial activity increased. This beneficial effect can be explained by the fact that the mother–liquor supplement improved the nutrient supply of microbes, especially N. The supplement provided 251.56 g/kg free amino acid and 78.69 g/kg urea and ammonium sulfate as N source for the microbial population (Table 1). Nitrite reduction results of 1.0 and 1.5 kg mother liquor supplementations were nearly identical; therefore it can be concluded that the elevated mother liquor dose did not increase further microbial activity.

Table 3. Development of microbial activity of rumen when feeding mother liquor (mean±SD)

Phase	Sampling time	Nitrite reduction (minutes)		
		0.2 ml KNO_2	0.5 ml KNO_2	0.7 ml KNO_2
Control	Before feeding	5.45±1.54^a	11.40±2.23^a	15.35±3.28^a
	3 hours after feeding	3.95±0.89^a	7.55±1.67^a	10.50±2.01^a
1.0 kg mother liquor	Before feeding	4.88±0.60^a	9.59±1.26^a	12.79±2.11^a
	3 hours after feeding	3.11±0.05^b	5.70±0.92^b	7.75±1.52^b
1.5 kg mother liquor	Before feeding	4.80±0.42^a	9.49±1.34^a	12.80±1.82^a
	3 hours after feeding	3.06±0.15^b	5.69±0.77^b	7.72±1.12^b
1.5 kg mother liquor + 1 kg molasses	Before feeding	3.55±0.69^a	6.65±1.50^a	8.95±1.90^a
	3 hours after feeding	3.10±0.31^b	5.15±0.99^b	7.05±2.44^b

^{ab} – different letters differ significantly ($P < 0.05$) in the same column and within the same phase

The role of rumen bacteria is the most important of the microorganisms involved in the degradation of the protein in the rumen, which is associated with the large number of them in the rumen ($10^{10–11}$ CFU/ml). The majority of the N present in the duodenum is rumen bacteria N. This can be explained by the fact that due to the self–destruction of protozoa, a significant amount of peptide and amino acid is introduced into the rumen fluid and about 65% of the nutrient from the dead protozoa is reused in the rumen [9, 20]. The proportion of protozoa in the microbial mass of the duodenum is relatively small which can be explained by the aforementioned recycling. N is present in the rumen as a variety of compounds (protein, peptide, amino acid, urea, ammonia), which may be derived from the feed, but may also be products of microbial protein breakdown and synthesis. According to Koenig et al. (2000) the amount of N from the latter source can reach up to 45% of rumen N–content. A set of microbe's urease does not limit the urea utilization of rumen microbes [16]. The amount of urea to be fed is influenced by the composition of the feed, its protein content and its rumen degradation. The important role of amino acids in the N supply of rumen bacteria is confirmed by a

previous in vitro experiment of Van Kessel and Russel (1996). The mixed bacterial population developed faster when the medium also contained an enzymatically hydrolyzed casein–soybean meal mixture, whereas only NH_3–N was available as N source.

The microbial protein synthesis in the rumen is significantly affected by the amount and composition of carbohydrates in the feed beyond the amount and composition of available N source. This is related to the energy need of the ATP synthesis necessary for metabolism of rumen microbes. It is primarily obtained by decomposing carbohydrates, but they can also use other materials for microbial energy recovery. The mother liquor supplementation improves the microbial activity not only by improving the N supply, but because the desaminated chain of amino acids is also used by the microbes to cover their energy requirements [4]. Feed carbohydrates can be structural (cellulose, hemicellulose) and non–structural carbohydrates (simple sugars, pectin and starch) [23]. Many bacteria can utilize different sources of carbohydrates, but many of them can only cover their energy needs by breaking down structural or non–structural carbohydrates [22]. The easily degradable carbohydrates cover the ATP needs of rumen microbes only for a short time (4–8 hours) [13], therefore they are hardly involved in the ATP supply of microbes [18].

Besides the nitrogen–containing materials of the mother liquor and the molasses, the carbohydrates of the molasses improved the energy supply of the rumen microbes and contributed to faster nitrite degradation. The results of in vitro and in vivo experiments unanimously confirm that the rate of carbohydrate digestion primarily determines the usable amount of energy required for the operation of rumen microbes [11]. The breakdown of structural carbohydrates (e.g. neutral detergent fibers) provides energy for ATP synthesis only 3–4 hours after the start of rumen fermentation, but can last longer than 90 hours [18].

The crude fiber content of some forages is characterized by considerable incorporation of lignin [17]. Therefore, the neutral detergent fibers of such feedstuffs are not completely decomposed after 72 hours in the rumen [12, 15]. An important limiting factor of functioning rumen microbes is when only limited rapidly degrading carbohydrates are present in the rumen. However, it is necessary to emphasize that in addition to rapidly degrading carbohydrates slowly decomposing ones are also required for the proper functioning of rumen microbes [10].

— **Microbial protein synthesis:**

The results of our experiments indicated that supplementing the feed with mother liquor and a mixture of mother liquor and molasses increases the activity of rumen microbes, therefore, we also examined the amount of microbial protein produced in the rumen and thus on the amino acids supply of the cows. The changes in rumen characteristics have crucial effects on microbial protein production, as well. Duodenal chyme protein was calculated using the multiplication factor for DAPA developed by Csapó et al. (2008). The results are summarized in Table 4. It can be stated that the crude protein content (g/kg) of chime significantly ($P<0.05$) increased due to the supplements; however, the DAPA content of chyme and the proportion of microbial protein were significantly ($P<0.05$) increased only by the combined mother liquor and molasses supplement compared to control. This may be related to the energy–intensive nature of microbial protein synthesis. In this, the carbohydrates of molasses could be involved. Daily supplementation with 1.5 kg mother liquor and 1.0 kg molasses increased microbial protein ratio in duodenal chyme by 24.3%, thus contributing to improved amino acid supply of the animals.

Table 4. Synthesis of microbial protein (mean±SD)

Phase	Control	1.0 kg mother liquor	1.5 kg mother liquor	1.5 kg mother liquor + 1 kg molasses
Chyme crude protein content (g/kg)	$237.33±23.65^{b}$	$266.04±17.97^{a}$	$261.65±17.03^{a}$	$273.98±31.72^{a}$
DAPA content of chime %	$0.07±0.01^{b}$	$0.08±0.01^{ab}$	$0.08±0.02^{ab}$	$0.09±0.01^{a}$
The proportion of microbial protein in the crude protein of chime %	$11.71±0.89^{b}$	$13.12±2.13^{ab}$	$13.59±3.63^{ab}$	$14.56±2.03^{a}$

^{ab} – values marked with different letters in the same row differ significantly ($P<0.05$)

— **Volatile fatty acids:**

Rumen volatile fatty acid production also plays vital roles in ruminant nutrition. Volatile fatty acid content of rumen fluid in different treatments is presented in Table 5. Compared to before feeding, 3 hours after feeding levels of i–valeric acid decreased ($P<0.05$), whereas levels of n–valeric acid increased ($P<0.05$) in the control phase, while other parameters remained similar ($P>0.05$). In each treatment (1.0, 1.5 kg mother liquor, and 1.5 kg mother liquor combined with 1.0 kg molasses, respectively), rumen fluid total volatile fatty acid content significantly ($P<0.05$) increased 3 hours after feeding compared to before feeding levels. Furthermore, individual levels of every volatile fatty acid increased significantly ($P<0.05$) except for i–butyric acid. Acetic: propionic acid ratio significantly ($P<0.05$) decreased in each treatment phase, which is considered beneficial in dairy cattle populations. The 1.5 kg mother liquor supplementation was the most efficient in samples taken before, and 1 or 3 hours after feeding, while the molasses combined supplement proved to be the most effective

at 2 hours after feeding. The volatile fatty acid content of the rumen fluid samples taken in similar times was different in the three experimental phases compared to the control.

Table 5. Development of the volatile fatty acid content of rumen fluid when feeding mother liquor, and mother liquor and molasses (mean±SD)

Phase	Sampling time	Acetic acid C_2 mmol/l	Propionic acid C_3 mmol/l	C_2/C_3	i–Butyric acid C_{4i} mmol/l	n–Butyric acid C_{4n} mmol/l	i–Valeric acid C_{5i} mmol/l	n–Valeric acid C_{5n} mmol/l	Total volatile fatty acids mmol/l
Control	Before feeding	77.02±14.11^{a}	17.08±3.90^{a}	4.59±0.61^{a}	0.47±0.14^{a}	9.48±2.53^{a}	1.00±0.29^{a}	0.44±0.27^{b}	105.49±20.36^{a}
	3 hours after feeding	81.35±16.44^{a}	18.70±3.87^{a}	4.39±0.48^{a}	0.44±0.12^{a}	10.56±2.53^{a}	0.83±0.25^{b}	0.66±0.28^{a}	112.53±22.23^{a}
1.0 kg mother liquor	Before feeding	62.78±12.27^{b}	17.04±4.89^{b}	3.77±0.40^{a}	0.36±0.04^{a}	7.62±1.80^{a}	0.61±0.12^{b}	0.33±0.03^{b}	88.74±18.34^{b}
	3 hours after feeding	80.02±6.67^{a}	26.19±3.80^{a}	3.11±0.33^{b}	0.38±0.03^{a}	12.09±1.53^{a}	0.89±0.18^{a}	0.93±0.25^{a}	120.47±10.62^{a}
1.5 kg mother liquor	Before feeding	64.89±11.36^{b}	17.49±4.35^{b}	3.79±0.39^{a}	0.35±0.04^{a}	8.10±1.91^{b}	0.75±0.19^{b}	0.30±0.04^{b}	91.88±17.19^{b}
	3 hours after feeding	77.16±4.41^{a}	24.78±3.34^{a}	3.18±0.31^{b}	0.38±0.03^{a}	11.58±1.43^{a}	0.90±0.16^{a}	0.75±0.17^{a}	115.55±8.25^{a}
1.5 kg mother liquor + 1.0 kg molasses	Before feeding	69.28±12.59^{b}	18.97±4.50^{b}	3.72±0.51^{a}	0.80±0.20^{a}	10.27±2.55^{b}	1.52±0.27^{b}	0.51±0.30^{b}	101.34±18.84^{b}
	3 hours after feeding	90.84±7.44^{a}	29.16±4.64^{a}	3.16±0.35^{b}	0.74±0.17^{a}	19.12±2.48^{a}	1.74±0.31^{a}	1.60±0.30^{a}	143.20±13.75^{a}

^{ab} – values marked in different letters differ significantly in the same column and within the same phase at a minimum $P<0.05$ level

In the control there was only in two of the six volatile fatty acids examined a significant ($P<0.05$) difference (i– and n–valeric acid) relating the two sampling times. In contrast to the control we found that in the three experimental phases (mother liquor and mother liquor + molasses supplement) significant differences for the tested fatty acids but these two volatile fatty acids. This confirms that the two supplements (mother liquor and molasses) had for 6 out of the 8 parameters studied (6 volatile fatty acids; C_2/C_3 ratio; and total volatile fatty acid content) a significant effect on the rumen volatile fatty acid composition that significantly influences the animals' production.

The mother liquor supplement significantly increased the amount of acetic acid, propionic acid and both isomers of valeric acid in the rumen fluids. Both concentration of the mother liquor (1.0 and 1.5 kg) was effective, but the 1.5 kg dose has resulted in a smaller but still significant increase. The same is true for all volatile fatty acid content.

For dairy cows, it is also important to know the proportion of acetic acid and propionic acid that is formed and present in the rumen. For lactating cows the optimum acetic acid: propionic acid ratio is 3:1. If the ratio is narrower, the fat content of milk is expected to decrease.

When the mother liquor supplement was combined with molasses, the amount of acetic acid and propionic acid increased the most, and the ratio of them was optimal.

Of the volatile fatty acids tested i– and n–valeric acid reacted positively and significantly in all three experimental stages for mother liquor and mother liquor and molasses supplements, like the acetic acid and propionic acid. Of the volatile fatty acids, only i–butyric acid did not react positively to treatments. Only the higher dose of mother liquor supplement and the combined mother liquor and molasses supplement resulted in a significant positive effect on n–butyric acid formation.

— Ammonia production and ruminal pH:

One important regulator of the microbial activity in the rumen is the pH of the rumen fluid, therefore we also monitored the effect of mother liquor and molasses supplementation on the pH of the rumen fluid. Rumen fluid pH is affected by both volatile fatty acid and ammonia production; changes in the NH_3 content of rumen fluid were also investigated: NH_3 content of rumen fluid was measured before feeding, and 1, 2, and 3 hours after feeding, respectively (Table 6). In each phase, highest NH_3 was detected 1 hour after feeding. Compared to before feeding levels, NH_3 content did not change significantly ($P>0.05$) over time in the control phase, whereas mother liquor and molasses combined mother liquor supplementations significantly ($P<0.05$) increased rumen NH_3 content, even at 3 hours after feeding. We found the lowest amount of NH_3 in case of the control, when the animals consumed corn silage and grass straw containing hardly biodegradable protein, as well as a grain mix of which the single protein rich component was the 4% extracted sunflower meal (Table 2).

Table 6. Development of NH_3 content of rumen fluid when feeding mother liquor, and mother liquor and molasses (mean±SD)

Sampling time		NH_3 (mmol/liter)			
		Control	1.0 kg mother liquor	1.5 kg mother liquor	1.5 kg mother liquor + 1 kg molasses
Before feeding		$3.94±2.21^{ab\ z}$	$6.11±1.91^{d\ y}$	$7.46±1.61^{d\ x}$	$7.38±2.05^{d\ xy}$
After feeding	1 hour	$5.81±2.95^{a\ z}$	$30.59±5.69^{a\ y}$	$40.64±9.25^{a\ x}$	$31.83±3.24^{ab\ y}$
	2 hours	$4.66±0.61^{a\ z}$	$19.70±9.00^{b\ y}$	$22.13±6.39^{b\ xy}$	$22.55±4.91^{b\ x}$
	3 hours	$2.38±0.55^{b\ y}$	$11.47±4.43^{c\ x}$	$14.06±5.72^{c\ x}$	$13.15±5.25^{c\ x}$

^{abcd} – values marked with different lowercase letters differ significantly ($P < 0.05$) in the same column
^{xyz} – values marked with different letters differ significantly ($P < 0.05$) within the same row

The low NH_3 content in the rumen fluid is due to the weak rumen degradation of the feed protein. According to literature data, the proteins' rumen degradability of the feed ration without supplement (mother liquor and molasses) is dg = 0.64 [26]. At the same time, the rumen degradability of N compounds of the mother liquor (free amino acids, ammonium sulphate and urea) and of the molasses is considerably better. This explains why the NH_3 content of the rumen fluid was high (30–40 mmol/l) in the experimental phases already 1 hour after feeding, which was in average 5.9 times higher than that in the case of control.

Although the feeding of mother liquor and mother liquor and molasses supplement resulted in a significant increase in NH_3 content of rumen fluid in the first hour after feeding, ammonia toxicities were not developed. According to Rechcigl (2017), this will only occur if the concentration of NH_3 in the rumen fluid exceeds 100 mmol/l. The risk of developing ammonia toxicities was also reduced by the fact that the ammonia level of the rumen fluid decreased significantly in 2–3 hours after feeding.

Changes in the pH of the rumen fluid during the experimental period are included in Table 7. It can be stated that the rumen fluid's pH has been continuously decreasing 3 hours following the morning feeding in all experimental phases, which is due to the increased volatile fatty acid production of the rumen microbes. The 1.0 and 1.5 kg mother liquor and molasses combined mother liquor supplementations resulted in significant ($P<0.05$) decrease in rumen pH; the greatest decrease (0.84) was observed in the molasses combined mother liquor phase. Overall, slightest decrease of the rumen fluid pH was observed in the control: three hours after the morning feeding it was above pH 6. In contrast to this in each experimental phase three hours after the morning feeding the pH of the rumen fluid was below 6.0, although the difference was minimal compared to the physiologically expected (pH 6.0) value (experimental phase 1: 0.07 pH; experimental phase 2: 0.03 pH; experimental phase 3: 0.17 pH).

Table 7. Development of pH of rumen fluid when feeding mother liquor and mother liquor + molasses (mean±SD)

Sampling time		pH			
		Control	1.0 kg mother liquor	1.5 kg mother liquor	1.5 kg mother liquor + 1 kg molasses
Before feeding		$6.63±0.18^{ab}$	$6.74±0.29^{a}$	$6.76±0.29^{a}$	$6.67±0.28^{a}$
After feeding	1 hour	$6.59±0.20^{b}$	$6.63±0.29^{a}$	$6.70±0.23^{a}$	$6.39±0.35^{b}$
	2 hours	$6.47±0.22^{a}$	$6.35±0.20^{b}$	$6.45±0.16^{b}$	$6.20±0.31^{b}$
	3 hours	$6.19±0.24^{c}$	$5.93±0.24^{c}$	$5.97±0.18^{c}$	$5.83±0.23^{c}$

^{abcd} – values in the same column marked with different letters differ significantly at a minimum $P<0.05$ level

Literature data suggest that a low pH, primarily a pH below 6, is detrimental to the functioning of rumen bacteria, but even more so to infusoria. Cerrato–Sanchez et al. (2007) found in a fermentation experiment with rumen fluid culture that digestibility of organic matter, NDF, and ADF was reduced when the pH of the medium was gradually reduced from 6.4 to 5.5 in 24 hours. The structure of the protein in the breakdown by the rumen microbes of the protein content of the feed is essential, but it is also influenced by the composition of the rumen fluid and the microbial population in the rumen. In the case of dairy cows consuming large amounts of roughage, the proteolytic activity of the rumen decreases due to the decrease in rumen fluid pH, but it does not occur when feeding cattle for fattening with a lot of grain fodder [1]. The energy available in the rumen serves not only to cover the energy required for the growth of rumen microbes, but it is also used for other purposes (e.g. to maintain neutral intracellular pH or membrane potential) [24]. Based on our experimental results conducted with 1 kg or 1.5 kg mother liquor and with a 1.5:1 mixture of mother liquor and molasses, we can assume that the negative physiological effects of rumen fluid pH below pH 6 mentioned in the literature occur only at a greater pH decrease.

4. CONCLUSION

In summary, based on its chemical composition the mother liquor is suitable for feeding cattle keeping certain rules in mind. The following benefits can be expected when feeding the mother liquor in an optimal amount: the activity of rumen microbes increases, resulting in an increase in the amount of fatty acids produced in the rumen. This improves the energy supply of the animals, as up to 70% of the ruminants' energy needs are

covered by the fatty acids formed in the rumen [3]. The absorbed fatty acids first enter the liver, then reach the adipose and muscle tissues via blood circulation, and in case of cows the udder, where they are utilized. The combined feeding of mother liquor and molasses significantly ($P<0.05$) increases the microbial protein ratio of chyme crude protein in the duodenum, improving the amino acid supply of the animals. At the same time, the use of mother liquor as a feed may present some hazards. The mother liquor contains several nitrogenous substances (urea, ammonium sulphate, free amino acids) which are degraded in the rumen, partly due to the operation of rumen microbes, and can cause ammonia toxicities; however, this can only occur when feeding more than 2 kg of mother liquor daily.

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