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INFLUENCE OF FORAGE ADDITION OF THREE KINDS OF BLOOD DRIED PREPARATES: LIVEXES (BLACK AND BROWN) AND MEAL ON CAECAL FERMENTATION IN SHEEP DURING PRELIMINARY IN VITRO INVESTIGATIONS

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ABSTRACT

The aim of the studies was the influence comparison of the three kinds of blood products: brown and black dried livexes and blood meal, added to sheep forage, on total protein level, energy value, volatile fatty acids (VFA) amount, levels of carbon dioxide and methane in caecal content of the animals. The samples of caecal content were taken from the sheep immediately after slaughtering, i.e. 2,5 hrs after morning feeding. The livex and blood meal were added to the forage in the amount of 2%. During in vivo application of these nonconventional dietary supplements, the significant inhibitory livex (black and brown) effect on methane level in the sum of caecal gases (45,5% increase for black and 33,4% for brown livex, respectively). Energy of caecal content was about 30% higher in sheep fed with black livex. Respective percentage was about 40% and about 20% for the brown

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livex and blood meal respectively. Percent increase of protein amount was as follows: 17,9% (for black livex), 23,1% (for brown livex) and 14,1% (for blood meal addition). Livex application as the fodder supplement resulted in the increase of either the caecal fermentation efficiency or the cell yield efficiency.

Key words: sheep, caecum, fermentation, livex, blood meal

INTRODUCTION

The earlier observations on the inhibitory livex effect on methanogenesis in the rumen of lamb and sheep (14, 15, 17, 18) suggested the idea of defining the levels of energetic value, total protein and volatile fatty acids in caecal content of sheep after supplementation the forage with black or brown dried livex instead of concentrate. Although either in vivo or in vitro influence of livex (black, brown and white) and blood meal on course of rumen fermentation was the subject of our experiments mentioned above, the mechanism of these nonconventional supplements in caecal fermentation is not understand till now. In present work we have compared the caecal action of livex and blood meal.

The main goal of actual publication was therefore to determine the role of black and brown livex and blood meal added to sheep forage as the products effecting three particular parameters of caecal fermentation: energetic value, protein level and formation of VFA sum, as well as interrelationship between acids, methane and carbon dioxide production, fermentation efficiency, NGGR factor, cell yield efficiency and ATP/glucose ratio.
Technological differences of the products subjected to the experiment are explained within the special bulletins and conference materials.

MATERIALS AND METHODS

The studies were performed on 3 sheep from own vivarium and the results were confirmed on the other 3 sheep belonging to the local Department of Immunology and Veterinary Prevention. Consequently, the studies were carried out on total number of 6 cross-breeding sheep (aged 3-4 years, about 45 kg of body weight). The samples of caecal content were obtained immediately after slaughtering, i.e. 2,5 hrs after morning feeding. Forage consumption ranged from 2,1 to 2,3 kg . The accurate percentage composition and energetic value of the forage for experimental sheep are presented in Table 1.

Table 1. The percentage composition and energetic value of forage in experimental sheep

Components	Type of forage		
	for control sheep	with addition of livexes	with addition of blood meal
Concentrates C-J	7,9	5,9	5,9
Green forage from a new lucerne	78,9	78,9	78,9
Barley straw	13,2	13,2	13,2
Livex	-	2,0	-
Blood meal	-	-	2,0
Total protein in forage (g)	214	235	228
Energy in forage (MJ)	5,28	5,29	5,27
Dry matter in fodder (kg)	1,53	1,53	1,53

Addition of livex (black and brown) or blood meal to forage constituted 2% of feeding ratio. Sheep were fed twice a day, between 6.30 and 7.30 and between 13.30 and 14.30. The forage was enriched with vitamins and mineral salts in accordance with feeding norms (7, 8, 11). Drinking water was given ad libitum. Chemical composition of black and brown livex was as follow: dry matter – 97,20% and 89,45% respectively, crude protein – 88,15% and 73,98%, crude fat – 0,95% in both the livexes, crude ash – 4,90% and 5,40%, N-free extractive substances – 3,20% and 9,13% respectively. The respective data for blood meal were: 90,51%, 64,15%, 7,09%, 15,35% and 5,58% respectively.

The calculations of fermentation efficiency (FE), cell yield efficiency (mg), non-glucogenic/glucogenic ratio of VFA (NGGR) and ATP/glucose ratio were performed on the basis of patterns given in the papers of Chalupa (3), Church (4), Czerkawski (5) and Orskov (10). Following parameters were determined for the caecal content after

preliminary preparation in vacuum evaporator and dryer: methane and carbon dioxide and VFA according to Zawadzki et al. (15), total protein level in caecal content according to Bradford (2) and Lowry (9), total energy in caecal content according to Gawęcki and Jeszka (6).

The resultes obtained were statistically analysed using the t-Student test (12) using a Texas Instruments 58/59 PC-100 C calculator.

RESULTS AND DISCUSSION

The results obtained in the studies are summarised in the Tables 2 and 3. Experiments discussed in the paper seem to confirm the preliminary in vitro results obtained for calves and sheep (14, 19, 20). It was noted (14) that livex added to rumen content in two doses (0,25 g and 0,50 g x 100 ml-1 of rumen content) and incubated for 1 hour in 39^{o}C causes the respective increase of energetic value of the content at the level of 12,9% and 22,6%. Moreover, the authors quoted claim, that dried brown livex (modified by whey) added in vitro to rumen content, inhibits significantly methanogenesis. This in vitro observation prompt us to test well-known in vitro data during in vivo studies with caecal fermentation. Accordingly, we have decided to define the influence of black and brown livex supplementation of the sheep forage on the level of protein, energy and VFA in caecal content. Simultaneously, the livex efficiency was compared with the blood meal activity.

All three diets (Tab. 1) exhibited well balanced level of total protein in forage (214-235 g), the similar energy level in forage (ranging from 5,27 MJ to 5,29 MJ) and dry matter level in fodder (1,53 kg). After the forage was supplemented with the black livex the total protein level in caecal content increased by about 17,9% (about 23,1% for brown livex and about 14,1% for blood meal addition, respectively).

Table 2. The influence of addition of three kinds blood's dried preparates: livexes (black and brown) and meal to forage on total protein and energy levels in caecum content taken 2,5 hrs after finished morning feeding

Parameters of caecum content	Type of forage			
	for control sheep	with addition of livex		with addition of blood meal
		black	brown	
Total protein mg * ml^{-1})	7,8 ± 0,6	9,2 ± 0,3	9,6 ± 0,1	8,9 ± 0,2
Increase of protein amount (%)	-	17,9	23,1	14,1
Energy of caecal content (kcal * g D.M. $^{-1}$)	2,0 ± 0,3	2,6 ± 0,1	2,8 ± 0,2	2,4 ± 0,1
Energy increase (%)	-	30,0	40,0	20,0

Simultaneously the increase energy level of about 30% (about 40% for brown livex and about 20% for blood meal addition, respectively) was noted. Interesting results were obtained in regards to volatile fatty acids level in caecal content. For forage, where 2% concentrate (C – J) was replaced by the blood meal, the sum of VFA changed only by about 6,9%. Black or brown livex supplementation caused the increase of VFA level by about 13,8% ($p \leq 0,05$) or 9,8% ($p \leq 0,05$) respectively. On the other hand, the amount of propionic acid in the sum of volatile fatty acids was on the increase by about 58,8% (the increase in rumen content by 52,2%), reaching 0,51 mmol x 100 ml^{-1} of caecal content for the control diet and 0,81 mmol x 100 ml^{-1} of caecal content for the diet supplemented with black livex ($p \leq 0,05$). In case of brown livex or blood meal addition the amount of propionic acid in caecal fermentation was higher by about 47,0 % and 37,2 % respectively ($p \leq 0,001$).
Black livex was 1,6 time more effective than blood meal and 1,25 time more efficient from the brown livex. Livex or blood meal addition to the forage did not changed the amount of acetic acid in total sum of VFA (tab. 3).

Table 3. The influence of addition of three kinds blood's dried preparates: livexes (black and brown) and meal on parameters of caecal fermentation in sheep during in vitro investigations. The samples were taken from 5 sheep immediately after slaughtering. Mean values are presented in mmoles · 100 ml^{-1} of caecal content.

Parameters	type of forage			
	for control sheep	with black liver addition	with brown liver addition	with blood meal addition
Methane	0,22	0,12**	0,14*	0,19
Carbon dioxide	0,62	0,52**	0,56*	0,60
Ratio CO_2:CH_4	2,82	4,33**	4,00**	3,16
Acetic acid	1,02	0,99	1,01	1,00
Propionic acid	0,51	0,81***	0,75**	0,70*
Butyric acid	0,11	0,10	0,09	0,08
Iso-butyric acid	0,03	0,02	0,01	0,02
Valeric acid	0,06	0,05	0,04*	0,05
Iso-valeric acid	0,01	0,01	0,01	0,01
Total ammount of VFA	1,74	1,98*	1,91*	1,86
Ratio acetic:propiomic acid	2,00	1,22**	1,35*	1,42*
Ratio acetic:propiomic: butyric acid	61,1:30,5:8,4	51,6:42,2:6,2	54,3:40,3:5,4	55,6:38,9:5,5
Fermentation efficiency (%)	83,30	87,48	86,02	85,20
NGGR factor	2,28	1,44**	1,56*	1,61*
Cell yield efficiency (mg)	51,00	58,5*	56,70	54,90
ATP/glucose	4,95	5,05	5,05	5,06

Explanations:
Extreme values:
Standard deviations - SD: 0,138 - 0,188
Standard deviation of the mean - SDM: 0,065 - 0,094
Confidence interval - CI: 0,100 - 0,128
Significant statistical differences when:
*****p ≤ 0,001 in relation to initial values,**
****p ≤ 0,01 in relation to initial values,**
***p ≤ 0,05 in relation to initial values**

The ratio of acetic / propionic acid decreased from 2,00 (in comparison with 3,33 in rumen content of control sheep) to 1,22-1,35 (for black and brown livex, respectively), as an effect of higher propionic acid level in caecal content of sheep fed with livex supplemented forage. Consequently, the ratio discussed was nearing the value for the diet with blood meal addition (1,42). It is possible to find out the afore mentioned effects from the relation between acetic and propionic acid (tab. 3). Livex addition to the forage increases fermentation efficiency by about 2,7 % to 4,2 % and cell yield by about 5,7 % to 7,5 %. Fermentation efficiency takes a normal course (tab. 3), when initially very good (2,28) NGGR factor reaches its low limits (1,44-1,61) after livex or blood meal supplementation.

According to Orskov (10) the normal level of NGGR is ranges from 2,25 to 3,00 and each value higher than 3,5 indicates the decrease of VFA utilisation.

In summary, it should have been noted that the energy increase after livex addition to the forage of experimental sheep could be explained as the result of either methane inhibition or increase in amount of total protein in caecal content and slight progression of total amount of VFA.

Antagonism between methane and propionic acid levels in rumen content described by Barej (1), Van Nevel et al. (13) and Zawadzki and Kollek (16) was confirmed also in case of caecal content of the sheep fed with forage with livex addition.

The data interpreted seem to confirm the results of in vitro investigations in sheep rumen (14), proving that livex plays an economical role in saving energy of caecal content.
Livex application in ruminant feeding is an important question for practical studies. In 1958 Konopinski (8) claimed about the role of blood in animal feeding recommending supplemented with blood meal feeds for calves, lambs and cows after habituation to such fodder. Also our results presented in the paper prompt us to study the livex role in ruminant feeding.

CONCLUSIONS

1. Livex addition to sheep forage in the amount of 2 % of nutritive dose causes the methane inhibition, as well as the slight increase in VFA sum and total protein amount and the increase of energy in caecal content.
2. The addition of black or brown livex to forage results in the increase of fermentation efficiency and cell yield efficiency in caecum.
3. Livex improves diet utilisation efficiency, increasing propionate production and decreasing methane production.

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