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CONTENT

THE EFFECT OF ANTI-MASTITIS MEASURES ON THE OCCURRENCE OF INTRAMAMMARY INFECTION IN DAIRY COWS

JULIANA ARVAIOVÁ, FRANTIŠEK ZIGO, ZUZANA FARKAŠOVÁ, ZUZANA LACKOVÁ, ANDREJ RÉCKY, ŠIMON HALÁS, LENKA BOSÁKOVÁ

Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, 040 01 Košice, Slovakia

Corresponding email address: juliana.arvaiova@student.uvlf.sk

ABSTRACT

Mastitis - inflammation of the mammary gland that affects all dairy herds, is a complex illness whose occurrence varies from herd to herd and whose number of ill cows is frequently unknown on most dairy farms. As a result, dairy cow herds need to constantly work on prevention and control. The aim of this study was to reduce the prevalence of mastitis by introduction of effective anti-mastitis measures in a herd of 125 dairy cows. The effectiveness of the relevant measures was monitored by six examinations conducted in two-month intervals during the one-year monitored period. A reduction in the prevalence of mastitis was recorded from 53.6% to 22.9%, i.e. by 26.0%. The prevalence of *Staphylococcus* spp. as the most frequently isolated pathogens of the mammary gland in the examined samples gradually reduced from 33.4% to 18.4%, 14.1%, 10.0%, 7.6%, and 8.1%. In contrast to the dynamics of mastitis, the monthly fluctuations in the values of somatic cell count (SCC) and total bacterial count (TBC) in bulk tank milk samples were irregular. However, a declining dynamics of SCC and TBC was evident during the last three samplings which reflected a reduction in the counts of udder pathogens after the treatment and introduction of mastitis suppression procedures.

***Keywords*:** dairy cows; milk quality; somatic cell count; *Staphylococcus* spp.

INTRODUCTION

Despite increasing advances in breeding technologies and veterinary measures nowadays, mastitis is one of three major health problems of dairy cows. The occurrence of mastitis in farms and their individual forms depends on the interaction of several factors such as the health status of dairy cows, stage of lactation, housing hygiene, nutrition and hygienic milking program (Abebe et al., 2016; Vargová et al., 2023).

Breeding and economic severity of this disease is determined by reducing milk quality and milk production, but also indirect losses such as the premature culling of dairy cows, treatment costs, medicines, disinfectants, and veterinary procedures. The total cost of treating one dairy cow affected by mastitis is in the range of € 120-180 (Hogeveen et al., 2021).

Taponen et al. (2017) reported that up to 95% of intramammary infections that occurred in dairy cows are caused by contamination of the mammary gland with bacterial pathogens through the teat canal. Many sources of udder pathogens have been identified in the milking parlor setting (Vargová et al., 2023). Because the infected mammary gland serves as the main reservoir of pathogenic microflora, keeping the udder clean and milking can shield the healthy cow from an infected cow, hence lowering the infection (Čobirka et al., 2020).

The aim of this study was to reduce mastitis in a herd of 130 dairy cows with the reduction in the incidence of *Staphylococcus* spp. during the annual application of damping procedures.

MATERIAL AND METHODS

Dairy herd and udder examination

The practical part of the study was carried out in dairy cows of Slovak spotted cattle in Eastern Slovakia during one year period 2021. The breeding technology is represented by free box stables covered with straw in two reconstructed stalls connected to the milking parlor with Westfalia tandem 2x8 equipment (Germany). The professional level of the staff as well as the correct observance of hygienic procedures during machine milking was within the limits of the current breeding standard with the application of a wet udder toilet with drying of the teats with

disposable towels before milking and dipping them into the post-dip after the end of the milking. For one year, the etiology and prevalence of mastitis was monitored in six herd examinations of 125 dairy cows at two-month intervals. Individual milk samples for bacteriological examination were taken following the clinical examination of the mammary gland, assessment of the first squirts of milk and California mastitis test (CMT) according to procedures indicated by the NMC (2021).

Sample examination

In addition, SCC, and total bacteria count (TBC) were evaluated in regular monthly intervals in the monitored dairy cows based on milk yield control by workers from The National Dairy Research Institute (Žilina, Slovakia). Microbiological diagnosis (cultivation and identification) of pathogenic bacteria was performed according to our previous study (Zigo et al., 2020). Bacteriological examination included culture in 5% blood agar, Medium No. 110 and Baird Parker agar (Oxoid, GB). Commercial kits, such as STAPHYtest 24, STREPTOtest 24, ENTEROtest 24 (Pliva-Lachema, Brno, Czech Republic) were used to identify individual spieces of udder pathogens.

Mastitis suppression procedures

Based on the results of the clinical examination of the udder of dairy cows supplemented by the California mastitis test, laboratory diagnostic of individual milk samples with the determination of the current sensitivity of bacteria to antibiotics and data from the control of milk utility were implemented the following measures:

- infected dairy cows were threated during lactation according to actually sensitive to antibiotics,
- cows with mastitis were separated from healthy cows and individually milked as the last,
- milk quality and composition was monitored from the treated animals after inclusion in the milking,
- dairy cows with chronic mastitis or atrophy of secretory tissue in udder quarters after unsuccessful treatment were discarded,

- milk samples for laboratory diagnosis were taken from all cows after parturition on the 5th day,
- all cows with high SCC (>400 x 10^3) during last three months of lactation were exanimated or treated and dried by applying selected intramammary preparations,
- the milking cups were disinfected with 5% H_2O_2 after changing each group of cows during the milking process,
- the bedding materials was cleaned frequently every time the cows left for the milking parlor,
- ground limestone was added to the bedding twice a week.

RESULTS AND DISCUSSION

The results of six microbiological examinations of milk samples performed at two-month intervals are evaluated in Table 1 and 2. The most significant representation of the isolated pathogens was represented by coagulase-negative staphylococci (22.8%) and *S. aureus* (10.6%), which were isolated during all 6 examinations.

Table 1. Overview of the isolated bacteria from individual cow´s milk samples during firs three examinations

Examinations/number of dairy cows	**January 123**		**March 125**		**May 121**	
Isolated bacteria	**n**	**%**	**n**	**%**	**n**	**%**
***Staphylococcus* spp.**	41	33.4	23	18.4	17	14.l
S. aureus	13	10.6	7	5.6	3	15
CNS	28	22.8	16	12.8	14	11.6
Others bacteria						
Streptococcus uberis	2	1.6	1	0.8	0	0
Streptococcus spp.	4	3.3	2	1.6	0	0
Aerococcus viridans	15	12.2	10	8.0	2	1.6
Enterococcus spp.	l	0.8	2	1.6	l	0.8
E. coli	0	0	7	5.6	4	3.3
Bacillus spp.	1	0.8	1	0.8	6	5.0
Arcanobacterium spp.	2	1.6	3	2.4		0.8
Proteus spp.	0	0	2	1.6	5	4.l
Findings	**66**	**53.6**	**51**	**40.8**	**36**	**29.8**

Table 2. Overview of the isolated bacteria from individual cow´s milk samples during last three examinations

Examinations/number of dairy cows	August 120		October 118		December 123	
Isolated bacteria	**n**	**%**	**n**	**%**	**n**	**%**
***Staphylococcus* spp.**	12	10.0	9	7.6	10	8.1
S. aureus	0	0	0	0	2	1.6
CNS	1 2	10.0	9	7.6	8	6.5
Others bacteria						
Streptococcus uberis	0	0	0	0	0	0
Streptococcus spp.	0	0	1	0.8	1	0.8
Aerococcus viridans	6	5.0	5	4.3	4	3.3
Enterococcus spp.	1	0.8	3	2.6	5	4.1
E. coli	0	0	l	0.8	3	2.4
Bacillus spp.	4	3.3	3	2.6	3	2.4
Arcanobacterium spp.	2	1.7	1	0.8	2	1.6
Proteus spp.	4	3.3	4	3.4	4	3.3
Findings	**29**	**24.1**	**27**	**22.9**	**32**	**26.0**

Note: CNS – coagulase-negative staphylococci

According to Vargová et al. (2023) Staphylococci are ubiquitous in the dairy cow's environment and *S. aureus* is recognized worldwide as a frequent cause of clinical or subclinical mastitis. Many sources of *S. aureus* have been identified in the skin of the teats, milking units and parlor setting (Rainard et al., 2018; Holko et al., 2020). Contamiantion sources of *S. aureus* was discovered by Bogdanovičová et al. (2014) in raw milk and milk processing tools (milk filters) from 50 dairy farms (from 2012 till 2014) in the Czech Republic. The author found *S. aureus* in 58 samples from 261 raw milk and milk filters, of which 37 (14.2%) were isolated from raw milk and 21 (8.1%) from milk filters.

When 42 dairy farms in the west of Slovakia were examined for the investigation, Holko et al. (2020) verified a significant incidence of intramammary infection brought on by coagulase-negative staphylococci and *S. aureus* isolated from contaminated milk samples. The coagulase-negative staphylococci were the most frequently found bacterium and accounted for 35.9% of positive findings.

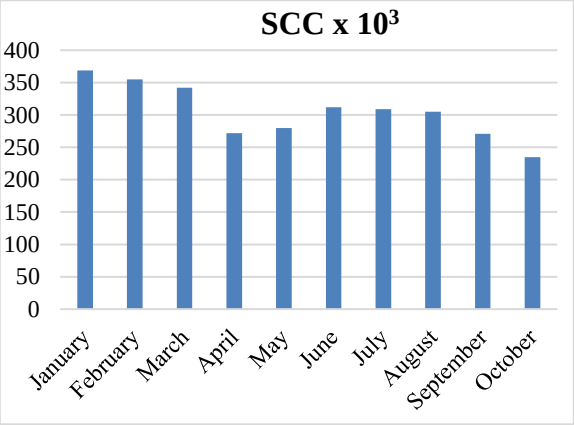
After the first examination were introduced methods to reduce mastitis and the occurrence of new udder infections which reflect examinations at regular two-month intervals in the farm. From the values of the findings, the decreasing dynamics of the prevalence of mastitis of breeding is evident from the value of 53.6% after the first examination to 22.9% in October, respectively 26.0% in December (Table 1).

The prevalence of mastitis at the level of 53.6% at the beginning of the monitored period and especially the effort of the breeder to maintain and improve the quality evaluation parameters of the produced milk were the prompt of the solution the entire complex or related tasks at the level of management and primary production workers in a long-term and systematic way. The analysis of the first examination revealed above all: optimize the implementation of hygienic milking program procedures, to create conditions for the treatment of clinical cases of mastitis in lactation with preparations containing antibiotics with a confirmed effect against isolated bacteria, respectively, it was to decide to treat dairy cows with an antibiotic preparation according to the current sensitivity of the bacteria. The result of each subsequent examination were a check on the effectiveness of the applied procedures and served to correct and possibly supplement the measures. As part of the reduction of the overall prevalence of mastitis in breeding, a significant reduction in the incidence of *Staphylococcus* spp. bacteria was recorded from 33.4% to 7.6% or 8.1% while the occurrence of *S. aureus* was reduced to a minimum (Table 1).

One of the methods of checking the established anti-mastitis measures and health status of the mammary gland of the dairy cows is the monitoring of somatic cells count (SCC) together with the total bacteria count (TBC) in raw milk (Zajác et al., 2012). SCC (fig. 1) and TBC values recorded in monthly intervals do not reflect the dynamics of mastitis prevalence. The discrepancy is also apparent at higher SSC values (369 and 350 x 10^3) a TBC (4.9 – 4.8 log.CFU.ml^{-1} from max. values 5), in the months of January to March, when the prevalence of mastitis was in the level of 53.6%-40.8% and should logically be related to their higher values above the maximum limit allowed. The mentioned contrast was explained in the anamnesis when the farmer used proactive

separation of milk during milking to solve the economic valorisation of production.

Figure 1. Overview of SCC from examination of pool samples of cows´ milk at monthly intervals



Note: SCC - somatic cells count

Based on the assessment of changes during the first squirts, the milk was milked separately into the tankard and was not part of the evaluated milk in the pool sample. The mentioned phenomenon of solving the quality at the expense of the quantity of milk in the absence of solving the causal links of the health of the udder of the part of the breeders is unfortunately becoming a reality.

CONCLUSION

The reduction in the prevalence of mastitis from 53.6% to 22.9%, or 26.0%, was achieved by applying preventive and mitigation procedures guided by the results of mastitis diagnosis. The occurrence of udder pathogens, mainly *Staphylococcus* spp. was reduced from 33.4%, gradually to 18.4%, 14.1%, 10.0%, 7.6%, or 8.1%. The optimal values of SCC and TBC, and obvious decreasing dynamics in pool samples were recorded after the third examination, when the effect of the reducing the occurrence of *Staphylococcus* spp. as the result of the targeted treatment of affected dairy cows based on the antibiogram and culling of chronically infected cows with less milk production.

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TMR QUALITY CONTROL WITH PENN STATE SEPARATOR FOR HIGH-YIELD DAIRY COW

VOJTĚCH BRABENEC, LUBOŠ ZÁBRANSKÝ, MILOSLAV ŠOCH, KANTOR MARTIN, ANNA ŠEBKOVÁ, DENISA POLÍVKOVÁ

Department of Animal Husbandry Science, Faculty of Agriculture and Technology, University of South Bohemia in České Budějovice, Czech Republic

Corresponding email address: Brabenecvojtech@seznam.cz

ABSTRACT

This work deals with feed control using Penn state particle separator and feed quality. The aim of the work was to determine the influence of the structure and quality of feed rations on the performance and health status of dairy cows. The work was processed on the agricultural company ZP Keblov a.s. where the necessary data for the monitored period were defined. Data production and changes in feeding were recorded several times a week during the study period. For feed control, PSPS was used to determine the structure of the feed rations and to match the standard. We also provided chemical analyzes of individual feeds and compared them with the previous feed in the feeding ration. The effect of feed changes on utility and control of continuity between two feed rations and production was evaluated. When swit-ching to a worse feed, the amount of fat in the milk was reduced by up to 0.3 %, and conversely, when switching to a better quality feed, there was an increase of up to 0.4 %. The quality of the feed has the same effect on the amount of milk milked, which ranges from 30 l to 35 l per stable dairy cow during the observed period. The least useful and first health problems were treated on hot days and in the change to high quality feeding.

Keywords: dairy cow, TMR, Penn state particle separator, nutrion, feed

INTRODUCTION

Penn state particle sepparator

A simple separator has been developed for the determination of forage particle sizes and TMR, which allows easy separation of the forage into three fractions and also allows plotting of the particle size distribution. The equipment was designed to mimic the laboratory forage particle size separator specified by the American Society of Agricultural Engineers standard S424. A comparison of the results with the standard device and the newly developed separator showed no difference in the ability to predict the fractions of particles with a maximum length of less than 8 and 19 mm. The separator requires a small amount of sample (1.4L) and is operated manually. The materials on the sieves and the bottom pan were weighed to obtain a cumulative percentage of the sample that was undersized for these two fractions (Lammers et. al., 1996). The PSPS was designed to allow the separation of input particles by a shaking motion duplicating vertical sieving. Two sieves, 19.0 mm and 8.0 mm, and a dish were originally used to estimate the mean particle size (Lammers et al., 1996). Since this publication, the PSPS system has been modified to include a third 1.18 mm screen (Heinrichs and Kononoff 2002).

Screen	Pore Size (inches)	Particle Size (inches)	Corn Silage	Haylage	TMR
Upper Sieve	0.75	> 0.75	3 to 8	10 to 20	2 to 8
Middle Sieve	0.31	0.31 to 0.75	45 to 65	45 to 75	30 to 50
Lower Sieve	0.16	0.16 to 0.31	20 to 30	30 to 40	10 to 20
Bottom Pan		< 0.16	< 10	< 10	30 to 40

Norm PSPS
(https://extension.psu.edu/penn-state-particle-separator#section-3)

Studies have been conducted regarding the structure of TMR on milk components and mallow dislocation. According to Simões et. al., (2013) the effect of unbalanced nutrition was detected when the percentage of

the lower, middle and upper fractions of TMR was determined on each farm using PSPS in feed particles. Maize silage represents about 70 % of TMR. The annual prevalence of dislocation abomasum (DA) was 9.1 %. A positive (r = 0.72; n = 13; P < 0.01) and negative (r = -0.90; P < 0.001) Pearson correlation was observed between the lower or upper TMR fraction and the incidence of DA. The middle fraction of TMR did not correlate with the incidence of mallow dislocation on farms. The dislocation estimate for occurrence from TMR fractions was determined using a multiple regression equation: DA (%) = 6.92 + 0.20 x lower TMR fraction (%) - 1.79 x upper TMR fraction (%) (R 2 adjusted = 0 .82; p <0.001). Different percentages of TMR fractions appeared to affect the incidence of mallow dislocation in these dairy farms. But he also says new studies from a larger population are needed to confirm the feasibility of using Penn State's particle separator to reduce the annual incidence of mallow dislocation in cattle farms.

Caccamão et. al., (2014) conducted a study regarding the amount of fractions in TMR on milk components and yield. And he reports that based on the study, particle size distribution in TMR was associated with small but significant effects on milk protein yield. The distribution most associated with increased protein yield was when the 19 mm fraction contained 10.4 to 17.4% TMR particles and the 1.18 mm screen and dish contained 45 to 59% by weight of TMR particles.

RESULTS AND DISCUSSION

During the experiment, several changes were noted, due to which it was necessary to change the composition of the feed ration. The first change took place in the 28th week due to the low percentage of fat in the milk. The change had to be made in order to reduce the fat percentage below 3.3%, which is the limit value for sale to the dairy. The main reason for the decrease in fat was the increase in temperature during this period, which resulted in a decrease in the amount of fat in milk (Bohmanova et al., 2005; Bohmanova et al., 2007; Gantner et al., 2011). In periods of heat stress, dairy cows clustered in places with a higher frequency of air movement and near drinkers. The dairy cows were also more restless and reacted worse than usual to the presence of the animal technician in the barn. There was therefore an increase in the concentration of nutrients/kg

of dry matter with reduced appetite during this period, the addition of hay to slow down the passage in the digestive tract and increase the frequency of rumination, an increase in the concentration of the amount of buffers in the feed mixture for sufficient salivation of the ruminant feed and a reduction in the likelihood of ruminal acidosis. The proportion of dry corn as well as dry grain that is digested in the rear part of the digestive tract compared to CCM that begins to be digested in the rumen has increased. 2 liters of water were added to the feed to increase the moisture content of the feed, improve deliciousness. It is also necessary to add that the stable in which the cows are stabled is of an older type. Therefore, the quality of lying boxes that are smaller than the ideal size, and it happens that dairy cows do not lie down in the box and are forced to either stand or lie down in the aisle. The cows are cooled by two rows of fans, which are placed in each group above the bedding and feeding corridor. Despite the maximum effort to cool down, it happens that on hot summer days, dairy cows are exposed to heat stress.

Another change in week 31 was the removal of hay due to the increase in milk fat %.

Change On the 32nd week, there was a transition to the 1st corn bag. silage, increase the amount of silage, decrease the amount of hay in feeding ration, increase corn in feed mixture and decrease buffers. The 36th week was the transition to clover hay without any change in production. In the 37th week, there was a transition to corn silage stored in the 2nd bag and a reduction in the proportion of fat in the milk. In this bag, an increased incidence of mold and temperature was detected during collection. Sensory, the corn silage had visible mold deposits and a pungent odor. Most studies report a reduction in milk quantity in the presence of molds and yeasts (Gotlieb 2002; Santos et al., 2015), but do not describe the effect of reduced quality on fat content.

During the 43rd week transition to 2019 silage, the percentage of fat increases. The transition resulted in stabilization of the feed ration.

In the 45th week, a drop in milk was announced due to the failure of the malt bloom, which could only be fed in limited quantities this week.

The last change in the feed ration took place in the 51st week, namely the transition to silage 2020. This change resulted in an increase in the amount of milk per stabled cow by 1.3 liters. During the entire monitored

period, there were no significant changes in the structure of the feed ration. In the months of November and December, there was a deterioration in the health conditions of cows in milking. The increase in the incidence of the disease apparently caused a deterioration in the quality of silage in this period and a subsequent decrease in the voraciousness of dairy cows. In the transit period and at the beginning of lactation, it is important to keep gluttony at a maximum, and the deterioration of quality caused the opposite (Windle 2013; Kung et al., 1998). Since corn silage made up 12 kg of the ration for parched dairy cows and 22 kg for lactating cows, it is the most important feed component. Any change results in an increase in health problems and a deterioration in the dairy cow's ability to achieve maximum production during the lactation period. The transit period is the most important in terms of future production.

week	amount	stable	Øl / stable	Cows milked	Øl / milked	Protein (g/100 g)	fat (g/100g)	SC (tis./ml)	urea (mg/100ml)	DIM	Change
55.	11 117	326	34,1	298	37,3	3,46	3,59	190	25		
54.	11 153	323	34,6	298	37,4	3,46	3,62	201	22		
53.	11 191	321	34,9	297	37,7	3,45	3,65	188	18		
52.	11 085	320	34,6	296	37,5	3,45	3,55	175	19	192	
51.	10 900	327	33,3	299	36,5	3,43	3,62	170	19		
50.	10 831	325	33,3	299	36,2	3,46	3,68	182	20		
49.	10 478	327	32	290	36,1	3,43	3,68	185	18		
48.	10 531	326	32,3	288	36,6	3,45	3,66	204	19	187	
47.	10 358	324	32	287	36,1	3,44	3,67	187	20		
46.	10 208	322	31,7	283	36,1	3,46	3,69	193	21		
45.	9 794	318	30,8	279	35,1	3,42	3,72	186	22		✓
44.	10 202	318	32	280	36,4	3,43	3,61	211	21	193	
43.	10 323	328	31,5	289	35,7	3,44	3,52	206	21		✓
42.	10 365	329	31,5	290	35,7	3,45	3,47	212	21		
41.	10 476	328	32	288	36,4	3,49	3,38	224	21		✓
40.	10 241	322	31,8	283	36,1	3,49	3,3	206	19		✓
39.	10 218	319	32	282	36,3	3,54	3,26	208	18	205	✓
38.	10 237	319	32,1	280	36,6	3,52	3,3	182	18		✓
37.	10 188	318	32	282	36,1	3,54	3,41	210	16		✓
36.	10 285	316	32,5	284	36,2	3,52	3,42	200	21		✓
35.	10 152	317	32	282	36	3,51	3,44	226	18	206	
34.	9 930	315	31,5	280	35,4	3,45	3,41	215	22		
33.	10 266	322	31,8	285	36	3,41	3,51	243	24		
32.	10 708	334	32,1	300	35,7	3,41	3,42	235	25		✓
31.	10 364	335	31	296	35	3,36	3,53	236	24	180	✓
30.	10 158	334	30,4	298	34,1	3,35	3,52	204	26		
29.	9 980	333	30	298	33,5	3,32	3,53	234	24		
28.	10 260	330	31,1	291	35,2	3,37	3,38	250	25		✓
27.	10 609	325	32,6	288	36,8	3,39	3,18	219	19		
26.	10 813	323	33,5	290	37,3	3,33	3,3	217	23	184	
25.	10 831	323	33,5	291	37,3	3,4	3,33	193	21		
24.	11 191	328	34,1	291	38,4	3,43	3,32	222	23		

23.	10 997	330	33,4	291	37,8	3,4	3,47	228	22		
22.	10 809	329	32,9	294	36,8	3,38	3,44	151	25	183	
21.	10 841	326	33,2	292	37,2	3,39	3,44	185	23		
20.	10 820	323	33,5	293	36,9	3,38	3,5	165	26		
19.	11 073	321	34,4	293	37,8	3,39	3,4	176	23		
18.	10 935	319	34,3	285	38,4	3,41	3,39	171	25		
17.	10 966	317	34,6	281	39,1	3,4	3,42	200	25	175	
16.	10 773	312	34,5	274	39,3	3,4	3,37	170	25		
15.	10 801	313	34,5	278	38,9	3,41	3,49	171	26		
14.	10 809	319	33,8	287	37,6	3,45	3,48	176	26		
13.	10 558	319	33,1	284	37,1	3,43	3,56	163	26	180	
12.	10 421	315	33	288	36,2	3,44	3,58	158	27		
11.	10 326	312	33,1	282	36,6	3,46	3,59	153	26		
10.	10 310	309	33,4	277	37,2	3,48	3,65	137	27		
9.	10 334	307	33,6	280	36,9	3,49	3,68	164	29	173	
8.	10 372	308	33,7	274	37,9	3,34	3,66	146	21		
7.	10 304	313	33	285	36,2	3,46	3,7	158	26		
6.	10 602	316	33,5	287	36,9	3,48	3,67	179	27		
5.	10 316	318	32,5	289	35,7	3,49	3,7	166	27		
4.	10 396	319	32,6	289	36	3,49	3,71	209	28	168	
3.	10 116	319	31,7	290	34,9	3,5	3,73	213	26		
2.	10 219	320	31,9	290	35,2	3,49	3,67	184	26		
1.	10 244	325	31,5	289	35,4	3,49	3,78	180	28		

CONCLUSIONS

The profitability on the farm varied during the observed period from 30 to 35 liters of milk per stabled dairy cow, depending on the quality of roughage, which changed over the year. Due to the high productivity on the farm, it is important to monitor all indicators, evaluate them regularly and focus on deficiencies in feeding. Inspection using PSPS is one of the methods, used together with faecal washing, for rapid inspection of feed. We are able to determine the structure of the feed ration within a few minutes, to find out whether the dairy cows are separating the feed and whether there is a higher percentage of longer particles needed for proper rumen function left in the trough.

When switching to poorer silage, a decrease in milk components and a deterioration in the health status of dairy cows were recorded. This change did not only result in a deterioration of production, but also a further deterioration of the economic indicators associated with the treatment of these diseases. Since dairy cows are most susceptible to changes in the feed ration during the transit period, the deterioration of feed quality and welfare is one of the reasons for the occurrence of postpartum diseases. Therefore, it is very important to focus on the

period around the birth and pay close attention to it in order to determine the subsequent production in lactation. The improvement occurred when switching to silage from 2020, which had a very good composition. After switching to this feed, increased production and a reduction in the number of health problems associated with feeding poor quality feed were noted.

For practice, I would recommend focusing on the quality of roughage, which largely determines our productivity in the following year. Adherence to technological procedures in the production of bulk fodder, such as the correct phenophase of maturity at harvest, adherence to optimal dry matter and the addition of homofermentative or heterofermentative bacteria for silage conservation. To reduce the occurrence of molds and yeasts, it is important to sufficiently expel the air from the material and quickly cover the silage pit. The selection of maize silage hybrids is also very important for the high proportion of starch and high digestibility of NDF.

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BLACK SOLDIER FLY LARVAE AS AN ALTERNATIVE PROTEIN SOURCE AFFECTING GROWTH PARAMETERS OF BROILERS

STANISLAVA DROTÁROVÁ, BRANISLAV GÁLIK, MILAN ŠIMKO, MIROSLAV JURÁČEK, MICHAL ROLINEC, ONDREJ HANUŠKOVSKÝ, MÁRIA KALÚZOVÁ, MÁRIA KAPUSNIAKOVÁ

Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak Agriculture University in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra

Corresponding email address: xdrotarova@uniag.sk

ABSTRACT

The use of insects as an alternative source of protein feed in broiler chick diets represents one possible solution to current issues aimed at efficient broiler rearing, with the least environmental burden on the planet, and at the same time to the ever-increasing consumer demand. Insects are very adept at converting waste into nutrient-rich feed. In particular, insects and their larvae represent an ideal natural feed for poultry. The aim of this study was to investigate to what extent feeding and replacing vegetable protein with insect meal can affect the growth performance of broilers. The experiment involved 131 one day old broilers of the hybrid Cobb 500. The whole experiment lasted 37 days, with 2 stages of broiler fattening taking place during the experiment. The broilers were divided into three experimental groups (control, 50% insect meal replacement of extracted soya bean meal and 100% insect meal replacement replacement of extracted soya bean meal experimental groups). Partially defatted insect meal from black fly (*Hermetia illucens*) larvae was used as insect meal. At the end of the experiment, all animals were weighed and 60 broilers were analysed to determine carcass, breast muscle and viscera weights. The highest average clean carcass weight was obtained by the group fed 100% insect meal (2047.63 ± 95.61 grams), with statistically significant differences between groups ($P>0.05$). Among viscera, no statistically demonstrated differences were observed between

stomach, heart and liver but on the contrary, statistically demonstrated differences (P> 0.05) were observed between abdominal fat and viscera overall. In addition, the highest average weight of viscera overall was obtained by the group fed diets containing 100% insect meal (235.41 ± 25.03 grams). A statistically significant difference (P>0.05) was observed among the pectoral muscle weights for all traits studied. The highest average weight in the breast muscle trait was obtained by the group fed diets containing 100% insect meal (322.20 ± 28.80 grams). Based on our obtained results, we conclude that feeding of partially defatted insect meal from black fly (*Hermetia illucens*) larvae results in positive changes and increases in carcass yield of broilers.

Keywords: insect meal; growth parameter; carcass yield; black soldier fly

INTRODUCTION

Black fly larvae and their sustainable breeding represent one solution to meet the increasing global demand for animal protein in animal and human nutrition (Raman et al.. 2022). The projected increase in world population to 9.6 billion by 2050, represents an increasing global need for food production (Fouilleux et al.. 2017), with the need to reduce emissions and environmental burdens (Raman et al.. 2022). Insects represent one of the possible alternative solutions used as feed not only in animal nutrition (Gasco et al.. 2020), but equally as food in human nutrition (Roos. 2018). Despite the potential of insects, in 2015 the European Food Safety Authority issued its first opinions on the potential risks associated with the rearing and consumption of insects as human food or animal feed. The risks mainly focus on factors associated with insect farming and subsequent consumption and possible allergic reactions and health problems or environmental risks (Mancini et al.. 2022). Despite the potential risks, the European Union has approved the consumption of insects that include both the Black Fly (Hermetia illucens) and its larvae (Gasco et al.. 2020).

The black fly (BSF) and its larvae have a high capacity to convert organic waste into high-protein feed, being able to inactivate some potential

bacterial risks arising from the quality of the rearing substrate (Barragan-Fonseca et al.. 2017) and are a potential alternative feed source in the diet of monogastric animals, especially poultry, pigs and fish (de Souza Vilela et al.. 2021). Nutrient composition and protein content largely influences the composition of the BSF breeding substrate (Spranghers et al. 2017). Larvae reared on fish waste achieved higher protein content (more than 78%) than larvae reared on a substrate composed of fruits and vegetables (less than 13% protein) (Hopkins et al.. 2021). In addition to protein and amino acid content, the substrate can also affect ash content at the same time, while no changes in fatty acid content were observed (Spranghers et al.. 2017. Ewald et al.. 2020). Changes in the content were equally observed in the content of macronutrients, especially calcium, phosphorus and potassium. Their concentrations varied with substrate composition (Chia et al.. 2020). Quality protein content and especially amino acid content is an important criterion in poultry nutrition (Cheng et al.. 2023). For this reason, the use of insect meal in poultry nutrition can be very effective, it contains a high digestible protein content, on average a crude protein content of 35-40% (Matin et al.. 2021), reaching similar or even higher values than soybean meal or fish meal. (Barker et al.. 1998). Feeding insect meal can lead to an increase in performance but also to an improvement in broiler immunity (de Souza Vilela et al.. 2021), but the fat contained in black fly larvae does not affect gut health (Schiavone et al.. 2018). Feeding insect meal to black fly larvae improves feed conversion and also affects the nutrient composition and fatty acid content of the final poultry meat product (Kim et al.. 2020).

MATERIAL AND METHODS

131 chickens of hybrid Cobb 500 were included in the experiment. The whole experiment lasted 37 days and during the whole experiment care was provided in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Care was provided by an experienced caretaker (feeding, watering, litter care and health check). At the beginning of the experiment, the chickens were divided into three experimental groups of control (C) - 44 chicks, group with

50% insect meal replacement of extracted soya bean meal (BR50%) - 44 chicks and group with 100% meal replacement of extracted soya bean meal (BR100%) - 43 chicks. For 18 days, each group was kept in a 90 x 200 cm straw bedding area where, in addition to the basic equipment (feeder, waterer), they also had a heater (UV lamp). For the next 19 days, the chicks were moved to a 300 x 300 cm brooding area without a heating element (UV lamp). Broiler weights were recorded throughout the experiment.

Each group had a separate feed formula, and the feed intake of all groups was ad libitum. Individual feeding mixtures differed between groups in composition and insect meal content of BSF larvae. The nutrient composition of the insect meal is presented in table 1.

Table 1. Basic nutrient composition of insect meal black soldier fly larvae (BSF) (*Hermetia illucens*) at 100% dry matter (g/kg)

Component	
Dry matter	1000
Protein	520.80
Fat	131.10
Fiber	92.89
Ash	58.88
Nitrogen free extracts	196.34
Nonstructural carbohydrates	135.33
Organic matter	941.12
Starch	83.94
Acid-detergent fraction of fiber	113.4
Neutral-detergent fraction of fiber	153.89
Lignin	26.65
Celulose	86.76
Hemicelulose	40.49

Technological procedures and calculations using Evonik's 2010 QuickChick Calculator software were used to calculate and prepare the experimental feed formulations. We report the amino acid content of the experimental feed formulas as standardized ileal digestible amino acids (amino acids digestible in the poultry small intestine). We divided the experimental feed formulas into two feeding stages. In the first stage, the feed mixture was fed to broilers from one to twelve days of fattening

(days 1-12) (Table 2). In the second stage, the experimental feed mixture was fed to the broilers from twelve days to the time of termination of the experiment (day 12-37) (Table 3).

Table 2. Composition of the diet in individual groups stage 1 (%)

Component	C	BR50%	BR100%
Wheat	19.5	48.40	50.45
Maize	40	20	19
Extracted Soya bean meal	29	12.40	-
Wheat DDGS	-	-	3.0
Albumex 102-Broiler	3.15	3.5	7
Insect meal (*Hermetia illucens*)	-	12.40	17.5
Rapeseed Oil	3.50	0.20	-
Calcium 37.8%	1.03	0.80	0.52
Salt	0.27	0.28	0.3
Su Minfos 22.7% P, Ca, L	0.6	0.35	0.3
Premix Treonin 20	0.45	-	-
Premix Lysine 40	0.95	0.60	0.85
Premix Methionie 40	0.55	-	-
NTR Chicken uni	1	1	1

Table 3. Composition of the diet in individual groups stage 2 (%)

Component	C	BR50%	BR100%
Wheat	20.55	40.70	45.60
Maize	48	33.0	28.0
Extracted Soya bean meal	20	10.80	-
Wheat DDGS	-	-	4.0
Albumex 102-Broiler	3.40	0.42	2.50
Insect meal (*Hermetia illucens*)	-	10.80	17.0
Rapeseed Oil	3.50	1.60	0.40
Calcium 37.8%	0.94	0.85	0.72
Salt	0.25	0.28	0.28
Su Minfos 22.7% P, Ca, L	0.55	0.35	0.35
Premix Treonin 20	0.65	-	-
Premix Lysine 40	0.83	0.20	0.15
Premix Methionie 40	0.33	-	-
NTR Chicken uni	1	1	1

For proper development of the musculoskeletal system, fat-soluble vitamins A, D3 and E were administered to all broiler chickens along with clean and hygienic drinking water throughout the duration of the experiment. By administering the vitamins in drinking water, we ensured the best and sure intake of these vitamins involved in the proper

development of the musculoskeletal system and the promotion of immunity of the experimental animals.

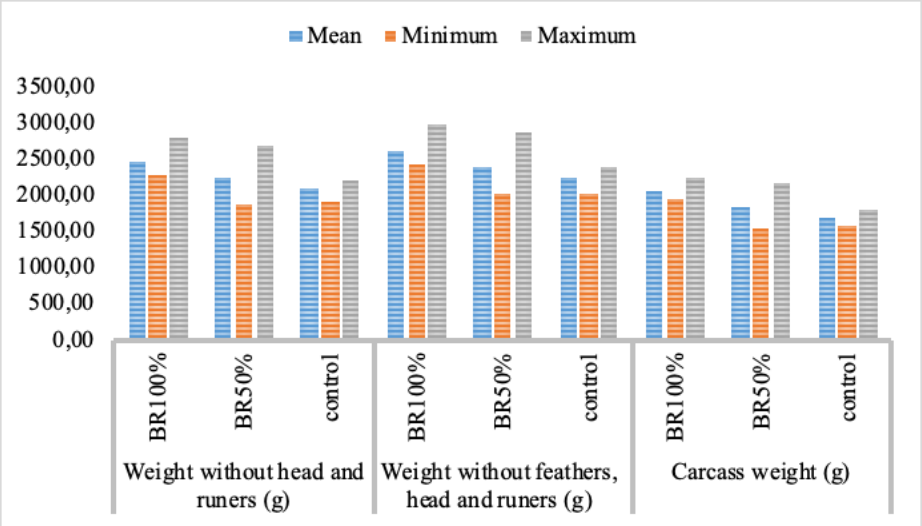
At the end of the experiment, all experimental animals were weighed. For subsequent laboratory analyses, 10 animals of each sex were selected from each experimental group (20 from each group: 10 roosters, 10 hens. 60 animals in total). They were weaned from the experimental feed mixture and transported to the Slovak Agricultural University in Nitra to the Institute of Animal Nutrition and Genomics, where the animals were killed, carcasses processed and samples processed. During the processing of the samples, the total weight and the weight of the individual carcass parts (breast muscle and viscera - stomach, heart and liver) were recorded.

Basic variation-statistics (arithmetic mean, standard deviation and coefficient of variation) were used for the statistical processing of the results, evaluated in SPSS 20.0 (IBM). ANOVA - Tukey's HSD test (SPSS v. 20.0) and Student's t-test (MS Excel) will be used to test the statistical significance of differences between the variants.

RESULTS AND DISCUSSION

After 37 days of feeding, the carcass weights of the 60 experimental animals were the first to be determined at the end of the experiment. Differences in weight without head and runners (g), weight without feathers, head and runners (g) and carcass weight (g) were determined. The highest premeasured weight without head and runners (g) was obtained by the BR100 % group (2467 ± 134.33 grams), the lowest weight was obtained by the control group (2104 ± 91.31 grams). The same results were observed for weight without feathers, head and runners (g) with the highest weight in the BR100% experimental group (2626 ± 153.49 grams) and the lowest in the control group (2259 ± 106.60 grams). The lowest total average carcass weight (g) was obtained by the control group, only 1689.36 ± 73.22 grams. On the other hand, the highest mean weight was obtained by the experimental group BR100% 2047.63 ± 95.61 grams (Figure 1).

Figure 1. Average weights at the end of experiment (g)



The average difference between the groups was 358.27 grams. Significantly significant differences (P > 0.05) were observed between experimental groups and mean weights, (Table 4)

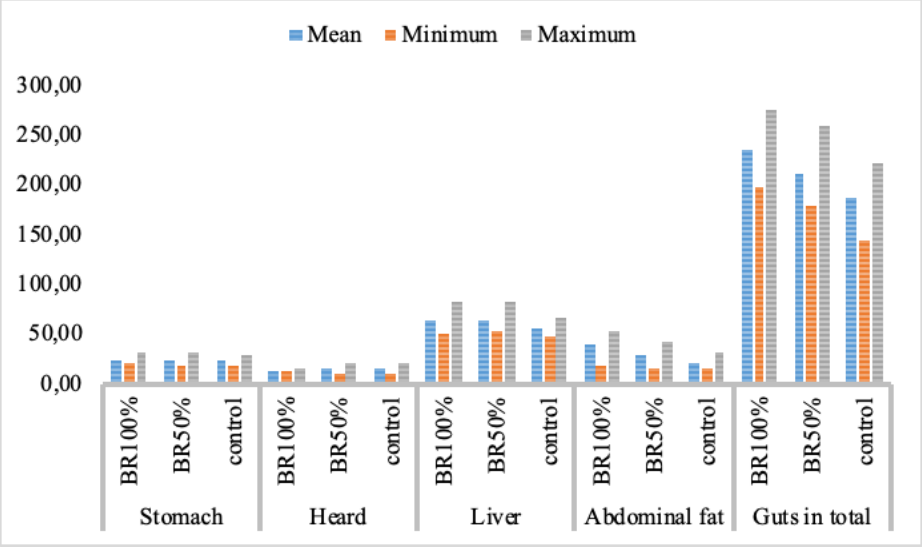
Table 4. Average weights at the end of the experiment (g)

		Mean	Minimum	Maximum	Sig.
Weight without head and runers (g)	BR100%	2467.00	2290.00	2790.00	0.0060
	BR50%	2227.00	1880.00	2680.00	
	control	2104.00	1910.00	2210.00	
Weight without feathers. head and runers (g)	BR100%	2626.00	2430.00	2990.00	0.0110
	BR50%	2378.00	2020.00	2860.00	
	control	2259.00	2030.00	2390.00	
Carcass weight (g)	BR100%	2047.63	1947.72	2257.61	0.0010
	BR50%	1817.59	1554.70	2152.00	
	control	1689.36	1566.55	1782.06	

No significant differences were observed between the experimental groups in the weights of viscera (stomach, heart) of the experimental animals. Differences between groups were observed in the case of liver,

where the highest measured mean weight was observed in the BR100% experimental group (64.61 ± 10.10 grams) compared to the control group 57.07 ± 6.96 grams. Similarly, we also observed the highest measured values for abdominal fat for BR100% group (39.17 ± 10.32 grams) at BR50% group (28.83 ± 9.021) grams and control group 21.18 ± 4.194 grams. The control group had the lowest mean total visceral weight (186.71 ± 24.18 grams) and the BR100% group had the highest (235.41 ± 25.03 grams). The mean difference between the groups was 48.7 grams (Figure 2).

Figure 2. Average gut weights at the end of the experiment (g)



No significant differences were observed between the mean weights for viscera such as stomach, heart and liver, but for weights between visceral fat and viscera overall, a statistically significant difference was observed between the groups ($P > 0.05$), (Table 5).

Table 5. Average gut weights at the end of the experiment (g)

		Mean	Minimum	Maximum	Sig.
Stomach	BR100%	24.58	19.90	32.81	0.201
	BR50%	24.87	18.32	32.19	
	control	22.96	17.24	29.09	
Heard	BR100%	14.18	12.17	16.86	0.087
	BR50%	15.85	10.00	21.98	
	control	15.30	10.50	21.66	
Liver	BR100%	64.61	50.67	83.75	0.130
	BR50%	62.96	51.94	81.60	
	control	57.07	47.17	67.10	
Abdominal fat	BR100%	39.17	19.73	53.55	0.019
	BR50%	28.83	16.36	42.90	
	control	21.18	15.71	30.85	
Guts in total	BR100%	235.41	198.48	276.66	0.018
	BR50%	210.83	178.78	259.51	
	control	186.71	143.72	223.01	

Last, differences in breast muscle weights, specifically breast with skin and fat, skin and subcutaneous fat from the breast and breast muscle total, were examined. In all the traits studied, the highest values obtained were recorded in the experimental group fed with 100% insect meal - BR100%. They achieved breast weight with skin and fat 345.66 ± 30.75 grams, skin and subcutaneous fat from the breast 32.54 ± 6.83 grams and breast muscle total 322.20 ± 28.80 grams. On the contrary, the lowest observed results were observed in the control group (C)- breast with skin and fat 278.75 ± 23.76 grams, skin and subcutaneous fat from the breast 25.13 ± 6.46 grams and breast muscle total 253.84 ± 22.10 grams (Figure 3).

Figure 3. Average breast weights at the end of the experiment (g)

Among all the traits studied, we observed significant differences in mean weights between groups (P > 0.05), (Table 6).

Table 6. Average breast weights at the end of the experiment (g)

		Mean	Minimum	Maximum	Sig.
Breast with skin and fat	BR100%	354.66	315.12	418.55	0.0000
	BR50%	326.94	266.32	394.73	
	control	278.75	251.38	331.24	
Skin and subcutaneous fat from the breast	BR100%	32.54	23.14	43.62	0.0170
	BR50%	27.23	12.42	39.14	
	control	25.13	19.54	42.07	
Breast muscle	BR100%	322.20	285.79	375.92	0.0000
	BR50%	299.65	253.93	355.64	
	control	253.84	229.91	307.91	

Similar positive results with insect feeding with addition to broiler feed mix were also reported by Khan et al. (2018), reporting improvements in growth parameters and weight gain with no observed reduction in feed

intake. Conversely, no change in differences in weight and average daily gains between groups fed with or without insect meal content was reported by Leiber et al. (2017). However, they reported that the use of insect meal as an alternative source and substitute for soybean meal extracted is a possible solution. A similar experiment with growth parameters and similar results with positive weight gain was also reported by Benzetiha et al, (2020), where in addition to the observed weight gain by feeding small amounts of insect meal (Tenebrio molitor), they also noted positive traits in the body's immune response. An experiment with complete replacement of vegetable protein was also carried out by Pietras et al. (2021), (2021), during the whole fattening period they did not observe significant changes in growth parameters and carcass yield. Like us, they did not observe a change in internal weight differences (heart, stomach and liver), on the contrary, they observed negative growth traits in the case of feeding on the blan wing insect (Zophobas morio). Schiavone et al. (2019), similarly, used BSF (Hermetia illucens) larvae as a source of insect meal. They used the same partially defatted insect meal in the same experiment and observed the same positive improvements in growth traits, but also a positive increase in breast muscle without negative effects on meat quality parameters. Opposite results with the replacement of soybean-extracted meal with insect meal from black fly larvae were reported by Murawska et al. (2021). The authors reported negative inherent and reduced weight gain when replacing more than 50% of soybean extracted meal due to reduced feed intake. The difference compared to our experiment consisted in feeding full-fat insect meal from black fly larvae. However, Barragan-Fonseca et al. (2017) report possible risks associated with feeding insect meal specifically from black fly larvae. Their nutrient composition is highly variable as the nutrient content of insect meal depends on the nutrient content of the feeding substrate. However, in the case of uniformity of rearing practices, they indicate insect meal as a suitable alternative feed for poultry, pig and fish nutrition.

CONCLUSION

Our results indicate the suitability of feeding insect meal from black fly larvae in broiler diets. We observed a positive increase in total carcass yield, an increase in breast muscle, and a concomitant increase in abdominal fat, which may have a positive effect in terms of consumer interest. However, from an economic and environmental point of view, the feeding of black fly larvae meal may pose some risk in the conditions of Central Europe, the temperature and nutrient requirements and the legislative conditions do not yet allow black flies to be reared on waste, and thus they are not yet a competitor in terms of feed for our farm animals in the conditions of the European Union. At the same time, the import of insect meal may represent a certain health risk for the time being at a high price. In the future, however, insect meal represents a suitable alternative protein feed, especially for monogastric animals.

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THE EFFECT OF MILK PRODUCTION AND ENERGY BALANCE ON PREGNANCY SUCCESS AFTER THE 1ST INSEMINATION IN DAIRY COWS

BEÁTA FRAŇOVA, IVETA MASKAĽOVÁ

Department of Animal Nutrition and Husbandry, University of veterinary medicine and pharmacy in Košice, Komenského 73, Košice Slovakia

Corresponding email address: beata.franova@student.uvlf.sk

ABSTRACT

The milk yield and reproductive performance are the standard economic barometers of dairy production. In this intention the aim of the work was to verify the influence of milk production, the energy balance and nitrogen transformation on success pregnancy after the 1st insemination. Milk production with a standard content of milk fat and protein with the analyzed negative energy balance at the level -23.7 MJ/day in multiparous dairy cows or -9.4 MJ/day in primiparous dairy cows did not confirm a significant effect on the success of conception after the 1st insemination.

***Keywords*:** energy balance, utilization, insemination, pregnancy

INTRODUCTION

Increased milk production in high-producing dairy cows was made possible by genetic selection, which was supported by an increased ability to mobilize fat from adipose tissue and by an improved level of nutrition. Higher milk production, however, has a negative impact on reproductive parameters with a tendency to decrease reproductive performance that is manifested by prolonged time to first insemination, poor oestrous behaviour, increased number of open days, reduced artificial insemination success and high culling rates due to poor reproductive performance (Lucy 2019, Thatcher et al., 2006). Reduced fertility in modern dairy cows includes delayed resumption of normal ovarian cyclicity (Crowe et al., 2015), uterine health (Crowe et al., 2014), lower expression of heat symptoms and lower pregnancy rates to first

and subsequent inseminations. The peripartum period in dairy cows has a major impact on their health and further fertility. Due to high energy losses, most cows reach a state of negative energy balance (NEB), when significant metabolic changes occur and these directly affect the recovery of the estrous cycle and the success of further inseminations (Wathes et al., 2007). NEB leads to lipomobilisation and thus burdens the liver. Important metabolic processes take place in the liver that affects liver function (gluconeogenesis, oxidation of fatty acids, production of insulin-like growth factor (IGF-I) (Butler 2005). Decreased IGF-I concentration in NEB affects ovarian function and affects the development of embryos. Recovery of the estrous cycle after calving and subsequent successful insemination depends on several factors associated with the function of organs that undergo a significant load in the peripartum period (Wathes et al., 2007). In the period at the end of pregnancy, hormone levels increase significantly, which subsequently negatively affects the recovery of ovarian activity. The recovery the activity of the ovaries and the production of hormones for the development of follicles, which is necessary for the estrus. Before the end of pregnancy, high levels of hormones suppress the production of gonadotropins, so after parturition, it is necessary to restore the secretion of LH, which is necessary for stimulating the development of follicles. NEB through lipomobilization and the increased concentration of non-esterified fatty acids in the body delays the increase in the secretion of gonadotropins, thereby delaying the maturation of ovarian follicles. Decreased levels of insulin in the blood affect the production of IGF-I in the liver and this causes a decreased sensitivity of the ovaries to gonadotropins. These connections cause a delay in the recovery of ovarian activity of the ovaries, a delay in the onset of the first postpartum estrus and, ultimately, in the recovery of the overall capacity for further reproduction (Butler 2005). Unbalance of protein nutrition can affect the reproduction of dairy cows through the toxic effect of ammonia and its metabolites on gametes and early embryos. Urea concentration in the uterine was positively associated with ammonia and blood urea concentration when animals were fed feed rations with high content of nonprotein nitrogen. Studies also report that a higher concentration of urea lowers uterine pH (Sammad et al., 2022). The harmful effect has an

unbalanced intake of energy and protein in the first phase of lactation. NEB could be exacerbated by the addition of excess protein due to the extra energy demands required for urea detoxification and excretion. Each gram of excess nitrogen can increase energy requirements by 13.3 kcal of digestible energy (Hammon at al., 2005). Several studies have shown links between protein nutrition and reproductive performance. It has been shown that feeding excessive amounts of protein can cause significant fertility problems in cows (Rajala-Schultz et al. 2001). On the contrary, with a low concentration of milk proteins, which were associated with an increased or decreased concentration of urea, various reproductive problems were recorded, such as silent estrus or increased embryonic mortality (Noordhuizen 2012).

The goals of the work are based on the hypothesis of a published negative relationship between the genetically determined increased milk production, with the simultaneous reduction of reproductive performance and the higher manifestation of metabolic disorders in dairy cows. In this intention the aim of the work was to verify the influence of milk production, the energy balance and nitrogen transformation on success pregnancy after the 1st insemination in primiparous and multiparous dairy cows in breeding conditions on a farm with milk production over 10 000 kg.

MATERIAL AND METHODS

The evaluations were carried out on the selected farm with a controlled nutritional level system and an average annual production over 10 000 kg per cow. Cows were housed in a freestall barn in a separate group and fed a TMR that was formulated to meet NRC (2001) recommendations for 1st phase of lactation of multiparous and primiparous dairy cows. Primiparous and multiparous dairy cows (n = 235) were evaluated for the efficiency of utilizing N and energy balance in relation to pregnancy success at first insemination. Samples of prepared TMR in the monitored farms were taken from the feed manger on the control day and there were analysed for dry matter (DM), crude protein (CP), acid and neutral detergent fibre (ADF, NDF), starch and ether extract (EE) contents according to conventional methods according to the Commission Regulation (EC) no. 691/2013. NEL and non-fibrous carbohydrate

(NFC) values were calculated using regression equations (NRC 2001). All cows were enrolled in a Presynch Ovsynch protocol. Controlled reproduction was applied from the 70th day after parturition. The success of reproduction was evaluated in dairy cows by transrectal ultrasonography of the ovaries and uterus.

Analysis of production parameters on the control day on individually collected milk samples was evaluated for milk production levels in dairy cows, milk components and milk urea. Milk samples were analysed the total protein content, fat, lactose, and urea concentration by near infrared spectrophotometric assay using MilkoScan FT^{+} and BENTLEY FTS at the Central Analytical Laboratory of Milk with accreditation under registration number 096/5878/2015/2. The analysed urea in milk (MU) was converted to urea nitrogen in milk (MUN) using the equation by Oudah (2009).

Efficiency of Nitrogen Utilization (EUN) for group of dairy cows was estimated according to the analysed content of MUN and the amount of milk produced by using regression equation by Huhtanen et al (2015) from meta-analytical assessments of the balance experiments.

The achieved results were processed by mathematical and statistical methods using the Tukey-HSD test at the significant levels of $P \leq 0.01$ and $P \leq 0.05$ by the statistical program GraphPad Prism9. Each parameter was presented as its mean (x), and standard deviation (SD).

RESULTS AND DISCUSSION

1.) Evaluation of nutritional composition

The average concentration of nutrients in TMR at the evaluated farm are presented in Tables 1. The feed ration based on corn, clover and grass silage with the addition of concentrated feed was formulated for multiparous dairy cows for milk production 39.3 kg (3.28% protein and 3.6% fat) or 36.5 kg (3.22% protein and 3.66% fat) for primiparous dairy cows.

Table 1. Nutritional composition of TMR

	Primiparous cows X ± SD	Multiparous cows X ± SD
CP g/kg DM	160.4 ± 7.5	157.2 ± 1.9
Fat g/kg DM	43.5 ± 1	43.6 ± 2.3
ADF g/kg DM	215.8 ± 5.0	215.8 ± 3.8
NDF g/kg DM	349.2 ± 4.5	347.7 ± 3.5
Starch g/kg DM	254.8 ±4.3	268 ± 2.4
NFC g/kg DM	378.2 ± 4.1	380.4 ± 3.7
NEL MJ/kg DM	6.59 ± 0.1	6.59 ± 0.1
Dry matter intake kg	21.3	24.5
NEL intake MJ/d	140.4	161.5
N intake g/d	615	517
Composition: corn silage 19-33 kg, legume silage 6-17 kg, grass (oat) silage 2-3 kg Grass/alfalfa hay 0.7–1 kg, wheat straw 0.5-0.8 kg DDGS 4 kg, wheat 0.8–3.5 kg, rapeseed meal 2.2 – 3.8 kg Feed mixture 4.2 kg		

2., Evaluation of success pregnancy after 1st insemination

Over a period of 5 multiparous and primiparous dairy cows (n 235) were evaluated after the first insemination with an average success of conception (success pregnancy) at the level of 30.6%, of which 23.6% in multiparous dairy cows (14.8 – 31.3%) and 41.8% in primiparous dairy cows (29.4- 66.7%).

3., Evaluation of milk production, energy balance and N utilization on reproduction

The evaluated indicators in the group of multiparous dairy cows are summarized in Table 2. The group of multiparous dairy cows achieved an average milk production of 46.5 ± 1.7 kg per day with a standard proportion of milk components (3.61% fat and 3.08% protein).

Table 2. Production parameters, energy balance, EUN and success pregnancy after 1st insemination in multiparous dairy cows

	multiparous cows (n 144) X ± SD	1st insemination positive (n 34) X ± SD	1st insemination negative (n 110) X ± SD
Milk production kg/d	46.5 ± 1.7	46.0 ± 1.8	47.1 ± 1.5
ECM kg/d	47.3 ± 2.1	47.2 ± 2.7	47.4 ± 1.7
Milk fat %	3.61 ± 0.3	3.66 ± 0.3	3.56 ± 0.2
Milk protein %	3.08 ± 0.1	3.11 ± 0.2	3.04 ± 0.1
Fat/Protein	1.17 ± 0.1	1.18 ± 0.1	1.17 ± 0.1
Lactose %	4.92 ± 0.1	4.95 ± 0.1	4.89 ± 0.1
Energy Balance MJ/d	-23.7± 7.2	-23.7± 8.7	-23.6 ± 6.3
NEL intake MJ/d	161.5	161.5	161.5
NEL in milk MJ/d	138.9 ± 6.3	138.9 ± 7.7	138.8 ± 5.6
EUN[1] %	32.5 ± 0.2	32.5 ± 0.2	32.5 ± 0.2
Milk Urea mg/dl	28.1 ± 2.1	27.6 ± 1.3	28.6 ± 2.7
MUN mg/dl	13.1 ± 1.0	12.9 ± 0.6	13.3 ± 1.2
Feed efficiency	1.93	1.93	1.93
% Of animals	-	23.6 (14.8 – 31.3)	76.4 (68.7 – 85.2)

ECM – energy corrected milk (3,2% protein and 3,5% fat)
EUN – Efficiency of nitrogen utilisation (Huhtanen et al.2015)
MUN – milk urea nitrogen

The achieved milk production in multiparous dairy cows with a dry matter intake of 24.5 kg/day compared to the calculated balance of nutrients shows a negative energy balance at the level of -23.7 MJ/day for the evaluated period of the 2nd and 3rd month of lactation, which corresponds to the confirmed difference between the actual measured milk production (39.3 kg) by predicting milk production when formulating TMR. The efficiency of N utilization calculated from the analyzed content of milk urea N (MUN) and milk production using the regression equation according to Huhtanen et al. (2015) reaches the upper limit of recommended values (31.2 ±0.7 %). In dairy cows with positive and negative results of conception after the 1st insemination, no statistically significant differences were confirmed in milk production, milk composition, energy balance, urea content, and efficiency of N utilization and they do not confirm the hypothesis of high production on reproductive success.

Table 3. Production parameters, energy balance, EUN and success pregnancy after 1st insemination in primiparous dairy cows

	primiparous cows (n 91) X ± SD	1st insemination positive (n 38) X ± SD	1st insemination negative (n 53) X ± SD
Milk production kg/d	34.9 ± 2.7	34.7 ± 2.4	35.0 ± 3.3
ECM kg/d	36.8 ± 2.3	36.9 ± 2.3	36.7 ± 2.6
Milk fat %	3.87 ± 0.2	3.91 ± 0.3	3.84 ± 0.2
Milk protein %	3.14 ± 0.1	3.18 ± 0.1	3.10 ± 0.1
Fat/Protein	1.23 ± 0.1	1.23 ± 0.1	1.24 ± 0.1
Lactose %	5.03 ± 0.1	5.03 ± 0.1	5.03 ± 0.1
Energy Balance MJ/d	-9.4± 1.9	-9.7± 1.7	-9.0 ± 2.3
NEL intake MJ/d	140.4	140.4	140.4
NEL in milk MJ/d	108.8 ± 6.9	109.1 ± 7.2	108.4 ± 7.5
EUN[1] %	31.2 ± 0.7	31.2 ± 0.8	31.2 ± 0.6
Milk Urea mg/dl	26.7 ± 2.3	26.8 ± 3.0	26.7 ± 1.7
MUN mg/dl	12.5 ± 1.1	12.5 ± 1.4	12.5 ± 0.8
Feed efficiency	1.73	1.73	1.71
% of animals	-	41.8 (29.4 – 66.7)	58.2 (33.3 - 70.6)

ECM – energy corrected milk (3,2% protein and 3,5% fat)
EUN – Efficiency of nitrogen utilisation (Huhtanen et al.2015)
MUN – milk urea nitrogen

In primiparous cows (Table 3) compared to multiparous dairy cows with a dry matter intake of 21.3 kg/day and analyzed the content of nutrients, the minimal difference was confirmed between the currently measured milk production (34.9 kg) and the predicted production for formulation feed ration (36.5 kg) and represent a lower level of NEB on average at the level of -9.7 MJ per day, due to the higher need for energy to complete growth. The tendency of non-significant differences between primiparous groups and unconfirmed effect on reproduction (conception success after the 1st insemination) is the same as in multiparous cows. The studies by authors (Garverick et al., 2013, Patton et al., 2007) showed that the pregnancy rate at the first insemination is higher in proportion to the decrease in negative energy balance in dairy cows in the postpartum period. Both the decrease and the increase in milk urea levels may also reflect an imbalanced intake of other nutrients, and the decrease in reproductive performance is the result of energy deficit and

reduced N balance (Raboisson et al., 2017). In addition, the alteration in the uterus environment including uterine pH changes and alterations in the uterine secretory activity, as reported for high urea concentrations (Melendez et al., 2003), cannot be excluded.

CONCLUSION

Our results did not confirm the negative effect of milk production, negative energy balance and utilization efficiency on pregnancy success after the 1st insemination. Evaluated NEB based on selected indicators (milk production and NEL intake) requires accurate analysis and confirmation of NEB based on direct markers (non-esterified fatty acids, glucose). The ongoing study will be supplemented with the results of the evaluation in the second half of the year with a seasonal analysis of the impact and action of heat stress. It remains a challenge for farmers to select cows with persistent lactation to encourage milk production and maintain reproductive performance.

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THE USE OF AMINO ACIDS IN PIG NUTRITION: A REVIEW

ŠIMON HALÁS, FRANTIŠEK ZIGO, ZUZANA FARKAŠOVÁ, ZUZANA LACKOVÁ, ANDREJ RÉCKY, JULIANA ARVAIOVÁ, LENKA BOSÁKOVÁ

Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, 040 01 Košice, Slovakia

Corresponding email address: simon.halas@student.uvlf.sk

ABSTRACT

This paper is devoted to the use of functional amino acids in pigs. Currently, there is an effort in pig farming to reduce the use of antibiotics and to find alternative solutions that can replace them. We start from the definition that functional amino acids are classified as replaceable amino acids, but with extra biological functions, i.e. they are not only used to make proteins, but are involved in the regulation of essential metabolic pathways in order to improve health, survival, growth and development. Therefore, in the paper, we discuss functional amino acids and their role in the overall health of sows and their offspring, the use of functional amino acids in piglets after weaning, and the effect of fattening pigs on meat quality.

Keywords: sow; pregnancy; weaning; oxidative stress; immunity

INTRODUCTION

The substitution of functional amino acids is considered to be an important topic mainly because of the proper nutritional and physiological status of pigs, as well as to reduce antibiotic resistance and the cost of (Kim et al., 2007). Their main role in the body is their use for the synthesis of proteins and other unnecessary substances, their oxidation serves as an energy source and regulates key metabolic pathways including oxidative stress, immunity and protection of the intestinal barrier (Wu 2013; Le Floc'h et al., 2018; Prates, 2021).

In this paper we discuss about the role of amino acids in the overall health of sows and their offspring, their functional use in piglets after weaning, and the effect of fattening pigs on meat quality.

Functions of amino acids

Amino acids are generally categorized into essential, semi-essential (conditionally essential) and non-essential amino acids depending on their dietary essentiality and role in protein synthesis (Mou et al., 2019). Amino acids provide the body with substrates for the synthesis of tissue proteins and regulate their degradation, affect the synthesis and secretion of hormones, regulate endothelial function, vasodilation and blood flow, affect nutrient metabolism, maintain acid-base balance and whole-body homeostasis (Wu, 2009; Nuntapaitoon et al., 2018). Amino acids and fatty acids with special functions include arginine, branched-chain amino acids (leucine, isoleucine and valine), glutamate, glutamine, tryptophan, glycine, taurine (Kim et al., 2007; Blavi et al., 2021).

L-arginine is one of the most desirable amino acids for pregnant sows and newborn piglets. It stimulates the secretion of insulin, growth hormone, prolactin, glucagon and placental lactogen (Blachier et al., 2013). Positive results on the number of live births of piglets were obtained when 0.5% or 1% of L-arginine was given to pregnant sows from day 85 of gestation until farrowing. Supplementation of pregnant sows with 0.5% L-arginine from day 85 of gestation to the day of farrowing also resulted in an increase in oxygen saturation and an increase in body weight of newborn piglets. It was also found that L-arginine is among the most important even for breastfed piglets (Nuntapaitoon et al., 2018).

Positive effects on the immune system and antioxidant status were also observed after administration of tryptophan and leucine. They support the function of the intestinal barrier and regulate the intestinal microflora. It has been found that in the inflammatory process in the body, there is an increased catabolism of tryptophan by the affected tissue (Trevisi et al, 2009; Li, et al., 2018).

Glutamate, glutamine and aspartate, in turn, provide energy for the development of metabolic processes in the intestines, which use energy in the form of adenosine triphosphate. After the addition of glutamate,

better development of the intestinal mucosa was observed and influence the support of the intestinal barrier (the so-called close connection). Glutamine supplementation prevents intestinal atrophy, also supports the activity of intestinal enzymes and improves the growth and development of weaned piglets (Hanczakowska and Niwinska, 2013). L-carnitine, promotes placental development, causes higher postnatal weight of piglets due to greater muscle development and an overall increased amount of muscle fibers. It also causes higher amounts of insulin growth factor (Ramanau et al., 2005).

Amino acids in pig diet

In newborn piglets, adequate threonine levels are critical to maintaining the necessary mucin and IgM production. The increase in threonine content from 8.5 g/kg to 9.0 g/kg above the required requirement resulted in increased secretion of protective IgM in newborn piglets infected with enterotoxigenic *Escherichia coli* (*E. coli*) (Le Floc'h et al., 2018). However, when the dietary ratio of threonine to lysine changed from 65 to 70%, weaners were infected with enterotoxigenic *E. coli*. The lack of action of complementary threonine can be explained by mucin-13, which is expressed in pig jejunum and is not rich in threonine, unlike other mucins that are dominant in the gastrointestinal segment (Wu, 2013; Le Floc'h et al., 2018).

Serine is classified as a nutritionally non-essential amino acid and is related to metabolism, as well as its synthesis pathway from glucose, whose metabolism, in turn, is influenced by the need for serine. Its deficiency can result in impaired glycine synthesis, leading to nutritional imbalance of other amino acids (Ji et al., 2020; Blavi et al., 2021). Serine is reported as the main amino acid needed for the synthesis of a regenerative protein that targets gram-positive bacteria and is abundantly produced in the small intestine of pigs (Blachier et al., 2013; Le Floc'h et al., 2018).

Amino acids as primary components of defense proteins are very rare because they perform defensive and protective functions in the intestine. It is necessary to ensure that certain amino acids do not restrict the synthesis of protective proteins for gut health. Some of the proteins consist as structural complexes and therefore undergo posttranscriptional

modifications and fulfill different biological functions, such as reducing the intercellular degradation of proteins. In this case, the presence of asparagine, serine and threonine sequences for N-glycosylation in the endoplasmic reticulum, as well as for O-glycosylation in the Golgi complex, is also very important.

Certain amino acids give these functional proteins functional properties. Protein is important for the development of various diets to stimulate protein synthesis (Qi, 2020). Prates et al. (2021) have previously tested whether a mixture of selected functional amino acids lipopolysaccharides induces infection in piglets. The mixture included arginine, branched amino acids and cysteine at a concentration 0,3 % higher than the feeding base. Evaluation methods included intestinal morphology observed microscopically, determination of inflammatory, immune and hormonal (i.e. tumour necrosis factor alpha, IGF-1, immunoglobulins, haptoglobin, cortisol) markers, and proteomic analysis from serum. Functional amino acid supplementation revealed moderate effects on gut morphology when villi height and crypt depth increased and cortisol decreased. Li et al. (2018) tested the effect of glutamate and aspartate mainly in a 21-day experiment. The control group was fed a diet containing 2.9% glutamate and 1.5% aspartate; while the experimental group had 2.6, 3.2 and 3.5% glutamate and 1.3 or 1.7% aspartate in their diet. Methods for assessing growth performance were biometric parameters (feed intake and weight), amino acid chromatographic assay from blood serum and gene expression analysis from liver tissue samples. The results showed that higher doses of aspartate and glutamate reduced growth performance as opposed to low doses of aspartate. Similarly, high doses also reduced amino acids, while low doses (3.2%) of glutamate increased amino acids.

Amino acids in piglets

Diarrheal diseases of piglets after weaning can be influenced by dietary measures. The main thing is to feed in the post-weaning period with a lower protein diet, supplement with essential amino acids, administer probiotics and ensure a sufficient amount of quality water. The use of feed with a combined content of digestible and indigestible fiber also has a positive effect, which favorably affects the composition of the

intestinal microflora, improves the digestibility of nutrients and supports the function of the intestinal barrier. Therefore, some authors recommend using easily digestible and absorbable feed with a sufficient amount of high-quality nutrients and a low anti-nutritional effect in the post-weaning period. The use of crude protein in feed with a total content exceeding 18 % has been reported to result in reduced absorption by the stomach and small intestine (Cemin, 2022). Organic acids are used as additives for an even more significant change in the pH of the stomach (Wu, 1995).

It was also found that piglets infected with enterotoxigenic *E. coli* had an increased need for tryptophan (Trevisi et al., 2009). Endogenous arginine production is also reduced in enterocytes at weaning, which could lead to arginine deficiency (Wu et al., 1996). According to Wu (2009), dietary glutamine supplementation (1.0%) prevented jejuna atrophy during the first week after weaning and increased the increment-to-food ratio by 25% during the second week after weaning. In another study conducted by Hsu et al. (2010), the results showed that supplementation of 1% or 2% of glutamine could be beneficial in villi morphology of the small intestine and the ability to absorb xylose in weaned piglets. Glutamine supplementation has also been helpful in reducing the severity of *E. coli* infection in weaned piglets by altering intestinal barrier function and reducing the cytokine response of the mucous membrane (Ewaschuk et al. 2011).

Another functional amino acid used to improve the intestinal barrier is arginine. Promotes the development of the intestinal mucosa and its regeneration (Nuntapaitoon et al., 2018). For example, Hanczakowska and Niwinska (2013) found that supplementation of L-arginine (6 g/kg) in the diet improved pig performance after weaning; i.e. average daily feed intake and average daily increment increased. The positive effects of arginine supplementation have been noted especially on the intestinal tract of piglets, since it has a positive effect on intestinal morphology.

For example, arginine supplementation in weaned piglets contributed to an increase in villi in the jejunum and ileum, an increased ability to reduce iron in plasma, and a lower incidence of oxidized form of glutathione by regulating oxidative stress with arginine (Prates et al.,

2021). In general, withdrawal stress can lead to oxidative damage to macromolecules, i.e. lipids, proteins and DNA, which can lead to adverse changes in growth and increased susceptibility to disease. Oxidative stress is the result of an imbalance between endogenous production of reactive oxygen species and antioxidants (Qi et al., 2020). Tryptophan itself acts as an antioxidant, but several tryptophan metabolites also act as traps with antioxidant properties. Its antioxidant properties have been reported when administered at doses above recommended requirements, i.e. 3.0 g/kg (Martinez-Montemayor et al., 2008).

CONCLUSION

Food replenishment of functional amino acids to sows during pregnancy has a positive effect on the development of the placenta, increases fertility and promotes fetal growth and reduces oxidative stress, increases meat quality. They can improve immunity and ensure the balanced activity of the immune system, as well as create a protective film on the mucosa of the epithelium of the gastrointestinal tract against infection and toxins or prevent water loss through the intestine, which is often used in the treatment of diarrhea, dyspepsia and intoxications in pigs.

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A COMPARISON OF ORGANIC AND INORGANIC SELENIUM SOURCES AND THEIR INFLUENCE ON PERFORMANCE PARAMETERS OF LAYING HENS AND SELENIUM CONCENTRATIONS IN EGGS

LUCIE HORÁKOVÁ[1], JAKUB NOVOTNÝ[1], MICHAL ŘIHÁČEK[1], DANA ZÁLEŠÁKOVÁ[1], ONDŘEJ ŠŤASNÍK[1], LEOŠ PAVLATA[1]

[1]Department of Animal Nutrition and Forage Production, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: luciehora@seznam.cz

ABSTRACT

The aim of this study was to compare the effect of organic and inorganic selenium (Se) sources and a combination of both forms with the main influence on performance parameters of laying hens and selenium concentrations in eggs. The effect of different selenium sources and levels was evaluated. Our results suggest that the difference between egg production, feed consumption and chemical analysis of eggs was not significant in any parameters. The significantly highest value of Haugh units was found in Organic group and in the group with the addition of both selenium sources group. These values were significantly different from Control group. Another significant difference was also found in the Organic group and the combination of both forms of selenium, namely in the concentration of selenium in egg yolks and egg albumens.

Keywords: poultry nutrition; *Saccharomyces cerevisiae*; sodium selenite

INTRODUCTION

Dietary selenium supplementation is crucial for maintaining animal health, productivity, and reproductive performance. Selenium plays a vital role in protecting cell membranes from oxidative damage, especially in conjunction with vitamin E (Chen et al., 2014). Otrubová (2018) highlights how higher selenium concentrations in meat, milk, and eggs can lead to increased selenium intake in the human diet, potentially offering various health benefits. Indeed, selenium (Se) is a trace element

that plays vital biological roles in numerous organisms, including animals and humans. It is classified as an essential micronutrient due to its critical functions in various physiological processes (Umysova et al., 2009).

Saccharomyces cerevisiae, commonly known as baker's yeast or brewer's yeast, is a well-known yeast strain that has various industrial and biotechnological applications. In the context of producing selenium-enriched yeast (Se-yeast), *Saccharomyces cerevisiae* is often utilized in a controlled aerobic fermentation process (Esmaeili and Khosravi-Darani, 2014). Asadi et al. (2017) found that organic selenium supplementation had positive effects on deposition of selenium in the egg and increased the overall quality of the eggs, especially when compared to other sources of selenium. This indicates that organic selenium likely contributes to a higher utilization and absorption by the animal.

A study by Surai et al. (2018) mentioned the advantages of organic selenium in poultry diets compared to traditional sodium selenite. This concept concerns about using selenomethionine (SeMet) as a storage form of selenium in chicken bodies. As poultry cannot synthesize SeMet, its necessary to be added to feed. The presence of SeMet maintain an effective antioxidant defense. Several selenium compounds have been authorized as feed additives in the EU, including sodium selenite, sodium selenate, Se-yeast, LSeMet, DL-SeMet, and OH-SeMet. The European Union (EU) regulates the total dietary selenium content to a maximum of 0.5 mg/kg, with organic selenium compounds being limited to an addition of 0.2 mg Se/kg, as per EFSA (2014) guidelines.

Further research into the various forms of selenium in poultry nutrition is necessary to gain a more comprehensive understanding of their benefits and limitations. Its important to investigated different selenium forms to determine which forms offer the most significant advantages in terms of animal health, growth, and stress management. It could contribute to better management of animal diets and potentially enhance the nutritional quality of poultry products for human consumption.

MATERIAL AND METHODS

An experiment was performed with laying hens of Dominant hybrid (n = 32) which were stabled in individual balance cage batteries. The experiment started at the age of 18 weeks and lasted until the 26th week of age. Laying hens were randomly divided into four groups. The Control group was fed with a diet without addition of selenium – Se was supplied only with its natural content in the feed. Organic group was fed with an organic source of selenium (Sel-Plex – Saccharomyces cerevisiae CNCM I-3060). Inorganic group was fed with an inorganic source of selenium (Sodium selenite – Na2SeO3). In these both groups (Organic and Inorganic) selenium sources were added to the feed with natural content of selenium already present. The last group (Org.+Inorg.) was fed a diet with mix of organic and inorganic selenium. These four isocaloric and isonitrogenous diets were formulated according to the recommended nutrient content for Dominant laying hens (Dominant CZ, 2020). The animals had free access to water and feed. Health status, feed consumption and egg production were monitored daily.

Table 1 shows the composition and chemical analysis of used experimental diets. Diets were made in unformed form and consist of the same components, differing only in the source of selenium.

Table 1. Composition and chemical analysis of experimental diets

Component	Unit	Control	Organic	Inorganic	Org.+Inorg.
Wheat	g/kg	310	310	310	310
Maize	g/kg	270	270	270	270
Soybean meal	g/kg	264	264	264	264
Premix[1]	g/kg	30	30	30	30
Rapeseed oil	g/kg	43	43	43	43
Monocalcium phosphate	g/kg	3	3	3	3
Limestone milled	g/kg	45	45	45	45
DL-Methionin	g/kg	2	2	2	2
Sodium chloride	g/kg	3	3	3	3

Limestone grit	g/kg	30	30	30	30
Sel-Plex*	%	-	0.5	-	0.25
Sodium selenite	%	-	-	0.5	0.25
Dry matter	%	100	100	100	100
MEN**	MJ/kg	11.77	11.77	11.77	11.77
Crude protein	%	20.09	16.65	19.46	19.74
Ether extract	%	6.25	6.51	6.16	6.35
Crude fibre	%	5.43	7.22	5.39	5.92
Crude ash	%	14.04	14.18	13.88	14.49

[1]Premix contains (per kg): L-lysine 0,41 g; DL-Methionine 7,35 g; calcium 8,91 g; phosphorus 2,07 g; sodium 1,38 g; copper 9 mg; iron 69 mg; zinc 54 mg; manganese 72 mg; iodine 0, 9 mg; retinol 9,900 lU ((international units); calciferol 3,000 lU; tocopherol 15 mg; phylloquione 1,2 mg; thiamine 1,2 mg; riboflavin 3, 6 mg; pyridoxin 1,62 mg; cobalamin 12 g; biotin 0,09 mg; niacinamid 12,6 mg; folic acid 0,9 mg; calcium pantothenate 7,5 mg; cholin chloride 180 mg.

*Sel-Plex – *Saccharomyces cerevisiae* CNCM I-3060

**MEN – Apparent metabolizable energy.

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). We used one-way analysis at variance (ANOVA). To ensure evidential differences Scheffe's test was applied and differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

During the 60-day experiment, observations were made on a daily basis to track the number of eggs laid and their weight. Yolks and albumens were sampled at regular intervals. Subsequently, lyophilized and then subjected to chemical analysis and analysis on the amount of selenium. Feed consumption was recorded at the same time every day.

As shown in Table 2, there was no significant difference in egg production and feed consumpton per trial (average egg weight, feed consumption per hen and day, feed conversion ratio, laying intensity).

Table 2. Egg production and feed consumption

	Average egg weight (g)	Feed consumption per per hen and day (g)	Feed conversion ratio	Laying intensity (%)
Group	Mean ± SEM			
Control	60.36 ± 0.97	107.23 ± 3.95	2.00 ± 0.08	88.85 ± 1.59
Organic	58.05 ± 0.98	111.42 ± 6.40	2.20 ± 0.14	87.71 ± 1.59
Inorganic	66.53 ± 6.76	108.05 ± 5.95	1.92 ± 0.10	86.77 ± 1.59
Org.+Inorg.	59.08 ± 1.01	104.83 ± 4.21	2.11 ± 0.10	84.58 ± 2.61

SEM = standard error of the mean

Table 3 illustrates chemical analysis of egg parts (yolks and albumens). No statistically significant differences were found in these parts.

Table 3. Chemical analysis of eggs (100% dry matter)

				Crude ash	Crude protein	Ether extract
	Group	n	Unit	Mean ± SEM		
Yolk	Control	6	%	3.66 ± 0.11	30.05 ± 0.31	54.31 ± 0.61
	Organic	6	%	3.41 ± 0.10	30.05 ± 0.33	54.57 ± 0.52
	Inorganic	6	%	3.99 ± 0.23	29.38 ± 0.56	54.26 ± 0.38
	Org.+Inorg.	6	%	3.98 ± 0.30	30.84 ± 0.33	54.01 ± 0.51
Albumen	Control	6	%	6.06 ± 0.13	77.23 ± 0.45	–
	Organic	6	%	6.02 ± 0.22	77.50 ± 0.27	–
	Inorganic	6	%	5.99 ± 0.14	77.81 ± 0.27	–
	Org.+Inorg.	6	%	6.05 ± 0.23	77.22 ± 0.58	–

SEM = standard error of the mean; n = the number of eggs from each group

The qualitative analysis of eggs is shown in Table 4. The highest value of Haugh units was found in Organic group with value 94.48 b ± 1.44 and in Org.+Inorg. group 94.84 ± 1.30. These values were significantly different from Control group. Patton's (2000) findings indicated that the supplementation of organic or inorganic selenium did not influence Haugh unit values in eggs when compared to eggs from hens that were fed the basic diet. Our results disagree with Patton's.

Table 4. Qualitative analysis of eggs

Group	Control	Organic	Inorganic	Org.+Inorg.
	Mean ± SEM			
Albumen weight ratio (%)	69.09 ± 0.33	67.35 ± 0.66	67.23 ± 0.27	67.58 ± 0.38
Yolk weight ratio (%)	23.79 ± 0.35	23.58 ± 0.54	23.34 ± 0.27	23.09 ± 0.33
Haugh units	86.22 ^{a} ± 1.58	94.48 ^{b} ± 1.44	90.78 ^{ab} ± 1.74	94.84 ^{b} ± 1.30
Eggshell weight ratio (%)	9.13 ± 0.16	9.07 ± 0.24	9.43 ± 0.24	9.33 ± 0.17
Eggshell strenght (N)	40.16 ± 2.08	38.16 ± 1.53	45.12 ± 2.90	44.17 ± 2
Eggshell thickness (mm)	0.44 ± 0.02	0.44 ± 0.02	0.45 ± 0.02	0.45 ± 0.02

SEM = standard error of the mean; Means in the row not sharing a common letter (a - b) are statistically different (P < 0.05)

The effect of adding different selenium sources on Se concentration in egg yolk and albumen is shown in Table 5. Compared with the Control group, the Se content in the egg yolk and albumen was significantly increased in Organic and Org.+Inorg. groups.

Table 5. Selenium concentrations in egg parts

			Yolk	**Albumen**
Group	n	Unit	Mean ± SEM	
Control	6	(mg/l)	676.58 ^{a} ± 29.87	506.32 ^{a} ± 36.07
Organic	6	(mg/l)	2525.38 ^{b} ± 158.96	4406.92 ^{b} ± 238.63
Inorganic	6	(mg/l)	2086.55 ^{ab} ± 142.73	1466.95 ^{ab} ± 268.57
Org.+Inorg.	6	(mg/l)	2771.63 ^{b} ± 82.02	4269.85 ^{b} ± 408.87

SEM = standard error of the mean; n = the number of eggs from each group;
Means in the row not sharing a common letter (a - b) are statistically different (P < 0.05)

The transfer of selenium to the egg depends on its source and level in the diets. Urso et al. (2015) demonstrated that the selenium content in egg yolk elevated upon incorporating 0.15 to 0.3 mg/kg of selenium into the diet. According to Surai et al. (2014), organic selenium at concentrations spanning from 0.3 to 0.5 mg/kg in the diet led to approximately 30% higher selenium accumulation compared to equivalent concentrations of inorganic selenium.

CONCLUSION

In our study, using various sources of selenium did not found any significant parameters in egg production, feed consumption and chemical analysis of eggs. Nevertheless, the experimental groups with organic source of selenium (Organic group and Org.+Inorg. group) resulted in higher value of Haugh units and higer level of selenium in yolks and albumens. In conclusion, adding a high level of organic selenium and mix of both sources of selenium in the diet significantly improved to the production of Se-enriched eggs, which might be a valuable source of Se to support optimal human health.

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THE EFFECT OF THE ADDITION OF DRONE BROOD ON THE GENE EXPRESSION OF SELECTED CYTOKINES IN PIGS

HUDEC ERIK[1], KARAFFOVÁ VIERA[1], REVAJOVÁ VIERA[1], LEVKUT MARTIN[1], LEVKUTOVÁ MÁRIA[1], RUDOLF ŽITŇAN[2], RAJČÁKOVÁ ĽUBICA[2], PATRÁŠ PETER[2]

[1]Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy, 04181 Košice, Slovakia
[2]National Agriculture and Food Centre, Research Institute for Animal Production Nitra, 951 41 Lužianky, Slovakia

Corresponding email address: erik.hudec@uvlf.sk

ABSTRACT

Honey bees and bee products (honey, pollen, propolis, royal jelly, wax, bee venom) have long been used in the treatment of human, animal and plant diseases as "apitherapeutic applications". The aim of the study was to observe the immunomodulatory effect of the addition of drone brood homogenate to the feed on the relative expression of genes for interleukins (IL-8, IL-10, IL-18) and olfactomedin 4 (OLFM4) in the ileum of pigs. 12 hybrid weaned piglets at the age of 8 weeks were included in the experiment lasting 21 days, which were divided into 3 groups: control, experimental group no. 1 (100 mg/kg) and experimental group no. 2 (200 mg/kg). The relative gene expression of selected immune parameters was evaluated using the quantitative Real-Time-PCR method. Our results revealed upregulation of relative expression for all parameters in the group with the addition of 200 mg/kg of drone brood homogenate. We assume that the addition of drone brood homogenate in a concentration of 200 mg/kg can have an immunomodulatory effect on selected parameters of non-specific immunity (interleukins, OLFM4) in the ileum of pigs.

Keywords: drone brood homogenate, interleukins, immunomodulation, pigs, bee products

INTRODUCTION

Albert Einstein: "If the bee disappeared off the surface of the globe, then man would have only four years of life left. No more bees, no more pollination, no more plants, no more animals, no more man."

The importance of bees and their products such as honey, propolis, royal jelly, beebread, pollen collected by bees, beeswax and venom have been known from Sumerian civilization for thousands of years (Sawczuk, Karpinska, Miltyk, 2018). These products are consumed for them high nutritional value as well as because of them antioxidant, bacteriostatic, anti-inflammatory, and antimicrobial properties. They also used to heal wounds and burns (Loukas and Maria, 2023). A less well-known product is drone brood homogenate. Drone brood homogenate is a bee product collecting of drone larvae from drone cells, from 3 to 11 days after hatching. Is a milky, creamy consistency with a high content of nutrients: proteins, lipids, fatty acids, carbohydrates, vitamins (A, B, E and D) and minerals. Moreover, when collected in the early stages of larval development, it is a rich source of sex hormones (testosterone, progesterone and estradiol) (Sidor and Dżugan, 2020). In many scientific reports have been confirmed the androgenic and anabolic effects. Drone brod homogenate is using in treating urgent global health problems, including ovarian dysfunction in women and male infertility, thyroid and immunity disorders, as well as malnutrition (Kistanova et al., 2020; Sidor,Dzugan, 2020). However, for the practical use is necessary a detailed study of its effect on the immune system of animals. The aim of this study was to observe the immunomodulating effect of the addition of drone brood homogenate to the feed on the relative expression of genes for interleukins (IL-8, IL-10, IL-18) and olfactomedin 4 (OLFM4) in the ileum of pigs.

MATERIAL AND METHODS

Experimental animals

An experiment was performed with twenty one hybrid weaned piglets (White meat x Landras). Weaned piglets were 8 weeks old and the experiment lasted 21 days. The animals were divided into 3 groups, where C was the control group fed with an isoenergetic standard feed mixture. The experimental group E1 was fed twice a day with the drone

brood homogenate in a dose of 100 mg/kg of live weight per feed. In the group E2 was fed the drone brood homogenate twice a day in the dose of 200 mg/kg of live weight per feed. All piglets were slaughted in an approved manner on the 21st day. Ileum samples were taken for RNA later for nucleic acid fixation until analysis.

RNA isolation and Real-Time PCR method

Total RNA was isolated from the samples using the RNEasy mini kit (Qiagen, Germany). The isolated RNA was transcripted into cDNA using the iScript cDNA Synthesis Kit and oligo DT-primers. The relative gene expression for interleukins (IL-8, IL-10, IL-18) as well as for olfactomedin 4 (OLFM4) was evaluated using the quantitative Real-Time-PCR method using the SsoAdvancedTM universal SYBR green supermix kit (Bio-Rad, USA) and specific primers on a LightCycler 480 II Instrument (Roche, USA) according to a predefined temperature program. The obtained Cq values of the genes were normalized to the average Cq value of the reference gene (HPRT), and the relative expression of each gene was calculated mathematically as $2^{-\Delta Cq}$.

Statistical analysis

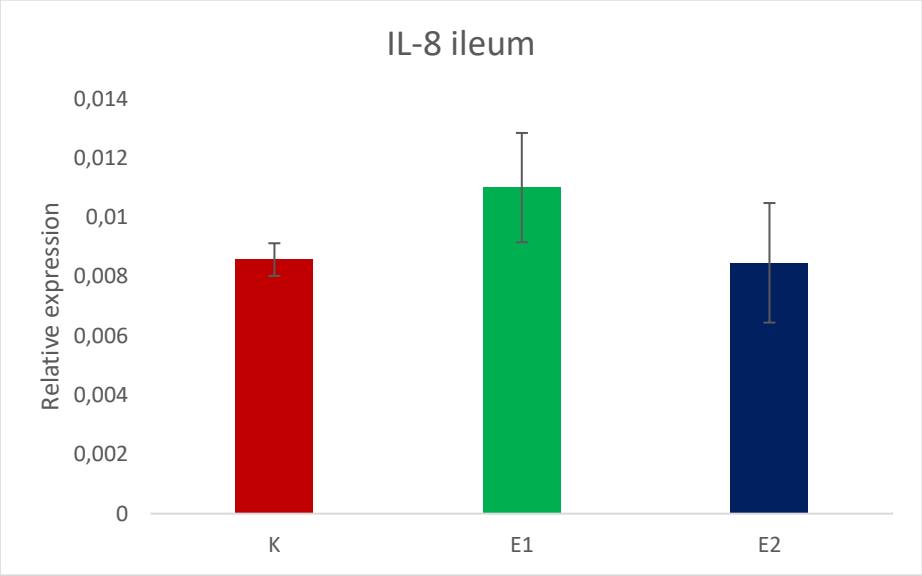
Statistical analysis of data was performed using Tukey test in one way Anova in GraphPad Prism 9 software. Differences between group values were considered statistically significant at $P^{ab} < 0,05$; $P^{ac} < 0,01$; $P^{ae} < 0,0001$.

RESULTS AND DISCUSSION

In our results, we did not observe a significant upregulation of the relative gene expression for IL-8 in any experimental group (Figure 1). This may be related to the fact that geographical location, climate, species and stage of development affect the composition of the larvae (Rutka et al., 2021). On the contrary, we recorded a statistically significant upregulation of relative gene expression for IL-18 in experimental groups 2 in comparison to other groups ($P < 0,05$; $P < 0,01$), IL-10 ($P < 0,05$; $P < 0,01$) and OLFM4 ($P < 0,0001$) (Figure 2, 3, 4). The results show that the administered drone brood homogenate in the amount of 200mg/kg had a stimulating effect on the gene expression of all tested parameters of non-specific immunity. A similar effect was

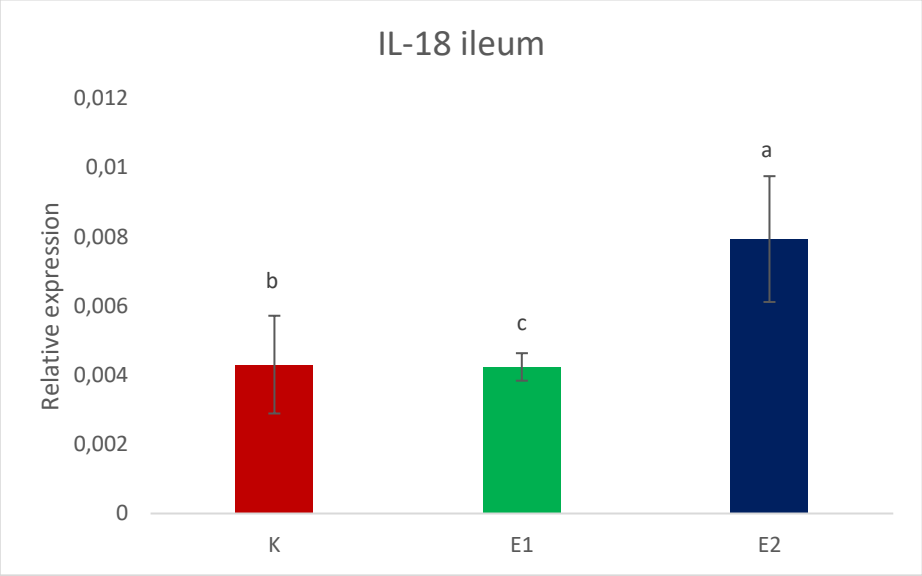
noted by the authors Sawczuk, Karpinska, Miltyk (2018), where drone brood homogenate was able to stimulate production of antibodies by the spleen and the immune response of T lymphocytes. Also, Wyszyńska et al. (2008) observed the immunostimulating as well as the hepatoprotective effect of the administered drone brood homogenate. Interestingly, Hamamci et al. 2020 found, that the number of degenerated neurons due to sepsis decreased as apilarnil dose increased in rats. Apilarnil reduced the elevated levels of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β) induced by sepsis. Apilarnil prevented sepsis-related apoptosis in the brain. The influence of drone brood homogenate on immunity may be related to the content of amino acids. It is a source of eight of the nine essential amino acids. Whereas amino acid deficiency impairs immune function and increases susceptibility to disease (Gosh, 2020; Wu, 2009).

Figure 1: Relative expression of IL-8 gene



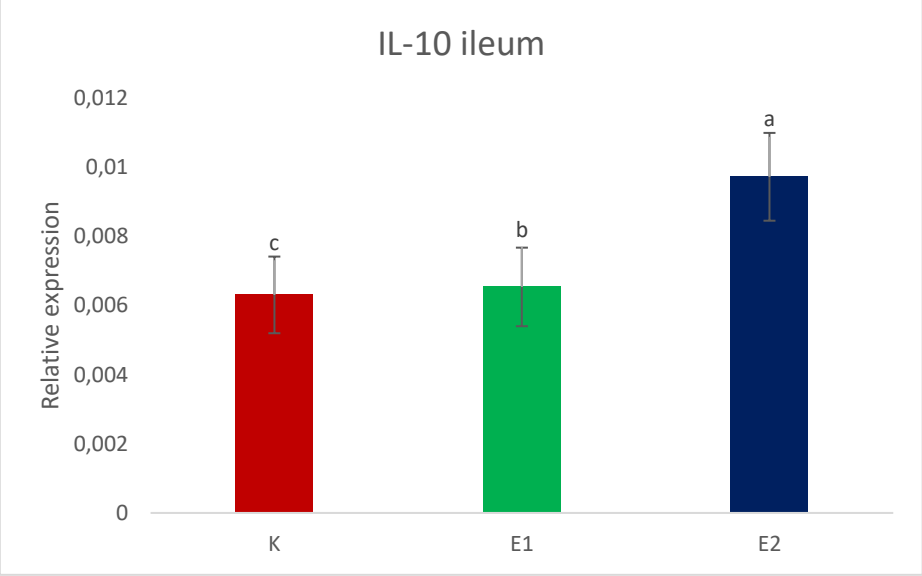
Legend: C – control, E1 - experimental group no. 1 (100 mg/kg), E2 - experimental group no. 2 (200 mg/kg)

Figure 2: Relative expression of IL-18 gene



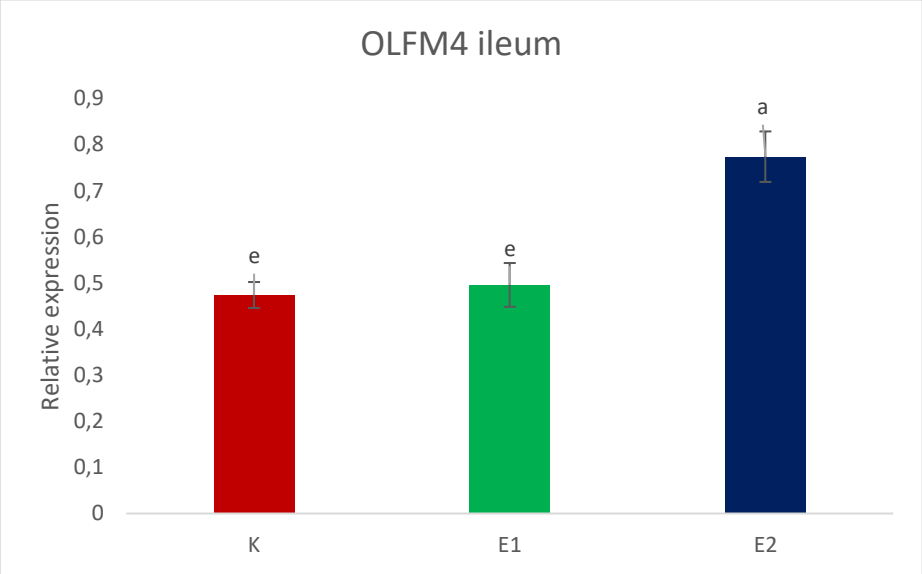
Legend: C – control, E1 - experimental group no. 1 (100 mg/kg), E2 - experimental group no. 2 (200 mg/kg)

Figure 3: Relative expression of IL-10 gene



Legend: C – control, E1 - experimental group no. 1 (100 mg/kg), E2 - experimental group no. 2 (200 mg/kg)

Figure 4: Relative expression of OLFM4 gene



Legend: C – control, E1 - experimental group no. 1 (100 mg/kg), E2 - experimental group no. 2 (200 mg/kg)

CONCLUSION

On the basic of our results, we assume that the addition of drone brood homogenate in a concentration of 200 mg/kg may have an immunomodulatory effect on selected parameters of non-specific immunity (interleukins, OLFM4) in the ileum of pigs.

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THE EFFECT OF RUMINATION TIME ON MILK PRODUCTION OF DAIRY COWS

MÁRIA KAPUSNIAKOVÁ, MILAN ŠIMKO, MIROSLAV JURÁČEK, ONDREJ HANUŠOVSKÝ, MICHAL ROLINEC, BRANISLAV GÁLIK, ANDREJ DUCHOŇ, STANISLAVA DROTÁROVÁ

Department of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovakia

Corresponding email address: milan.simko@uniag.sk

ABSTRACT

The aim of this study was to show the relationship between rumination time and milk yield (MY). The effect of rumination time (RT) of high producing Holstein-Friesian dairy cows in the top production group (n=142) on milk production was evaluated. The monitoring of rumination time was realised 24 hours a day using collars from BouMatic. Daily milk yield data of dairy cows (milked 3 × daily - herringbone type of parlour) were recorded and then downloaded using the HerdMetrix program. Statistical analysis showed results regarding increasing rumination time which was statistically significant with milk yield ($p<0.05$). The overall effect of rumination on MY (0.347; $P<0.01$) was on average correlated.

Keywords: dairy cows; rumination; nutrition; milk yield

INTRODUCTION

Rumination, a cyclical process characterised by regurgitation, remastication, and reswallowing has also been described by many authors as a main indicator of welfare, correct digestion and passage of feed through the gastrointestinal tract of the dairy cow and also as a factor influencing the overall health status of the cow or herd (pH) (Soriani et al., 2012; Cocco et al., 2021). Authors Bar and Solomon (2010); Paudyal (2021); Ning et al. (2022) report that adequate time spent ruminating is in the range of 8-9 hours. Rumination as described by Beauchemin

(2018); Cook et al. (2021); Paudyal (2021) and Ning et al. (2022) is sufficiently influenced by health status, nutrition or ration composition and structure and last but not least feeding frequency. Wadhwani et al. (2023) describe a statistically positive correlation between milk yield of dairy cows and rumination time. However, it must be taken into account as described by Beauchemin (2018) that for high milk yield it is necessary to provide not only a nutrient balanced feed but also a feed of adequate length (physically effective neutraldetergent fibre). Fibre is an important component of the ration supporting the rumination process and its amount depends on the production group. Dairy cows producing >40 kg milk day should have 300 $g.kg^{-1}$ dry matter in their ration (Chamberlain 1996). The aim of this study was to show the relationship between rumination time and milk yield. It was hypothesised that rumination time would have a positive effect on milk yield.

MATERIAL AND METHODS

The experiment was realised on the university farm Kolíňany - farm Oponice, where rumination and milk production of high producing Holstein-Friesian dairy cows in the top production group (n=142) were observed. The realisation of the experiment was 13 weeks (>30-120 days of lactation). The dairy cows on the above mentioned Oponice farm are provided with collars from BouMatic, whose task is to monitoring and recording the total time spent by the dairy cows on feed intake and subsequent rumination (24 $h.day^{-1}$). The rumination time (RT) monitoring was carried out using a device on the collar and a digital receiver for this data. Data transmission between the collar-mounted device and the receiver installed in the barn was done in 15-minute time intervals, and then the data were sent at two-hour intervals to the RealTime Activity program. Data downloads were performed every 24 hours throughout the experiment. Daily milk yield data of dairy cows (milked 3 × daily - herringbone type of parlour) were recorded and then downloaded using the HerdMetrix program. The MY records contained information regarding milking date and time, MY (liters), collar number, and cow identification number. High producing dairy cows at Oponice Farm were fed a mixed ration (TMR), which was fed 1 × daily with the

implementation of feed addition every 6 hours. The composition of the TMR together with the content of selected nutrients for the top lactation group is given in Table 1. Statistical processing of the results was carried out using IBM SPSS ver. 26.0 software. Descriptive statistics (mean, standard deviation, minimum and maximum values) with one way ANOVA analysis. Statistical significance of the differences between RT and the amount of MY was expressed by Post Hoc Tukey test ($p<0.05$). Pearson's correlation coefficient was used to determine the effect of RT on MY.

Table 1. Composition of the mixed ration and content of selected nutrients

Component	**Feed dry matter**
Corn silage [kg]	8.16
Alfalfa silage [kg]	6.16
CCM [kg]	5.28
Straw [kg]	0.18
Feed Mixture [kg]	5.90
Dry matter intake [kg]	25.67
Nitrogenous substances [g]	174.03
Fat [g]	21.77
Fibre [g]	157.25
ADF [g]	148.99
NDF [g]	253.07
Starch [g]	291.10
Total sugars [g]	25.21
NEL [MJ]	6.80

*CCM = Corn Cob Mix, ADF = Aciddetergent fibre, NDF = Neutraldetergent fibre, NEL = Netto energy lactation, Kg = Kilograms, g = grams, MJ = Megajoule

RESULTS AND DISCUSSION

The results of the effect of rumination time on milk production are shown in Table 2.

The minimum mean MY (9.85 ± 6.79 l^{-1} daily) was observed at RT<1 $h.day^{-1}$ (n=4) and on the other hand, the maximum mean (45.97 ± 8.81 l^{-1} daily) was observed at RT>13 $h.day^{-1}$ (n=3). The longest RT that was >13 $h.day^{-1}$ compared to the lowest RT<1 $h.day^{-1}$ and its difference in MY in terms of percentage was 21.42%. It can be evaluated that the MY is in the relationship with increasing RT according to the Post Hoc Tukey test ($p<0.05$). A positive correlation between RT and MY was also found by Antanaitis et al. (2018) in their study where they observed first lactation ($r=0.471$), second lactation ($r=0.302$) and multiple lactation ($r=0.561$) dairy cows ($p<0.001$). In a study by Moretti et al. (2016), they reported the maximum mean MY (33.59 ± 9.18 l^{-1} daily) at RT of 8.55 $h.day^{-1}$. In a study by Johnston & DeVries (2018), they found a statistically significant effect of RT on MY ($p<0.001$) and the authors also reported that an extra hour at ruminating time increased milk yield by 1.26 $kg.day^{-1}$. The authors Codl et al. (2023) also found through their research that cows where RT was increased by 1 minute also had an increase in MY of 0.03 kg. Here, the importance of the fact that for high MY it is necessary to provide adequate conditions for dairy cows regarding housing, nutrition, rest and also suitable conditions regarding climatic data (Cocco et al., 2021; Paudyal, 2021; Ning et al., 2022) can be mentioned. RT≤3 $h.day^{-1}$ and MY, which was 9.85 ±6.79 l^{-1} daily within one hour (n=4), 21.90 ±3.00 l^{-1} daily within two hours (n=3) and 15.21 ±7.97 l^{-1} daily of milk within three hours (n=9) was not statistically significant ($p>0.05$). DeVries et al. (2009); Lindgren (2009); Schirmann et al. (2009) describe that dairy cows that are either disturbed from their routine, resting, or suffering from various diseases or metabolic disorders are more susceptible to a reduction in RT. The relationship between health status and RT reduction is also indicated by Antanaitis et al. (2019) who confirmed a statistically significant effect ($P=0.001$) in their research. A finding regarding increasing MY based on increasing RT was also found by Kaufman et al. (2018) who, observed RT in dairy cows at each lactation: 1 lactation ($p<0.05$), 2 ($p<0.001$) and ≥3 ($p<0.001$). A similar observation is described in the study by Stone et al. (2017), who

assessed the results regarding RT and MY as a positive correlation ($P<0.01$; $r=0.30$). Dairy cows in this study and their values related to rumination, which were from 1 to 7 h.day^{-1} were not statistically significant ($p>0.05$), however, when comparing RT.day^{-1} from 1 to 2 (n=3) and >7 (n=386), were observed a significant increase in MY of 15.55 l^{-1} daily ($p<0.05$). For RT representing >13 h.day^{-1} (n=3) compared to time representing rumination <1 h.day^{-1} (n=4), was determined a significant difference in MY of up to 36.12 l^{-1} daily and can assess that RT was positively correlated with MY ($p<0.05$). RT depends on many factors such as a properly formulated feed ration in terms of the content of individual components and their length, which is very necessary to induce the regurgitation process (Beauchemin and Yang, 2005; Beauchemin 2018; Brandstetter et al., 2019; Ning et al., 2022). The minimum MY (2.50 l^{-1} daily) was recorded in cows that ruminated between four and five hours (n=29) which compared to the highest recorded MY (74.20 l^{-1} daily at RT 9-10 h.day^{-1}) (n=1131) was a difference of 71.7 l^{-1} daily of milk. The overall effect of rumination on MY (0.347; $P<0.01$) was on average correlated.

Table 2. Effect of rumination time on milk production

Rumination time	N	Mean	Std. Deviation	Mininum	Maximum
0 to 1	4	9.85^{a}	6.79	11.80	15.60
from 1 to 2	3	21.90^{abc}	3.00	18.90	24.90
from 2 to 3	9	15.21^{ab}	7.97	8.80	26.20
from 3 to 4	12	23.97^{bc}	12.26	2.70	42.50
from 4 to 5	29	30.46^{cd}	8.85	2.50	49.60
from 5 to 6	108	32.85^{cde}	10.46	2.90	50.60
from 6 to 7	192	33.77^{cdef}	8.35	10.00	53.80
from 7 to 8	386	37.45^{def}	9.04	11.30	68.50
from 8 to 9	756	40.16^{def}	8.76	8.60	71.30
from 9 to 10	1131	42.54^{def}	9.64	4.60	74.20
from 10 to 11	931	43.76^{ef}	10.06	10.10	72.80
from 11 to 12	463	43.85^{ef}	8.42	11.20	68.90
from 12 to 13	66	43.38^{ef}	7.36	25.10	61.20
over 13	3	45.97^{f}	8.81	36.90	54.50
total	4093	41.15	9.99	2.50	74.20

a-f = Different letters indicate statistical significance ($p<0.05$). *N-number of observation

The data on RT and MY (Table 3) were further divided into groups based on RT.day^{-1}: under 8 hours (n=743), from 8 to 10 (n=2818) and over 10 hours (n=532). The maximum mean MY (43.81 ± 8.28 l^{-1} daily) was significantly observed in the RT over 10 hours group ($p<0.05$). A study by Codl et al, (2023) significantly recorded the highest MY (30.20 kg) for the RT >8.6 h group of dairy cows ($p<0.01$). As opposed, in this study, the minimum MY was observed in RT under 8 which was 34.86 ±9.90 ($p<0.05$). A study by Codl et al, (2023) reported the lowest MY (27.20 kg) at RT <7.56 h ($p<0.01$). From the above table number 3, it can be evaluated that the different groups showing RT and MY are statistically significant among each other and RT was statistically positively correlated with MY ($p<0.05$). The difference between the minimum (34.86 ±9.90 l^{-1} daily) and maximum MY (43.81 ±8.28) was around 8.95 l^{-1} daily. In the study of Codl et al, (2023) the difference between the maximum (30.20 kg) and minimum (27.20 kg) MY was 3 kg. The lowest amount of MY was observed in the RT under 8 group and was 2.50 l^{-1} daily and on the other hand, the maximum MY (74.20 l^{-1} daily) was observed in the RT from 8 to 10 group.

Table 3. Daily milk production based on formed groups of rumination time

Rumination time	N	Mean	Std. Deviation	Minimum	Maximum
under 8	743	34.86[a]	9.90	2.50	68.50
from 8 to 10	2818	42.30[b]	9.66	4.60	74.20
over 10	532	43.81[c]	8.28	11.20	68.90
total	4093	41.15	9.99	2.50	74.20

a-c = Different letters indicate statistical significance ($p<0.05$). *N-number of observation

CONCLUSION

The conclusion summarises the main points of the article and outlines its contribution to the current state of research in the field. Of the innovative methods of rumination monitoring, rumination time has an impact on milk production. This was evident when comparing groups of rumination

times where cows with higher rumination times produced statistically significantly higher milk yields.

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EFFICACY OF INOCULANT ON THE FERMENTATION PROCESS AND NUTRITION VALUE FROM ALFALFA SILAGE WITH DIFFERENT DRY MATTER UNDER FARM CONDITIONS

JAN KOLEČKÁŘ[1], EVA MRKVICOVÁ[1], PETR DOLEŽAL[1]

[1]Department of Animal Nutrition and Forage Production, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: xkolecka@mendelu.cz

ABSTRACT

The objectives of this study were to evaluate the effects of dry matter content and two commercial inoculants on the fermentation process and nutrition value of wilted alfalfa stored in commercial farm silos in the Czech Republic. The enzymatic-bacterial inoculant „A“ contained selected homofermentative lactic acid bacterial strains and enzymes of xylanase and β-glucanase. As effective substances of enzymatic-bacterial water–soluble inoculant „B“ were selected homofermentative and heterofermentative lactic acid bacterial strains and enzymes of xylanase and β-glucanase. Freshly chopped alfalfa was wilted to dry matter contents <320 g (LDM), 320-400 (MDM), and >400 (HDM) g/kg fresh weight. There was no significant difference between the groups in nutrient composition, pH value, or amount of lactic, propionic, and butyric acid. The LDM B group had the highest levels of acetic acid, the HDM groups had the lowest levels with no difference in treatment, and the MDM A group had the widest LA/AA ratio.

Keywords: alfalfa, fermentation process, dry matter, inoculants, lactic acid bacteria

INTRODUCTION

Silage preservatives composed of lactic acid bacteria are a common part of silage production under farm conditions. Silage preservatives composed of lactic acid bacteria have a demonstrable effect on the resulting silage quality (Blajman et al. 2020; Oliveira et al. 2017) and

reducing hazards are associated with poorly fermented silages (Driehuis et al. 2018). Homofermentative inoculants increase lactic acid concentrations and decrease acetic acid and butyric acid concentrations (Oliveira et al. 2017). Heterofermentative inoculants have higher acetic acid levels and higher dry matter losses than inoculants composed of homofermentative lactic acid bacteria (Arriola et al. 2021). Higher acetic acid levels can have a negative effect on dry matter intake (Gerlach et al. 2021). Inoculants composed of heterofermentative lactic acid bacteria are used because of prolonged aerobic stability. To reduce the negative effect of heterofermentative lactic acid bacteria, it is recommended to use a combined inoculant with homofermentative lactic acid bacteria (Arriola et al. 2021). Low dry matter is another factor that increases acetic acid concentrations and the amount of fermentation products (Kung et al. 2018). Low dry matter decreases the nutritional value of the resulting silage (Liu, Dong, and Shao 2018). The aim of this study was to compare the treatment with homofermentative inoculants with enzymes versus the treatment with a combination of homofermentative and heterofermentative lactic acid bacteria with enzymes on the quality of the fermentation process and the nutritional composition of alfalfa wilted silages of different dry matter under farm conditions.

MATERIAL AND METHODS

In the course of 2022 and 2023, research was conducted in the Czech Republic and Slovakia to evaluate the effect of the use of silage inoculants "A" and "B" on the resulting fermentation and nutritional composition of alfalfa wilted silages with various dry matter under farm conditions. In the survey, 62 samples were collected from silos on 30 dairy farms. Samplings were carried out according to Decree No. 415/2009 which establishes the requirements for sampling and principles of methods of laboratory testing of animal feed. The samples were divided into six groups according to the treatment type and the wilting rate. The enzymatic-bacterial inoculant „A“ contained 500,000 CFU/g treated forage selected homofermentative lactic acid bacterial strains and enzymes of xylanase and β-glucanase. The enzymatic-bacterial inoculant „B“ contained 250,000 CFU/g treated forage selected homofermentative and heterofermentative lactic acid bacterial strains and enzymes of

xylanase and β-glucanase. Freshly chopped alfalfa was wilted to dry matter (DM) contents <320 g (LDM), 320-400 (MDM), and >400 (HDM) g/kg fresh weight. Silage was analyzed for dry matter, crude protein, neutral-detergent fiber, acid-detergent fiber, ash, lactic acid, acetic acid, propionic acid, butyric acid, and pH. Analysis was carried out according to AOAC 2005. Statistica 14.0 (CZ) software was used for the statistical evaluation. Data were analyzed by one-way ANOVA and Scheffe´s multiple comparison post hoc test ($p<0.05$) was used.

RESULTS AND DISCUSSION

The results of monitoring the effect of silage preservatives on the quality of the fermentation process and nutrition value are presented in Table 1.

Table 1. Effects of LAB inoculant and different DM on the fermentation process and nutrition value alfalfa silage. (%/100% DM)

ITEM	LDM A (n=8)	LDM B (n=9)	MDM A (n=12)	MDM B (n=10)	HDM A (n=10)	HDM B (n=13)
DM	28.9^{a}	28.4^{a}	36.3^{b}	36.6^{b}	46^{c}	47.9^{c}
CP	19.5^{a}	19.6^{a}	18.7^{a}	18.5^{a}	17^{a}	18.1^{a}
NDF	40.3^{a}	39.3^{a}	37.8^{a}	38.4^{a}	43.1^{a}	40.2^{a}
ADF	32.2^{a}	34.1^{a}	30.1^{a}	30.1^{a}	34.7^{a}	32^{a}
ASH	11^{ab}	13.6^{b}	11^{ab}	10.1^{a}	11.8^{ab}	11.1^{ab}
LA	8.6^{a}	7.3^{a}	9.3^{a}	7.7^{a}	7.2^{a}	7^{a}
AA	5.5^{ab}	6.5^{a}	3.8^{bc}	4.3^{bc}	2.6^{c}	2.7^{c}
PA	0.7^{a}	0.7^{a}	1.1^{a}	0.6^{a}	0.4^{a}	0.4^{a}
BA	1.1^{a}	0.6^{a}	0.2^{a}	0^{a}	0^{a}	0^{a}
LA/AA	2.2^{ab}	1.3^{a}	3^{b}	1.9^{ab}	2.9^{ab}	2.7^{ab}
Acid	15.8^{a}	14.9^{a}	14.4^{a}	12,5^{ab}	10.2^{b}	10.1^{b}
pH	4.5^{a}	4.8^{a}	4.4^{a}	4.5^{a}	4.7^{a}	4.6^{a}

Different letters in one row mean statistically significant differences ($p<0.05$). LDM - dry matter contents <320 g/kg fresh weight, MDM - 320-400 g/kg fresh weight, HDM - >400 g/kg fresh weight. A – treated with inoculant containing homofermentative lactic acid bacteria and enzymes. B - treated with inoculant containing homofermentative and heterofermentative lactic acid bacteria and enzymes.

The monitoring does not indicate any significant differences in nutrient composition. No response to the type of inoculant used and the amount of nutrients was consistent with the results of the meta-analysis by Irawan et al. 2021. The only conclusive difference was found in the ash content in the LDM B group of 13.6 ± 4.2%. Higher ash content could be associated with ash contamination at harvest or higher dry matter losses during fermentation (Borreani et al. 2018). Higher dry matter losses could be associated with heterofermentative bacteria in the combined inoculant (Arriola et al. 2021). There was no demonstrable difference in pH, propionic acid, lactic acid, and butyric acid between the groups. Measurable butyric acid levels were 1.1 ± 2.9%, 0.6 ± 1.7%, and 0.2 ± 0.46% in the LDM A, LDM B, and MDM A groups, respectively. The measurable amount of butyric acid is referred to as an indicator of poor fermentation regardless of its quantity (Kung et al. 2018). The highest demonstrable acetic acid content compared to the other groups was 6.5 ± 1.5% in the LDM B and 5.5 ± 2.5% in the LDM A groups compared to groups with the highest dry matter. The groups with the highest dry matter had the lowest amounts of fermentable acids with a demonstrable difference to the LDM A, LDM B, and MDM A groups. The narrowest lactic to acetic acid ratio was 1.3 ± 0.9% in the LDM B group and the widest in the MDM A group was 3 ± 1.5%.

CONCLUSION

Our results show that treatment with combined silage inoculants compensated for the negative effect of using heterofermentative lactic acid bacteria on acetic acid production. There were no demonstrable differences in acetic acid content, lactic/acetic acid ratio, and the sum of acids between groups of different dry matter. There was no demonstrable difference in nutrient content between groups according to preservatives and dry matter content. This result may indicate nutrient preservation with the use of preservatives, but research needs to be done under laboratory conditions with a control group.

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MODULATION OF RUMEN FERMENTATION BY MEDICINAL PLANTS IN LAMBS WITH HAEMONCHOSIS – AN *IN VITRO* STUDY

KLÁRA MIKULOVÁ[1], DANIEL PETRIČ[1], DOMINIKA BATŤÁNYI[1], MARIÁN VÁRADY[2], ZORA VÁRADYOVÁ[1]

[1]Institute of Animal Physiology of Centre of Biosciences, Slovak Academy of Sciences, Šoltésovej 4-6, 040 01 Košice, Slovak Republic
[2]Institute of Parasitology of Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovak Republic

Corresponding email address: mikulova@saske.sk

ABSTRACT

The aim of the experiment was to determine the effect of medicinal plants on the ruminal fermentation parameters of lambs with haemonchosis *in vitro*. Twelve lambs were divided into two groups: uninfected and infected with third-stage larvae of the gastrointestinal nematode (GIN) *Haemonchus contortus*. On days 48, 49 and 50 *post*-infection, two lambs in each group were killed and rumen contents were collected. For the fermentation of substrate with inoculum in fermentation vessels for 24 hours at 39°C under anaerobic conditions, an *in vitro* gas production technique was used. Meadow hay, Herbmix (*Malva sylvestris, Matricaria chamomilla, Fumaria officinalis, Artemisia absinthium*) and chicory (*Cichorium intybus*) were used as substrates for *in vitro* experiment. Phytochemical compounds were quantified using ultra-high-resolution mass spectrometry. Substrate and infection significantly affected ammonia-N, total gas and methane concentrations, which were higher in the infection group than in the control. Chicory is a promising substrate for mitigating methane production and may probably modulate the ruminal fermentation of GIN-infected lambs due to its chemical and phytochemical composition.

Keywords: *Haemonchus contortus*; phytochemicals; ruminal fermentation; methane

INTRODUCTION

Gazing ruminants are at constant risk of getting infected by larvae of parasitic gastrointestinal nematodes (GIN). Control of GIN is usually limited to the repeated use of anthelmintic drugs. However, their excessive use has led to the development of anthelmintic resistance in parasite populations, which develops relatively quickly within a few years. Medicinal plants produce a wide range of phytochemicals such as phenols, alkaloids, and terpenes, which are responsible for the therapeutic effect as herbal nutraceuticals against GIN (Váradyová et al., 2018; Mravčáková et al., 2019). Chicory (*Cichorium intybus*) from the *Asteraceae* family is interesting for its anthelmintic effect in ruminants (Peña-Espinoza et al., 2018), but it can probably also modulate rumen fermentation and influence rumen methanogenesis. However, studies on the effect of bioactive compounds of medicinal plants on methanogenesis in infected lambs are limited. We hypothesized that medicinal plants with antiparasitic properties would also affect the rumen microbial communities of GIN-infected lambs. We used traditional medicinal plants with antiparasitic properties to investigate their effect on ruminal fermentation *in vitro* using inoculum from GIN-infected animals.

MATERIAL AND METHODS

Ruminal and abomasal contents were collected from slaughtered uninfected (Control) and infected (Infection) lambs and dispensed in volumes of 35 ml into serum bottles (35 ml) containing 0.25 g of a substrate. Meadow hay (MH), plant mixture (Herbmix - *Malva sylvestris*, *Matricaria chamomilla*, *Fumaria officinalis*, *Artemisia absinthium*) and chicory (*Cichorium intybus L.*) were used as substrate (Agrokarpaty, Plavnica, Slovak Republic). Substrates were ground using a Molina grinder (Mipam bio s.r.o., České Budějovice, Czech Republic) and sieved through a 0.15-0.40 mm sieve. The volume of accumulated gas released from the recorded pressure, or the volume of gas produced after 24 hours of fermentation was determined using the *in vitro* gas-producing technique. The gas samples were collected using a gas-tight syringe. Short-chain fatty acids and methane were analyzed on a PerkinElmer Clarus 500 gas chromatograph (Perkin Elmer, Shelton,

USA). The concentration of ammonia was determined using the phenol-hypochlorite method. Fermentation parameters were analyzed using a two-way ANOVA in a 3 × 2 × 3 factorial arrangement in a completely randomized design. The model included effects for substrate, inoculum, and interaction as fixed factors, and each consecutive run was considered as a random factor.

RESULTS AND DISCUSSION

Quantitative analyzes of polyphenols in Herbmix identified three main groups: flavonoids (23 mg/g), phenolic acids (15 mg/g) and alkaloids (3 mg/g), and in chicory flavonoids (9 mg/g), phenolic acids (17 mg/ g) and coumarins (5 mg/g). Substrate and infection significantly affected the concentrations of methane ($p = 0.001$), ammonia N ($p = 0.021$), and total gas ($p = 0.001$), and which were higher in the infection group than in the control (Figures 1-3). Changes in the rumen concentrations of methane and ammonia-N in the infection group were probably caused by a change in the ruminal microbiota. In the infection group, the optimal level of ammonia in the rumen was exceeded (20-100 mg/l). The increased concentration of ammonia in the rumen of infected lambs could be due to lower consumption of ammonia by microorganisms. In our experiment, the substrates also affected the concentrations of *n*-butyrate ($p = 0.048$), *iso*-butyrate ($p < 0.001$), *n*-valerate ($p < 0.001$) and *iso*-valerate ($p < 0.001$). These branched-chain fatty acids are formed in the rumen by amino acid deamination. Our results probably point to a direct effect of plant bioactive compounds in chicory on methanogens, or indirectly to the reduction of hydrogen production as a substrate for microorganisms (Petrič et al., 2020). Chicory bioactive compounds may have the potential to inhibit methane production in ruminal fermentation in GIN-infected animals without adversely affecting fermentation. Changes in the rumen microbiome, fermentation kinetics, anti-methanogenic inhibitors, and dietary substrates may be important factors influencing the efficacy of polyphenols in fermentation (Patra et al., 2017). Efficacy may vary depending on the type of polyphenols, source, molecular weight, and their content in the diets (Petrič et al., 2020).

Figure 1. Effect of substrates (S) and inocula (I) on methane emission

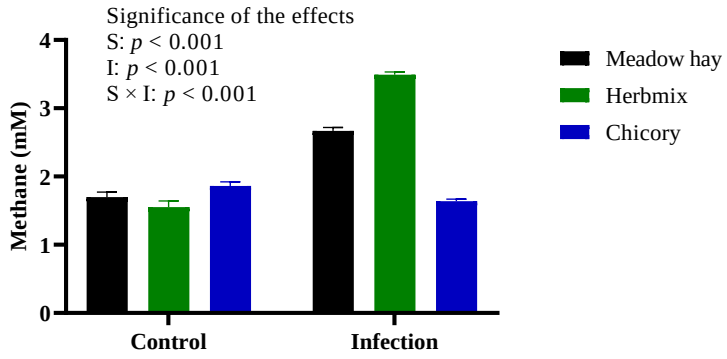


Figure 2. Effect of substrates (S) and inocula (I) on ammonia N

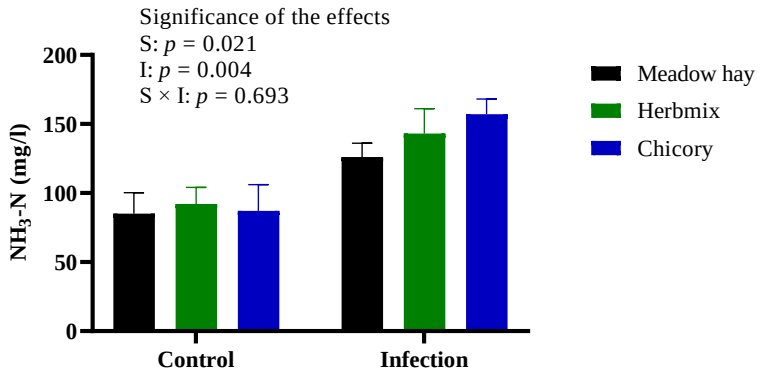
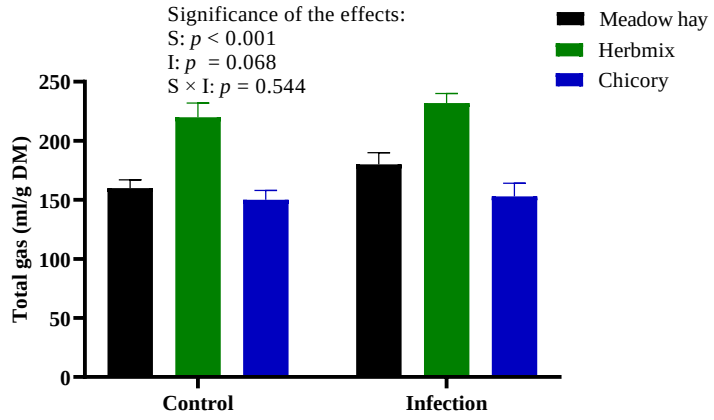


Figure 3. Effect of substrates (S) and inocula (I) on total gas



CONCLUSION

Our results suggest an association between GIN infection and increased methanogenesis in the rumen. Chicory mitigated methane *in vitro* and may modulate rumen fermentation in GIN-infected lambs.

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EVALUATION OF THE FERMENTATION PROCESS IN CORN SILAGES

ANDREJ MITRÍK[1], TOMÁŠ MITRÍK[2], IVETA MASKAĽOVÁ[1]

[1]Department of Animal Nutrition and Husbandry, University of veterinary medicine and pharmacy, Komenského 73, 041 81 Košice, Slovak Republic

[2]FEEDLAB s.r.o., Magurská 3, 052 01 Spišská Nová Ves, Slovak Republic

Corresponding email address: andrej.mitrik@feedlab.sk

ABSTRACT

Propane-1,2-diol (propanediol) represents an important glucoplastic substance for the nutritional requirements of highly productive cows, and its natural synthesis during silage fermentation is of great importance. The aim of this paper was to monitor the fermentation activity of two combinations of homo and hetero-fermentative lactic acid bacteria (LAB) in two preparations (preparation 1: L. buchneri, L. rhamnosus, L. plantarum; preparation 2: L. buchneri, L. rhamnosus, L. diolivorans). Propanediol production was monitored in maize silages prepared at the following combinations levels: cutting technology, hybrid, vegetation development, type of silage fermentation and length of fermentation time. Preparation 1 had a demonstrably positive influence on the production of propanediol in maize silages from whole plants (average content of 4.93 $g.kg^{-1}$). Propanediol production in the silage alternative with preparation 2 (average content of 1.33 $g.kg^{-1}$) was higher when compared to the negative control (average content of 0.11 $g.kg^{-1}$). The production of propanediol decreased with increasing dry matter content, which is directly related to the advancing vegetative development of plants. The type of silage fermentation and vegetation development had a statistically demonstrable effect on the level of propanediol production in maize silages. The fermentation activity of the Lactobacillus buchneri strain used depended on its combination with other homofermentative and/or heterofermentative LAB strains.

Keywords: *Zea mays, hybrids, harvesting technology, vegetation stages, dry matter content, silage, 1,2-propanediol, Lactobacillus buchneri*

INTRODUCTION

The development and course of silage fermentation is fundamentally influenced by the fermentation's microflora. It is primarily formed by epiphytic bacteria. Targeted inoculation and guidance of the course of silage fermentation is achieved by adding various additives, in which different types and strains of lactic acid bacteria (hereinafter LAB) are most often used. These are isolated from nature and their selection depends on their unique fermentation characteristics, and their potential to interact with one another.

The aerobic stability of silage is one of the primary goals of successful silage fermentation. The dominant factor for increasing this is acetic acid (Danner et al., 2003), which is produced to varying degrees and in different ranges by LAB (Mitrík, 2021). Some strains of heterofermentative LAB have the ability to produce acetic acid primarily from water-soluble sugars, and secondarily through the fermentation of lactic acid (Oude Elferink et al., 2001), which creates the basis for the synergistic action of homofermentative and heterofermentative LAB. During the transformation of lactic acid to acetic acid, propane-1,2-diol (herein- after propanediol) and ethanol are also formed (Oude Elferink et al., 2001, Danner et al., 2003). Propanediol is also used in the food industry to positively influence the palatability of food (Patent EP2822399A1) and it is assumed that it also improves the palatability of silages (Mitrík, 2021). It has no direct effect on increasing aerobic stability (Danner et al., 2003), but is a potential precursor of 1-propanol, which is effective against yeasts (Nishino and Touno, 2005) and is a precursor for the subsequent formation of propionic acid (Krooneman et al., 2002), which is effective against fungi. From a nutritional point of view, propanediol is an important glucoplastic substance in the nutrition of high-production cows (Wilkinson and Rinne, 2017; Lau et al., 2018), which is purposefully added to feed rations to compensate for the negative energy balance of cows, especially in the postpartum period.

The aim of this work was to monitor and evaluate the dynamics of the production of propanediol and other fermentation metabolites in silages

of whole plants of different maize hybrids (*Zea mays*) using two combinations of homofermentative and heterofermentative LAB, which contained one homofermentative and one heterofermentative strain (Table 1). We monitored changes in the composition of the fermentation profile at the following levels: 7 different silage maize hybrids, 2 material cutting technologies, 4 points of vegetation development in over a time interval of 34 days and 3 lengths of the fermentation process.

Table 1 Characteristics of preparations

PREPARATION	**0**	**1**	**2**
L.buchneri 1k2075		+	+
L.diolivorans 1k20752			+
L.plantarum 1k2079		+	
L.rhamnosus 1k20711		+	+
KTJ/1g		min. $3.0x10^{11}$	min. $2.5x10^{11}$
dosing		1g/1t	1g/1t

** $g.kg^{-1}$; grey background: matched strains of lactic acid bacteria*

MATERIAL AND METHODS

Seven (7) different silage maize hybrids (FAO 200 – 530) from KWS SEMENA s.r.o. were sown on 28/04/2021 in four repetitions on the plot in Bátka: altitude 182 m above sea level - 48°21'45.9"N 20°11'55.7"E (Fig. 1). Sampling was carried out at an interval of 34 days on four dates (12/08/2021; 19/08/2021; 2/9/2021; 13/09/2021). From each cut sample, a roughly 30 kg coarse sample was taken from at least 10 places. The coarse samples were transported to the laboratory immediately after collection, where each of them was again thoroughly mixed and laboratory samples were taken from this material for nutrient analysis, and at the same time three silage alternatives (Tab. 2) were ensiled in the laboratory, each in two replications for three different periods of silage fermentation (90, 150 and 240 days). After the prescribed fermentation time, the samples were opened.

After opening the samples, the silage was thoroughly mixed. Preparation of aqueous solution: 100 g of silage in 2000 ml of distilled water by mixing (30,000 revolutions/1 min.) for 1 minute and subsequent filtering through a paper filter. The pH value was immediately measured on a Seven Compact S220 instrument (Mettler Toledo). Leachate samples were prepared by standard purification (clarification via updated Carrez solution I and II, dilution, centrifugation and ultra-filtration) prior to UHPLC measurement. Fermentation characteristics were measured on a UHPLC Dionex UltiMate 3000 Series with an AGILENT Hi-Plex H 300 x 7.7 mm column. Mobile phase 0.01M H2SO4 with a flow rate of 0.7 ml/min. and with a sample injection of 20 µl. Lactic acid and volatile fatty acids were measured on a UV-VIS 210 nm detector at a temperature of 40°C, and alcohols, propane-1,2-diol (propanediol) and monosaccharides on an RI detector at a temperature of 55°C.

RESULTS AND DISCUSSION

The dry matter content in all three silage alternatives (Tab. 2) did not show statistically significant differences. Each of the groups consisted of an extensive set of 168 silage samples (Tab. 2), which testifies to a broad and standard starting base for each group. The addition of silage additives affects the presence of L. buchneri in maize silages (Mitrík et al., 2019; Kaluzová et al., 2022). We found very significant statistical differences ($P<0.01$) in the content of propanediol between the silage alternatives. Preparation 1 reached significantly the highest content of 4.9 $g.kg^{-1}$, which is also in accordance with the results of other authors (Weiss et al., 2005; Kleinschmit and Kung, 2006; Arriola et al., 2021; Huang et al., 2021), indicating very good performance of the L. buchneri strain in combination with two homofermentative strains. Despite using the same strain of L. buchneri in preparation 2, the production of propanediol was almost 3.8 times lower. The control contained only trace amounts of propanediol, which did not exceed 1.00 $g.kg^{-1}$ even in the maxima, which is also in accordance with the results of Kleinschmit and Kung (2006). The results show that the use of a particular strain/species and its combination with other LAB affects the success of propanediol production. We found a statistically significant correlation ($P<0.01$)

between propanediol and lactic acid (r -0.356), acetic acid (r 0.415) and ethanol (r -0.381).

Table 2 Silage alternative – fermentation parameters**

PREPARATION	0	1	2
n	168	168	168
DRY MATTER*	346.2 ±80.2	335.0 ±82.8	335.9 ±80.8
pH	3.87 ±0.10 12	3.92 ±0.10 02	3.81 ±0.11 01
LACTIC ACID*	20.9 ±4.8 1	15.6 ±6.0 02	20.1 ±4.5 1
ACETIC ACID*	5.9 ±2.8 12	14.7 ±8.6 02	12.1 ±6.2 01
BUTYRIC ACID*	0.0 ±0.1	0.0 ±0.1	0.0 ±0.1
PROPIONIC ACID*	0.0 ±0.0 1	0.1 ±0.3 02	0.0 ±0.1 1
FORMIC ACID*	0.6 ±0.2 12	0.5 ±0.2 02	0.6 ±0.2 01
ETHANOL*	4.9 ±3.3 12	3.2 ±2.7 0	2.9 ±2.6 0
1,2-PROPANEDIOL*	0.1 ±0.5 12	4.9 ±4.4 02	1.3 ±1.8 01
1-PROPANOL*	0.1 ±1.1 12	0.8 ±1.7 02	1.4 ±1.7 01
1-BUTANOL*	0.1 ±0.7	0.1 ±0.5	0.1 ±0.5

** $g.kg^{-1}$; ** average of 90, 150 and 240 days of fermentation; indices: statistically significant differences in the row (P<0.01)*

The fermentation parameters and their ratios when using preparation 1 point to the successful course of the secondary production of acetic acid from lactic acid, which is also confirmed by:

- the lowest lactic acid content,
- the highest acetic acid content,
- higher ethanol content than preparation 2,

which is in agreement with the description of fermentation pathways characteristic of L. buchneri (Oude Elferink et al., 2001; Krooneman et al., 2002; Rooke and Hatfield, 2003). Homofermentation supported by the inclusion of L. plantarum in preparation 1 most likely supported higher lactic acid formation in the first stages of fermentation, thus

creating the basis for its secondary fermentation by the L. buchneri strain. At the level when using the silage alternative with preparation 1, we also found statistically significant (P<0.01) correlations between dry matter content and the following fermentation products: propanediol (r -0.590), lactic acid (r 0.629), acetic acid (r -0.627).

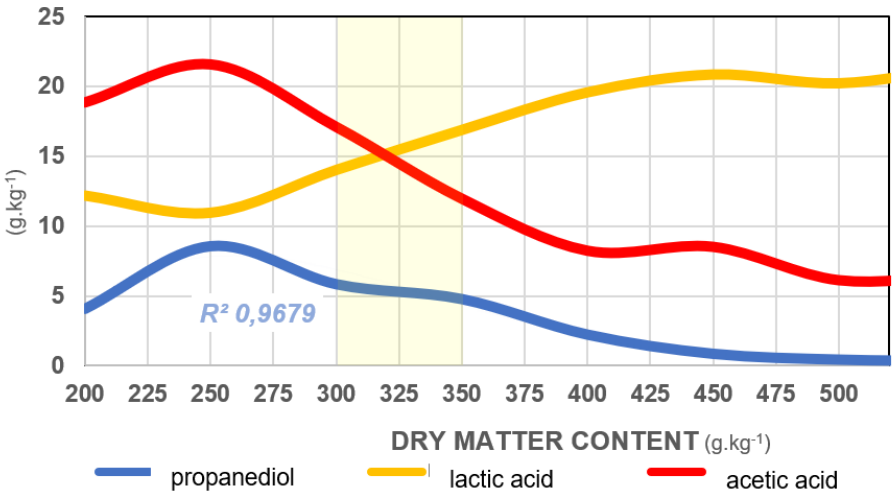
Propanediol peaked (8.58 $g.kg^{-1}$) at a dry matter content of 250 $g.kg^{-1}$ (Table 3) and dropped at a dry matter content of 300 to 350 $g.kg^{-1}$ to a level of *approximately* 5.0 $g.kg^{-1}$. At a dry matter content of 400 $g.kg^{-1}$, propanediol production dropped to 2.21 $g.kg^{-1}$ and continued to drop below 1.00 $g.kg^{-1}$ thereafter. These results again indicate that the intensity of the secondary fermentation of lactic acid to acetic acid and propanediol decreases with increasing dry matter content.

Table 3 Dry matter content (group) – fermentation parameters** in the silage alternative with PREPARATION 1

group	1	2	3	4	5	6	7	8	average
DM content*	200	250	300	350	400	450	500	550	
replication (n)	14	94	61	65	33	51	17	1	
LA *	12.18 567	10.95 3 4567	14.02 2 567	16.89 26	19.59 123	20.87 1234	20.24 123	21.44	15.57
AA*	18.84 567	21.56 3 4567	17.08 2 4567	11.94 23	8.21 1 23	8.49 12 3	6.10 1 23	6.47	14.65
ETH*	5.13 2	2.06 167	2.76 6	2.73 6	2.95 6	5.43 23 45	4.38 2	5.45	3.17
PPD *	4.07 2	8.58 134 567	5.84 256 7	4.77 2 67	2.21 2 3	0.83 23 4	0.41 2 34	0.30	4.92

** $g.kg^{-1}$; ** average of 90, 150 and 240 days of fermentation; indices: statistically significant differences in the row (P<0.01) DM – dry matter; LA – lactic acid; AA – acetic acid; ETH – ethanol; PPD – 1,2-propanediol*

Chart 1 **Propanediol and dry matter content**



CONCLUSION

The same L. buchneri strain was able to increase propanediol production almost 3.8-fold under the same conditions if it was inoculated in combination with two homofermentative LAB strains. The combination of two heterofermentative LAB strains (L. buchneri and L. diolivorans) with one homofermentative strain (L. rhamnosus) did not produce increased amounts of acetic acid. The results achieved and the differences between the preparations indicate that the performance of the same L. buchneri strain in the production of propanediol depends, with great probability, on its action in combination with other Lactobacillus species.

Preparation 1 had a demonstrably positive influence on the production of propanediol in maize silages (average content 4.93 $g.kg^{-1}$), fulfilled the declared properties and is strongly assumed to positively influence the health of highly productive cows. The production of propanediol in the silage alternative with preparation 2 (average content 1.33 $g.kg^{-1}$) was higher than in the negative control (average content 0.11 $g.kg^{-1}$), but only approximately at a third of the level compared to preparation 1. The results of this work significantly indicate that the nutrient characteristics of silage hybrids in individual vegetation stages can create different

fermentation starting points for the course of the silage process, and this issue will require further monitoring.

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THE EFFECT OF FEED PARTICLE SIZE IN SLOW-GROWING BROILER CHICKENS ON PERFORMANCE PARAMETERS, DIGESTIVE TRACT MORPHOLOGY, ILEAL VISCOSITY AND NITROGEN RETENTION

JAKUB NOVOTNÝ, LUKÁŠ ČUMPLÍK, LUCIE HORÁKOVÁ, MICHAL ŘIHÁČEK, DANA ZÁLEŠÁKOVÁ, ONDŘEJ ŠŤASTNÍK, LEOŠ PAVLATA

[1]Department of Animal Nutrition and Forage Production, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: jakub.novotny@mendelu.cz

ABSTRACT

The influence of different feed particle size was evaluated on 120 slow-growing Hubbard JA57 broiler chickens. Three types of diets were used, which differed in particle size - fine, medium, coarse. The Geometric Mean Diameter was 0.66 vs. 0.89 vs. 1.07 mm, respectively. The positive effect of the coarse particles on the apparent digestibility of the crude protein in the finisher mixture was observed. Performance parameters, as well as digestate viscosity and weight or length of organs of the digestive tract, were not affected.

Keywords: poultry nutrition; Hubbard JA57; animal diets; Geometric Mean Diameter; Geometric Standard Deviation

INTRODUCTION

The proportion of coarse particles in the mixture shows a positive effect on the proper functioning of the gastrointestinal tract and its health (Gabriel et al. 2008). Supporting the development of a gizzard is one of the interests of nutrition, which improves digestion and the subsequent utilization of nutrients (Svihus 2011). The feed particle size also affects the viscosity of the digesta. A higher digestive viscosity was found in birds fed the fine feed mixture compared to the viscosities of the ones fed the medium and coarsely ground particles in the mixture.

This higher viscosity was associated with reduced feed intake (Yasar 2003).

Feed particle size has also been found to affect the gizzard and other segments of the digestive tract, but the results are contradictory. Amerah et al. (2007a) noted a decrease in the relative length of all parts of the digestive tract as wheat particle size increased. A decreased intestinal weight or length may result in improved feed efficiency due to reduced maintenance costs (Xu et al. 2015).

MATERIAL AND METHODS

Hubbard JA57 slow-growing broilers were selected for this experiment, it was a male individual. Fattening was planned for 49 days. In total, there were 120 animals, which were subsequently divided into three groups of five repetitions (8 pcs for one repetition). Chickens were housed in balance cages. Slow-growing broilers had unlimited access to feed and drinking water during the experiment. In the room in which the experiment takes place, the light program, temperature and humidity set were controlled according to the technological manual related to the Hubbard JA 57 hybrid. During the observation period, the individuals were weighed every week and the measured values were recorded. The amount of feed consumed was also monitored.

Diets

The observed individuals were all fed a non-pelleted mixture that corresponded to the nutrient needs of broilers. The feed mixture given to all three groups was composed of the same components and all the presented feed mixtures also had the same nutrient composition (Table 1).

Table 1. Composition of experimental diets

	Fine, Medium, Coarse		
Component (g/kg)	Starter	Grower	Finisher
Rapeseed oil	33.6	37.6	40
Limestone milled	0.8	-	-
Monocalcium phosphate	10	5.6	4.9
DL-Methionine	2.4	2.2	1.5
Soybean meal	341	295	252
Maize	330	356.6	396.1
Wheat	252.2	270	272.5
Chromium oxide	-	3	3
Premix	30	30	30

Legend: **Premix for starter contains** (per kg): L-lysine 2.34 g; DL-Methionine 2.4 g; Threonine 0.99 g; calcium 5.25 g; phosphorus 1.95 g; sodium 1.44 g; copper 15 mg; iron 84 mg; zinc 99 mg; manganese 99 mg; iodine 0.99 mg; selenium 0.18 mg; retinol 13,500 IU (international units); calciferol 5,001 IU; tocopherol 45 mg; phylloquinone 1.5 mg; thiamine 4.2 mg; ri-boflavin 8.4 mg; pyridoxin 6 mg; cobalamin 30 µg; biotin 0.21 mg; niacinamid 36 mg; folic acid 1.8 mg; calcium pantothenate 13.5 mg; cholin chloride 180 mg. **Premix for grower and finisher contains** (per kg): L-lysine 2.58 g; DL-Methionine 2.52 g; Threonine 1.47 g; calcium 5.04 g; phosphorus 1.65 g; sodium 1.38 g; copper 15 mg; iron 75 mg; zinc 99 mg; manganese 99 mg; iodine 0.9 mg; selenium 0.36 mg; retinol 9,900 IU (international units); calciferol 5,001 IU; tocopherol 45 mg; phylloquinone 1.5 mg; thiamine 4.2 mg; ri-boflavin 8.4 mg; pyridoxin 6 mg; cobalamin 28.8 µg; biotin 0.18 mg; niacinamid 36 mg; folic acid 1.71 mg; calcium pantothenate 13.35 mg; cholin chloride 180 mg. * Apparent metabolize energy, calculated value

In Table 2, there are shown chemical analysis values of used diets. The difference between the mixtures consisted only in the difference in structure - fine, medium and coarse. The feed mixture was presented *ad libitum* to slow-growing broilers.

Table 2. Chemical analysis of used diets (88% dry matter)

	Starter			Grower			Finisher		
(g/kg)	Fine	Med.	Coar.	Fine	Med.	Coar.	Fine	Med.	Coar.
Crude protein	215.2	220.1	212.9	194.5	198	197.4	178.9	175.3	175.2
Crude fiber	29.9	28.9	29.7	25.1	25.9	24.9	19.8	18.7	20.4
Ether extract	49.6	50.7	48.8	62.4	58.1	58.2	59.9	61.1	60.0
Crude ash	57.1	60.7	57.0	52.9	52.2	55.0	52.2	51.8	50.7

Sieve analysis

The structure of the diets was evaluated by dry sieving using a separator Retch AS 200 Control. A representative sample of 100 g of each diet was passed for 10 minutes through the set of sieves with 3 mm, 2 mm, 1.5 mm, 1 mm and 0.3 mm mesh sizes. An amplitude was set to 1.8 mm/g. After the shaking process, the amount of particles retained on each sieve was determined by subtracting the weight of the sieve and the retained feed from the blank weight of the sieve. The GMD and GSD were calculated by Asabe 2008 and their values are in Table 3.

Table 3. GMD and GSD values of used diets

Group	n	GMD (mm) mean ± SE	GSD (mm) mean ± SE
Fine	10	0.66 ± 0.01^{a}	0.56 ± 0.01^{a}
Medium	10	0.89 ± 0.01^{b}	0.83 ± 0.02^{b}
Coarse	10	1.07 ± 0.03^{c}	1.10 ± 0.05^{c}

^{a,b,c} means statistically significant differences ($p < 0.05$)

Measurements of the digestive tract

The 10 chickens from each group (three of each replicate) were selected and slaughtered by decapitation. The entire digestive tracts were removed and divided into the following sections: crop, proventriculus, gizzard, duodenum, jejunum, ileum, ceca, and colon. These sections were emptied and the remaining fat and mesenteries were removed. The segments removed from the small intestine were the region from the gizzard junction to the pancreatic and bile ducts (duodenum), the area between the end of the duodenum and Meckel's diverticulum (jejunum), and the segment between Meckel's diverticulum and the ileo-ceco-colic junction (ileum). The lengths (or widths) and empty weights of each segment were recorded. Gizzard height was measured as the maximum distance between the proximal (distal limit of the proventriculus) and distal (proximal limit of the duodenum) part of the gizzard. Gizzard width was measured as the maximal distance at right angles of the gizzard height. Gizzard depth was measured as the maximum distance between tendineal centers on the 2 flat sides of the gizzard and the gizzard muscle height was measured as the maximum height at the main muscle measured along the maximal extension of the muscle. All gizzard

measurements were measured using a slide calliper. The obtained values were recalculated and expressed in the live weight of the chickens.

Chromium oxide determination

The excreta were lyophilized and then homogenized (ground to pass a 1-mm sieve) before analyses. Chromium oxide (Cr2O3) content was then determined in the feed and faeces as well. The principle is that the chromium content is determined by titration after oxidation to dichromate. A 1 g sample (to 3 decimal places) of feed of faeces was weighed into a porcelain crucible, which was then burned in a muffle furnace at 550±20 °C for 4 hours. The resulting ash was melted on burner with 2-3 g of melting mixture (KClO3 + Na2CO3; 4:1, respectively). After cooling, the crucible with the melt was poured into the beaker with hot distilled water. It was then covered with a watch glass and leached for 30 minutes while heating. The contents of the beaker were quantitatively transferred to a 100 mL volumetric flask after cooling, made up to the mark, mixed and filtered through a thick filter paper. For the titration, 50 mL of filtrate were pipetted off, 10 mL of potassium iodide (30% solution) and 5 mL of 25% sulfuric acid solution were added. A few drops of dissolved starch (2% solution) were added as an indicator. The solution was stirred and titrated with a standard solution of sodium thiosulfate (0.1 N) until the colour changed. Each sample was performed duplicate. The proportion of chromium oxide was calculated from the measured consumption of thiosulphate.

Equation (1): chromium oxide content (X) g/kg in sample

$(X) = (cv*F*V_0*2.533)/(V_1*w)$

cv: consumption of a standard volumetric solution of thiosulphate

F: volumetric solution factor

V_0: leachate volume (100 mL)

V_1: pipetted volume

w: sample weight in g

The content of dry matter and crude protein in feed and excreta were determined according to the Commission regulation (EC) 152/2009.
The apparent digestibility of crude protein was calculated as follows:

Equation (2): 100 – [(% Cr_2O_3 in the diet × % nutrient in the excreta content) / (% Cr_2O_3 in the excreta content × % nutrient in the diet)] ×100.

Statistical analysis

Data has been processed by Microsoft Excel (USA) and StatSoft Statistica (USA). It was used one-way analysis of variance (ANOVA). For evaluate statistically differences between groups was used the Sheffé´s test and $P < 0.05$ was regarded a level of statistically significant difference.

RESULTS AND DISCUSSION

The experiment showed no effect of the structure of the feed mixture on the weight of the organs of the gastrointestinal tract (see Table 4). The same conclusion was reached by Lv et al. (2015) and Hossein et al. (2019). Even in their experiments, the influence of the structure of the feed on the weight of the organs of the digestive tract was not proven. The opposite results were obtained by Amerah et al. (2007b). They report that higher weights were achieved in the crop, proventriculus, gizzard, duodenum, jejunum, ileum, and cecum when fed the unformed mixture with medium particle size. The increase in the weight of the gizzard when fed with coarse particles is confirmed by the research of Ege et al. (2019).

Table 4 Weight and length of gastrointestinal tract segments

	Group		
n	10	10	10
(g/kg of body weight)	Fine mean ± SE	Medium mean ± SE	Coarse mean ± SE
Live weight	2387.60 ± 198	2470.80 ± 205	2406.60 ± 177
Crop	2.83 ± 0.48	2.63 ± 0.79	2.63 ± 0.47
Proventriculus	3.24 ± 0.47	3.13 ± 0.43	3.58 ± 0.51
Gizzard	12.16 ± 1.60	13.90 ± 1.90	14.33 ± 2.18
Duodenum	3.43 ± 0.56	3.49 ± 0.53	3.47 ± 0.36
Jejunum	7.06 ± 1.15	7.03 ± 0.92	7.06 ± 0.57
Ileum	6.56 ± 1.65	5.87 ± 1.44	6.59 ± 0.64
Colon	1.12 ± 0.17	1.17 ± 0.26	1.18 ± 0.14
Caeca	3.22 ± 0.62	3.22 ± 0.66	3.16 ± 0.62
(mm/kg of body weight	Fine mean ± SE	Medium mean ± SE	Coarse mean ± SE
Duodenum	110.57 ± 12.52	105.72 ± 10.82	111.69 ± 6.87
Jejunum	236.24 ± 52.30	249.86 ± 55.23	230.50 ± 28.78
Ileum	212.03 ± 67.91	208.33 ± 43.03	224.97 ± 25.74
Colon	27.48 ± 4.39	28.82 ± 3.09	30.88 ± 4.41
Caeca	76.63 ± 5.27	68.69 ± 9.69	71.93 ± 8.62

no statistically significant differences; n – nubmer of cases; SE – standard error

In addition to considering the organs of the gastrointestinal system, the lengths of the individual parts - duodenum, jejunum, ileum, colon and cecum - were also measured. However, this measurement did not show a significant influence of the structure of the feed mixture on the length dimensions of the intestinal sections. Amerah et al. (2008) reported that feeding a coarse feed during their trial reduced the length of all parts of the intestine compared to feeding a fine feed. The opposite trend was shown in the experiment of Bozkurt et al. (2019), where feeding a coarse feed mixture had an effect on caecal elongation compared to a fine feed mixture. The aforementioned studies show a contradictory effect of the structure of the feed on the length of the individual sections of the intestines.

The results show (Table 5) that when feeding with grower, the structure of the feed had no significant effect on the resulting coefficient

of apparent digestibility. In broilers fed with the finisher in the final stage of fattening, the effect of structure on digestibility of crud protein was demonstrated. When feeding the mixture that contained the largest proportion of coarse particles, the highest digestibility was achieved. The feed mixture with a medium particle size was demonstrably the lowest coefficient of digestibility. This is probably due to the fact that fine particles are more accessible to digestive enzymes and are therefore more digestible, while coarse particles pass through the digestive tract more slowly and are therefore digested longer. And the average particle size loses these properties.

Table 5. Apparent digestibility of grower and finisher diets

Group	n	Grower apparent digestibility of (%) mean ± SE	Finisher apparent digestibility (%) mean ± SE
Fine	6	61.19 ± 3.25	59.44 ± 1.23^{ab}
Medium	6	57.33 ± 3.74	55.42 ± 3.46^{a}
Coarse	6	60.33 ± 3.18	60.71 ± 3.34^{b}

^{a,b} means statistically significant differences ($p < 0.05$); n – nubmer of cases; SE – standard error

Similar results were obtained by the study by Marx et al. (2021), who investigated the effect of feed structure on the performance of broilers. In this case, soybean was the component investigated. Digestibility was highest for the fine and coarse feed mixture. At the same time, they state that feeds with coarse particles are the most suitable for the final stage of fattening, thereby confirming the results of this experiment. Measurements by Pacheco et al. (2013) also showed that the highest digestibility of protein is when fed with coarse particles. When compared with other studies, it can be stated that the highest nitrogen retention can be achieved when feeding with feed mixtures with coarse particles in the final stage of fattening, and the lowest with feed mixtures with medium-sized particles.

In Table 4, there are shown values concerning the viscosity of digesta. There were not found any statistically significant differences among groups. In contrast to our experiment, Yasar (2003) recorded a high value

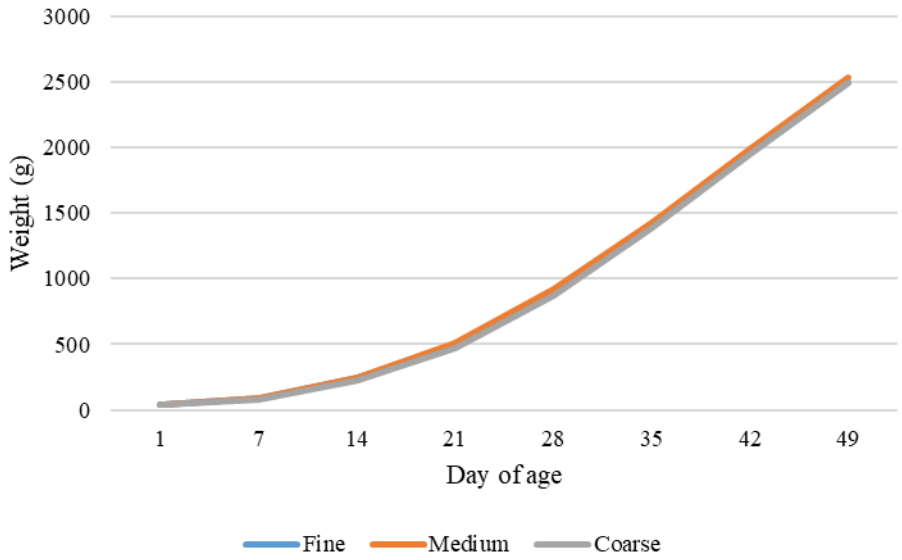
of digesta viscosity in birds fed a fine mixture compared to feeds containing medium or coarse wheat particles in diets.

Table 6. Digesta viscosity

Group	n	Viscosity (mPa·s) mean ± SE
Fine	6	4.49 ± 0.23
Medium	6	4.13 ± 0.21
Coarse	6	4.71 ± 0.14

no statistically significant differences; n – nubmer of cases; SE – standard error

Figure 1. Broilers´ body weight during the trial



no statistically significant differences

The body weight (Figure 3) of all three broiler groups were similar during the trial, no differences were observed. Also, feed consumption and conversion were not affected by the feed structure, when the conversion reached values of 1.70 vs. 1.72 vs. 1.75, respectively (Fine vs. Medium vs. Coarse, respectively). The Table 7 shows values of carcass yield and yield of main meat parts. There were found no statistically significant differences among the evaluated carcass traits.

Nir et al. (1995) stated that broiler chickens fed a coarse mixture of wheat and sorghum had a higher body weight and better feed conversion compared to broilers fed a finely ground mixture. Amerah et al. (2007c) confirmed that chickens fed a mixture with finely ground wheat had reduced weight gain and lower feed intake than groups given a diet with a high proportion of medium and coarse particles.

Table 7. Performance parameters

8	Fine	Medium	Coarse
n	8	8	8
		Mean ± SE	
Body weight (g)	2569 ± 34.21	2674 ± 52.80	2630 ± 40.96
Carcass weight (g)	1837 ± 26.28	1847 ± 54.09	1839 ± 28.03
Carcass yield (%)	71.51 ± 0.60	69.13 ± 1.73	69.95 ± 0.30
Breasts (%)	19.76 ± 0.38	18.30 ± 0.61	18.39 ± 0.40
Thighs (%)	16.05 ± 0.31	16.30 ± 0.55	15.84 ± 0.15

no statistically significant differences; n – nubmer of cases; SE – standard error

CONCLUSION

In the experiment, it was found that the different feed particle size affected the digestibility of crude protein when broilers fed with the finisher. The different sizes of the feed particles had no effect on the other observed parameters (performance, digesta viscosity, weight and length of the organs of the digestive tract).

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EFFICIENCY OF USING BIOGENIC METALS FOR FEEDING PIGLETS

SHKROMADA OKSANA,[1] PIKHTIROVA ALINA,[2] HREK ROMAN,[1] SHKROMADA OLEKSANDR,[3] ZIGO FRANTIŠEK[4]

1Department of Obstetrics and Surgery, Sumy National Agrarian University, H. Kondratiev 160, 40021 Sumy, Ukraine

2Department of Public Health, Sumy State University, Rymskogo-Korsakova 2, 40007 Sumy, Ukraine

3Faculty of Veterinary Medicine, Sumy National Agrarian University, H. Kondratiev 160, 40021 Sumy, Ukraine

4Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, Komenského 73, 04181 Košice, Slovakia

Corresponding email address:a.pikhtirova@med.sumdu.edu.ua

ABSTRACT

Our study was aimed to investigat the impact of the use of biogenic metals (iron, zinc, copper, manganese) as a feed additive in the composition of compound feed for piglets after weaning. Two groups of piglets - control (C, n = 7) and experimental (E, n = 12) - were involved in the experiment. The experiment lasted 20 days. Blood samples in a volume of 1 ml were taken from the eye sinus before the start of the experiment and after its completion. Biochemical parameters and the content of microelements were determined in the blood samples (iron, zinc, copper, manganese). It was established that in the blood serum of piglets from E group there was more copper by 26.7% and iron by 7.9%, compared to animals of the control group. In addition, the use of biogenic metals in the diet of piglets after weaning did not have a significant effect on the biochemical parameters of the blood, which remained within the physiological norm throughout the experiment. Positive effect of the use of metal citrates as a feed additive on the microelement composition of piglet's blood at weaning has been proven. It can be argued that biogenic

metals in the compound feed do not have a negative or toxic effect on the body of piglets and can be used as a feed additive.

Keywords: piglets; biogenic metals; inorganic substances; biochemical indicators

INTRODUCTION

Pigs experience biological stress during weaning from the sow, transfer to another room, change of diet and even change of supervisor. All these processes lead to the deterioration of the pig's health, growth and feed consumption, especially during the first to two weeks after the change of previous conditions (*Genova et al., 2020;* Lallès et al., 2004).

It is proved that iron is a one of the most important component of the blood. It is involved in enzymatic processes in the electron transport chain such as cytochrome oxidase and cytochrome enzymes. Iron is very important in preventing iron deficiency anemia and promotes healthy animal's growth. The main cause of iron deficiency is the peculiarities of the physiological development of piglets (Ding et al., 2020). The physiological importance of zinc has been known for a long time. Recently, its importance in immunomodulation has emerged. Zinc plays a modulatory role in the immune response through its availability, which is regulated by several regulators and transporters. Zinc deficiency affects the cells of the innate and adaptive immunity at all levels of their functioning (Bonaventura et al., 2015).

We know that copper is a trace element that contributes to the normal growth of pigs. Many previous studies have proven its effect on improving the performance of pigs due to its antibacterial capabilities. It has also been shown that the addition of high levels of copper leads to low copper absorption (Gonzales-Eguia et al., 2009). The significant role of manganese in the processes of metabolism, enzyme activation, exchange of nitrogenous substances, calcium and phosphorus has been determined. It promotes the growth of young animals, affects hematopoiesis (Cao et al., 2022).

Many different feed additives based on metal chelates have been proposed in the world (Perevozchikov et al., 2017; Acda and Chae, 2002). We decided to conduct a study of the effect of compound feed with the addition of a mixture of chelated metals (iron, zinc, copper,

manganese) on piglets, which was proposed by the regional enterprise "Kronos Agro".

The aim of our study was to compare the effect of feed additive with biogenic metals on blood parameters and mineral profil of piglets.

MATERIAL AND METHODS

Animals

The research was carried out in the SE "EFIANE" of the NAAS of Ukraine, Sumy region (Ukraine) on piglets of the large white breed 3 weeks age. 2 groups were formed: control (C) - 7 heads, which were fed the usual ration based on compound feed, and experimental (E) - 12 heads, which were fed the main ration with the addition of premix + metal sulfates for 20 days. The experiment was conducted in accordance with the ARRIVE guidelines (Percie du Sert et al., 2020).

Selection and analysis of samples

For laboratory studies, blood samples were taken from the eye sinus in piglets in a volume of 1 ml before the start of the experiment and after its completion, and immediately sent to the laboratory. The content of: total protein, albumin, globulin, urea, total cholesterol, glucose, creatinine was determined in the blood samples; activity of aspartate aminotransferase (ALT), alanine aminotransferase (AST) and alkaline phosphatase (ALP). The content of zinc, copper, manganese and iron was also determined. Laboratory tests were conducted in accordance with generally accepted requirements (Official method of analysis, 2000) using general purpose photometer Stat Fax 1904 Plus (Awareness Technology, USA) and biochemical photometer Humalyzer Junior (Human GMBH, Germany).

Statistical analysis

The results of the study were elaborated statistically using one-factor analysis of variance ANOVA in Statistica 13.0 software. $p<0.05$ and $p<0.01$ were considered significant and the differences between the means with $0.05<p<0.10$ were regarded as tendencies.

RESULTS AND DISCUSSION

According to the dates of the study (Table 1), it can be noted that the introduction of a premix with metal sulfates into the diet of piglets of the E group didn't have a negative effect on the protein metabolism in the animal's body.

Table 1. The level of the main biochemical indicators in the blood serum of piglets

Parameters	Period	C (n=7)	E (n=12)	Reference level
Total protein, g/l	before	53.45±0.1	56.74±0.2	70.0 - 80.0
	after	62.01±0.07	58.72±0.26	
Albumin, g/l	before	29.677±0.09	28.12±0.1	28.0 - 44.0
	after	36.64±0.06	30.34±0.11	
Total globulin, g/l	before	23.77±0.08	26.14±0.2	32.9 - 52.0
	after	**25.37±0.08**	**28.39±0.1***	
Urea, mmol/l	before	3.2±0.09	4.1±0.05	3.3 - 7.0
	after	**5.7±0.09**	**4.8±0.07***	
Total cholesterol, µmol/l	before	1.34±0.05	1.37±0.02	1.56 - 2.86
	after	1.24±0.06	1.31±0.02	
Glucose, mmol/l	before	3.7±0.02	4.4±0.04	3.33 - 5.55
	after	4.1±0.04	4.5±0.02	
ALT activity, mmol/l	before	0.43±0.01	0.37±0.01	0.3 - 1.2
	after	**0.52±0.02**	**0.39±0.01***	
AST activity, mmol/l	before	0.81±0.01	0.89±0.02	0.6 - 2.1
	after	0.84±0.01	0.85±0.01	
Creatinine, µmol/l	before	130.18±1.25	132.49±0.95	100 - 200
	after	145.26±0.9	141.73±1.2	
ALP activity, units/l	before	90±0.87	100±1.1	30 - 150
	after	**50±0.5**	**140±1.09****	

*Trend; ** significant difference, $p<0.05$.

A slightly higher level of total protein and a lower content of urea in the blood serum of piglets of the C group was observed. The level of cholesterol and glucose in the blood serum of piglets of the C and E groups remained almost at the same level before and after our experiment. The activity of ALT in the piglet's blood serum from E group was 25% lower than that of animals of the C group. The results of laboratory studies revealed a 2.8 times higher activity of ALP in piglets of the E group.

All other biochemical indicators of blood serum of piglets of both groups were within the physiological norm. Therefore, we can state the fact that the animals received a sufficient amount of protein with well-digested feed.

The analysis of the microelement content in the blood serum of piglets from the E and C groups let us to establish that their level before the experiment and after its end remained within the physiological norm (table 2).

Table 2. The content of inorganic elements in blood serum samples of pigs

Parameters	Period	C (n=7)	E (n=12)	Reference level
Zinc, µg	before	130.93±2.1	123.84±1.96	100.0 - 160.0
	after	126.11±1.9	130.96±2.03	
Copper, µg	before	172.72±2.05	189.81±1.07	100.0 - 212.0
	after	**153.47±1.84**	**209.50±3.5****	
Manganese, µg	before	4.90±**0.08**	5.20±0.1	4.0 - 6.0
	after	4.70±0.1	5.00±0.09	
Iron, µg	before	158.87±3.1	162.86±4.02	160.0 - 200.0
	after	**178.18±2.8**	**193.53±2.65***	

*Trend; ** significant difference, $p<0.05$.

It was found that in the blood serum of piglets of the E group, the content of copper (by 26.7%) and iron (by 7.9%) was higher than in animals of the C group.

An important role in the bioavailability of microelements plays molecular form of the mineral and its valence in the diet. These specific characteristics of the mineral responsible for the complexes it forms with other components in the gut, which may effect mucosal absorption, transport, metabolism of the mineral in tissues (Mantovani, 2017).

CONCLUSION

The positive effect after using of biogenic metals (iron, zinc, copper, manganese) in the form of a feed additive on the content of inorganic substances in the blood of piglets at weaning has been proven. It was established that in the blood serum of piglets of the experimental group there was more copper by 26.7% and iron by 7.9%, compared to animals of the control group. In addition, the use of biogenic metals in the diet of piglets after weaning did not have a significant effect on the biochemical parameters of the blood, which remained within the physiological norm throughout the experiment.

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POSSIBILITIES OF USING FEED ADITIVES IN PREVENTION AND CARE OF CALF HEALTH

DENISA POLÍVKOVÁ, LUBOŠ ZÁBRANSKÝ, MILOSLAV ŠOCH, MARTIN KANTOR, ANNA ŠEBKOVÁ, VOJTĚCH BRABENEC

Faculty of Agriculture and Technology, University of South Bohemia in České Budějovice, Studentská 787/13, 370 05 České Budějovice

Corresponding email address: polivd00@fzt.jcu.cz

ABSTRACT

This work consists in the implementation of an experiment based on the administration of various probiotic feed preparations to calves during the period of colostrum nutrition. Subsequent collection of blood samples, their evaluation and comparison in relation to individual probiotic preparations. Evaluation of the effect of preparations on health status, frequency of occurrence of diarrheal diseases and microbial profile of faeces. Based on the resulting values of the Brix correlation coefficients, it can be said that this is indeed a positive correlation. However, the dependence can be characterized as weak, considering the significance test of the correlation coefficient, it can be said at the same time that the statistical significance is inconclusive. However, it was proven that both groups of probiotic feed additives were able to reduce the incidence of diarrheal diseases. On the basis of the mentioned results from the hematological and biochemical analysis carried out through various tests, it can be stated that the blood count for the experimental group B and BEL does not differ from the control group. As part of the microbiological analysis of the droppings, statistical processing by ANOVA analysis did not show a conclusive, statistically significant difference in the occurrence of pathogenic microorganisms. Based on the evaluation of the data, it can be concluded that the probiotic feed additives had no demonstrable effect on the weight gain of the calves or on the blood parameters of the monitored experimental groups.

Keywords: nutrition; probiotic; cattle; hematology

INTRODUCTION

Calves are a very sensitive category in dairy farm systems. Since they are born devoid of their own defense substances and do not have a developed immune system like adult animals, they are exposed to a high infectious pressure from the external environment from birth. For the correct development of its own passive immunity, the newborn calf is dependent on receiving antibodies in the form of immunoglobulins, which it only receives together with the first infusion of colostrum (Blum 2006). The necessity to receive immunoglobulins immediately after birth is due to the type of bovine placenta, which does not allow the passage of immunoglobulins to the fetus during uterine development. Due to the increasing impermeability of the intestinal wall to immunoglobulins, colostrum must always be provided to the calf in time and of sufficient quality (Conneely et al., 2014; Strapák et al., 2013). In the early postnatal period, diarrheal diseases are the most significant rearing problem. The occurrence of diarrheal diseases leads to clear direct and indirect economic losses in farms. They manifest themselves not only in the deaths of the animals themselves, but also in the reduction of weight gain, the increase in the costs of veterinary care, treatment, prevention and selection of animals. Probiotics, prebiotics or synbiotics are promising helpers in solving, and above all preventing the occurrence of diarrheal diseases. Thanks to their mechanism of action, it is possible to develop and stabilize the intestinal microflora in newborn calves in the shortest possible time, which stimulates the development of local intestinal immunity and positively affects the health of the host (Illek 2007; Muktar et al., 2015).

MATERIAL AND METHODS

To implement the project, 2 groups of calves were assembled, while each group was further divided into an experimental group and a control group. The first group was given probiotics containing the strain *Bifidobacterium bifidum* (B) and is represented by a total of 70 calves, equally divided into control and experimental groups. The second group was given probiotics containing a mixture of *Bifidobacterium bifidum,*

Enterococcus faecalis and Lactobacillus sporogenes (BEL). This group is also equally divided into control and experimental groups.

All calves included in the experiment received the same care. After leaving the calf with its mother for a short time, but no later than two hours after birth, it was moved to a clean, disinfected outdoor box with straw bedding and weighed. Within two hours after calving, the calf was given its first drink of colostrum, fresh, but more often frozen, with a volume of at least 2.5 liters. The quality of freshly obtained colostrum was always carefully measured with a refractometer. Calves in experimental group B were given a probiotic preparation containing 3 g of Bifidobacterium bifidum before the first feeding with colostrum and subsequently for 21 days, always in the morning feeding. In the experimental group BEL, calves were given a probiotic mixture until the first feeding and then for 5 days until life in a dose of 3 g, always before the morning feeding**.**

Between the third and fifth days, blood was taken from the jugular vein of the calves, in order to obtain samples for checking the level of immunoglobulins in the blood plasma, as well as for the laboratory determination of the blood count test. Farm diagnostics consists in centrifugation of the blood at 2000 whirls per minute and subsequent reading of the total protein level from the blood plasma, with the help of a digital refractometer. In order to determine the blood count test is also collected between the third and fifth day from the jugular vein into a set of tubes containing the anticoagulant sodium EDTA and sodium fluoride and a second set of tubes containing Heparin. Immediately after collection, the blood samples were mixed with anticoagulants, placed in a cooling box and transported to the laboratory for processing. Biochemical analysis was performed on an Ellipse Dialab device, and a blood count was performed on an Exigo LABtechnik device.

An important aspect of the experiment was monitoring the weight and weight gain of the calves. They were weighed when they were moved from the farrowing box to an outdoor individual box and when they were moved to group pens in the calf house at the age of approximately 150

days. A two-wheeled cart with built-in tensometric scales is used. Fecal samples were also taken from each calf for microbiological analysis, always on the 5th and 56th day of life for the analysis of nitrogenous substances. Samples were collected from the rectum, at a depth of approximately 5 cm, with sterilized cotton swabs and placed in a plastic, sterilized tube with a cap. Immediately after collection, the samples were stored in a refrigerator with a constant remaining temperature of 4 °C and transported to the laboratory. A modern statistical approach [2] using principal component analysis was used to assess the difference in measured values between experimental groups and groups, which transforms multivariate observations into a set of linearly uncorrelated variables, which are subsequently assessed using other methods, specifically, they are used to test the difference MANOVA, t - test, the Mann-Whitney nonparametric U test (M-W), and modified variants of the t-test and M-W.

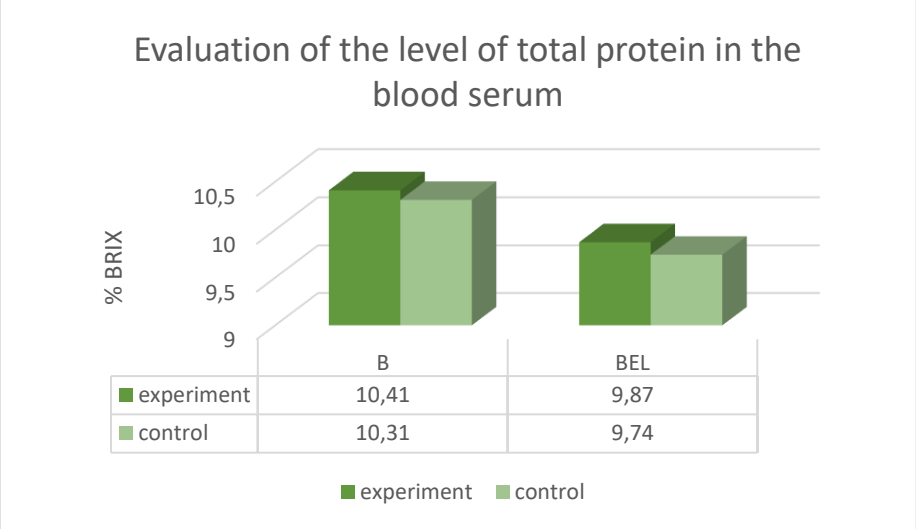
This approach has the advantage that it automatically identifies the difference it explains, and is not dependent on the distributional properties of the analyzed samples. Furthermore, in order to assess the linear relationship between the Brix value and weight gain over the observed period, the test for the significance of the correlation coefficient was used, together with its non-parametric variant (Speraman's correlation coefficient). All statistical tests are interpreted at a significance level of 0.05, i.e. with 95% confidence. Numerical calculations were performed in the programming environment R ver. 4.0.1

RESULTS AND DISCUSSION

Evaluation of the level of total protein in the blood serum

According to Deelen et al., (2014), physiologically normal values are 8.4 or 8.3% Brix. On average, all groups significantly exceeded the given value limits. Gaspers et al., (2014) in their study mention a negative correlation between serum Immunoglobulin G and birth weight of calves. As a result, calves with a higher birth weight may have lower serum immunoglobulin G concentrations than calves with a lower birth weight during the first 24 hours after birth**.**

Figure1. Evaluation of the level of total protein in the blookd serum



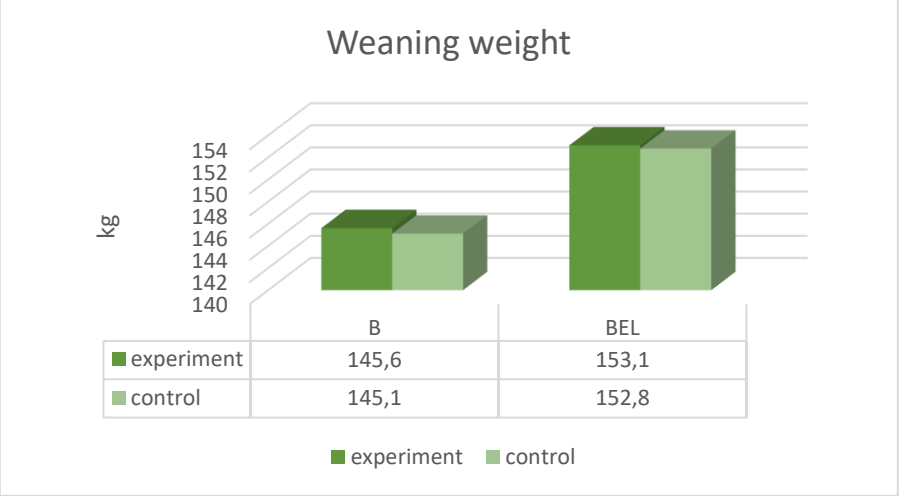
Growth assessment

According to Alaweneh et al., (2020) there are enough studies and evidence, thanks to which it is possible to state that after supplementation with probiotics there was a more or less significant improvement in calf performance parameters. Also in this experiment, both experimental groups outperform the experimental group on average. In their study, Liu

et al., (2022) also demonstrates a positive effect on the intestinal microbiome and the subsequent performance of calves.

Based on the resulting values of the correlation coefficients, it can be said that this is indeed a positive correlation (with increasing Brix values, the increment increases). However, the dependence can be characterized as very weak, moreover, with regard to the significance test of the correlation coefficient, it can be said at the same time that the statistical significance is again inconclusive (i.e. it is not statistically significantly different from linear independence H0: r=0 vs. HA: r 0). In this case, the monitored variables (Brix and weight gain) can be considered linearly independent.

Figure 2. Weaning weight

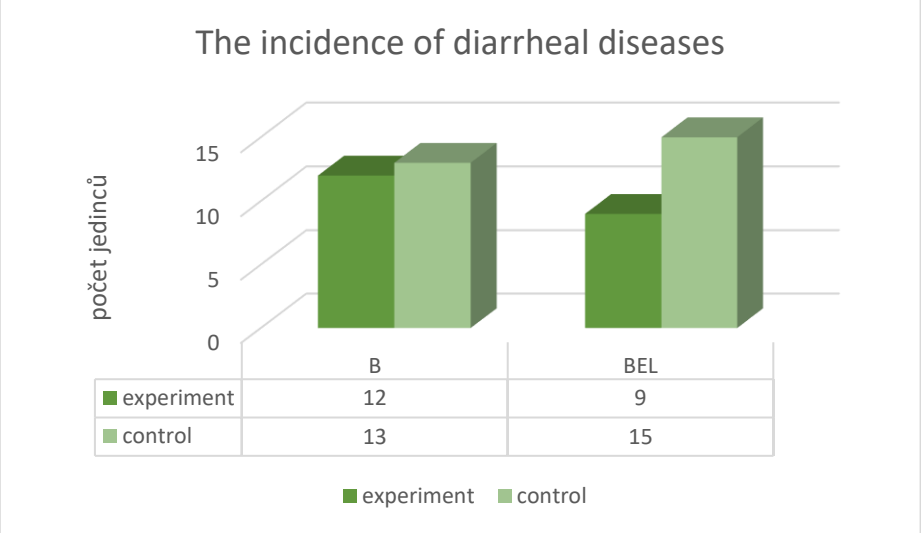


Health assessment

The evaluation of the health status of the calves included in the experiment was based on counting the incidence of diarrheal diseases and monitoring their progress. It can be seen from the graph that the feeding of probiotic preparations had an effect on the incidence of diarrheal diseases, especially when a combination of probiotic strains wasincluded. Liu et al., (2022) and Wu et al., (2021) also agree with the

statement about the positive effect of probiotics in their studies. However, Alawneh et al., (2020) are skeptical of this claim in their study and recommend paying increased attention to hematological parameters and the mechanimechanism of rumen development.

Figure 3. The incidence of diarrheal diseases



Bifidobacterium bifidum

On the basis of the above results performed through various tests (MANOVA on 4 principal components, t-test for the first principal component and Mann-Whitney test on the first principal component, or possibly their adjusted versions (t-test, Mann-Whitney test)) to say that the blood count for the experimental group does not differ from the control group in the case of the first measurement. The obtained significance values, i.e. p-value, are 0.894223 (MANOVA), 0.6749672 (t-test), 0.8430127 (M-W), and for the adjusted versions then 0.68079 (t-test) and 0. 8502857 (M-W).

Mixture of probiotics BEL

On the basis of the above results performed through various tests (MANOVA on 4 principal components, t-test for the first principal component and Mann-Whitney test on the first principal component, or

possibly their adjusted versions (t-test, Mann-Whitney test)) to say that the blood count for the experimental group does not differ from the control group in the case of the first measurement. The obtained significance values, i.e. p-value, are 0.344334 (MANOVA), 0.4165041 (t-test), 0.2815568 (M-W), and for the adjusted versions then 0.4919547 (t-test) and 0. 3325613 (M-W).

Microbiological analysis of feces

The types of microorganisms found in the samples were: *Escherichia coli, Escherichia fergusonii, Proteus mirabilis, Proteus vulgaris, Morganella morganii, Campylobacter jejuni, Klebsiella pneumoniae, Citrobacter freundii, Citrobacter kooseri, Citrobacter braakii, Citrobacter amalonaticus, Providencia stuartii, Enterobacter kobei*. The occurrence of all microorganisms decreased with age and there were no statistically significant differences between them. The most represented species was *E. coli*, which occurred in all calves of the monitored groups. According to Martin et al. (2003) and Nagy and Fekete (2005), E. coli is still considered to be the main infectious disease causing neonatal diarrhea in calves. Compared to the author of Luginbühl et al. (2005) who states that *E. coli* is less important in the monitored populations compared to Cryptosporidiosis and rotavirus infection. Younis et al. (2009) in their study found less *E. coli* infection in calves that received colostrum directly from their mothers compared to hand-fed calves.

The second most frequently found species was *Campylobacter jejuni*, which also occurred in abundance in all groups in the observed groups of calves. Klein et al. (2013) in their experiment found the occurrence of *C. jejuni* in the majority of monitored individuals without dependence on the occurrence of diarrhea. He states that calves act more like a reservoir and can therefore infect other animals, or humans. Besser et al. (2005) reported a high incidence of *C. jejuni* in calves under 4 months of age. It is believed that the occurrence is promoted by transmission between the calves themselves.

The species *Citrobacter spp*. and *Klebsiella pneumoniae* was also present in increased numbers. Windeyer et al. (2014) reported that these bacteria cause serious problems in calf rearing, e.g. neonatal septicemia, which is the cause of serious illness and death of calves. Fecteau et al. (2009) states that infections are caused by the faecal-oral route and often during the first days after birth. He also states that the transmission of infection is facilitated by the failure of passive immunity in calves.

For the other microrganisms found, only a rare occurrence was recorded and they had no influence on the results of the experiment. Statistical processing by ANOVA analysis did not show a statistically significant difference between the experimental groups and the control with a value of p=0.167.

CONCLUSION

Taking into account the processed results as part of the evaluation of the correlation between the BRIX level and weight gains, it can be stated that this is a positive correlation. As the BRIX values increase, so does the weight gain of the calves. Based on this finding, it is possible to confirm the necessity of providing high-quality colostrum in the optimal amount and time interval. The results of monitoring the effects of probiotics administered to calves both in a mixture containing strains of *Bifidobacterium bifidum, Enterococcus faecium and Lactobacillus sporogenes*, or as a separate strain of *Bifidobacterium bifidum* do not differ in any way when monitoring the hematological profile. As part of the evaluation of the occurrence of all microorganisms, it was confirmed that their number decreased along with age and there were no statistically significant differences between them. However, the sampling was mainly carried out to check the health status of the calves.

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EFFECT OF ADDING HERBAL PLANTS TO PIGEON DIETS ON GROWTH, PRO-OXIDANT STATUS AND BLOOD PARAMETERS

ANDREJ RÉCKY, ŠIMON HALÁS, JULIANA ARVAIOVÁ, LENKA BOSÁKOVÁ, FRANTIŠEK ZIGO, ZUZANA FARKAŠOVÁ, ZUZANA LACKOVÁ

Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, 040 01 Košice, Slovakia

Corresponding email address: andrejrecky@gmail.sk

ABSTRACT

This study aimed to evaluate the effect of adding *Origanum majorana* (OM) powder to domestic pigeon diets on growth performance; blood hematology; blood biochemical parameters; blood inflammatory and oxidative markers; carcass characteristics. Fifty-four unsexed pigeon squabs (average body weight 321g and 30 days old) were randomly divided into three groups. The first group was fed the grower basal diet without adding OM powder, while it was added at the levels of 0.5 and 1% into the basal diets of the 2nd and 3rd groups, respectively. Obtained result show that, there are significant increase in globulin level, and glutathione peroxidase enzyme, While heterophils, heterophil to lymphocyte ratio, malondialdehyde level was significantly (P<0.05) reduced. Moreover, blood examination showed positive responses to OM powder as red blood cells, hemoglobin, hematocrit, mean corpuscular volume, white blood cells, and lymphocytes. These results indicated that adding OM powder to the pigeon diet may improve their immunity, increase their antioxidant status, and correct some hematological disorders.

Keywords: *Origanum majorana* powder; performance; antioxidant; malondialdehydehematology

INTRODUCTION

Compared to other animal outputs, poultry production is distinguished by reduced costs, greater feed conversion, and fewer associated environmental and health issues (Daghir et al., 2021; Puvaca et al., 2022). It was crucial for researchers to look into new feed additives and incorporate them into chicken diets in order to improve the health, growth performance, and immune response of poultry. The use of herbal plants and their extracts as growth promoters, antioxidant and immunological modulators in place of antibiotics, which have negative effects on poultry, is seen to be a promising additive in chicken production (Seidavi et al., 2021; Sakr et al., 2022). *Origanum majorana* (OM), also known as sweet marjoram, is aaromatic, medicinal, creeping herb plant (Cala et al., 2021) that belongs to the *Lamiaceae* family and is widely used in North Africa and Western Asia (Yen et al., 2021). Because of its high content of flavonoids, phenolic compounds, and essential oils containing pinene, terpinene, borneol, sabinene, and terpineol.*Origanummajorana* has antibacterial, antioxidant, immune modulator, antifungal, analgesic, antiseptic, and metabolism properties (Banchio et al., 2008; Bina et al., 2017; Kordali et al., 2022). Additionally, using of OM extract may guard against liver and kidney damage (Yen et al., 2021) and hyperlipidemia (El-Ghany et al., 2010).

In study Ahmed et al. (2009) were broilers fed diets supplemented with probiotics, prebiotics, or herbal blends (*Carum carvi*, *Origanum majorana*, and *Foeniculum vulgare*) as antibiotic alternatives. The groups supplemented of herbal blend outperformed the others.Additionally, when Saleh et al. (2021) supplemented the diets of laying hens with OM and another medicinal herbal plant, they observed an improvement in the hens' utility performance, including the the quality and quantity of their eggs with feed conversion ratio. Domestic pigeons are frequently sold in Egypt due to its extremely marketable and delectable flesh, which suggests that it has a good nutritional value, easy maintenance and raising, and quick weight gain (Salem et al., 2022). To our knowledge, there haven't been many research done to assess the OM powder's impacts on the pigeon diet's nutritional, behavioral, antioxidant, and immunomodulatory components.

In this study, we hypothesize that adding OM powder to pigeon diets may modulate their growth performance, immune response, and antioxidant status.Thus, the effect of adding OM powder to the pigeon diet on fattening, feeding and drinking behavior, blood biochemical and hematology parameters, inflammatory and antioxidant markers was investigated in this study.

MATERIAL AND METHODS

The experiment was carried out in the research animal house belonging to the Faculty of Veterinary Medicine, Assiut University, Egypt. Ethical bird management and treatment were followed according to the guides by the Animal Care Committee of Assiut University, Egypt. 6102017Yahya.

Origanum majorana Powder

Origanum majorana (OM) powder was obtained from a commercial source (Organic, Natural Oil Factory, Assiut, Egypt), organized, and analyzed in accordance with the AOAC (AOAC., 2012) strategies. Herein, 95.5% dry matter (DM, AOAC official method 930.15), 17.5% crude fiber (CF, AOAC method 978.10), 14% crude protein (CP, AOAC official method 984.13), 10.3% ash (AOAC official method 942.05) and 3.3% ether extract (EE, AOAC official method 920.39) made up the chemical composition.Metabolizable energy (ME) (2712 Kcal/Kg diet) was calculated using NRC (15's chemical composition). Furthermore, the active principles of OM powder were examined in the Chemistry Lab at Assiut University's Faculty of Science in Assiut. The active principles included thymol (4.2%), terpineol contents (alpha-terpinene 6.8%, gamma-terpinene 5.5%), alpha-terpineol 3.6%, alpha-phellandrene 2.07% andalpha-terpinolene 1.5%, (1-Pyrrolyl) phenol 1.7%, caryophyllene 1.5%, carene 1.1%, fluoro-5-(1-hydroxy-2-(methylamino)ethyl) phenol 0.2%, aminopropyl phenol 0.09%, 5-methyl phenol 0.14%, and cathine 0.02%. Previous studies (Ahmed et al., 2009; Saleh et al.,2021) were used to determine the amount of OM powder to include in the diets.

Birds, Diets, and Experimental Design

In the practical part of the study, 54 unsexed pigeons squabs (30 days old, average body weight; 321g) were included anddivided into three groups (n=18), with three replicates of 6 birds per replicate. The first group was fed the grower basal diet without supplementing it with OM powder, whereas the second and third groups' basal diets were supplemented with it at 0.5 and 1%, respectively. The diet was in the form of mash. Table 1 shows the ingredients and chemical composition of the grower basal diet. The temperature was adjusted to meet the needs of the birds (18-23 °C). There was both natural and mechanical ventilation. There was free access to both water and feed.

Table 1. Ingredient and chemical composition of the grower basal diet of pigeon squabs.

Item	**Control diet***
Ingredient, % DM	
Yellow corn	75.94
Soybean meal	19.72
Supplement	4.34
Chemical composition	
CP, % DM	16.0
CF, % DM	2.88
EE, % DM	2.97
Available Ph, % DM	0.40
Ca, % DM	1.20
Lysine, % DM	0.80
Methionine, % DM	0.30
ME, Kcal/Kg diet[1]	2988

*Grower basal diet was fed to the three groups of pigeons with supplementation of Marjoram powder at the level of 0.5% and 1% to the 2nd and 3rd groups of pigeons. [1]ME - Metabolizable energy was calculated using ME of the ingredients according to NRC (1994).

Growth Performance Parameters

Each bird's body weight was recorded at the start of the experiment. Following that, pigeon squabs' individual body weight, cumulative body weight, and feed intake were all recorded weekly. The feed conversion ratio (FCR) was calculated using the body weight gains and feed

consumption. The relative growth rate (RGR) and European production efficiency index (EPI) were calculated according to studies Marcu et al. (2013) and Fattah et al. (2019), respectively.

Carcass Characteristics and Blood Examination

The experiment lasted 45 days. At the experimental end were randomly selected three birds from each group, weighed, and euthanized by slaughtering. During slaughtering, blood samples were taken from the *cervical vein*. Heparinized and non-heparinized tubes (Vacutainer, Becton Dickinson, USA) were used to store the blood.

Blood Hematology

The tubes containing heparin were utilized for assessing the number of red blood cells (RBCs), white blood cells (WBCs), concentration of hemoglobin (Hb) in the blood, average size of red blood cells (MCV), concentration of hemoglobin in each red blood cell (MCH), volume of packed red blood cells (HCT), and the breakdown of different types of white blood cells. The ratio of heterophils to lymphocytes was calculated. RBCs and WBCs were counted with a hemocytometer, and blood smears were stained with the Wright-Giemsa stain.

Blood Biochemical Parameters

The blood samples in the remaining tubes were spun (for 15 min, at 3000 rpm, 4 °C) and stored at -20 °C until additional examination. The levels of complete proteins, albumin, globulin, overall cholesterol, urea, and creatinine were measured utilizing commercially available kits (Egyptian Company for Biotechnology, Cairo, Egypt).

Serum Inflammatory and Oxidative Markers

TNF-α and interleukin 6 (IL6) were measured for inflammation using an ELISA Kit for chicken (Egyptian Company for Biotechnology, Cairo, Egypt). The levels of glutathione peroxidase (GPx) and malondialdehyde (MDA) as anti-oxidative markers were determined using commercial colorimetric kits (Egyptian Company for Biotechnology, Cairo, Egypt) and a spectrophotometer (Unico UV 2000; Spectra Lab Scientific Inc., VA, USA).

Statistical analysis

Statistical analysis of the effect of OM on fattening, carcass characteristics, behavior, blood biochemistry and hematology parameters, and anti-oxidative markers in pigeons was performed using the Duncan's Multiple Range Test (Statistical Package for Social Science 26.0) for treatment means comparison, in significance level at 5%.

RESULTS AND DISCUSSION

1- Growth Performance Parameters

Table (2) show that, there are non-significant effect of *Origanummajorana* powder's using in pigeon squab diet on growth performance parameters (body weight, weight gain, feed intake, feed conversion, production index, or relative growth rate)this data agreed with previouslyobtained data of Khattab et al. (2018).

Table 2. The effect of incorporating *Origanum majorana* powder into the diet of pigeons on growth performance parameters

Item	Groups*			SEM	P-value
	Control	0.5M	1M		
Initial BW, g	322	321	324	10.3	0.98
Final BW, g	424	438	439	13.8	0.68
BWG, g	102	115	117	10.5	0.57
Total FI, g	1178	1207	1237	41.0	0.62
FCR, g/g	4.7	5.8	5.2	0.87	0.73
EPI	12.8	12.5	12.2	1.97	0.98
RGR	17.5	19.2	19.1	2.34	0.85

Means within the same row with different superscripts differ significantly ($P < 0.05$).*Groups: Pigeons fed only basal diet - control group, 0.5M - pigeons fed with addition of Marjoram powder at the level of 0.5%, 1.0M - pigeons fed with addition of Marjoram powder at the level of 1%, BW, Bodyweight; BWG, Bodyweight gain; FCR,

Feed conversion ratio; FI, Feed intake;EPI;European production indexRGR; Relative growth rate.

2. Hematological Parameters

The impact of the powder on blood hematology is presented in Table 3. With OM powder, red blood cells (RBCs), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), white blood cells (WBCs), and lymphocyte percentage all showed elevated values ($p < 0.05$). In contrast, the percentage of heterophils and the ratio of heterophils to lymphocytes were significantly lower ($p < 0.01$).

Origanummajorana powder greatly boosted both red blood cell (with 1% OM) and white blood cell counts, potentially due to thymol (active ingredient in OM), which enhances the immune response (Shad et al., 2016). Meanwhile, the beneficial effect of OM on hemoglobin levels may be attributed to its higher iron content, which is a vital nutrient for the production of hemoglobin (AlGarni et al., 2015).

Table 3. The effect of adding *Origanummajorana* powder to pigeon diet on blood hematology

Item	Groups*			SEM	P-value
	Control	0.5M	1M		
RBCs ($x10^6/mm^3$)	4.27^b	4.43^b	5.37^a	0.145	< 0.01
Hb (g/dl)	11.70^c	12.30^b	14.83^a	0.135	< 0.01
HCT (%)	38.90^c	40.00^b	48.70^a	0.149	< 0.01
MCV (fl)	89.15^c	92.10^b	96.55^a	0.648	< 0.01
MCH (pg)	27.63	27.76	27.96	0.652	0.93
WBCs ($x10^3/mm^3$)	38.33^b	43.33^a	44.00^a	0.981	0.01
Monocyte%	7.00	7.00	8.00	0.577	0.42
Heterophil%	42.33^a	27.33^b	27.33^b	2.769	0.01
Lymphocyte%	45.00^c	73.00^a	65.66^b	1.981	< 0.01
H/L ratio	0.95^a	0.385^b	0.42^b	0.049	< 0.01

Means within the same row with different superscripts differ significantly ($P < 0.05$). *Groups:Pigeons fed only basal diet - control group, 0.5M - pigeons fed with addition of Marjoram powder at the level of 0.5%, 1.0M - pigeons fed with addition of Marjoram powder at the level of 1%;H/L ratio, Heterophil to Lymphocyte ratio; WBCs, White Blood Cells; MCH, Mean Corpuscular Hemoglobin; MCV, Mean Corpuscular Volume; HCT, hematocrit value; Hb,blood hemoglobin; RBCs, red blood cells count.

3. Blood Biochemical Parameters, Serum InflammatoryMarkers, and OxidativeMarkers

Table (4) illustrated that, OM supplements are available. The powder had no effect on serum total protein, albumin, creatine, or urea levels. In contrast, globulin was higher ($p < 0.05$), and the albumin-to-globulin ratio was lower ($p < 0.05$) in the treated groups. This data agreed with finding of Shawky et al. (Shawky et al., 2020) Moreover, hypocholesterolemia data of OM wasagree with (Saleh et al., 2021) and may be due to carvacrol and thymol present in OM could reduce cholesterol levels by inhibiting hepatic 3-hydroxy-3-methyl-glutaril198 CoA reductase (Shad et al., 2016).

Table 4. The effect of adding *Origanum majorana* powder to pigeon diet on blood biochemical parameters as well as serum inflammatory and oxidative markers

Item	Groups*			SEM	P-value
	Control	0.5M	1M		
Total protein, g/dl	5.05	5.37	5.30	0.16	0.37
Albumin, g/dl	3.40	3.23	3.25	0.08	0.34
Globulin, g/dl	1.65^{b}	2.13^{a}	2.05^{a}	0.11	0.04
A/G ratio	2.06^{a}	1.53^{b}	1.59^{b}	0.08	0.01
Cholesterol, mg/dl	194^{a}	159^{b}	148^{b}	9.46	0.03
Urea, mg/dl	37.1	34.1	37.7	2.07	0.47
Creatinine, mg/ dl	0.39	0.48	0.43	0.02	0.18
Interleukin 6 ng/l	273	284	279	23.2	0.95
TNFα Pg/ml	222^{b}	230^{a}	236^{a}	6.90	0.09
MDA nmol/ml	8.95^{a}	6.17^{b}	4.50^{c}	0.32	< 0.01
GPx mu/ml	38.5^{c}	102^{b}	124^{a}	5.57	< 0.01

Means within the same row with different superscripts differ significantly ($P < 0.05$). *Groups:Pigeons fed only basal diet - control group, 0.5M - pigeons fed with addition of Marjoram powder at the level of 0.5%, 1.0M - pigeons fed with addition of Marjoram powder at the level of 1%;GPx, Glutathione Peroxidase; MDA, Malondialdehyde; TNFα, Tumor Necrosis Factor α; A/G ratio, Albumin/Globulin ratio.

Finally, Interleukin 6 remained unaffected, while tumor necrosis factor appeared to be ($p = 0.09$) elevated by adding OM powder. Furthermore, serum anti-oxidative markers, specifically glutathione peroxidase enzyme and malondialdehyde, increased and decreased significantly,

respectively. Substances that stimulate leukocytosis may induce cytokine secretion from these cells, including interleukin 6 and TNF (Sevimli et al., 2013). Thus, the tendency for TNF to increase may be a compensatory response to leukocytosis. In contrast to our findings, Arranz et al. (2015) proposed that the essential oil derived from OM has anti-inflammatory properties because it contains terpineol and sabinene hydrate, both of which have a negative impact on cytokine production.

In the present investigation, OM powder significantly enhanced the GPxlevel and reduced the MDA level. The relationship between the abundance of phenolics and flavonoids in OM (such as carnosic acid, carnosol, and hydroxycinnamic acid) and its antioxidant activity has been studied (Yen et al., 2021). Consequently, OM can have a crucialfunction in upholding the regular physiology, health, production and welfare ofanimals.

CONCLUSION

Supplementation of OM powder into the grower diet for pigeons increased the body weight, serum globulin level, lymphocyte and WBCs count. These findings indicate that OM powder could potentially boost bird immune system. The elevated levels of Hb, HCT, and MCV suggest its potential to promote blood cell production. The reduction in H/L ratio, MDA, and increase in GPx levels suggest that OM powder may possess antioxidant properties. To summarize, incorporating OM powder into pigeon diet could be crucial in reducing stress, addressing certain blood-related issues, and maintaining overall bird health and well-being. However, further research is still needed to fully understand its effects.

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NUTRITIVE EVALUATION OF SELECTED VARIETIES OF SORGHUM GROWN IN DIFFERENT SOIL CONDITIONS

MICHAL ŘIHÁČEK[1], JAKUB NOVOTNÝ[1], LUCIE HORÁKOVÁ[1], DANA ZÁLEŠÁKOVÁ[1], EVA MRKVICOVÁ[1], LEOŠ PAVLATA[1], LENKA PORČOVÁ[2], MICHAL RÁBEK[2]

[1]Department of Animal Nutrition and Forage Production, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

[2]Department of Agrosystems and Bioclimatology, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: xrihace2@mendelu.cz

ABSTRACT

The aim of this study was to evaluate selected sorghum varieties and their nutritional potential in relation to different types of soil locations. The comparison was done at the Field Experimental Station in Žabčice at two different locations Obora (clay loam soil – fluvial soil) and Písky (light sandy soil). Ten sorghum varieties were analyzed for basic laboratory parameters – ash, crude protein, acid detergent fibre (ADF), neutral detergent fibre (NDF) and digestibility of dry matter (DMD) and organic matter (OMD). The highest ash content was achieved for the variety Pamapa Triunfo XLT BMR (8.37%) at the Písky location. The highest crude protein content was recorded for the variety Buffalo Grain BMR at the locations Obora (10.63%) and Písky (10.28%). In the content of NDF the best results showed KWS TARZAN (65.97%) variety at the location Písky and KWS KALLISTO (60.77%) at the location Obora. Also, in the content of acid-detergent fibre these varieties achieved higher values, especially KWS TARZAN (44.18%) at Obora and KWS KALLISTO (41.49%) at Písky sites. The DMD values achieved in varieties Buffalo Grain BMR (58.11%), KWS SOLE (46.03%) and Pampa Centurion (64.93%) showed a higher content at the Písky location. For OMD values, the same varieties Buffalo Grain BMR (54.86%), KWS SOLE (41.08%) and Pampa Centurion (62.94%) had

also higher content at the Písky location and the varieties Ruzrok (38.56%), Latte (54.87%) and Pampa Triunfo XLT BMR (59.98%) at the location Obora. The results of this research prove that the differences in selected nutritional parameters between the compared locations are not very high, thus both locations could be considered suitable for sorghum cultivation.

Keywords: biomass; digestibility; climate change; drought; acid detergent fibre; neutral detergent fibre

INTRODUCTION

Sorghum, C4 plant, belongs to one of the most widely grown cereals in the world and mostly thrives in regions characterized by dry and semi-arid climates (Ratnavathi et al. 2012, Vittal et al. 2010). Various plant tissue types among fodder crops determine variances in nutritional value and digestibility (Wilson 1993). The difference in nutritive value is reflected in differences of tissue structure and distribution of leaves, stem, and ears/head, even though forage sorghum and corn have same photosynthehic pathaway (C4). Due to global warming, the possibility of using sorghum as feed, but also as human nutrition for gluten-free diet, interest in growing sorghum is extending. Grain sorghum is usually grown for grain, which is used as food, especially for people who are intolerant to gluten (celiac disease) (Al-Naggar et al. 2018). It can also be used as a forage (green biomass or silage) (Hermuth et al. 2012). The plant has significant adaptation potential to drought, high salinity, high temperatures, these are important characteristics ofgenotypes growing in extreme environments (Tari et al. 2013). The resistance of sorghums to dry conditions is supported by an extensive root system and a waxy layer on the surface of the leaves (Brink et al. 2006). From this point of view, there is a reduction of water loss and the ability to stop growing in times of drought and subsequently resume their growth under appropriate conditions (Brink et al. 2006). An advantageous characteristic of sorghum is its versatility in adapting to various soil types. Sorghum displays greater resilience to shallow soils and drought conditions compared to corn, although it thrives optimally in deep, fertile, and well-drained clay soils. Sorghum is grown on soils with an optimal pH > 6.5

(Butchee et al. 2012). Environmental requirements are quite low, for germination minimal temperature is 12–15 °C and the annual requirement for the sum of temperatures is 2500–3000 °C. The lenght of the vegetation period without frosts requires 120–180 days. Sorghum has the ability to thrive without the need for irrigation in regions where the average annual precipitation ranges from 400 to 700 mm (Hermuth et al. 2012). Seeds of forage sorghum are planted 25–50 mm deep at rates that can be from 5 to 15 kg/ha.

MATERIAL AND METHODS

Characteristic of experimental location

The field experiment took place at the Field Experimental Station (49°00′50.3″N, 16°36′03.6″E) in Žabčice located 179 meter above sea level in maize production area of South Moravia in the Czech Republic. The annual average temperature is 9.2 °C and the thirty-year annual average precipitation is 480 mm. Two habitats were used for the experiment (Obora and Písky), differing in soil quality. While Písky are locations with lighter, sandy and dry soils (chernozem), Obora is a location characterized by heavier soils with higher groundwater levels (fluvisol). All varieties were grown in both localities.

Plant material and trial establishment

A total of 10 varieties of sorghum were used in the experiment (KWS FREYA, KWS KALLISTO, KWS SOLE, KWS TARZAN, Buffalo Grain BMR, Latte, Nutri Honey, Pampa Centurion, Pampa Triunfo XLT BMR, Ruzrok). Sowing material was obtained from companies SEED SERVICE s.r.o. and KWS OSIVA s.r.o. To achieve optimal agronomic sowing conditions, sowing was conducted at both sites in the end of May. The varieties were sown using the pneumatic seeder HALDRUP SP-35 at a depth of 3 cm, with a row spacing of 45 cm and a sowing rate of 245,000 seeds per hectare. The harvest date was determined by the value of the dry matter from samples of plants (28% of dry matter and above) as the important indicator. From each variety, 2 kg of fresh matter was taken. Above-ground biomass from all varieties was harvested and chopped. Samples were chopped (VIKING GE 375), pre-dried in oven Venticell 707 (BMT Medical Technology s.r.o., CZ) at the temperature

65 °C for 24 hours and grinded FRITSCH (Pulverisette, Germany) through a 1 mm sieve.

Analyses of sorghum samples

For all sorghum sorghum varieties were determined qualitative laboratory parameters such as ash, crude protein, acid-detergent fibre (ADF), neutral detergent fibre (NDF) and *in vitro* digestibility of dry matter (DMD) and organic matter (OMD). The analyses were performed according to the relevant standards. According to commision regulation (EC) No. 152/2009 for determination of methods used in sampling and laboratory testing for the official control of feeding stuffs, the analysis are carried out according to current methodics of Central Agricultural Inspection and Testing Institute. Ash (macro-elements and micro-elements) can be determined from the dry matter by incineration at temperature of 550 ± 20 °C to constant weight in a muffle furnace. Crude protein was determined by the Kjeldahl method and then multiplied by factor of 6.25, expressed as nitrogen content. After hydrolysis, the fibre remains in diluted sulfuric acid and diluted lye of potassium hydroxide. After washing of the fibre with an organic solvent and after deducting the value of the ash was determined. The method of Henneberg and Stohmann is used to determine the value. The nitrogen-free extractives are determined by calculation after deducting the values of fat, crude protein, ash and fibre obtained from dry matter. The digestibility of dry matter and organic matter was determined by the pepsin cellulase method *in vitro* using an ANKOM Daisy Analyzer incubator (Ankom Technology, NY) was monitored. The digestibility of dry matter and organic matter by pepsin cellulas method *in vitro* is based on incubation of the sample in acidic pepsin solution, the hydrolysis of starch at elevated temperature and the subsequent incubation in a buffered cellulase solution.

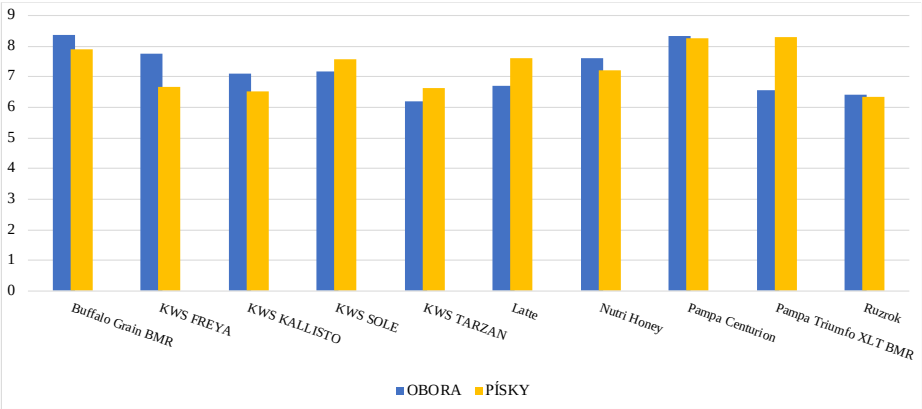
RESULTS AND DISCUSSION

Based on the results obtained from the laboratory analyses, attention was focused mainly on the comparison of selected sorghum varieties in terms of nutrient composition.

Ash

The evaluation of the amount of ash in the selected varieties is shown in Figure 1.

Figure 1. Overview of the results of ash



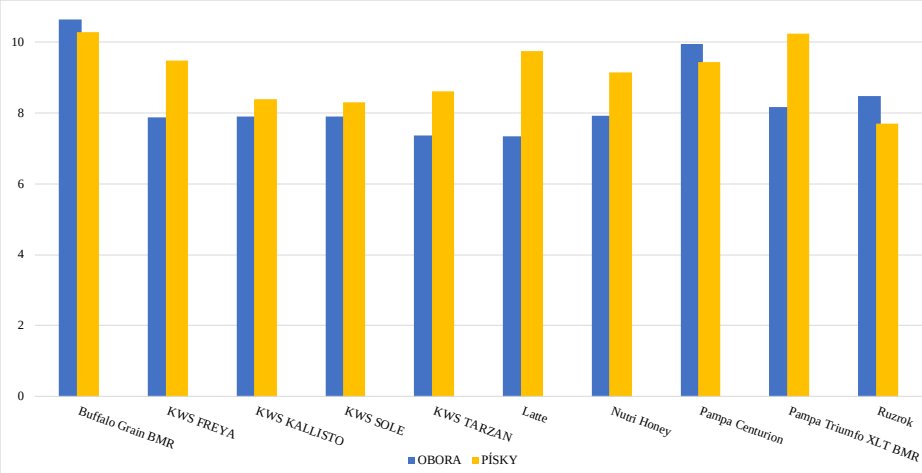
The highest ash content was achieved by the variety Pampa Triunfo XLT BMR (8.29%) at the Písky location. On the other hand, the Bufalo Grain BMR variety (8.37%) had the highest ash content in the second location Obora.

Crude protein

Figure 2 shows achieved values of crude protein in selected varieties of sorghum. The highest content of crude protein was recorded in the Bufalo Grain BMR variety at the Obora (10.63%) and Písky (10.28%) sites among all varieties. This may correspond with the fact that the soil at Písky location is well supplied with nitrogen. However, low values are not a problem, due to crude proteins can be increased in crop by higher fertilization. According to Doležal (2014) range of crude protein in sorghum is from 13% to 18%. This correspond with the study of

Rajčáková (2005), where she determined the range of crude proteins 13.1–18.6%.

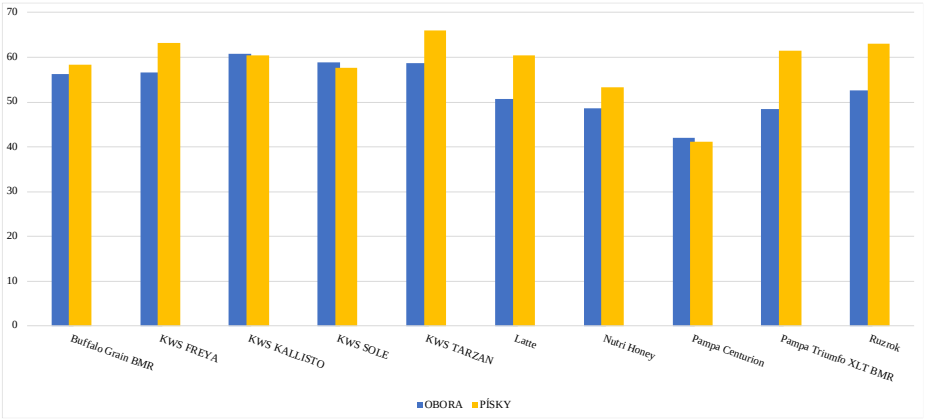
Figure 2. Overview of the results of crude protein



Neutral detergent fibre

In terms of the absolute amount of NDF, the best results are for the KWS TARZAN variety (65.97%) at the Písky site (Figure 3). Within the second site of the Obora, the KWS KALLISTO variety showed a higher amount in NDF content (60.77%). Differences are also visible in the Latte, Ruzrok and Pampa Triunfo XLT BMR varieties, where all these varieties have a higher NDF content from plants grown at the Písky, compared to the Obora. It can therefore be assumed that these varieties, depending on the NDF content, are more suitable for the Písky location-soils that are lighter, sandy and dry. For the other varieties, the differences in values are not very significant in comparison between locations.

Figure 3. Overview of the results of neutral detergent fibre

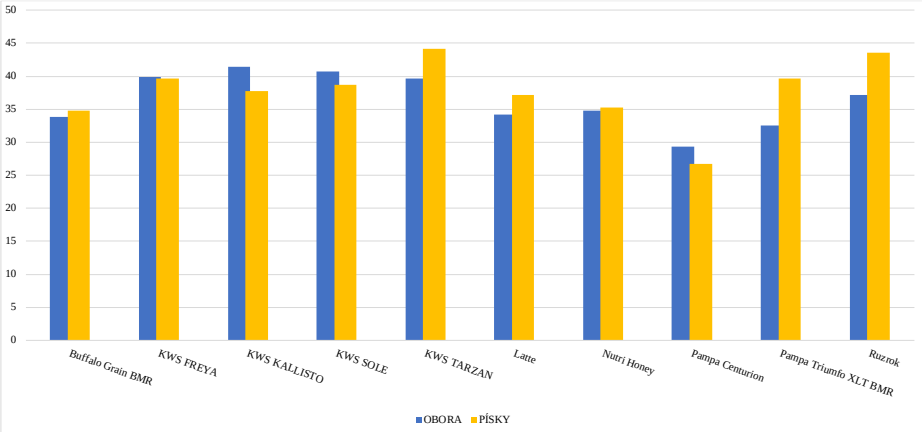


According to Carita et al. (2016) NDF is an indispensable indicator of the digestibility and consumption of the plant by livestock. Better forage quality exhibits low content of NDF and ADF because these indicators are negatively correlated with the ruminants' nutrient intake and food digestibility (Guretzky et al. 2011, Bean et al. 2013). Rajčáková (2005) reports a range of NDF values from 54–55.2%. Baholet et al. (2018) achieved NDF values of 41.72–54.12% for some sorghum varieties. As reported by Fritz et al. (1990) and Grant et al. (1995), BMR sorghum hybrids and Sudan grass hybrids exhibit greater levels of NDF digestion compared to other varieties. Dann et al. (2008) discovered that the NDF content in BMR sorghum silage is higher than of maize silage. Moreover, the *in vitro* digestibility of NDF for BMR sorghum silage was noted to be around 10% higher compared to the maize hybrid. Agricultural practices also have the potential to enhance the nutritional quality of forage sorghum. The spacing between plants within a row has been shown to lead to a reduction in NDF concentration and an increase of 8.7% *in vitro* digestibility. However, it's important to consider that this improvement could be accompanied by a decrease in dry matter yield, which could go as far as a 25.7% reduction (Caravetta et al. 1990).

Acid detergent fibre

The following Figure 4. compares the values of acid-detergent fibre of individual varieties.

Figure 4. Overview of the results of acid detergent fibre

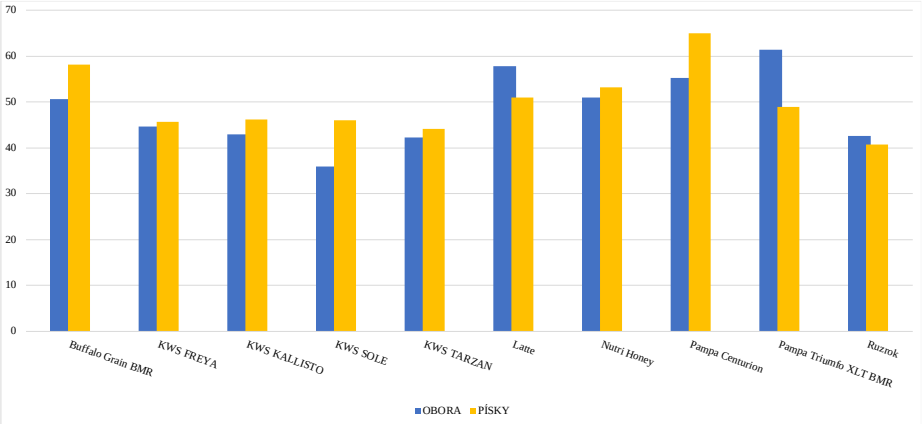


The highest content of acid-detergent fibre was recorded at the Písky location in the variety KWS TARZAN (44.18%). At the Obora location, the highest content of acid-detergent fibre was found in the variety KWS KALLISTO (41.49%). In the comparison of individual varieties, the higher content of acid-detergent fibre was found in the varieties Buffalo Grain BMR (34.79%), Latte (37.13%), Nutri Honey (35.23%), Ruzrok (43.56%) and Pampa Triunfo XLT BMR (39.65%) at the Písky site opposite Obora. According to Přikryl (2014), the differences in nutritional values in the ADF content of sorghum are 30–40% compared to maize with 20–25%. In a study by Schmid et al., (1976) an investigation was carried out to assess the nutritional benefits of silage derived from corn and forage sorghum. The researchers collected samples from eleven corn hybrids and fourteen forage sorghum cultivars, which were then divided into leaves, stems, and ears. On average, corn exhibited a higher proportion of leaves and ears, while the stem content was lower compared to forage sorghum. Higher ADF concentration found in non-bmr forage sorghum in comparison to corn could potentially correlate with reduced digestibility. The rigid composition of stems, containing significant amounts of lignified tissue, restricts their availability for digestion (Akin 1989). According to Cummins (1981) while ADF concentration in forage sorghum leaves increases with maturity, it remains relatively constant in stems or tends to decrease.

Digestibility of dry matter

With regard to the digestibility of dry matter, when comparing the varieties at two different sites, the values of the Buffalo Grain BMR (58.11%), KWS SOLE (46.03%) and Pampa Centurion BMR (64.94%) differ the most, while higher values for given variety were determined for samples from the Písky locality. Conversely, varieties such as Ruzrok (42.57%), Latte (57.83%) and Pampa Triunfo XLT BMR (61.44%) showed higher DMD values in samples from the Obora location.

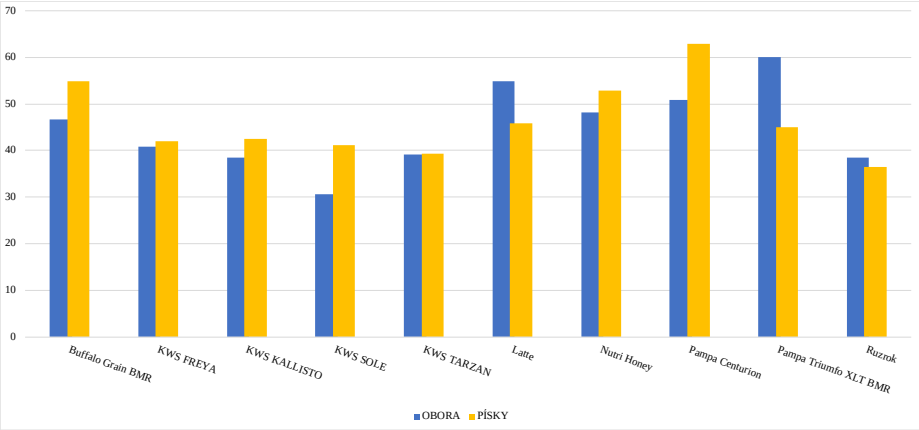
Figure 5. Overview of the results of DMD



Digestibility of organic matter

In vitro organic matter digestibility (OMD) was also evaluated (Figure 6).

Figure 6. Overview of the results of OMD



The results correspond to the results for DMD. The varieties Buffalo Grain BMR (54.86%), KWS SOLE (41.08%) and Pampa Centurion (62.94%) have higher OMD values from the Písky site compared to the same varieties at the Obora site. From the point of view of OMD, the site of Obora is apparently more favorable for the varieties Latte, Ruzrok and Pampa Triunfo XLT BMR. Digestibility of dry matter and organic matter belong to important parameter in animal nutrition. Statistically significant differences were found among tested varieties in the study of Koláčková et al. (2020). The lowest values were observed in KWS KALLISTO (DMD 73.21 ± 1.57%, OMD 70.58 ± 2.12%), Ruzrok (DMD 73.99 ± 3.11%, OMD 71.46 ± 3.71%) and KWS SOLE varieties (DMD 74.13 ± 2.03%, OMD 71.91 ± 2.48%). The highest digestibility was observed in Triumfo BMR (DMD 79.57 ± 0.72%, OMD 77.90 ± 0.64%), Sweet Susana (DMD 82.11 ± 0.94%, OMD 80.62 ± 0.97%) and DSM 45-480 (DMD 86.70 ± 1.22%, OMD 85.67 ± 1.41). The average digestibility of organic matter is in the range of 45–60% (Přikryl 2014). Sriagtula et al. (2017) claims that the digestibility of organic matter was affected by mutant lines of sorghum. In their study BMR sorghum achieved higher digestibility of organic matter (66.59%). Traditional

varieties without BMR mutation contained lower digestibility of organic matter (60.59%).

CONCLUSION

A total of 10 sorghum varieties grown in different soil conditions and their qualitative nutritional parameters were compared. The results of this study show that the differences in the selected nutritional parameters of the individual varieties between the comparative locations are not too high. Each variety thrived differently at the selected habitats. It is therefore possible to state that both localities are suitable for their cultivation.

ACKNOWLEDGEMENT

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IMPORTANCE OF PREBIOTIC, PROBIOTIC AND PHYTOBIOTIC FEED SUPPLEMENTS IN CALF NUTRITION

ANNA ŠEBKOVÁ, LUBOŠ ZÁBRANSKÝ, MILOSLAV ŠOCH, BRABENEC VOJTĚCH, POLÍVKOVÁ DENISA, KANTOR MARTIN

Department of Animal Husbandry Sciences, Faculty of Agriculture and Technology, University of South Bohemia in České Budějovice, Studentská 1668, 370 05 České Budějovice, Czech Republic

Corresponding email address: sebkovaAnna@seznam.cz

ABSTRACT

The work evaluates the frequency of occurrence, course, duration of diarrhea and the subsequent effect on growth in calves fed probiotic feed supplements compared to calves not fed probiotics. During the experiment, calves were divided into three groups. The first group was given RumiForm Digest. The second group was fed RumiForm Digest in combination with a mixture of bacteria and the third group was the control group. Newborns weight in seven days after birth was similar (54 kg) in every of three groups. Significant diferences were registered at the end of these monitoring, in ninety days after birth. First group which consumed RumiForm Digest had average wight 126 kg. Groups which consumed RumiForm Digest with bacteria mixture, had average weight 146 kg. In every monitored groups diarrheal was detected. The biggest frequency was detected at group, which consumed RumiForm Digest with bacteria mixture. There was detected 9% diarrheal diseases in total 48 calfs. Deads were detected only in the group of calfs whitche eat RumiForm Digest with probiotics during the experiment. Total mortality was less than 2%. There wasn´t shown influence of probiotics to calfs growth p-value (calves weight on the 7th day after birth) = 0,63; p-value (calves weight on the 90th day after birth) = 0,31, p-value greater than 0,5), either to calfs health (p-value (comparison of diarrhea rates) = 0,67; p-value (death) = 0,82, p-value greater than 0,5). Can not be said, that the eating of probiotics supplements had positive influence to calfs grow and health.

Keywords: calf; nutrition; probiotics; diarrhea; growth

INTRODUCTION

Only a healthy and resilient individual can provide the required high level of meat or milk production (Rincker et al., 2011). Not only future production but also the level of health quality begins to take shape at the time of rearing immediately after birth (Nowak et al., 2012). Proper and quality management of breeding will ensure a viable and healthy individual that is able to cope with the pathogenic microorganisms to which it is constantly exposed. Such an animal will be able to produce a high quality of production and reproductive performance as an adult (Rincker et al., 2011).

Calves need to be cared for immediately after birth. Cows have a type of placenta (syndesmochorial placenta) that does not allow the transfer of antibodies during intrauterine development (Marvan, 2011). The young are born without immunity. Therefore, the acquisition of the necessary antibodies (immunoglobulins) and the establishment of the own defence (immunological) mechanisms (Illek, 2019). Colostrum or immature milk is the first secretion produced by the mammary gland. It contains many substances, including the aforementioned immunoglobulins, very important for the young (Weaver et al., 2000).

During breeding, calves are constantly exposed to an "infectious" environment. The most common diseases are gastrointestinal problems (Cho and Yoon, 2014; Smulski et al., 2020). Diarrhoea is caused by many factors. One of the factors causing diarrheal diseases are representatives of various pathogenic microorganisms - virus, bacteria or protozoa. Other risk factors, and more common reasons, for diarrhoea in calves include errors caused by the breeder - dietary errors and poor zoohygiene of the breeding stock (Cho et Yoon, 2014; Illek, 2018). Diarrhoea causes not only losses in the form of deaths in breeding farms, but also large economic losses associated with treatment costs (Cho et Yoon, 2014; Katsoulos et al., 2020).

Gaggia et al. (2010) define probiotics as living organisms that can provide health benefits to the host. Reid (2016) defines them as cultures

from one or more strains of microorganisms that can be combined with prebiotics or synbiotics.
The use of probiotics in calf nutrition could be a suitable alternative method for the prevention of diarrhoeal disease and in reducing their progression (Soto et al., 2011). Given the fact that the use of antibiotics as growth promoters is banned, their additional importance could lie in their effect on weight gain (Frizza et al., 2010; Gaggia et al., 2010; Ülger, 2019).
Determine whether feeding probiotic feed supplements makes a difference is the aim of this study.

MATERIAL AND METHODS

The experiment took place on a farm of Holstein-Friesian dairy cattle in the northern Pilsen region. During the experiment, the calves were housed in outdoor individual sheds immediately after birth. Here, they were kept until they had two months of age. After that, the calves were placed in outdoor group shelters from seven to ten calves. They remained in these groups until they were four to five months of age. And then they were transported to the young stock nursery. The calves were fed for the first five days after birth twice a day with 3 to 4 litres. Sixth day after birth, they were fed with native milk twice a day with 4 to 5 litres per feeding There were added a water and a starter ad-libitum. The milk feed mix was fed to them from the 13th day after birth until about three months of age.
Only heifers were included in the experiment. Probiotic feed supplements were fed from the first day to the seventh day after birth.
During the experiment, the subjects were divided into three groups. The first group was fed twice a day a probiotic feed supplement consisting of *Lactobacillus sporogenes*, *Bifidobacterium bifidum* and *Enterococcus faecalis* in the amount of 5 mg and the feed supplement RumiForm Digest (yeast, sorbitol, sodium chloride) - Supplementary feed for calves at a dose of 20 ml for seven days. The second experimental group was fed only the feed supplement RumiForm Digest in a dose of 20 ml twice a day for seven days. The third group control and no probiotic feed supplement was fed to these animals during the experiment.

Each tested subject was placed individually during the experiment to ensure that all subjects received the exact dose.

All tested animals were weighed (using a tape measure). Calves were weighed on the day of birth, at seventh days and on the day of weaning (90 days).

All the experimental animals were given the same treatment during the experiment, between the second and the fifth day. A blood sample (from the vena jugularis) was taken by using of hemos (HEMOS H-02 for cattle with needle). The blood was left for 24 hours and precipitated at room temperature. The level of total protein was determined from the resulting blood serum by using an optical refractometer. The refractometer was calibrated before each use. Further blood collections were made on the day of weaning.

The health status and weight gain of all individuals was monitored and recorded throughout the experiment (from birth to approximately 90 days). For diarrhoeal diseases, a detailed summary (duration, course, colour and consistency of faeces) was recorded. The course of treatment and drugs used were also recorded.

Tested subjects who showed any signs of disease during the study were treated according to the standard farm protocol. This treatment protocol is determined by the veterinarian. The course of treatment was guided by the calf's current state of health.

For diarrhoea, the most frequently used preparations were Lectade Plus (oral rehydration solution containing glucose and a mixture of electrolytes), Duphalyte (rehydration solution with a complex composition, containing vitamins, electrolytes, amino acids and nutritive components), which was applied subcutaneously (s.c.) and in acute cases together with physiological solution NaCl - infusion therapy. In addition, antibiotic and anticoccidial drugs were applied.

Data were processed in GRAPHPAD INSTANT 3. Among the tests, chi-square, Bartlett's test and non-parametric Kruskall-Wallis test were used.

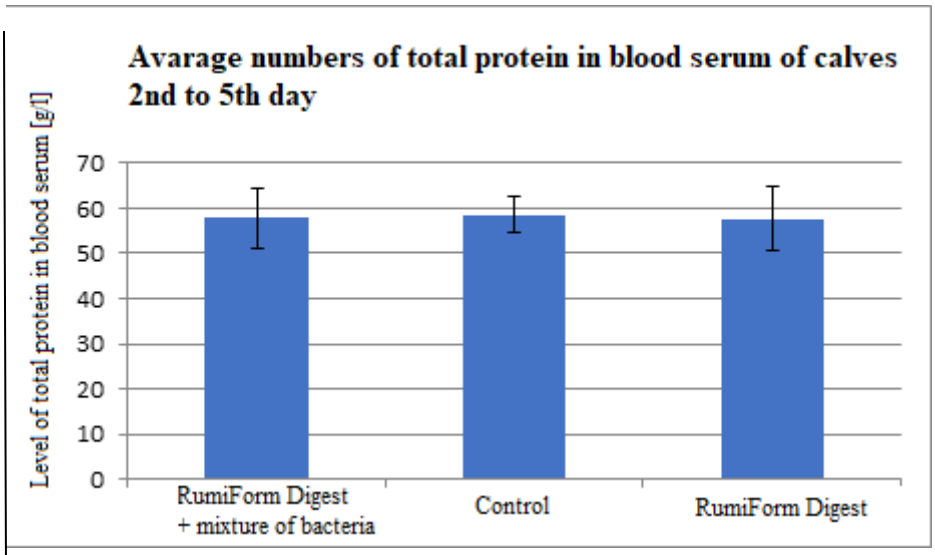
RESULTS

A total of 48 calves were included in the experiment and divided into two experimental groups and one control group.

EVALUATION OF THE LEVEL OF TOTAL PROTEIN IN BLOOD SERUM

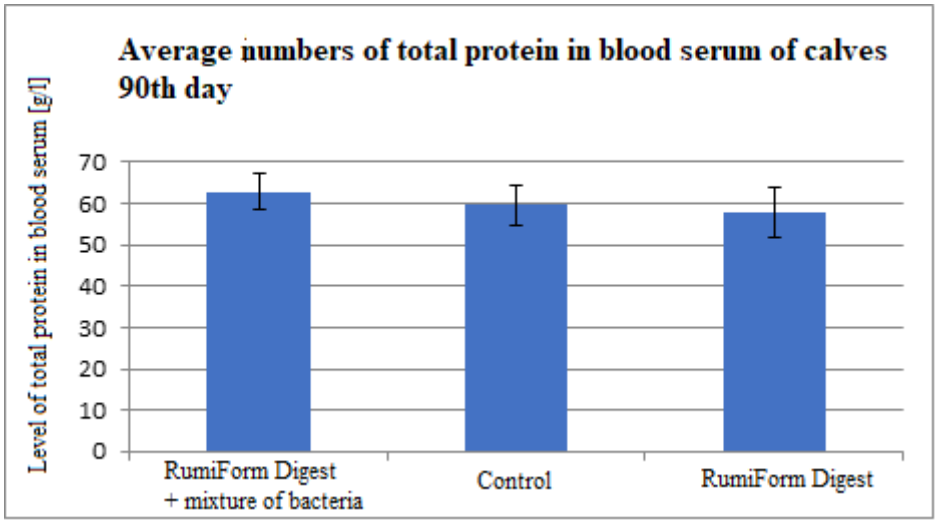
During the experiment, blood was collected from all animals between days 2nd and 5th after birth and on the day of weaning. Then, the total protein level was determined from the blood serum using an optical refractometer.

Graph 1. Average numbers of total protein in blood serum of calves 2nd to 5th day



The average numbers of total proteins in the blood serum (Graph 1) were on the 2nd to 5th day at the same value of approximately 58 g/l. The minimum value found was 45 g/l, the maximum value was 72 g/l. Therefore, the difference based on the parametric Bartlett's test was not significant (N = 16; Bartlett = 4,806; p = 0,0904).

Graph 2. Average numbers of total protein in blood serum of calves 90th day

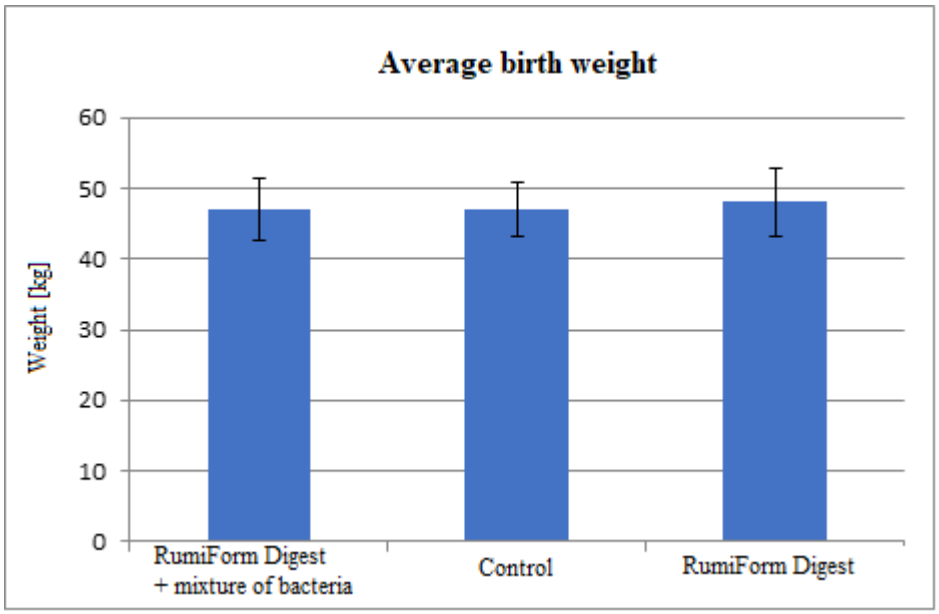


The average numbers of total proteins in blood serum (Graph 2) ranged between 63 g/l in the group given a combination of RumiForm Digest feed supplement and a mixture of bacteria to 58 g/l for the group that was fed only the RumiForm Digest feed supplement. The minimum detected value was 45 g/l, the maximum value was 70 g/l. Therefore, the difference based on the parametric Bartlett's test was not very significant (N = 16; Bartlett = 1,785; p = 0,4096).

GROWTH ASSESSMENT

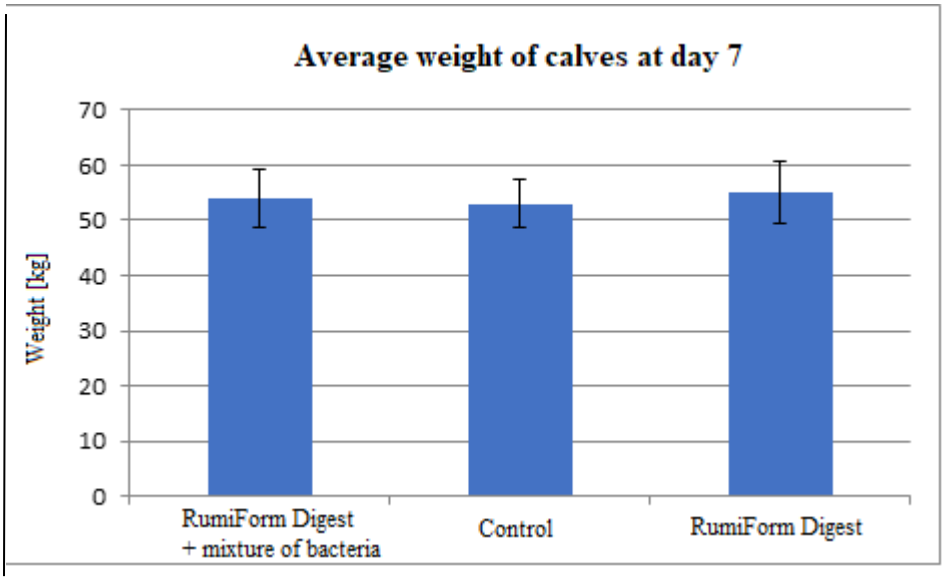
In the experiment, the weight was measured at day of birth, at 7th day and at day of weaning (90 days after calving).

Graph 3. Average birth weight of calves



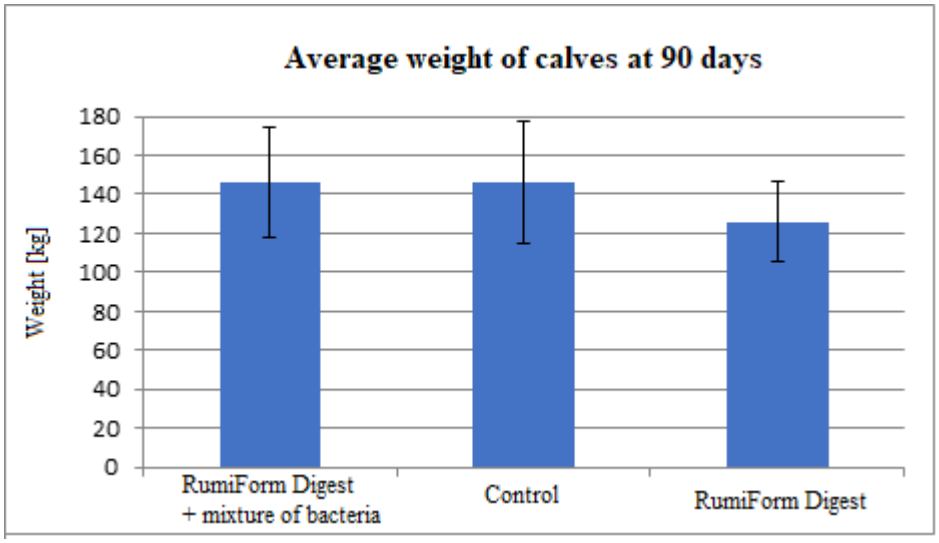
Birth weight (Graph 3) was approximately the same in all tested groups. In the group which was fed with a combination of the RumiForm Digest feed additive and the bacteria mixture and the control group had an average birth weight of 47 kg. In the case of the group which was fed only RumiForm Digest the average was 48 kg. The lowest measured weight was 39 kg, while the maximum recorded weight was 57 kg. Based on the parametric Bartlett's test, it was verified that there was no significant difference between the tested groups at the beginning of the weighting (N = 16; Bartlett = 0,9975; p = 0,6073).

Graph 4. Average weight of calves at day 7



As shown in Graph 4, the weight gain trend did not change even after seven days. All three groups remained at approximately the same average values. The lowest recorded value was 45 kg, while the highest was 65 kg. Based on the non-parametric Kruskall-Wallis test, no significant difference was observed even after seven days (N = 16; KW = 0,9134; p = 0,6334).

Graph 5. Average weight of calves at 90th days

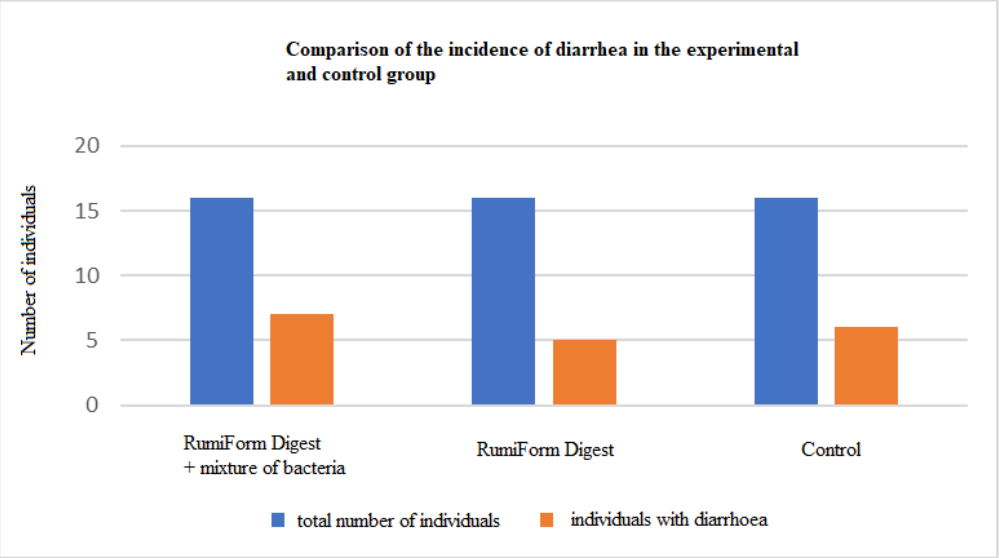


A more significant difference between the tested groups was observed only at the end of the experiment at the last weighing (Graph 5), when the average weight of the group fed the RumiForm Digest feed supplement was 126 kg, while the average weight of the group fed the RumiForm Digest feed supplement together with the bacterial mixture and the control group was 146 kg. The lowest recorded value was 95 kg, the highest 190 kg. Even in this case, no significant difference was noted between the tested groups using the Bartlett's parametric test (N = 16; Bartlett = 2,298; p = 0,317).

HEALTH ASSESSMENT

Throughout the study, the health status and treatment progress of the subjects was recorded. The course and treatment of diarrhoeal diseases were recorded in detail.

Graph 6. Comparison of the incidence of diarrhea between the experimental and control groups



Diarrhoea occurred in all three tested groups including the control group (Graph 6). The highest incidence of diarrhoea was recorded when was used the feed supplement RumiForm Digest in combination with the bacterial mixture, with 7 out of 16 individuals suffering from diarrhoea. When was used the feed supplement RumiForm Digest without the bacterial mixture the occurrence of diarrhea was recorded in five individuals. When we compared these groups with control group, the use of chi-square test, there was a significant difference between the tested groups ($N = 16$; $\chi 2 = 0,17$; $p = 0,67$).

Table 1. Course of diarrhoeal diseases in calves

Group in the experiment	When diarrhea appeared	Duration	Colour, consistency
RumiForm Digest and mixture of bacteria	10th day after birth	3 days	yellow colour, mushy consistency
	2nd day after birth	3 days	light yellow colour, thin consistency
	12th day after birth	4 days	yellow colour, very thin consistency
	10th day after birth	3 days	light brown colour, watery consistency
	7th day after birth	5 days	light brown colour, thin consistency
	10th day after birth	7 days	yellow-brown colour, thicker consistency
	around the 60th day after birth	6 days	light brown colour with an admixture of blood, very watery consistency
Control	10th day after birth	1 day	light brown colour, thinner consistency
	2nd day after birth	2 days	light yellow colour, mushy consistency
	around the 60th day after birth	3 days	light brown colour with an admixture of blood, very watery consistency
	around the 60th day after birth	7 days	light brown colour with an admixture of blood, very watery consistency
	around the 60th day after birth	5 days	light brown colour with an admixture of blood, very watery consistency
	around the 60th day after birth	2 days	light brown colour with an admixture of blood, very watery consistency

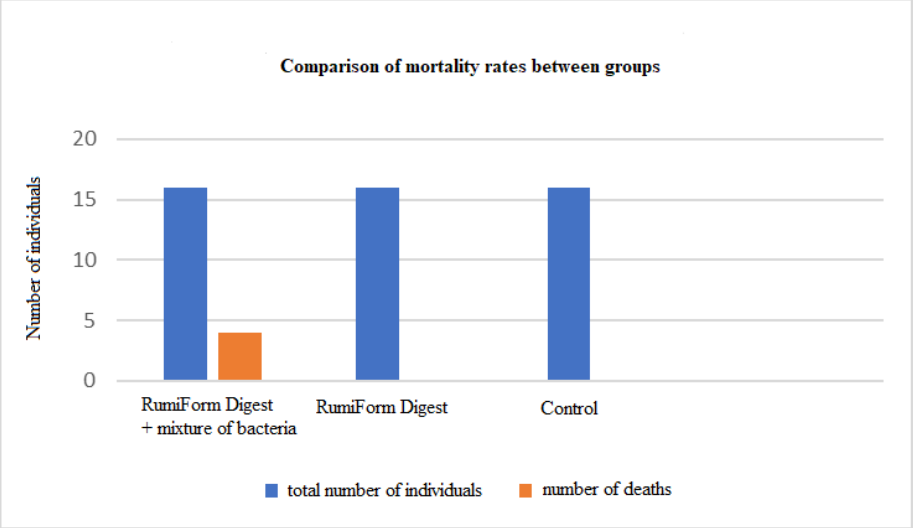
RumiForm Digest	9th day after birth	8 days	brown colour, very thin consistency
	12th day after birth	2 days	brown colour, very mild course
	25th day after birth	7 days	yellow-brown colour, watery consistency
	14th day after birth	3 days	yellow-brown colour, thicker consistency
	9th day after birth	6 days	brown colour, thinner consistency

Diarrhea was observed (Table 1) in individuals on day 2 after birth and in calves 60 days after birth. Diarrhoeal diseases were the most common in animals around 10th day after birth. Diarrhoea lasting 1 day was the shortest with the mildest course. The longest persisted diarrhea was 8 days. The colour of the faeces ranged from light yellow to brown and 5 individuals had admixture blood. The most watery stools had calves that had diarrhea around the 60th day after birth.

MORTALITY

The deaths that occurred during the experiment were each caused by a different cause. One of them, according to the veterinarian, was a clostridial infection. The calf died without symptoms at 23 days of age. Another reason was diarrhea in an 11 days old heifer, which was immediately started on standard treatment - live water (Lectade) twice a day, 60 ml Duphalite under the skin. Antibiotics and infusion therapy were administered the next day. The calf did not respond to the treatment. It died in 4 days. The third cause of death was acute pneumonia. Treatment with antibiotics combined with infusion therapy was started. The final reason for losses during the study was injury. One of the animals dislocated its pelvic limb. After consultation with the veterinarian it was sent to the necessary slaughter.

Graph 7. Comparison of mortality rates between groups



Mortality (Graph 7) was recorded only in the group given the combination of feed supplement and bacterial mixture, namely in four cases. No mortality was recorded when using the feed supplement alone and in the control group. Based on statistical analysis using the chi-square test, no significant difference was recorded in this case either ($N = 16$; $\chi 2 = 0{,}3983$; $p = 0{,}8194$).

DISCUSSION

From the data collected for this study, there is no evidence of increased health problems or deaths in calves with serum total protein levels below 55 g/l. This value is according to Bielmann et al. (2010) considered sufficient. Urban (1997) claims that diarrheal diseases are a very common problem during the milk feeding period. They usually arrive between the fifth and seventh day. In the study, diarrheal diseases were observed most often around the 10th day.

From the experiment conducted in this study, it can not be shown that probiotic feed supplements have a beneficial effect on health or weight gain. This fact is confirmed by the authors Simon et al. (2001). They claim that the improvement in weight gain and feed conversion are only sporadic. Simon et al. (2001) agree with Uyeno et al. (2015) and Renaud et al. (2019),

who add that the effect of probiotics on improving health status is inconclusive. Studies on this topic are insufficient.

Diarrhoeal disease was the most common and most serious health problem in calves in this study. Exceptionally, it was necessary to deal with a cold, cough or pneumonia. Diarrhoea occurred most frequently in animals fed probiotic feed supplements, specifically RumiForm Digest and a mixture of bacteria. All mortalities that occurred during the test period are also from this experimental group. Studies conducted (Cho et Yoon, 2014; Smulski et al., 2020; Katsoulos et al., 2020) confirm the claim that diarrheal diseases are one of the most serious and common health problems in calf rearing. Cho et Yoon (2014) add that there are significant economic losses due to deaths, loss of growth and increased medical expenses.

CONCLUSION

The calf rearing period is the most important part of an animal's life. Poorly managed rearing, which includes nutrition, housing, zoohygiene, etc., has a negative impact not only on the calf's viability but also on its future production. With proper breeding management and quality nutrition, the individual will be healthy and strong. We will also get a quality animal that will perform well.

As part of the experiment, it was found that the average level of total protein in the blood serum between 2nd and 5th day was 58 g/l. This value is sufficient for the transmission of passive immunity. A level of total protein in blood serum above 55 g/l was measured in 79% of cases. Individuals with a level below 55 g/l also appeared among the animals. The minimum detected value was 45 g/l. In these calves, there was insufficient transfer of immunoglobulins from the colostrum (insufficient transfer of passive immunity). Average total protein counts in blood serum at 90 days after calving ranged from 58 g/l to 63 g/l. A total serum protein level of 55 g/l was recorded in 83% of calves. The lowest measured value was 45 g/l and the maximum was 70 g/l. These values, rather than the transfer of passive immunity, are indicative of the quality of nutrition and actual health status. Total serum protein values below 55 g/l indicate

a deficiency of protein in the feed and values above 75 g/l indicate dehydrated calves with overly concentrated blood.

The data showed that the average weight of newborn calves was 47,5 kg. After seven days, the values in all tested groups remained at approximately the same average values. The lowest value was measured in a calf weighing 45 kg. On the contrary, the highest value was recorded in an individual weighing 65 kg. A more significant difference between the groups was observed only at the last weighing at 90 days. The average weight of the group fed the probiotic feed supplement RumiForm Digest was 126 kg. The average weight of the group fed RumiForm Digest in combination with the bacterial mixture and the control group was 146 kg.

Diarrhea was observed in all tested groups. The most frequent occurrence was recorded when the feed supplement was used in combination with a mixture of bacteria. Diarrhoea was suffered by 7 calves out of a total of 16 individuals. When feeding RumiForm Digest alone, 5 animals out of a total of 16 individuals became ill. In the control group, 6 calves fell ill out of a total of 16 individuals. Calves most often fell ill with diarrhea around the 10th day after birth. The course of treatment was guided by the actual health status of the animals. The most serious course of the disease was in calves around the 60th day. These individuals had to be given anticoccidials in combination with antibiotics and supportive care.

Losses occurred only in individuals fed the probiotic feed supplement RumiForm Digest with a mixture of bacteria. Calves that died included individuals with clostridial infection, diarrhea, acute pneumonia, and an individual with a dislocated pelvic limb. Total mortality was less than 2%. In this study, a positive effect of probiotic feed supplements on the growth and health status of calves could not be statistically demonstrated. This fact is possibly due to the small number of tested individuals. For a more accurate proof of the results, it would be advisable to expand the study.

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THE EFFECT OF CRUDE PROTEIN DEFICIENCY IN DIET ON BLOOD BIOCHEMICAL PARAMETERS IN LAYING HENS

DANA ZÁLEŠÁKOVÁ, JAKUB NOVOTNÝ, MICHAL ŘIHÁČEK, LUCIE HORÁKOVÁ, ONDŘEJ ŠŤASTNÍK, LEOŠ PAVLATA

Department of Animal Nutrition and Forage Production, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: xzalesa4@mendelu.cz

ABSTRACT

The influence of crude protein deficiency on blood biochemical parameters of laying hens of the Dominant hybrid combination was investigated. The study involved two groups of hens: a control group fed a diet with recommended level of crude protein for layers, and test group with a 30% deficiency of crude protein compared to the control. The average feed consumption wasn't affected by different diets, as well as certain egg quality traits such as egg weight, shell strength and thickness, proportion of yolk and albumen or Haugh units. In blood biochemical parameters, some indicators showed statistically significant differences (p<0.05): lactate dehydrogenase levels were observed at 10.78 µkat/l in the control group, contrasting with 7.39 µkat/l in the test group, while uric acid levels measured 231.57 µmol/l in the control group compared to 132.05 µmol/l in the test group.

Keywords: poultry; nutrition; plasma; metabolism; nitrogen depletion

INTRODUCTION

Limitation of nutrient intake can cause body stress in birds, resulting in an increase in plasma hormones e.g., T3, T4, somatotropin (Bruggeman et al., 1997; Decuypere et al., 2005) and corticosterone (De Jong et al., 2002). Blood biochemistry analysis can be valuable for poultry veterinarians and nutritionists as it can identify metabolic conditions that may be overlooked through conventional diagnostic methods (Adams et al., 2022). However, avian clinical pathology

is a field with real scarcity in reference intervals (Silva et al., 2007; Tang et al., 2013; Board et al., 2018), and compared to their use in larger animals, laboratory ranges are infrequently established in avian medicine (Arzour-Lakehal and Boudebza, 2021). While precise reference intervals for blood parameters in hens are unknown, it's known that total plasma protein can serve as an indicator of bird body condition (Rajman et al., 2006). Albumins function as a protein source during periods of reduced nitrogen intake, and their proportion decreases in layers (Dibner and Ivey, 1990) and broilers (Yaman et al., 2000) when dietary nitrogen intake is limited. Uric acid is a major waste product of nitrogen metabolism in birds (Harr, 2002) and its concentration in blood plasma should increase with crude protein intake (Donsbough et al., 2010). On the contrary, some studies indicate that with a lower intake of crude protein in diet, there is no influence on plasmatic parameters, especially total protein, albumin, globulin, cholesterol, or alkaline phosphatase (Hussein et al., 2018; Raja et al., 2021). Regarding the representation of triglycerides, other studies claim that their content in the blood increases together with the restriction of crude protein in the diet (Ghasemi et al., 2014; Torki et al., 2015).

The objective of this study is to investigate the potential effect of dietary crude protein deficiency on selected blood biochemical parameters in laying hens.

MATERIAL AND METHODS

A total of 64 layers of the Dominant hybrid combination were included in the experiment. These 32-week-old hens were randomly divided into two different groups. They were housed in enriched cage batteries (8 layers per battery). Constant temperature and humidity were monitored and maintained in the experimental room daily based on the technological instruction for Dominant hybrids (Dominant CZ, 2020). Furthermore, the health status of the laying hens, daily feed consumption and egg parameters (weight, shell strength and thickness, Haugh units, etc.) were monitored and measured.

Two different feed mixtures were prepared for feeding. The nutritional composition of the control mixture (C) corresponded to the nutritional recommendations for Dominant hybrids. The nutritional composition of the second experimental mixture (N) exhibited an approximate 30% crude protein deficiency when compared to control mixture. A comprehensive breakdown of component and nutritional parameters is shown in Table 1.

Table 1. Component and nutritional composition of feed mixtures for C (control) and N (nitrogen deficiency) group (g/kg)

Components, nutritional composition (g/kg)	C	N
Wheat	300.0	380.2
Maize	288.7	350.0
Soybean meal	264.0	128.0
Rapeseed oil	40.0	30.0
Premix N1[1]	30.0	30.0
Limestone grit	30.0	30.0
Finely ground limestone	44.0	44.5
Monocalciumphosphate	3.3	3.3
DL-Methionine	-	0.5
L-Lysine	-	3.5
ME_N (MJ)[2]	11.8	12.0
Dry matter	880.0	880.0
Crude fiber	41.9	42.4
Crude protein	176.6	126.7
Ether extract	53.0	45.2
Crude ash	120.2	119.9

Legend: [1]Vitamin and mineral premix (per kg): L-lysine 0.41 g; DL-methionine 1.35 g; calcium 8.91 g; phosphorus 2.01 g; sodium 1.38 g; copper 9 mg; iron 69 mg; zinc 54 mg; manganese 72 mg; iodine 0.9 mg; selenium 0.24 mg; retinol 9,900 IU (international units); calciferol 3,000 IU; tocoferol 15 mg; phylloquinone 1.2 mg; thiamine 1.2 mg; riboflavin 3.6 mg; pyridoxin 1.62 mg; cobalamin 12 µg; biotin 0.09 mg; niacinamid 12.6 mg; folic acid 0.9 mg; calcium pantothenate 7.5 mg; cholin chloride 180 mg.

[2]ME_N – apparent metabolize energy (calculated value)

The trial lasted for 21 days. In the 35th week of age hens were slaughtered, and their blood was collected. Blood samples were gathered in heparinized tubes after slaughtering. Plasma was collected after centrifugation (15 minutes, 3,000 rpm) and then it was frozen (-20 °C) until biochemical examination.

Standardized biochemical procedures were used to evaluate various parameters in the blood plasma samples, utilizing Erba Lachema (Czech Republic) commercial sets and the Ellipse automatic biochemical analyzer (AMS Spa, Italy). The enzymatic activities of AST (aspartate aminotransferase), GGT (gamma-glutamyl transferase), ALT (alanine aminotransferases), ALP (alkaline phosphatase), LD (lactate dehydrogenase) and CK (creatine kinase) were determined.

In addition to the enzymatic markers, various other indicators of hepatic metabolism, nitrogen metabolism, and fat metabolism were evaluated. These included measurements of total bilirubin (TBili), triglycerides (TG), cholesterol, urea, creatinine, total protein (TP), albumin, and the calculated value of globulin (obtained by subtracting albumin from total protein).

The collected data were processed in StatSoft Statistica, version 14.0 and Microsoft Excel. An analysis of variance (ANOVA) with a one-way design through the general linear model was conducted, and the level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

No significant differences were observed in average daily feed consumption, which was 106.87 g for C and 109.31 g for N group.

Egg quality traits, including egg weight, shell weight, strength and thickness, yolk weight, albumen weight, Haugh units and egg shape index were measured and calculated three times during the trial. Obtained results are shown in Table 2.

Table 2. Qualitative parameters of eggs (n=24 per each group)

	Egg weight (g)	Egg shape index	Shell weight (g)	Shell strength (N)	Shell thickness (mm)	Yolk (%)	Albumen (%)	Haugh units
C	59.58	1.27	5.50	41.40	0.37	25.26	65.08	92.13
N	58.61	1.32	5.42	43.72	0.37	26.06	64.68	93.25
SEM	0.55	0.01	0.06	1.18	0.00	0.31	0.34	0.86
p	0.38	0.05	0.55	0.33	0.50	0.19	0.24	0.52

Legend: C – control group; N – test group with 30% crude protein deficiency; SEM – Standard Error

There were no statistical differences in monitored parameters ($p>0.05$) which suggests that a 30% crude protein deficiency in diet does not affect egg quality traits. Similar findings for these parameters are confirmed by Zeweil et al. (2011).

Blood biochemical parameters were measured in the end of the trial, in 35th week of the hen's age. The values of selected basic enzymes are described in Table 3.

Table 3. Blood biochemical parameters – enzymes (n=6 per each group)

	ALP (µkat/l)	ALT (µkat/l)	AST (µkat/l)	GMT (µkat/l)	LD (µkat/l)	CK (µkat/l)
C	4.84	0.26	3.21	0.47	10.78^{a}	22.61
N	4.69	0.53	2.85	0.36	7.39^{b}	20.56
SEM	0.980	0.148	0.286	0.081	0.874	2.120
p	0.346	0.380	0.559	0.537	0.045	0.652

Legend: C – control group; N – test group with 30% crude protein deficiency; SEM – Standard Error; ALP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate aminotransferase; GMT – gama-glutamyltransferase; LD – lactate dehydrogenase; CK – creatine kinase
$^{a:b}$ – different characters show statistically significant differences $p<0.05$

There were no significant differences in monitored parameters except the lactate dehydrogenase, which was higher in C group. Abnormally increased plasma LD could indicate oxidative damage of cell membranes and permeability (Patel et al., 2019). In contrast to our findings, Khajali and Qujeq (2005) recorded higher LD plasma activity in broilers with

feed restriction. The values of other indicators agree with findings of Raja et al. (2021).

Parameters of nitrogen metabolism are described in Table 4.

Table 4. Blood biochemical parameters – nitrogen metabolism (n=6 per each group)

	UA (µmol/l)	Crea (µmol/l)	TP (g/l)	Alb (g/l)	Glob (g/l)	Urea (mmol/l)
C	231,57[a]	8,42	50,32	19,46	30,85	0,30
N	132,05[b]	12,89	45,98	16,46	29,53	0,37
SEM	21,130	2,097	1,969	0,884	1,656	0,445
p	0,009	0,309	0,292	0,088	0,709	0,462

Legend: C – control group; N – test group with 30% crude protein deficiency; SEM – Standard Error; UA – uric acid; Crea – creatinine; TP – total protein; Alb – albumin; Glob – globulin (calculated value)

No differences were found in creatinine, total protein, albumin, globulin or urea. The absence of significant changes in albumin, globulin and total protein is supported by studies of Hussein et al. (2018) and Raja et al. (2021).Uric acid, on the other hand, were significantly higher in C group which is consistent with the findings of Donsbough et al. (2010).
Remaining parameters, related to energy metabolism and bilirubin, are present in Table 5.

Table 5. Blood biochemical parameters – energy metabolism and bilirubin (n=6 per each group)

	Glu (mmol/l)	Chol (mmol/l)	TG (mmol/l)	TBili (µmol/l)
C	9,37	3,15	21,32	1,85
N	9,51	3,48	18,30	3,03
SEM	0,478	0,508	2,420	0,917
p	0,894	0,758	0,558	0,546

Legend: C – control group; N – test group with 30% crude protein deficiency; SEM – Standard Error; Glu – glucose, Chol – cholesterol; TG – triglycerides; TBili – bilirubin total

There were no significant disparities in glucose, cholesterol, triglycerides or bilirubin. As mentioned by Hussein et al. (2018), the cholesterol level didn't change with the restriction of crude protein in the diet. In contrast, some studies mention triglycerides increased in nitrogen-deficient groups in layers (Ghasemi et al., 2014; Torki et al., 2015). However, our experiment didn't confirm this trend.

CONCLUSION

In this study, the influence of crude protein deficiency in diet of Dominant hybrid laying hens was examined. Despite the dietary deficit, average feed consumption and egg quality traits remained relatively stable, suggesting that the crude protein deficiency didn't significantly impact these aspects of hen physiology and egg production.

However, blood biochemical parameters revealed distinct changes in specific indicators. LD levels exhibited a considerable reduction in the test group compared to control group. Similarly, uric acid levels demonstrated decrease in the test group, indication a potential alteration in nitrogen metabolism due to the dietary manipulation.

In conclusion, the obtained data indicate that significant nutrient reduction could affect metabolism and some indicators of blood and further research in this area could provide valuable information for poultry nutritionist and veterinarians.

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