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EFFECT OF FINISHING DIET WITH EXCLUDED SILAGE ON AMINO-ACID, FATTY-ACID, AND MINERAL COMPOSITION OF MEAT (*M. LONGISIMUS DORSI)* IN CALVES

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Abstract

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To study the composition of amino acids, fatty acids, and minerals in beef as affected by silage in the diet, ten male calves were allotted by the analog method into two groups for a period of four months. During the first sub-period (2 mo) the two groups were subjected to one and the same diet, while during the second silage was excluded from the diet fed to the experimental group. At the end of the period, three animals of each group were slaughtered and meat test samples from the ribeye area within ribs 11 and 12 of *m. longisimus dorsi* taken from each carcass. Higher composition of essential amino acids was established in the meat of the calves fed silage-free, the relative difference in methionine and lysine being respectively 27.8 and 11.1 per cent. Compared to the control group, the meat from the calves fed diet with excluded silage is characterized with more favorable composition of the main omega-3 acid, C18:3, the UFA C18:1, as well as the SFA's palmitic and stearic acid. With regard to mineral composition, the meat produced by the calves from the experimental group has shown to have lower levels of K and higher of Mg, Fe, and Cu than the control group.

Key words: beef, silage, amino acids, fatty acids, minerals

Introduction

Ruminants have the unique ability to transform the relatively low-quality diet protein to high-quality meat protein. The requirements for the amino-acid deposition are normally to a great extent satisfied by the ruminal microbial protein synthesis. Nevertheless, for economic reasons in achieving fast development of muscle tissue and hence fast growth rate, feeding high levels of exogenous protein, especially of the limiting amino acids methionine and lysine, is prerequisite in intensive beef production to meet the higher requirements.

In contrast, the attention towards lipid profile in meat is guided by the concern about human vitality with an emphasis on omega-3 fatty acids and their adequate ratio with omega-6 (Jump et al., 1997; Kris-Etherton et al., 2002), having important role for cell function regulation and hence for health status (Devon, 1990; Pedersen, 1990; Skjervold, 1992).

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Fatty acids are also subject to dramatic transformations caused by hydrogenation in the rumen that alters the profile of diet lipids to be deposited as body tissue, mostly by diminishing the polyunsaturated fraction (Doreau and Ponset, 2000; Givens, 2005; Talpur et al., 2007). Despite of these gross changes, feeding has been proved to exert effect on fatty acids in beef in an array of studies, recommending roughage and especially pasture (French et al., 2000; Pflimlin and Todorov, 2003; Nuernberg et al., 2005; Aldai et al., 2005; Ponnampalam et al., 2006). Such farm management is stimulated by EC, FAO (Silvestri, 1997), and the World Review of Nutrition and Dietetics (Simopoulos, 1998) for solving the issue of optimization of the diet of meat consumers. That implies disregarding the systems for intensive, high-protein feeding and adopting extensive or semi intensive systems with as much pasture feeding involved as possible.

Comparative studies on types of roughage show negative effect of silage, corn in particular, on lipid absorption and fatty-acid profile of animal tissues (French et al., 2000; O'Sullivan et al., 2002; Dewhurst et al., 2003; Evans et al., 2009; Kennedy et al., 2010). This is due to the reduction of the portion of the unsaturated acids in the diet because of ensiling, though the omega ratio is not skewed (Khan et al., 2009, 2011; Alves et al., 2011). On the other hand, such forage processing is associated with protein degradation and deterioration of utilization of amino acids (Thomas and Chamberlain, 1982; Chamberlain et al., 1986; Flores et al., 1986).

The present trial was initiated with the objective to study the effect of the exclusion of corn silage from the finishing diet on amino acid, fatty acid, and mineral composition of meat in bovine calves.

Material and Methods

For the aim of the study, a research trial was initiated assigning ten male calves of the *Bulgarian Black and White* breed on the farm of Agricultural Research Centre, Targovishte. The animals were allotted by the analog method into two groups – control (n= 5) and experimental (n= 5) group. They were housed in loosebox group sheds enclosed from three sides and provided with exercise yards.

The experimental period was 120 d, divided in two. During the first sub-period (60 d) the two groups were fed corn silage *ad libitum* as well as 1 kg meadow hay and 3 kg concentrate meal per capita per day. The component composition and the nutritional value of the meal are presented in Table 1.

During the second sub-period the calves in the control group were still fed corn silage *ad libitum* supplemented with 1 kg wheat straw and 4 kg concentrate meal per capita per day, while in the experimental group the silage was excluded from the diet and the animals were fed 1 kg meadow hay, 1 kg alfalfa hay, 2 kg wheat straw and 4 kg concentrate meal per capita per day.

At the start of the experimental period, the calves from the control group were at the average age of 522.0 d and live weight 317 kg, and those from the experimental group – at 524.6 d and 319 kg. Three animals of each group were slaughtered at the end of the period – the control group at 373 kg, and the experimental at 432 kg.

Table 1
Composition of the concentrate meal

Components		%
Maize		60.0
Barley		22.0
Sunflower cake		16.2
Dicalcium phosphate		0.4
Common salt		1.0
Microelement premix for cattle		0.2
Vitamin premix for ruminants #27-86CM		0.2
Nutritional value of 1 kg		
Energy for growth, MJ	5.726	
Intestinally digestible protein, g	86.0	

From each carcass meat test samples from the ribeye area within ribs 11 and 12 of *m. longisimus dorsi* were taken. The analyses carried out at the Research Laboratory of Trakia University – Stara Zagora concerned the following components and the corresponding instruments:

- Amino-acid composition – by an automatic amino acid analyzer *T339M*;
- Lipid composition – by gas chromatographers *Unicom* and *Carlo-Erba*;
- Mineral matter – by an atomic absorption spectrophotometer *Perkin-Elmer*.

The data were processed by the software program STATISTICA for Windows, 1994.

Results and Discussion

The results of the amino-acid analysis of the meat samples from the calves from the two studied groups are presented in Table 2. The calves fed finishing diet with excluded silage (experimental group) have shown to deposit higher levels of all studied essential amino acids (EAA) in meat than the control group, the values for the limiting methionine and lysine being 0.46 and 2.21 g respectively – by 27.8 and 11.1 per cent higher. With regard to the relative portion of the different EAA, highest is the percentage of lysine (over 19 %), followed by leucine (over 17 %). Noteworthy is the higher portion of methionine out of the total fraction of EAA in the experimental group (4.09 %) compared to the control (3.55 %), which to some extend applies to arginine as well. In turn, the percentage of threonine, valine, and histidine in the calves fed silage-free diet is relatively low.

The data in the table indicates that of all amino acids in both groups the fractions of the essential and the non-essential acids (NAA) are practically equal. The silage-free diet has also shown to result in higher levels of the majority of NAA, except for cysteine and glycine. Significant ($P< 0.05$) are the differences concerning glutamic (4.38 versus 3.81 g) and aspartic acid (2.01 versus 1.87 g), having highest portion of all NAA. Glutamic acid is of relatively higher percentage in the experimental animals (38.66 %) compared with the control (37.17 %). The amino acids serine and tyrosine also have well-expressed higher levels in the calves fed finishing diet with excluded silage (relatively by 16.7 and 13.0 per cent), while the differences concerning proline and alanine are smaller (respectively 9.4 and 6.8 per cent).

Despite of the differences in the composition of the studied amino acids between the groups, there are no substantial differences concerning the ratios of tyrosine with the large neutral amino acids (valine, leucine and isoleucine) and with phenylalanine, in response to the diminished ones in silage in comparison to other types of roughage (Ohshima et al., 1979; Flores et al., 1986).

The lower composition of amino acids in the calves from the control group can be attributed to the diminished microbial protein synthesis and the degradation of larger portion of the diet protein (amino acids) in the rumen when silage is the main forage (ARC, 1984; Veira et al., 1991), keeping in mind the important role of metionine and lysine in particular for productivity (Thomas and Chamberlain, 1982). The results for the EAA are commensurate with the tendency of changes in the biomass as a result of ensiling, i.e. the observed decreased content of histidine, arginine, and threonine (Flores et al., 1986); nevertheless, this does not apply to the most important amino acid, methionine, having higher levels established in silage. As for the NAA, this tendeny concerns serine, glutamic acid, and tyrosine (Flores et al., 1986). All this suggests that the different amino acids have different fate during roughage processing to silage, storing, as well as during degradation and microbial synthesis, apparently affecting methionine to greatest extent.

The data about the fatty-acid composition of meat (Table 3) show that major part of the saturated fatty acids (SFA) is palmitic (C16:0) and stearic acid (C18:0), which is normal for the red meats (Fink-Gremmels, 1993; MAFF, 1998) and in keeping with a very recent, unpublished study of our team (Dimov et al., submitted). The meat of the experimental calves has lower percentage of C12:0 (1.13 %) and C16:0 (27.35 %, P< 0.01) and higher of C18:0 (14.85 %) compared to those fed

Table 2
Amino-acid composition in meat

Amino acids, g/100g meat	Silage finishing (control group, n= 3)			Silage-free finishing (experimental group, n= 3)			Significance of differences
	x̄ ± SE	%	CV	x̄ ± SE	%	CV	
Essential (EAA)	10.14	100.00		11.26	100.00		
Threonine	0.76 ± 0.036	7.50	8.29	0.82 ± 0.078	7.28	15.97	n.s.
Valine	1.13 ± 0.063	11.14	9.65	1.23 ± 0.036	10.92	5.12	n.s.
Metnionine	0.36 ± 0.018	3.55	8.89	0.46 ± 0.040	4.09	15.00	n.s.
Isoleucine	1.06 ± 0.043	10.45	6.98	1.17 ± 0.046	10.39	6.92	n.s.
Leucine	1.74 ± 0.087	17.16	8.62	1.95 ± 0.063	17.32	5.59	n.s.
Phenylalanine	0.86 ± 0.046	8.48	9.42	0.94 ± 0.022	8.35	4.15	n.s.
Histidine	1.03 ± 0.058	10.16	9.71	1.11 ± 0.018	9.86	2.88	n.s.
Lysine	1.99 ± 0.096	19.63	8.34	2.21 ± 0.056	19.63	5.09	n.s.
Arginine	1.21 ± 0.079	11.93	11.41	1.37 ± 0.029	12.17	1.43	n.s.
Non-essential (NAA)	10.25	100.00		11.33	100.00		
Aspartic acid	1.87 ± 0.058	18.24	5.40	2.07 ± 0.064	18.27	5.36	P< 0.05
Serine	0.60 ± 0.041	5.85	11.83	0.70 ± 0.053	6.18	13.14	n.s.
Glutamic acid	3.81 ± 0.069	37.17	3.15	4.38 ± 0.153	38.66	6.05	P< 0.05
Proline	0.96 ± 0.101	9.37	18.23	1.05 ± 0.110	9.27	18.19	n.s.
Cysteine	0.18 ± 0.018	1.76	17.78	0.18 ± 0.018	1.59	17.78	n.s.
Glycine	0.97 ± 0.058	9.46	10.31	0.93 ± 0.040	8.21	7.53	n.s.
Alanine	1.32 ± 0.129	12.88	16.97	1.41 ± 0.052	12.45	6.31	n.s.
Tyrosine	0.54 ± 0.022	5.27	7.22	0.61 ± 0.048	5.38	13.77	n.s.
Sum	20.39			22.59			

Table 3
Fatty-acid composition in meat

Fatty acids, %	Silage finishing (control group, n= 3)		Silage-free finishing (experimental group, n= 3)		Significance of differences
	x̄ ± SE	CV	x̄ ± SE	CV	
Lauric C12:0	1.35 ± 0.097	12.37	1.13 ± 0.117	17.88	n.s.
Myristic C14:0	2.70 ± 0.198	12.70	2.73 ± 0.160	10.12	n.s.
Palmitic C16:0	29.50 ± 0.472	2.77	27.35 ± 1.107	7.01	P< 0.01
Palmitoleic C16:1	1.82 ± 0.109	10.33	1.82 ± 0.203	19.34	n.s.
Margaric C17:0	0.86 ± 0.145	29.19	0.99 ± 0.121	21.21	n.s.
Stearic C18:0	14.09 ± 1.062	13.05	14.85 ± 1.987	23.18	n.s.
Oleic C18:1	42.93 ± 0.742	2.99	44.53 ± 0.973	3.78	n.s.
Linoleic C18:2	4.49 ± 0.457	17.62	4.49 ± 0.050	1.94	n.s.
Linolenic C18:3	1.67± 0.092	9.47	1.81 ± 0.091	8.73	n.s.

silage throughout the whole period (1.35, 29.50, and 14.09 %). As far as myristic acid (C14:0) is concerned, the difference between the groups was established to be negligible. All this implies that the meat of the calves fed silage-free finishing diet would be a healthier element of the human diet in view of the less atherogenic effect of stearic compared to palmitic and myristic acid (Ovesen et al., 1998), and their relative effect on cholesterol (Grundy, 1994; Judd et al., 2002; Givens, 2005).

Of all studied fatty acids in the calves on a silage diet during the whole period the unsaturated fatty acids (UFA) consist 51.2 %, while in those with removed silage they are 52.8 %. Proven to be a healthy component in human diet, e.g. to obtain protection against age-related cognitive decline (Solfizzi et al., 1999), oleic acid (C18:1) shows to be the major MUFA component, as observed earlier in cattle (Varela et al., 2004; Ponnampalam et al., 2006). It has higher percentage in the experimental group (44.53 %) than in the control (42.93 %), which is a relative difference of 3.7 per cent. The other monounsaturated acid, palmitoleic (C16:1), is 1.82 % of all fatty acids in both groups. These two fatty acids have shown to be desirable in the human diet because of their favorable effect on cholesterol levels (Danke, 1994), the values for C18:1 and C16:1 established herein being respectively higher and lower then those in the previous study (Dimov et al., submitted).

As for the polyunsaturated fatty acids (PUFA), remarkable is the higher value of linoleic acid (C18:3) in the group with excluded silage from the finishing diet in comparison to the control group (by 8.4 per cent), with no difference concerning the content of linoleic acid (C18:2). Of all PUFA, the essential C18:2 is considered most nutritionally valuable for human health (Parodi, 1999; AbuGhazaleh et al., 2002), the values established in the present study being little lower than the results by Sami et al. (2004). Despites, as major representatives respectively of omega-6 and omega-3 PUFA, the ratio between C18:2 and C18:3 renders the meat of the calves from the experimental group dietetically more valuable because of its favorable effect on health and vitality (Jump et al., 1997; Kris-Etherton et al., 2002), playing effect on productive performance and carcass quality in ruminants as well (Mattos et al., 2000; Ponnampalam et al., 2002).

The established less favorable fatty-acid profile, in particular the C18:1 and C18:3 UFA, of the meat of the calves fed silage finishing diet is not to be accounted much to the fodder texture itself, as changes take place not only during ensiling but also in the process of hay wilting (Dewhurst and King, 1998; Dewhurst et al., 2003; Boufaïed et al., 2003). It is the sooner due to the effect of corn silage on the utilization of fatty acids and the profile of the lipids deposited in ruminants' tissues (French et al., 2000; O'Sullivan et al., 2002; Lee et al., 2009; Kennedy et al., 2010).

The results of the mineral analysis of the meat of the calves from the two studied groups (Table 4) show no substantial differences between the calves fed different finishing diets. Of the macro minerals noteworthy is the higher content of magnesium in the group fed silage-free finishing diet – nearly 10 percent more ($P< 0.05$). Mg is important for the protein deposition in muscles and for the metabolism of substances and minerals (Toscani, 1945; NRC, 1989, 2000), which suggests that it is to be associated with the higher levels of amino acids (Table 2) and the noticeably higher weight gain of the animals in the experimental group (see Material and methods). No substantial difference in the Ca/P ratio is observed between groups.

Of the micro minerals greatest difference is observed between the levels of potassium, which is essential for muscle function (NRC, 1989), showing to be by 12.8 per cent relatively higher in the control group ($P< 0.05$). The other microelements have higher levels in the meat of the calves fed silage-free diet. Zink is known to be important for

Table 4
Mineral composition in meat

Minerals	Silage finishing (control group, n= 3)			Silage-free finishing (experimental group, n= 3)			Significance of differences
	x̄ ± SE		CV	x̄ ± SE		CV	
Ca, g/100g	0.0048	± 0.0006	20.83	0.0054	± 0.001	38.89	n.s.
P, g/100g	0.188	± 0.001	1.17	0.201	± 0.013	10.95	n.s.
Na, g/100g	0.038	± 0.0009	4.21	0.037	± 0.004	18.92	n.s.
K, g/100g	0.238	± 0.008	5.92	0.211	± 0.006	4.74	P< 0.05
Mg, g/100g	0.0142	± 0.0005	6.27	0.0156	± 0.00004	0.45	P< 0.05
Fe, mg/100g	2.210	± 0.088	6.92	3.137	± 0.722	39.88	n.s.
Cu, mg/100g	0.180	± 0.0150	14.44	0.197	± 0.009	8.02	n.s.
Zn, mg/100g	3.50	± 0.162	8.00	3.63	± 0.069	3.28	n.s.
Sum, mg/100g	488.89			476.96			

skeletal muscle formation and development (NRC, 1989) as well as for utilization of Ca and growth (Hambridge et al., 1986; Wyness et al., 2011), the relative difference between the groups being 3.7 per cent. The other trace element, copper, is by 9.4 per cent higher in the experimental group, also contributing for the Ca-metabolism (Davis and Mertz, 1987). In addition, it is to be associated with the much higher content of iron (by ~42 per cent), in view of its effect on Fe-metabolism and hence on growth and immune response (Arredondo and Nuñez, 2005; Wyness et al., 2011). The levels of iron in the control and the experimental group are respectively lower and higher than the results for beef reported by Wyness et al. (2011) in a global-scale study, while that concerning K, Na, and Zn are markedly lower.

Conclusions

In the present study, higher composition of essential amino acids was established in the meat of the calves fed diet with excluded silage two months from slaughter, the relative difference in methionine and lysine being 27.8 and 11.1 per cent.

Compared to the control group, the meat from the calves fed silage-free finishing diet is characterized with more favorable composition of the main omega-3 acid, C18:3, of C18:1 UFA, as well as of palmitic and stearic SFA.

With regard to mineral composition, the meat produced by the calves from the experimental group has shown to have lower levels of K and higher of Mg, Fe, and Cu than the control group.

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