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DIETARY INFLUENCE ON FATTY ACID CHARACTERISTICS OF LAMB CARCASS IN RELATION TO PROTEIN SOURCE

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Abstract: The aims of our study were to evaluate the effect of different protein supplements on fatty acid (FA) composition (%), profile (ratios and indices), etc. of carcass in lambs fed iso-caloric, iso-nitrogenous and equal in PDI and Ca: P ratio high- concentrate rations. Regional breed (Bulgarian Synthetic Dairy Population) lambs were fed cereal-based diets with different protein supplement - control diet with sunflower meal (SFM) or a DDGSc diet containing dried distillers' corn grains with solubles (DDGSc). Animals were slaughtered after 87-d feedlot period. Fat tissue extracted from carcass was analyzed for FA profile. There were significantly higher ($p< 0.01$) performance (FBW= 38.90 vs. 35.1 kg and HCW= 5.24 vs. 4.67 kg) of DDGSc diet on lamb performance. Feeding 37.6 % (DM basis) DDGSc significantly increased the content of C18:2 ($p< 0.05$) but decreased n_3 PUFA and total long chain n_3 FA ($p< 0.05$) compared with control group. DDGSc increased n_6 ($p= 0.06$), PUFA ($p= 0.07$) and PUFA / SFA ratio ($p= 0.10$), but decreased MUFA ($p= 0.10$). Examined relationships between ingested FA and carcass FA in slaughtered lambs shows good parity and are characterized with significant Pearson's correlation coefficients ($R> 0.56$). In regards to obtained results, dietary DDGS inclusion altered the fatty acid profile and indices of lipids of lamb carcass.

Key words: Lamb, Protein Source, Carcass, DDGSc, Fatty Acids Profile and Indices

Introduction

The Bulgarian Synthetic Dairy Population (*BSDP*) is the major breed of total sheep population – more than 1 million head or over 70 % (*www.noa.bg*).

Protein supplements, fed to improve growth rate at intensive feeding system, affecting dietary lipid composition in concentrate- based diets (*Webb and O'Neill, 2008*), e.g. influenced meat quality (*Wood et al., 2003*). Ruminants disposed gather of feedstuffs enrished in PUFA with different protection against rumen biohydrogenation. Such forage is dried distillers' corn grain with solubles – nutrient-dense by-product, rich in protected amino and fatty acids. Latter, by-passing the rumen, were in regards to increasing proportions of monounsaturated (MUFA), polyunsaturated (PUFA) FA and n_6/n_3 PUFA ratio in ruminant meat (*Williams, 2000; Palmquist, 2009*).

The fatty acid profile of animal products plays an important role in human nutrition. High- quality meat, stamped as healthful, is high in UFA/SFA and DFA/OFA ratios. Simultaneously, the demand for low-fat meat poor in SFA and rich in CLA has been increased in order to avoid health risk associated with excessive fat intake (*Scollan et al., 2006*). The FA with a potential negative effect on human health is saturated FA (SFA), unlike favourable proportions of beneficial FA such as C18:1, conjugated linoleic acid (CLA) and PUFA (especially n_3).

The present study is an extension of our wide investigation on DDGSc and its nutritive, metabolic and productive peculiarities as a protein and / or energy supplement and report effects on fatty acid profile and indices of lamb meat (*Yossifov, 2012; Yossifov, 2012a; Yossifov and Kozelov., 2012; Yossifov and Kozelov., 2012a; Yossifov et al., 2012; Yossifov, 2013; Yossifov, 2013a; Yossifov, 2014*).

Analyses on the fatty acid profile and indices of carcasses obtained from ruminants, fed DDGSc- based diets are still limited and needs more thoroughness. In this regards, our hypothesis was that DDGSc lipid profile would be affected the fatty acid content of lamb carcass.

Material and methods

Animals and management. Detailed descriptions of experimental design, animals and diets composition were reported in a companion paper (*Yossifov et al., 2012*). Briefly, weaned lambs (Bulgarian Synthetic Dairy Population, n= 32, age = 59 d, initial BW= 16.69±2.53 kg) were randomly allotted by BW, sex, type of litter. Dietary treatments (table 1) were iso-caloric, iso-nitrogenous and equal in PDI and Ca: P ratio – 1./ control (CON) – with sunflower meal (SFM), and 2./ experimental (EXP) – with DDGSc. Lambs were fed twice daily to approximately 5 % weigh-back to ensure *ad libitum* consumption. The concentrate (offered at 8.00 and 14.00 h) and forage (offered at 10.00 and 16:00 h) were fed separately throughout the experimental period.

Slaughter and sample collection. On d 87,5 male lambs per diet were randomly selected, weighted in two consecutive days (to calculate final BW) and slaughtered (*Yossifov et al., 2012*). Dressed carcass was weighted (hot carcass weight (HCW), kg) and after 24 h cold storage (at 4 ^{o}C) was divided into halves. The right carcass half was weighed and dissected into compound tissues (meat, fat and bone), expressed as absolute and relative values of the right half. Dissected meat and fat were mixed, ground and sampled for consecutive FA analysis. Concentrates and forage were also evaluated for individual FA.

Table 1. Diet composition[1] and nutrient content (% DM) of sunflower meal (CON) and DDGSc (EXP) protein supplemented diets

	CON	EXP
Meadow hay	36.6	35.6
Sunflower meal	26.3	-
DDGSc	-	37.6
Triticale	17.2	16.7
Corn	17.2	6.7
Vitamin-mineral premix	2.7	3.4
Feed units for gain, FUG	1.2	1.2
CP, %	19	18
PDI, % CP	62	62
Ca/P ratio	2	2

[1] Yossifov et al., 2012

DDGSc-dried distillers' corn grains with solubles; CP-crude protein; PDI-protein digestible in small intestines; Ca-calcium; P-phosphorus.

Measurements and calculations. Total lipids extraction (*Bligh and Dyer, 1959*) and fatty acid methyl esters (FAME) isolation (*Christie, 1973*) of the samples were described in details at *Yossifov* (*2014*). Samples lipids were extracted by homogenising in chloroform: methanol: water (1:2:8 v/v/v) and FAME were prepared for 14 h with 0.01 % solution of sulphuric acid in dry methanol. The FA profile of triacylglycerols was determined by GLC analysis with chromatograph C Si 200 equipped with a 60 m capillary column (TR-FAME) with 0.25 mm inner diameter and coating thickness of 0.25 μm. Hydrogen was used as a carrier gas. The temperature programme started at 160 ^{o}C (held for 0.2 min) and increasing at a rate of 5 ^{o}C.min^{-1} to 220 ^{o}C, where it's maintained for 5 min. Injector' and detector' temperature set points were stated at 200 ^{o}C. Individual FAME peaks were identified by comparison with reference methyl esters. FAs were expressed as a weight percentage of total FAs (*Christie, 1973*). A number of indices (fatty acid and healthy) and enzyme activities were calculated (*Yossifov, 2014*): total of saturated FA (SFA), hypercholesterolemic FA (OFA), total amount of

monounsaturated fatty acids (MUFA), n_6 fatty acids, n_3 fatty acids, total of polyunsaturated fatty acids (PUFA); total of long-chain n_3 fatty acids (*total LC* n_3), total of unsaturated fatty acids (UFA), sum of desirable fatty acids (DFA), atherogenicity index (AI), thrombogenic index (TI), hypocholesterolemic / hypercholesterolemic *index* (h/H), index of D_9 desaturase enzyme activity on the conversion of C16:0 and C18:0 to C16:1 n_9 and C18:1 n_9 ($IDSA_{16:0}$ and $IDSA_{18:0}$), stearoyl CoA desaturase (SCD) and elongase activity (EAI).

Statistical analyses. Carcass FA data were analysed by Statistical Package (*Microsoft Office, 2007*). Diet was used as the treatment effect, with individual animal as the experimental unit. All obtained data are offered as mean, standard deviation (SD), and simple variance (Var). The results were submitted to calculate standard error of mean (SEM) to assess the influence of dietary protein source (DDGSc vs. SFM) on the meat FA profile. Means were compared throughout the Student t-test and differences with level of significance below $p< 0.05$ were considered as significant. Pearson's correlation coefficient between variables was also calculated as a measure of the strength and direction of the linear relationship between two variables.

Results and discussion

Animal performance. A summary of diet composition (table 1) and animal performance (table 2 and 3), as reported by *Yossifov et al.* (*2012*), illustrated the effect of DDGSc inclusion. Feed intake was increased (6 %), but protein intake (CP) was similar (22 %). Nutrient differences between the protein supplements affected fat intake – 1.8 vs. 7.3 % for CON and EXP, respectively.

Table 2. Mean daily intake of diet DM and nutrients (g)

Item	Forage		Concentrate		Total ration	
	CON	EXP	CON	EXP	CON	EXP
DM	327.31	353.81	764.36	799.16	1091.70	1152.98
CP*	32.68	35.32	188.40	180.40	221.10	215.70
EE*	4.21	4.55	13.30	68.70	17.50	73.20
CF*	138.20	149.40	95.63	51.92	233.80	201.30

* g.kg DM^{-1}

DM-dry matter; CP-crude protein; EE-ether extract; CF-crude fiber; CON-control diet; EXP-experimental diet

Final body weight ($p<0.01$) and hot carcass weight ($p<0.05$) were significantly increased (35.10 and 16.29 vs. 38.90 and 18.45 kg for CON and EXP,

respectively). Meat (5.24 vs.4.67 kg) and fat yield (1.45 vs. 1.15 kg) were higher in DDGSc- based diet compared with CON (table 3).

Table 3. Lamb performance fed DDGSc vs. SFM- based diets (kg)

Item	Treatment								SEM	P-value
	SFM				DDGSc					
	Avr.	%	SD	Var	Avr.	%	SD	Var		
Final body weight	35.10	100.0	3.13	9.80	38.90	110.8	1.98	3.92	1.01	<0.01
Hot carcass weight	16.29	100.0	2.25	5.06	18.45	113.3	1.04	1.08	0.63	<0.05
Meat yield[1]	4.67	58.3	0.64	0.41	5.24	58.4	0.35	0.04	0.05	0.98
Separable fat[1]	1.15	15.2	0.18	0.03	1.45	16.2	0.27	0.07	0.10	0.56

[1] Right side slaughter weight ; SFM-sunflower meal; DDGSc-dried distillers' corn grains with solubles

Diet FA composition. Fatty acid composition of diets is presented in table 4. Percentages of FAME were similar among the diets, but greater levels of EE (1.8 vs. 7.3 %) increased the differences at ingested FA. Weights of C16:0, C18:1, C18:2, as well as SFA, DFA, OFA, MUFA, PUFA, UFA and C18:2/C18:3 ratios were greater in DDGSc- based diets. Established concentrations of the weight percentages of FAME are presented in table 5. The lipid content of the lamb carcass for the respective diets was 3.9 g/100 g (CON) and 4.6 g/100 g (EXP), but the differences were not significant (p= 0.12, SEM= 2.3) (*Yossifov, 2013*).

There was a tendency for EXP diet (table 5) to increase PUFA levels compared to CON (p=0.07). C18:2 concentrations were the most eminent PUFA member, significantly affected by DDGSc supplement (p<0.05) than the other treatment. DDGSc altered rumen environment and higher amounts of C18:2 escaping biohydrogenation in rumen, resulting in less C18:0 and C18:1 in carcass. Thus confirmed the other reports (*Kim et al., 2007*). There was also a hardy trends of increased n_6 elongation and desaturation products, e.g. increased n_6 FA when feeding DDGSc (p= 0.06) compared to the SFM (table 5). It's in agreement with some authors (*Gill et al., 2008*). In contract, n_3 FA and total LC n_3 FA contents of the CON diet were significantly higher (p<0.05) compared to the EXP. The numeric decreases of C18:0 in EXP differed from data obtained by others (*Gill et al., 2008; Depenbusch et al., 2009a*).

DDGSc increased C18:3, MUFA/SFA, PUFA/SFA, C18:2/C18:3, C18:2/CLA but decreased C18:1, MUFA and SCD.

Table 4. Fatty acid content of sunflower meal (CON) and DDGSc (EXP) protein supplemented diets

Item	FA profile (% identified FA)			Ingested FA ($g.head^{-1}.d^{-1}$)	
	Hay	CON	EXP	CON	**EXP**
C14:0	1.16	ND	ND	0.05	**0.06**
C16:0	22.05	12.12	14.80	2.49	**9.56**
C18:0	2.18	2.30	2.86	0.38	**1.74**
C18:1	10.38	28.67	29.65	3.93	**17.44**
C18:2	29.76	54.90	50.99	7.98	**30.60**
C18:3	34.47	2.01	1.71	1.85	**2.72**
SFA	25.39	14.42	17.66	2.92	**11.36**
OFA	23.21	12.12	14.80	2.54	**9.62**
MUFA	10.38	28.67	29.65	3.93	**17.44**
PUFA	64.23	56.91	52.70	9.84	33.32
UFA	74.61	85.58	82.35	13.76	50.76
MUFA/SFA	0.41	1.99	1.68	1.35	1.54
PUFA/SFA	2.53	3.95	2,98	3.37	2.93
UFA/SFA	2.94	5.93	4.66	4.71	4.47
DFA	76.79	87.88	85.21	14.14	52.51
(C18:0+C18:1)/C16:0	0.57	2.56	2.20	1.73	2.01
C18:2/C18:3	0.86	27.31	29.82	4.30	11.25

CON-control diet; EXP-experimental diet; FA-Fatty acids; SFA-saturated FA; OFA-Undesirable FA; DFA-Desirable FA; MUFA-Monounsaturated FA; PUFA-Polyunsaturated FA; UFA-Unsaturated FA; LC-Long-chain; IDSA16:0-Index of D9 desaturase enzyme activity on the conversion of C16:0 to C16:1 n9; IDSA18:0-Index of D9 desaturase enzyme activity on the conversion of C18:0 to C18:1 n9; CLA-Conjugated linoleic acid; AI-Atherogenicity Index; TI-Thrombogenic index; h/H-Hypocholesterolemic/hypercholesterolemic index; SCD-Stearoyl CoA desaturase; EAI-Elongase activity; ND – not detectable

The values of human healthy indices, as AI and TI, were higher in EXP (0.81, 1.09 and 0.99, 1.30, respectively) compared with CON. Contrary, h/H value was lower (1.70 vs. 1.51, respectively).

The indices of D_9 desaturase enzyme activity on the conversion of C16:0 and C18:0 to C16:1 and C18:1 was greater in CON than EXP. The lower desaturase activity and higher CLA supported lower substrate (C18:1) availability (Table 5). EAI and SCD values were lower in EXP (0.49 and 0.58) than CON (0.52 and 0.66). In regards to obtained FA profile and indices, more or less carcass FA reflects ruminal FA profile (*Vasta et al., 2009*).

Table 5. Effect of protein supplement on FA profile and indices of lamb carcass

	CON			EXP			SEM	P-value
	Avr.	± SD	Var.	Avr.	± SD	Var.		
C14:0	4.30	0.70	0.49	5.36	1.70	2.88	0.43	0.23
C15:0	0.77	0.25	0.06	0.83	0.25	0.06	0.07	0.72
C16:0	24.59	2.41	4.82	26.89	2.19	3.55	1.09	0.32
C16:1	1.93	0.90	0.82	1.78	0.52	0.27	0.22	0.75
C17:0	1.90	0.41	0.17	1.54	0.35	0.12	0.13	0.18
C18:0	15.94	2.21	4.90	14.95	2.80	7.85	0.77	0.55
C18:1	42.80	2.13	4.53	38.07	1.99	2.88	1.55	0.13
C18:2	5.44	1.04	1.07	8.32	2.21	4.90	0.70	< 0.03
C18:3	0.43	0.07	0.01	0.51	0.08	0.01	0.03	0.15
Total CLA	0.71	0.09	0.01	0.78	0.20	0.04	0.05	0.48
C20:4	0.98	0.40	0.16	0.83	0.35	0.12	0.12	0.55
C20:5	0.11	0.03	0.01	ND			0.01	< 0.001
C22:5	0.15	0.06	0.01	0.13	0.08	0.01	0.02	0.71
SFA	47.50	2.39	5.70	49.57	3.61	13.04	0.98	0.31
OFA	31.55	1.47	2.10	34.62	2.30	4.73	1.60	0.37
DFA	67.78	3.37	11.30	64.59	6.24	38.94	1.59	0.34
MUFA	44.73	1.90	3.60	39.85	1.55	3.84	1.48	0.10
n_6	6.85	1.31	1.72	9.66	2.52	6.33	0.76	0.06
n_3	0.26	0.09	0.01	0.13	0.08	0.01	0.03	< 0.04
PUFA	7.11	1.36	1.84	9.79	2.53	6.40	0.75	0.07
(C18:0+C18:1)/C16:0	2.41	0.33	0.11	2.05	0.62	0.39	0.16	0.28
Total LC n_3	0.26	0.09	0.01	0.13	0.08	0.01	0.03	< 0.04
UFA	51.84	2.27	5.16	49.64	3.54	4.56	0.96	0.28
MUFA/SFA	0.95	0.09	0.01	0.81	0.17	0.03	0.04	0.15
PUFA/SFA	0.15	0.03	0.01	0.20	0.04	0.01	0.01	0.10
UFA/SFA	1.10	0.11	0.01	1.01	0.14	0.02	0.04	0.31
C18:2/C18:3	12.58	1.89	2.59	16.49	2.25	3.07	1.18	0.10
C20:4/C20:5	11.27	1.85	2.42	NA			0.58	< 0.001
C18:2/CLA	7.70	1.16	1.34	11.48	4.66	21.70	1.19	0.12
C18:3/CLA	0.62	0.07	0.01	0.68	0.19	0.03	0.04	0.48
AI	0.81	0.12	0.01	0.99	0.30	0.09	0.07	0.24
TI	1.09	0.16	0.02	1.30	0.34	0.11	0.09	0.24
h/H	1.70	0.22	0.05	1.51	0.39	0.15	0.10	0.37
IDSA16:0	7.13	3.17	10.10	6.10	0.94	0.89	0.72	0.51
IDSA18:0	72.93	2.73	7.45	71.87	1.60	2.58	0.69	0.47
SCD	0.52	0.02	0.01	0.49	0.04	0.00	0.01	0.12
EAI	0.66	0.13	0.02	0.58	0.19	0.04	0.05	0.48
ND – not detectable; NA-not available								

Carcass concentration of C18:1, C18:2 and C18:3 have been shown to be highly correlated (*Nuernberg et al., 2005; Aldai et al., 2009*).

Pearson's correlation coefficients. The results of the regression analysis of the experimental data are presented graphically (Fig. 1, 2, 3 and 4). Examined relationships between ingested FA and carcass FA in slaughtered lambs shows good parity and as could be seen below, are characterized with significant Pearson's correlation coefficients (R> 56).

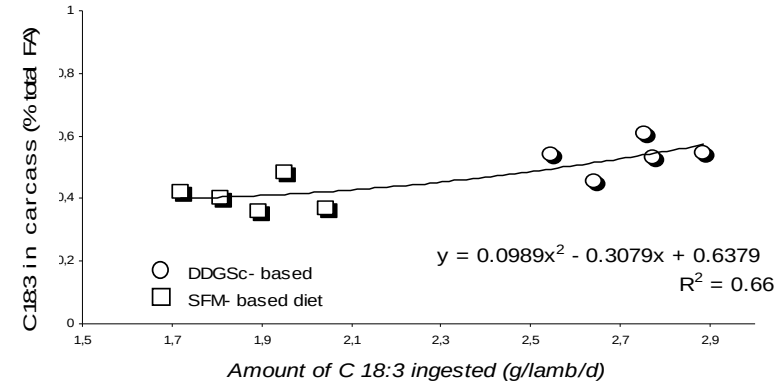

Figure 1. Relationship between the level of carcass C18:3 (% total FA) and amount of C18:3 ingested (g.lamb^{-1}.d^{-1})

In the first graph (Fig. 1) the amount of C18:3 as percentage of total FA in carcass displayed high coefficient of determination (R= 0.66) with concentrations of ingested C18:3 (g.lamb^{-1}.d^{-1}).

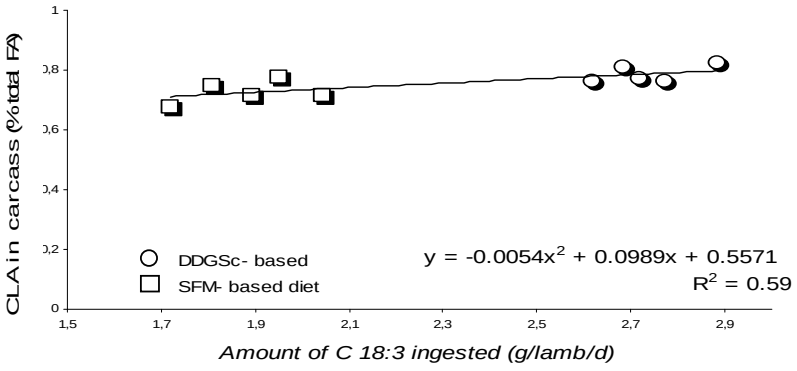

Figure 2. Relationship between the level of CLA in lipids of carcass (% total FA) and amount of C18:3 ingested (g.lamb^{-1}.d^{-1})

Similar trends were observed between total amount of carcass CLA (% total FA) and level of ingested C18:1 (R= 0.56) or C18:3 (R= 0.59) – fig. 2 and 3.

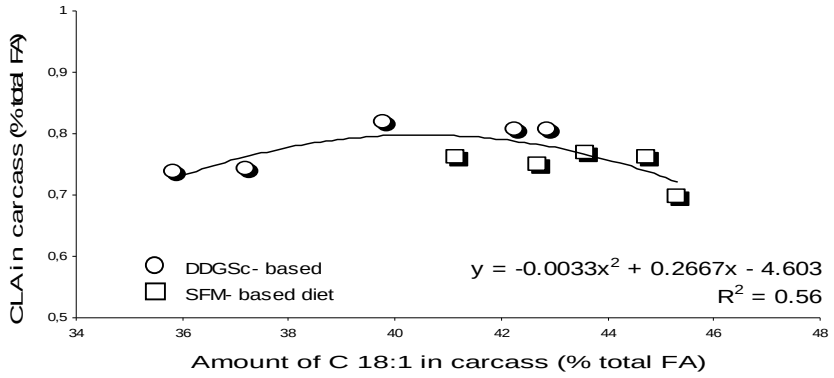

Figure 3. Relationship between the level of CLA in lipids of carcass (% total FA) and C18:1 (% total FA)

The relationship between the values of CLA in lamb carcass and index of D_9 desaturase enzyme activity on the conversion of C18:0 to C18:1 n_9 is revealed with high coefficient of determination (R= 0.73) – fig. 4.

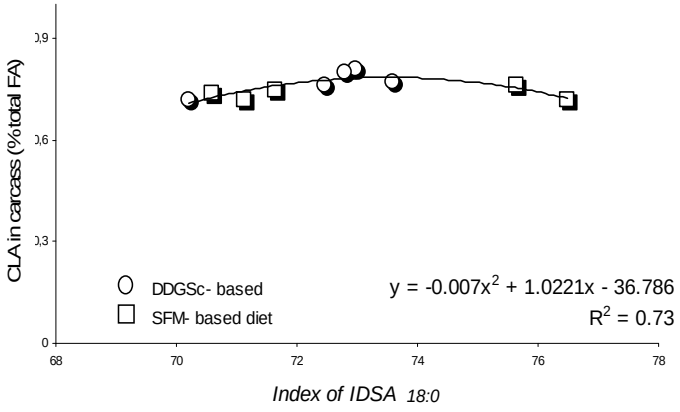

Figure 4. Relationship between the level of CLA in lipids of carcass (% total FA) and IDSA $_{18:0}$

Conclusion

The tested protein supplements altered fatty acid profile and indices (% and ratios) of lamb carcass. In this regards, differences in ruminal biohydrogenation

were related to the supplement source' FA profile as well as factors altered rumen condition, e.g. rumen environment. DDGSc- based diet significantly increased the content of C18:2 ($p< 0.05$) but decreased n_3 PUFA and total long chain n_3 FA ($p<0.05$) compared with control. DDGSc increased n_6 ($p= 0.06$), PUFA ($p= 0.07$) and PUFA / SFA ratio ($p= 0.10$), but decreased MUFA ($p= 0.10$). Examined relationships between ingested FA and carcass FA in slaughtered lambs shows good parity and are characterized with significant Pearson's correlation coefficients ($R> 0.56$). In regards to obtained results, dietary DDGS inclusion altered the fatty acid profile and indices of lipids of lamb carcass.

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Uticaj obroka na profil masnih kiselina jagnjećih trupova u odnosu na izvor proteina

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Rezime

Cilj našeg istraživanja je bio da se ispita uticaj različitih proteinskih dodataka na sastav masnih kiselina (FA) (% , profil (koeficijenti i indeksi), itd, trupa jagnjadi hranjenih izo-kaloričnim, izo-azotnim koncentratnim obrocima, obrocima sa jednakim PDI i odnosom Ca : P. Jagnjad regionalna rase (Bugarska sintetička mlečna populacija) su hranjena obrokom na bazi žitarica sa različitim proteinskim dodacima - kontrolni obrok sa suncokretovom sačmom (SFM) ili obrok sa DDGSc koji sadrži sušenu destilovana kukuruza zrna sa rastvorljivim materijama (DDGSc). Životinje su zaklane posle 87 dana tova. Masno tkivo ekstrahovano iz trupa je analizirano na FA profil. Proizvodne performanse jagnjadi na DDGSc ishrani su bile značajno veće ($p< 0,01$) (FBV = 38,90 u poređenju sa 35,1 kg i HCW = 5,24 prema 4,67 kg). Ishrana sa 37,6 % (na bazi SM) DDGSc značajno je povećala sadržaj C18:2 ($p<0,05$), ali je smanjena n_3 PUFA i ukupne masne kiseline dugog lanca n_3 FA ($p<0,05$) u poređenju sa kontrolnom grupom. DDGSc je uticao na povećanje n_6 ($p=0,06$) PUFA ($p=0,07$) i PUFA/SFA odnosa ($p=0,10$), ali je uticao na smanjenje MUFA ($p=0,10$). Ispitivani odnosi između unete FA i FA u trupovima zaklane jagnjadi pokazuje dobar paritet i karakterišu ih značajni koeficijenti Pearsonov-e korelacije ($R>0,56$). U vezi sa dobijenim

rezultatima, uključivanje DDGS u obrok je uticalo na menjanje profila masnih kiselina i indeksa lipida jagnjećeg trupa.

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