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Impact of Inclusion Dried Sugar Beet Pulp in Ruminant's Ration on Rumen Parameters *in vitro*

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ABSTRACT

The purpose of this study was to determine effect of partial or complete replacement of corn grains by dried sugar beet pulp on rumen parameters. The yellow corn was substituted by dried sugar beet pulp at 25 (D1), 50 (D2), 75 (D3), and 100% (D4). Results of chemical composition analyses indicated that DM, OM, ash and CP contents did not differ among treatments. Crude fiber content increased linearly with increased dried sugar beet pulp percentage in rations, with significant differences in D2, D3 and D4. Content of NDF was significantly higher in all experimental groups when compared with control group, whereas non-fibrous carbohydrates content decreased in all groups as the effect of corn grains replacement by dried sugar beet pulp. Complete substitution of yellow corn by sugar dried beet pulp in D4 significantly increased number of bacteria. D3 and D4 diets resulted in higher ($P<0.001$) concentration of *Holotrichs*. The ratio of acetate to propionate increased linearly with inclusion of dried sugar beet in the diet, however statistically significant differences were found only in D4. On basis of presented data, it could be concluded that yellow corn could be partially of fully substituted by dried sugar beet pulp without negative impact on rumen parameters including dry matter digestibility.

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Authors' Contribution
AEMM, AC and MSS conceived and designed the study. AEMM and PS performed the experiments and collected the samples. PS and AŁ performed the analyses and prepared the description. AEMM, MSS and AC interpreted the data and wrote the manuscript.

Key words
Bacteria, Beet pulp, Dried sugar, *In vitro*, Protozoa, Rumen.

INTRODUCTION

Increasing population of animals in Mediterranean countries is usually constrained by the insufficient and fluctuating natural supply of feeds *e.g.* grains or forages (Molina-Alcaide *et al*., 2003; Crosby-Galván *et al*., 2018). Reduction of cereals utilization in ruminant diets requires their replacement by high-energy non-cereal by-products such as dried sugar beet pulp (DSBP). Sugar beet is the second important source of sugar in many countries (Sarwar *et al*., 2008). Beet pulp is the primary by-product of sucrose extraction (Fadel, 1999). This residue comprises 6% of the total fresh matter of harvested sugar beet pulp (Kjaergaard, 1984). Since 1982, there has been developed the tendency in Egypt to increase the sugar production from beets. FAO statistics showed that production of sugar beets increased from 253.74 million tones in 2005 to 278.83 million tons in 2011 (FAO, 2005, 2011). Sugar beet pulp is fed as ruminants' dietary component in the wet and dry forms and is categorized as an energy source ingredient (Bath, 1981).

The nutritive value of DSBP can be compared with that of high energy grains like barley, corn and oat. The TDN (total digestible nutrients) value of DSBP was reported to range from 68-74%, with metabolizable energy averaging 2.99 Mcal/kg DM (Mandebvue and Galpraith, 1999).

Partial substitution of cereal grains with pectin-rich feedstuffs, such as sugar beet pulp, leads to lower rumen lactate and propionate production (Hall *et al*., 1998). This can be preventive against digestive disorders such as ruminal acidosis.

On the basis of above data, we can state that sugar beet pulp could be used as a source of energy for growing and fattening ruminants in Mediterranean countries, like in Egypt (Mohamed, 1998).

Although the possibilities of ruminant diet supplementation with dried sugar beet pulp are scientifically justified, the most effective dose as well as the effect on rumen metabolism has not been previously considered.

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Determination of the optimum amount of dried sugar beet pulp supplement will depend on the diet composition and the type of dietary ingredient that is replaced by this feed.

Thus, the objective of the present study was to evaluate the effect of partial and complete substitution of yellow corn by dried sugar beet pulp in ruminants ration on ruminal fermentation and microbial populations in vitro. The evaluated diet is typical for the animals raised in Mediterranean climate.

MATERIALS AND METHODS

Experimental design

In an *in vitro* experiment a batch culture system was used to simulate a rumen environment. Rumen fluid was collected from 3 ruminally cannulated Polish Holstein-Friesian cows (weight 680±23 kg) fed with the diet (kg. day^{-1}) composed of lucerne silage, 46.0; meadow hay, 1.80; maize meal, 0.90; dry brewer's grains, 0.60; protein concentrate (35% crude protein), 1.50; wheat bran, 0.60; and commercial concentrate (19% crude protein), 5.50. The rumen fluid was sampled before morning feeding, squeezed through 4-layers cheesecloth into a Schott Duran® bottle (one litter) with an O_2-free headspace, transported within 30 minutes under anaerobic conditions to the laboratory at 39°C and used as a source of inoculum. The cows were fitted with ruminal cannulas by surgical procedure approved by the guidelines of Local Ethical Board for animal treatment. The batch culture method was adopted from Szumacher-Strabel *et al*. (2004). As a basal substrate (control) mixture of meadow hay and yellow corn in the ratio 60:40 diet was used. The control diet consisted of 240 mg of meadow hay (dry matter – 897 g kg^{-1}, crude protein – 164 g kg^{-1} DM, crude fiber – 256 g kg^{-1} DM, ether extract – 19 g kg^{-1} DM) and 160 mg of yellow corn (dry matter – 856 g kg^{-1}, crude protein – 138 g kg^{-1}DM, crude fiber – 44 g kg^{-1} DM, ether extract – 19 g kg^{-1} DM), both substrates grounded to 1 mm. Weight of sample from each experimental ration was 400 mg. Substitution of yellow corn by dried sugar beet pulp (DSBP) was tested in five treatments: control, 0 % of DSBP; D1, 25% DSBP; D2, 50% DSBP; D3, 75% DSBP and D4, 100% DSBP in four replicates for each repetition. Each repetition comprised of four vessels for each substitution, a control without extract and four vessels as blanks (without any substrate). The experiment was repeated within two consecutive days. The incubation flasks, sealed with rubber stoppers and aluminium caps, were placed in an incubator for 24 h and periodically mixed every few hours.

Sampling procedures and analytical method

Dry matter was determined by oven drying at 110ºC for 48 h. Standard methods were used for ash (No. 942.05), crude protein (No. 968.06), crude fiber (No. 942.05) and crude fat (No. 942.05) according to AOAC (1990). The content of non-fiber carbohydrates (NFC) was calculated according to following equation: 1000 – (crude protein + crude fat + ash + NDF) and expressed as g/kg DM (Cozzi *et al*., 2002). Neutral detergent fiber (NDF) (with amylase treatment), acid detergent fiber (ADF) and acid detergent lignin (ADL) excluding residual ash were determined according to the methods of van Soest and Wine (1967). The cellulose and hemicelluloses were calculated by difference, where, cellulose = ADF-ADL and hemicelluloses = NDF-ADF. Chemical composition and fiber fractions of corn grain, meadow hay and sugar beet pulp are presented in Table I, whereas the percentage composition of concentrate components in Table II.

Table I.- Chemical composition of corn grains, meadow hay and dried sugar beet pulp (g/kg).

Item	Corn grains	Meadow hay	Dried sugar beet pulp
DM	890	900	910
OM	982	910	940.7
Ash	18.0	38.9	59.3
CP	86.5	135	96.2
CF	29.2	27.7	219.8
EE	48.3	300	6.60
NFC	757.2	388.7	407.9
NDF	90.0	409.7	430
ADF	22.0	313.2	270
ADL	10.1	83.7	20.2
Cellulose	20.2	229.6	250
Hemicellulose	68.3	96.40	160

DM, dry matter; OM, organic matter; CP, crude protein; CF, crude fiber; EE, ether extract; NFC, non-fibrous carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

Table II.- Percentage composition of concentrate components.

Item	Ingredients in rations				
	Control	D1	D2	D3	D4
Corn grains	60.00	45.00	30.00	15.00	0.00
Sugar beet pulp	0.00	15.00	30.00	45.00	60.00
Soybean meal	17.50	17.50	17.50	17.50	17.50
Wheat bran	20.00	20.00	20.00	20.00	20.00
Limestone	1.00	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00	1.00
Vitamins and mineral mix	0.50	0.50	0.50	0.50	0.50

Control, 0 % of DSBP; D1, 25% DSBP; D2, 50% DSBP; D3, 75% DSBP; D4, 100% DSBP.

Gas and methane production

After 24 h of incubation, gas production was estimated by the displacement of gastight syringe piston, which was connected by needle to the serum flasks. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank vessels (without substrate) from total gas produced in the vessels containing buffered rumen fluid and substrate. The gas and methane production measurements were adopted from Szumacher-Strabel *et al*. (2004).

Ammonia concentration

Quantitative analysis of ammonia concentration was carried out by a modified Nessler's method modified by Cieslak *et al*. (2016).

Volatile fatty acids (VFA)

At the end of incubation, 3.6 ml of fermented rumen fluid was stabilized with 0.4 ml of a 46 mM $HgCl_2$ solution and frozen until analyses by HPLC. After thawing the mixture was centrifuged at 12000 rpm for 10 min, filtered through 0.22 µm and 10 µl of clear supernatant was injected to the High Performance Liquid Chromatograph Waters 2690 equipped with Waters 2487 Dual λ detector and Aminex HPX-87H column (300 mm x 7.8 mm) according to Cieslak *et al*. (2016).

Table III.- Chemical composition and fiber fractions of experimental rations.

Item	Experimental rations					P value
	Control	D1	D2	D3	D4	
Chemical composition (%)						
DM	91.48	91.34	91.97	91.78	92.18	0.424
OM	92.79	92.43	92.42	92.39	91.42	0.325
Ash	7.21	7.57	7.58	7.61	8.58	0.120
CP	15.23	15.39	15.42	15.49	16.02	0.525
CF	11.27[c]	12.58[c]	14.56[b]	17.18[a]	18.80[a]	0.021
EE	3.86[a]	2.66[ab]	2.54[ab]	2.40[ab]	1.56[b]	<0.011
NFC	43.97[a]	36.55[b]	35.8[b]	34.75[b]	31.03[c]	0.011
Fiber fractions						
NDF	29.73[c]	37.83[b]	38.66[ab]	39.75[b]	42.81[a]	<0.001
ADF	25.05[c]	26.25[c]	28.64[b]	30.51[a]	32.57[a]	<0.001
ADL	5.03[d]	8.38[c]	8.61[c]	13.11[b]	16.80[a]	<0.001
Cellulose	20.03[a]	17.88[b]	20.03[a]	17.40[bc]	15.76[c]	<0.001
Hemicellulose	4.68[c]	11.57[a]	10.03[ab]	9.24[b]	10.24[ab]	0.004

a and b, values within a row with different letters differ P <0.05. For abbreviations, see Table I. For treatment details, see Table II.

In vitro *dry matter digestibility*

For the *in vitro* dry matter digestibility (IVDMD) the same experimental design was used as during the batch culture. The buffered rumen fluid was incubated with 400 ± 1 mg of substrate for 24 h at 39°C. After incubation, the incubation vessels content was transferred to the previously weighed crucibles. The residues of incubation were washed with 50 ml distilled water and dried at 105°C for 3 days. The percent loss in weight of dry matter (DM) of the feed was determined and presented as IVDMD. After that, crucibles were fired to determine the organic matter digestibility.

Rumen microflora

Samples of the rumen fluid after fermentation for counting of ciliate protozoa or bacteria were collected in duplicates after 24-h fermentation. The population of bacteria was obtained with Thoma counting chamber (Blau Brand®, Wertheim, Germany; Ericsson *et al*., 2000). Counts of protozoa (*i.e., Entodiniomorphs* and *Holotrichs*) were determined according to Michalowski *et al*. (1986).

Statistical analyses

Batch culture experiment (24 h incubation) was performed in four replications (4 incubation vessels) for each of the treatments (n = 5). Data were analyzed by ANOVA using the GLM procedures of SAS (Version 6.0; SAS Inst. Inc. Carry, NC, 1989) with treatment as a factor. Treatment means were calculated using the LSMEANS option of SAS.

RESULTS

Chemical composition

The chemical composition of DSBP used in the present study is presented in Table I. The percentage composition of concentrate components is presented in Table II, whereas the chemical analyses of the five experimental diets are given in Table III. Results of DM, OM, ash and CP contents did not differ among treatments. The main difference among rations relates to carbohydrate content. Crude fiber content increased linearly with increasing dried sugar beet pulp percentage in diets, what was reflected in significant differences in D2, D3 and D4 groups. Content of NFC was statistically higher in control compared with all experimental rations. The group with the highest level of DSBP (D4) was characterized by the lowest NFC value (31.0%). Concentration of neutral detergent fiber (NDF) was significantly higher in all experimental diets compared with control diet. Acid detergent fiber (ADF) and acid detergent lignin (ADL) increased linearly with increasing level of DSBP supplementation.

In vitro *ruminal fermentation*

Results of the effect of corn grains substitution by beet pulp on rumen pH, ammonia, total gas production,

Table IV.- Effect of corn grains substitution by dry beet pulp on *in vitro* ruminal fermentation.

Item	Experimental rations					SE	P value
	Control	D1	D2	D3	D4		
Rumen kinetics							
pH	6.09	6.09	6.07	6.07	6.10	0.007	0.515
NH_3 (mM)	12.4	11.7	11.8	12.2	12.2	0.103	0.171
Total gas production (ml)	127	129	131	129	127	0.611	0.444
Methane (mM)	8.30	8.66	9.85	9.50	9.92	0.238	0.08
IVDMD (%)	38.3	41.4	44.2	38.1	36.6	1.327	0.405
Microbial analysis (cells/ml)							
Rumen bacteria (x 10^8)	21.6 bc	16.0 c	17.5 bc	24.5 ab	27.8 a	1.30	0.001
Total protozoa (x 10^1)	5681 ab	6498 a	2928 ab	3348 ab	2730 b	425.1	0.021
Entodinimorphs (x 10^1)	5250 ab	6183 a	2450 ab	2800 ab	2100 b	448.5	0.016
Holotrichs (x 10^1)	431 bc	3150 c	478 bc	548 a	630 a	30.1	<0.001
Volatile fatty acids (VFA; mM)							
Total VFA	37.55	36.37	25.61	25.82	31.56	1.429	0.164
Acetate (A)	15.79	15.67	11.73	12.08	17.12	0.582	0.104
Propionate (P)	11.65	10.68	7.62	7.50	7.82	0.473	0.112
Isobutyrate	0.53	0.51	0.26	0.28	0.22	0.039	0.174
Butyrate	7.20	7.11	4.81	4.85	5.17	0.309	0.166
Isovaleriate	1.05	1.01	0.51	0.49	0.57	0.069	0.091
Valeriate	1.34	1.38	0.70	0.66	0.67	0.092	0.071
A/P	1.36 b	1.47 ab	1.54 ab	1.64ab	2.30 a	0.082	0.024

IVDMD, *in vitro* dry matter digestibility. a and b, values within a row with different letters differ P <0.05. For treatment details, see Table II.

methane concentration, *in vitro* dry matter digestibility, microbial counts, and volatile fatty acids are shown in Table IV. No significant differences were observed in rumen pH among the rations. Rumen ammonia content indicated no differences among rations, that may suggest the similar direction of protein metabolism in control and experimental rations. Rumen bacteria counts presented also in Table IV indicated that the complete replacement of corn by sugar beet pulp (D4 ration) had a significant effect on bacteria number in comparison with the control diet. Bacteria count was statistically higher in D4 group. No statistically significant difference in IVDMD was reflected in the lack of differences either in total VFA or the individual VFA. However, the acetate: propionate ratio was the lowest for the corn grain treatment (control group, 1.36) and the highest for D4 ration where 100 % of DSBP was used (2.30).

DISCUSSION

Chemical composition

Chemical composition of the beet pulp ranged usually from 83.80 to 92.49% for DM 9.33-10.71% CP, 0.10-2.40% EE, 18.40-22.37% CF (Mansfield *et al.*, 1994). In the present study the observed differences were mainly due to changes in proportion of corn to DSBP diets. Corn was characterized by a lower crude fiber content compared with DSBP (29 *vs.* 220 g/kg). Changes in carbohydrate fractions content that mean increased NDF, ADF and ADL and simultaneous linear decrease of NFC in experimental groups when compared with control agree with experiment by Voelker and Aleen (2003), when high moisture corn fed to dairy cattle was replaced by dried sugar beet pulp up to 24%. Also, Mojtahdi and Danesh-Mesgaran (2011) showed that increasing level of sugar beet pulp (up to 33% DM of concentrate) resulted in higher concentration of NDF and ADF. The increase of structural carbohydrates concentration in ruminant's low-forage diets may improve the chewing behavior, ruminal environment and nutrient digestibility.

In vitro *ruminal fermentation*

In the research reported by Bodas *et al*. (2007) partial replacement of barley grain with sugar beet pulp (12% DM) in the basal concentrate markedly increased ruminal pH 5.5 *vs.* 6.7. In another study ruminal pH increased linearly with the substitution of sugar beet pulp for barley grain in the diet of late lactation cows (Mahjoubi *et al*., 2009). The high level of NDF and non-starch polysaccharides (*e.g.* pectin) that are present in sugar beet pulp usually results in a higher rumen pH compared to barley grain (Mahjoubi *et al*., 2009). One of the reasons for higher pH in cows fed the diets consisting sugar beet pulp is, that pectin was not fermented to lactate, which is acidifying agent (Strobel and Russell, 1986). Contrary, ruminal pH was not affected in studies by Mandebvue and Galpraith (1999) when sugar beet pulp was replaced by barley. Stable level of rumen pH in the study by Mandebvue and Galpraith (1999) could be obtained by molasses sugar beet pulps which have similar level of non-structural carbohydrates like barley.

Ammonia concentration was not affected by the experimental factors because the level of available energy in the rumen was at the same level regardless the applied dose, as it was previously observed by Mojtahedi and Danesh-Mesgaran (2011). That may also suggest the similar tendency of protein metabolism in control and experimental rations. Various reports have indicated no effect of DSBP on ruminal ammonia concentration (Bodas *et al*., 2007; Mahjoubi *et al*., 2009). Mojtahedi and Danesh-Mesgaran (2011) found that inclusion of sugar beet pulp in the diet resulted in a linear decrease in minimum and maximum ammonia concentrations, but the range and variance of ammonia concentration were similar among treatments. The ammonia concentration in the rumen is directly dependent on carbohydrate availability (Russell *et al*., 1983).

Diet 3 and diet 4 (75 or 100% of DSBP) had positive effects on *Holotrichs* populations; however total protozoa and *Entodiniomorphs* number numerically decreased. The rumen protozoa population is influenced by a number of interacting factors, like the nature of the diet consumed. *Holotrichs* number in the rumen are usually increased when the diet contains a source of readily available soluble carbohydrates content *e.g.* dry sugar beet pulp. Hence, the increased number of *Holotrichs* in the present study where the DSBP was used. On the other hand, the increasing numbers of bacteria and *Holotrichs* population, in the present study, did not affect *in vitro* dry matter digestibility (IVDMD). Silva and Ørskov (1988) showed that feeding DSBP might promote growth of cellulolytic and hemicellulolytic microorganisms and this, in turn, should increase the extent of nutritive components digestion. The sugar beet pulp is a rich source of pectin, which can directly influence on concentration of acetic and propionic acids. Marounek *et al*. (1985) reported a greater acetate:propionate ratio for fermentation of component rich in pectin (6.4) than for starch (1.9).

CONCLUSION

The results of this *in vitro* study demonstrated that dried sugar beet pulp can be used as feed ingredient of the ruminant diets in Mediterranean countries and can replace even up to 100% of high-energy yellow corn. However, the suggested doses are 75% because this amount does not alter the basic rumen fermentation parameters. The practical utilization of this dietary supplement should be verified in *in vivo* study.

Statement of conflict of interest

The authors declare no conflict of interest.

REFERENCES

AOAC, 1990. *Official methods of analysis*, 13th ed. Association of Official Analytical Chemists, Washington, DC.

Bath, D.L, 1981. Feed by-products and their utilization by ruminants. In: *Upgrading residues and by-products for animals* (ed. J.T. Huber). CRC Press, Boca Raton, FL, pp. 16.

Bodas, R., Giraldez, F.J., Lopez, S., Rodrıguez, A.B. and Mantecon, A.R., 2007. Inclusion of sugar beet pulp in cereal-based diets for fattening lambs. *Small Rumin. Res*., **71**: 250-254. https://doi.org/10.1016/j.smallrumres.2006.07.006

Cieslak, A., Zmora, P., Matkowski, A., Nawrot-Hadzik, I., Pers-Kamczyc, E., El-Sherbiny, M., Bryszak, M. and Szumacher-Strabel, M., 2016. Tannins from *Sanguisorba officinalis* affect *in vitro* rumen methane production and fermentation. *J. Anim. Pl. Sci.,* **26**: 54-62.

Cozzi, G., Burato, G.M., Berzaghi, P. and Andrighetto, I., 2002. Evaluation of pellets from different industrial processing of dehydrated lucerne in dairy cattle feeding. *Anim. Feed Sci. Technol*., **99**: 13-24. https://doi.org/10.1016/S0377-8401(02)00111-6

Crosby-Galván, M.M., Espinoza-Velasco, B. and Ramirez-Mella, M., 2018. Effect of Chihua pumpkin residue (*Cucurbita argyrosperma*) in ruminal gas production and digestibility *in vitro*. *Pakistan J. Zool.,* **50**: 1-3. http://dx.doi.org/10.17582/journal.pjz/2018.50.3.sc1

Ericsson, M., Hanstorp, D., Hagberg, P., Enger, J. and Nystrom, T., 2000. Sorting out bacterial viability with optical tweezers. *J. Bacteriol*., **182**: 5551-5555. https://doi.org/10.1128/JB.182.19.5551-5555.2000

Fadel, J.G., 1999. Quantitative analyses of selected plant by-product feedstuffs, a global perspective. *Anim. Feed Sci. Technol*., **79**: 255-268. https://doi.org/10.1016/S0377-8401(99)00031-0

FAO, 2005. *Production-crops*. Food and Agriculture Organization, Rome, Italy. Available at: http://www.fao.org/faostat/en/#data/QC (Accessed on 20 March, 2019).

FAO, 2011. *Production-crops*. Food and Agriculture Organization, Rome, Italy. Available at: http://www.fao.org/faostat/en/#data/QC (Accessed on 20 March, 2019)

Hall, M.B., Pell, A.N. and Chase, L.E., 1998. Characteristics of neutral detergent-soluble fiber fermentation by mixed ruminal microbes. *Anim. Feed Sci. Technol*., **70**: 23-39. https://doi.org/10.1016/S0377-8401(97)00068-0

Kjaergaard, L., 1984. Examples of biotechnological utilization of beet and bagasse sugar. *Technol. Rev*., **10**: 183.

Mahjoubi, E., Amanlou, H., Zahmatkesh, D., Ghelich Khan, M. and Aghaziarati, N., 2009. Use of beet pulp as a replacement for barley grain to manage body condition score in over-conditioned late lactation cows. *Anim. Feed Sci. Technol*., **153**: 60-67. https://doi.org/10.1016/j.anifeedsci.2009.06.009

Mandebvu, P. and Galbraith, H., 1999. Effect of sodium bicarbonate supplementation and variation in the proportion of barley and sugar beet pulp on growth performance and rumen, blood and carcass characteristics of young entire male lambs. *Anim. Feed Sci. Technol*., **82**: 37-49. https://doi.org/10.1016/S0377-8401(99)00099-1

Mansfield, H.R., Stern, M.D. and Otterby, D.E., 1994. Effects of beef pulp and animal by-products on milk yield and *in vitro* fermentation by rumen microorganisms. *J. Dairy Sci*., **77**: 205-216. https://doi.org/10.3168/jds.S0022-0302(94)76943-5

Marounek, M., Bartos, S. and Brezina, P., 1985. Factors influencing the production of volatile fatty acids from hemicellulose, pectin and starch by mixed culture of rumen microorganisms. *Z. Tierphysiol. Tierernahr. Futtermittelkd*., **53**: 50-58. https://doi.org/10.1111/j.1439-0396.1985.tb00006.x

Michałowski, T., Szczepkowski, P. and Muszyński, P., 1986. The nutritive factors affecting the growth of the rumen ciliate *Diploplastron affine in vitro*. *Acta Protozool*., **25**: 419-426.

Mohamed, A.E., 1998. F*eeding ruminants with treated agricultural by-products to raise their nutritive value*. M. Sc. thesis, Faculty of Agriculture, Cairo University, Giza, Egypt.

Mojtahedi, M. and Danesh-Mesgaran, M., 2011. Effects of the inclusion of dried molassed sugar beet pulp in a low-forage diet on the digestive process and blood biochemical parameters of Holstein steers. *Livest. Sci.*, **141**: 95-103. https://doi.org/10.1016/j.livsci.2011.05.009

Molina-Alcaide, E., Ynez-Ruiz, D.R., Moumen, A. and Martn-Garca, I., 2003. Chemical composition and nitrogen availability for goats and sheep of some olive by-products. *Small Rumin. Res*., **49**: 329-336. https://doi.org/10.1016/S0921-4488(03)00148-2

Russell, J.B., Sniffen, C.J. and van Soest, P.J., 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. *J. Dairy Sci*., **66**: 763-775. https://doi.org/10.3168/jds.S0022-0302(83)81856-6

Sarwar, M.A., Hussain, F. and Chattha, A.A., 2008. After harvest qualitative and quantitative behaviour of some sugar beet varieties. *J. Anim. Pl. Sci*., **18**: 139-141.

Silva, A.T. and Ørskov, E.R., 1988. The effect of five different supplements on the degradation of straw in sheep given untreated barley straw. *Anim. Feed Sci. Technol*., **19**: 289-298. https://doi.org/10.1016/0377-8401(88)90091-0

Strobel, H.J. and Russell, J.B., 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. *J. Dairy Sci*., **69**: 2941-2947. https://doi.org/10.3168/jds.S0022-0302(86)80750-0

Szumacher-Strabel, M., Martin, S.A., Potkański, A., Cieślak, A. and Kowalczyk, J., 2004. Changes in fermentation processes as the effect of vegetable oil supplementation in *in vitro* studies. *J. Anim. Feed Sci*., **13**(Suppl. 1): 215-218. https://doi.org/10.22358/jafs/73843/2004

van Soest, P.J. and Wine, R.M., 1967. Use of detergent in the analysis of fibrous feed. IV. Determination of plant cell wall constituent. *J. Assoc. Off. Anal. Chem*., **50**: 50-55.

Voelker, J.A. and Allen, M.S., 2003. Pelleted beet pulp substituted for high-moisture corn: 1. Effects on feed intake, chewing behavior, and milk production of lactating dairy cows. *J. Dairy Sci*., **86**: 3542-3552. https://doi.org/10.3168/jds.S0022-0302(03)73960-5