INCLUSION OF SUGAR BEET PULP IN RUMINANT DIETS. 2 - CHANGES OF RUMEN FERMENTATION, MICROBIAL COUNT AND ENZYMATIC ACTIVITY ASSOCIATED WITH FEEDING DIFFERENT LEVELS OF UREATED SUGAR BEET PULP IN RATIONS OF GROWING SHEEP

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SUMMARY

Ruminal fluid of 18 (Ossimi X Rahmani) yearling male sheep was collected after 24 weeks of feeding on mixtures containing 0 , 50 and 100% 3% ureated-sugar beet pulp (USBP) to investigate changes in the rumen media that associated with feeding USBP. Experimental animals in three equal groups were fed individually on; 100% common feed mixture (CFM) for group I, 50% CFM + 50% USBP for group II and 100% USBP for group III. Rations were offered once daily at 4% of body weight and one kg/h/d of fresh berseem (*Trifolium alexandrinum*) was offered after 4hrs. of the morning meal. Samples of ruminal fluid (100ml/animal) were collected after 4 and 24 hrs. of feeding by a stomach tube. Rumen pH, NH_3-N and VFA's concentrations were immediately determined. Ruminal microbes were counted for cellulolytic, proteolytic, methanogenic, lactobacilli and streptococci bacteria and fungi. Enzymatic activity for cellulase, polygalacturonase (PG) and pectinestrase (PE) was estimated by using a liquid media of *Aspergillus niger*.

Daily feed intake was ($P<0.01$) decreased with feeding 100% USBP ration, however, no significant difference of DM intake was detected in comparison with control feeding USBP at 50% replacement level. Rumen pH at 4 and 24 hrs. after feeding showed higher ($P<0.05$) values for USBP rations. Ammonia - N concentration was ($P<0.05$) lower after 4 hrs. of feeding on 50% USBP ration, while it was higher ($P<0.05$) than that of control after 24hrs. of feeding on 100% USBP ration. Total VFA's concentration at 4 and 24 hrs. of feeding was comparable among groups. Microbial count was drastically lowered on 100% USBP ration, meanwhile most of microbial strains count was nearly two times greater on ration containing 50% USBP in comparison with those of control ration. Ruminal enzymes (U/100ml fluid) were generally higher ($P<0.05$) after 4 than 24 hrs. of feeding. Cellulase enzyme was lower ($P<0.05$) for rations containing USBP. Polygalacturonase (PG) activity showed similar values with experimental rations after 4 hrs., while PG sustained at a higher level ($P<0.05$) after 24 hrs. of feeding with 100% USBP ration. Pectinesterase (PE) was poorly produced in the rumen media of animals fed the USBP-free ration (control). The highest level of PE was reached after 24 hrs. of feeding with ration containing 100% USBP.

It is concluded that, the inclusion level of 3% ureated-SBP at 50 or 100% to replace common CFM had a remarkable influences on ruminal fermentation pattern, microbial count and enzymatic activities. The partial replacement of CFM with 50% USBP is highly recommended in rations of growing sheep, while the complete replacement with USBP was

associated by a dramatic fall of rumen microbial count and enzymatic activity and a subsequently significant decrease of the daily feed intake.

Keywords: sugar beat pulp, sheep, *feed intake, rumen fermentation, polygalacturonase, pectinesterase.*

INTRODUCTION

Beet pulp is the solid by-product left after sugar extraction from beet. It is normally pressed, dried and processed in cubes either with or without molasses supplementation to be used as an animal feedstuff. The sugar content of the manufactured dried beet pulp is low (< 5% of the dry weight) and its starch content is zero, which make it unsuitable energy source for monogastrics (Hanksson, 1982). Meanwhile, beet pulp is a rich material of polysaccharides containing in average 6% galactan, 28% arabun, 25% cellulose and 25% pectin (McCready, 1966). The cellulose structure of sugar beet pulp (SBP) is mainly amorphous, which make it easily hydrolyzable (Kjaergaard, 1984) and its pectin content is not covalently linked to a lignified matrix, which make it available source of readily fermentable carbohydrate to enhance the microbial biosynthesis in the rumen (McCready, 1966; Matwally and Stern, 1989 and Mansfield *et al.*, 1994). The impact of feeding dried SBP on rumen fermentation was investigated in many studies (Bhattacharya *et al.*, 1985; Gilad *et al.*, 1988; Mansfield *et al.*, 1994; Chilliard *et al.*, 1996 and Molina *et al.*, 2000); however, the results did not show clear trends and they were contradictory. On the other hand, the effect of feeding beet pulp on rumen microbial population and microbial enzymatic activity was poorly studied. Chilliard *et al.* (1996) concluded that the microbial protein production was improved on rations containing SBP. Molina *et al.*, (1999) found no effect of feeding rations containing 25 or 50% SBP on protozoal counts of sheep. At the same time, none of the previous studies gave attention to certain ruminal enzymes needed to hydrolyze the polysaccharides of beet pulp. However, polygalacturonase and pectinesterase were noted to have a specific role in hydrolyzing SBP-pectin (Mohamed *et al.*, 1988), which is the major readily fermentable carbohydrate source providing energy for microbial biosynthesis.

Therefore, this study was conducted to investigate the possible changes in the rumen media that associated with feeding different levels of untreated sugar beet pulp in replacement of common feed mixtures fed local growing sheep.

MATERIALS AND METHODS

After a feeding period of 24 successive weeks, ruminal fluid of 18 (Ossimi x Rahmani) yearling male sheep in three groups was collected to investigate possible changes in the rumen media due to feeding beet pulp at different levels. Dry untreated sugar beet pulp supplemented with 3% urea (USBP) was included in feed mixtures to replace 0, 50 and 100% of the common concentrate feed mixt on grains and grain by products. Proportional replacement of USBP in experimental mixtures and their chemical composition according to A.O.A.C. (1990) methods are given in Table 1. Experimental feed mixtures of each group (I, II and III) were offered once daily and rumen liquor was individually collected at 4 and 24 hrs. after feeding. Samples of 100 ml of rumen liquor were collected from animals of each group at sampling times on two successive days. Rumen liquor was withdrawn by a rubber stomach tube and

strained through four layers of cheese cloth and kept warm at 35-37°C for immediate tests, while the rest of samples were kept frozen at -4°C until further analysis.

Ruminal pH was immediately measured using pH-meter (Model 201 Orion Research Digital). Ammonia -N concentration was determined according to Conway (1962) and total VFA's concentration was analysed according to Warner (1964).

Counts of ruminal microbes were estimated in its selective media for total and cellulolytic bacterial count according to Hungate (1957), proteolytic bacteria (Smith *et al.*, 1952), methanogenic bacteria (Smith and Hungate, 1958), lactobacilli (Rogosa *et al.*, 1959), streptococci (Medrek and Barnes, 1962), and fungal count was estimated at 30°C for 2-7 days using the technique of colony forming unit (CFU) according to Allen (1959).

Cellulase assay:

Cellulase activity was measured according to Fischer and Kohtes (1951) using CM-cellulose (1,4-B-D-glucanhydrolase, Ec 3.2.7.2) with rumen enzyme preparation and acetate buffer (pH5.5) and the mixture was incubated at 39°C for one hour. Dinitrosalicytic acid (DNS) reagent was added to the mixture with boiling for 15 min. where the colour developed indicating the concentration of the reducing sugars that had been liberated. The colour density was measured at 560nm and the concentration of the enzymatically liberated reducing sugars was calculated in comparison with a standard curve of glucose. Cellulase activity was expressed in term of unite that defined as the amount of enzyme producing 1.0 μ mole of reducing sugar per hour.

Polygalacturonase (PG) assay:

Polygalacturonic acid was used as a substrate for PG assay according to Valsangiacomo and Gessler (1992) by measuring the formation of reducing sugar using DNS reagent. Reducing sugar was calculated from a previously established standard curve using galacturonic acid as a standard. One unit of enzyme activity was defined as the amount of enzyme producing 1.0 ? mole of reducing sugar per hour.

Pectinesterase (PE) assay:

Pectin was used as a substrate for PE assay according to Wood and Siddiqui (1971) by measuring the formation of methanol. Methanol was calculated from a previously established standard curve using methanol as a standard. One unit of enzyme activity was defined as the amount of enzyme producing 1.0 μ mol methanol per hour under a standard assay conditions.

Collected data of ruminal fermentation, microbial population and enzymatic activity within the two sampling times (4 and 24 hrs. after feeding) were subjected to statistical analysis applying the one way analysis of variance by using the General Linear Models Procedure adapted by SAS (1988) for PC computers. Significant means were separated using the L.S.D. test according to Duncan (1955).

RESULTS AND DISCUSSION

Daily offered, refused and consumed amounts of feeds by sheep in experimental groups are given in Table (2). It was clear that inclusion of 3% ureated sugar beet pulp (USBP) at 50% of the feed mixture did not influence the DM intake of sheep in comparison with that of control ration (0% USBP). While, daily DM intake significantly ($P<0.01$) decreased in group (III) fed on 100%

Table 1. Chemical composition of experimental feeds.

Item	DM %	DM composition, %				
		CP	EE	CF	NFE	Ash
100% CFM[i] (I)	88.61	15.20	2.50	14.61	60.28	7.41
50% CFM + 50% USBP[ii] (II)	89.30	15.26	1.82	17.08	59.90	5.94
100 % USBP (III)	89.95	15.30	1.40	19.68	59.20	4.42
Berseem fodder	10.00	13.09	2.00	24.76	43.09	17.06

i Commercial concentrates mixture consisting (as fed basis) of: 30% undecorticated cotton seed meal, 30% yellow corn, 30% wheat bran, 7% cane – molasses, 2% lime stone and 1% sodium chloride.
ii Weekly prepared, by spraying a solution of 30 g urea dissolved in 100 ml water per kg of sugar beet pulp.
DM = dry matter, CP = crude protein, EE = ether extract and NFE = nitrogen free extract.

Table 2. Daily offered, refused and consumed amounts of feeds by sheep in experimental groups.

Item	Experimental groups			SE
	I (Control)	II (50% USBP)	III (100% USBP)	
Experimental feeding period, wk	24	24	24	
No. of animals	6	6	6	
Mean body weight, kg	34.17±7.88[a]	39.21±8.34[a]	27.90±8.13[b]	3.31*
Mean amount of feeds (DM basis),kg/d				
Offered:				
Feed mixture	1.198	1.348	0.984	
Berseem	0.100	0.100	0.100	
Refused:				
Feed mixture	0.00	0.081	0.325	
Berseem	0.00	0.00	0.00	
Consumed:				
kg/d	1.30±0.27[Aa]	1.37±0.26[Aa]	0.76±0.11[B]	0.09**
of body weight, %	3.81±0.09[Aa]	3.50±0.17[Aa]	2.80±0.38[B]	0.10**

NS = non-significant difference. * P<0.05 ** P<0.01
a,b means with different superscripts in the same row are different at P<0.05
A,B means with different superscripts in the same row are different at P<0.01

Table 3. Rumen fermentation patterns at 4 and 24 hrs. after feeding for sheep in experimental groups.

Item	Experimental groups			SE
	I (control)	II (50% SBP)	III (100% USBP)	
4hrs. after feeding				
PH	6.67[b]	6.72[b]	7.07[a]	0.10*
VFA's, m.eq./dl	10.83	10.48	11.20	0.19 NS
NH_3-N, mg/dl	35.03[a]	25.77[b]	33.20[a]	2.17*
24hrs. after feeding				
PH	6.97[b]	7.18[ab]	7.38[a]	0.09*
VFA's, m.eq./dl	8.02	8.13	8.18	0.21NS
NH_3-N, mg/dl	10.13[b]	9.65[b]	16.47[a]	2.32*

NS = non-significant difference. * P<0.05
a,b Means with different superscripts in the same row are different at P<0.05.

USBP ration. Voluntary DM intake of 100% USBP ration was in average 2.80% of body weight and about 33% of daily offered USBP was uneaten until the end of the feeding period. The lower DM intake with the increasing level of SBP over than 50% of the total ration was confirmed by findings of Bhattacharya *et al.* (1975) and Mandebvu and Galbraith (1999) on sheep and Mohsen *et al.* (1999) on growing Angora goats. The lower DM intake on ration containing 100% USBP might be explored by investigating some changes that taken place in the rumen media.

Rumen fermentation parameters determined after 4 and 24 hrs. of feeding for sheep in experimental groups are shown in Table 3. Ruminal pH was higher ($P<0.05$) on ration containing 100% USBP at the two sampling times, however, corresponding values for 0 or 50% USBP rations were not statistically different. Total VFA's concentration was generally influenced by the time after feeding rather than type of feed. Ruminal VFA's was higher after 4 than 24 hrs. of feeding in all groups, while no statistical difference was detected between groups for VFA's due to feeding different levels of USBP. Ammonia-N concentration after 4 hrs. of feeding was lower ($P<0.05$) on 50% USBP than on the other two rations, while NH_3-N concentration remained higher ($P<0.05$) after 24 hrs. on ration containing 100% USBP. The present results are in favour with the findings of Castle (1972), Rymer and Armstrong (1989) and Molina *et al.* (2000), who stated that ruminal pH tended to be higher and more stable with increasing the replacement level of grains with SBP in rations of sheep, beef bulls and dairy cattle. It was also mentioned that, total or proportional VFA's concentration was not significantly influenced by chainging the carbohydrate source from grains to SBP (Bhattacharya and Sleiman, 1971; Mohsen *et al.*, 1999 and Molina *et al.*, 2000). However, higher acetate and lower butyrate and volatile branched-chain fatty acids were noticed for dairy cattle fed corn based rations partially replaced with SBP (Sabri *et al.*, 1988; Metwally and Stern, 1989 and Mansfield *et al.*, 1994). The effect of feeding SBP on ruminal NH_3-N concentration has not been confirmed yet. Gihad *et al.* (1989) and Chikunya *et al.* (1996) found that NH_3-N decreased with increasing the level of SBP in ration of sheep. On the contrary, Rouzbehan *et al.* (1994) with Suffolk wethers fed on 50 or 80% SBP rations, found that ruminal NH_3-N was higher ($P<0.01$) for ration contained the higher proportion level of SBP. Similar results were reported by Mohsen *et al.* (1999), who experienced a significant ($P<0.05$) increase in ruminal NH_3-N on rations contained 25 or 50% SBP in comparison with control ration (0% SBP) by Angora goat kids. Such unstable ruminal fermentation patterns with feeding SBP in previous studies are eventually due to differences of; animal type, formulation and processing of rations, SBP feeding level, supplementation level with urea and / or molasses, sampling time after feeding ... etc. From the present results the higher ruminal pH and NH_3-N after 24 hrs. of feeding on 100% USBP ration is revealing the presence of more unutilizable nitrogen in the rumen which might be due to failure of rumen microbes to get use of hydrolyzable carbohydrates of SBP when it was fed alone.

Microbial count of some bacterial strains and fungi in the rumen fluid from animals in experimental groups are given in Table 4. The number of cellulose digester bacteria was ($P<0.01$) higher after 4 hrs. of feeding on USBP-free ration (control), while it was ($P<0.01$) higher for USBP containing rations after 24 hrs of feeding, suggesting the presence of substances covalently linked with beet

Table 4. Bacterial and fungal counts (10^6 CFU/ml) in rumen fluid at 4 and 24 hrs. after feeding for sheep in experimental groups.

Item	Experimental groups			SE
	I (control)	II (50% USBP)	III (100% USBP)	
4hrs. after feeding				
Cellulose digesters	13.3^{A}	10.3^{B}	2.7^{C}	0.52^{**}
Methanogenic	6.2^{B}	14.0^{A}	3.1^{C}	0.64^{**}
Proteolytic	12.9^{Ab}	16.6^{Aa}	2.3^{B}	0.84^{**}
Lactobacilli	152.4^{ABb}	281.0^{Aa}	54.2^{Bb}	35.18^{**}
Streptococci	226.2^{ABb}	520.9^{Aa}	98.0^{Bb}	77.00^{*}
Fungi	3.2^{Aa}	4.5^{Aa}	1.0^{B}	0.42^{**}
24 hrs. after feeding				
Cellulose digesters	3.1^{Bb}	7.3^{Aa}	6.0^{Aab}	0.83^{*}
Methanogenic	4.2^{Aa}	4.0^{Aa}	2.2^{B}	0.26^{**}
Proteolytic	2.0^{b}	3.1^{a}	3.0^{a}	0.22^{*}
Lactobacilli	54.3^{Ba}	122.5^{A}	25.1^{Bb}	7.69^{**}
Streptococci	93.0^{Ba}	227.0^{A}	27.4^{Ba}	28.10^{**}
Fungi	4.0^{Aa}	5.1^{Aa}	1.0^{B}	0.44^{**}

* Each value is the mean count of 6 samples / time/group. * P< 0.05 ** P < 0.01
a,b Means with different superscripts in the same row are different at P<0.05.
A,B,C Means with different superscripts in the same row are different at P<0.01.

Table 5. Enzymatic yield (U/dl) of rumen fluid at 4 and 24 hrs. after feeding for sheep in experimental groups.

Item	Experimental groups			SE
	I (control)	II (50% USBP)	III (100% USBP)	
4hrs. after feeding				
Cellulase	5340^{Aa}	2977^{ABb}	1956^{Bb}	621.57^{*}
Polygalacturonase (PG)	2123	2491	2232	106.27^{NS}
Pectinesterase (PE)	71^{B}	527^{Aa}	554^{Aa}	38.80^{**}
24 hrs. after feeding				
Cellulase	1493^{Aa}	1489^{Aa}	681^{B}	74.56^{**}
Polygalacturonase (PG)	70^{C}	170^{B}	358^{A}	19.38^{**}
Pectinesterase (PE)	81^{C}	550^{B}	971^{A}	25.40^{**}

NS = non-significant difference. * P<0.05 ** P <0.01
a,b Means with different superscripts in the same row are different at P<0.05.
A,B,C Means with different superscripts in the same row are different at P<0.01.

pulp cellulose (may be pectin) delayed its hydrolysis. Methanogenic, proteolytic, lactobacilli and streptococci bacterial numbers, particularly after 4 hrs. of feeding were nearly two times greater for ration containing 50% USBP in comparison with those for control. Fungal cells count was almost similar with 0 or 50% USBP rations at 4 and 24 hrs. after feeding. In the contrast, all microbial numbers counted after 4 or 24 hrs. of feeding drastically fell down on 100% USBP ration. These results are in agreement with results mentioned by Sorokin (1983) who found that beet pulp supplemented rations increased the entry of microbial bacterial and protozoal protein into the abomasum, in sheep. Moreover, Huhtanen (1988) found that microbial protein synthesis and microbial N entering the small intestine of male cattle increased with feeding molassed-SBP as concentrate feed portion in silage based diet. In more recent study with sheep, Chikunya *et al.* (1996) found that source of N had no influence on microbial numbers or yields, but viable bacteria more than doubled and microbial protein flow increased with feeding ureated beet pulp. The present results point out, that the lower inclusion level of USBP (50%) is enhancing the bacterial number in the rumen of sheep, while the higher level of USBP (100%) is disrupting the population of most rumen microbes.

Values of ruminal enzymatic activities evaluated at 4 and 24 hrs. of feeding are shown in Table 5. Ruminal cellulase activity after 4 hrs. of feeding was higher ($P<0.01$) for USBP - free ration (control) than those containing either 50 or 100% USBP (5340 vs. 2977 and 1950 U/dl liquor). Such result might be due to the higher number of cellulose digester bacteria counted on the control ration. However, the difference in cellulase production between 0 and 50% USBP rations was depleted into a non-significant level after 24 hrs. of feeding. Polygalacturonase (PG) production was similar after 4 hrs. of feeding in all groups, while it was ($P<0.01$) higher after 24 hrs. of feeding for USBP rations (see Table 5). Pectinesterase (PE) production was ($P<0.01$) higher on USBP rations. The PE production level (U/dl liquor) was very close after 4 hrs. of feeding on 50 or 100% USBP rations (527 and 554, resp.). While PE level was maintained at 550 U/dl after 24 hrs. of feeding on 50% USBP ration, it was significantly raised up to 97/ U/dl on 100% USBP ration. At the two sampling times, PE production was extremely low on the control feed mixture (71 and 81 U/dl after 4 and 24 hrs. of feeding). The present results revealed that pectinesterase performed more specific enzyme than polygalacturonase or cellulase for beet pulp pectin hydrolysis, as it turned out from its higher production level on ration containing SBP. However, PE production was poorly liberated in the rumen media in comparison with the other two measured enzymes particularly, cellulase. Hungate (1966) demonstrated that only *Lachnospira multiparous* was classified as pectin digester in the rumen. Such illustration might give reason for the relatively limited production of PE in the rumen of sheep fed for 24 weeks on 100% USBP ration. On the other hand, the higher production of PE on USBP rations is suggesting that a qualitative modification of some ruminal enzymes was taken place due to feeding beet pulp. However, such modification might be acted more efficiently with feeding USBP in partial replacement of concentrates (50%) rather than feeding USBP alone. Spagnuolo *et al.* (1999) found that xylanase and arabinases in combination were essential enzymes to fractionate sugar beet pulp into pectin, cellulose and arabinose. Similarly, Mohamed *et al.* (2000) found that PG+ PE + xylanase at

2000 + 240 + 600 units respectively, had significant effects on decreasing the water absorptive capacity and increasing the in-vitro DM disappearance of SBP.

It is concluded that, the inclusion level at 50 or 100% of SBP supplemented with 3% urea to replace concentrates, had a remarkable influence on DM intake and ruminal activities. The partial replacement with USBP 50% is highly recommended in rations of growing sheep, while the complete replacement with USBP was associated by a dramatic fall of rumen microbial count and enzymatic activities and a subsequently significant decrease in the daily feed intake.

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إحلال تفل بنجر السكر في تغذية المجترات. ٢- تغير التخمر والعد الميكروبي والنشاط الإنزيمي في الكرش المصاحب لتغذية الأغنام النامية على علائق ذات مستويات مختلفة من تفل بنجر السكر المعامل باليوريا.

علاء الدين يحيى البدوي[١]، رشدي إبراهيم القاضي[٢]، طه محمد البدوي[١]، محمد زكريا صديق[٣]

١- قسم تغذية وإنتاج الحيوان والدواجن – المركز القومي للبحوث – الدقي – الجيزة – مصر.

٢- قسم الإنتاج الحيواني – فرع تغذية الحيوان – كلية الزراعة – جامعة القاهرة – الجيزة – مصر.

٣- قسم الميكروبيولوجيا – كلية الزراعة – جامعة القاهرة – الجيزة – مصر.

أجريت هذه الدراسة بهدف تقييم بعض التغيرات في بيئة كرش الأغنام المغذاة على مخاليط علفية مركزة ذات مستويات إحلال مختلفة بتفل بنجر السكر الجاف المعامل بـ ٣% يوريا، حيث تم جمع سائل الكرش بعد ٤ ساعات و ٢٤ ساعة من التغذية اليومية لعدد ١٥ حولي (أوسيمي × رحماني) مقسمين إلى ثلاث مجموعات متساوية عددياً. غذيت حيوانات المجموعة الأولى (الضابطة) على ١٠٠% من مخلوط العلف المعتاد (١٤% بروتين خام)، والمجموعة الثانية على ٥٠% مخلوط العلف المركز + ٥٠% تفل بنجر سكر معامل باليوريا والمجموعة الثالثة على ١٠٠% تفل بنجر السكر المعامل باليوريا. وكانت التغذية فردية في جميع المجموعات والمخاليط العلفية التجريبية تقدم مرة واحدة يومياً بمعدل ٣% من وزن الجسم بالإضافة إلى ١ كجم دريس برسيم للرأس يومياً بعد ٤ ساعات من الوجبة الصباحية. وقد استمرت التغذية على العلائق التجريبية لمدة ٢٠ أسبوعاً متصلة تم بعدها جمع عينات سائل الكرش من حيوانات كل مجموعة.

- أوضحت نتائج الدراسة أن الإحلال الكامل بتفل بنجر السكر (١٠٠%) محل المخلوط المركز قد صاحبه انخفاضاً معنوياً (P<0.01) في كمية المادة الجافة المأكولة ولم يظهر هذا التأثير عند الإحلال الجزئي من مخلوط العلف المركز بـ ٥٠% تفل بنجر السكر.
- أشارت مقاييس التخمر في بيئة الكرش إلى ارتفاع أيون الأيدروجين السالب وكذلك تركيز الأمونيا (P<0.05) في سائل الكرش للحيوانات المغذاة على العليقة المحتوية على ١٠٠% تفل بنجر السكر سواء بعد ٤ ساعات أو ٢٤ ساعة من التغذية بينما كان تركيز الأمونيا منخفض معنوياً (P<0.05) بعد ٤ ساعات من التغذية على العليقة المحتوية على ٥٠% تفل بنجر السكر مقارنة بتلك العليقة الضابطة. ولم يكن هناك اختلاف بين المجموعات التجريبية الثلاث من حيث تركيز الأحماض الدهنية الطيارة.
- أعداد بكتريا الكرش المحللة للسليلوز والبروتين والبكتريا المنتجة للميثان والمحللة للكاتات وكذلك أعداد الفطريات انخفضت بشدة في مجموعة الحيوانات المغذاة على العليقة المحتوية على ١٠٠% من تفل بنجر السكر، في حين ارتفعت أعداد بكتريا الكرش بمقدار الضعف تقريباً للحيوانات المغذاة على العليقة

المحـــتوية على ٥٠% مخلوط العلف المعتاد + ٥٠% تفل بنجر السكر مقارنة بتلك المغذاة على العليقة الضابطة.

- إنـــتاج أنزيم السليوليز انخفض ($P<0.05$) فى الحيوانات المغذاة على المخاليط العلفية المحتوية على تفل بـــنجر السكر، بينما كان إنتاج أنزيم البولى جلاكترونيز متماثلاً تقريباً فى المجموعات التجريبية الثلاث بعـــد مـــرور ٤ ساعات من التغذية، فى حين احتفظت المجموعة المغذاة على ١٠٠% تفل بنجر السكر بمســـتوى مـــرتفع معـــنوياً ($P<0.05$) مـــن إنـــتاج الأنـــزيم بعد مرور ٢٤ ساعة من التغذية مقارنة بالمجموعات التجريبية الأخرى.
- إنـــتاج أنـــزيم البكتيـــن اســـتيريز كان مرتفعاً ($P<0.01$) فى بيئة كرش الحيوانات المغذاة على العلائق المحـــتوية على تفل بنجر السكر، وواصل الانزيم ارتفاعه ($P<0.01$) فى المجموعة المغذاة على العليقة المحـــتوية على ١٠٠% تفل بنجر السكر بعد مرور ٢٤ ساعة من التغذية مقارنة بالمجموعات التجريبية الأخرى.

أشارت نتائج الدراسة إلى أن الإحلال الجزئى بـــ ٥٠% تفل بنجر السكر فى مخاليط الأعلاف المركزة قـــد كـــان له تأثير إيجابى واضح على زيادة إحياء الكرش الدقيقة واعتدال النشاط الإنزيمى فى كرش الأغنام مما صـــاحبه ارتفاع كمية المادة الجافة المأكولة، بينما كانت التغذية على العليقة الكاملة بتفل بنجر السكر تأثير معاكس تمامـــاً على جميع المقاييس، ولم تستطيع الأغنام التأقلم مع عليقة تفل بنجر السكر الكاملة حتى مرور ٢٤ أسبوعاً من التغذية عليها.