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Seasonal rumen fermentation pattern of Egyptian sheep in health and field digestive disorders

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Abstract

Rumen fermentation pattern of sheep was greatly affected by several factors. This investigation was conducted to evaluate the effect of seasons and digestive disorders on rumen physical, cellular, biochemical constituents and rumen fermentation efficiency in Egyptian sheep. A total number of 97 native breed sheep were classified according to clinical presentation into apparently healthy 58 and clinically diseased 39. The apparently healthy sheep were divided according seasons into: autumn 15, spring 16, summer 17 and winter 10. Diseased sheep were suffering clinically from different digestive disorders; simple indigestion 19, rumen acidosis 12 and rumen alkalosis. All animals were subjected to detailed case history, comprehensive clinical examination and rumen liquor sampling for analysis. Obtained results revealed that autumn had significant increase in pH and acetic acid with significant decrease in TPC, *Epidinium*, NH_3-N and calcium. Winter had significant increase in *Epidinium*, TPC, lactic, butyric acids, calcium and magnesium with significant decrease in *Entodinium*. Spring had significant increase in ALT and GGT activities. Summer had significant decrease in TVFAs, lactic, butyric acids, CH4 and magnesium. Sheep in spring and winter seasons had better rumen fermentation efficiency than sheep in autumn and summer. Simple indigestion, rumen acidosis and rumen alkalosis were common digestive disorders affecting Egyptian sheep causing significant changes in rumen liquor constituents. Simple indigestion had highly significant increase in *Diplodinium* with highly significant decrease in TPC. Rumen acidosis had highly significant increase in *Entodinium*, TVFAs, propionic, acetic acids, FE %, calcium and magnesium with highly significant decrease in pH, TPC, *Diplodinium*, *Epidinium*, *Holotricha*, NH_3-N, A/P ratio, CH_4/TVFAs % and AST activity. Rumen alkalosis had highly significant increase in pH with highly significant decrease in TPC, TVFAs, lactic, propionic, butyric, acetic acids, CH_4, calcium and magnesium.

Key words: rumen fermentation, digestive disorders, seasons, sheep, rumen enzymes, rumen methane, rumen ciliates.

Introduction

Sheep are able to optimally convert browse, forages and other feedstuffs hardly usable for more commonly faced livestock species into usable animal products as meat, milk, hide and fiber to reach ultimate performance **(Pugh and Baird, 2012).**

The reticulorumen is a structure unique to the ruminant species. Digestion of feedstuffs within this chamber is accomplished by microbial fermentation. The mucosal epithelium is capable of absorption and exchange of certain products of fermentation, but it performs essentially no secretory function **(Dirksen *et al.*, 1985)**.

The importance of digestive of diseases is underscored by the fact that forestomach digestion supplies the ruminants not only with energy but also with a balance of essential amino acids and most of the vitamins requirements. In addition, forestomach motility and digestion are secondarily affected by many diseases of other body systems, and an accurate assessment of such problems can be very important in arranging therapeutic measures that support an optimum recovery of normal function (**Smith *et al.* (1992)**.

From an economical point of view, forestomach diseases result in great losses to the producers through death, wasted feed (e.g. rumen acidosis), delayed marketing, premature marketing (e.g. repetitive bloaters), unthriftness of the recovered animals and extralabour coasts of therapeutic and preventive measures **(Kimberling, 1988)**.

Therefore, evaluation of the activity of the rumen is an important aid in the study of ruminant digestion. Substantial information is available on the subject from a strictly nutritional point of view, but relatively little is known about the interaction between the disease and rumen digestion **(Alonso, 1979)**

Diagnosis of such group of rumen dysfunction diseases is difficult using only routine clinical methods of examination. So, in order to establish an objective diagnosis, we have to examine the rumen fluid adjacent to the blood analysis **(Hofirek *et al.*, 1989)**.

The objectives of the present investigation are to study rumen fermentation pattern of apparently healthy sheep within different seasons; autumn, winter, spring and summer and evaluate the effect of field digestive disorders; simple indigestion, rumen acidosis and rumen alkalosis on rumen fermentation pattern of sheep to confirm field diagnosis.

Material & Methods

1. Material:

a. Animals:

A total number of 97 native breed adult Egyptian sheep their ages ranged between 1 to 3 years, there weight ranged between 35 to 50 kilograms belonged to different private farms in Giza government, farms of Faculty of Veterinary Medicine, Faculty of Agriculture, Cairo University and clinical cases in Teaching Hospital of Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University. These animals were divided into 58 apparently healthy sheep and 39 clinically diseased cases. Apparently healthy sheep according to the seasons of (2015) were divided into 15 sheep in autumn, 16 in spring, 17 in summer and 10 in winter. They were 26 rams and 32 non-lactating ewes. Apparently healthy sheep in dry seasons (summer and autumn) were fed on wheat straw and a concentrate mixture of yellow corn, cottonseed meal, wheat bran, sodium chloride, molasses and calcium carbonate powder with manufacturer's chemical analysis of crude protein not less than 14 %, crude fiber not more than 15 %, crude fat not less than 2 %, ash not more than 9 % and moisture not more than 12 % with water ad libitum, while in green seasons (winter and spring) they were fed on barseem in addition to the concentrate mixture. Diseased cases were divided into 19 cases of simple indigestion (SI), 12 cases of rumen acidosis (RA) and 8 cases of rumen alkalosis (RK), these clinically diseased cases were diagnosed carefully via complete case history and comprehensive clinical examination.

b. Samples:

A total number of 97 rumen fluid samples each of 50 ml were collected during the present investigation using a stomach tube connected to a suction pump through a mouth gag. Color, odor, consistency, pH and protozoal activity were examined immediately after sampling. They were sieved through 4 folds of gauze and divided into 4 portions: the first portion: 2 ml for determination of ammonia nitrogen level after preservation by adding 2 ml hydrochloric acid 0.1 N then centrifugation and taking supernatant, the second portion: 4 ml; 2 ml for Total volatile fatty acids(TVFA) and 2 ml for differentiation of volatile fatty acids after preservation by adding 2 ml orthophosphoric acid and 1 ml hydrochloric acid 0.1 N, the third portion: 2 ml for total protozoal count(TPC) and ciliate proportions after adding equal volume of methyl green formal saline (MFS) and keeping in dark place till examination by high power, the fourth portion was centrifuged for 15 minutes at 3000 rpm and the supernatant were collected for determination of biochemical constituents.

2. Methods

The rumen fluid pH, color, odor and consistency were determined according to **Alonso (1979), Dirksen and Smith (1987)**and **Radostits *et al.* (2007)**.Rumen protozoal activity was determined according to method described by **El-Saifi (1969)** and **Alonso (1979)**. TPC and ciliate proportions were determined using Fuchs-Rosenthal haemocytometer chamber according to method described by **Ito *et al.* (1994)**. (TVFAs) was determined by steam distillation method as described by **Eadie *et al.* (1967)**, VFAs concentrations were analyzed using Gas Chromatography according to the method described by **Mathew *et al.* (1997)**. Fermentative methane **(**CH4) was calculated according to the equations of **Wolin (1960)**which has been validated recently by**Blümmel *et al.* (1993)**. Fermentation efficiency (FE) was calculated on the basis of the equation worked out by**Orskov (1975)**and modified by**Baran and Zitnan (2002)**. Rumen ammonia nitrogen (NH_3-N) was determined calorimetrically using specific kits produced by Egyptian Company for Biotechnology (SPECTRUM), Egypt, according to the method described by **Chaney and Marbach (1962)**. Rumen lactic acid concentration was determined calorimetrically using specific kits produced by Egyptian Company for Biotechnology (SPECTRUM), Egypt. Activity of aspartate amino-transferase, (AST), alanine amino-transferase (ALT) and gamma glutamyl transferase (GGT) were determined using specific kits produced by STANBIO Laboratory, USA for AST and ALT and SPINREACT Company, Spain for GGT with specific spectrophotometer (Apple 302, USA). All kits were performed according to the manufacturer's recommendations. Magnesium level was determined according to **Young and Friedman (2001)** using specific kits produced by QCA Company, Spain. Inorganic phosphorus level was determined according to **Young and Friedman (2001)**using specific kits produced by Egyptian Company for Biotechnology (SPECTRUM), Egypt. Calcium level was determined according to **Young and Friedman**

(2001). Using specific kits produced by SPINREACT Company, Spain.

3. Statistical analysis:

Statistical analysis of obtained data was carried out by SPSS program version 20 using independent samples T test, Mann-Wittney U and one way ANOVA with LSD as post hock test, normality of distribution and equality of variances were checked using Shapiro-Wilk test and Leven's test respectively according to method described by **Nie *et al.* (1975)**and obtained results were recorded as mean value ± stranded error of mean (SE).

Results

Results of physical examination of freshly obtained rumen fluid including color, odor, consistency, pH and protozoal activity of apparently healthy sheep within different seasons and sheep suffering from different digestive disorders were tabulated in tables 1 and 2 respectively.

Results of statistical analysis of obtained data of rumen TPC and protozoal proportions; *Entodinium, Diplodinium, Epidinium* and *Holotricha* of apparently healthy sheep within different seasons and sheep suffering from different digestive disorders were tabulated in tables 3 and 4.

Results of statistical analysis of obtained data of rumen fermentation characteristics including NH_3-N, TVFAs, lactic acid, acetic acid, propionic acid, butyric acid, A/P ratio, fermentative CH4, CH4/TVFAs and fermentation efficiency of apparently healthy sheep within different seasons and sheep suffering from different digestive disorders were summarized in tables 5 and 6.

Results of statistical analysis of obtained data of rumen biochemical constituents including calcium level, inorganic phosphorus level, magnesium level and rumen enzyme activities of AST, ALT and GGT of apparently healthy sheep within different seasons and sheep suffering from different digestive disorders were summarized in tables 7 and 8.

Table 1. Physical characters of rumen fluid of apparently healthy sheep under effect of different seasons (mean ± SE)

Variables	Seasons				Overall mean (58)
	Autumn (15)	Winter (10)	Spring (16)	Summer (17)	
Color	yellowish-brown	dark green	greenish-yellow	Faint yellow	differs according to feed
Odor	aromatic	aromatic	aromatic	aromatic	aromatic
Consistency	slimy	slimy	slimy	slimy	slimy
Protozoal activity	+++	+++	+++	+++	+++
pH	6.92 ± 0.05 [a]	6.44 ± 0.07 [c]	6.53 ± 0.06 [c]	6.76 ± 0.04 [b]	6.68 ± 0.04

Values have the same symbols within the same raw are not significantly different at P value less than 0.05

Table 2. Physical characters of rumen fluid of sheep under effect of some digestive disorders in Egypt (mean ± SE)

Variables	Disorders			Apparently healthy control (58)
	Simple indigestion(SI) (19)	Rumen acidosis (RA) (12)	Rumen alkalosis (RK) (8)	
Color	differs according to causative feed	grayish white to milky white	differs according to causative feed	differs according to feed
Odor	aromatic to slight sour	sour	putrefied	aromatic
Consistency	slimy to watery	viscid in early and watery in late stage	watery	slimy
Protozoal activity	+	zero	zero	+++
pH	6.37 ± 0.11 [c]	5.12 ± 0.16 [a]	7.39 ± 0.04 [a]	6.68 ± 0.04

a: P value less than 0.001 b: P value less than 0.01 c: P value less than 0.05

Table 3. Rumen protozoa of apparently healthy sheep under effect of seasons (mean ± SE)

Variables	Seasons				Overall mean (58)
	Autumn (15)	Winter (10)	Spring (16)	Summer (17)	
TPC ($\times 10^4$/ml)	21.73 ± 2.24 [d]	77.70 ± 2.87 [a]	56.69 ± 6.31 [b]	36.53 ± 2.05 [c]	45.36 ± 3.24
Entodinium %	90.33 ± 0.55 [a]	71.00 ± 1.03 [c]	75.00 ± 1.02 [b]	89.12 ± 0.40 [a]	82.41 ± 1.15
Diplodinium %	2.83 ± 0.06	3.20 ± 0.42	3.56 ± 0.33	2.82 ± 0.06	3.09 ± 0.12
Epidinium %	3.00 ± 0.22 [d]	10.70 ± 0.40 [a]	8.69 ± 0.31 [b]	3.82 ± 0.06 [c]	6.14 ± 0.43
Holotricha %	3.83 ± 0.27 [b]	15.10 ± 0.46 [a]	12.75 ± 0.66 [a]	4.24 ± 0.30 [b]	8.35 ± 0.68

Values have the same symbols within the same raw are not significantly different at P value less than 0.05

Table 4. Rumen protozoa of sheep under effect of some digestive disorders in Egypt (mean ± SE)

Variables	disorders			Apparently healthy control (58)
	Simple indigestion (SI) (19)	Rumen acidosis (RA) (12)	Rumen alkalosis (RK) (8)	
TPC (×10^4/ml)	8.89 ± 1.66 ^{a}	2.50 ± 1.04 ^{a}	5.00 ± 0.65 ^{a}	45.36 ± 3.24
Entodinium %	78.58 ± 2.23 ^{c}	95.00 ± 0.74 ^{a}	81.25 ± 3.17	82.41 ± 1.15
Diplodinium %	3.95 ± 0.24 ^{a}	0.83 ± 0.11 ^{a}	2.50 ± 0.19	3.09 ± 0.12
Epidinium %	7.26 ± 0.83	2.67 ± 0.45 ^{a}	6.75 ± 1.26	6.14 ± 0.43
Holotricha %	10.21 ± 1.18 ^{c}	1.50 ± 0.29 ^{a}	9.50 ± 2.10	8.35 ± 0.68

a: P value less than 0.001 **b: P value less than 0.01** **c: P value less than 0.05**

Table 5. Rumen fermentation characteristics of apparently healthy sheep under effect of different seasons (mean ± SE)

Variables	Seasons				Overall mean (58)
	Autumn (15)	Winter (10)	Spring (16)	Summer (17)	
NH_3-N (mmol/L)	3.33 ± 0.41 ^{b}	5.33 ± 0.24 ^{a}	6.05 ± 0.58 ^{a}	5.82 ± 0.73 ^{a}	5.15 ± 0.32
TVFAs (mmol/L)	63.90 ± 2.34 ^{a}	60.60 ± 1.83 ^{a}	64.06 ± 1.52 ^{a}	43.82 ± 1.30 ^{b}	57.53 ± 1.46
Lactic acid (mmol/L)	0.61 ± 0.02 ^{b}	0.92 ± 0.05 ^{a}	0.61 ± 0.02 ^{b}	0.31 ± 0.06 ^{c}	0.58 ± 0.03
Propionic acid (mmol/L)	11.78 ± 0.33 ^{a}	10.58 ± 1.48 ^{ab}	12.17 ± 0.71 ^{a}	8.22 ± 0.28 ^{b}	10.77 ± 0.51
Butyric acid (mmol/L)	9.46 ± 0.40 ^{c}	18.77 ± 1.33 ^{a}	13.15 ± 0.46 ^{b}	6.34 ± 0.39 ^{d}	12.00 ± 1.08
Acetic acid (mmol/L)	42.69 ± 0.70 ^{a}	31.25 ± 0.91 ^{c}	38.76 ± 0.73 ^{b}	29.27 ± 0.63 ^{c}	35.15 ± 1.16
A/P ratio	3.63 ± 0.16	3.11 ± 0.55	3.21 ± 0.25	3.57 ± 0.20	3.35 ± 0.13
CH4 (mmol)	23.13 ± 0.24 ^{a}	22.36 ± 1.11 ^{a}	22.91 ± 0.53 ^{a}	15.75 ± 0.21 ^{b}	20.88 ± 0.69
CH4 /TVFAs (%)	36.19 ± 0.38	36.90 ± 1.83	35.77 ± 0.82	35.94 ± 0.47	36.05 ± 0.54
FE %	73.87 ± 0.31 ^{b}	75.99 ± 0.65 ^{a}	75.03 ± 0.41 ^{ab}	73.94 ± 0.40 ^{b}	74.85 ± 0.27

Values have the same symbols within the same raw are not significantly different at P value less than 0.05

Table 6. Rumen fermentation characteristics of sheep under effect of some digestive disorders in Egypt (mean ± SE)

Variables	disorders			Apparently healthy control (58)
	Simple indigestion (SI) (19)	Rumen acidosis (RA) (12)	Rumen alkalosis (RK) (8)	
NH_3-N (mmol/L)	3.70 ± 0.49 ^{c}	1.33 ± 0.13 ^{a}	4.56 ± 1.53	5.15 ± 0.32
TVFAs (mmol/L)	58.39 ± 4.25	88.00 ± 5.36 ^{a}	25.75 ± 1.82 ^{a}	57.53 ± 1.46
Lactic acid (mmol/L)	0.47 ± 0.08	0.71 ± 0.11	0.20 ± 0.07 ^{a}	0.58 ± 0.03
Propionic acid (mmol/L)	11.27 ± 2.14	31.03 ± 0.46 ^{a}	0.73 ± 0.12 ^{a}	10.77 ± 0.51
Butyric acid (mmol/L)	10.51 ± 1.20	15.49 ± 0.92	0.32 ± 0.07 ^{a}	12.00 ± 1.08
Acetic acid (mmol/L)	36.57 ± 3.31	41.51 ± 0.61 ^{a}	1.52 ± 0.05 ^{a}	35.15 ± 1.16
A/P ratio	3.60 ± 0.95	1.34 ± 0.02 ^{a}	2.27 ± 0.55 ^{c}	3.35 ± 0.13
CH4 (mmol)	20.72 ± 1.60	20.74 ± 0.33	0.74 ± 0.09 ^{a}	20.88 ± 0.69
CH4 /TVFAs (%)	35.49 ± 2.74	23.57 ± 0.37 ^{a}	28.72 ± 3.63	36.05 ± 0.54
FE %	74.70 ± 1.77	81.06 ± 0.13 ^{a}	77.71 ± 1.58	74.85 ± 0.27

a: P value less than 0.001 **b: P value less than 0.01** **c: P value less than 0.05**

Table 7. Rumen biochemical constituents of apparently healthy sheep under effect of different seasons (mean ± SE)

Variables	Seasons				Overall mean (58)
	Autumn (15)	Winter (10)	Spring (16)	Summer (17)	
Calcium (mmol/L)	0.07 ± 0.02 ^{d}	4.42 ± 0.39 ^{a}	2.07 ± 0.35 ^{b}	0.19 ± 0.03 ^{c}	1.43 ± 0.24
Inorganic phosphorus (mmol/L)	7.35 ± 0.16 ^{a}	4.84 ± 0.49 ^{b}	5.93 ± 0.71 ^{b}	8.03 ± 0.04 ^{a}	6.71 ± 0.26
Magnesium (mmol/L)	0.21 ± 0.03 ^{c}	2.46 ± 0.04 ^{a}	0.39 ± 0.05 ^{b}	0.09 ± 0.01 ^{d}	0.62 ± 0.11
AST (Ukat/L)	0.15 ± 0.01 ^{b}	0.10 ± 0.02 ^{b}	0.27 ± 0.03 ^{a}	0.28 ± 0.05 ^{a}	0.22 ± 0.02
ALT (Ukat/L)	0.35 ± 0.03 ^{b}	0.59 ± 0.14 ^{b}	0.74 ± 0.06 ^{a}	0.36 ± 0.01 ^{b}	0.51 ± 0.04
GGT (Ukat/L)	0.05 ± 0.01 ^{c}	0.10 ± 0.03 ^{bc}	0.18 ± 0.02 ^{a}	0.10 ± 0.01 ^{b}	0.11 ± 0.01

Values have the same symbols within the same raw are not significantly different at P value less than 0.05

Table 8. Rumen biochemical constituents of sheep under effect of some digestive disorders in Egypt (mean ± SE)

Variables	Disorders			Apparently healthy control (58)
	Simple indigestion (SI) (19)	Rumen acidosis (RA) (12)	Rumen alkalosis (RK) (8)	
Calcium (mmol/L)	0.83 ± 0.15	4.26 ± 0.30 [a]	0.06 ± 0.02 [a]	1.43 ± 0.24
Inorganic phosphorus (mmol/L)	7.26 ± 0.28	6.97 ± 0.33	7.76 ± 0.11	6.71 ± 0.26
Magnesium (mmol/L)	0.85 ± 0.18	1.94 ± 0.03 [a]	0.03 ± 0.01 [a]	0.62 ± 0.11
AST (Ukat/L)	0.15 ± 0.02	0.09 ± 0.01 [a]	0.15 ± 0.02 [c]	0.22 ± 0.02
ALT (Ukat/L)	1.18 ± 0.28	0.55 ± 0.18	0.31 ± 0.03 [b]	0.51 ± 0.04
GGT (Ukat/L)	0.13 ± 0.02	0.10 ± 0.04	0.25 ± 0.08 [c]	0.11 ± 0.01

a: P value less than 0.001 **b: P value less than 0.01** **c: P value less than 0.05**

Discussion

1. **Physical characters of rumen fluid of Egyptian sheep in health and diseases:**

a. Color, odor, consistency and protozoal activity:

Regarding apparently healthy sheep, obtained findings were in complete agreement with **Kubesy (1983), Fouda (1995), Jackson *et al.* (2002), Kelany (2002), Radostits*et al.* (2007), Al-Shami (2008), Anderson and Rings (2008), Karapinar*et al.* (2008), Pugh and Baird (2012)** and**Orabi (2015)**.

Regarding the effect of seasons, color in dry seasons; summer and autumn was faint yellow to yellowish brown, this may be due to nature of feed, while in green seasons; winter and spring was greenish yellow to dark green due to nature of feed, this observation was in agreement with **Radostits*et al.* (2007)**.

Regarding the effect of digestive disorders, SI group showed varied color; yellowish brown, greenish yellow, dark brown and dark green according to nature of causative feed, aromatic to slight sour odor, slimy to watery consistency and (+) protozoal activity. Similar finding was reported by **Kubesy (1983)** and **Radostits*et al.* (2007)**. RA group showed grayish-white color, sour odor, viscid or watery consistency and (0) protozoal activity. Similar observation was reported by **Kubesy (1983)**, **Fouda (1995), Radostits*et al.* (2007), Anderson and Rings (2008)** and**Karapinar*et al.* (2008)**. RK group showed varied color; dark brown, grayish brown and grayish black according to nature of causative feed, putrefied odor, watery consistency and (0) protozoal activity. Similar observation was reported by **Radostits*et al.* (2007)** and **Anderson and Rings (2008)**.

b. Rumen pH

Regarding apparently healthy sheep, obtained result was in agreement with **Fouda (1995),Patra*et al*. (1996), Jackson *et al.* (2002), Radostits*et al.* (2007), Santra *et al.* (2007), AL-Shami (2008), Anderson and Rings (2008), Karapinar*et al.* (2008), Baraka and abdl-Rahman (2012)** (in-vitro)**, Smith (2014)**and**Orabi (2015),** while **Kubesy (1983), Lu and Jorgensen (1987), Kelany (2002), Brossard *et al.* (2003), Carro*et al.* (2005), Chaves *et al.* (2008), Baraka (2012), Chegeni*et al.* (2013)** and **Santos *et al.* (2015)** recordedlower mean values, meanwhile **Pugh and Baird (2012)** recorded higher range and these differences may be referred to different feeding conditions.

Regarding effect of seasons, autumn was significantly (P<0.05) higher than all seasons, summer was significantly (P<0.05) higher than spring and winter which had the lowest pH among seasons, similar finding was recorded by **Maas *et al.* (2001)** in autumn and spring and **Wang *et al.* (2007)** in autumn and summer while the latter authors recorded lower values within autumn and summer with different feeding conditions. The highest rumen pH obtained in autumn could be attributed to highest *Entodinium* % which engulf starch and utilize lactic acid keeping rumen pH away from acidosis as mentioned by **Baraka (2006)**and **Chaucheyras-Durand *et al.* (2012)**in addition to positive correlation between rumen pH and *Entodinium* % recorded by **Baraka (2011).**

Regarding the effect of digestive disorders in comparison with control group, SI group was significantly (P<0.05) lower, this finding was in agreementwith **Kubesy (1983), Pugh and Baird (2012)** and**Smith (2014)**and this change can be explained as result of ruminal atony affecting the rate of fermentation and/or hydrolysis and thereby the production of acid or alkaline intermediates **(Hungate, 1966)**and **(Bradford, 1990)**, whiledisagreement was with **Anderson and Rings (2008)** which could be attributed to nature of the causative feedstuff and its resultant fermentative degradation **(Smith, 2014)**. RA group showed highly significant (P<0.001) decrease. Similar observation was reported by **Kubesy (1983), Fouda (1995), Jackson *et al.* (2002), Radostits*et al.* (2007), Anderson and Rings (2008) Karapinar*et al.* (2008), Pugh and Baird (2012)** and **Smith (2014)** who explained this finding on basis of excessive fermentation of carbohydrate, rabidly continuous production of VFAs and inhibition of lactate utilizing bacteria; *Megaspheara elsdenii* and *Selenomonas ruminatium*. RK group showed highly significant (P<0.001) increase. Similar observation was reported by **Jackson *et al.* (2002), Radostits*et al.* (2007)** and **Anderson and Rings (2008)** but with higher ranges.

2. **Rumen cellular constituents of Egyptian sheep in health and diseases:**

a. Rumen TPC

Regarding apparently healthy sheep, obtained result disagreed with **Fouda (1995)** and **Kelany (2002)** as they recorded higher values, meanwhile **Kubesy (1983), Brossard *et al*. (2003), Santra *et al.* (2007), Baraka (2012)** and **Mousa (2014)** recorded lower values which could be referred to variation in nature of feed; as **Lu and Jorgensen (1987)** recorded both higher and lower values than obtained results in roughage and concentrate diets respectively.

Regarding the effect of seasons, winter was significantly (P<0.05) higher than all seasons, spring was significantly (P<0.05) higher than summer and autumn and summer was significantly (P<0.05) higher than autumn which had the lowest TPC among seasons. These differences among seasons may be attributed to nature and composition of diets.

Regarding the effect of digestive disorders in comparison with control group, all diseased groups showed highly significant (P<0.001) decrease. Finding of SI group was in agreement with **Kubesy (1983)**. Finding of RA group was also reported by **Kubesy (1983), Fouda (1995)** and **Smith (2014)**who attributed it to highly acidic pH and high rumen osmotic pressure leading to killing of rumen protozoa reducing its total count, formerly, **Nagaraja *et al.* (1992)** also explained it on basis of low ruminal pH, hypertonicity and a faster passage rate of ruminal contents from the forestomach to the lower GIT.

b. Rumen Entodinium

Regarding apparently healthy sheep, obtained results revealed that *Entodinium* spp. was the major components of true ruminant ciliates up to more than 80 % and this observation was in agreement with **Dehority (2003)**, **Santra *et al.* (2007)**, **Tsankova *et al.* (2010)** and **Baraka (2011)**, while **Fouda (1995)** recorded lower value, meanwhile **Baraka (2012)** recorded higher values and these variations could be attributed to different nature and composition of feed.

Regarding the effect of seasons, autumn and summer was significantly (P<0.05) higher than spring and winter, spring was significantly (P<0.05) higher than winter which showed the lowest *Entodinium* % among seasons. This variation could be explained on basis of more availability of concentrate diet in dry seasons as *Entodinium* reaches up to 90 % in high concentrate diets **(Williams and Withers, 1993)** and **(Franzolin and Dehority, 1996)**.

Regarding the effect of digestive disorders in comparison with control group, SI group was significantly (P<0.05) lower.RA group showed highly significant (P<0.001) increase, **Fouda (1995)** reported similar observation and this could be explained on basis of ability of *Entodinium* to survive under pH of 6 and engulf starch grains very rabidly for a few minutes and much more slowly thereafter **(Coleman, 1992)**. RK group showed slight decrease.

c. Rumen Diplodinium

Regarding apparently healthy sheep, obtained result agreed with **Baraka (2012)**. Regarding the effect of seasons, *Diplodinium* % showed slight differences among seasons; spring had the highest while summer had the lowest value among seasons. Regarding the effect of digestive disorders in comparison with control group, SI group showed highly significant (P<0.001) increase, meanwhile RA group showed highly significant (P<0.001) decrease. RK group showed slight decrease.

d. Rumen Epidinium

Regarding apparently healthy sheep, obtained result disagreed with higher value recorded by **Fouda (1995)**, while **Baraka (2012)** recorded lower value. Regarding the effect of seasons, winter was significantly (P<0.05) higher than all seasons, spring was significantly (P<0.05) higher than autumn and summer which was significantly (P<0.05) higher than autumn which had the lowest *Epidinium* % among seasons. This variation among seasons could be attributed to nature of available diet. This observation was in agreement with negative correlation between *Epidinium* % and *Entodinium* % reported by**Baraka (2011)**.

Regarding the effect of digestive disorders in comparison with control group, SI group and RK group showed mild increase.RA group showed highly significant ($P<0.001$) decrease and this observation was reported by **Fouda (1995)**.

e. Rumen Holotricha

Regarding apparently healthy sheep, obtained result was in disagreement with higher value reported by **Fouda (1995)**, while **Baraka (2012)** recorded lower value and these differences could be attributed to different composition of diet. Regarding the effect of seasons, winter and spring was significantly ($P<0.05$) higher than summer and autumn which had the lowest *Holotricha*%while winter had the highest *Holotricha*% among seasons. This observation was in agreement with positive correlation between rumen TPC and *Holotricha* reported by **Baraka (2011)**.Regarding the effect of digestive disorders in comparison with control group, SI group was significantly ($P<0.05$) higher.RA group showed highly significant ($P<0.001$) decrease and this observation was reported by **Fouda (1995)**. RK group showed mild increase.

3. **Rumen fermentation characteristics of Egyptian sheep in health and diseases**

a. Rumen ammonia nitrogen concentration

Regarding apparently healthy sheep, obtained result was in agreement with **Santra *et al.* (2007)** and **Baraka and abdl-Rahman (2012) (**in-vitro**)**, meanwhile **Lu and Jorgensen (1987)**, **Fouda (1995), Kelany (2002), Brossard *et al.* (2003), Carro*et al.* (2005), Mousa (2014), Orabi (2015)** and **Santos *et al.* (2015)** recordedhigher mean value, while **Chaves*et al.* (2008)** reported lower values and these variations could be attributed to amount of available dietary protein degraded in rumen, recycled nitrogen via saliva or across rumen wall **(Mehrez *et al.*, 1983),** composition of adapted feed and season of sampling.

Regarding the effect of seasons, spring, winter and summer was significantly ($P<0.05$) higher than autumn which had the lowest value while spring had the highest value among seasons. **Maas *et al.* (2001)** reported higher values in autumn and spring. **Wang *et al.* (2007)** reported similar finding in autumn and summer but the authors also recorded varied values within autumn and summer with different feeding conditions. These interrupted values among seasons could be attributed to amount of available dietary protein degraded in rumen and recycled nitrogen via saliva or across rumen wall **(Mehrez *et al.*, 1983)**.

Regarding the effect of digestive disorders in comparison with control group, SI group was significantly ($P<0.05$) lower and this observation was in agreement with **Kubesy (1983).**RA group showed highly significant ($P<0.001$) decrease and similar observation was reported by **Kubesy (1983)**and **Fouda (1995)** and this could be explained on basis of decreased TPC and reduced rumen organic matter **(Ushida *et al.*, 1986)**, also, the deaminase activity in the ruminal microbial population was higher in presence of protozoa **(Wallace *et al.*, 1987)**. RK group showed mild decrease.

b. Rumen TVFAs concentration

Regarding apparently healthy sheep, obtained findings were in agreement with **Kelany (2002)** and**Orabi (2015),** while**Lu and Jorgensen (1987), Fouda (1995), Brossard *et al.* (2003), Carro*et al.* (2005), Chaves *et al.* (2008)** and **Chegeni*et al.* (2013)** reportedhigher values, meanwhile **Patra*et al.*(1996), Santra *et al.*(2007), Baraka and abdl-Rahman (2012) (**in-vitro**)** and **Mousa (2014)** reported lower values. These variations could be attributed to different feeding conditions and level of carbohydrate supplemented in ration as increasing the concentrate portion in the ration led to increasing VFAs concentration in rumen fluid **(Mehrez *et al.*, 1983)**.

Regarding the effect of seasons, spring, autumn and winter was significantly ($P<0.05$) higher than summer which had the lowest TVFAs concentration while spring had the highest TVFAs concentration among seasons. **Wang *et al.* (2007)** reported similar finding in autumn but higher in summer, meanwhile the authors also recorded varied values within autumn and summer with different feeding conditions.

Regarding the effect of digestive disorders in comparison with control group, SI group showed mild increase.RA group showed highly significant ($P<0.001$) increase and this observation was in agreement with **Patra*et al.*(1996)** and **Brossard *et al.* (2003)**in experimentally-induced acidosis and **Radostits*et al.* (2007)**, while disagreement was with **Fouda (1995)** in experimentally induced rumen acidosis. RK group showed highly significant ($P<0.001$) decrease.

c. Rumen lactic acid concentration

Regarding apparently healthy sheep, obtained findings disagreed with higher values recorded by **Fouda (1995)** and **Brossard *et al.* (2003)**, while **Patra*et al.* (1996)** reported lower mean value. These variations may be attributed to different feeding conditions.

Regarding the effect of seasons, winter was significantly ($P<0.05$) higher than all seasons, spring and autumn were significantly (P<0.05) higher than summer which had the lowest lactic acid concentration. These interrupted values among seasons could be attributed to different diet composition.

Regarding the effect of digestive disorders in comparison with control group, SI group showed slight decrease. RA group showed moderate increase, **Fouda (1995)** and **Patra*et al.* (1996)** reported similar observation in experimentally-induced rumen acidosis. RK group showed highly significant (P<0.001) decrease.

d. Rumen acetic acid concentration

Regarding apparently healthy sheep, obtained findings was also recorded by **Lu and Jorgensen (1987)** and**Carro*et al.* (2005),** while **Fouda (1995), Brossard *et al.* (2003) and Chegeni*et al.* (2013)** reported higher values, meanwhile **Chaves*et al.* (2008), Baraka and abdl-Rahman (2012) (**in-vitro**),** and **Santos *et al.* (2015)** reported lower values. These variations may be attributed to different feeding conditions and different roughage to concentrate ratio in ration.

Regarding the effect of seasons, autumn was significantly (P<0.05) higher than all seasons and spring was significantly (P<0.05) higher than winter and summer which had the lowest lactic acid concentration among seasons. **Maas *et al.* (2001)** reported similar values in autumn and higher values in spring. **Wang *et al.* (2007)** reported lower values in summer and autumn within different feeding conditions.

Regarding the effect of digestive disorders in comparison with control group, SI group showed slight increase.RA group showed highly significant (P<0.001) increase and this finding was in disagreement with **Fouda (1995)** in experimentally-induced rumen acidosis. RK group showed highly significant (P<0.001) decrease.

e. Rumen propionic acid concentration

Regarding apparently healthy sheep, obtained findings were in agreement with **Lu and Jorgensen (1987), Brossard *et al.* (2003), Carro*et al.* (2005)** and **Santos *et al.* (2015),** while **Fouda (1995), Chaves*et al.* (2008)** and **Baraka and abdl-Rahman (2012) (**in-vitro**),** reported higher values, meanwhile **Chegeni*et al.* (2013)** recorded lower value. These variations may be attributed to different feeding conditions and different roughage to concentrate ratio in ration.

Regarding the effect of seasons, autumn and spring showed mild increase than winter but significant (P<0.05) increase than summer which had the lowest propionicacid concentration while autumn had the highest value among seasons. **Maas *et al.* (2001)** reported similar values in autumn and spring. **Wang *et al.* (2007)** reported similar values in summer and higher values in autumn. The latter authors also reported varied values in summer and autumn within different feeding conditions.

Regarding the effect of digestive disorders in comparison with control group, SI group showed mild increase. RA group showed highly significant (P<0.001) increase than and this observation was in disagreement with **Fouda (1995)** in experimentally-induced rumen acidosis. RK group showed highly significant (P<0.001) decrease.

f. Rumen butyric acid concentration

Regarding apparently healthy sheep, obtained findings were in agreement with **Lu and Jorgensen (1987),** while **Fouda (1995), Brossard *et al.* (2003), Carro*et al.* (2005), Chaves*et al.* (2008), Baraka and abdl-Rahman (2012) (**in-vitro**), Chegeni*et al.* (2013)** and **Santos *et al.* (2015)** reported lower values. These variations may be attributed to different feeding conditions and different roughage to concentrate ratio in ration.

Regarding the effect of seasons, winter was significantly (P<0.05) higher than all seasons, spring was significantly (P<0.05) higher than autumn and summer and autumn was significantly (P<0.05) higher than summer which had the lowest butyricacid concentration among seasons. **Maas *et al.* (2001)** reported lower values in autumn and spring. **Wang *et al.* (2007)** reported similar values in summer and autumn and authors also reported varied values in summer and autumn within different feeding conditions.

Regarding the effect of digestive disorders in comparison with control group, SI group showed mild decrease. RA group showed moderate increase and this finding was in agreement with **Fouda (1995)** in experimentally-induced rumen acidosis. RK group showed highly significant (P<0.001) decrease.

g. Rumen A/P ratio

Regarding apparently healthy sheep, obtained findings were in agreement with **Brossard *et al.* (2003), Carro*et al.* (2005)** and **Santos *et al.* (2015),** while**Chegeni*et al.* (2013)** reported higher ratio, meanwhile **Chaves *et al.* (2008)** and **Baraka and abdl-Rahman (2012) (**in-vitro**),** recorded lower ratio. These variations may be attributed to different feeding conditions and different roughage to concentrate ratio in ration.

Regarding the effect of seasons, A/P ratio showed mild differences among seasons; autumn had the highest while winter had the lowest A/P ratio among seasons. **Maas *et al.* (2001)** reported similar values in autumn and spring. **Wang *et al.* (2007)** reported lower values in summer and autumn within different feeding conditions.

Regarding the effect of digestive disorders in comparison with control group, SI group showed mild increase, while RA group showed highly significant (P<0.001) decrease. RK group was significantly (P<0.05) lower.

h. Rumen fermentative methane

Regarding apparently healthy sheep, obtained findings disagreed with **Baraka and abdl-Rahman (2012)** as they recorded lower value using in-vitro rumen microbial fermentation.

Regarding the effect of seasons, autumn, winter and spring were significantly (P<0.05) higher than summer which had the lowest while autumn had the highest methane produced among seasons. These findings could be attributed to lowest concentration of acetic acid in summer but highest in autumn because methanogenesis involved mainly reduction of CO_2 produced during acetic acid production by methanogens in presence of NADH+H, coenzyme F420 and coenzyme M **(Lovley *et al.*, 1984).**

Regarding the effect of digestive disorders in comparison with control group, SI group and RA group showed slight decrease, while RK group showed highly significant (P<0.001) decrease.

i. Rumen fermentative methane/TVFAs %

Regarding apparently healthy sheep, obtained finding disagreed with lower values reported by **Baraka and abdl-Rahman (2012)** using in-vitro rumen microbial fermentation.

Regarding the effect of seasons, fermentative methane/TVFAs % showed slight differences among seasons; winter had the highest while spring had the lowest fermentative methane/TVFAs % among seasons.

Regarding the effect of digestive disorders in comparison with control group, SI group and RK group showed mild decrease.RA group showed highly significant (P<0.001) decrease.

j. Rumen fermentation efficiency (FE) %

Regarding apparently healthy sheep, obtained finding agreed with **Baraka and abdl-Rahman (2012)** using in-vitro rumen microbial fermentation. Regarding the effect of seasons, winter was slightly higher than spring but significantly (P<0.05) higher than summer and autumn which had the lowest fermentation efficiency% among seasons.Regarding the effect of digestive disorders in comparison with control group, SI group showed mild decrease.RA group showed highly significant (P<0.001) increase. RK group showed mild increase.

4. Rumen biochemical constituents of Egyptian sheep in health and diseases

a. Rumen calcium level

Regarding apparently healthy sheep, obtained finding was in agreement with **Abdel-Salam (1981)**. Regarding the effect of seasons, winter was significantly (P<0.05) higher than all seasons and spring was significantly (P<0.05) higher than summer and autumn, while summer was significantly (P<0.05) higher than autumn which had the lowest rumen calcium level among seasons, this could be explained on basis of highest rumen pH in autumn and lowest pH in winter as decreased rumen pH caused increased concentration and solubility of calcium in rumen fluid **(Van't Klooster, 1967)** and **(Huber, 1971)**.

Regarding the effect of digestive disorders in comparison with control group, SI group showed moderate decrease.RA group showed highly significant (P<0.001) increase, while RK group showed highly significant (P<0.001) decrease, this could be explained on basis of decreased rumen pH caused increased concentration and solubility of calcium in rumen fluid **(Van't Klooster, 1967)** and **(Huber, 1971)**.

b. Rumen inorganic phosphorus level

Regarding the effect of seasons, summer and autumn was significantly (P<0.05) higher than spring and winter which had the lowest rumen inorganic phosphoruslevel while summer had the highest rumen inorganic phosphoruslevel among seasons and this observation could be attributed to increase salivation in summer and saliva rich in phosphorus increasing rumen phosphorus level **(Nokata *et al.*, 1977)**.

Regarding the effect of digestive disorders in comparison with control group, all diseased groups showed mild increase. Finding of RA could be explained on basis of increase saliva for buffering excess rumen lactate and saliva rich in phosphorus increasing rumen phosphorus level **(Nokata *et al.*, 1977)**.

c. Rumen magnesium level

Regarding apparently healthy sheep, obtained finding agreed with **Abdel-Salam (1981)**. Regarding the effect of seasons, winter was significantly (P<0.05) higher than all seasons and spring was significantly (P<0.05) higher than summer and autumn, meanwhile autumn was significantly (P<0.05) higher than summer which had the lowest rumen magnesiumlevel among seasons. The possible explanation of these interrupted values of ruminal magnesium level is that the changes of sodium and potassium levels were attributed to the levels of them in the saliva; and in the ration**(Hungate, 1966)**.

Regarding the effect of digestive disorders in comparison with control group, SI group showed mild increase.RA group showed highly significant (P<0.001) increase and this observation could be explained on basis of decreased rumen pH caused increased concentration and solubility of magnesium in rumen fluid **(Van't Klooster, 1967)** and **(Huber, 1971)**. RK group showed highly significant (P<0.001) decrease.

d. Rumen AST activity

Regarding apparently healthy sheep, obtained finding disagreed with**Faixová and Fiax (2002), Faixová *et al.* (2004)** and **Mousa (2014)** as they reported higher values. Regarding the effect of seasons, summer and spring was significantly (P<0.05) higher than autumn and winter which had the lowest rumen AST activity while summer had the highest rumen AST activity among seasons.Regarding the effect of digestive disorders in comparison with control group, SI group showed mild decrease. RA group showed highly significant (P<0.001) decrease and this could be referred to decreased TPC and reduced rumen organic matter **(Ushida *et al.*, 1986)**.RK group was significantly (P<0.05) lower.

e. Rumen ALT activity

Regarding apparently healthy sheep, obtained finding was in agreement with **Faixová *et al.* (2004),** while **Faixová and Fiax (2002)** and **Mousa (2014)** reported lower values. Regarding the effect of seasons, spring was significantly (P<0.05) higher than all seasons. Autumn had the lowest rumen ALT activity among seasons.Regarding the effect of digestive disorders in comparison with control group, SI group and RA group showed mild increase.RK group was significantly (P<0.01)lower.

f. Rumen GGT activity

Regarding apparently healthy sheep, obtained finding was in disagreement with **Faixová and Fiax (2002), Faixová *et al.* (2004)** and **Mousa (2014)** who reported higher values. Regarding the effect of seasons, spring was significantly (P<0.05) higher than all seasons and winter showed mild increase than autumn, meanwhile summer was significantly (P<0.05) higher than autumn which had the lowest rumen GGT activity among seasons.

Regarding the effect of digestive disorders in comparison with control group, SI group and RA group showed mild increase and decrease respectively, while RK group was significantly (P<0.05) higher.

Conclusion

It could be concluded that seasons had variable significant effect on rumen fermentation pattern of sheep. Autumn had significant increase in pH and acetic acid with significant decrease in TPC, *Epidinium*, NH_3-N and calcium. Winter had significant increase in *Epidinium*, TPC, lactic, butyric acids, calcium and magnesium with significant decrease in *Entodinium*. Spring had significant increase in ALT and GGT activities. Summer had significant decrease in TVFAs, lactic, butyric acids, CH_4 and magnesium. Sheep in spring and winter seasons had better rumen fermentation efficiency than sheep in autumn and summer. Simple indigestion, rumen acidosis and rumen alkalosis were common digestive disorders affecting Egyptian sheep causing significant changes in rumen liquor constituents. Simple indigestion had highly significant increase in *Diplodinium*, with highly significant decrease in TPC. Rumen acidosis had highly significant increase in *Entodinium*, TVFAs, propionic, acetic acids, FE %, calcium and magnesium and marked increase in lactic acidwith highly significant decrease in pH, TPC, *Diplodinium*, *Epidinium*, *Holotricha*, NH_3-N, A/P ratio, CH_4/TVFAs % and AST activity. Rumen alkalosis had highly significant increase in pH with highly significant decrease in TPC, TVFAs, lactic, propionic, butyric, acetic acids, CH_4, calcium and magnesium.

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الملخص العربي

كفاءة التخمر الموسمية في الكرش في الأغنام المصرية في الصحة والإصابات الهضمية الحقلية

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تتأثر عملية التخمر في كرش الأغنام بعوامل متعددة. أجريت هذه الدراسة بهدف تقييم تأثير مواسم السنة والإصابات الهضمية على الخواص الفيزيائية والمكونات الخلوية والبيوكيميائية لسائل الكرش وكفاءة التخمر في كرش الأغنام المصرية. ولهذا الغرض تم استخدام عدد ٩٧ من الأغنام المصرية محلية السلالة تم تقسيمها حسب الصورة الإكلينيكية إلى حيوانات سليمة ظاهريا (٥٨) وحيوانات مصابة إكلينيكيا (٣٩)، وتم تقسيم الحيوانات السليمة ظاهريا حسب مواسم السنة إلى الخريف (١٥)، الربيع (١٦)، الصيف (١٧) والشتاء (١٠) وتم تقسيم الحيوانات المصابة إلى عسر الهضم البسيط (١٩)، حمضية الكرش (١٢) وقلوية الكرش (٨). تم أخذ تاريخ حالة مفصل وعمل فحص إكلينيكي كامل وسحب عينات سائل كرش من كل حيوان لتحليلها معمليا. أوضحت النتائج أن فصل الخريف قد تميز بزيادة معنوية في الأس الهيدروجيني وحمض الخليك مع انخفاض معنوي في عدد الأوليات الكلي و أوليات *Epidinium* وتركيزات الأمونيا والكالسيوم. تميز فصل الشتاء بزيادة معنوية في عدد الأوليات الكلي و أوليات *Epidinium* وحمضي اللاكتيك و البيوتيرك والكالسيوم والماغنسيوم مع انخفاض معنوي في أوليات *Entodinium*. كما تميز فصل الربيع بزيادة ملحوظة في إنزيمي الأنين امينو ترانسفيريس، جاما جلوتاميل ترانسفيريس. وتميز فصل الصيف بانخفاض ملحوظ في مجموع الأحماض الدهنية المتطايرة وحمضي اللاكتيكوالبيوتيرك وغاز الميثان والماغنسيوم. وتبين إن الأغنام في فصلي الربيع والشتاء لها كفاءة تخمرفي الكرش افضل منها في فصلي الصيف والخريف. وتبين أيضا إن عسر الهضم البسيط، حمضية الكرش و قلوية الكرش هي أكثر الأمراض الهضمية شيوعا في الأغنام على الترتيب والتي تسبب تغيرات ملحوظة في مكونات سائل الكرش. تسبب عسر الهضم البسيط في زيادة معنوية كبيرة في أوليات *Diplodinium* مع انخفاض معنوي كبير في عدد الأوليات الكلي. تسببت حمضية الكرش في زيادة معنوية كبيرة في أوليات *Entodinium*ومجموع الأحماض الدهنية المتطايرة وحمضي البروبيونيك و الخليك و نسبة كفاءة التخمر والكالسيوم والماغنسيوم مع انخفاض معنوي كبير في الأس الهيدروجيني و عدد الاوليات الكليو أوليات *Diplodinium*و *Epidinium* و *Holotricha*والأمونيا ونسبة الخليك/البروبيونيك ونسبة الميثان/مجموع الأحماض الدهنية المتطايرة إنزيم اسبرتات امينو ترانسفيريس. تسببت قلوية الكرش في زيادة معنوية كبيرة في الأس الهيدروجيني مع نقص معنوي كبير في عدد الأوليات الكلي ومجموع الأحماض الدهنية المتطايرة وأحماض اللاكتيك والبروبيونيك والبيوتيريك والخليك وغاز الميثان والكالسيوم و الماغنسيوم.