EFFECT OF YEAST CULTURE AND PRONIFER SUPPLEMENTATION ON SOME PHYSIOLOGICAL AND REPRODUTIVE RESPONSES IN RAHMANI RAMS

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Received on: 23/2/2006 *Accepted on: 22/3/2006*

ABSTRACT

Sixteen healthy male Rahmani lambs, with average body weight of 29± 0.5kg, were divided into four equal groups .Control group$_1$ (G_1), was fed untreated bagasse silage plus 600 gm concentrate feed mixture (CFM). Urea group$_2$ (G_2),was fed on 3% urea treated bagasse silage plus 600 gm CFM .Yeast group$_3$ (G_3), was fed as group$_2$ (G_2) in addition to 10 gm yeast/animal/day. Pronifer group$_4$ (G_4), was fed as group$_2$ in addition to 2 gm pronifer/animal/day. The present work aimed to study the effect of live yeast and pronifer supplementation on some blood constituents, testes size and semen quality. Results showed that rams of urea group(G_2) had significantly ($P<0.05$) higher values for total serum protein, globulin, cholesterol, urea-N, AST, ALT, bilirubin and creatinine compared with the control group (G_1). On the other hand, urea group (G_2) showed the lowest values ($P<0.05$) in albumin and glucose, while the control group (G_1) gave the highest of these values .The obtained values with yeast and pronifer groups were intermediate between values of control and urea groups expect for transaminases. However, rams serum testosterone levels of pronifer group were higher ($P<0.05$) than those of the other groups . However, no significant differences were detected among treatments for testes size. Rams of G_3 or G_4 had significantly ($P<0.05$) higher ejaculate volume , sperm concentration and total number of sperm / ejaculate compared with (G_1) or (G_2). Moreover, rams fed diet with pronifer (G_4) had lower ($P<0.05$) total abnormalities and higher ($P<0.05$) mass motility than those of the control group. Also, pronifer and yeast groups had the highest values for live sperm percentage compared to those fed G_1 and G_2. However, no significant differences were found between G_1 and G_2 in semen quality. This study indicated that supplementing yeast and pronifer was more effective than urea diet alone or control diet to alleviate the physiological response and enhance the reproductive performance in Rahmani rams.

Keywords: Rams, yeast, pronifer, blood constituents , testosterone, semen quality.

INTRODUCTION

Dietary yeast (*Saccharomyces cerevisiae*) is one of the feed additives used by commercial dairies. Pronifer, as probiotic, contains *Lactobacillus plantarum, L. brvis, L.fermentum, L. casei and Pediococcus acidilacticcii* (approx. 10^6 CFU/gram). Sugarcane is one of the most popular cash crops grown in Egypt. A huge quantity of sugarcane bagasse is produced during sugar production. Preserving it as a silage with urea enhanced feeding value for animal feeding (Abd El-Hafez *et al.*, 1997 and Megahed *et al.*, 2000). Supplementation of yeast culture to fibrous diets increased the molar proportion of propionic acid and improved animal performance (Orskov and Ryle, 1990). Also, Wohlt *et al.* (1991) reported that addition of yeast increases nutrients digestibility in dairy cattle. Recently, Chauchyras-Durand and Fonty (2001) observed that dietary yeast stimulated the development of cellulolytic bacteria which improved fiber digestion and enhanced microbial activity, that could be beneficial in preventing microbial imbalance and reduction of rumen function efficiency. Also, Abd El-Ghani *et al.* (2004) found that the combined effect of diet plus yeast was more effective than diet alone to enhance the physiological responses and milk production performance of lactating Friesian cows. It was reported that pronifer regulates the microbial environment, decreases digestive disturbance, inhibits pathogenic microorganism and improves feed efficiency (Peter, 1991; Windschitl, 1992 and Dhingra, 1993). It also improves health performance and increases growth rate in Egyptian buffaloes and cattle (Bohm and Srour, 1995).The most positive effect is expected especially in suboptimal hygienic measures with indoor animals and under stress ful conditions such as inferior feed quality, heat or unfavorable weather and parasites which may be better managed by using probiotics (Games, 1987 and Sisson, 1988). Therefore, the main objective of this work was to study the effect of live yeast and pronifer supplementation on some blood constituents, testosterone level, testes size and semen quality in Rahmani rams fed sugarcane bagasse silage with or without urea.

MATERIALS AND METHODS

The present study was carried out at the Animal Production Research Farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt.

Sixteen healthy Rahmani male lambs above five months of age (29 ± 0.5 kg average body weight) were used during a six-month experimental period. They were randomly divided into 4 groups: 1-control group (G_1) was fed on untreated sugarcane bagasse silage (*ad libtum*) plus 600 gm concentrate mixture; 2-urea group (G_2) was fed on 3% urea treated bagasse silage (*ad libtum*) in addition to 600 gm concentrate mixture; 3-yeast group (G_3) was fed as G_2 plus 10 gram live yeast/ animal/ day; 4-pronifer group (G_4) was fed as G_2 in addition to 2 gm pronifer/animal/day. Approximate analyses of the experimental diets are shown in Table (1). Blood samples were taken monthly. Serum samples were analyzed for total protein by

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Bio-Adowic Kits (Egypt) according to Doumas (1975) method. Serum albumin by Bio-Merieux Kits(France) according to Drupt (1974) method. Serum globulin was calculated by difference between total protein and albumin concentration. Glucose was determined by Diamond Diagnostics Kits (Egypt) according to Trinder (1969). Serum urea–N by Diamond Diagnostic Kits (Egypt) according to Patton and Crouch (1977). Serum cholesterol by Diamond Diagnostics Kits (Egypt) according to Watson (1960) method. Total bilirubin by Diamond Diagnostics Kits (Egypt) according to Jendrassik (1938). Serum creatinine by Diamond Diagnostics Kits (Egypt) according to Henrey (1974) method. Serum aspartate aminotransferase (AST) and alanine amiotransferase (ALT)enzymes by Wienerlab (Argentina) according to Reitman and Frankel (1957). Serum testosterone hormone by using commercial ELISA Kits (Biosource, Belgium). Also, the size of testes (two testes and scrotum) were measured monthly by using the volume displacement method. Semen samples were collected weekly by using an artificial vagina during the last 6 weeks of this trial in the morning at 9.00 a.m. Semen was examined for ejaculate volume, mass motility, live sperm %, total abnormalities % and sperm concentration according to Zemjanis (1970) procedures.

Table(1):Chemical composition of the experimental diets (on DM basis)

Items	Diets		
	CFM	Bagasse silage	Urea treated bagasse silage
Organic matter, %	87.00	95.66	88.00
Crude protein, %	14.13	3.70	8.91
Ether extract, %	5.11	1.80	1.90
Crude fiber, %	16.33	45.27	38.78
Nitrogen - free extract, %	51.43	44.89	38.41
Ash, %	13.00	4.34	12.00

CFM = Concentrate Feed Mixture.

All data were analyzed by ANOVA using General Linear Models Procedure of SAS (1985).

The statistical model for blood parameters and size of testes was:

$Yijk = M + Ai + Bj + Eijk$

M - common mean.

Ai = treatment effect, where i = 1 – 4

Bj = month effect, where j = 1 – 6

Eijk = residual error.

The statistical model for semen evaluation was:

$Yij = M + Ai + Eij$

M = common mean.

Ai = treatment effect, where i = 1 – 4

Eij = residual error.

RESULTS AND DISCUSSION

1- Serum metabolites:

The effect of sugarcane bagasse silage treated with urea (G_2) on some serum constituents are shown in Table (2). Results indicated that treated groups (G_2, G_3 and G_4) had significantly ($P<0.05$) higher values for total serum protein, globulin, cholesterol, urea-N, AST, ALT, bilirubin and creatinine compared with the control group (G_1) except for total protein and globulin in group G_3. On the other hand, treated groups showed the lowest values ($P<0.05$) for serum albumin and glucose than the control group (G_1). However, the obtained values with yeast and pronifer groups were intermediate between values of control and urea groups except for transaminases. The above mentioned results indicated that yeast or pronifer can modulate serum critical values and consequently alleviate the toxic effect of urea on blood constituents. Similar trend was observed by Joshi and Rangnekar (1979) who found insignificant decrease in serum albumin and higher globulin values for calves fed diets containing 1% urea treated bagasse than for those fed control diet. They are also in agreement with those of Akbar *et al.* (1999) who found high blood protein levels in rams fed live yeast culture. Similar trend of total protein was observed by El-Nor and Kholif (1998) and Kholif *et al.* (2000) in lactating buffaloes. Recently, Abd El-Ghani *et al.* (2004) found that plasma total protein was increased by 17.8% for cows receiving yeast culture (10 gm/cow/day). Yeast culture may stimulate rumen microbes thus altering microbial protein synthesis and increasing protein passage as well as protein yield (Puntam and Schwab, 1994). Abd El-Ghani *et al.* (2004) also reported that yeast culture acts by exerting stimulating effect on rumen fermentation patterns and tissue enzyme reactions (may be through increasing rumen pH, concentration of rumen bacteria, total viable cells and cellulolytic bacteria).

Table (2): Effects of yeast and pronifer supplementation on some serum constituents in rams

Items	Treatment groups				
	G_1 Control	G_2 Urea	G_3 Yeast	G_4 Pronifer	± SE
Total protein (g/dl)	7.18 c	7.47 a	7.24 bc	7.30 b	0.03
Albumin (g/dl)	2.92 a	2.52 d	2.74 b	2.72 c	0.01
Globulin (g/dl)	4.26 c	4.98 a	4.42 bc	4.58 b	0.06
Glucose (mg/dl)	67.01 a	54.72 c	62.20 b	62.88 b	1.05
Cholesterol (mg/dl)	62.34 c	78.41 a	74.90 b	73.82 b	0.38
Urea-N (mg/dl)	9.38 c	17.61 a	16.06 b	16.39 b	0.11
AST (U/L)	13.38 c	21.58 ab	19.79 b	22.67 a	0.77
ALT (U/L)	9.96 b	13.71 a	13.71 a	13.25 a	0.25
Total bilirubin (mg/dl)	0.29 c	0.47 a	0.36 b	0.35 b	0.003
Creatinine (mg/dl)	1.45 c	1.84 a	1.69 b	1.68 b	0.03

a,b,c,d Means with different letters in the same row are significantly different ($P \leq 05$).

Swanson (1989) found negative energy balance by feeding excess protein because it requires energy to metabolize the excess protein (Tyrrell *et al.*, 1970). This explains the presence of low blood glucose in rams fed urea diet in this studies . Addition of yeast culture to feeds treated with urea can enhance serum glucose which agrees with those reported by Piva *et al.* (1993). This improvement may be due to the presence of cellulolytic bacteria in the yeast added and producing more serum glucose (Maynard *et al.* 1983 and Abd El-Ghani *et al.* 2004). Also, it may be due to low acetate to propionate ratio in the rumen (Harrison *et al.* 1988 and Williams *et al.* 1991). Furthermore, Kobeisy and Hussein (1995) found that dietary live yeast increased ($P>0.01$) serum glucose concentration in fish. Addition of live yeast to animal's diet may act as a source of chromium (Schwarz and Mertz, 1959) which markedly decreases cholesterol concentration (Bunting *et al.*,1994) compared with the urea group. Serum urea-N in groups fed urea, especially of urea group (G_2), showed higher concentrations ($P<0.05$) than control group. These results agree with those of Harris *et al.* (1992) and Abd El-Hafez *et al.* (1997). Addition of yeast culture, (yeast group), decreased significantly ($P<0.05$) serum urea-N compared with that of urea group (G_2) but still higher than the control group (G_1). These results agree with those of Piva *et al.* (1993) and Akbar *et al.* (1999). This reduction in serum urea-N concentration in the yeast group may be due to the decrease in ammonia production in rumen and assimilation in blood (Tiwari and Yodava, 1989 and Bhagwat and Srivastava, 1993) or increased efficiency of utilization of amino acids absorbed post-ruminally (Bhagwat and Srivastava, 1993) for yeast diets.

Concerning the enzymatic activities, Mohamed (1998) and Abd El-Aziz (2001) found higher concentrations of AST and ALT in blood of lambs fed silage plus 1% urea than those fed silage free of urea, which agree with results of the present study (Table 2). However, Daghash and Mousa (1994) found that blood serum AST level trended to be lower in sheep fed urea diets than those fed urea free diet. Kobeisy and Hussein (1995) and Abd El-Hafez *et al.* (1997) found no significant effect of yeast on AST and ALT concentrations in fish and sheep serum. However, Abd El-Aziz (2001) reported that lambs fed urea treated silage plus yeast had no effect on serum creatinine level but lowered AST than those fed the same diet without yeast. These results showed that yeast and pronifer groups (G_3 and G_4) had serum bilirubin value within that of control and urea groups (G_1 and G_2). The high serum bilirubin concentration in urea diets may be attributed to a destruction in the red blood cells . However, Piva *et al.* (1993) found no significant effect of yeast culture on serum bilirubin level. Such differences in response may be due to differences in animal species, level of yeast and/or urea, feeding period and/or associative effect of diet composition (Mehrez, 1989).

2- Serum testosterone hormone:

Results regarding the effect of dietary yeast and pronifer supplementation on testosterone level of Rahmani rams are presented in Table (3). Results showed that serum testosterone levels of live yeast and pronifer groups were higher ($P<0.05$) than those of control and urea groups, and that pronifer group achieved the highest value. These events indicated that pronifer and live yeast may have beneficial effects on reproductive performance and NPN assimilation in sheep. These results also showed no significant effect of urea on increasing serum testosterone level. However, Megahed *et al.* (2000) indicated that 1% urea treated bagasse silage had significant ($P<0.05$) positive effect on serum testosterone level in sheep. These differences may be due to urea level intake, feeding period and/or crude protein of the basal diet.

Table (3): Effects of yeast and pronifer supplementation on serum testosterone (ng/ml) in rams.

Experimental Period (month)	Treatment groups				
	G_1 Control	G_2 Urea	G_3 Yeast	G_4 Pronifer	± SE
1	1.34	1.33	1.51	1.66	0.154
2	1.79	1.80	1.88	2.12	0.154
3	2.13	2.13	2.47	2.91	0.154
4	2.20	2.29	2.85	3.11	0.154
5	2.27	2.34	3.15	3.35	0.154
6	2.48	2.45	3.37	3.74	0.154
Overall Mean	2.03c	2.06b	2.54b	2.82a	0.154

a,b,c Overall means with different letters in the same row are significantly different ($P \leq 0.05$).

3- Testes size:

The effects of dietary yeast and pronifer supplementation on testes size are shown in Table (4). Results showed no significant differences among treatements for testes size. On the other hand, Megahed *et al.* (2000) found higher testicular growth for sheep fed sugarcane bagasse or tops treated with 1% urea than those fed the same diet without urea. Differences may be due to differences in urea intake, ration composition, feeding period, level and source of nitrogen (Thwaites, 1994).

Table (4): Effects of yeast and pronifer supplementation on testes size* (ml) in rams.

Experimental Period (month)	Treatment groups				
	G_1 Control	G_2 Urea	G_3 Yeast	G_4 Pronifer	± SE
1	255.00	275.00	290.00	262.50	10.45
2	312.50	327.50	342.50	310.00	10.45
3	387.50	377.50	390.00	355.00	10.45
4	445.00	432.50	462.50	396.50	10.45
5	500.00	527.50	520.00	480.00	10.45
6	552.50	597.50	547.50	567.50	10.45
Overall Mean	408.75	422.87	425.42	395.42	10.45

There were no significant differences among groups.

* Testes size = the two testes and scrotum size .

4- Semen quality:

Results in Table (5) showed the effect of yeast and pronifer on ram semen quality. Results revealed no significant differences between control and urea groups in sperm concentration, total abnormalities, ejaculate volume, total number of sperm/ ejaculate, mass motility or live sperm percentage . Results in control group are in agreement with the findings of Megahed *et al.* (2005) regarding the values of live sperm and total number of sperm/ ejaculate in Egyptian Rahmani rams. Yeast and pronifer groups showed higher ($P<0.05$) sperm concentration, ejaculate volume and total sperm/ ejaculate than those of control (G_1) and urea (G_2) groups. Moreover, pronifer group had lower ($P<0.05$) total sperm abnormalities but higher ($P<0.05$) mass motility than those of control and urea groups. Although no significant differences were detected among all treatments for live sperm% , but pronifer and yeast groups had the highest values than other groups. The present results revealed that urea treated bagasse silage had no significant beneficial effect on semen quality. On the other hand, addition of yeast and pronifer can enhance ($P<0.05$) semen quality in rams fed urea-treated bagasse silage. Similar results were observed in previous studies by Abd El-Aziz,(2001) who found higher ($P<0.05$) ejaculate volume in rams fed yeast diet. The increase in ejaculate volume of animals fed live yeast and pronifer may be due to the enhancement of testosterone hormone level which activates the secretory function of the accessory glands (Salisbury *et al.* 1978) and consequently increases semen volume.

Table (5): Effects of yeast and pronifer supplementation on semen quality in rams

Items	Treatment groups				
	G_1 Control	G_2 Urea	G_3 Yeast	G_4 Pronifer	± SE
Sperm conc. ($x10^9$/ml)	2.66 b	2.77 b	3.73 a	3.88 a	0.21
Total abnormalities (%)	15.64 a	15.57 a	14.46 ab	14.05b	0.39
Ejaculate volume (ml)	0.97 b	1.00 b	1.77 a	1.87 a	0.09
Total no. of sperm ($x10^9$)/ejaculate	2.58 b	2.77 b	6.60 a	7.26 a	0.15
Mass motility (0-5)	4.00 b	4.33 ab	4.67 ab	5.00 a	0.24
Live sperm (%)	82.31	83.74	85.04	86.67	1.64

a,b Means with different letters in the same row are significantly different ($P \leq 0.05$).

With regard to the efficiency of yeast dose used in this study, several studies applied on ruminants indicated that the mode of action of yeast is to enhance ruminal activity and milk production at a dose of 10 gm/ head/ day (Abd El-Ghani *et al.* 1995; Kholif *et al.* 2000 and El-Barody *et al.* 2001).

In conclusion, supplementation of yeast and pronifer had beneficial effects on the reproductive performance by increasing level of testosterone hormone, improving the semen quality and alleviating the toxic effects of urea on blood constituents in Rahmani rams.

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الملخص العربى

تأثير إضافة الخميرة والبروبيوفير على بعض الإستجابات الفسيولوجية والتناسلية فى الكباش الرحمانى

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إستخدم فى هذه الدراسة ستة عشر ذكراً من الحملان الرحمانى بمتوسط وزن ٢٩ ± ٠,٥كجم. قسمت بالتساوى إلى أربع مجموعات. المجموعة الأولى (الكنترول) غذيت على سيلاج مصاصة القصب غير المعامل + ٦٠٠ جم مخلوط علف مركز؛ بينما المجموعة الثانية (مجموعة اليوريا) غذيت على سيلاج مصاصة القصب المعامل بـ ٣% يوريا+ ٦٠٠جم مخلوط مركز والمجموعة الثالثة (مجموعة الخميرة) غذيت على عليقة المجموعة الثانية مع إضافة ١٠ جم خميرة حية/ حيوان/ يوم بينما المجموعة الرابعة (مجموعة البروبيوفير) غذيت أيضاً على عليقة المجموعة الثانية مع إضافة ٢جم بروبيوفير/ حيوان/ يوم . إستمرت هذه الدراسة لمدة ١٨٢ يوماً بهدف دراسة تأثير الخميرة والبروبيوفير على بعض مكونات سيرم الدم ومستوى هرمون التستسترون وحجم الخصية وجودة السائل المنوى. ودلت النتائج على أن حيوانات المجموعات المعاملة (الثانية والثالثة والرابعة) أظهرت ارتفاعا معنوياً (P<0.05) فى بعض مكونات الدم مثل البروتين الكلى والجلوبيولين والكوليسترول وأزوت اليوريا وإنزيمى AST و ALT والبايروبين والكرياتينين وإنخفاضا معنويا فى قيم الألبيومين والجلوكوز مقارنة بالمجموعة الكنترول . ومن ناحية أخرى أعطت مجموعتي الخميرة والبروبيوفير(الثالثة والرابعة) قيما وسطية بين مجموعتى المقارنة واليوريا (الأولى والثانية) فى جميع مكونات سيرم الدم المدروسة عدا إنزيمي AST و ALT ، وبالتالى قللت الخميرة والبروبيوفير من سمية اليوريا فى العليقة . كما أظهرت النتائج زيادة معنوية (P<0.05) لمستوى هرمون التستسترون فى كباش مجموعة البروبيوفير مقارنة بالمجموعات الأخرى . ولم تظهر النتائج فروقاً معنوية فى حجم الخصية بين جميع المعاملات. سجلت كباش مجموعتى الخميرة والبروبيوفير ارتفاعاً معنوياً (P<0.05) فى حجم القذفة المنوية وتركيز الحيوانات المنوية وعدد الحيوانات المنوية فى القذفة مقارنة بمجموعتى الكنترول واليوريا. وتميز السائل المنوى لكباش مجموعة البروبيوفير بالإنخفاض المعنوى (P<0.05) فى النسبة المئوية للشواذ الكلية والإرتفاع المعنوى (P<0.05) فى الحركة الكلية مقارنة بالمجموعة الكنترول . كما أعطت مجموعتى البروبيوفير والخميرة أعلى قيماً فى النسبة المئوية للحيوانات المنوية الحية مقارنة بمجموعتى الكنترول واليوريا . ولم تظهر فروق معنوية فى مقاييس جودة السائل المنوى بين كباش المجموعة الأولى والثانية.

نستنتج من هذه الدراسة أن إضافة الخميرة والبروبيوفير لعلائق الكباش الرحمانى أدت إلى تحسين الإستجابة الفسيولوجية والكفاءة التناسلية لهذه الحيوانات مقارنة بتلك التى غذيت على عليقة اليوريا فقط أو التى غذيت على عليقة الكنترول دون أى إضافات.