THE IMPACT OF PRONIFER[®] SUPPLEMENTATION ON PERFORMANCE OF LACTATING EWES AND NEWBORN LAMBS UNDER UPPER EGYPT CONDITIONS

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SUMMARY

The effects of Pronifer[®] on the performance of lactating ewes and their offspring were investigated in the present study. Twelve single-type of birth Saidi ewes with their lambs were divided into two similar groups according to their lambing date and weight of lamb. Animals were assigned randomly to either the control diet (20% wheat straw and 80% concentrate mixture) or the experimental diet (the control diet plus Pronifer[®] at a rate of 3 kg/ton of the concentrate mixture). The experiment lasted for 10 weeks starting at the 5th day after lambing time. Milk yield as well as its fat and protein content were determined in the 3rd, 4th and 5th week of lactation. Weighing lambs at birth and biweekly thereafter assessed their growth performance. At the end of experiment, blood samples were collected from ewes and lambs to determine some serum metabolites. Results indicated that ewes supplemented with Pronifer[®] in their diet tended to produce higher milk yield, protein percent and protein yield than untreated ones by 9.3, 4.7 and 14.6%, respectively. However, fat percent and its yield tended to be lower by 24.9 and 13.2%, respectively in the tested than the control group. Average daily gain of tested lambs was numerically higher (about 12%) as compared with control lambs. Concentration of serum glucose and total cholesterol increased significantly (P < 0.05) in the Pronifer[®]-supplemented ewes and their offspring. Albumin concentration significantly (P<0.05) decreased while globulin concentration significantly (P<0.05) increased in blood serum of Pronifer[®]supplemented animals. It could be concluded from the present investigation that the use of the probiotic Pronifer[®] leads to improve the health status and feed utilization of both ewes and their lambs.

Keywords: Pronifer[®], ewes, lambs, milk, daily gain, blood metabolites

INTRODUCTION

Pronifer[®] is a probiotic agent, which composed of microorganisms or substances that contribute to the improvement in intestinal microbial balance (Lilly and Stillwell, 1965; Parker, 1974 and Fuller, 1989). Probiotics regulate the microbial environment of the intestine, decrease disturbances, inhibit pathogenic intestinal microorganisms and improve feed conversion efficiency (Windschitl, 1992 and Dhingra, 1993) with consequent improvement of animal health (Games, 1987 and Sisson, 1988) and increases of growth rates (Bohm and Srour, 1995).

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In addition, probiotics improve milk production (both yield and fat content) as a result of fungal supplementation such as *S. cerevisiae* or *A. oryzae* (Williams and Newbold, 1990). This may be a consequence of the observed effect on rumen metabolism.

Currently, considerable attention is being given to the use of probiotics in animal programs. Traditional probiotics are lactic acid bacteria such as *Lactobacillus casein*, *Lactobacillus acidophilus* and streptococci. Freter (1992) and Hughes and Heritage (2002) stated that probiotics are used when the indigenous microflora is incomplete, as in newborns, or disturbed by stress such as the administration of antibiotics.

In ruminants, the potential benefits of probiotics may be obtained by enhancing the rate at which the rumen flora and fauna develop in young ruminants or by stimulating fermentation in adult ruminants (Wallace and Newbold, 1992).

Nahashon *et al.* (1992) pointed out that composition of probiotic is mono or mixed culture of living microorganisms applied to the animal or man, which beneficially affect the host by improving the properties of indigenous microflora.

Commercial probiotics (Pronifer[®]) as a feed additive during the growth stage of lambs and ewes (after parturition) was not examined for growth performance, milk yield and its constituents and some blood serum metabolites under Upper Egypt conditions. Therefore, the objective of this trial was undertaken to throw a light on the effect of supplemental Pronifer[®] on growth performance and some blood serum metabolites of ewes and their offspring. In addition, ewes milk yield and its constituents were also studied.

MATERIALS AND METHODS

Pronifer[®] was identified by P.G.E. (EGGER, GmbH, Milter labill, Austria) as a feed additive made by specific lactic acid fermentation of heat-treated soybean meal and malt, using a multiple-strain mixture of Lactobacilli and Pediococcus, selected from their natural habitat. It contains: 1) viable lactic acid bacteria of approx. 10^6 colony forming units (CFU) per gram (*L. plantarum*, *L. fermentum*, *L. brevis*, *L. casei and Pediococcus acidilactici*); 2) lactic acid fermentation metabolites and enzymes; and 3) free amino acids and short-chain peptides. Pronifer[®] contains 48% CP, 44% DCP, 1% ether extract and 7% ash on DM basis.

Animals and experimental design:

The experiment was carried out at the Experimental Farm of Animal Production Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Twelve single-type of birth Saidi ewes with their lambs were divided into two similar groups according to their lambing date and weight of lamb and randomly assigned to the control and experimental group. Each group of ewes was housed with their lambs in a 8 x 12 m pen. The daily energy and protein requirements were calculated as explained by the NRC (1985). Ewes were fed 20% of their daily allowance as wheat straw (WS) and 80% as a concentrate mixture (CM). The concentrate mixture consisted of 77% ground yellow corn, 20% soybean meal, 2% lime stone, 1% common salt (sodium chloride) and trace minerals mixture at a rate of 3 kg/ton of the CM. Each 100g of the mineral mixture contains 25.6g Na, 4.6g Ca, 1.8g P, 1.6g K, 300mg Fe, 40mg Mg, 32mg Mn, 15mg I, 10mg Mo, 9mg Ni, 5mg Zn, 1.5mg Cu, 1mg Co and 1mg Se. Proximate analysis of CM and WS was determined

according to A.O.A.C. (1975). The chemical composition and nutritive value of WS and CM are shown in Table (1). The control group fed WS and CM as described above, while the experimental group supplemented with Pronifer[®] at a rate of 3 kg/ton of the CM. Each group was fed the assigned diet at the 5th day after lambing (to allow the newborn lambs suckling colostrum) and continued for 10 weeks thereafter. Both groups were kept under the same environmental and managerial conditions. Clean drinking water was freely available at all times.

Item	9	6
	СМ	WS
Chemical analysis (on DM basis):		
Crude protein	16.83	4.62
Crude fiber	4.57	44.72
Ether extract	2.63	1.89
Ash	5.90	9.25
Nitrogen free extract	70.07	39.52
Nutritive value ¹ :		
TDN	81.91	36.84
DCP	12.94	-2.26

 Table 1. Chemical composition and nutritive value of the concentrate mixture (CM) and wheat straw (WS)

1- nutritive values of CM were calculated from nutritive values of its ingredients while those of WS were as reported by Soliman *et al.* (2002).

Milk sampling and analysis:

Three milk samples were taken from each ewe in the 3rd, 4th and 5th week of lactation. Milk yields of ewes were estimated using the weighing-suckling technique as described by Economides (1987). According to this technique, lambs were separated from their dams and allowed to suckle 3 times daily. At each suckling, lambs were weighed before and after suckling. The difference in weights representing the amount of milk consumed. After the last suckling, ewes were milked by hand to remove any surplus milk. The milk suckled plus the amount of surplus milk is the total daily milk yield of ewes. Milk fat percentage was determined by the standard Gerber method, while milk protein content was determined by following the Kjeldahl procedure (A.O.A.C., 1975). Feed efficiency was calculated as Mcal energy value of milk (EV₁) per Mcal digestible energy intake (DEI). The EV₁ was calculated as a function of fat content and days in lactation utilizing the equations recommended by the AFRC (1993) while the DEI was calculated according to the NRC (1985).

Lamb growth performance:

Lambs were fasted overnight then weighed before the morning feeding. Weights were recorded at birth and biweekly thereafter. Total and daily live weight gains were calculated for the whole experimental period. Feed efficiency was calculated as Mcal energy value of gain (EV_g) per Mcal EV_l . Energy value of gain (EV_g) was calculated according to the AFRC (1993).

Blood sampling and analysis:

At the end of experiment, blood samples (5 ml) from each ewe and lamb were collected from the jugular vein into dry glass tube and allowed to clot at room temperature for 30 minutes. Blood sera were separated by centrifugation at 3000 rpm for 15 minutes, decanted into plastic vials and stored at -20°C until the time of assay. Some metabolic profiles such as glucose, total cholesterol, total protein, albumin, urea-N, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by using calorimetric assay kits. Total globulin was calculated by difference between serum total protein and albumin.

Statistical analysis:

Data on ewes' milk and lambs growth performance were tested using the twosample t-test procedure provided by the STATISTIX program (Analytical Software, 1994). Blood serum constituents of ewes and lambs were statistically analyzed using the GLM procedure of SAS (1996). Treatment and ewe-lamb effects were examined utilizing the two-way ANOVA (Steel and Torrie, 1980). The statistical model was:

 $Yijk = \mu + Ti + Aj + TAij + Eijk$ Where:

Yijk = Serum constituent of animal in its treatment.

 μ = General mean

Ti = Treatment effect; i=1, 2

Aj = Animal effect; j= Ewe, Lamb

TAij = Interaction between treatment effect and animal effect

Eijk = Random error

Differences between means of the main effects and interaction were tested utilizing the GLM procedure of SAS (1996) for mean separation.

RESULTS AND DISCUSSION

Milk yield and its constituents:

Results in Table (2) indicate that ewes fed the experimental diet tended to produce higher milk yield, protein percent and protein yield than the other group by 9.3, 4.7 and 14.6%, respectively. Abou'l Ella *et al.* (2003) obtained similar results on Rahmani ewes fed diets supplemented with Pronifer[®] at a rate of 10 gm/head/day. However, fat percent and its yield tended to decrease by 24.9 and 13.2%, respectively. Elevation in milk and protein yields are considered to be an indicator that treated ewes were in a good health and better condition than control ewes.

Sisson (1988) reported that supplementation with probiotic organisms gives a daily protection against any pathogens that may get entrance to the animal gut. This protection improves the digestion and absorption processes of the essential nutrients giving rise to better feed utilization by the animal. Improved feed utilization enhances milk secretion and increases milk yield. Since both of groups consumed similar amounts of feed (Table 2), the control group showed higher body weight change (+2.1 kg) than Pronifer[®] -supplemented group (+0.04 kg). This means that Pronifer[®] - supplementation was in favor of milk production while it was at expense of body weight gain.

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Item	Control	Pronifer [®]	
Milk yield, g/d	894.33±52.90	977.67±5.33	
Fat, %	6.46 ± 0.91	5.17±0.36	
Fat yield, g/d	57.77± 5.83	50.55±3.35	
Protein, %	4.67 ± 0.24	4.89±0.47	
Protein yield, g/d	41.76± 3.43	47.81±4.84	
Feed intake, kg DM/d	1.41	1.41	
Feed efficiency:			
Kg milk/kg DMI	0.63	0.69	
Mcal EV ₁ / Mcal DEI	0.21	0.21	
Body weight change, kg	+2.1	+0.04	

Table 2. Effect of Pronifer[®] supplementation on milk yield and composition, feed efficiency and body weight change of ewes throughout the experimental period

DMI = dry matter intake; EV_1 = energy value of milk; DEI = digestible energy intake.

Lambs growth performance:

Results illustrated in Table (3) and Figure (1) show that growth rate of lambs affected insignificantly (P>0.05) by Pronifer[®] supplementation. However, body weight and daily gain increased gradually during the experimental period (10 weeks). At the end of the trial, average body weight and daily gain of lambs fed the supplemented diet were numerically higher by 8.9% and 11.9%, respectively than those fed the control diet. High body weight and daily gain of treated lambs may be attributed to the higher intake of milk from their mothers (Table 3), where data of milk yield showed that milk yield of treated ewes increased by 9.3% as compared to untreated ewes. The probiotic Pronifer[®] is composed mainly of *lactobacillus* bacterial cocktail that improved the balance of intestinal microflora and consequent improvement in the digestion process reaching to an efficient digestion and feed utilization. These effects were reflected on the overall performance of treated lambs in the form of good appearance, higher activity and an increase in live body weight and daily gain.

Fuller and Booker (1974) mentioned that lactobacilli affected the balance of enteric organisms and improved weight gain. Manickam *et al.* (1994) found that performance (weight gain and feed conversion) of broilers given lactobacilli was significantly better than untreated controls.

Assuming that lambs are the target of production during the experimental period, the economic return could be calculated utilizing the data of feed intake (Table 2) and total gain (Table 3). Pronifer[®] costs 5.03 L.E. ($1.41 \times 3 \times 70 \times 1.7$) while the price of total gain due to the treatment is 16.8 L.E. (1.2×14). Therefore, the economic return from Pronifer[®] supplementation is 11.77 L.E. per lamb (9.81 L.E. / kg live body weight gain).

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Item	Control	Pronifer [®]	
Initial body weight, kg	3.12 ± 0.33	3.18 ± 0.25	
Final body weight, kg	13.20 ± 1.87	14.38 ± 1.27	
Total gain, kg	10.08 ± 1.68	11.28 ± 1.17	
Daily gain, g	144.00 ± 24.02	161.14±16.71	
Feed efficiency:			
Kg gain/kg milk	0.16	0.17	
Mcal $EV_g/Mcal EV_1$	0.28	0.34	
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Table 3. Effect of Pronifer[®] supplementation on live weight, daily gain and feed efficiency of lambs

 EV_{g} = energy value of gain; EV_{1} = energy value of milk.



Figure 1. Lambs' growth curve throughout the experimental period.

Serum metabolites changes:

Studies on the effect of Pronifer[®], as a probiotic supplement, on serum metabolites of ewes and lambs are lacking. In the present trial, concentration of serum glucose for treated ewes and lambs was significantly (P<0.01) higher by 11.8 and 19.2%, respectively than untreated ones (Table 4). This increase may be due to that Pronifer[®] feeding may increase propionate production in the rumen (McDonald *et al.*, 1995). Much of propionate produced in the rumen is absorbed and transformed to glucose in the liver. Higher levels of glucose are then released into the peripheral circulation. Bergman (1983) reported that propionate is a potent glycogenic compound and can account for about two-thirds of total glucose produced in animals fed large amount of concentrates. In general, the increase in both serum levels of glucose and growth rate indicated that treated ewes and their offspring were in better condition than untreated ones.

In the present study, concentration of serum total cholesterol in Pronifer[®]-treated ewes and their offspring increased (P<0.05) by 50.8 and 61.4%, respectively. The higher values of total cholesterol levels in treated group may be due to the improvement of ether extract digestibility by Pronifer[®] supplementation. Jin *et al.*

(1997) reported that the use of probiotics stimulate appetite and digestion and eventually increase fat retention. Another explanation for increasing total cholesterol concentration may be attributed to the higher absorption of dietary lipids and hence energy available for anabolism and fat synthesis processes. This is confirmed by Stullwbeam *et al.* (1969) who reported that lower energy intake by beef heifers resulted in a lower serum cholesterol level.

Table 4. Effect of Pronifer[®] supplementation on some blood serum metabolites of ewes and lambs

Item	E	wes	Lambs		Р	SEM
	Control	Pronifer [®]	Control	Pronifer [®]		
Glucose, mg/dl	30.34c	33.93 <i>bc</i>	45.22 <i>ab</i>	53.90a	< 0.01	6.22
Cholesterol, mg/dl	64.93 <i>ab</i>	97.89a	30.00 <i>b</i>	48.41 <i>ab</i>	< 0.05	25.23
Total protein, g/dl	12.09	11.14	10.91	12.03	NS	0.78
Albumin, g/dl	4.89 <i>a</i>	3.43 <i>bc</i>	4.40ab	3.31 <i>c</i>	< 0.05	0.50
Globulin, g/dl	7.20 <i>ab</i>	7.71 <i>ab</i>	6.51 <i>b</i>	8.72 <i>a</i>	< 0.05	1.01
Urea-N, mg/dl	45.79 <i>ab</i>	39.51 <i>b</i>	56.06 <i>a</i>	52.56ab	< 0.05	7.79
AST, U/l	70.82	68.61	72.36	84.40	NS	8.21
ALT, U/l	6.53 <i>bc</i>	5.07 <i>c</i>	11.18 <i>ab</i>	13.19 <i>a</i>	< 0.01	2.43

^{*a.b.c*} Interaction means in the same row followed by different letters differ significantly (P < 0.05).

Results in Table (4) indicate that serum total protein concentration increased insignificantly (P>0.05) by 10.27% in treated lambs while the reverse effect was observed in treated ewes as compared to control ones. In respect to lambs, such increase may be attributed to the good effect of Pronifer[®] in preventing diseases that usually lead to a great loss of body proteins. Sisson (1988) pointed out that probiotic addition improved animal health by preventing diarrhea and increasing animal growth rate. In addition, *lactobacillus* bacteria, which presented in the Pronifer® enhanced growth and improved animal health (Chateau et al., 1993). Another explanation is that the addition of $Pronifer^{\text{\tiny (B)}}$ to the lambs ration increases the rumen microbial protein synthesis (Abou'l Ella et al., 2003) and crude protein digestibility, which possibly increased total protein in serum of Pronifer[®]-treated lambs. It is not surprising to notice that concentration of total globulin increased significantly (P<0.05) by 6.6 and 33.9% in treated ewes and their offspring, respectively (Table 4). For this reason, Pronifer[®] supplementation had a positive effect on the health status of animals (Chateau et al., 1993) due to the elevation of immune system in the body. Shoeib et al. (1996) found an enhancement effect to the humoral immune response in broiler chicks treated with Pronifer® as it increased the number of activated Blymphocytes and plasma cells in the spleen and bursa of the treated birds. Also, Miake et al. (1985) stated that lactobacilli are potent immuno-stimulants through enhancement of humoral and cellular immune response.

Elevation in AST and ALT are considered to be a good indicator of liver dysfunction (Sheriock, 1975). Lambs fed Pronifer[®]-supplemented diet had higher serum transferases (AST and ALT) concentrations than those fed control diet (Table 4) by 16.6 and 17.99%, respectively while the reverse effect was observed in ewes. This increase may be attributed to the increased synthesis of these enzymes in response to the increased need for glyconeogenesis. AST and ALT enzymes are

important in glucose synthesis from non-carbohydrate metabolism sources (Harper *et al.*, 1977).

Results in Table (4) revealed that ewes and lambs fed Pronifer[®] supplemented diet tended to have lower (P<0.05) serum urea-N concentration by 15.89 and 6.66% than control ewes and lambs, respectively. The decrease in serum urea-N concentration in response to Pronifer[®] supplementation may be attributed to the decrease in ammonia production in the digestive tract. Chateau *et al.* (1993) recorded that *lactobacillus* bacteria present in the Pronifer[®] suppress ammonia production in the intestine.

CONCLUSION

It could be concluded from the present investigation that the use of the probiotic Pronifer[®] leads to improve the health status and feed utilization of both ewes and their lambs. Increasing both milk production of ewes and body weight gain of lambs manifested this improvement. In addition, immune systems of ewes and lambs were in better conditions, which reflected in protection against pathogens. However, a recent report from the Scientific Committee for Animal Nutrition (SCAN, 2001), concerning the safety of Pronifer[®], found that two of the principal strains within the product, *Pediococcus acidilactici* and *Lactobacillus plantarum*, were resistant to tetracyclines. Because of the possible dissemination of tetracycline resistance genes in animal bacterial populations, the food chain and the environment, SCAN (2001) considered that Pronifer[®] poses a risk when used in animal nutrition.

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تأثير إضافة البرونيفير على أداء النعاج الحلابة والحملان حديثة الولادة تحت ظروف صعيد مصر

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أجريت هذه الدراسة على ١٢ نعجة بعد ولادتها ولادات فردية وحملانها (٦ذكور و ٦إناث). قسمت إلى مجموعتين متشابهتين تبعاً لتاريخ الولادة ووزن الحملان بحيث ضمت كل مجموعة ٦نعاج مع ٣حملان ذكور و ٣حملان إناث. غذيت إحدى المجموعتين على عليقه مقارنه (٢٠% تبن قمح و٨٠% مخلوط مركز) بينما غذيت المجموعة الأخرى على عليقه تجريبية (العليقه المقارنة مضافاً إليها البرونيفير بمعدل ٣كجم / طن من المخلوط المركز). استمرت التجرية لمدة ١٠ أسابيع ابتداءً من اليوم الخامس بعد الولادة. تم تسجيل إنتاج اللبن وأخذ ثلاث عينات من اللبن من كل نعجة في الأسابيع الثالث والرابع والخامس من الحليب لتقدير محتوى اللبن من الدهن والبروتين. تم تقدير أداء النمو للحملان عن طريق وزنها عند الميلاد ثم كل أسبوعين بعد ذلك. وفي نهاية التجربة أخذت عينات دم من النعاج والحملان لتقدير بعض مكونات سيرم الدم. وقد أوضحت النتائج أن مجموعة النعاج التجريبية كانت أعلى من مجموعة المقارنة في كمية اللبن ونسبة البروتين وكمية البروتين بحوالي ٩.٣ و ٤.٧ و ١٤.٦% على التوالي. ومن ناحية أخرى فقد كانت نسبة الدهن وكميته أقل في المجموعة التجريبية بحوالي ٢٤.٩ و ١٣.٢% على التوالي عن مجموعة المقارنة. أما بالنسبة لأداء الحملان ، فقد كان متوسط الزيادة اليومية في وزن حملان المجموعة التجريبية أعلى بحوالي ١٢% بالمقارنة بحملان مجموعة المقارنة. أظهر تحليل سيرم الدم زيادة تركيز الجلوكوز والكوليسترول والجلوبيولين بينما قل تركيز الألبيومين معنوياً في دم نعاج وحملان المجموعة المغذاة على العليقه المضاف إليها البرونيفير. وتخلص الدراسة إلى أن استخدام المنشط الحيوي برونيفير في علائق النعاج والحملان خلال فترة الرضاعة يؤدي إلى تحسين حالتها الصحية وزيادة معدل الاستفادة من الغذاء.