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EFFECT OF UREA SUPPLEMENTATION AND ANTHELMINTIC TREATMENT ON THE PRODUCTIVE AND REPRODUCTIVE PERFORMANCES OF SUFFOLK EWES INFECTED BY GASTROINTESTINAL NEMATODE

(With 3 Tables and 2 Charts)

By

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تأثير اضافة اليوريا والعلاج بمضادات الطفيليات على الإنتاجية والأداء التناسلى فى نعاج السافولك المصابة بالديدان الإسطوانية

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لدراسة تأثير اضافة اليوريا وعلاج الديدان الأسطوانية أثناء المراحل المتأخرة من الحمل فى النعاج السافولك المصابة بالديدان الإسطوانية على قدرتها الانتاجية والتناسلية، تم استخدام عدد عشرون من نعاج السافولك فى مراحل متأخرة من الحمل ومصابة بالديدان الإسطوانية، من مزرعة سخا محافظة الغربية. تم تقسيم النعاج الى مجموعتان رئيسيتان على حسب التغذية كل مجموعة = ١٠ نعاج، اول مجموعة نم تغذيتها على عليقة ضابطة، والمجموعة الاخرى =١٠ نم تغذيتها على نفس العليقة مضاف اليها اليوريا. قسمت كل مجموعة مرة أخرى الى مجموعتين كل منها تتكون من ٥ نعاج، الأولى منها تم حقنها تحت الجلد بالأيفوماك ١مل/٥٠ كيلوجرام من وزن الجسم، أما الأخرى فتركت بدون علاج. وعلى هذا فيكون هناك أربعة مجاميع كلاتى: مجموعة معالجة بالأيفوماك مع اضافة يوريا فى العليقة الخاصة بها(المجموعة الأولى) ومجموعة مضاف يوريا الى العليقة الخاصة بها فقط (المجموعة الثانية) ومجموعة معالجة بالأيفوماك فقط (المجموعة الثالثة) والمجموعة الأخيرة غير معالجة بالأيفوماك وغير مضاف يوريا (المجموعة المصابة الضابطة) الى العليقة الخاصةبها. مع الفحص الميكروسكوبى لبراز جميع المجاميع أسفرت النتائج عن اختفاء بويضات الديدان الإسطوانية من براز المجموعة الأولى والمجموعة الثالثة كما انخفضت نسبة عدد البويضات من براز المجموعة الثانية (يوريا فقط) انخفاضا ثابتا الى نهاية فترة

الدراسة، أما المجموعة الضابطة فقد سجل عدد البويضات فى البراز زيادة ملحوظة الى أن وصـــلت أعلى معدلاتها فى نهاية الدراسة. لوحظت زيادة معنوية فى نسبة البروتينات الكلية والزلال (الألبيومين) والجلوبيولين فى المجموعة الأولى والثانية والثالثة عنها فى المجموعة الضـــابطة. أمـــا نســـبة اليوريا فسجلت انخفاضا معنويا فى المجموعة الأولى عنها فى بقية المجامـــيع وان كانـــت أعلـــى فى المجموعة الضابطة عنها فى بقية المجاميع. كانت أوزان الحملان المولودة من جميع النعاج المضاف اليوريا والمعالجة الى علائقها تزيد زيادة معنوية عن أوزان الحملان المولودة من الأمهات المصابة بالديدان الإسطوانية. أيضا لوحظت زيادة معـــنوية فى نسبة الاناث العشار والوالدة فى المجموعة الأولى عنها فى بقية المجاميع، كما ســـجلت نسبة الخصوبة وكذلك انتاج التوائم زيادة ملحوظة فى المجموعة الأولى والمجموعة الثانية عنها فى بقية المجاميع.

SUMMARY

To study the effect of urea supplementation and or ivomec treatment on the productivity and reproductive performance of infected ewes with gastrointestinal nematodes, twenty mature Saffolk ewes, naturally infected with gastrointestinal nematodes and in their late stages of pregnancy were used. At first, ewes were divided according to the mode of nutrition into two main groups (n=10 in each group). The first group was fed on a basal diet (low protein diet) without supplementation while, the second group (n=10) was fed on the same diet supplemented with urea (high protein diet). Then each group was further divided into two subgroups (n=5 each). The first subgroup was treated with subcutaneous injection of ivomec at 1 ml/ 50kg B.Wt, while the second subgroup was left untreated. Hence, four groups were formed: urea/ivomec gp, urea gp, ivomec gp and positive control gp (without urea or ivomec). Faecal examination of all groups revealed the absence of gastrointestinal nematode ova from the faeces of the first and the third groups, while there was steady decrease and increase of the faecal egg count of the second and fourth groups respectively, till the end of the study. There werer significant improvement in the levels of total protein, albumin and globulin of the first three groups than the control ones. While, there was significant decrease in urea level of the first group as well as non significant decrease in the second and third groups than the control ones. Urea supplementation and or ivomec treatment affect positively on the birth weights of the newly born lambs, as the birth weights of the newly born lambs of the first, second and third groups were significantly higher than those of the positive control ewes. Pregnancy % was higher in the first group (100%), while in the other groups it was 80%. However, lambing % tended to be higher in the first group (100%), and in the

second group, it was 80%, while it was 60% in the third and control ones. Meanwhile, fertility% was also enhanced in ewes of the first group then the second group, while it was equal in both third and control groups. In addition, urea supplementation improved prolificacy%, the recorded results were 140, 150, 133.3, and 133.3% in the first, second, third and positive control groups respectively.

Key words: *Urea, anthelmintic, ewes, gastrointestinal nematode*

INTRODUCTION

The interaction of parasitism and reproductive activity in pregnant ewes, characterized by the "periparturient" (PPR) or "spring" (Dunn, 1978) rise in egg worm output, this resulted in a decline in the expression of immunity which will be manifested as "breakdown or relaxation in immunity" (Barger *et al.*, 1973; Barger and Southcott, 1975). In general gastrointestinal nematode (GIN) infection reduces nutrient availability to host through both reductions in voluntary feed intake and/or reduction in the efficiency of absorbed nutrient, although the underlying mechanisms of the depression in appetite have not been fully elucidated (Dynes *et al.*, 1998). The extent of metabolic impairment by a parasite is influenced predominantly by the level of larval challenge and the number of species of worms which established in the host (van Houtert & Sykes, 1996). This impairment resulted in an increase in protein requirements of the parasitized animal and the need for a high dietary content is accentuated by the reduction in the voluntary feed intake that accompanies such parasitism (Symons 1985). The regulation of nematode populations and the ability of the host to withstand the pathophysiological effects of infection are influenced by nutritional and immune status of the host (Coop & Holmes, 1996 and Coop & Kyriazakis, 1999). The host under severe under nutrition would never overcome parasitic infections (Behnke *et al.*, 1992). Recently, there was a nutritional base or manipulation of pregnant or lactating ewes can alter the onset of the periparturient relaxation in immunity into before (McAnulty *et al*,. 1991) or after parturition (Houdijk *et al.*, 2001). The ewes on the higher level of protein supplementation were better able to regulate the incoming larvae of *Otertagia cercumcincta and Trichostrongylus colubriformis*, can moderate the periparturient breakdown in resistance of ewes (Donaldson, 1998). Periparturient ewes, have demonstrated significant improvements in resistance and resilience

to infection with GIN by dietary metabolizable protein supply as a chemotherapeutic alternative premise to the management of GIN, which was obvious in his experimental studies of Kahn, (2004) and Sykes & Greer (2004).

The following trial is carried out to assess the influence of urea supplementation and/or ivomec treatment of the pregnant Suffolk ewes on the course of natural nematode infections, and its reflection on the reproductive and productive status of these ewes.

MATERIALS and METHODS

Animals

To determine the effect of urea supplementation and/ or ivomec treatment on the reproductive and productive performance of ewes infected with GIN, the present study was carried out on twenty adult Suffolk ewes (3-4 years of age) from Sakha farm (Gharbia province) flock. Before the beginning of the trial, all ewes were pregnant and chosen to be naturally infected with different species of GIN after individual examination of their faecal samples.

Experimental design and parasitology

The infected animals were allocated firstly into two main groups (n = 10 each). The first group was fed on a basal diet (low protein diet) without supplementation while the second group was fed on the same diet supplemented with urea (high protein diet). Ingredients and chemical composition of the used diets are shown in table 1. Then each group was further divided into two subgroups (n=5 each). The first subgroup was treated with subcutaneous injection of ivermectin (Bomectin$^{R.}$ product of BoMAC Laboratories, 1% W/V) at 1 ml/ 50kg B.Wt, while the second subgroup was left untreated. Hence, four groups were formed: urea/ivomec group, urea group, ivomec group and positive control group (without urea or ivomec).

Table 1: Ingredients and chemical composition of the experimental diets.

	Basal diet	*Urea supplemented diet*
Ingredients (g/2kg fresh weight):		
Berseem hay	1648	1648
Corn	273	275
Wheat straw	52	47
Salt	5.5	5.5
Dicalcium phosphate	6.3	6.3
Urea	-	12.8
Mineral mixtute*	3.4	3.4
Vitamin mixture**	1.8	1.8
chemical composition (As DM basis):		
DM (%)	85.7	58.8
Crude protein (%)	13.1	14.99
TDN (%)	63.7	63.3
Crude fiber (%)	21.1	21.14
Ca (%)	1.00	1.1
P (%)	0.32	0.30

*each 2 kg contain 10000 mg iron, 500 mg iodine, 7000 mg copper, 100 mg cobalt, 40000 mg manganese, 45000 mg zinc and 150 mg selenium.

** each 1 kg contain 4000000 iu vitamin A, 1000000 iu vitamin D3 and 15000 mg vitamin E.

Sampling

Blood and faecal samples were obtained from each ewe before the trial and five times during gestation period with twenty days intervals. Jugular blood samples were collected individually from all ewes in the four tested groups. All blood samples, were maintained at room temperature for coagulation, and then centrifuged (at 2,000 r.p.m for 20 minutes). Sera were collected and were stored at -20^0 C until biochemical analysis.

Faecal examination

Faecal samples were collected from rectum of each ewe before treatment and five times (twenty days intervals) throughout the gestation period till the end of the trial, and examined for the presence of nematode eggs by saturation concentration floatation technique using saturated sodium chloride solution (Soulsby, 1982). Degree of infestation was performed by egg counting (FEC) using the McMaster

technique according to Urquhart *et al.*, (1988). A coproculture (faecal culture for identification of the infected larvae) for 21 days at 22° C was performed and the larval identification determined according to Georgi *et al.*, (1985).

Biochemical analysis

Serum samples from all ewes were analyzed for total protein, albumin, and urea using standard diagnostic kits according to the method of Kato, 1960; Rodkey, 1964; Fawcett and Scott, 1960 respectively. Globulin was measured by subtraction of albumin from total proteins values; albumin/globulin ratio was also calculated.

At lambing, number of newly born lambs, incidence of twinning as well as lamb birth weight was recorded.

Statistical analysis

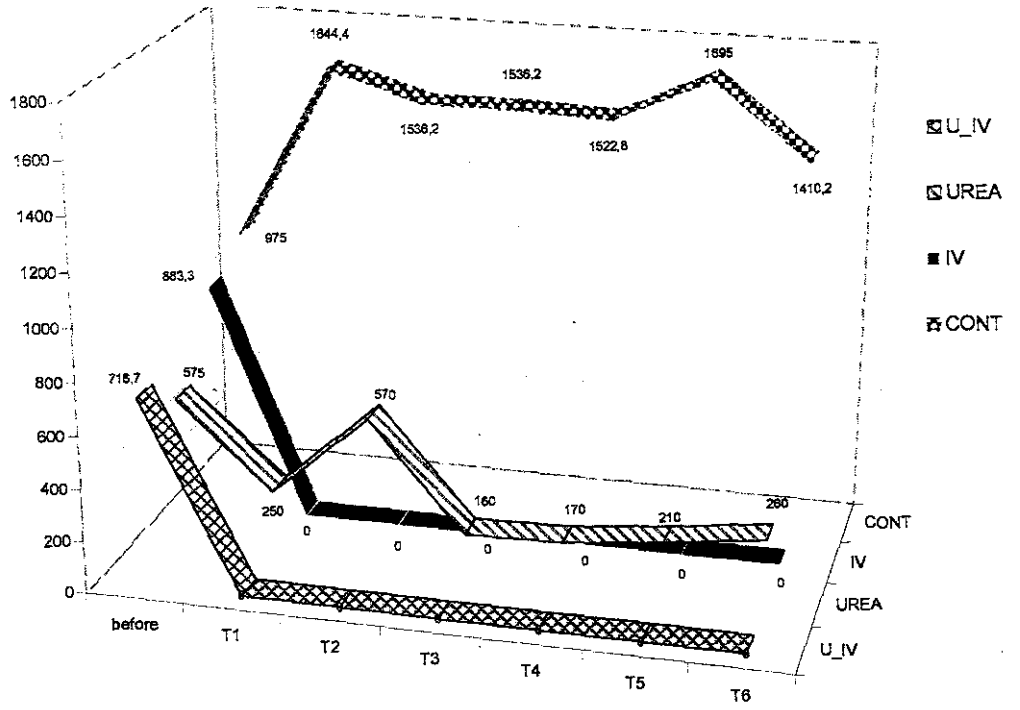
Categorial data including percent of conception (number of ewes lambed per ewes exposed), percent of fertility (number of lambs per ewes exposed), percent of prolificacy (number of lambs born per ewe lambed), were analyzed using Chi-square for counted data. Live birth weight of lambs, faecal egg count (FEC) as well as concentration of all biochemical parameters were submitted to one way analysis of variance. Such statistical analysis were preferred according to Sendecor and Cochran (1987) using the 1984 version of MICROSTAT (Ecosoft, inc, USA) computer program.

RESULTS

Faecal examination revealed that, all ewes were naturally infested with mixed Trichostrongylid and Strongylide species. Coproculture revealed that, the predominant nematode genera were *Trichostrongylus sp., Haemonchus sp., Strongyloioedes papillosus and Nematodirus sp.* Faecal egg count (FEC) of the four ewe groups under investigation are shown in chart (1). At the beginning of the trial, the mean FEC of urea supplemented and ivomec treated revealed (716.7 ± 221.2), urea supplemented group was (575 ± 153.7), ivomec treated group was (883.3 ± 177.7), while the control positive group recorded (975 ± 184.2). Then after the treatment, no eggs were detected in each of the urea supplemented/ivomec group and ivomec treated groups throughout the experimental period. Meanwhile in the urea supplemented group, there was a steady decrease of the FEC, allover the trial period until it reached, (260 ± 85.7) at the end. Concerning the positive control group, it recorded steady increasing in the FEC from the

beginning of the trial (975 ± 184.2) until it reached (1466.8 ± 125.7) before lambing (periparturient rise in egg count).

Chart 1: Effect of urea supplementation and/or ivomec treatment on the Feacal egg count of infected pregnant Suffolk ewes with GIN.



Biochemical analysis of all groups is shown in table (2).

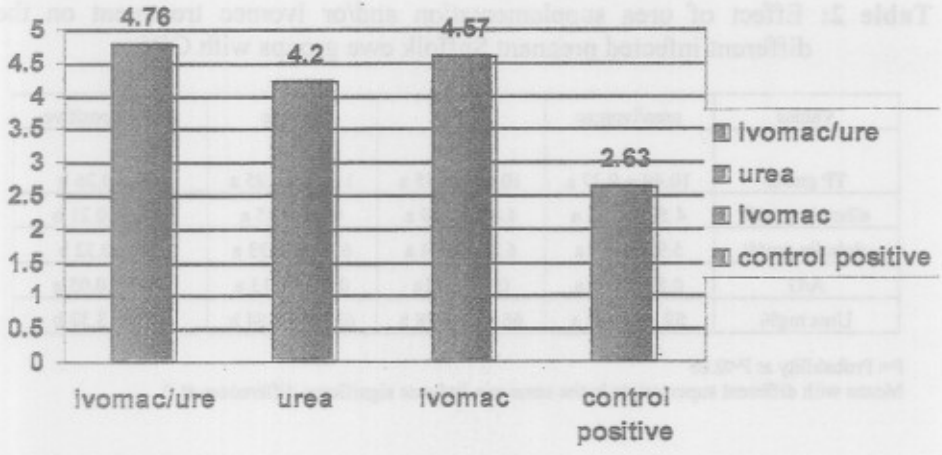
Table 2: Effect of urea supplementation and/or ivomec treatment on the different infected pregnant Suffolk ewe groups with GIN.

Values	urea/ivomac	urea	ivomac	control positive
TP gm/dl	10.49 ± 0.37 a	10.67 ± 0.45 a	11.13 ± 0.25 a	6.8 ± 0.26 b
albumin gm/dl	4.57 ± 0.32 a	4.45 ± 0.29 a	4.8 ± 0.35 a	2.75 ± 0.21 b
globulin gm/dl	5.92 ± 0.31a	6.28 ± 0.44 a	6.22 ± 0.33 a	4.1 ± 0.32 b
A/G	0.83 ± 0.11a	0.77 ± 0.1a	0.81 ± 0.13 a	0.69 ± 0.05 a
Urea mg%	59.3 ± 4.69 a	66.92 ± 3.78 b	63.56 ± 2.84 b	67.9 ± 3.32 b

P= Probability at P<0.05
Means with different superscripts in the same row indicate significant differences at P

There was a significant improvement ($P<0.05$) in the total protein concentrations between each of urea supplemented/ivomec treated, urea supplemented, ivomec treated groups and the control positive group, as they recorded (10.49 ± 0.37), (10.67 ± 0.45), (11.13 ± 0.25) and (6.8 ± 0.26) respectively, and the positive control group was lower in their concentrations. In addition, the level of albumin concentration recorded significant ($P < 0.05$) higher levels in supplemented and treated groups than the positive control group as it recorded (4.57 ± 0.32), (4.45 ± 0.29) (4.8 ± 0.35), and (2.75 ± 0.21) for urea supplemented/ivomec treated, urea supplemented, ivomec treated groups and the control positive group respectively. While, the levels recorded non-significant changes within urea supplemented and/ or ivomec treated groups. Concerning the globulin levels, recorded significant ($P < 0.05$) higher levels in the urea supplemented and/ or ivomac treated groups than the control positive group, while there were no changes within the urea supplemented and/ or ivomec treated groups. The albumin /globulin ratio showed non-significant changes between the all groups of the trail. Concerning the urea concentration level in the different groups, it recorded significant ($P < 0.05$) higher levels in the control positive groups second, third groups respectively, while it was in its lower level in the first group. There was a significant increase in the birth weights of the first three groups than the control positive group as shown in chart (2).

Chart. 2: Effect of urea supplementation and/or ivomec treatment on the birth weight of the newly born lambs from the different ewe groups. (Kg BW.)



The group means being (4.76 ± 0.6), (4.2 ± 0.69), (4.57 ± 0.48) and (2.6 ± 0.18) Kg BW. for urea supplemented/ivomec treated, urea supplemented, ivomec treated groups and the control positive group respectively while, there was non-significant differences in lambs birth weight within the urea supplemented and /or ivomec treated group .

The effect of dietary urea supplementation / ivomec treatment during the gestation period on the subsequent reproductive performance of Suffolk ewes is shown in table (3).

Table 3: Effect of urea supplementation and/or ivomec treatment on the productive and reproductive performance of infected pregnant Suffolk ewes with GIN.

	U &Ivomec	Urea	Ivomec	Control +ve	P
no of ewes exposed	5	5	5	5	
pregnancy %	5/5(100%)	4/5(80%)	4/5 (80%)	4/5(80%)	p< 0.01
No. of ewes lambed	5/5 (100%) a	4/5 (80%) b	3/5 (60%) b	3/5 (60%) b	p< 0.05
No. of lambs born	7	6	4	4	p< 0.01
fertility %	7/5 (140) a	6/5 (120) a	4/5 (80) b	4/5 (80) b	p< 0.05
Prolificacy%	(7/5) 140 a	(6/4) 150 a	(3/4) 133.3 b	(3/4) 133.3 b	p< 0.05

P = probability
Means with different superscripts in the same row indicate significant differences at P

It was found that, pregnancy % was higher in ewes supplemented with urea / ivomec treated (100%) than those in the other three groups, as it revealed the same percentage (80%). However, lambing % tended to be higher in both urea supplemented / ivomec treated (100%) and urea supplemented groups (80%) than ivomec treated group (60%) or positive control group (60%). The number of lambs born from the urea supplemented/ivomec treated ewes (7 lambs), were more than the lambs born from urea supplemented group (6 lambs) or from the ivomec treated and the positive control ones (4 lambs each). Meanwhile, fertility % was also enhanced in urea supplemented/ivomec treated and in urea treated ewes than those ivomec treated or the positive control groups. The urea supplementation tended to improve prolificacy%. The recorded results were 140, 150, 133.3, and 133.3 % in urea supplemented/ivomec treated, urea supplemented; ivomec treated and control positive groups respectively.

DISCUSSION

In the present study, FECs were moderate to low in all groups, then with urea supplementation and/or ivomec treatment, absence of eggs in faecal samples occur throughout the experimental period as indicated by Familton *et al.*, (1995) and Umima & Dalal (2004), who indicated that, anthelmintic treatment of ewes during gestation period virtually eliminated ewe parasite faecal egg counts and consequently eliminated the expected spring rise in FECs of ewes associated with lambing which lead to significantly higher lamb weaning weights. The urea supplemented group showed that, FECs were lower than those in the control positive group suggesting that, resistance was enhanced by protein supplementation. These results coincided with those of Singh *et al.*, (1995); Etter *et al.*, (2000); Waruiru *et al.*, (2003) and Kahn (2004). Meanwhile, in the positive control group, FECs increased reaching its higher levels at the end of the trial. This periparturient rise in egg count (PPR) is strongly supported by Dunn (1978), Dunsmore (1965) and Thomas & Ali (1983), this is attributed to a consequence of either increased nutrient requirements due to depression in appetite, or decline in nutrient supply, there will be a decline in the expression of immunity which will be manifested as "breakdown in immunity" (Barger *et al.*, 1973) and (Dynes *et al.*, 1998). Both total protein and albumin concentration in the control parasitized group were significantly decreased than the other supplemented or treated groups These results tend to support the previous findings of Ibrahim *et al.*, (1983); Taha *et al.*, (1986); Ismail *et al.*, (1990); Mandour and Omaima (1994) ; Radostitis *et al.*, (1994) and Maiti *et al.*, (1999). They concluded that, the significant decrement in total protein and albumin levels may be attributed to involvement of the liver by metabolic products of GIN, also alteration of protein absorption and metabolism which ending by hypoproteinaemia, also may be attributed to the haematophagic parasite *Haemonchus sp.*, or may be due to leakage of protein to the gastrointestinal tract (Barker, 1973) and (Holmes 1985). Regarding to the influence of the urea supplementation and/or ivomec treatment, there were significant increase in the levels of total protein and albumin between the urea supplemented and / or ivomec treatment, and urea supplemented group versus the control positive one, a similar reduction in serum protein and albumin levels which improved after anthelmintic treatment has been described in pregnant ewes by other workers (Rahman & Collins, 1990; Mousa *et al.*, 1998 and Umima & Dalal,

2004). This improvement may be due to elimination of the parasites, stimulation in feed intake, enhancement ruminal digestion, elevated NH3-N levels which would be expected to have increased rumen microbial protein synthesis and availability to intestines (Wallace *et al.*, 1998 and Knox & Steel, 1999) also, may be due to the enhancement of the innate resistance of sheep or accelerate the development of immunity to GIN (Fox, 1997).

Concerning serum urea concentration, there was a higher level in the control positive group than the other supplemented and or treated groups which coincided with Abott *et al.*, 1985 and Brar *et al.*, 1991, who found increase in urea level concentration in lams and in sheep infected with gastrointestinal nematodes respectively. Urea increase may be due to the excess protein intake, leading to the increased circulating concentration of ammonia and urea, since urea is the end result of protein metabolism, the non-essential amino acids will be deaminated in the absence of the essential amino acids leading to increase of plasma urea concentration. (Canfield *et al.*, 1990 and Larson *et al.*, 1980). The dietary urea supplementation enhanced pregnancy rate, fertility %, and prolificacy%. In addition, the lamb birth weight was higher in all groups versus the positive control ones. As general results, the effect of urea supplementation and treatment in the diet of periparturient Suffolk ewes can increase resilience and resistance to GIN, thereby improving reproductive and productive performance of them. These results were in agreement with those of Blackburn *et al.*, (1991) in goats infected with *Haemonchus contortus*, Wallace *et al.*, (1996) ; Knox& Steel (1999); Datta *et al.*, (1998); McKellar *et al.*, (2000); Chartier *et al.*, (2000); Waruiru *et al.*, (2003); Sykes & Greer (2004) and Kahn (2004). The observed effect of urea supplementation was seemingly due to greater food consumption as well as the better diet Wallace *et al.*, (1998). Valderrabano *et al.*, (2002) showed that, both female worm size and their fecundity decreased significantly with the increased level of nutrition in the diet of infected lambs.

On conclusion, this work suggests that, potential exists to reduce GIN infection through manipulation of protein level in diet. It means that, urea supplementation and/or anthelmintic treatments may be beneficial in improvement the resistance and/or resilience to nematodes infection, through getting rid of or reduction of the periparturient rise in FEC.

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