LAMBS RESPONSES TO DIETARY SUPPLEMENT OF RUMEN UNDEGRADABLE NITROGEN AS AFFECTED BY NIGELLA SATIVA FEED ADDITIVES

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ABSTRACT

The effect of two levels of rumen undegradable nitrogen (7 and 10 g UDN / kg DM) and two levels of *Nigella sativa* (0 and 7.5 g NS / kg DM) upon live-weight gain ,feed conversion ratio and some blood parameters were investigated in a 2x2 factorial experiment . Twenty four individual Karadi male lambs were used .They were weighing approximately 34±1.22 kg and 7 months of age. Two lambs were randomly allocated from each diet in order to determine some blood parameters. There were no differences among diets in daily DM,OM,ME, RDN, NDF,ADF, lignin, hemicellulose and cellulose intake when expressed as g/day or g/kg $W^{0.75.}$ The live weight gain and feed conversion ratio differences were not significantly affected by increased level of UDN ., However, live weight gain and FCR were significantly improved with those lambs fed with high UDN diet supplemented with NS as compared with those fed diets without NS . Lambs fed high level of UDN significantly reduced blood sugar (BS) and increase blood urea nitrogen (BUN) as compared with those fed low level of UDN. Whereas ,level of UDN had no effect on serum uric acid (SUA) and growth hormone (H). The results also indicated that the lambs fed diets supplemented with NS significantly increased BS,SUA and GH and reduced BUN as compared with those fed diets without NS .It was concluded that higher responses to NS additives was associated with higher level of UDN .We recommend that 7.5 g NS and 10 g UDN achieved butter live weight gain and feed conversion ratio .

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استجابه الحملان الكراديه لنتروجين الغذاء غير المتحلل في الكرش باستخدام الحبه السوداء كأضافات غذائيه

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المستخلص

تمت دراسة تأثير مستويين من النتروجين غير المتحلل في الكرش(7و10 غم/كيلوغرام مادة جافة) ومستويين من الحبة السوداء (0و7.5 غم /كغم مادة جافة)في معدل الزيادة الوزنية اليومية ،كفاءة التحويل الغذائي وبعض صفات الدم في 2x2 تجربة عاملية.تم استخدام 24 حمل عواسي ذكر في حضائر مفردة وبوزن تقريبي 34 كغم وبعمر 7 اشهر .تم اختيار اثنين من الحملان عشوائيا من كل معاملة لتقدير بعض قياسات الدم. اظهرت النتائج عدم وجود اختلافات معنوية في كمية المتناول من المادة الجافة،المادة العضوية الطاقة المتأيضة ، النتروجين المتحلل في الكرش،الالياف المستخلص المتعادل والحامضي ، الكلين ،الهيموسليلوزو السليلوز بين المعاملات المختلفة عند حسابها على اساس غم/يوم او غم /كغم جسم ايضي . معدل الزيادة الوزنية وكفاءة التحويل الغذائي لم تتأثر معنوياً بمستوى النتروجين غير المتحلل في الكرش. مع ذلك ،فأن معدل الزيادة الوزنية اليومية وكفاءة التحويل الغذائي قد تحسنت معنوياً في الحملان المغذات على مستوى عالي من النتروجين غير المتحلل في الكرش عند اضافة الحبة السوداء مقارنة بالحملان المغذات على عليقة لاتحوي على الحبة سوداء . ان الحملان المغذات على مستوى عالي من النتروجين غير المتحلل في الكرش اظهرت انخفاضاً معنويا في سكر الدم ونتروجين يوريا الدم مقارنة بالحملان المغذات على مستوى واطئ من النتروجين غير المتحلل في الكرش . في حين لم يؤثر مستوى نتروجين غير المتحلل في الكرش على مستوى حامض اليوريك وهرمون النمو . اظهرت النتائج ان الحملان المغذات على الحبة السوداء زيادة معنوية في سكر الدم ويلازما حامض اليوريك وهرمون النمو يقابلها انخفاض معنوي في نتروجين يوريا الدم مقارنة بالحملان المغذات على عليقة لاتحوي على الحبة السوداء . ان اعلى استجابه للحبه السوداء كانت مرتبطه مع اعلى مستوى للنتروجين غير المتحلل في الكرش .نوصي باستخدام 7.5 غم حبه سوداء و 10 غم نتروجين غير متحلل في الكرش /كغم ماده جافه للحصول على افضل زياده وزنيه وكفاءة تحويل غذائي .

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***Key word*: Rumen undegradable nitrogen , live weight gain and blood parameters**

Introduction

All animal diets have the same basic goal; to provide nutrients in adequate amounts and proportions to meet their maintenance and production requirements while avoiding waste and over feeding. Providing nutrients in excess of animal requirements results increase costs of production and contribute environmental problems (12).Therefore, protein systems were efforts to maximize efficiency, and minimize the loss of nutrients in particular term by ARC,(2) and NRC (29) were recommended ., that the protein requirement for ruminant animals is a combination of the need of rumen micro-organism (Rumen degradable N ,RDN) and of the host animal (Rumen un degradable N,UDN).In the specific case of lambs weighing more then 30 kg , ARC (2) has proposed that the nitrogen requirement may , in most instances, be met by microbial protein only, and thus, only RDN is required in the diet .However , a number of previous reports indicated response to a supplement of UDN when lambs were given roughage (20) or concentrate (3 , 15) basal diets. Also lambs respond to UDN supplementation was associated with available energy at small intestine (12) . Protein supplementation and natural feed additives are very important material that can improve, growth rate, feed efficiency utilization and carcass characteristics of growing lambs (3, 13, 14,15, 21,22,23, 25, 28, 30) .In contrast , blood urea nitrogen (BUN) analyses can be used as a signal red to point out potential problem in the feeding program. The BUN level in excess of 18 to 20 mg/dl can be associated with lower reproductive performance , and higher feed costs, health problems , and poor production (12) . Therefore, the observed responses to feed additives need more explanation and some possible reasons has this responses may need to explain the beneficial of additives feed in the diet. There is still a need to investigate other factors which may affect this response to UDN supplementation .

The objective of this experiment was to maximize the utilization efficiency of UDN by providing feed additives such as *Nigell Sativa* (NS) in quantities sufficient to ensure productivity (14) from diets containing similar and adequate amount of RDN (2) such as live weight gain , feed conversion ratio and some blood parameters .

Materials and Methods

Design of experiment and diets

The effect of two levels of rumen undegradable nitrogen (7 and 10 g UDN / kg DM) and two levels of *Nigella Sativa* (0 and 7.5 g NS / kg DM) upon live-weight gain ,feed conversion ratio and some blood parameters were investigated in a 2x2 factorial experiment using a randomized block design with six replicates per cell of the design .Diets were formulated to provide two levels of UDN and two levels of NS and a constant daily intake of RDN g/MJ of ME and metabolizable energy across treatments. This was achieved by using both untreated soybean meal (SBM) and formaldehyde- treated soybean meal (FTSBM) in the diets and substituting FTSBM for untreated SBM as UDN level increased .Yellow corn was chosen as the basal ingredients for the diets because it has low N concentration. SBM (all of USA origin) was chosen as the source of RDN because the N content is reputed to be largely rumen degradable . FTSBM was used as the source of rumen undegradable nitrogen (UDN) ,because its N content is reputed to be largely un degradable N .The disappearance of N from the feedstuffs in the rumen was estimated by using the values reported by (16,17) . The barley , yellow corn , soybean meal ,other supplements and minerals were mixed and offered as a concentrate fed separately from the treated barley straw. The diets were formulated to contain a 40 parts NaoH-treated barley straw DM and 60 parts concentrate DM .Chemical composition of the feedstuff and formulation and chemical composition of experimental diets are shown in table 1and 2 respectively.

Formaldehyde-treated soybean meal

Formaldehyde treatment of SBM was done by spraying formaldehyde solution onto the meal at the ratio of 10 ml /100 g SBM DM, equivalent to 1 g formaldehyde per 100 g crude protein (15), and mixing well then packing in

polyethylene bags . Bags were sealed and left at room temperature (25 C^0) for 48 h and were shaken occasionally .After 48 h , treated SBM was air-dried in trays for 24 h to remove excess formaldehyde.

Preparation of NaoH - treated barley straw

The barley straw used in this experiment was ground and treated with NaoH at rate of approximately 40 g/kg DM as following **:** NaoH was applied by spraying equal weight of NaoH solution on straw to provide a treatment level of 40 g NaoH per kg straw DM . The sprayed straw was mixed well to bring NaoH solution into contact with straw as completely as possible .The freshly- made material was covered with polyethylene nylon for approximately 2-3 weeks to absorb moisture that formed during the heating process . The chemical composition of treated barley straw is presented in table (1).

Animals and management

Twenty four individual Karadi male lambs were used .They were weighing approximately 34 kg live weight and 7 months old at the start of the experiment .Six lambs were randomly allocated from live weight block to each treatment . Two lambs were randomly allocated from each group in order to determine some blood parameters . The lambs were individually housed in pens (1x1.3 m) that allowed access to diets supplied in mettle bucket fixed in side the pen .Water was available at all times .The diets were gradually introduced to the lambs over a period of 3 weeks before the start of the experiment . During this time the lambs were vaccinated against clostridia diseases. The diets were offered once daily at about 08.00 hour (h) in quantities calculated to support maintenance and daily gain of 200 g (3). Allowances were recalculated each 2 weeks according to live weight. NaoH-treated barley straw and feeds refusal were collected and weighed back daily. Offered and refusal feeds were sampled and stored at -15C^0 for subsequent chemical analysis .

The lambs were weighed each two weeks to nearest 0.5 kg , at the same time each day .Recording of daily intake and live weight gain was maintained for 9 weeks .

Determination of some blood parameters

Within 2-3 days before ending the feeding trail , blood samples were taken from half (10 lambs) of the experimental animals to determine plasma growth hormone , blood sugar and urea nitrogen concentration. Animals were fitted with jugular canella and blood samples (3 ml) were drawn into heparin zed syringe before morning feeding (zero time) and 3 ,6 ,9 12 and 24 h after morning feeding. Blood samples were centrifuged and plasma was removed and stored at -20 C^{o} until analysis for growth hormone (GH), blood sugar (BS) , blood urea nitrogen (BUN) and serum uric acid (SUA) using a radioimmunoassay technique, international, France. Mean plasma concentration were calculated for all times for each animal within each treatment group.

Chemical analysis

Samples of feedstuffs, feed offered and refusals were dried at 50 C^{o} until constant weight before chemical analysis .Samples than ground through a 1mm screen for chemical analysis. DM,OM,TN,EE,CF and NFE were determined for all feedstuffs according to (6). Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and lignin were determined by the method of (10) . In Vitro DM and OM digestibility of NaoH-treated barley straw was determined by the method of (34) .

Statistical analysis

Data were statistically analyzed using Completely Randomized Design Model (CRD) procedure by (32). Duncan's multiple range test was used to determine the significance of differences among treatments means (7).Analysis of variance was carried out on all data. The treatment was partitioned into main effects and their interactions.

Results and Discussion

Intake

The lambs consumed all the diets offered .The overall daily intake of DM , OM, ME,TN, RDN , UDN , NDF,ADF ,lignin ,hemi cellulose and cellulose, are presented in Table 3. There were no differences among the daily dietary intake of DM,OM, ME, RDN, NDF,ADF, lignin, hemi cellulose ,cellulose

when expressed as g/day or g/kg $W^{0.75}$. The TN ,and daily intake of total UDN were followed the intended treatments in composition.

Live-weight gain

Live –weight gain values are presented for the first 4 weeks, the second 4 weeks and the overall experimental period (Table 4.). Live-weight gain in the second part of the experiment was greatly higher than that in first part for all treatments ;except the lambs given high level of UDN with out NS (Diet 3) .The pattern of responses to UDN and NS varied between the first and second parts and overall of the experiment .

In the first part ,the lambs fed low UDN either with or with out NS (diet 1and 2) grew below the predicted value of 200 g/day; while the lambs fed high UDN (with or with out NS ,diet 3 and 4) grew above or close to the predicted value of 200 g/day. Differences in live weight gain were statistically significant ($P<0.05$) between lambs fed low and high UDN .There were no interaction between level of UDN and NS ($p>0.05$) . During the second part of the experiment , the lambs on all treatments grew above or close to the predicted value of 200 g/day , except the lambs fed diet 3 grew 31g/day below the target value. Lambs responses to dietary supplement of UDN in this period was not statistically ($p>0.05$)significant . While, lambs received diets supplemented with NS(Diets 2 and 4) grew significantly ($P<0.01$) faster than those lambs fed diets without NS. Higher responses to NS was associated with lambs fed high level of UDN (Diet 4) . but, there was a level of UDN x NS interaction .

For overall period , live weight gain and feed conversion ratio differences were not significantly affected by increasing level of UDN ;However, live weight gain and FCR were significantly ($P<0.05$) improved with those lambs fed high UDN diet supplemented with NS (Diet 4) as compared with those fed diets with out NS. Interaction between level of UDN and NS was statistically ($p<0.05$) significant .

Blood parameters

Blood sugar (BS) , Serum uric acid (SUA), Growth hormone (GH), and Blood urea nitrogen (BUN) of lamb fed the experimental diets are presented in table 5 . BS and BUN were significantly affected by the levels of UDN .Lambs fed high level of UDN showed significant ($p<0.05$) BS reduce and BUN increase as compared with those fed low level of UDN. However ,level of UDN had no effect on SUA and GH . Lambs fed diets supplemented with NS either containing low or high level of UDN significantly increased BS,UAS and GH .In contrast ,NS supplementation caused significant reduce of BUN in lambs fed low or high level of UDN as compared with those fed diets with out NS . Interaction between level of UDN and NS for all parameters was not significant.

Since the lambs consumed similar amount of ME and RDN and non significant main effect in the levels of UDN across treatments , so any change in responses to dietary supplementation of UDN in this experiment is mainly related to NS additives . Greater gains during the late part compared to the early part of the growth periods have been agree with results reported by (21,22) and different from other results reported by (19 ,20); and since they are generally common to all diets may merely represent changes in gut fill . Therefore, in the absence of evidence for the contrary results , it would seen safer to accept the results of overall growth period as a fairer representation of UDN and NS feed additives effects on live weight gain and feed conversion ratio .

The results of LWG and FCR indicated that the lambs did not response to dietary supplement of UDN ,or at least to formaldehyde-treated SBM , after dietary requirements for RDN have been satisfied .Similarly (2) proposed that the N requirement of lambs weighing over 30 kg may in most instances be met by microbial protein only and thus that only RDN is required in the diet .In contrast , another studies (15 ,18)clearly indicated that the lambs responded to dietary supplement of UDN after dietary requirements for RDN have been satisfied and with range of 40:60 forage to concentrate ratio and similar to

the ratio used in this experiment. Thus , with the range of forage –to-concentrate ratio used in the present study , consistent responses to dietary supplement of UDN were not found and the hypothesis that greater response to protein supplements will be seen with diet containing high roughage rather than with a high concentrate content was not proved (12) .Thus ,No responses to dietary supplement of UDN in this experiment might be due to available adequate level of UDN (7g/kg DM) in basal diets used (T1 and T2) a. Whereas ,lambs fed diet content high level of dietary supplement of UDN showed great response to NS additive ,this response was not related to energy intake .However ,there are some reasons which may explain the beneficial effects of NS in the diet.

One explanation for the response may be reduce the rate of nutrient passage in elementary tract and give more time for utilization and absorption of nutrients (33 ,35); Moreover ,the lower BUN associated with higher response to UDN for those lambs fed diets supplemented with NS was sport the hypothesis that, blood urea nitrogen (BUN) analyses can be used as a signal , or red flag to point out potential problem in feeding program . BUN level in excess of 18 to 20 mg/dl in cow can be associated with lower reproductive performance , higher feed costs, health problems , and poor production (12) .Similar improvement in protein utilization and reduction in BUN was associated with NS and rosemary officinal additives reported by (14,21,22).

Second explanation , It must also be acknowledged that supplementation of the diet with feed additives such NS provided additional minerals, of those minerals , phosphorus was most likely to have been in deficit . (2) proposes a daily requirement of phosphorus of 2.1 g/ kg DM for a lamb gaining 200 g . The control diet contained 3.8 g/kg DM . It therefore seems unlikely that phosphorus was limiting .

Third explanation , (1,5,8) reported that the feed additives (medicinal plants) improved rumen activity and nutrient digestibility .This improvement might be increases the efficiency utilization of rumen un degradable protein. Similar results were reported by (28) who calculated that the nutritive values such as TDN ,ME and DCP were improved significantly as a result of NS supplementation .These results are in agreement with results obtained by (27,31) who reported that the medicinal plants (NS and Metrical chamomile) additives improved the digestion coefficient and nutritive value during feeding sheep.

Alternative explanation cited by (21,28), may be that feed additive such as medicinal plants used as alternative growth promoters ,such include NS ,has some properties as antiseptic, antibacterial activities against microorganism treatment ,of gastro-intestinal complaints and tonic .Moreover ,(26) recorded that Matricaria chamamilla has anti-inflammatory ,antiseptic and spasnolytic activities against microorganisms treatment of gesture-intestinal complaints and tonic .(11) reported that NS seeds extracts inhibited gram-positive and gram- negative bacteria .(9) indicated that the oil of NS seeds have therapeutic potential for the treatment of diarrhea caused by 37 isolates of shigella species and 10 strain of V. cholera and E .coli

Table 1.Chemical composition of the feedstuff (g /kg DM) .

	Ingredients[#]			
	Soybean meal	FSBM[$]	Yellow corn	*Nigella Sativa*
Chemical compositic				
Dry matter g/kg fresh	946	944	937	919
Organic matter (OM)	881	880	927	913
Total nitrogen (TN)	70	70	13	41
Crude fiber (CF)	50	50	36	67
Ether extract (EE)	22	22	34	115
Nitrogen free extract (NFE)	245	245	812	433
Metabolizable energy (ME, MJ)[+]	9.6	9.6	13.6	15.5

Treated barley straw containing (DM basis): 87% OM , 0.59 % N, 8% NDF, 5% ADF, and 45% organic matter digestibility ,OMD.

$ FSBM= Formaldehyde-treated soybean meal .

[+] ME (MJ/ kg DM) = 0.012 CP +0.031 EE+0.005 CF +0.014 NFE (24).

Table 2 . Formulation and chemical composition of experimental diets .

Level of UDN (g/kg DM)	Low UDN		High UDN	
Feed additives	No NS	With NS	No NS	With NS
Diet no.	1	2	3	4
Ingredients (g / kg DM)				
NaOH-treated straw	400	400	400	400
Yellow corn	400	392.5	360	352.5
Soybean meal(SBM)	175	175	130	130
Formaldehyde-treated SBM	-	-	85	85
Nigella sativa	-	7.5	-	7.5
Urea	5	5	5	5
Min. and vit. mixture	20	20	20	20
Chemical composition (g/kg DM)				
DM g/kg fresh	919.5	919.4	918.3	918.2
OM	876.0	875.9	873.3	873.2
Total nitrogen (TN)	23.39	23.49	26.82	26.92
Rumen degradable N(RDN) *	16.35	16.35	16.82	16.82
RDN g / MJ of ME	1.401	1.401	1.446	1.448
Rumen un degradable N(UDN)	7.04	7.14	10.00	10.10
Metabolizable energy (MJ)	11.67	11.65	11.63	11.61
Neutral detergent fiber (NDF)	310.98	310	311.54	311.54
Acid detergent fiber (ADF)	215.92	215.93	219.04	219.04
Hemi cellulose	95.06	95.06	92.5	92.5
Cellulose	153.5	153.6	154.35	154.35
Lignin	62.42	62.42	64.69	64.69

* (16 ,17)

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