



Effect of Fenugreek (*Trigonella foenum graecum*) Seeds supplementaion on Feed Intake and Some Biochemical Blood Parameters of Lactating Nubian Goats

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Abstract

The study aimed to investigate the effect of supplementing concentrate rations with four levels of crushed fenugreek seeds on feed intake and some biochemical blood parameters in Nubian goats' at their early lactation stage. Twelve lactating Nubian goats were randomly distributed into four groups, three animals in each group using completely randomize design. Based on the supplement levels four rations were formulated: 0% (R0), 5% (R5), 10% (R10) and 15% (R15). The results indicated that feed intake was enhanced significantly ($P \leq 0.001$) with the increasing levels of supplement, accompanied with concomitant increase in the concentration of insulin hormone, whereas the level of glucose and leptin were decreased. Highly negative significant ($P \leq 0.001$) correlation between feed intake, glucose ($r = -0.81$) feed intake and leptin ($r = -0.75$) were recorded. On the other hand, highly positive significant ($P \leq 0.001$) correlation were evident between feed intake and Insulin hormone ($r = 0.83$). Insulin correlated negatively with leptin ($r = 0.63$) and glucose ($r = -0.68$) at ($P \leq 0.001$), while leptin correlated positively with glucose ($r = 0.61$) at ($P \leq 0.001$). In conclusion, the increasing level of crushed fenugreek seeds in the concentrate ration of goats at their early lactation stage boosted feed intake, secretion of insulin hormone, regulated glucose physiologic secretion, while negatively affected leptin concentration.

Key words: feed intake, fenugreek seed, glucose, insulin, leptin

المستخلص

هدفت الدراسة لمعرفة تأثير إضافة مسحوق بذور الحلبة إلى العليقة المركزة علي المتناول من الغذاء وبعض قياسات الدم الكيميائية أثناء فترة الحلابة المبكرة. تم إختيار إثني عشر رأس من إناث الماعز النوبي مكتملة النضوج بصوره عشوائية، وتم توزيعها إلي أربع

مجموعات كل ثلاثة حيوانات في مجموعة وذلك باستخدام التصميم كامل العشوائية. تمت إضافة مسحوق بذور الحلبة للعليقة الشاهد على النحو الآتي: (0%، 5%، 10% و 15%) أوضحت النتائج علي أن المتناول من الغذاء تحسن معنويًا ($P \leq 0.001$) بزيادة مستويات بذور الحلبة وصاحب ذلك زيادة في إفراز هرمون الأنسولين. بينما تناقص مستوى كل من الجلوكوز واللبتين. وجدت علاقة ارتباط سلبية عالية المعنوية ($P \leq 0.001$) بين الغذاء المتناول والجلوكوز، ($R = -0.81$) وبين الغذاء المتناول واللبتين ($R = -0.75$)، بينما هناك علاقة ارتباط إيجابية عالية المعنوية ($P \leq 0.001$) بين المتناول من الغذاء والأنسولين ($R = 0.83$). سجل الأنسولين علاقة ارتباط سلبية مع اللبتين والجلوكوز ($R = -0.63$)، ($R = -0.68$) علي التوالي، بينما لللبتين علاقة إيجابية مع الجلوكوز ($R = 0.61$). خلصت التجربة إلي أن زيادة مستويات مسحوق بذور الحلبة في العليقة المركزة للماعز الحلوب ارتبطت بتحفيز الغذاء المتناول وزياده إفراز هرمون الأنسولين، و تنظيم الإفراز الفسيولوجي للجلوكوز وقللت من تركيز اللبتين.

Introduction

Fenugreek (*Trigonella foenum graecum* Linn) belongs to the family of Papilionaceae-Leguminosae, extensively cultivated in India, Mediterranean region and Yemen. Fenugreek seeds activities are associated with the defatted fraction, and implicating a saponin-rich sub fraction. In addition to that, the seeds and some of its fractions have a hypoglycaemic effect in experimentally induced diabetes and in diabetic patients (Al-Habori *et al.*, 1998). Petit *et al.*, (1993) reported that oral administration of fenugreek seed extract (10 and 100 mg/day per 300 g body weight) enhanced feed intake with motivation to eat in rats.

Metabolic status is defined as the amount of nutrients and energy available to the animal's tissues at a given time, and it depends on three factors: the mass of food consumed the mass of body reserves and the rate of expenditure of energy (Margetic *et al.*, 2002). Dramatic changes in the energy metabolism throughout lactation are well recognized in dairy animals. Since the demand for energy in dairy animals due to high milk yield is at a high-level in early lactation, the large deficit in energy intake in relation to energy requirements during early

lactation period may not be met by the intake of feed (Jouany, 2006). Because the energy balance is often negative in the early lactation period, the high-yielding dairy animals are negatively affected by a negative energy balance (Fricke, 2004). Block *et al.* (2001) stated that mobilization of endogenous lipids meets approximately 33% of the animal's energy requirements between parturition and the third week of lactation. Moreover, decreased insulin sensitivity, and hyperlipidaemia are major features during the early lactation period (Jouany, 2006). The energy metabolism of domestic animals is under the control of hormonal factors (Bartha, *et al.*, 2005).

The differences in body adipose tissues, due to the energy requirements for milk production in dairy animals through the lactation period, can be determined by the variations in plasma level of leptin secreted by the adipose tissue (Accorsi *et al.*, 2005). Leptin, mainly produced in adipose tissue, inhibits feed intake and down-regulates adipose tissue deposition (Fruhbeck *et al.*, 1998 and Bartha *et al.*, 2005). As leptin affects fat deposition, it could play an important

role in the processes occurring during the lactation period in dairy animals.

Feeding fenugreek seeds promote appetite; feed intake and milk yield (Attalmanan *et al.*, 2013). Therefore, the relationship between fenugreek seeds and leptin, which has the opposite role of fenugreeks, is going to be the main objective of this study, through studying the fluctuations of leptin, insulin, glucose concentrations and feed intake during the first two months of the lactation period of Nubian goats.

Materials and methods

Experimental site

The study was conducted at the experimental farm of the Department of Animal Nutrition: Faculty of Animal Production, University of Khartoum (Shambat).

Experimental animals

Twelve mature Sudanese Nubian goats were purchased from a local livestock market (Abu Zaid) in Western Omdurman. To ensure homogeneity within the selected goats, some criteria were adopted e.g. body weight, breed, age and late gestation stage.

Management of experimental flock

On arrival to the experimental site, (1-2 weeks pre-partum) each doe was identified by an ear-tag, treated against internal and external parasites, divided into four groups three animals each, using complete randomized design. Then, they were housed individually in

pens partially shaded and were allowed free drinking. **Feed and Feeding:**

Group (R0) was fed the control diet Table (1); groups (R5), (R10) and (R15) were fed the control diet supplemented with 5%, 10% and 15% of crushed fenugreek seeds, respectively. Green roughages were offered three times weekly. Feeding programme continued for 2 consecutive months, and feed intake was recorded daily. Percent of determined chemical composition of experimental diets and chemical composition of fenugreek seed were shown in Tables 2 and 3, respectively.

Blood sampling and analysis

Blood samples were collected every two weeks at 10 a.m. from jugular vein using a 5ml plastic disposal syringe. Ten ml of blood were obtained from each animal into clean dry heparinized *Vacutainer*. Serum was separated by centrifugation at high speed of 4000 (rpm) at 30 °C for 5 minutes and stored at -20° C. Blood glucose was determined according to method described by (Burrin and Alberti *et al.*,1990). Serum insulin was analyzed by insulin commercial kit, measured by radioimmunoassay (RIA) using insulin iodination (iodine-125) and standard following the procedure of (Midgley *et al.*, 1969).

Blood sampling for leptin determination

Plasma leptin concentration was analyzed by leptin commercial kit, measured by Enzyme-Linked Immuno sorbent Assay (ELISA) standard following the procedure of (Compfield

et al. (1995) New Biolab, <http://www.NeoBiolab.com>.

Reagent preparation

The reagent was prepared in the following sequence:

All kit components and samples were brought to room temperature before use.

Microtiter plate was brought to room temperature before opening. The desired number of well strips were taken and immediately resealed and stored at 2-8° C.

Five µl of balance solution was dispensed into 100 µl experimental samples.

Ten ml of wash solution concentrate (100×) was diluted with 990 ml of deionized or distilled water.

Assay procedure

Hundred µl of sample or standard was added to the appropriate wells in the supplied microtiter plate.

Incubation was conducted 1 hour at room temperature.

Wells were emptied and washed 3-5 times with 300-400 µl 1×wash solution per well.

Final wash was emptied and 50 µl of conjugate per well was added and mixed well, covered and incubated for 1 hour at 37° C in a humid chamber.

Each well was washed 5 times with 1× wash solution and after the last wash the plate was (leptin, serum insulin, and glucose) were calculated using correlation procedure of the statistix 8.0.

inverted and blotted dry by tapping on absorbent paper. The liquid at each step was completely removed for good performance.

Fifty µl substrate *a* was added to each well followed by addition of 50 µl substrate *b*, covered and incubated for 10-15 minutes at room temperature. The substrate being light sensitive was covered with foil and kept out of direct sunlight.

Fifty µl of stop solution was added to each well and mixed well.

The optical density was immediately read at 450 nm.

The mean blank value was subtracted from each sample or standard value and the mean for duplicate (or greater) wells was calculated.

The standard curve was constructed on graph paper.

Statistical analysis

The data obtained from feed intake, insulin, glucose and leptin were subjected to Statistical analysis of variance (ANOVA) for completely randomized design, using computerized program known as statistix^{8.0}. A least significant difference (LSD) was carried out to test significant difference between the treatment means. Pearson product moment correlations between the variables (feed intake, plasma,

Table 1: Ingredient composition (%) of the experimental diets

Ingredients	R0	R5	R10	R15
Sorghum	55	53	50	51
G .N.C	5	5	5	3
S .C	5	5	5	3
Wheat bran	14	11	9	7
Molasses	20	20	20	20
Fenugreek	0	5	10	15
L. stone	0.5	0.5	0.5	0.5
NaCl	0.5	0.5	0.5	0.5
Total	100	100	100	100

R0: (0% fenugreek seed): R5: (5% fenugreek seed): R10: (10% Fenugreek seed): R15: (15%

Fenugreek seed): G.N.C: Ground Nut Cake: S. C: Sesame Cake and L. stone: Limestone.

Table 2: Calculated chemical analysis of experimental diets

Ingredients	R0	R5	R10	R15
CP (%)	14.59	15.25	15.95	15.47
ME (Mj/kg)	11.21	11.11	10.92	10.95

R0: (0% Fenugreek seed), R5: (5% Fenugreek seed).

R10: (10% Fenugreek seed), R15: (15% Fenugreek seed).

Table 3: Chemical composition (% DM) of fenugreek seeds

Component	Percent
DM	94.11
CP	28.60
CF	14.04
EE	6.16
Ash	4.65
N F E	40.66

ME: calculated according to the equation of Lodhi *et al.* (1976)

Results

Effect of supplementation of different levels of fenugreek seeds on feed intake (g/day) and glucose (mg/dl) of lactating Nubian goats:-

The results of the effect of inclusion of different levels of fenugreek seeds 0% (R0), 5% (R5), 10% (R10) and 15% (R15) on feed intake and blood glucose were illustrated in Table 4. There was a highly significant

difference ($P \leq 0.001$) in feed intake and glucose concentration between the control and treated groups. While the feed intake was enhanced significantly ($P \leq 0.001$) with increasing the level of fenugreek seeds, the blood glucose concentration was decreased. The R15 had the lowest value of glucose concentration which was significantly ($P \leq 0.001$) differed from R5 and numerically from R10.

Effect of supplementation of different levels of fenugreek seeds on plasma leptin (ng/ml) and serum insulin (ml U/L) of lactating Nubian goats

The results of plasma leptin and serum insulin of goats fed concentrate ration supplemented with different levels of fenugreek seeds were shown in Table 4. Generally the results indicated that the increasing levels of fenugreek seeds were associated with a highly significant ($P \leq 0.001$) increase of serum insulin concentration and decrease of plasma leptin concentration between the control and the supplemented groups. Among the treated groups the highest value of insulin concentration was recorded by R15 (13.64 ml U/L) and the lowest value was recorded by R0 (7.97 ml U/L). Contrary, the R15 had the

lowest value of leptin concentration (2.04) and R0 had the highest value of the leptin concentration (6.04).

The correlation

The correlations between feed intake, serum glucose, insulin and plasma leptin as affected by inclusion of different levels of fenugreek seeds were presented in Table 5. The highly negative correlation ($r = -0.81$) between the feed intake and serum glucose concentration, feed intake and plasma leptin concentration ($r = -0.76$) were obtained when the percentage of fenugreek seed increased in the diet. On the other hand, the correlation was highly significantly positive ($r = 0.83$) between Feed intake and serum Insulin, as can be noticed that the increase in feed intake was associated with increasing serum Insulin concentration.

The correlation between serum glucose and plasma leptin concentration was significantly positive ($r = 0.61$), while the correlation between serum glucose and serum Insulin was significantly negative ($r = -0.68$). Furthermore, there was a significant negative correlation between serum insulin and plasma leptin ($r = -0.63$).

Table 4: Effect of supplementation of different levels of fenugreek seeds on feed intake (g/day/animal), serum glucose (mg/dl), serum insulin (ml U/L) and plasma leptin (mg/ml) of lactating Nubian goats

Item	R0%	R5%	R10%	R15%	SEM
Feed intake	546.28 ^d	910.11 ^c	1124.2 ^b	1437.2 ^a	20.17
Glucose	50.47 ^a	48.73 ^b	48.13 ^{bc}	47.67 ^c	0.21
Insulin	7.97 ^c	10.36 ^b	11.32 ^b	13.64 ^a	0.43

Leptin	6.04 ^a	4.04 ^b	3.00 ^{bc}	2.04 ^c	0.45
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R0 (0% Fenugreek seed), R5 (5% Fenugreek seed) R10, (10% Fenugreek seed), R15 (15% Fenugreek seed), SEM: stander error of the means. Letters a-d: values with in row within the common superscript differ significantly ($P \leq 0.001$).

Table 5: Correlation between the feed intake, glucose, insulin and leptin

Item	Feed intake	Glucose	Insulin	Leptin
Feed intake	1	-0.81	0.83	-0.75
Glucose	-0.81	1	-0.68	0.61
Insulin	0.83	-0.68	1	-0.63
Leptin	-0.75	0.61	-0.62	1

Discussion

The present study indicated that there was a significant increase ($P \leq 0.001$) in feed intake among groups fed diet supplemented with fenugreek seeds. This might be attributed to the fact that fenugreek seeds improve diet palatability. Mamoun *et al.* (2014) stated that the carbohydrates contents of fenugreek and their main components (galactomannan) stimulate the appetizing and digestive processes. Similar results were also obtained by Ismail (2000) who fed growing Barki lambs fenugreek seeds and found that DM intake increased gradually as the level of fenugreek seed increased and he attributed this to the saponins content in fenugreek seeds which increased feed intake. The present results were also in agreement with the findings of Petit *et al.* (1995), Tomar *et al.* (1996), Atta Elmnan and Mangara (2012) and Atta Elmnan *et al.* (2013) who found that the fenugreek seed stimulates feed intake and they attributed that to

the effect of saponin content in fenugreek seeds. Borca *et al.* (2000) suggested that the boosting effect of fenugreek seeds might be due to the fact that fenugreek seeds increase the appetite for food and that these seeds might have an effect on hypothalamus gland which stimulates hungriness center in the brain and increases the desire for eating (Abo El-Nor, 1999). Similar results on dairy cattle found that (DM) intake was increased when lactating buffaloes fed different levels of fenugreek seeds (Petit *et al.*, 1993) resulting in a significant increase in milk production (Tomar *et al.*, 1996).

The results of this study indicated that, when the feed intake was enhanced significantly ($P \leq 0.001$) with increasing the level of fenugreek seeds, the glucose concentration was decreased. This was in agreement with the results of Mohammad *et al.* (2006) who stated that the administration of *Trigonella foenum-graecum* seed powder to diabetic animals has

been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrates and lipid metabolism to near normal levels in various animal models. Also Raju *et al.* (2001); Vats *et al.*, (2003) and Baquer *et al.* (2011) reported that the fenugreek seed extracts have the potential to slow enzymatic digestion of carbohydrates, reduce gastrointestinal absorption of glucose, and thus reduce post-prandial glucose levels by the presence of steroid saponins in *Trigonella* seeds that inhibit intestinal glucose uptake. Al-Habori *et al.* (2001) had reported that the amino acid 4-hydroxyisoleucine in fenugreek seeds increased glucose-induced insulin release in goat's pancreatic islet cells.

The increased level of fenugreek seeds in the experimental diets resulted in a significant increase ($P \leq 0.001$) in Insulin hormone secretion. This finding is supported by the earlier results demonstrated by Broca *et al.* (2000) and Baquer *et al.* (2009) who reported that fenugreek seeds contain amino acid called 4-hydroxy iso leucine, which appears to act on pancreatic beta cells to enhance insulin production. The result was also in agreement with the findings of Abajnoor and Tilmisany (1988), Devi *et al.* (2003) and Yadav *et al.* (2008) who found that the extract of the whole seed of fenugreek seeds, stimulate pancreatic insulin secretion. Moreover, the effect of fenugreek on feed intake is related to its well-documented ability to increase insulin sensitivity because fenugreek interferes with intestinal glucose absorption as a result of local

effects at the gastro-intestinal level mainly due to dietary fibers contained in fenugreek seeds (Gad *et al.*, 2006). Insulin sensitivity and glucose metabolism are involved in the complex endocrine regulation of feeding behavior (Hannan *et al.*, 2007).

Leptin functions as a feedback mechanism has a signal key that regulatory center in the brain to inhibit food intake and to regulate body weight and energy homeostasis (Klok *et al.*, 2006). In this study, there was a significant ($P \leq 0.001$) decrease of plasma leptin concentration between the control and the supplemented groups associated with the increasing the level of fenugreek seeds. These results were in agreement with Kumar *et al.* (2014) who mentioned that fenugreek seeds treatment caused significant reduction of leptin levels. After parturition leptin concentrations seemed to rise, this appeared to be constant until at least 70 days after parturition (Liefers *et al.*, 2003). During this period the cow mobilizes her fat reserves (i.e. adipose tissue) and it appears that all her energy is going to the production of milk.

In the present study the goats fed on the control diet which contained zero levels of fenugreek seed showed the same pattern. On the other hand the goats fed on the progressive levels of fenugreek seed showed opposite pattern to the pervious mentioned fact. The decrease of plasma leptin may be due to the effect of dietary fenugreek seeds which was found to stimulate feed intake resulting in positive energy balance for goats fed fenugreek

seed diets. Complete food deprivation caused a rapid fall in plasma leptin (Marie *et al.*, 2001). The satiety effects of leptin were also observed in sheep, by administration of leptin in ewe's diet for 3 days. This treatment resulted in a decrease of voluntary feed intake (Henry *et al.*, 1999). However, these effects were lost when sheep were underfed and leptin was administered (Morrison *et al.*, 2001). The pervious results indicate that another signal blocks, the effect of leptin on feed intake when the body is in a negative energy balance. Moreover, acute or long-term changes in food composition or food restriction caused changes in plasma leptin in ruminants. A complete food deprivation caused a rapid fall in plasma leptin (Marie *et al.*, 2001) while long-term food restriction decreased plasma leptin concentration in sheep (Delavaud *et al.*, 2000; Morrison *et al.*, 2001). From the current study there was a highly negative correlation ($P \leq .001$) between feed intake and serum glucose concentration ($r = -0.81$), this was in agreement with (Tahir, 2008). Glucose is generally regarded as the least significant metabolic regulator of voluntary intake in ruminants. It is significant for the voluntary control of DMI in non-ruminants but not in ruminants (Allen, 2000). Glucose is not the main energy-yielding substrate for maintaining the body energy balance in ruminants and is found in relatively small concentrations in the blood plasma when compared to non-ruminants (Forbes, 1995). Although it does play a key role in many functions such as brain metabolism,

nourishment of the foetus and milk synthesis (Banerjee, 1991).

The correlation was highly significantly positive ($r = 0.83$) at ($P \leq 0.001$) between feed intake and serum Insulin. This result was similar to the result obtained by Bassett, (1975) who found that the plasma insulin concentration was increased after feeding in ruminant animals. Woods *et al.* (1998) suggested that Insulin secretion is stimulated acutely in response to the intake of a meal. Lofgren and Warner (1972) reported a sustained increase in plasma insulin concentrations which peaked at 30 minutes in sheep fed with a high concentrate diet. Also Strubbe *et al.* (1977) showed that insulin is low up to 5 min before a meal but increases after the ingestion of the food.

The correlation between serum glucose and serum insulin was significantly negative. The progressive increase of feeding of fenugreek seeds to the experimental animals resulted in an increase in insulin hormone secretion; this may be due to the presence of the amino acid 4, hydroxyisoleucine in fenugreek seeds. This amino acid appeared to act on pancreatic beta cells to enhance insulin production. This result agree with Broca *et al.* (2000) and Baquer (2009) who reported that fenugreek seeds contain amino acid called 4 hydroxy iso leucine, which appears to increase the body's production of insulin. Also Ajabnoor and Tilmisany (1988), Devi *et al.* (2003) and Yadav *et al.* (2008) found that the extract of the whole seed of fenugreek seeds, stimulate pancreatic insulin secretion. Moreover, the effect of

fenugreek on feed intake related to its well-documented ability to increase Insulin sensitivity because fenugreek interferes with intestinal glucose absorption as a result of local effects at the gastro-intestinal level mainly due to dietary fibers contained in fenugreek seeds (Gad *et al.*, 2006). Insulin sensitivity and glucose metabolism are involved in the complex endocrine regulation of feeding behavior (Hannan *et al.*, 2007).

The negative correlation between the feed intake and plasma leptin in the current study was in agreement with Prunier *et al.*, (2001) who found that the high leptin concentrations inhibit appetite. Leptin, mainly produced in adipose tissue, inhibits feed intake (Morrison *et al.*, 2001) this may be attributed to fact that leptin is secreted by fat cells, and circulating leptin triggers receptors on hypothalamic nerve cells which leads to decreased hunger (satiety) and thus decreased food-seeking behavior as well as increased energy expenditure in peripheral cells.

Leptin showed significant positive correlation with glucose, Block *et al.* (2003) reported that leptin concentrations tend to correlate positively with plasma glucose levels in dairy cattle. Contrary subcutaneous administration of leptin was reported to decrease plasma glucose concentration in rats (Sivitz, 1997). In the present result a negative correlation between serum insulin and plasma leptin was observed, this result was in agreement with Lichnovskaet *et al.* (2005) who found that the leptin takes part in the influence of insulin resistance factor.

Conclusion

In conclusion the increasing level of fenugreek seeds in the concentrate ration was associated with enhancing the feed intake and secretion of insulin hormone. Furthermore, the induced elevating insulin hormone resulted in the normal physiologic secretion of glucose. On the other hand feeding fenugreek seeds resulted in decreased leptin concentration

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