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EVALUATION OF DIETARY INFLUENCES ON ESCHERICHIA COLI O157:H7 SHEDDING BY CATTLE

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SUMMARY

The effect of diet type and an abrupt change on shedding of *Escherichia coli* O157:H7 was investigated, first group of animals were fed on hay which was low in nutrient and digestible energy and high fiber, the second group mixture of concentrated feed (grains) and barseem which is height in nutrient and digestible energy and low in fiber content, third group fed on barseem moderate in nutrient and digestible energy and moderate in fiber content.

After a single oral administration of virulent *E.coli* O157:H7 all animals shed *E.coli* O157:H7.

The number of culture positive animals in specific media increased according to the type of diet and also after abruptly changing of diet the shedding of microorganism increases by variable amount.

Cattle fed on hay still shedding *E.coli* O157:H7 for 55 days, animals fed on barseem and grains shed it for 52 days but animals fed on barseem only shed it for 44 days whereas control group shed microorganism for 31 days only.

The concentrations of microorganism at the 5th day post-inoculation were $3x10^4$, $2.4x10^7$ and $2.1x10^6$ for the first, second and third group respectively, whereas the concentrations of the microorganism at the 25th day post-inoculation were $5x18^8$ n, $5.8x10^{13}$ and $6x10^{12}$ respectively. The groups reach the last detectable concentrations ($2.2x10^4$, $3.4x10^2$ and $2x10^3$) at the day (40th , 50th and 45th) post-inoculation for the (first, second and third) group respectively.

It can be concluded that the grains and barseem fed animals shed *E. coli* O157:H7 longer and more concentrated than barseem or hay fed animals.

Animals were feeding on grains and barseem or barssem may increase chance of human infection with *E.coli* O157:H7.

INTRODUCTION

Cattle management significantly impacts public health. Cattle transiently harbor *E.coli* O157:H7 in their gastro-intestinal tracts and many human infections result from ingestion of contaminated bovine products. *E.coli* has an enormous economic impact. Human infections with *E.coli* O157:H7 result in hemorrhagic colitis that can progress to hemolytic uremic syndrome that is the most common cause of acute renal failure in children (Kaper and Brien, 1998). Diez-Gonzalez et al. (1998) reported that cattle fed grains diet have large number of acid resistant total generic E.coli organisms in their feces while cattle fed hay diets do not. They suggest that feeding cattle hay diets would reduce the risk of food borne *E.coli* infection for human.

E.coli O157:H7 is the pro-type of entero-hemorrhagic *E.coli* and has been the cause of most outbreaks of colitis (Boyee et al. 1995 & Su and Brandt, 1995) . About 5-10 % of hemorrhagic colitis progress to hemolytic uremic syndrome characterized by hemolytic anemia, thrombocytopenia and renal failure lead to mortality rate of 3-5 % (Boyee et al. 1995 & Griffin and Tauxe. 1991).

The pathogenesis of entero-hemorrhagic E.coli infection is associated with production of one or both shiga toxins (Stx1 and/or Stx2) and probably the information of attaching effacing lesions in the intestinal mucosa (Griffin, 1995). Most human infections with *E.coli* O157:H7 are caused by consumption of contaminated improperly cooked beef, un-pasteurized milk or fecally contaminated vegetables and water (Su and Brandt, 1995). Direct transmissions from animals to humans have been reported (Griffin, 1995). Recent studies have established ruminant animals as reservoir for this human pathogen [Chapman et al. 1993; Hancock et al. 1994; Kudva et al., 1996 & 1997].

Our goals were to study the effect of different diets (I)Hay (II)Mixture of grains and barseem (III) Barseem and (IV) animal fed on preservative ration on shedding of *E.coli* O157:H7.

MATERIAL AND METHODS

1- 60 healthy 1.5 years old heifer used in this experiment, the cows were housed in 4 groups (Table 1) without contact in raised, grated floor pens without bedding.

The animals had water supply and were fed twice daily, the groups were identified by ear tags with letter / number combination indicating the diet (letter) and number referring to individual animals.

Table (1) : Groups of animals and type of feeding

No.of groups	Type of diet	No. of animals
I	Hay	15
II	Grains and barssem	15
III	Barseem	15
IV	Preservative ration	15

2- Bacterial strains: *E.coli* O157:H7 which was sensitive to nalidixic acid, or spontaneous nalidixic acid resistance mutant of bovine *E.coli* (O157:H7 strains) were used. Both strains produce Stx1 and Stx2 toxin.

Within a group of penned animals, the strains were alternatively administered.

The inoculum was prepared by culturing each *E.coli* O157:H7 strain in separate flasks of Luria-Bertani (LB) broth.

The cultures were grown at 37°C with aeration until the culture densities reached 10^8 CFU of *E.coli* /ml. Then the cells were harvested by centrifugation and re-suspended at conc. of 10^9 CFU/ml in sterile saline viable cell counts were estimated by spread plate culture of triplicate serial dilutions on L.B agar.

Sterile 10 ml plastic syringes were used to administer 10^9 CFU/ml of *E.coli* O157:H7 to each cow in a single oral infusion.

3- Dietary differences and dietary changes :-

Hay: which is a relatively high in fiber content and low in nutrient content and digestible energy.

Table (2) : Mean values of feed ingredients of hay.

Feed ingredients	Percentages (%)
Dry matter	90 %
Dig, energy(Cal./Kg)	2.65 %
Metabolisable energy	2.17 %
Net energy	2.17 %
TDN	60 %
Crude protein	18 %
Ether extract	3 %
Ash	9.6 %
Crude fiber	23 %
Cell wall	42 %

TDN = Total digestable nutrient.

b- Concentrated feeds (Grains) (80%) and barseem (20%): have a relatively high nutrient and digestible energy content and low fiber content.

Table (3): Constituents formula of feed ingredients and percentage of concentrated mixture (Misr Com. For Oil and Soap).

Feed ingredients	Percentages (%)
Wheat bran	20%
Decorticated cotton seed cake	40%
Yellow corn	20%
Soya bean cake	10%
Rice polish	4%
Molase	3%
Lime stone	2%
Common salt	1%

Table (4): Concentrated ration analysis (dry weight).

Feed ingredients	Percentages (%)
Protein	16.1 %
Nitrogen	2.89 %
Fat	2.4 %
Fibers	14.77 %
Ash	12.47 %
Moisture	8.6 %

C- Barseem: has a high nutrient, digestible energy and fiber content.

Table (5) : Mean values of feed ingredients of Trifolium alexandrium (Barseem).

Feed ingredients	Percentages (%)
Dry matter	21-25%
Dig, energy(Cal./Kg)	2.78 - 2.43 %
Metabolisable energy	2.28 - 1.99%
Net energy	1.41 - 1.14 %
TDN	63 - 55 %
Crude protein	20 - 14 %
Ether extract	2.6 - 3.1 %
Ash	9.8 - 8.5 %
Crude fiber	23 - 31 %
Cell wall	38 - 52 %

TDN = Total digestable nutrient

d- Preservative ration : mixture of barseem and hay once daily .

The animals were acclimated to their pen mates, housing and diet for approximately 3 weeks before inoculation of bacteria.

The effect of diet on the quantity and duration of shedding of microorganisms was investigated and the presence of the microorganism (quantity and duration) by none and enrichment media was measured.

Group I: gradual changes from hay to barseem , the feed was changed to 75% hay and 25% barseem for 2 days, 50% hay and 50% barseem for the next 2 days and 80% barseem and 20% hay for the last 2 days, the gradual increase was required to curtail sudden increase in grastro-intestinal anaerobic flora that can lead to intoxication and death of an animal [Church et al. 1975 & Woolcock 1991].

Group II: gradual changes on the diet after 30 days from first inoculation of the *E.coli* O157:H7

Group III: abrupt changes from barseem to hay . The gradual change was required to curtail sudden increase in the gastro-intestinal anaerobic flora that can lead to intoxication and death of animal (Griffin, 1995). Fecal samples were cultured prior to inoculation (pre-dose) and from the 2nd

to the 42nd with 4 days intervals (post-inoculation).

- As expected the grain diets as determined by standard techniques Association of Official Analytical Chemists (1990), Komarek (1993) and Van Soest et al., (1991).

  After 3 weeks adaptation to particular diet cattle were inoculated with *E.coli* O157:H7 which had been grown on LB broth at 37°C with aeration to cell density of 10^9 CFU/ml.
- Each animal received 10^9 CFU via gastric tube directly into rumen.
- Fresh fecal samples were obtained by rectal palpation every 4 days and were cultured by a height sensitive technique to monitor shedding of *E.coli* O157:H7 [Kudva et al., (1995) & Kudva et al., (1997)].

RESULTS

- Regardless of the diet, all pre-inoculated animal fecal samples were negative for *E.coli* O157:H7.
- After inoculation all animals initially shed *E.coli* in amounts detectable without enrichment culture.
- There were no differences in apparent duration of shedding but there is differences in quantity of microorganism shedding with regard to initial inoculums strain, the number of organism in fecal samples declined steadily over time until detection of organism in fecal samples required selective enrichment culture.

Table (6): Percentage of animals (feeding on preservative ration) shedding the microorganism without enrichment media after first inoculation.

Time by days	percentages of animal shedding *E.coli*
3	92 %
6	100 %
9	98 %
12	70 %
15	54 %
18	23 %
21	18 %
24	9 %

Effect of diet on shedding of *E.coli* O157:H7 by cattle :-

Animals fed GB shed microorganism longer than those fed on hay only or barseem only because feed differ significantly in nutrient and fiber content.

The duration over which H, GB and B. shed *E.coli* was approximately 30, 48, and 42 days, post-inoculation respectively.

Table (7): The duration over which H, GB and B fed animals shed fecal *E.coli* O157:H7 post-inoculation and isolated without enrichment media.

No. of group	Type of feed	Duration of shedding	(+) ve culture
1	H	30 days	8
2	GB	40 days	13
3	B	42 days	11
4	Preservative	28 days	7

- When the microorganism could not be detected in feces by direct plating.
- Animal in groups continued to shed bacteria at levels detectable by selective enrichment culture for about 10, 16 and 14 days for group 1, 2 and 3 respectively.
- 8 animals of 15, 13 of 15 and 11 of 15 from animals fed on H,GB and B respectively were culturally positive.
- Survival analysis were used to asses the effect of diet on duration of shedding of *E. coli* as detected by selective enrichment media.
- Two end points were examined, the first was that an animal was culturally negative on two consecutive samples and the second was the first time that an animal was culturally positive in two consecutive samples.

Effect of an abrupt dietary change on the shedding of *E.coli* O157:H7.

The comparison of the effect of abrupt changing the diet for 1 month (from H to B or from B to H or from GB to B) with that of no dietary change (group 4).

Table (8) : The number of positive animals by enrichment culture (L.B. broth) in 4 groups after abrupt changing of diet and the duration of shedding of fecal *E.coli* O157:H7.

No. of groups	No. of (+ve) animals after *E.coli* inoculation	Period of shedding
1	13	36 days
2	19	52 days
3	15	44 days
4	11	31 days

Table (9) : Bacterial concentration in feces of cattle fed on H, GB, and B post-inoculation of *E.coli* O157:H7.

Time by days	Group I	Group II	Group III	Group IV
5	$3x10^4$	$2.4x10^7$	$2x10^6$	$4.8x10^5$
10	$4x10^6$	$4x10^6$	$3.8x10^8$	$5.4x10^7$
15	$4.2x10^7$	$5.6x10^9$	$4x10^{84}$	$3.92x10^5$
20	$5.4x10^8$	$6.4x10^{11}$	$6.2x10^{10}$	$5.5x10^3$
25	$5x10^8$	$5.8x10^{13}$	$6x10^{12}$	$6.8x10^2$
30	$4.6x10^6$	$6x10^{10}$	$5.4x10^9$	$2.2x10^2$
35	$3.2x10^5$	$4.2x10^6$	$4.8x10^6$	$1.6x10^4$
40	$2.2x10^4$	$5x10^5$	$2.9x10^3$	$0.8x10^4$
45	$50.4x10^2$	$7.2x10^3$	$2x10^2$	-
50	$0.2x10^2$	$3.4x10^2$	$1.4x10$	-

DISCUSSION

Three conclusions about the relationship between the presence of *E.coli* O157:H7 and type of diet could be obtained from our results:-

1- Diet influences the amount and concentration of *E.coli* O157:H7 organisms shed and the duration of shedding.

2- An abrupt changes can induce an increase in the number of *E.coli* O157:H7 culturally positive animals.

3- High nutrient and moisture (barseem) induce shedding of *E.coli* O157.H7 longer and more concentrated than low nutrient and dry nutrient.

Cattle are naturally colonized by *E.coli* O157:H7 in a transient and seasonal manner and by other enterohaemorrhagic *E.coli* stranis as Stx producing (Kudva et al. 1996 & 1997).

Present results led to conclusion that the relationship between shedding of *E.coli* O157:H7 and animals culturally positive affected by diet which influence the concentration of microorganism shed and duration of shedding, an abrupt diet change can induce an increase in the number of *E.coli* O157:H7 culturely positive animals.

Cattle are naturally infected by *E.coli* O157:H7 in transient and seasonal manner and other enterohaemorrhragic *E.coli* strains and other Stx-producing *E. coli* strains (Kudva et al. 1996 & 1997).

To determine if abrupt diet change or withholding of feed and water had an effect on fecal *E.coli* O157:H7 the cattle were fed in a hay which was low in protein and digestible energy and high in fiber or mixture of grains and barseem which was high in protein and digestible energy and low in fiber.

In our study culturally positive animals higher in group 2 which was high in nutrient content and low in fiber content than groups 1 (high in fiber and low in nutrient content) (Kudva et al. 1995) while cattle fed on grains and barseem shed *E.coli* O157:H7 for the longest duration.

In the first group (fed on hay) animals shed fecal E.coli O157:H7 by concentration ranged from $3x10^4$ CFU/gm feces after 5 days pos-inoculation of 10^9 by gastric tube reaching maximum concentration $5x10^8$ CFU/gm after 25 days, then begin to decrease to reach undetectable count $2.2x10^4$ CFU/gm at the 40th day post-inoculation, while animals on group II (fed on barseem and grains) shed $2.4x10^7$ CFU/gm at the 5th day post-inoculation and reaching the maximum concentration $5.8x10^{13}$ CFU/gm at the 25th day post-inoculation and the last detectable concentration was $3.4x10^2$ CFU/gm at the 50th day post-inoculation this agree with Diez-Gonzalez et al. (1998) and Carolyn et al. (1999) who proved that cattle fed on grains diets have large numbers of acid resistance total generic *E.coli* O157:H7 while cattle fed on hay don`t, the group III (fed on barseem) shed concentration of microorganism ranged from $2x10^6$ CFU/gm at the 5th day post-inoculation reaching the highest concentration $6x10^{12}$ CFU/gm at the 25th day post-inoculation and the last detectable concentration was $2x10^2$ CFU/gm at the 45th day post-inoculation this agrees with Carolyn et al.(1999) who proved that the level of *E.coli* O157:H7 less than 10^2 detectable only by selective enrichment media.

Previous studies have established an association between the ruminal and gastro-intestinal tract volatile fatty acid concentration (VFA) and PH and the dietary fiber and nutrient quality [Brown-

lie and Gru (1967), Church (1975), Gru et al. (1969) and Wallace et al. (1989)] high fiber and low nutrient feeds decrease the VFA and increase the pH ,while low fiber and high nutrient feeds have the opposite effect [Church (1975), Gyles (1994) and Woolcock (1991)] this gives the suitable condition for the growth and survival of E.coli O157:H7 (Wallace et al. 1989).

Abrupt change of diet creates a more hospitable gastro-intestinal tract condition for *E.coli* O157:H7 which increased concentration of bacteria in the feces of cultured positive animals vise versa (Wallace et al., 1989).

So that shedding of *E.coli* O157:H7 increased when the diet abruptly changed from hay to grains or barseem and decreased when changed from grains and/or barseem to hay.

Because the cattle placed together in close contact and with common water troughs, we predicted the horizontal transmission of *E.coli* O157:H7 between pen mates would occur (Kudva et al., 1995).

Also, the water may be the source of infection of microorganism (Faith et al. 1996).

The low quality feed decreases the gastero-intestinal tract VFA and that would increase fecal *E.coli* O157:H7 counts in culture positive animals (Rasmussen et al. 1993).

From our study we concluded that hay diet and abrupt changes extend the duration for which culture fecal samples of cattle positive shed *E.coli* O157:H7 (Kudva et al. 1995 & 1997), the results of this study confirm that cattle shed *E.coli* O157:H7 longer when they are fed hay and barseem than when fed on grain, so that more scientific information about the relationship between the diet of cattle and *E.coli* O157:H7 is needed before management changes are advocated.

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تقييم تأثير إختلاف العليقة المفاجئ على إفراز الميكروب القولونى (العترة O157:H7) فى الماشية

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تم دراسة تأثير إختلاف العليقة المفاجئ من عليقة إلى أخرى على إفراز الميكروب القولونى حيث تم تغذية المجموعة الأولى دريس الذى يحتوى على نسبة علية من الألياف ونسبة بسيطة من المواد الغذائية والطاقة المهضومة والمجموعة الثانية تغذت على خليط من الأكل المركز (الحبوب) وبرسيم ويحتوى هذا الخليط على نسبة عالية من المواد الغذائية والطاقة المهضومة ونسبة بسيطة من الألياف بينما المجموعة الثالثة تغذت على برسيم فقط وهو يحتوى على نسبة معتدلة من المواد الغذائية والطاقة والمهضومة ونسبة من الألياف.

وبعد إعطاء العترة الضارية من الميكروب القولونى (*Escherichia Coli* O157: H7) عن طريق الفم وجد أن عدد الحيوانات الموجبة للزرع على وسط غذائى عادى قد إزدادت بنسب مختلفة حسب نوع العليقة وكذلك قد تغيير بعد التغيير المفاجئ للعليقة.

ولقد أستنتجنا أن التغذية على الحبوب والبرسيم تزيد مدة إفراز الميكروب القولونى (*Escherichia coli* O157:H7) 48 يوماً بينما إفراز القولونى فى الحيوانات التى تتغذى على البرسيم فقط 42 يوماً و 30 يوماً فقط مدة إفراز الميكروب القولونى فى الحيوانات التى تتغذى على دريس.

بينما تغير عدد الحيوانات الموجبة للزرع على وسط غذائى خاص وكذلك مدة إفراز الميكروب القولونى حيث أن الحيوانات التى تغذت على الحبوب والبرسيم تزيد مدة إفراز الميكروب القولونى (*Escherichia Coli* O157: H7) 52 يوماً بينما إفراز الميكروب القولونى فى الحيوانات التى تتغذى على البرسيم فقط 44 يوماً و 36 يوماً فقط مدة إفراز الميكروب القولونى فى الحيوانات التى تتغذى على دريس.

ووجد أن الحيوانات التى تتغذى على حبوب وبرسيم أو على برسيم فقط يمكن أن تزيد إصابة الإنسان بالميكروب القولونى.