Diarrhoeagenic *E.coli* in Kareish cheese manufactured by different methods with special reference to *E.coli* O_{157}:H_7

By
Ekbal M.A.Ibrahim*and Azza M.K.Sobeih**
* Department of Food Control, Fac. of Vet. Med., Moshtohor, Benha University
** Department of Food Hygiene, Fac. of Vet. Med., Kafr El-Sheikh, Tanta University

SUMMARY

Sixty random samples of Kareish cheese manufactured by traditional method (TM), direct acidification (DA) and lactic acid bacteria starter cultures (LAB). Twenty each collected from different farm-houses for traditional kareish cheese samples and supermarkets for DA and LAB samples at Kalyobia governorate and prepared for microbiological examination.

The mean pH values of the examined kareish cheese samples were 4.47 ± 0.05, 5.04 ± 0.05 and 4.78 ±0.04, with mean coliform counts (MPN/g) of $3.22 \times 10^7 \pm 0.46 \times 10^7$, $2.39 \times 10^6 \pm 0.41 \times 10^6$ and $7.21 \times 10^5 \pm 1.62 \times 10^5$ for examined samples made by TM, DA and LAB, respectively. Accordingly, there was direct relationship between the pH values and the coliform counts in the examined Kareish cheese samples. Moreover, 25%, 20% and 10% of the examined samples that made by TM, DA and LAB were contaminated with diarrhoeagenic E.coli, respectively. The isolated E.coli were serologically identified as O_{26}: K_{60} (B_6), O_{86}: K_{61} (B_7), O_{111}: K_{58} (B_9), O_{124}: K_{72} (B_{17}), O_{125}: K_{70} (B_{15}) and O_{128}:K_{67} (B_{12}) that found with variable percentages. In contrast, all examined kareish cheese samples were free from E.coli O_{157}: H_7. The survival rate of E.coli O_{157}:H_7 inoculated in Kareish cheese samples (10^6cfu/g) which stored at refrigeration temperature (2-5°C) was studied. The count of the organism was continuously decreased till completely disappeared after 4, 6 and 9 days of storage of inoculated samples made by LAB starter culture, TM and DA, respectively. The public heath significance and probable sources of contamination of kareish cheese with such serious pathogens were discussed.

INTRODUCTION

Kareish cheese is an acid coagulated skim milk soft cheese commonly made and consumed in Egypt, owing to its low fat and high protein content as well as its characteristic acidity resulting from lactose fermentation by the action of lactic acid either produced by the natural microflora present in raw

milk (traditional method) or by the action of lactic acid bacteria if a pasteurized milk is used *(Abou-Donia, 1991 and Salem et al., 1997).*

Lactic acid bacteria are used as starter culture due to their healthy and nutritional benefits resulting from production of lactic acid as a major end product of lactose fermentation and large number of metabolic activities that responsible for the acceptability and safety of fermented milks and cheese due to their antimicrobial effect *(El-Soda, 1997 and Tailliez, 1999).*

Direct acidification of milk is an another method used in cheese manufacture by addition of diluted organic acid such as lactic, acetic or citric acid to acidify the skim milk to the desired pH. It is not affected by antibiotics and /or bactriophage and can be applied in remote areas where skilled cheese makers and cultures are not available *(Wahba & EL-Abbassy, 1982a and Molder & Emmons, 1994).*

Several outbreaks of acute food poisoning traced to consumption of various types of soft cheese contaminated with enteropathogenic *E.coli* were reported by *Mariene et al. (1992).*

The pathogenic strains of *E.coli* are called diarrhoeagenic *E.coli (Dolye and Padyle, 1989).* There are more than 60 distinct strains which are categorized into five groups according to their virulence, clinical signs and their O (somatic) and H (flagellar) antigens. These groups are: Entero-pathogenic *E.coli* (EPEC), Entero-toxogenic *E.coli* (ETEC), Entero- invasive *E.coli* (EIEC), Enterohaemorrhagic *E.coli* (EHEC) and Entero Adherent-Aggregative *E.coli* (EA- Agg EC). These groups can induce intestinal and extra intestinal diseases *(ICMSF, 1996 ; Ryser, 1998 and Varnam & Evans, 1991).*

Escherichia coli O_{157}: H_7 is one of EHEC strains that associated with bloody diarrhoea. Since 1982, more than 100 outbreaks of E.coli O_{157}: H_7 infection have been reported in USA *(WHO,1997).*

The object of the current work was to study the effect of PH on coliform counts in kareish cheese manufactured by different methods, Detection of diarrhoeagenic *E . coli* specially O_{157}: H_7 and survival of the later strain in such type of cheese.

MATERIALS AND METHODS

Sampling:

Sixty random samples of kareish cheese, twenty samples were collected from farm- houses that were manufactured by traditional method (TM) and forty samples were collected from dairy shops and supermarkets in their retail packages. Twenty out of them were labeled to be made by direct acidification (DA) and the others by addition of starter culture (LAB).

Determination of pH:

The pH values of examined samples were measured according to *Pearson (1984)* by using pH meter (C.D. 2026, Fischer model, Germany).

Preparation of the samples for bacteriological examination:

Kareish cheese samples were homogenated with sodium citrate (2%) and tenth fold serial dilutions were prepared *(BSI, 1984).*

Determination of coliform count (MPN/g):

Mac Conkey broth tubes supplemented with Durham's tubes were used for enumeration of total coliform count (MPN) as described by *APHA (1992).*

Screening of Diarrhoeagenic *E.coli:*

The technique recommended by *APHA (1992)* was applied by using Mac Conkey broth and Eosin Methylene Blue (EMB) plates. The suspected green metallic colonies were identified biochemically and serologically according to *Krieg and Holt (1984)*. Diagnostic Antisera for typing *E. coli* were coli test sera poly I, II and Bacto *E. coli* antisera.

Detection of *E.coli* O_{157}: H_7:

According to *March and Ratnam (1986),* Sorbitol Mac conkey agar was used for detection of *E.coli* O_{157}:H_7 then confirmed by applying Latex agglutination test, using *E.coli* O_{157} antisera.

Survival of *E.coli* O_{157}: H_7 in kareish cheese:

A standard Escherichia coli O_{157}: H_7 strain was obtained from Animal Health Institute Dokki, Giza. Fifteen samples of Kareish cheese that made by TM, DA and LBA (5 of each) were inoculated with the organism at rate of 10^6 cfu/g. Inoculate samples were kept at refrigerator and the count was determined daily to determine the survival rate of the inoculated organism.

RESULTS AND DISCUSSION

Determination of pH is one of the most important parameters to control the quality of dairy products. In this respect, inspection of Table (1) declared that the mean pH values were 4.47 ± 0.05, 5.04 ± 0.05 and 4.78 ± 0.04 with average coliform counts of $3.22 \times 10^7 \pm 0.46 \times 10^7$, $2.39 \times 10^6 \pm 0.41 \times 10^6$ and $7.21 \times 10^5 \pm 1.62 \times 10^5$ for kareish cheese samples made by TM, DA and LAB, respectively.

Comparatively lower pH values were reported by *Zaki (1990) and Nawar (2001).* While, nearly similar coliform count were reported by *Ahmed et al., (1988); Moussa et al., (1989) and El-Leboudy (1998)* and relatively lower results were recorded by *Dardir (1999).*

Statistical analysis proved that there was direct relationship between the pH values and the coliform bacterial count with percentages of 59%, 62% and 69% for kareish cheese samples made by TM, DA and LAB, respectively.

The low pH values of the examined samples may refereed to uncontrolled natural fermentation especially to those made in farm- houses and/or bad or prolonged storage conditions that leads to fermentation of residual lactose in cheese *(Wahba and Abbassy, 1982b and Badawi and Kebary, 1996).*

On the other side, the relatively higher pH values of kareish cheese samples that made by DA may attributed to the technique of manufacture, as such cheese producer acidify the skim milk to the desired pH (5.4 – 5.8) to save the time, labor equipment and lactic culture cost *(Molder and Emmons, 1994).*
Concerning the coliform bacteria, their presence in kareish cheese with high counts may attributed to the unhygienic measures adopted during kareish cheese preparation at farm-houses either from the initial counts of raw milk used, cheese mat, farmers, who practice poor personal hygiene and/or the surrounding environment *(Ibrahim et al., 1994).* also, their presence in the examined Kareish cheese made by DA and added starter culture may be due to the unsatisfactory hygienic measures at plant level and/or post-pasteurization contamination as well as using of contaminated water in cleaning of pasteurization machine pipes *(Huynh & Huynh, 1985 and Greenwood et al., 1988).*
Good quality kareish cheese can be manufactured with addition of lactic acid to reach pH (5.6 – 5.8) and 0.5% starter culture under strict hygienic conditions *(Wahba and EL- Abbassy, 1982b).*
LAB can not be successfully used as biopreservative; owing to the anti microbial effect of their metabolite on contaminating bacteria especially coliforms; except the good hygienic standard during production and storage of the fermented product should be adopted. (*Ghita et al., 2004)*

Table (2) showed that 25%, 20% and 10% of the examined kareish cheese samples made by TM, DA and LAB, respectively were contaminated with diarrhoeagenic *E.coli.* While all examined kareish cheese samples proved to be free from *E.coli* O_{157}:H_7.
Serological identification of the *E. coli* isolated from different samples of kareish cheese was shown in table (3). Where 2 strains of O_{111}: K_{58} (B_9) and one strain for each of O_{26}: K_{60}(B_6), O_{124}: K_{72} (B_{17}) and O_{128}:K_{67} (B_{12}) were detected in TM *Kareish* cheese. While, O_{124}: K_{72} (B_{17}), O_{125}: K_{70} (B_{15}) and untypable strains were demonstrated in 10%, 5% and 5% of DA karsiesh cheese, respectively. The strains were belonged to O_{86}: K_{61} (B_7) and another to O_{125} : K_{70} (B_{15}) were isolated from kareish cheese samples that made by addition of LAB starter culture.
Nearly similar E . coli serovars were previously isolated from *kareish* cheese by *Ahmed et al. (1988) and El-Leboudy (1998).*
Escherichia coli O_{86}: K_{61} and O_{125}:K_{70} strains were incriminated in many cases of gastroenteritis, epidemic diarrhoea in infants and food poisoning outbreaks *(Antani, & Anozie, 1987).* While, *E.coli* O_{124} was the causative agent of shigella- like illness in causing dysentry or cholera like syndrome *(Small and Falkow, 1988).* E. coli O_{26}: K_{60} and O_{111}: K_{58} serovars were belonged to EHEC were associated with bloody diarrhoea in human *(Levine, 1987).*
While, O_{128}: K_{70} was mentioned by *David et al., (1990),* that it is enterotoxigenic strain produce either heat labile and /or heat stable toxins and ETEC may present in faeces of human carriers for several months *(Cliver, 1990).*

A large numbers of plants all over the world manufacturing cheese from raw milk or milk thermally processed at subpasteurization temperature *(D'aoust et al., 1987 and Kosikowski & Mistry, 1997).* In this respect, Escherichia coli serovars O_{157}: H_7 is widely found in raw milk that exhibit unusual acid tolerance and its presence even in low numbers may constitute a threatening potential to cheese consumers causing their infections and even fatalities *(Teagasc, 2001 and Farkye &Vedamuthu, 2002).*

Owing to the public health hazards of O_{157}: H_7, as it is an important cause of haemorrhagic colitis and haemolytic uraemic syndrome in human especially children under 5 years *(Griffin, & Tauxe, 1991 and Wang et al., 1997).* The survival rate of *E.coli* O_{157}: H_7 during storage of kareish cheese samples at refrigerator temperature (2-5°C) was studied and tabulated in table (4). The results revealed that there was daily decrease of *E.coli* count from an initial dose 1×10^6 cfu/g to $2.01 \times 10^2 \pm 0.35 \times 10^2$ at the 5th day for kareish cheese made by TM, $8.13 \times 10^2 \pm 1.75 \times 10^2$ at the 8th day to these samples made by DA and to $1.13 \times 10^2 \pm 0.21 \times 10^2$ at the 3rd day for kareish cheese samples made by LAB starter culture. Complete disappearance of the organism was occurred at the 4th, 6th and 9th day of refrigerated storage for cheese made by LAB, TM, and DA respectively.

The behavior of *E. coli* O_{157}: H_7 in the cheese may attributed to it's optimum survival pH value which ranged from 5 to 7 and increasing the acidity or alkalinity decreased it's count *(Blackburn et al., 1997).* However there are interaction factors including water activity, salt, acidulent used and storage conditions to increase inhibition of the growth of *E.coli* O_{157}: H_7 in the cheese *(ICMSF, 1996 and Guraya et al., 1998).*

Persistence of *E.coli* O_{157}: H_7 up to the 8th day in Kareish cheese made by DA, came in accordance to those reported by *Cutter & Siragusa (1994) and Conner & Kotrola (1995).* In this respect, *Blackburn et al. (1997)* found that cheese made by addition of acetic acid and/or lactic acid had more lethal effect on *E.coli* O_{157}: H_7 comparing to those made by Hcl, where as citric acid has a less effect.

On the opposite site, LAB- added kareish cheese samples leads to complete disappear of E. coli O_{157}:H_7 after 4 days of refrigeration storage. Nearly similar results were recorded by *Saad et al. (2001)* who reported that starter culture is important to control *E.coli* O_{157}: H_7 in cheese, regardless it is manufactured with raw or pasteurized milk, with or without salt with application of well established good manufacturing practices.

E.coli O_{157}: H_7 grew faster in the absence of starter culture. This result may attributed to the effect of LAB metabolites which are antimicrobial substances; organic acids, hydrogen peroxide, bactericins and reuterin that used mainly as food biopreservative and to extend their shelf-life with developing of desirable organoleptic properties *(De Vuyst & Vandamme, 1994; Klaenhammer et al., 1994, Navarro et al., 2000 and Zambou et al., 2004).*

In conclusion, the obtained results declared that *kareish* cheese samples that made by addition of LAB starter culture were the safest ones for controlling *E.coli* particularly O_{157}:H_7 as compared with those made by TM or DA. To

prevent *E.coli* O_{157}: H_7 to gain excess to raw milk cheeses, they should be made with heat treated milk at temperature equivalent to pasteurization ,avoid post-pasteurization contamination with applications of good manufacturing practices.

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Table (1): Effect of pH on coliform counts (MPN/g) in the examined Kareish cheese samples. (n= 60)

Kareish cheese samples according to the manufacture method	n	PH			Coliform count			Correlation coefficient (r)
		Min	Max	*Mean ±S.E*	Min	Max	*Mean ±S.E*	
TM	20	4.26	4.86	4.47±0.05	$2x10^6$	$1x10^9$	$3.22x10^7$ $\pm0.46x10^7$	+ 0.59*
DA	20	4.61	5.48	5.04±0.05	$9x10^4$	$1.1x10^7$	$2.39x10^6$ $\pm0.41x10^6$	+0.62*
LAB	20	4.37	5.16	4.78±0.04	$3x10^4$	$5x10^6$	$7.21x10^5$ $\pm1.62x10^5$	+0.69*

TM= traditional method
DA= direct acidification method
LAB= lactic acid culture method
* Significant differences
n= No. of the examined samples

Table (2): Incidence of diarrhoeagenic *E.coli* in the examined samples Kareish cheese .

Kareish cheese samples	n	Diarrhoeagenic *E .Coli*		*E .coli* O_{157}: H_7	
		No.	%	No.	%
TM	20	5	25	ND	0
DA	20	4	20	ND	0
LAB	20	2	10	ND	0

ND= Not detected
DA=direct acidification method
TM= traditional method
LAB= lactic acid culture method

Table (3): Serotyping and strain characteristics of diarrhoeagenic *E. coli* in the examined Kareish cheese samples. (n = 60)

E.coli strains	TM (n=20)		DA(n=20)		LAB(n=20)		Strain characteristic
	No.	%	No.	%	No.	%	
O_{26}: $K_{60}(B_6)$	1	5	-	-	-	-	EHEC
O86: K61 (B7)	-	-	-	-	1	5	EPEC
O111 : K58 (B9)	2	10	-	-	-	-	EHEC
O124 : K72 (B17)	1	5	2	10	-	-	EIEC
O125: K70 (B15)	-	-	1	5	1	5	EPEC
O128: K67 (B12)	1	5	-	-	-	-	ETEC
Untypable	-	-	1	5	-	-	-
Total	5	25	4	20	2	10	

EHEC= Enterohaemorrhagic *E.coli.* EPEC = Enteropathogenic *E.coli*
EIEC: Entero invasive *E.coli.* ETEC: Enterotoxigenic *E.coli*

Table (4): Survival of *E .coli* O_{157} : H_7 in the examined Kareish cheese samples stored at refrigerator temperature (n = 15)

Samples / Storage time	TM (n=5)	DA (n=5)	LAB (n=5)
	Mean ± S.E	Mean ± S.E	Mean ± S.E
Zero time*	$1x\ 10^6$	1×10^6	$1 \times 10^{6*}$
1st day	$6.53 \times 10^5 \pm 0.98 \times 10^5$	$8.14x\ 10^5 \pm 1.73x10^5$	$3.87x\ 10^5 \pm 0.71 \times 10^5$
2nd day	$9.79 \times 10^4 \pm 2.06x\ 10^4$	$3.81 \times 10^5 \pm 0.52 \times 10^5$	$2.66x\ 10^3 \pm 0.41\ x10^3$
3rd day	$4.38x10^4 \pm 0.63x10^4$	$1.03 \times 10^5 \pm 0.18 \times 10^5$	$1.13x\ 10^2 \pm 0.21 \times 10^2$
4th day	$8.85x\ 10^2 \pm 1.74x10^2$	$7.43 \times 10^4 \pm 1.12x\ 10^4$	ND
5th day	$2.01x10^2 \pm 0.35\ x10^2$	$5.67 \times 10^4 \pm 0.91x\ 10^4$	ND
6th day	ND	$2.55 \times 10^4 \pm 0.46x\ 10^4$	ND
7th day	ND	$6.36 \times 10^3 \pm 0.87x\ 10^3$	ND
8th day	ND	$8.13x\ 10^2 \pm 1.75x\ 10^2$	ND
9th day	ND	ND	ND

* Significant differences by ANOVA test ($p < 0.05$)
ND = Not detected

الملخص العربى

الإشيرشيا كولاى المسببة للإسهال فى الجبن القريش المصنع بطرق مختلفة مع التركيزعلىعترة $H_7 : O_{157}$

إقبال محمد عادل إبراهيم * -عزه محمود كامل صبيح **

* قسم مراقبة الأغذية – كلية الطب البيطري بمشتهر – جامعة بنها

** قسم مراقبة الأغذية- كلية الطب البيطري بكفر الشيخ- جامعة طنطا

تم جمع 60 عينة من الجبن القريش المصنع بطرق مختلفة مثل "الطريقة الفلاحى" "القديمة " وبالحموضة المباشرة و بإضافة البادئ (20 عينة من كل نوع) من منازل الفلاحين و محلات الألبان والسوبر ماركت المختلفة بمحافظة القليوبية وذلك لدراسة علاقة الأس الهيدروجيني للجبن بتواجد ميكروبات الكوليفورم وكذلك عزل وتصنيف ميكروبات الاشريشياكولاى وبالأخص العترة $O_{157}:H_7$ وأيضا دراسة نمو عترة الاشيريشياكولاي O157:H7 في عينات الجبن المخزن بالثلاجة. وقد دلت النتائج على أن متوسطات قيم الأس الهيدروجيني هي 4.47 ± 0.05، 5.04 ± 0.05 و 4.78 ± 04, لعينات الجبن القريش المصنع بالطريقة القديمة والحموضة المباشرة وبإضافة البادئ ، على التوالى0 كما كانت متوسطات عدد الميكروبات القولونية لهذه العينات بنفس الترتيب هي 3.22× 10^7 ± 46, × 2.39،10^7 × 10^6 ± 41, × 10^6و 7.21 × 10^5 ± 1.62 × 10^5/جم0

وقد تبين من نتائج التصنيف السيرولوجى لعترات الإشيرشياكولاى المعزولة من الجبن بأنها تواجدت بنسبة 25% في الجبن القريش المصنع بالطريقة القديمة ،20% من الجبن المصنع بالحموضة المباشرة و10% من الجبن القريش المصنع بإضافة البادئ وقد كانت العترات المعزولة تنتمي للأنواع الاتيه:
$O_{124} : K_{72} (B)_{17}$, $O_{111}: K_{58} (B_9)$, $O_{86} : K_{61} (B_7)$, $O_{26} : K_{60} (B_6)$, O128 : K67 (B_{12}), $O_{125} : K_{70} (B_{15})$ حيث تم عزلها بنسب مختلفة0 كما نوقشت النواحى الصحيحة لهذه العترات المعزولة ، هذا وقد أثبتت النتائج خلو جميع عينات الجبن القريش من عترة الاشيريشيا كولاي $H_7 : O_{157}$ 0

كما تم معمليا حقن عترة $H_7 : O_{157}$ (10 ^{6}خلية / جم) فى خمس عينات من كل نوع من أنواع الجبن القريش المصنع بطريقة مختلفة محل الدراسة مع التخزين بالثلاجة ومتابعة العدد يومياً وقد دلت النتائج على أن هناك إختزال تدريجى فى عدد الميكروب حتى أختفى تماماً عند اليوم الرابع لعينات الجبن المضاف له البادئ (LAB) وعند اليوم السادس للعينات المصنعة بالطريقة القديمة وعند اليوم التاسع للعينات المصنعة بالحموضة المباشرة0

لذلك فإن الجبن القريش المصنوع من لبن مبستر ومضاف له البادئ هو الأكثر أمنا من الناحية الصحية لما له من أثر فعال على ميكروبات الآشريشياكولاى وبالأخص العترة الخطيرةO157: H7 ، بالإضافة إلى ضرورة تطبيق النواحى الصحية عند مستوى المصنع للحصول على منتج خالى من مسببات الأمراض0

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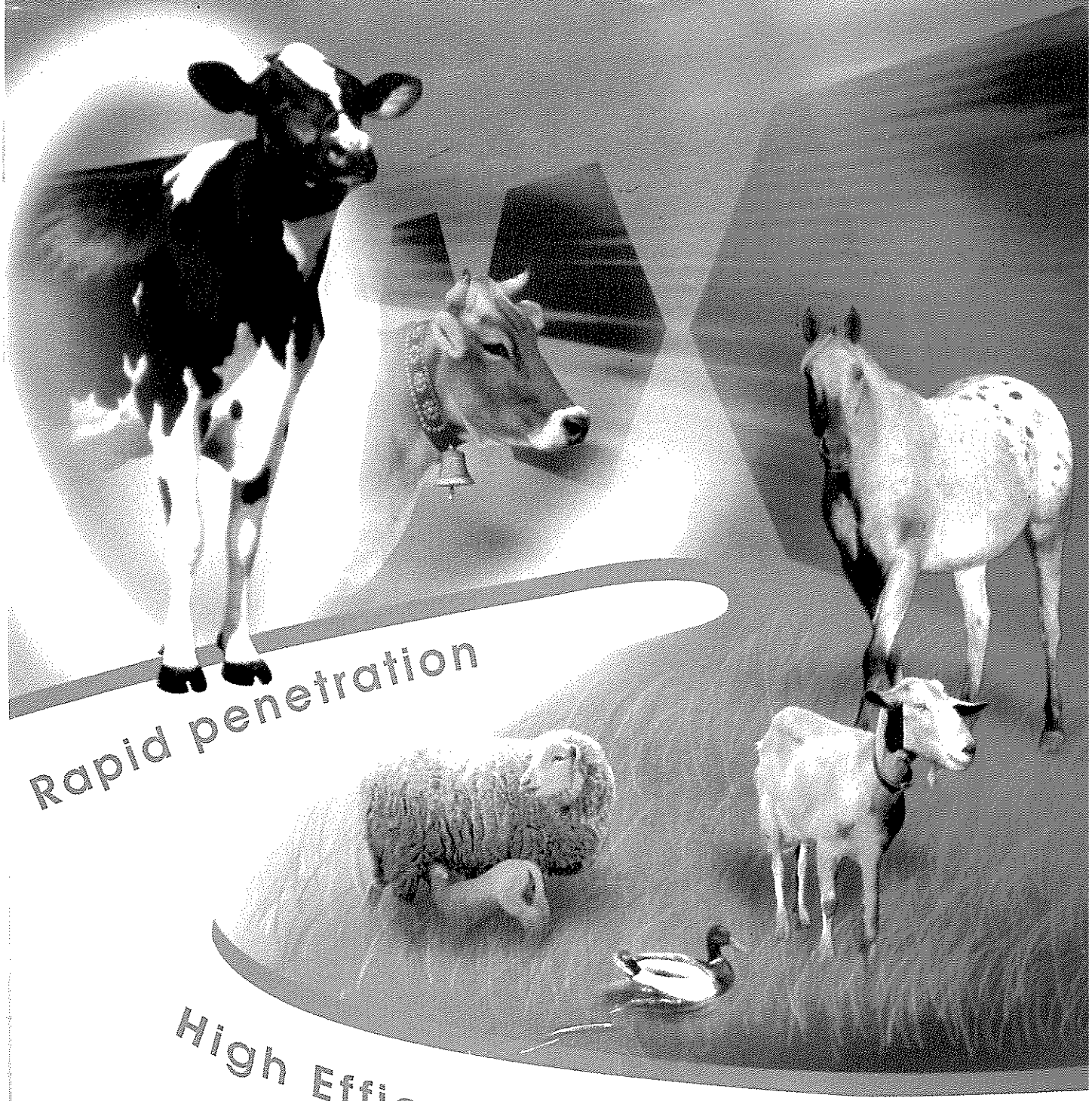

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