ANTIBACTERIAL AND ANTIFUNGAL IMPACT OF HONEY ON MILK AND YOGHURT QUALITY

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SUMMARY

Bee products especially honey constitute one of the most widely applied groups of natural products used by human beings from ancient times up till now. Honey proved to act as an anti-microbial agent against a very wide spectrum of pathogenic bacteria, fungi and viruses. sixty samples (18 of raw milk and 42 lab.- made yoghurt) were used to investigate the antibacterial and antifungal effect of honey on the microbial load of those products. Honey was added to milk and yoghurt samples at concentration of 1%, 2.5%, and 5%; all samples were analyzed microbiologically when fresh and for 2 and 5 days of storage refrigeration temperature respectively. The obtained results indicated that there were adequate difference in S.aureus, Enterobacteriace and fungal count in the examined samples treated with 1%, 2.5%, & 5% honey concentration from zero time till the 5th day of storage. S.aureus was completely eliminated by the 5th day of storage in yogurt samples made with 5% honey concentration.

INTRODUCTION

Milk and its products are preferred as an excellent food for human of all ages as they contain all the nutrients required for growth and maintenance of body health. However, such products may be found to contain various pathogenic microorganisms that may affect their safety. *Dahi (1986)* could detect S.aureus in milk samples implicated in cases of gastric disorder. Furthermore, *Varnam & Sutherland (1994)* and *Horner et al.(1995)* reported that certain species of yeasts and molds are implicated in several gastrointestinal disturbances, pulmonary infections, cardiac disorders and other affections. Milking utensils are recorded to be a dangerous source of contamination of milk with fungi *(El-Zayat et al.,1988).*

Honey proved to act as an anti-microbial agent against a very wide spectrum of pathogenic bacteria (Gram positive as well as gram-negative bacteria), fungi and viruses *(Molan,1992).* Honey is characteristically quite acidic; its pH being between 3.2 and 4.5, which is low enough to inhibit

growth of many animal pathogens, where the optimum pH for their growth falls between 7.2 and 7.4. Thus the honey acidity is considered a significant antibacterial factor. Hydrogen peroxide and acidity produced by the reaction; (glucose + H_2O+ O_2 --> gluconic acid + H_2O_2) were another factor make honey an anti-microbial agent *(Wortmann,1981; Patton et al., 2005* and *Schmidt ,1997).* Besides, both alive yoghurt bacteria (starter) and its own pH (near 4.5) support the bactericidal activity of yoghurt as concluded by *Kotz et al. (1990).*

The aim of this study is to investigate the antimicrobial activity of crude honey for control the microbial contamination of milk and yoghurt to ensure the quality and safety of such products.

MATERIALS AND METHODS

Sixty samples, 18 of raw milk and 42 lab.- made yoghurt. Milk samples were divided as follow, the first 6th considered as a control and the other 12th samples were divided into three groups (each was 4 samples) and mixed with honey concentration of 1%, 2.5%, & 5% respectively, treated milk samples were analyzed at zero time and after 1 and 2 days of treatment. In yoghurt samples the first six considered as a control and the others were divided into six groups mixed with honey concentration 1%, 2.5%, & 5% respectively. It is important to ensure that, honey was distributed evenly through the product, then yoghurt samples were analyzed at zero time and after 1, 2, 3, 4, & 5 days of treatment. Microbiological analysis of all samples was performed to determine:

a) Bacteriological counts: Aerobic plate count, Enterobacteriace, S. aureus, and Fungal counts were carried out according to *APHA (1992).*
b) Isolation and enumeration of some pathogen were done according to *Lodder, and Kreger, (1967), Samson et al.(1981)* and *APHA (1992).*

RESULTS AND DISCUSSION

It is evident from the obtained results in table (1) and figure (1) that, Aerobic plate count, S. aureus, Enterobacteriace, and Fungal counts in the milk and yoghurt control samples showed a relatively change at zero time till the fifth day of storage. For milk samples, nearly the same results were recorded by *Araujo(1984)* and *Hassan (1999)* for aerobic plate, S.aureus and Fungal counts respectively, while comparatively higher mean values of aerobic plate count, Fungal and S.aureus counts were reported by *Moustafa et al.(1987)* and *Adesiyun (1994).*

Presence of S.aureus in milk and its products is usually taken as an index for contamination either from animal, especially in cases of mastitis or human sources that make such products a good vehicle of food poisoning outbreaks. Enterobacteriaceae count is be taken as a definite index of fecal contamination of milk and its products, that besides the possible presence of

enteric pathogens which constitute health hazards to the consumers *(Jay, 1986).*

On the basis of microbiological analyses of milk and yoghurt samples carried out for enumeration of aerobic bacteria colonies, Fungi and other bacteria, approximately 25% (5.6% of milk and 16.7% of yoghurt), were not fit for human consumption *(Franco et.al, 2000).* Also *ZinEl-Din and El-Sawah (1997)* reported that yeasts could be detected in all examined yoghurt samples, and their occurrence in dairy products is significant because they can cause spoilage, desirable biochemical changes and they may adversely affect public health. A number of studies have clearly demonstrated that yoghurt proved to contain microorganisms that agreed, to some extent with the obtained results reported by *Ibrahim et al.(1989), Bahout and Moustafa(2003)* and *Nashwa & Rawia (2004).*

It is well established that honey could inhibit a broad spectrum of bacterial species; possessed bactericidal and bacteriostatic activity, and having antifungal activity *(Molan,1992).* In this study the effect of honey concentration, on the growth of microorganisms were evaluated for Aerobic plate count, S.aureus, Enterobacteriace and Fungal counts. It is clear from the results given in tables (1,2)and figures (1,2) that, nearly no difference in S. aureus and Fungal counts in control milk samples from zero time till second day of storage.

In table (2) and figures (3,4,5) there were differences in the concerned microbial counts of milk samples treated with 1%, 2.5%, & 5% ml. honey concentrations from zero time till second day of storage. Great differences in the some counts of yoghurt samples treated with 1%, 2.5%, & 5% honey concentrations from zero time till fifth day of storage were observed. Honey has an inhibitory effect on Gram positive bacteria and delayed the bacterial growth, but dose not had the same inhibitory effect on Gram negative bacteria *(Ensminger& Esminger, 1986 and Bogdanov, 1997).* The ethylacetate honey extract showed antibacterial, anti Candida and antifungal effects at low concentration, also honey propolis showed a weak activity against Gram-negative bacteria and Candida albicans *(Zaghloul et al., 2001 and Silici& Kutluca , 2005).* A possible explanation for reduction of micro-organisms by honey is due to a factor other than hydrogen peroxide, the association of high antibacterial activity with particular floral sources suggests that the non-peroxide antibacterial activity may be of floral origin, *Molan and Russell,(1995).*

There were adequate differences in these counts of yoghurt samples treated with 1%, 2.5%, & 5% honey concentrations from zero time till the fifth day of storage, table (3) and figures (6,7,8). S.aureus was completely disappeared by the 5^{th} day of storage in yogurt treated with 5% honey concentration.

The results obtained in tables (2&3) agreed with that obtained by *Silici and Kutluca (2005)* who mentioned that honey would be effective in

controlling the micro-organisms as S.aureus and Fungi. Inhibitory activity of honey against food borne pathogens is influenced by the presence of hydrogen peroxide and the level of antioxidant power and raw honey contains small amounts of the same resins found in propolis *(Patton et al.,2005).*It has been well established that honey would prevent growth of S. aureus if diluted by body fluids a further seven-fold to fourteen-fold beyond the point where their osmolarity ceased to be completely inhibited, the antibacterial action of the honey relied on release of hydrogen peroxide *(Cooper et al.,1999).*

Finally, it is clear that, traditional methods of preservation may not be adequate or non applicable or economic in controlling microorganisms where the obtained results indicated that, the use of honey alone introduced protection and preservation to yoghurt and milk to some extent. Although all honey will stop the growth of bacteria because of its high sugar content, it is also important that honey for use as an antimicrobial agent must be stored at low temperature and not exposed to light *(Ensminger and Esminger, 1986).*

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Table (1): The mean values of Microbial counts (Control group) Log 10 CFU /ml or g .

Storage time	Types of samples	Aerobic plate count	% of reduction	S. aureus	% of reduction	Enterobacteriace	% of reduction	Fungal count	% of reduction
0 time	*milk*	7.52	0.00	3.62	0.00	4.85	0.00	4.78	0.00
(fresh)	*yoghurt*	0	0.00	2.86	0.00	3.89	0.00	3.72	0.00
1st day	*milk*	7.5	0.27	3.6	0.55	4.79	1.24	4.61	3.56
	yoghurt	0	0.00	2.86	0.00	3.87	0.51	3.72	0.00
2nd day	*milk*	7.13	5.19	3.4	6.08	4.35	10.31	4.4	7.95
	yoghurt	0	0.00	2.85	0.35	3.8	2.31	3.7	0.54
3rd day	*yoghurt*	0	0.00	2.79	2.45	3.72	4.37	3.62	2.69
4th day	*yoghurt*	0	0.00	2.75	3.85	3.63	6.68	3.51	5.65
5th day	*yoghurt*	0	0.00	2.7	5.59	3.84	1.29	3.38	9.14

Table(2):The mean values of microbial counts in milk samples treated with honey Log10CFU/ml.

Storage time	Honey concent-ration	Aerobic plate	% of reduc-tion	S. aureus	% of reduc-tion	Enterob-acteriace	% of reduc-tion	Fungal count	% of reduc-tion
0 time	*milk*	7.52	0.00	3.62	0.00	4.85	0.00	4.78	0.00
1st day	1	7.52	0.00	3.62	0.00	4.85	0.00	4.78	0.00
	2.5	6.63	11.84	2.96	18.23	4.74	2.27	3.72	22.18
	5	5.9	21.54	2.61	27.90	4.63	4.54	3.52	26.36
2nd day	1	5.86	22.07	2.48	31.49	3.95	18.56	3.15	34.10
	2.5	4.7	37.50	2.15	40.61	3.0	38.14	2.38	50.21
	5	4.36	42.02	1.7	53.04	1.96	59.59	2.0	58.16

Table(3):The mean values of microbial counts in yoghurt samples treated with honey Log10CFU/g

Storage time	Honey concent-ration	S. aureus	% of reduc-tion	Enterobac-teriace	% of reduction	Fungal count	% of reductio
(0 time)	*yoghurt*	2.86	0.00	3.89	0.00	3.72	0.00
	1	2.86	0.00	3.89	0.00	3.72	0.00
1st day	2.5	2.85	0.35	3.85	1.03	3.7	0.54
	5	2.8	2.10	3.85	1.03	3.69	0.81
	1	2.8	2.10	3.85	1.03	3.68	1.08
2nd day	2.5	2.78	2.80	3.76	3.34	3.6	3.23
	5	2.75	3.85	3.7	4.88	3.51	5.65
	1	2.72	4.90	3.7	4.88	3.51	5.65
3rd day	2.5	2.36	17.48	3.7	4.88	3.08	17.20
	5	2.3	19.58	3.08	20.82	2.95	20.70
	1	2.0	30.07	2.9	25.45	2.8	24.73
4th day	2.5	2.0	30.07	2.6	33.16	2.36	36.56
	5	1.88	34.27	2.0	48.59	2.0	46.24
	1	1.85	35.31	2.0	48.59	2.0	46.24
5th day	2.5	1.49	47.90	1.8	53.73	1.95	47.58
	5	0	100.00	1.2	69.15	1.38	62.90

Figure (1) Microbial counts of examined milk samples (Control group)

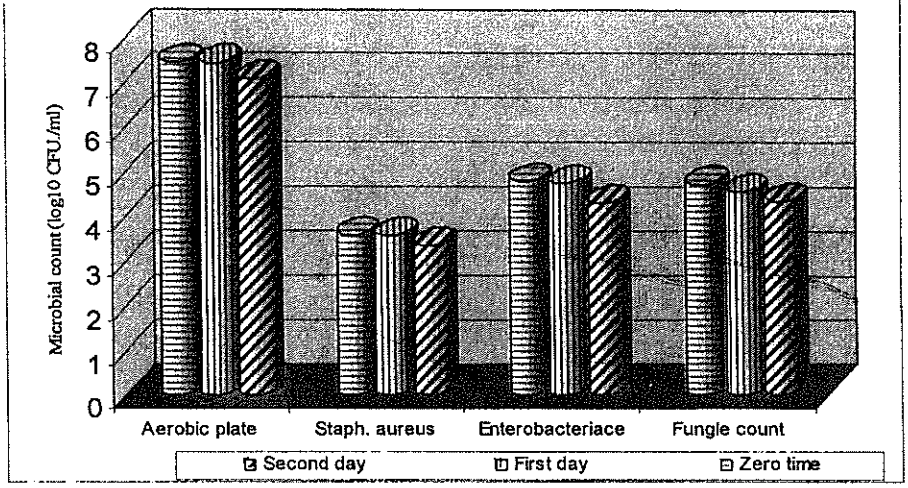


Figure (2) Microbial counts of examined yoghurt samples (Control group)

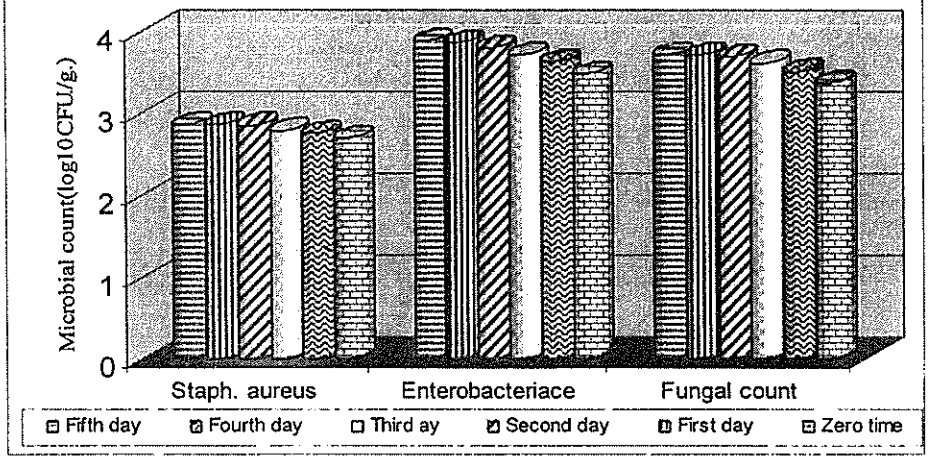
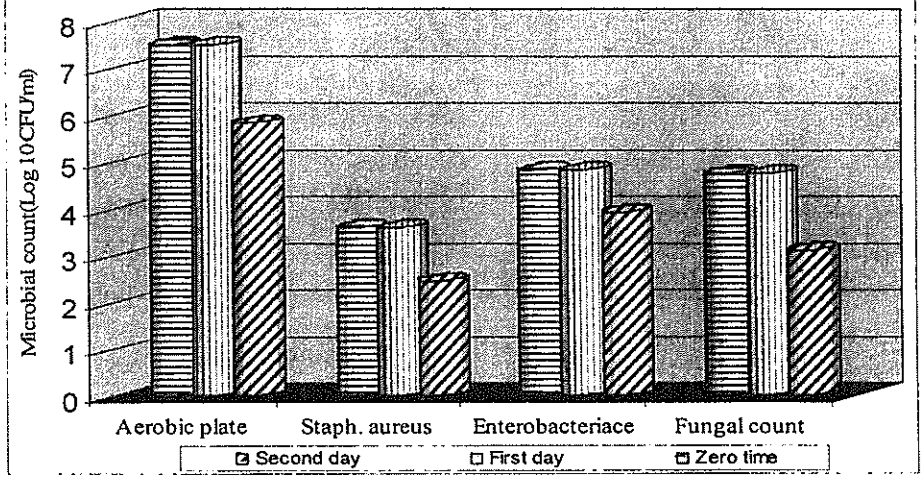


Figure (3) Microbial counts of examined milk samples (Honey Concenteation 1%)



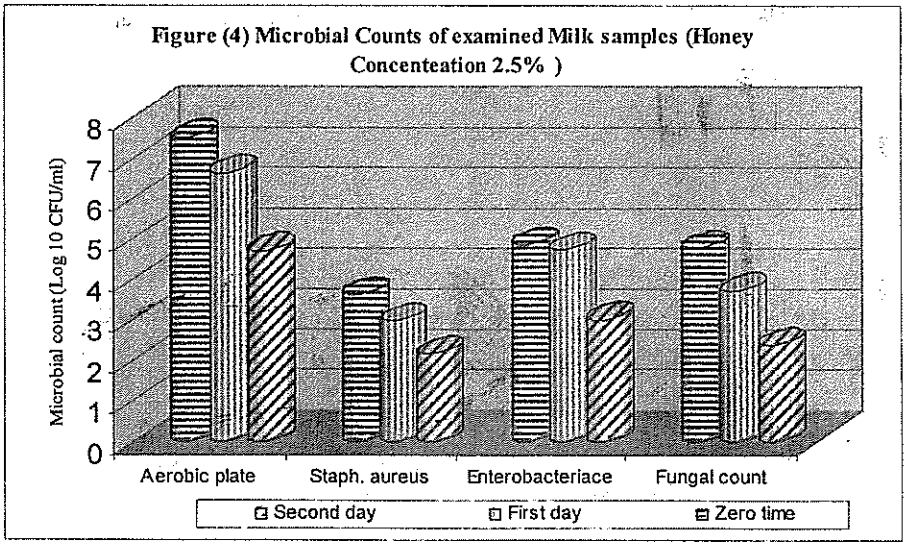


Figure (4) Microbial Counts of examined Milk samples (Honey Concenteation 2.5%)

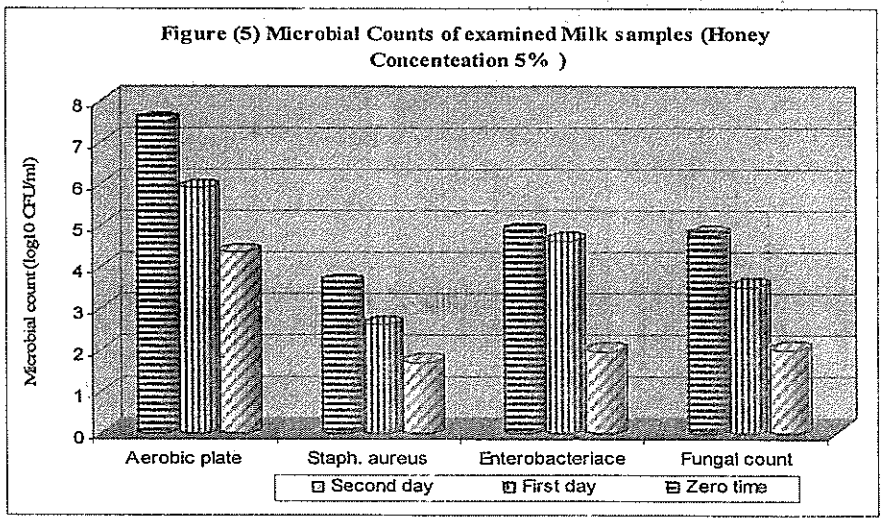


Figure (5) Microbial Counts of examined Milk samples (Honey Concenteation 5%)

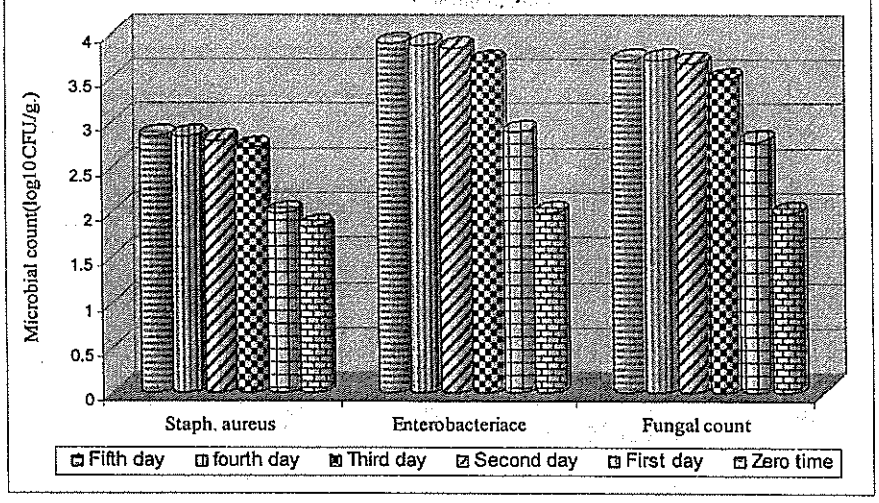


Figure (6) Microbial counts of examined yoghurt samples (Honey concentration 1%)

Figure(7) Microbial counts of examined yoghurt samples (Honey concentration 2.5%)

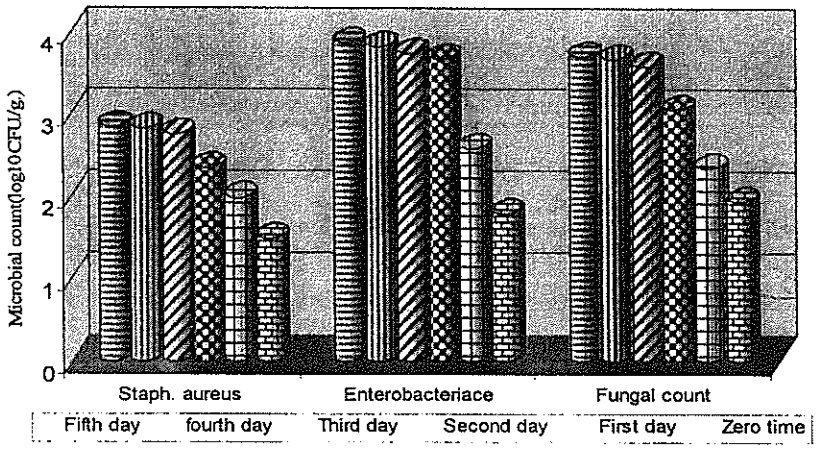
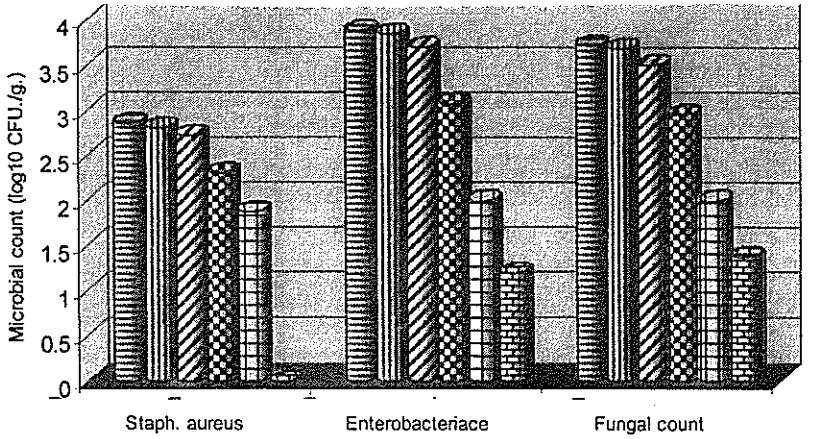


Figure (8) Microbial counts of examined yoghurt samples (Honey concentration 5%)



الملخص العربي

تأثير عسل النحل كمضاد للبكتيريا والفطريات على نوعية اللبن والزبادي

شهاب عبد الوهاب عبد الفتاح

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تم جمع عينات من الألبان الخام والزبادي الذي تم تصنيعه بالمعمل من هذه الألبان لدراسة تأثير تركيزات مختلفة من عسل النحل الخام على كل من عد البكتيريا الهوائية والميكروب العنقودي الذهبي والميكروبات المعوية وأيضا الخمائر والفطريات. كل نوع من العينات المختبرة تم تقسيمها مجموعات إحداها تم اعتبارها كمجموعة ضابطة وباقي المجموعات تم معاملتها بتركيزات مختلفة من عسل النحل (1، 2.5, 5%) ثم تم حفظها لمدة يومين بالثلاجة بالنسبة للألبان وخمسة أيام بالنسبة للزبادي.

وقد أظهرت العينات التي عوملت بتركيزات العسل المختلفة أظهرت انخفاضا ملحوظا في قيم العدد الكلي للميكروبات والميكروب المعوي والخمائر والفطريات، بينما العينات التي عوملت بتركيزات العسل (2.5, 5%) تراجعاً واضحا في قيم عدد الميكروب العنقودي الذهبي في الزبادي. وتم مناقشة أهمية عسل النحل كمثبط لنمو الميكروبات والخمائر والفطريات وكذلك الأهمية الصحية لتلك الميكروبات .

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