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Exogenous foliar application of $FeSO_4$ on enrichment of iron in rice grain and yield

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Abstract
A pot experiment was conducted at Department of Soil Science and Agricultural Chemistry, TNAU, Coimbatore during 2019 with rice var. CO 51 to study the impact of foliar nutrition of $FeSO_4$ on the enrichment of rice grain iron content and yield. The experiment was a factorial completely randomized design with two factors *viz*., a) levels of $FeSO_4$ (Five levels *viz.,* Water spray - control, 0.5% $FeSO_4$, 0.75% $FeSO_4$, 1.0% $FeSO_4$ and 1.5% $FeSO_4$) along with 0.1 % citric acid and b) Spray at different growth stages of rice (14 stages *viz.,* Panicle initiation, Flowering, Milking, Dough, Panicle initiation + Flowering, Panicle initiation + Milking, Panicle initiation + Dough, Flowering + Milking, Flowering + Dough, Milking + Dough, Panicle initiation + Flowering + Milking, Panicle initiation + Flowering + Milking, Panicle initiation + Milking + Dough, Panicle initiation + Flowering + Milking + Dough). The result revealed that foliar application of ferrous sulphate at various concentrations sprayed at different growth stages caused a significant increase in rice grain and straw yields besides, enrichment of iron in rice grain over control (water spray). The Foliar spray of 1.5 % $FeSO_4$ + 0.1% CA four times *i.e.*, Panicle initiation + Flowering + Milking + Dough recorded significantly highest grain yield (29.10 g pot^{-1}), straw yield (41.11 g pot^{-1}) and grain iron uptake (61.20 µg pot^{-1}) which is 66, 49 and 190 per cent increase over water spray respectively. However, it was on par with $FeSO_4$ at 1.0 % along with 0.1% CA sprayed at all the four growth stages.

Keywords: $FeSO_4$, foliar spray of Fe, rice iron enrichment and rice iron uptake

Introduction
Rice (*Oryza sativa* L.) is the staple food for three-fourth of the Indian population. Similarly, it feeds roughly half the planet's population and approximately three-quarters of a billion of the world's poorest people depend on the staple to survive (Zeigler, 2007) [16]. India produced 275.68 million tonnes of food grains with a major contribution from rice *i.e.* 110.15 million tonnes (Anonymous, 2017) [3] but the productivity of milled rice is still much lower *i.e.* 2,404 kg/ha (Anonymous, 2016a ; Anonymous, 2016b) [1, 2]. Iron is a necessary element for plant growth and since the formation of chlorophyll depends on iron, plant deficiency or deactivation of iron shows chlorosis. The decrease of chlorophyll leading to the reduction of the plant food processor and finally the yield is reduced. Iron (Fe) is an essential micronutrient in biological systems and is receiving growing concern worldwide because of increasing reports about Fe deficiencies in humans (Welch and Graham, 2004) [14]. According to a report by the WHO, Fe deficiency affects over three billion people in the world, especially in developing countries (Organization, 2002) [10]. Iron deficiency causes impairments in mental and psychomotor development in children and diminished productivity in adults, and represents the most common cause of anemia (Neumann *et al*., 2002) [9]. Foliar application of Fe was considered as a short time tool for bio-fortification of rice with Fe because soil application of most Fe sources are generally ineffective because of the rapid conversion of soluble Fe into plant unavailable solid Fe (III) forms (Fageria *et al*., 2009) [5]. Foliar applied Fe can be absorbed by the leaf epidermis, remobilized and transferred into the grain via the phloem. The time of foliar application may differentially influence grain Fe concentration. It is well established that foliar Fe application after the flowering stage more distinctly increases the grain Fe concentration (Zhang *et al*., 2009) [17]. With this background, a pot experiment was conducted to study the effect of foliar nutrition of $FeSO_4$ sprayed at different stages of crop growth on rice yield and enrichment of iron in rice grain.

Materials and methods
The experiment was conducted in glass house of Department of Soil Science and Agricultural Chemistry, TNAU, Coimbatore during 2019. Bulk soil was collected from the TNAU farm field and was shade dried and powdered to pass through 2 mm sieve. The soil was neutral (pH- 7.91), non-saline (EC - 0.18 dS m^{-1}) sandy loam in texture with a bulk density of 1.50 Mg

m^{-3}. The organic carbon content was 3.3 g kg^{-1} with Low – Low – High fertility status (190, 10.5 and 346 kg ha^{-1} of available N, P and K respectively). The DTPA- Fe, Zn, Mn and Cu were 3.51 1.25, 6.70, and 0.85 mg kg^{-1} respectively. The treatment imposed consisted of two factors *viz.*, $FeSO_4$ levels (Five levels - water spray-control, 0.5%, 0.75%, 1.0%, and 1.5%) along with 0.1 % citric acid and sprayed at 14 different growth stages of rice *viz.*, T_1- Panicle initiation, T_2- Flowering, T_3- Milking, T_4- Dough, T_5- Panicle initiation + Flowering, T_6- Panicle initiation + Milking, T_7- Panicle initiation + Dough, T_8- Flowering + Milking, T_9- Flowering + Dough, T_{10}- Milking + Dough, T_{11}- Panicle initiation + Flowering + Milking, T_{12}- Panicle initiation + Flowering + Dough, T_{13}- Panicle initiation + Milking + Dough and T_{14}- Panicle initiation + Flowering + Milking + Dough. The test crop was rice var. CO 51. The design of experiment was FCRD with two replications. Fertilizer was applied in soil as per STCR recommendation of 191, 110, and 26 kg ha^{-1} of N, P_2O_5, and K_2O respectively. Foliar spray of ferrous sulphate was imposed as per the treatments. The flooded condition was maintained throughout the experiment period. Grain and straw yield were recorded at harvest. The grain samples were powdered and triple acid digestion was done to estimate iron content through AAS. Iron uptake in grain was computed as per the treatment by multiplying respective grain yield with corresponding iron content. The data were subjected to statistical analysis using SPSS (16.0 version) software. The difference among the treatments was compared using Duncan at a 5% probability level.

Result

Grain yield: The effect of foliar application of $FeSO_4$ in various concentrations sprayed at different growth stages of rice caused significant improvement in rice grain yield over control (Table1). The grain yield ranged from 17.53 to 29.10 g pot^{-1}. However, the grain yield increased due to different levels of foliar spray of $FeSO_4$ along with 0.1% CA ranged from 24.21 to 27.16 g pot^{-1}. Significantly maximum grain yield recorded with 1.5% $FeSO_4$ + 0.1% CA (27.16 g pot^{-1}) however, it was comparable with 1.0% $FeSO_4$ + 0.1% CA (26.49 g pot^{-1}) but superior to 0.75%, 0.5% and water spray (control) treatments. The minimum grain yield was observed in water spray treatment (17.53 g pot^{-1}). The per cent increase in grain yield due to levels of $FeSO_4$ spray over control ranged from 38 to 55.

With respect to spray stages of foliar $FeSO_4$, grain yield ranged from 21.81 to 26.28 g pot^{-1}. The grain yield was significantly higher when applied four times *viz.*, T_{14}- Panicle initiation + Flowering + Milking + Dough stages (26.28 g pot^{-1}) and it was on par with three times application *i.e.*, T_{11}- Panicle initiation + Flowering + Milking (26.16 g pot^{-1}) and T_{12} - Panicle initiation + Flowering + Dough stages (25.84 g pot^{-1}) but superior to rest of the treatments. The lowest grain yield was noticed with foliar spray of $FeSO_4$ at T_4 – Dough stage (21.81g pot^{-1}).

The interaction between levels of $FeSO_4$ and spraying at different growth stages were significant on grain yield. The foliar spray of $FeSO_4$ @ 1.5% + 0.1% CA sprayed at four times (T_{14}- Panicle initiation + Flowering + Milking + Dough) was recorded significantly maximum grain yield (29.10 g pot^{-1}) and it was comparable with $FeSO_4$ @ 1.0 % but superior to rest of the treatments. The minimum grain yield was noticed with water spray at all stages (17.53 to 17.55 g pot^{-1}).

Table 1: Effect of foliar nutrition of $FeSO_4$ on rice (CO 51) grain yield (g pot^{-1})

Spray at different growth stages of rice	Levels of $FeSO_4$ (%)					Mean
	Water spray	0.5	0.75	1.0	1.5	
T_1- Panicle initiation	17.54	22.55	23.00	25.53	26.05	22.93
T_2- Flowering	17.55	22.91	23.43	25.90	26.15	23.19
T_3- Milking	17.53	21.25	22.06	24.25	25.55	22.13
T_4- Dough	17.53	20.80	21.74	24.00	25.00	21.81
T_5- Panicle initiation + Flowering	17.55	25.40	26.34	27.78	28.00	25.01
T_6- Panicle initiation + Milking	17.54	23.74	24.97	26.13	27.40	23.96
T_7- Panicle initiation + Dough	17.54	23.29	24.65	25.98	26.85	23.66
T_8- Flowering + Milking	17.54	24.10	25.40	26.50	27.50	24.21
T_9- Flowering + Dough	17.54	23.65	25.08	26.25	26.95	23.89
T_{10}- Milking + Dough	17.53	21.99	23.71	25.91	26.35	23.10
T_{11}- Panicle initiation + Flowering + Milking	17.55	27.55	28.04	28.64	29.00	26.16
T_{12}- Panicle initiation + Flowering+ Dough	17.55	27.10	27.72	28.39	28.45	25.84
T_{13}- Panicle initiation + Milking + Dough	17.53	25.44	26.40	26.74	27.85	24.79
T_{14}- Panicle initiation + Flowering + Milking + Dough.	17.55	27.80	28.15	28.80	29.10	26.28
Mean	17.54	24.11	25.05	26.49	27.16	
	$FeSO_4$ Levels		Spray Stages		Levels x stages	
SE_d	0.210		0.351		0.784	
CD @ 5%	0.415		0.694		1.552	

Straw yield

Perusal of rice straw yield data (Table 2) underlined that significant influence of different levels of $FeSO_4$ applied at different stages on straw yield over control (water spray). The straw yield ranged from 27.62 to 41.11 g pot^{-1}.

The straw yield increased due to foliar spray of $FeSO_4$ at various concentrations along with 0.1 % citric acid ranged from 37.24 to 39.97 g pot $^{-1}$. The maximum straw yield was recorded with 1.5% $FeSO_4$ + 0.1% CA (39.97 g pot^{-1}) and it was comparable with 1.0 % spray (39.84 g pot^{-1}), but superior to 0.75%, 0.5% and water spray (control) treatments. The minimum straw yield was observed in water spray treatment (27.64 g pot^{-1}). The per cent increase in grain yield due to levels of $FeSO_4$ spray over control ranged from 35 to 49.

With respect to spray stages, foliar spray of $FeSO_4$ on straw yield ranged from 35.37 to 37.99 g pot $^{-1}$. The straw yield was maximum when a foliar application of $FeSO_4$ sprayed at four times *viz.*, T_{14}- Panicle initiation + Flowering + Milking + Dough stages (37.99 g pot^{-1}) and it was comparable with T_{11}- Panicle initiation + Flowering + Milking (37.94 g pot^{-1}) and T_{12} - Panicle initiation + Flowering + Dough stages (37.79 g pot^{-1}) but superior to rest of the treatments. The lowest straw yield was noticed at T_4 – Dough stage with 35.37 g pot $^{-1}$.

The interaction between levels of $FeSO_4$ and spraying at different growth stages were significant on straw yield. However, the foliar spray of $FeSO_4$ @ 1.5% + 0.1% CA at four times (T_{14}- Panicle initiation + Flowering + Milking + Dough) was registered significantly maximum straw yield (41.11 g pot^{-1}) and it was on par with $FeSO_4$ @ 1.0 % but superior to rest of the treatments. The minimum straw yield was noticed with water spray at all the stages (27.62 to 27.65 g pot^{-1}).

Table 2: Effect of foliar nutrition of $FeSO_4$ on rice (CO 51) straw yield (g pot^{-1})

Spray at different growth stages of rice	Levels of $FeSO_4$ (%)					Mean
	Water spray	0.5	0.75	1.0	1.5	
T_1- Panicle initiation	27.64	35.22	37.18	38.92	39.00	35.59
T_2- Flowering	27.64	35.59	37.53	39.42	39.51	35.94
T_3- Milking	27.65	35.00	37.10	38.84	38.90	35.50
T_4- Dough	27.62	34.85	36.92	38.70	38.75	35.37
T_5- Panicle initiation + Flowering	27.63	37.41	38.30	40.24	40.40	36.80
T_6- Panicle initiation + Milking	27.64	36.82	37.87	39.66	39.79	36.36
T_7- Panicle initiation + Dough	27.64	36.67	37.69	39.52	39.64	36.23
T_8- Flowering + Milking	27.63	37.19	38.22	40.16	40.30	36.70
T_9- Flowering + Dough	27.63	37.04	38.04	40.02	40.15	36.58
T_{10}- Milking + Dough	27.64	36.45	37.61	39.44	39.54	36.14
T_{11}- Panicle initiation + Flowering + Milking	27.63	39.98	40.05	40.95	41.08	37.94
T_{12}- Panicle initiation + Flowering+ Dough	27.63	39.83	39.87	40.71	40.93	37.79
T_{13}- Panicle initiation + Milking + Dough	27.65	39.24	39.44	40.26	40.42	37.40
T_{14}- Panicle initiation + Flowering + Milking + Dough.	27.64	40.06	40.15	40.98	41.11	37.99
Mean	27.64	37.24	38.28	39.84	39.97	
	$FeSO_4$ Levels		Spray Stages		Levels x stages	
SE_d	0.210		0.351		0.785	
CD @ 5%	0.415		0.695		1.553	

Grain iron Content

Grain iron content was significantly influenced by foliar application of $FeSO_4$ at different concentrations at various stages over control (Table 3). The iron content ranged from 117 to 220 mg kg^{-1}. However, the iron content in rice grain increased due to different levels of $FeSO_4$ ranged from 175 to 198 mg kg^{-1}. The highest concentration of iron in rice grain was noticed with 1.5 % $FeSO_4$ + 0.1% CA (198 mg kg^{-1}). However, it was comparable with 1.0% $FeSO_4$ spray (194 mg kg^{-1}) but significantly superior to other levels and minimum at water spray (119 mg kg^{-1}). The per cent increase in iron content in rice grain over control was 67.

Grain iron content was increased due to the application of $FeSO_4$ at different growth stages of rice crop ranged from 159 to 190 mg kg^{-1}. Among the various treatments, the significantly maximum iron content in rice grain was recorded when foliar application of $FeSO_4$ sprayed at four times *viz.*, T_{14}- Panicle initiation + Flowering + Milking + Dough stages (190 mg kg^{-1}) and it was on par with three times spray during (T_{13}) Panicle initiation + Milking + Dough stages (185 mg kg^{-1}) but significantly superior to rest of the treatments. The minimum grain iron content was noticed with foliar spray of $FeSO_4$ at (T_1) Panicle initiation stage (159 mg kg^{-1}).

Interaction effect between levels of $FeSO_4$ and time of application was significant on grain iron content. Among the treatments, foliar spray of $FeSO_4$ @ 1.5 % + 0.1% CA sprayed at four times *viz.*, T_{14} - Panicle initiation + Flowering + Milking + Dough stages was recorded significantly maximum grain iron content (220 mg kg^{-1}) and it was at par with $FeSO_4$ @ 1.0 % but superior to rest of the treatments. The minimum grain iron content was noticed with water spray at (T_1) panicle initiation stage (117 mg kg^{-1}).

Grain iron uptake

Scrutiny of analytical data of rice grain iron uptake depicted in Table 4 revealed that significant on, time of application of different concentration of $FeSO_4$ on grain iron uptake over control. The grain iron uptake ranged from 20.51 to 61.20 µg pot^{-1}.

Iron uptake in rice grain increased due to different levels of $FeSO_4$ ranged from 42.26 to 53.68 µg pot^{-1}. The highest uptake of iron in rice grain was noticed with 1.5% $FeSO_4$ + 0.1% CA treatment (53.68 µg pot^{-1}) and it was on par with 1.0% but significantly superior 0.75%, 0.5% and water spray treatments. The minimum grain iron uptake was recorded in water spray application (20.81 µg pot^{-1}). The per cent increase in iron content in rice grain over control was 158.

Rice grain iron uptake was increased due to the application of $FeSO_4$ at different growth stages ranged from 36.16 to 50.99 µg pot^{-1}. The significantly maximum iron uptake was recorded in the treatment which received foliar spray of $FeSO_4$ four times *viz.*, T_{14}- Panicle initiation + Flowering + Milking + Dough stages (50.99 µg pot^{-1}) and it was comparable with three times sprayed treatment *i.e.*, T_{11}- Panicle initiation + Flowering + Milking stages (49.49 µg pot^{-1}) but superior to rest of the treatments. The lowest grain iron uptake was noticed with the foliar spray of $FeSO_4$ at (T_1) Panicle initiation stage (36.16 µg pot^{-1}).

Interaction effect between levels of foliar spray of $FeSO_4$ and time of application was significant on rice grain iron uptake. However, the foliar spray of $FeSO_4$ @ 1.5% + 0.1% CA sprayed at four times *viz.*, T_{14}- Panicle initiation + Flowering + Milking + Dough stages was observed significantly maximum grain iron uptake (61.20 µg pot^{-1}) and it was at par with $FeSO_4$ @ 1.0 % but superior to rest of the treatments. The minimum grain iron content was noticed with water spray at (T_1) Panicle initiation stage (20.51 µg pot^{-1}).

Table 3: Effect of foliar nutrition of $FeSO_4$ on rice (CO 51) grain iron content (mg kg^{-1})

Spray at different growth stages of rice	Levels of $FeSO_4$ (%)					Mean
	Water spray	0.5	0.75	1.0	1.5	
T_1- Panicle initiation	117	157	166	176	179	159
T_2- Flowering	119	159	169	178	182	161
T_3- Milking	119	164	175	183	187	166
T_4- Dough	118	161	173	180	184	163
T_5- Panicle initiation + Flowering	118	171	180	191	194	171
T_6- Panicle initiation + Milking	118	176	186	196	199	175
T_7- Panicle initiation + Dough	119	173	184	193	196	173
T_8- Flowering + Milking	118	178	189	198	202	177
T_9- Flowering + Dough	118	175	187	196	199	175
T_{10}- Milking + Dough	119	180	193	201	204	179
T_{11}- Panicle initiation + Flowering + Milking	120	186	198	206	209	184
T_{12}- Panicle initiation + Flowering+ Dough	119	183	195	203	206	181
T_{13}- Panicle initiation + Milking + Dough	119	188	201	208	211	185
T_{14}- Panicle initiation + Flowering + Milking + Dough.	120	192	207	212	220	190
Mean	119	175	186	194	198	
	$FeSO_4$ Levels		Spray Stages		Levels x stages	
SE_d	1.050		1.758		3.930	
CD @ 5%	2.079		3.479		7.779	

Table 4: Effect of foliar nutrition of $FeSO_4$ on rice (CO 51) grain iron uptake (µg pot^{-1})

Spray at different growth stages of rice	Levels of $FeSO_4$ (%)					Mean
	Water spray	0.5	0.75	1.0	1.5	
T_1- Panicle initiation	20.51	33.49	37.61	43.20	46.00	36.16
T_2- Flowering	20.88	36.43	39.60	46.10	47.59	38.12
T_3- Milking	20.86	34.85	38.61	44.38	47.78	37.29
T_4- Dough	20.70	35.40	38.18	44.93	46.63	37.17
T_5- Panicle initiation + Flowering	20.71	43.43	47.41	53.06	54.32	43.79
T_6- Panicle initiation + Milking	20.70	41.78	46.44	51.21	54.53	42.93
T_7- Panicle initiation + Dough	20.87	40.29	45.36	50.14	52.63	41.86
T_8- Flowering + Milking	20.70	42.90	48.01	52.47	55.55	43.92
T_9- Flowering + Dough	20.70	41.39	46.90	51.45	53.63	42.81
T_{10}- Milking + Dough	20.86	39.58	45.76	52.08	53.75	42.41
T_{11}- Panicle initiation + Flowering + Milking	21.06	51.24	55.52	59.00	60.61	49.49
T_{12}- Panicle initiation + Flowering+ Dough	20.88	49.59	54.05	57.63	58.61	48.15
T_{13}- Panicle initiation + Milking + Dough	20.86	47.83	53.06	55.62	58.76	47.23
T_{14}- Panicle initiation + Flowering + Milking + Dough.	21.06	53.38	58.27	61.06	61.20	50.99
Mean	20.81	42.26	46.77	51.60	53.68	
	$FeSO_4$ Levels		Spray Stages		Levels x stages	
SE_d	0.461		0.772		1.727	
CD @ 5%	0.913		1.528		3.417	

Discussion

Rice is the staple food all around the world, it supplies most of the micronutrients, protein, carbohydrates, etc. (Jiang *et al.*, 2007) [7]. The foliar application of Fe nutrients revealed that an efficient approach to increase the Fe content in cereal crops (Fang *et al.*, 2008; Wei *et al.*, 2012; Yuan *et al.*, 2012) [6, 13, 15]. In the present study, it was observed that 1.5% $FeSO_4$ spray along with 0.1% citric acid four times at Panicle initiation + Flowering + Milking + Dough stages recorded significantly maximum rice grain yield (29.10 g pot^{-1}) and minimum with water spray treatments (17.53 to 17.55 g pot^{-1}). The thinkable reasons may be that due to foliar $FeSO_4$ application could have increased the chlorophyll content, antioxidant enzymes and their activities and finally increased the yield parameters followed by grain yield. Also, increased the absorption and their by easily loading the iron nutrients into the edible parts of the plants and this was contributed to improve the grain yield. This finding is comparable with the previous studies by Shaygany *et al.*, (2012) [11] and Singh *et al.*, (2013) [12] who have found that $FeSO_4$ foliar spray at various growth stages of rice crop increased the grain yield by effective translocation of Fe to economic parts.

Similarly, the maximum straw yield recorded in the superior treatment *i.e.*, 1.5% $FeSO_4$ + 0.1 % CA spray at four times at (T_{14}) Panicle initiation + Flowering + Milking + Dough stages (41.11 g pot^{-1}) might be due to improving enzymatic activities and the process of photosynthesis and their by resulted in increased the straw yield upon $FeSO_4$ application. Similar finding was also observed by Mathpal *et al.*, (2018) [8].

The present study was also reveals that, significantly higher rice grain iron content (220 mg kg^{-1}) and uptake (61.20 µg pot^{-1}) was recorded in 1.5% $FeSO_4$ + 0.1% CA four times sprayed treatment *viz.*, Panicle initiation + Flowering + Milking + Dough stages. This is because the leaf tissue translocates the Fe into vascular sap which helps in transfer the Fe into grains. However, mere foliar application of $FeSO_4$ alone became inefficient due to poor translocation within the plants. There are two reasons for which $FeSO_4$ sprayed along with citric acid; one reason that applied citric acid helps to maintain the iron insoluble forms within the plants (iron acidified by citric acid keeps it in active form), another reason was formation of Fe-citrate complex makes it easier for absorption and translocation and to move faster through the xylem. Thus, foliar application of $FeSO_4$ along with citric acid could enrich the nutritive value of rice grains and also increasing the Fe content. This is in line with finding of Zhang *et al*., (2009) [17] and Singh *et al*., (2013) [12] who have reported that, foliar application of $FeSO_4$ at maximum tillering, pre-anthesis, post-anthesis and maturity stages increased the iron concentration in rice grains. Dhaliwal *et al*., (2010) [4] also found that Fe spray improves the grain iron uptake in rice grains. The plausible reason that increased concentration and uptake of Fe nutrients in grain due to the synthesis of photosynthetic products with specific absorption and transport sites and it also increases the plant biomass. Negatively charged citric acid helps to induce the photochemical reduction of Fe^{3+}, which act as prerequisite for the uptake of iron.

Conclusion

In recent days, bio-fortification technique is an effective tool to improve the nutritive quality of crop plants. Furthermore, foliar method of application of nutrients makes it to absorb and translocate quickly within the plants and its timely application result its accumulation in edible parts. Thus, a pot culture experiment was conducted to assess the effects of $FeSO_4$ to enriching the rice grain iron content and yield. The results revealed that foliar spray of $FeSO_4$ at 1.5 % along with 0.1% CA at four times *viz.*, panicle initiation + flowering + milking + dough recorded significantly highest grain and straw yield and also improved the Fe content and uptake in rice grains. However, it was at par with $FeSO_4$ at 1.0 % along with 0.1% CA sprayed at all the four stages. Based on this finding, $FeSO_4$ along with CA as foliar spray could be used as an effective and easy technique for improving the Fe content in rice grains to overcome Fe malnutrition in human.

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