**Moringa oleifera** Diets Effect on Haematological Parameters of Rat (*Ratus norvegicus*)

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**Abstract**

With aim of place at the disposal of the laboratories nonconventional food with food values and which do not have impact on the system haematopoietic of the rats of laboratory in particular the rat of the species *Rattus norvegicus*, the study of the hematologic parameters of rats nourished with nonconventional food makes containing powder of *Moringa oleifera* was undertaken. Indeed, analysis of the blood taking away carried out following the administration of five diets container various rates of powder of *Moringa oleifera* (0% (Control food), 25%, 50%, 75% and 100%) respectively codified L3P, L3P25, L3P50, L3P75 and L3P100. These different food formulations were administered to rats (*Ratus norvegicus*) during three months. The weight of these animals was daily taken, followed by blood samples every 30 days for the determination of the blood count. The results of the study were shown differences between the various food formulations for each period of blood samples in biological parameters. These differences were more significant for platelets. During the three months of study, the hematocrit, platelets, MCV and outside the control group, were reported a slight progressive increase. The leukocyte parameters of all the rats in the investigation, indicated an increase in the second month. In conclusion, the study shows that during the three months of investigation, the tested foods do not disturb the haematological parameters except for thrombocytes which reveal a modification.

**Keywords**

*Moringa oleifera*, Food’s Formulation, Haematological Parameters, Rat (*Ratus norvegicus*)

**1. Introduction**

For some time, a new event has appeared in food and feed [1]. These are the leaves of a plant species, *Moringa oleifera* [2] that man uses in various forms,
usually in powder form with meals, drinks and others in several countries of the world [3]. This plant is even grown and spread in many homes of our country. Individuals trade it and even extol the extraordinarily excellent nutritional and therapeutic properties of *Moringa oleifera* powder. In terms of food, *Moringa oleifera* powder is considered a dietary supplement. In view of its popular use, it would be wise to explore its nutritional potential. To do this, a study in laboratory animals should be considered. In this sense, a food trial should be conducted to assess the nutritional quality of the plant’s powder. In addition, this study could contribute to animal nutrition in the context of breeding in laboratories. The high costs of food proteins partly constitute a barrier to the development of the breeding in Africa [4]. This report orders the search for other proteinic sources cheap and accessible to all. The nutritionists tried to locally use animal and vegetable proteins available, in order to substitute them completely or partially for conventional proteins [5] [6]. These nonconventional resources played a significant role in the development of the breeding, allowing a strong increase in the production of meat [7]. The composition of certain products as their uses already was the subject of many studies. Their nutritional value today is relatively well described. It is the case of *Moringa oleifera* which, thanks to its exceptional nutritional qualities, was proposed in the human consumption like proteinic complement [8] [9]. These nutritional qualities constitute for us a great interest from his use in the food of the rats. Introduced, this plant passes in blood circulation and could have consequences on the organization of the consumer. This is why the main objective of this work is to evaluate the effect of food at base of *Moringa oleifera* on the blood hematologic parameters in the rat.

Specifically, this work reports the changes of each haematological parameter of the different formulations for each month of study. In addition, our investigation reveals the evolution of each haematological parameter for each food submitted to the rats during the study period.

2. Material and Methods

2.1. Animals

The rats used during these experiments are albino white rats male and female of the species *Ratus norvegicus* and Wistar stock. These rats were aged of four weeks old with body mass mean of 30.25 ± 2.8 g. These young rats are acclimatized in cages ten days before the beginning of the experiment in the Laboratory of Physiology, Pharmacology and Pharmacopeia (LPP) of Training and Research Unit of Nature Sciences (UFR-SN) of Nangui Abrogoua University (Abidjan, Côte d’Ivoire). During the first ten days, all animals received control food (L3P) and water at will. They are subjected daily to the ambient temperature, 12 o’clock in light and 12 hours of darkness. The rats of other stocks or having an apparent immobility and parasitic signs of diseases (nasal flow or ganglia) were not selected in this study. It is the same for the male and female rats old of more or less four weeks.
The equipment, handling and sacrificing of the animals were in accordance with the European Council legislation 87/609/EEC for the protection of experimental animals [10].

2.2. Different Foods Formulation Based on *Moringa oleifera*

The conventional ingredients used during this study were: powders of soya (PS), fish (PP), dead bread (PM) of bakery commonly called “pain godio” in Cote d’Ivoire dried and finely crushed, of cooking salt (S) and the corn (M). As for non-conventional food, it is composed of the sheets of *Moringa oleifera*. They were collected in various cities of Cote d’Ivoire (Abidjan, Bouaké and Bondoukou) in September 2016. The method used, was described by some investigations [11]. It consisted in transporting to the Laboratory of Physiology, Pharmacology and Pharmacopeia of Nangui Abrogoua University, the branches layers of *Moringa oleifera* Lam. These branches layers are dried during approximately five days under a temperature ranging between 18°C and 20°C until they become crusty, friable and cracking. These dried sheets, were finely pulverized with an electric crusher of mark RETSH, type SM 100 (Haan, Germany). The powder obtained was conditioned in small bags of approximately 5 kg to be used for the preparation of food. From the various ingredients, five diets were formulated. They were modes L3P, L3P25, L3P50, L3P75 and L3P100 where the powder of the sheets of *Moringa oleifera* was respectively built in to 0, 25%, 50%, 75% and 100% in partial or total substitution for the soya bean oil cake. Distilled water was then added at a rate of 640 ml/kg of compound feeding stuff, so as to form a malleable and homogeneous paste more or less round. The composition of each experimental diet was consigned in Table 1 and Figure 1. Moreover, with regard to our prospection study [12], the L3P food was chosen as a control.

2.3. Food Tests

Forty rats including 20 males and 20 females, were divided randomly into five groups of eight animals combining four groups tests and a control group. Each group was composed of four rats of male and females. Group 1 (control) was

| Table 1. Composition of diets. |
|--------------------------------|
| **Different diets** | L3P | L3P<sub>25</sub> | L3P<sub>50</sub> | L3P<sub>75</sub> | L3P<sub>100</sub> |
| Bread powder         | 44.50 | 44.50 | 44.50 | 44.50 | 44.50 |
| Crushed maize        | 25    | 25    | 25    | 25    | 25    |
| Fish powder          | 16    | 16    | 16    | 16    | 16    |
| Soy powder           | 14    | 10.50 | 7     | 3.50  | 0     |
| Moringa powder       | 0     | 3.50  | 7     | 10.50 | 14    |
| Salt                 | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  |
| Total                | 100   | 100   | 100   | 100   | 100   |

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received food not containing powder of *Moringa oleifera* (food L3P). The groups 2, 3, 4 and 5, they were respectively received the food which contains the powder of *Moringa oleifera* with the percentage of 25 (food L3P25), 50 (food L3P50), 75 (food L3P75) and 100% (food L3P100). Each group of rats were placed individually in polystyrene cages and were received a meal per day for three months. The blood was collected at the end of each month aseptically over a period of three months in fasting rats the night before. On these animals anaesthetized beforehand in a bell containing of the cotton soaked with ether during 2 to 3 minutes, blood was taken on the level of the orbital retro sine according to the technique of a study [13]. Approximately 5 ml of blood was collected in tubes containing tetraacetic ethylene diamine anticoagulant (EDTA) and transported to the laboratory for a blood count with Sysmex Xl 2000i.

All the experiments were carried out in accordance with the recommendations of the French Republic, relating to the protection of animals used for scientific purposes.

**Statistical analysis**

The statistical analysis of the data was carried out thanks to the software GraphPad PRISM 5.01 (San Diego, California, the USA). The results were given in the form of mean followed by the standard error on the mean (M ± SEM). The test of the analysis of the variance to one factor (ANOVA 1) to check the normality of the variables was used. When differences between the mean of the parameters were significant, the ANOVA 1 is supplemented by multiple comparisons of the mean values of the various parameters by using the *post hoc* test of Turkey-Kramer. A probability level $p < 0.05$ was chosen for the significance of all analyzes.
3. Results
3.1. Effect of Various Foods on Monthly Haematological Parameters

In the first month, the consumption of food L3P25, L3P50, L3P75 and L3P100 by the rats did not induce any significant change (p > 0.05) between hematocrit, hemoglobin, red blood cells and their indices (MCV, MHC and MCHC) of rat groups respectively with L3P25, L3P75, L3P100 food and those which were received L3P food (control group) (Table 2). As for the evolution of the leucocyte parameters, no significant modification (p > 0.05) between mean values of the groups submitted to L3P25, L3P75 and L3P100 food and those of the control group during the three months of study was observed. On the other hand, during the first month of study, the Platelets L3P25, L3P75 and L3P100 foods were decreased significantly (p < 0.05) in comparison with the control (Table 2). In the second month of study, a significant (p < 0.05) increase of the Platelets was indicated in L3P25, L3P75 and L3P100 foods compared to control food. This increase was profound in animals submitted to L3P100 food (Table 3). As far as concern the third month of study, the increase of the observed Platelets in L3P25, L3P75 and L3P100 groups during the second month have significantly (p < 0.05) persisted compared to the control rats. However, this increase was less marked (p > 0.05) that observed in the second month of study (Table 4).

Table 2. Haematological parameters of rats fed different foods enriched with *Moringa oleifera* leaf powder during 1 month.

| Haematological parameter | L3P | L3P25 | L3P50 | L3P75 | L3P100 | p values |
|--------------------------|-----|-------|-------|-------|--------|----------|
| Red blood cells (10^6/µL) | 7 ± 0.57 | 6.80 ± 0.18 | 6.85 ± 0.12 | 7.46 ± 0.45 | 7.02 ± 0.16 | >0.05 |
| Platelets (10^3 µL) | 214.88 ± 26.60 | 172.33 ± 12.70 | 172.86 ± 14.50 | 191 ± 27.50 | 181.29 ± 13.90 | <0.001 |
| Hemoglobin (g/dl) | 14.54 ± 1.01 | 14.65 ± 0.50 | 14.88 ± 0.29 | 15.60 ± 0.92 | 15.01 ± 0.56 | >0.05 |
| Hematocrit (%) | 38 ± 3.29 | 35.27 ± 1.22 | 36.85 ± 1.10 | 39.91 ± 2.51 | 36.56 ± 0.71 | >0.05 |
| MCV (fl) | 53.80 ± 0.68 | 51.90 ± 0.62 | 53.90 ± 1.37 | 53.51 ± 0.46 | 52.24 ± 0.51 | >0.05 |
| MHC (pg) | 21.03 ± 0.73 | 21.52 ± 0.48 | 21.68 ± 0.21 | 20.87 ± 0.25 | 21.31 ± 0.40 | >0.05 |
| MCHC (g/dl) | 38.91 ± 1.63 | 41.50 ± 1.16 | 40.53 ± 1.22 | 39.11 ± 0.67 | 40.94 ± 1.02 | >0.05 |
| White blood cells (10^3/µL) | 12.09 ± 2.33 | 11.18 ± 0.67 | 11.89 ± 1.42 | 10.87 ± 0.99 | 10.66 ± 1.30 | >0.05 |
| Neutrophils (10^3 µL) | 1.17 ± 0.18 | 1.03 ± 0.37 | 0.98 ± 0.23 | 1.62 ± 0.32 | 1.18 ± 0.22 | >0.05 |
| Eosinophils (10^3 µL) | 9.83 ± 1.53 | 8.60 ± 1.54 | 8.17 ± 1.76 | 9.67 ± 3.40 | 9.29 ± 3.50 | >0.05 |
| Lymphocytes (10^3 µL) | 8.77 ± 1.78 | 6.75 ± 0.52 | 6.49 ± 1.10 | 8.48 ± 1.17 | 8.06 ± 1.10 | >0.05 |
| Monocytes (10^3 µL) | 0.63 ± 0.11 | 0.30 ± 0.07 | 0.51 ± 0.116 | 0.59 ± 0.11 | 0.54 ± 0.14 | >0.05 |

The values of the same line are statistically different from the control (rats fed the L3P food) to p < 0.05, n = 6 rats. Lot L3P: 0% M. oleifera powder; lot L3P25: 25% M. oleifera powder; lot L3P50: 50% M. oleifera powder, lot L3P75: 75% M. oleifera powder and lot L3P100: 100% M. oleifera powder.
### Table 3. Haematological parameters of rats fed different foods enriched with *Moringa oleifera* leaf powder during 2 months.

| Haematological parameters          | Different food rations | p values |
|-----------------------------------|------------------------|----------|
|                                   | L3P  | L3P25 | L3P50 | L3P75 | L3P100 |          |
| Red blood cells (10^6/µL)         | 7.54 ± 0.36    | 6.88 ± 0.67 | 7.24 ± 0.25 | 6.47 ± 0.68 | 7.14 ± 0.22 | >0.05    |
| Platelets (10^4 µL)               | 98.83 ± 15.70  | 172 ± 18.30 | 152.80 ± 12.50 | 205 ± 13 | 215 ± 50 | <0.001   |
| Hemoglobin (g/dl)                 | 15 ± 0.84      | 14 ± 1.52  | 14.74 ± 0.40 | 14.33 ± 0.39 | 13.97 ± 0.38 | >0.05    |
| Hematocrit (%)                    | 42.59 ± 2.08   | 39.14 ± 4.17 | 41.38 ± 1.50 | 40.72 ± 1.55 | 39.53 ± 0.96 | >0.05    |
| MCV (fl)                          | 56.65 ± 1.25   | 56.64 ± 0.81 | 57.18 ± 0.99 | 56.92 ± 1.40 | 55.50 ± 0.95 | >0.05    |
| MCHC (pg)                         | 19.81 ± 0.44   | 20.26 ± 0.91 | 20.34 ± 0.50 | 20.42 ± 0.074 | 19.47 ± 0.033 | >0.05    |
| MCHC (g/dl)                       | 35.10 ± 0.58   | 35.76 ± 1.43 | 35.66 ± 0.92 | 35.30 ± 0.98 | 35.27 ± 0.47 | >0.05    |
| White blood cells (10^3/µL)       | 14.58 ± 1.16   | 15.88 ± 1.46 | 15.76 ± 3.47 | 14.32 ± 1.77 | 14.83 ± 1.28 | >0.05    |
| Neutrophils (10^3 µL)             | 1.52 ± 0.35    | 2.36 ± 0.56  | 1.56 ± 0.38  | 2.40 ± 0.17  | 1.05 ± 0.03  | >0.05    |
| Eosinophils (10^3 µL)             | 17.71 ± 4.54   | 22 ± 2.74    | 15.80 ± 3.87 | 38.40 ± 5.75 | 14.33 ± 3.84 | >0.05    |
| Lymphocytes (10^3 µL)             | 13.13 ± 1.08   | 12.20 ± 1.31 | 16.94 ± 2.75 | 16.36 ± 1.48 | 11.87 ± 1.08 | >0.05    |
| Monocytes (10^3 µL)               | 1.18 ± 0.22    | 1.28 ± 0.12  | 1.24 ± 0.33  | 1.33 ± 0.29  | 0.90 ± 0.17  | >0.05    |

The values of the same line are statistically different from the control (rats fed the L3P food) to p < 0.05, n = 6 rats. Lot L3P: 0% *M. oleifera* powder; lot L3P25: 25% *M. oleifera* powder; lot L3P50: 50% *M. oleifera* powder, lot L3P75: 75% *M. oleifera* powder and lot L3P100: 100% *M. oleifera* powder.

### Table 4. Haematologic parameters of rats fed different foods enriched with *Moringa oleifera* leaf powder during 3 months.

| Haematological parameters          | Différentes rations alimentaires | p values |
|-----------------------------------|-----------------------------------|----------|
|                                   | L3P  | L3P25 | L3P50 | L3P75 | L3P100 |          |
| Red blood cells (10^6/µL)         | 6.96 ± 0.70    | 5.87 ± 0.97  | 6.41 ± 0.74  | 5.80 ± 0.69  | 6.50 ± 0.83  | >0.05    |
| Platelets (10^4 µL)               | 144.2 ± 21.10  | 182.50 ± 29.40 | 184.80 ± 30  | 191.60 ± 24.3 | 181.63 ± 29.30 | <0.001   |
| Hemoglobin (g/dl)                 | 15.13 ± 0.28   | 15.08 ± 0.78  | 15.15 ± 0.42  | 14.737 ± 0.82 | 14.74 ± 0.23  | >0.05    |
| Hematocrit (%)                    | 41.2 ± 2.19    | 43.44 ± 5.22  | 40.58 ± 4.10  | 42.89 ± 3.69  | 43.06 ± 4.77  | >0.05    |
| MCV (fl)                          | 55.69 ± 1.17   | 57.28 ± 1.41  | 57.23 ± 1.15  | 57.25 ± 3.23  | 58.825 ± 0.72 | >0.05    |
| MCHC (pg)                         | 20.73 ± 1.82   | 23.02 ± 3.92  | 22.5 ± 3.42   | 23.14 ± 3.53  | 21.37 ± 5.04  | >0.05    |
| MCHC (g/dl)                       | 42.09 ± 5.09   | 43.38 ± 7.16  | 39.26 ± 3.77  | 43.44 ± 4.76  | 46.11 ± 8.36  | >0.05    |
| White blood cells (10^3/µL)       | 11.13 ± 1.16   | 12.14 ± 1.89  | 12.55 ± 1.77  | 11.29 ± 3.14  | 11.81 ± 1.66  | >0.05    |
| Neutrophils (10^3 µL)             | 1.66 ± 0.39    | 2.24 ± 0.45   | 2.07 ± 0.34   | 2.52 ± 0.40   | 1.74 ± 0.25   | >0.05    |
| Eosinophils (10^3 µL)             | 20 ± 3.24      | 22.20 ± 4.62  | 24.17 ± 7.15  | 21.13 ± 8.50  | 24.63 ± 3.61  | >0.05    |
| Lymphocytes (10^3 µL)             | 8.55 ± 0.69    | 8.67 ± 1.64   | 9.67 ± 1.47   | 8.55 ± 2.34   | 8.31 ± 1.33   | >0.05    |
| Monocytes (10^3 µL)               | 0.90 ± 0.12    | 1.40 ± 0.218  | 0.80 ± 0.10   | 1.76 ± 0.40   | 0.86 ± 0.14   | >0.05    |

The values of the same line are statistically different from the control (rats fed the L3P food) to p < 0.05, n = 6 rats. Lot L3P: 0% *M. oleifera* powder; lot L3P25: 25% *M. oleifera* powder; lot L3P50: 50% *M. oleifera* powder, lot L3P75: 75% *M. oleifera* powder and lot L3P100: 100% *M. oleifera* powder.
3.2. Changes in Haematological Parameters within Each Food during the Food Trial Period

The different figures below represent the evolution of haematological parameters of each type of food after three months of food testing. Hemoglobin, MCH and red blood cells have been no real change in their content during the three months of their use in all experiments in rats lots (Figure 2(a), Figure 2(b) and Figure 2(e)). The hematocrit and VGM content outside the control group were shown a slight progressive increase during the different months of the experiment (Figure 2(c) and Figure 2(d)). All rats experience declined from MCHC rate in the second month with no significant difference during the period of experimentation (Figure 2(f)). For platelets, outside the control group, there was a slight increase in the second month of L3P75 and L3P100 foods to decrease in the third month. Conversely, the food L3P50 revealed a decrease in the second month and an increase in the third month, while L3P25 has indicated a high rate of platelets in the third month of study (Figure 2(g)). The leukocyte parameters of all the rats in the investigation, indicated an increase in the second month (Figure 3). This increase is observed without significant difference (p > 0.05) in white blood cells (Figure 3(a)), while it is significant (p < 0.05) in neutrophils of rats fed 25% of *Moringa* leaves (Figure 3(b)), highly significant (p < 0.001) followed by a significant decrease (p < 0.05) in Eosinophils (Figure 3(c)) L3P75 group. This increase in the other rats of the experiment is progressive without

**Figure 2.** Changes in red cells indices and platelets for each tested food during three months. (a) Red blood cells, (b) Hemoglobin, (c) Hematocrit, (d) MCV, (e) MHC, (f) MCHC, (g) Platelets.
significant value. In the same vein, a high level of lymphocytes in the second month with a highly and highly significant difference respectively at the L3P50 and L3P75 groups, followed by a decrease in the third month with a very significant difference in the L3P50 and L3P75 groups (p < 0.01) was observed (Figure 3(d)). Monocytes also were shown an increase with significant difference (p < 0.05) in L3P25 group. However, their rate decreased in the control, L3P50 and slightly for L3P100 foods, unlike the L3P25 and L3P75 groups (Figure 3(e)).

4. Discussion

The evaluation of the haematological parameters following the daily administration of four food containing of Moringa oleifera (L3P25, L3P50, L3P75 and L3P100) over one three months period to rats, made it possible to show that this food does not disturb the haematological parameters except for thrombocytes which have undergone a modification during three months of study.

During the three months of the study, the rate of all observed red blood cells in rats fed Moringa leaf powder (apart from lots L3P75 and L3P100 in the first month) has always remained low compared to controls. However, this rate is in reference value of wistar rats which is (5 - 12, 5 × 10⁶/µL).

The food consumption was uniform as well in the animals treated at the control. These four food formulas have improved appetite treated rats. With regard to the effect of four food on the haematological parameters, no modification of erythrocytes was observed in the treated rats. Moreover, no significant difference of hemoglobin, was observed in the animals compared to the witnesses. This absence of effect on these haematological parameters states clearly that this food (L3P25, L3P50, L3P75 and L3P100) does not interfere with the hematopoietic system of the rats on the one hand and do not cause anemia in a repeated administration on the other hand. These results are similar to those of some inves-
tigations [14] [15]. Indeed, these authors did not observe any disturbance of the haematological parameters with the substitution of the soya bean oil cake by the flour of sheet of *Moringa oleifera* in the food of table fowls. The erythrocyte indices such as MCV, MCH and MCHC of the rats nourished with four food did not indicate modification compared to the control. These results corroborate those obtained by some authors [16] which showed that the administration of the aqueous extract of *Syzigium aromaticum* (L.) Merr. and Perry (Myrtaceae) with rats with the amount of 300 and 700 mg/kg of body weight over one month period does not disturb the erythrocyte indices. However, the blood smear of rats fed with this food would be necessary to confirm or refute this hypothesis. In the first month of the study, a fall of thrombocytes was recorded. This fall was corrected during the second and third month of the study when the rats were nourished with food (L3P25, L3P50, L3P75 and L3P100) containing the powder of *Moringa oleifera*. This means that the blood clotting factors disappeared during the first month, but this deterioration was corrected during the second and third months of the study. The major function of thrombocytes is to ensure hemostasis [17]. This food (L3P25, L3P50, L3P75 and L3P100) containing the *Moringa oleifera* powder, by stimulating the proliferation of thrombocytes would facilitate the hemostasis.

The white blood cell count was not disturbed in animals fed four foods. It is well known that lymphocytes play an important role in the formation of disease barriers and may therefore be involved in the formation of the antibody to protect the body against pathogens [18]. As no case of diseases even of mortality was observed during this study one could suggest that the powder of *Moringa oleifera* did not have negative impact on the immune function of the rats. These results are in agreement with those which announced that the aqueous extract of seed of *Moringa oleifera* does not induce any toxic effect on the leucocytes and the leucocytes in the Wistar rats [19]. These results are in conformity also with the results in which the seed extract of *Moringa oleifera* employed with the high amounts of 400, 800 and 1600 mg/kg is without negative effect on the haematological parameters in particular the leucocytes [20]. On the other hand, these results are not similar to those of some studies which showed that the administration repeated of the extract methanolic of the roots of *Clerodendrum myricoides* to the amount of 400 mg/kg of body weight to albino Swiss mice during 44 days would involve a significant reduction in the white globules [21]. The absence of neutrophil modification observed in rats in this study would indicate that the suppression of leukopoiesis in the bone marrow does not result in the immunizing and phagocytic activity of blood cells in animals. The analysis of these results shows clearly that the powder of *Moringa oleifera* can be used in the food of the animals of laboratory in particular at the rat wistar without damaging of the hematopoïetic system. Moreover, the reduction of the loads related on the food, the breeding what would contribute to the valorization of this plant that we meet in almost each court in Cote d’Ivoire. Thus, partial substitution of conventional foods with *Moringa oleifera* powder is possible and advantageous.
5. Conclusion

The daily administration of four foods containing *Moringa oleifera* (L3P25, L3P50, L3P75 and L3P100) over a period of three months to rats is harmless, no adverse effects being demonstrated during hematological investigations. However, other studies including blood smears and biochemical coupled histopathological observations of the liver of rats fed these four foods are needed to confirm this harmless action.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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