Inclusion of Hermetia illucens larvae meal on rainbow trout (Oncorhynchus mykiss) feed: effect on sensory profile according to static and dynamic evaluations

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Inclusion of *Hermetia illucens* larvae meal on rainbow trout (*Oncorhynchus mykiss*) feed: effect on sensory profile according to static and dynamic evaluations

RUNNING TITLE Sensory profile of rainbow trout fed insect meal

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ABSTRACT

BACKGROUND: Diet implementation with insect meal arouses increased attention in aquaculture considering the advantages of this new protein source. The effect of *Hermetia illucens* meal (HI) inclusion in diets on rainbow trout physical-chemical and sensory properties was evaluated. Three diets were prepared: HI0, HI25, HI50, with 0, 25 and 50% of HI replacing fish meal, respectively. Fillet sensory profiles were described by descriptive analysis (DA) and Temporal Dominance of Sensation (TDS) methods. Cooking Loss, WB-Shear Force, proximate analysis, fatty acid composition were also determined.

RESULTS: Diets significantly affected fillets sensory profile. DA indicated significant changes in perceived intensity of aroma, flavour and texture descriptors as a function of diet composition. TDS evaluations provided information on dominance and evolution of sensations perceived in fillets from different diets. The first sensations perceived as dominant were related to texture attributes, followed by flavours. Dominance of fibrousnesses decreased with the increasing of HI in diet. Boiled fish, algae flavours and umami taste clearly dominated the HI0 dynamic profile. The onset of metallic flavour dominance characterized HI25 and HI50. No differences in physical parameters were detected. Principal component analysis highlighted the relationship between sensory attributes and physico-chemical parameters.

CONCLUSION: Sensory description of fillets indicated that HI inclusion induces significant differences in the perceived profile.

Key words

Hermetia illucens, insect feeding, rainbow trout, Descriptive Analysis, Temporal Dominance of Sensation.

INTRODUCTION

The rising demand and consumption for aquaculture feeds have generated a rapid decline in the availability of fish meal (FM) and a concurrent price increase.[1] FM is the optimal animal protein source used in commercial fish feeds.[2] However, the use of FM is both environmentally and economically unsustainable.[1] Alternative protein sources have been investigated to replace FM in livestock feeds, especially in aquaculture. Nowadays, insects are being considered as a novel protein source both for humans and livestock.[3-5] Insects grow and reproduce easily, have high feed conversion efficiency, can be grown on low quality organic waste, do not compete with humans and other farmed animals for nutrients and are particularly suitable for many freshwater and marine fish feeding as a part of their natural diet.[6] Moreover, they are a rich source of protein, lipids, minerals and vitamins.[7] Different insect species have been considered for their possible use in fish feeds and some studies have been carried out.[8-10] Among the different species, *Hermetia illucens* seems to be very interesting as sustainable alternative to replace FM in aquaculture feeds.[8] *H. illucens*, is suitable to be reared on organic wastes by converting them into protein-rich and lipid-rich biomass, therefore it can be used for various purposes including animal feeding, biodiesel and chitin production.[6]

Changes in fish diet affect fish flesh sensory characteristics[11,12] such as texture[13,14] and volatile compounds.[15,16] Previous studies showed that the replacement of FM with insect protein in aqua feeds determines changes of chemical composition of fish muscle, especially for lipid content and fatty acid profile.[17,18] In several studies relationships were found between fish flavour, muscle chemical composition[19] and fatty acid profiles.[20-22] Considering the growing interest of *H. illucens* as alternative protein source to replace FM in the fish feeds, it can be highlighted that until now the studies on the related effects on sensory properties of fish meat are scarce.[17,18,23]

Modifying feeding practice without taking into account possible changes in fish sensory properties appears a risky option[24], since modifications on fish flavour and aroma can affect the perceived quality[25] and the acceptability by consumers.[16]

Descriptive Analysis (DA) and Temporal Dominance of Sensations (TDS) have been found as methods providing complementary information to describe food sensory properties.[26] DA permits identification, quantification, and description of food sensory attributes.[27] It is useful when a detailed description of the sensory properties is desired and provides a picture of the perceived differences among products in terms of intensity of sensory attributes.

TDS tracks the evolution of "dominant" sensations (the ones catching the attention) during the product evaluation.[28] The dynamic of perception has important consequences for a better understanding of the processes used by consumers to assess acceptability and sensory properties of food products.[29]

In this work, DA and TDS were utilised to investigate the effect of replacing part of diet proteins with *H. illucens* on sensory properties of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). The physical-chemical characteristics and sensory properties of rainbow trout fed regular diet with FM as exclusive source of protein or fed diet including *H. illucens* in partial replacement of FM were described and compared. Moreover, the relationships between physico-chemical characteristics and sensory profile of fillet samples were investigated.

MATERIALS AND METHODS

Sample characteristics

Diet formulation

Three isoproteic and isolipidic diets were formulated (Table 1), in which *Hermetia illucens* prepupae meal (HI; Hermetia Deutschland GmbH & Co. KG (HDKG), Baruth/Mark, Germany) substituted 0% (HI0), 25% (HI25) and 50% (HI50) of FM

Fish feeding and sampling

The experimental trial was performed at the experimental facility of the Department of Agricultural, Forest, and Food Sciences (Italy). A total of 360 rainbow trout (*Oncorhynchus mykiss*), with an average initial weight of 178.9 ± 9.81 g, was individually weighed and randomly distributed in twelve fiberglass tanks (1 m^3; T: 13±1°C; dissolved oxygen: 7.6 - 8.7 mg L^{-1}; water flow: 8 L min^{-1}), in an indoor openwater system (30 fish per tank). Experimental diets were randomly assigned to the tanks.

Fish were fed by hand twice a day at 1.5% of body weight, and fish were weighted in bulk every 15 days to adjust the feeding rate.

At the end of the trial (92 days), fish were individually weighed and 30 fish for each diet were slaughtered, accurately packaged inside polystyrene boxes with ice covering, and brought to the Department of Agri-Food Production and Environmental Sciences (Florence, Italy). After the arrival, all the fish were weighed, dissected and filleted; the fillets were vacuum packaged, and frozen at −80°C until analyses.

Sample selection and preparation for sensory evaluations

Fish were ranked within each diet according to their weight. Individuals with the highest weights within the diets and with comparable weights across diets were selected for sensory evaluations (Table 2). In total, 15 individuals were selected from each diet, four were utilised for Descriptive Analysis (DA) and three were utilised for Temporal Dominance of Sensation (TDS) analysis. The remaining individuals were used to set up the evaluation conditions and for sensory panel training purpose.

Before evaluations, fillets were thawed at 4°C for 24 h, washed and accurately dried with paper, and skinned. The part close to tail was discharged and the ventral fish bones removed. Samples were prepared by cutting the cleaned fillets in several portions of 4±0.2 g each, and around 22 portions from each individual were obtained. Each portion was wrapped in an aluminium foil, and stored at 6-8°C until the evaluation session started.

Wrapped samples were steam cooked for approximately 1.30 min, until reaching a temperature of 62°C at the heart and immediately presented to subjects for evaluation.

General sensory evaluation conditions

Samples were presented monadically and identified by a three-digit code. The presentation order was randomized between subjects and sessions. The order of attributes was balanced between subjects to minimize a possible ‘‘proximity’’ effect and was always the same for a given assessor. After each sample, subjects rinsed their mouth with water for 30 s, had some plain crackers for 30 s and finally rinsed their mouth with water for a further 30 s. Subjects took a fifteen min break after every session. Data were collected with the software Fizz (ver. 2.47.B, Biosystemes, Couternon, France).

Subjects of the sensory evaluation

Ten subjects, 8 males and 2 females, aged from 20 to 30 years, regular fish consumers, were recruited. The subjects were informed that the aim of the evaluation was the description of sensory properties of fish fed diets containing also proteins from insects. Before starting with the experiment, a written informed consent was obtained from each subject. The subjects had no history of disorders in oral perception.

Sensory evaluation by Descriptive Analysis

Training sessions: sensory vocabulary development and subject training

Panellists participated in three training sessions of about 60 min each. The subjects developed a vocabulary describing differences and similarities between experimental samples in two different sessions, according to a simplified version of the repertory grid method.[30] The initial list of attributes was reduced to achieve a list that comprehensively and accurately described the product space: redundant and/or less-cited terms were grouped on a semantic basis and/or eliminated according to the subjects' consensual decisions. A main list of attributes was developed (Table 3) which described the texture, taste and flavour of fish samples. Some standards were prepared, as reported in Table 4, to induce a weak/moderate intensity. A nine point category scale labelled at the extremes with ‘‘extremely weak’’ (corresponding to 1) and ‘‘extremely strong’’ (corresponding to 9) was utilised for evaluation. Two repetitions of the whole set of samples were performed in individual booths. Assessor and panel performance were validated by evaluating two sets of samples used for the study. Panel and assessors data were analysed using Panel Check software (ver. 1.4.0, Nofima, Trømso, Norway).

Evaluation

The evaluation of fish meat from each diet was replicated four times in four sessions. In each session, each panellist evaluated three individuals, one from each diet. Two samples from each individual were tested. The first sample was utilised for aroma (ortho-nasal odour) and texture assessment, while the second sample was utilised for taste and flavour evaluation. The overall aroma and flavour descriptors were always presented as the last attribute of the relevant list.

Sensory evaluation by Temporal Dominance of Sensations

Subjects participated in three training sessions. In the first session, the concepts of dominance and temporal evolution of sensations were explained to the subjects. Then, the most relevant attributes for describing the temporal evolution of sensory properties were selected from the attribute lists used for DA. Nine attributes were selected: Melt in mouth, Tenderness, Juiciness, Fibrousness, Metallic, Boiled fish and Algae flavours, Umami, and Astringency. Two sessions were performed for training subjects with the use of the computer system for TDS data acquisition. Panellists were trained to click on the ‘‘Start’’ button as soon as the sample was in the mouth and to immediately start the evaluation. Performance of panellists and eventual artefacts were evaluated by visual inspection of individual out-put of training session evaluations.

Panellists participated in six evaluation sessions. Two sessions per day were performed and three individuals, one from each diet, were evaluated twice in the same day, in two independent sessions. In total, three individuals per each diet were evaluated. The total evaluation time was 90 sec.

Sample presentation and evaluation conditions were the same described for DA evaluations.

Physical analyses

A number of 4 fish for each diet was randomly weighed and slaughtered. Analyses on physical and chemical properties were performed on the cooked fillets of each sample. The cooking loss (CL) was calculated by measuring the difference in weight of the fillet before the cooking process and after, according to the formula:

$$100 \times [\text{raw fillet weight - cooked fillet weight (g) / raw fillet weight (g)}]$$

Texture analyses were carried out using a Zwick Roell® 109 texturometer (Ulm, Germany) with the Text Expert II software, equipped with a 1 kN load cell. The Warner-Bratzler shear force test (WB-SF) was performed on the cranial part of the fillet epaxial region (two measurement for each fillet). A straight blade (width of 7 cm), perpendicular to muscle fibre direction, was utilised at a crosshead speed of 30 mm/min to 50% of total deformation. Maximum shear force, defined as maximum resistance of the sample to shearing[31] was determined from the plot of force (N) compared with deformation (%) and expressed as mean.

Chemical analyses

Proximate composition of HI meal, experimental diets and cooked freeze-dried fillets from the three groups of fish differently fed was determined according to AOAC procedures.[32] Dry matter, ash, crude protein and ether extract were determined according to 950.46, 920.153, 976.05, and 991.36 methods, respectively.

The fatty acid (FA) group composition was analysed on total lipid extract[33] of HI meal, experimental diets samples and cooked muscle samples obtained from fish fed different diets. The FAs composition was determined by gas chromatography (Varian GC 430; Agilent, Palo Alto, CA, USA) equipped with a flame ionization detector and a Supelco Omegawax™ 320 capillary column (30 m × 0.32 mm i.d., 0.25 μm film and polyethylene glycol bonded phase; Supelco, Bellefonte, PA, USA). FAs were identified by comparing the FAME retention time with the standard Supelco 37 component FAME mix (Supelco). Individual FAs were quantified using tricosanoic acid (C23:0) (Supelco) as internal standard. FAs were expressed as a percentage of total FAME.

Statistical analysis

Intensity data from the trained panel were analysed by multi-block PCA (Tucker-1) and by *P**MSE plot (Panel Check software, ver. 1.4.0, Nofima, Norway) to assess panel calibration and assessor performance, respectively.[34] Based on the *P**MSE plots and Tucker-1 plot, 2 out of 10 subjects were considered unreliable and were taken out from further data analysis.

Principal Component Analysis (PCA) was carried using the mean data for each repetition, in order to geometrically represent the variability associated to diets and individuals by using The UnscramblerX 10.3 software (Norway). Samples were included as dummy variables (downweighted in the data matrix) to improve the visual interpretation.[35] The full cross validation was computed to validate the interpretation of the first two components.

Every sensory attribute was analysed following a two-way factorial design in which the diets and panellist were treated as a fixed effect and as a random variable, respectively.

The mean dominance curves for each treatments and six repetitions were computed from raw software coding (1 selected; 0 not selected). The data of TDS were analysed by the software Fizz (ver. 2.47.B, Biosystèmes, Couternon, France). When the TDS curves were plotted, two additional lines were drawn for the chance and significance levels. The chance level refers to the dominance rate that an attribute could obtain by chance. Its value is inversely proportional to the number of attributes (P_0=1/p, where p is the number of attributes). The significance level (*Ps*) is the minimum value this proportion should be equal if it is to be considered significantly ($p<0.05$) higher than P_0. Rosner[36] recommended that $np\ (1-p)>5$ (where n=number of trials and p=probability of success). In the present study, 10 panellists performed six replications of each product and nine attributes were utilised, thus the number of observation was satisfied ($np = 5.87$).

Normality of data distributions was tested by the Kolmogorov-Smirnov test on cooking loss, WB-shear force, proximate analysis and fatty acid composition. One-way ANOVA was performed on physical-chemical results, considering the diets as main effect. The Bonferroni post-hoc test was applied to check the significance of the differences among diets, using SPSS version 17.0 software (SPSS Inc. Illinois, USA).

A PCA was calculated after standardization of variables in order to assess the relationship among the sensory, physical and chemical dataset, using The UnscramblerX 10.3 software (Norway).

RESULTS

Descriptive analysis

The analysis of principal components (PCA) was performed using all tested individual as independent samples, in order to evaluate the differences due to both the different diets and those relevant to biological variability among of individuals reared following the same diet. The PCA correlation loading plot (Figure 1) showed that samples were mainly discriminated along the first component (PC1: 42% explained variance) according to the diets. Fish fed with the control diet were positioned on the left side of the map, while those with 50% of HI meal were located on the right. The HI25 samples were located closed to the origin of the component. Along the second component (18% of explained variance), sample position reflects the sensory variability due to the biological variability of individuals within the same diet. Considering the distribution of samples in the perceptual space, it appears that the differences among samples due to different diets are more evident than those perceived between different individuals fed the same diet. Thus, the individual evaluations belonging to the same diet were treated as repetition.

The mixed ANOVA model on the intensity data of the sensory attributes were performed, in order to estimate the sample effect (three levels: HI0, HI25, HI50) (Table 5). A significant sample effect of the diets was found for 12 out of 19 attributes evaluated. No significant effects of assessor × product interactions were found for the significant attributes (data not reported). Results reported in Table 5 showed that the main differences were found between the control (HI0) and HI50 samples, while HI25 expressed some similarities with HI0 for some attributes and with HI50 for the others. Considering the aroma-related attributes, the perceived intensities of boiled fish, algae and overall aroma were significantly higher in HI0 than HI50 samples ($p < 0.001$). On the other hand, the fresh fish aroma showed a significantly higher intensity in HI25 than HI0 and HI50 ($p < 0.05$). Metallic aroma was higher in samples from FM partially replaced with insect proteins diets. Texture attributes resulted significantly more intense in HI50 than HI0 and HI25 ($p < 0.05$). Indeed, samples obtained by fish fed the 50% of insect meal inclusion diet were juicier, more tender and melting more in mouth than HI0 samples. Overall aroma intensity tended to significantly decrease with the increasing of insect protein inclusion.

Boiled fish flavour and sweet taste were perceived as more intense in HI0 samples, with respect to individuals fed insect meal diets ($p < 0.05$). The addition of *Hermetia illucens* prepupae meal also induced a significant increase in overall flavour intensity, independently from HI concentration. Moreover, metallic flavour intensity increased with the increase of HI meal content in the diets.

Temporal Dominance of Sensations

Mean TDS curves of the samples from the three diets are reported in Figures 2–4 for HI0, HI25 and HI50 groups, respectively. In general, the curves showed that the texture attributes dominated the first part of evaluation (0 to 15 seconds), followed by flavour and taste attributes. In HI0 samples (Figure 1) tenderness and fibrousnesses clearly dominated the first part of evaluation. On the other hand, only tenderness clearly dominates the dynamic profile of HI25 (Figure 3) and HI50 samples (Figure 4). Furthermore, it appears that the dominance of juiciness is mainly related to FM partial replacement with insect proteins. Flavour of HI0 samples was dominated by boiled fish and algae flavours even at a lower extent, umami taste was the sensations mostly dominating the after taste. In samples HI25, boiled fish clearly dominated the profile together with algae, metallic flavours and umami. The dynamic profile of HI50 appears complex with several descriptors perceived as dominant at the same time (boiled fish, algae, metallic, umami). Even though umami resulted as the most important attribute in the aftertaste of all samples however some differences among diets have been observed. Indeed, while in HI0 the umami was the only dominant attribute, in HI25 the metallic flavour was dominant and in HI50 the boiled fish flavour persisted until the end of evaluation.

Physical and chemical characterization of fish fed different diets

Table 6 reports results of physical and chemical parameter analyses. No significant effect of diets was observed for both cooking loss and WB shear force, indicating that, from an instrumental standpoint, the samples lose the same amount of water during cooking and were equally soft. Proximate composition of cooked samples was not significantly affected by diets of fish ($p>0.05$). On the contrary, the sum of the principal groups of the fatty acids showed differences associated with the experimental diets. It is of note that HI50 fish have the significantly highest level of saturated fatty acids (SFA), followed by HI25. The HI0 had the significantly lowest content of SFA and the significantly highest content of PUFAω3, showing an inverse relationship with fish fed *Hermetia illucens* inclusion diets. MUFA and PUFA showed a similar trend, significantly decreasing with the increase of HI meal concentration in diets.

Relationship between instrumental and sensory analyses

The correlation loadings plot in Figure 5 summarizes the main trend of sensory, chemical and physical variables of the samples obtained from fish fed different diets, highlighting the relationship between sensory and instrumental parameters. The explained variance after the first two components (PC) account of 53%. PC1 (37% of explained variance) separated samples without *Hermetia illucens* inclusion in diet from samples fed including HI prepupae meal. PC2 seemed to further separate samples that have different content of HI. The predominant differences between the samples were due to the fatty acids (FAs), mainly SFA that were negatively related to PUFAs. SFA resulted positively correlated to metallic aroma/flavour, overall flavour and tenderness (negative part of PC1), as well as protein and ash content. The positive part of PC1 showed the relationship between PUFA, PUFAω3, MUFA and boiled fish flavour and overall aroma. PUFAω6 seemed highly related to algae flavour, loaded on the positive part of the second component. At the same time, juiciness and melt in mouth attributes were strongly related to PUFAω6 and moisture content. WB-shear force did not play a relevant role in this PCA, as expected considering the lacking of significant differences detected with analysis of variance.

DISCUSSION

Sensory evaluation

Terms freely generated by assessors to describe fish sensory properties are not associated to negative hedonic valence, thus indicating that FM partial replacement with insect meal did not induce the perception of sensory defects or off-flavours. According to sensory results, differences among diets have been observed. DA showed significant differences in terms of aroma, flavour and texture. These results disagree with previous findings reported in literature. For example, no sensory significant differences have been found with inclusion of insect meal in diets on Atlantic salmon.[18] Performing a triangle test on rainbow trout fed diet with different content of insect meal, Sealey et al.[17] did not find any significant differences. In these studies, differences in FA composition were detected, and the lacking of significant differences in sensory proprieties was quite unexpected since differences in FA composition affect the sensory profile.[20,21] Possibly these results reflect the lack of power of the adopted sensory techniques. The results of the present work further confirm DA as a powerful sensory descriptive technique, providing the accurate description of sample sensory properties.

The dynamic analysis of sensory proprieties confirmed the differences between the groups of fish fed different diets. TDS results partially confirmed the results obtained by DA, and allowed a better understanding of the perception of sensory proprieties during all the chewy process. Fibrousnesses intensity was not significantly different amongst trout samples but this sensation appears to be much more important in HI0 than in HI25 and HI50 samples. The inclusion of HI prepupae meal in diets led to the perception of a more complex sensory profile with several flavour sensations dominating the perception at the same time. Dominance of metallic flavour characterized HI25 and HI50 samples in respect to HI0. This sensation can be seen as unfamiliar or as unexpected in fish thus catching the assessor attention despite its moderate intensity value. Dominance values indicated that the importance of a sensation during food consumption is not necessarily the same as that indicated by intensity ratings from static sensory profiles.[37] Thus, the use of the TDS method for the sensory characterization of fish samples provides information which complements those from DA studies.

Physical and chemical characteristics and relationship with sensory profile

The partial replacement of FM with HI meal in diets for fish feeding, as alternative source, seems to have effects on qualitative aspects of fillet, in terms of sensory and physico-chemical characteristics.[16,17,23] In the present study, the instrumental physical differences concerning the parameters investigated were not identified. The *H. illucens* meal is a high-value feed source, rich in protein and fat. The fat amount of black soldier fly larvae is extremely variable and depends on the feeding substrate and development stage of the insect. Further, their FA composition depends on the FA composition of the diet utilised for larvae rearing.[3] The lipid content of HI affected the chemical composition of fish fillet, when the FM was partially replaced by the insect meal. In this study, it seems that the inclusion of HI in diets implies a change in FA profile of fish fillet, especially increasing the incidence of SFAs. On the other hand, the PUFA incidences diminish when HI inclusion increases compared to control samples. This trend was also observed in previous studies on Atlantic salmon[18] and rainbow trout,[17] where the amount of whole-body SFAs increased employing diets containing increased amount of HI. Regarding proximate composition, in our trial no significant differences in fillets from different groups of fish were noted. Contrariwise, Sealey et al. observed that fillet moisture and lipid composition were significantly altered by replacement of dietary FM with black soldier fly prepupae meal in rainbow trout.[17] They reported that fish fed diets containing HI had significantly greater moisture and lower lipid in muscle in comparison with fish fed the control diet. Even though these analyses were conducted on raw muscle, while in our study the analysis was performed on cooked fillets, the results of this work are partly in line with these previous findings, since a trend for a lower lipid content in fillet with increasing inclusion of insect meals, even if not significant, was also observed.

Relationship between sensory and instrumental analyses

The compositional differences of the diets, i.e. lipid content, FA profile and proximate composition, can have affected the sensory properties. These variations in diet modify, in particular, lipid content and composition of fish muscle. Overall aroma and flavour intensity are both dependent on final product lipid content.[12] This study has highlighted the relationship between sensory and physico-chemical parameters (Figure 5). FAs, flavour and texture attributes showed the main relationships. SFA increase in the fish fillet with the increased inclusion of HI in the diets, and it seems to be correlated to flavour and texture in fish flesh, in agreement with previous study.[15] The rise of fatty acids had an effect on tenderness of fish meat as confirmed also by Grigorakis et al.[11] and Rincón et al.[12] findings. Valente et al.[38] found a significant relationship between lipid content and both fatty flavour and perception of fatty texture. Additionally, lipid content and FA profile of fillets have a connection to flesh texture[39] and they affect texture attributes, mainly juiciness and tenderness.[11] However, this relationship with tenderness measured with Warner-Bratzler shear force was not revealed in the case of this trial samples. Water contents contributed to juiciness and melting in mouth of the fish samples, in agreement with a previous work findings.[12] Concerning the flavour modification, Grigorakis et al.[11] showed that fat content strongly affects the mouth impression and volatile compounds that were also correlated to differences in sensory taste.

CONCLUSION

Sensory description of fish samples indicated that HI inclusion induces significant differences in the perceived profile. Furthermore, HI inclusion in the diet did not induce the perception of sensations relevant to defects or off-flavours. The effect of diets was highlighted both by DA and TDS descriptions and the information obtained appear complementary. The strict relationship between sensory profile and fatty acid composition was also confirmed by the results obtained. Further study will be necessary to understand if the highlighted differences in sensory properties of fish fed diets characterized by different protein sources would be reflected in liking judgements by consumers.

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