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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(2): 1166-1175. **OPEN ACCESS**

Fermented Wheat *Hamoum* improves the recovery of intestinal mucosal and the short-chain fatty acids profile of colonic bacterial flora in malnourished rats

Djamila Yssaad[1*], Ben Mehel Benakriche[1,2], Philippe Gérard[3], Philippe Pochart[4], Omar Kheroua[1]

[1]Laboratory of Physiology of Nutrition and Food Safety, Department of Biology, Faculty of Natural and Life Sciences, University Oran 1 Ahmed Ben Bella, 31000 Oran, **Algeria**
[2]Laboratory of Applied Animal Physiology**,** University of Mostaganem, 27000 Mostaganem, **Algeria**
[3]Micalis Institute, INRAE, AgroParisTech, University Paris-Saclay, 78350, Jouy-en-Josas, **France**
[4]Microbial Ecology Laboratory (MIEL), Conservatoire National des Arts et Métiers, F-75003 Paris, **France**

***Correspondence:** djamilabio31@yahoo.fr **Received 24-02-2020, Revised: 08-06-2020, Accepted: 10-06-2020 e-Published: 11-06-2020**

The aim of the present study was to investigate whether a re-feeding protocol with fermented wheat *Hamoum* based-diet (FWH) had a beneficial effect on the recovery of weight growth as well restoration of intestinal organs morphometry and production of short-chain fatty acids (SCFA) in young malnourished rats during the weaning period. Male Wistar rats (*n* = 36) aged 4-weeks and weighing 66 ± 0.17g were divided into five groups. The first group constitute the control group (C) subjected to an experimental balanced diet with 20% casein; the second is the malnourished group (Mal) received a protein-deficient diet (2% casein) for 28 days; The other three groups constitute the malnourished and refed groups, they were refed for 28 days with FWH (Mal/FWH), non-fermented wheat-based diet (Mal/NFW) and control diet (Mal/C). We evaluated the weight growth of rats, the intestinal organs morphometry (small bowel, cecum and colon), the pH level and SCFA content. The results obtained show that the refeeding with FWH was sufficient to induce a recovery in the weight growth and the intestinal morphometry. It also contributed to the enhancement of the pH levels in the intestinal lumen and increased SCFA content in particular acetic acid at the ileal and colic level compared to Mal group. This recovery was strongly observed in Mal/FWH than for Mal/NFW and Mal/C groups. These findings indicated that FWH based diet could effectively improve the physiological functioning of the intestinal tract after weaning in situations of protein malnutrition.

Keywords: Protein malnutrition, FWH, Intestinal morphometry, SCFA.

INTRODUCTION

Poor intake of nutrients by an organism may result in an active pathological condition known as malnutrition, with different degrees and manifestations. Malnutrition includes both the deficiency and the imbalance of energy, protein and other nutrients (Corware et al., 2014; Jeejeebhoy and Duerksen, 2018). It is a leading cause worldwide of mortality among children less than 5 years old (Rodriguez et al., 2011; Bhutta and Salam, 2012). Protein malnutrition can cause undesirable effects that affect the proper functioning of organs. Therefore, metabolic and morphologic changes which occur in every organ are different and deserve specific studies so that the mechanisms involved in this phenomenon can be understood (Franco et al., 2010). For children, the development, the structure and the functioning

of the intestinal tract can be disrupted in protein malnutrition situations. In this case, the balance and regulation of the intestinal microbiota can be affected with a decrease in the number of *lactobacilli* and strict anaerobes (Nieto et al., 2007; Million et al., 2017). However, some studies have shown that a diet, rich in lactic fermentation ingredients resulting in a relatively high synthesis of SCFA, has a positive effect on the intestinal mucosa architecture (Scholten et al., 1999). Bousbahi et al. (2018) reported that fermented wheat "*Hamoum*" supplementation acts positively on the modulation of the gut microbiota. It protects from bacterial translocation and intestinal damages after protein malnutrition phase. For the purpose of this study, we chose durum wheat (*Triticum durum*) of *"Hamoum"* fermented variety, historically considered as a food with medicinal properties in the prevention and treatment of many intestinal physiopathological complications. Through the natural underground storage system, fermented wheat undergoes a fermentation process in a biotope rich in natural nutrients. The natural fermentation of wheat is regulated through the action of probiotic bacteria and yeasts. Recent studies have shown that fermented wheat contains a bacterial flora rich in lactic acid bacteria such as: *Lactobacillus plantarum, Lactobacillus pentossus, Lactobacillus brevis, Lactobacillus Lactis, Lactococcus Lactis subsp cremoris, pediococcus acidilactici, pediococcus pentosueus, Streptococcus Bovis, streptococcus thermophilus* and *Lactococcus raffinolactis* (Benakriche et al., 2016). The objective of this study was to evaluate the effects of an experimental diet, based on fermented wheat *Hamoum* rich in lactic acid bacteria, on the restoration of the morphometry of intestinal organs and the evaluation of the SCFA content in young Wistar rats subjected to protein malnutrition during the weaning period.

MATERIALS AND METHODS

Animals and diets

The general guidelines on the use of living animals in scientific investigation (Council of European Communities, 1987) were followed, and the protocol and use of rats were approved by the institutional committee on animal care and use. Thirty-six young male Wistar rats at weaning period purchased from Pasteur Institute of Algiers were used. Rats were aged 4 weeks at the start of the experiment and weighing 66 ± 0.176 g. During a 6-days adaptation period, they were submitted to a balanced experimental control diet with a protein contribution of 20% casein + 0.3% methionine, methionine was added because it is the limiting amino acid of casein (Bertrand et al., 1992). Twelve rats continued to receive the control diet until the end of the experiment d56 (C), the rests 24 rats were submitted to a low-protein diet with 2% casein + 0.3% methionine, for 28 days from d0 to d28, it's the malnourished group (Mal). After the protein malnutrition phase (PM), rats were distributed into 3 groups of 6 rats each. They were refed for 28 days from d28 to d56 with 3 different diets; control diet with 20% casein + 0.3% methionine (Mal/C), fermented wheat diet *Hamoum* (Mal/FWH), and non-fermented wheat diet (Mal/NFW). Each diet is complemented by a blend rich in minerals and vitamins. The composition of the diets is presented in Tables 1 and 2 (Bertrand et al., 1992). The diets containing casein are semi-synthetic and performed in the Laboratory of Physiology, Nutrition and Food Safety (Department of Biology, University of Oran1, Algeria). The FWH was collected from a rural Mediterranean region of Mostaganem city in west Algeria (February 2015), our samples were taken under aseptic and hygienic conditions in sterile bags to avoid any contamination that may affect the endogenous bacterial flora. The sample was taken from the peripheral part of the "Matmora" in contact with the ground. The sample was stored in the dark in a food package at 4°C until needed. The method of storage of wheat in Matmora was described by Benakriche et al., (2016).

The animals were placed individually in conventional cages at the animal house of the Laboratory of Physiology, Nutrition and Food Security (Department of Biology, University of Oran1, Algeria) under conventional breeding conditions with a normal circadian cycle, a constant temperature at 22 °C and humidity of about 60%. The daily intake of the different diets is 25 g per rat. Animals in all groups were weighed daily during the 56 days of the experimental protocol to ensure weight kinetics.

The experiments described in this study comply with the current Algerian legislation covering the protection of animals.

Table 1: Composition of the experimental diets in weighted percentages (Bertrand et al., 1992)

	Control[a] (C)	Deficient diet (Mal)
	%	%
Casein[b]	20	2
Starch[c]	59	75.6
Saccharose[d]	5	6.4
Oil [e]	5	5
Cellulose[b]	5	5
Vitamin supplement[f]	2	2
Mineral supplement[g]	4	4
L-Methionine[b]	0.3	0.3
Lipids (%)	5	5
Proteins (%)	19.69	1.9
Fibres (%)	5	5
Carbohydrate (%)	70.11	87.1

C; Control; Experimental isocaloric diet (20% casein), **Mal**; Malnourished; Protein deficient diet (2% casein). [a]The energy value of the control diet is 15.92 (MJ/Kg of diet). The diets were given in powder form; [b]Prolabo (Paris, France); [c]ONAB (Sidi Bel Abbes, Algeria); [d]Enasucre (SfiseF, Algeria); [e]Sunflower oil, Cevital (Bejaia, Algria); [f]UAR 200 (villemoisson, 91360, Epinary, France); Composition of the vitamin supplement (mg/kg): retinol, 12; inositol, 300; cyanocobalamin, 0.1; ascorbic acid, 1600; dl-αtocopherol, 340; menadion, 80 ; nicotinic acid, 200; para-aminobenzoic acid, 100; folic acid, 10; biotin, 0.6; [g]URA 205 B (villmoisson, 91360, Epinary/Orge, France). Composition of the mineral supplement (g/kg diet): Ca, 4; K, 2.4; Mg, 0.4; Fe, 0.12; elements (tracks): Mn, 0.032; Cu, 0.005; Zn, 0.018; Co, 0.00004; I, 0.00002, completed at 40000 with cellulose.

Table 2: Composition of FWH and NFW diets in weighted percentages

	FWH	NFW
	%	%
Casein[a]	10.12	8.92
Fermented wheat Hamoum[b]	79.99	-
Non- fermented wheat[b]	-	76.81
Saccharose[c]	-	5.2
Oil[d]	2.19	3.81
Cellulose[a]	4.87	4.1
Vitamin supplement[e]	2	2
Mineral supplement[f]	0.81	1.5
Lipids (%)	2.81	1.19
Proteins (%)	11.90	13.1
Fibres (%)	0.13	0.9
Carbohydrate (%)	69.31	75.54
Mineral salts (%)	3.19	2.5
Humidity (%)	11.67	6.73

FWH; Fermented wheat *Hamoum*, **NFW**; Non-fermented wheat.The diets were given in powder form; [a]Prolabo (Paris, France); [b]Negmaria, Mostaganem, Algeria; [c]Enasucre (SfiseF, Algeria); [d]Sunflower oil, Cevital (Bejaia, Algria); [e]UAR 200 (villemoisson, 91360, Epinary, France); Composition of the vitamin supplement (mg/kg): retinol, 12; inositol, 300; cyanocobalamin, 0.1; ascorbic acid, 1600; dl-αtocopherol, 340; menadion, 80; nicotinic acid, 200; para-aminobenzoic acid, 100; folic acid, 10; biotin,0.6; [f]URA 205 B (villmoisson, 91360, Epinary/Orge, France); Composition of the mineral supplement (g/kg diet): Ca, 4; K, 2.4; Mg, 0.4; Fe, 0.12; elements (tracks): Mn, 0.032; Cu, 0.005; Zn, 0.018; Co, 0.00004; I, 0.00002, completed at 40000 with cellulose.

Preparation of tissues

On the other days of dissection, animals were fed individually. For every rat, dissection started exactly 1.5 h after feeding. Rats in the different experimental lots were anaesthetized with pentobarbital sodium intraperitoneally (6 mg per 100 g body weight).

The entire small bowel, cecum and colon were dissected, freed from the mesentery, and weighted after the contents were gently removed. The segment from the pylorus to the ligament of Treitz was considered as the duodenum and the rest of the small intestine was divided into two equal segments, the proximal segment was considered the jejunum and the distal segment as ileum. After flushing with phosphate buffered saline (PBS 10%) solution, the length of the small intestine (duodenum, jejunum and ileum) and colon were measured using a graduated ruler. The pH of the separate parts of the intestinal tracts was measured immediately after dissection, using a pH meter by inserting a glass electrode directly in the opening made in the organs with digesta and the content of each segment was weighed and divided into samples stored at – 80 °C for short chain fatty acids analysis.

Short-chain fatty acids (SCFAs) analysis

The analysis of SCFAs was carried out at the Microbiology Research Unit for Food and Health (MICALIS) INRAE Jouy-en-Josas, France, using the method described by Lan et al. (2007). Samples were water extracted and proteins were precipitated with phosphotungstic acid. A volume of 0.1 ml supernatant fraction was analysed for SCFA on a gas–liquid chromatography (Autosystem XL; Perkin Elmer, Saint-Quentinen-Yvelines, France) equipped with a split-splitless injector, a flame-ionisation detector and a capillary column (15 m X 0.53 mm, 0.5 mm) impregnated with SP 1000 (FSCAP Nukol; Supelco, Saint-Quentin-Fallavier, France). Carrier gas (He) flow rate was 10 ml/min and inlet, column and detector temperatures were 175, 100 & 280 degrees, respectively. 2-Ethylbutyrate was used as the internal standard (Rabot et al., 2000). Samples were analysed in duplicate. Data were collected and peaks integrated using the Turbochrom v.6 software (Perkin Elmer, Courtaboeuf, France).

Statistical analysis

All data were expressed as mean values with their standard errors (SE). Independent Student's t-test was used to compare continuous variables between two groups in each phase. Differences between means were tested for significance using one-way ANOVA. P values less than 0.05 ($p < 0.05$) were considered significant.

RESULTS

Growth performance of rats

The results obtained showed that protein malnutrition induced a very significant loss in the total body weight of rats compared to controls ($p < 0.001$) (Fig.1). This suggests the deficient effect of the hypo-protein diet (2% casein) for 28 days. Furthermore, the results of the refeeding phase during 28-day showed a significant weight gain in Mal group, this period does not perfectly correct the body growth of malnourished rats compared to controls (Fig.1). However, the refeeding with FWH diet (Mal/FWH) provided satisfactory results and seems to improve body weight recovery ($p < 0.001$), this recovery was significantly more important compared to Mal/NFW and Mal/C groups ($p < 0.01$).

Morpho-metric characteristics of the intestinal tract

The morpho-metric analysis data of the small intestine segments, cecum and colon are presented in Table 3. The results indicated that protein malnutrition caused a very significant decrease of the total weight of the small intestine (duodenum, jejunum and ileum) compared to C group ($p < 0.001$). It has also induced a decrease of the cecum weight ($p < 0.001$). Likewise, a highly significant decrease of the colon weight was observed ($p < 0.0001$). In addition, our data have also reported that protein malnutrition induced a very significant decrease of the length of the small intestine and colon compared to C group ($p < 0.001$). Moreover, the refeeding with FWH diet increased significantly the total weight of the small intestine segments compared to Mal group ($p < 0.001$), as well, it induced a very significant increase in the weight of the cecum and colon ($p < 0.001$, $p < 0.0001$). In Mal/NFW group, no significant difference in the weight of the small bowel and cecum was observed. Except for a very significant increase of the colon weight compared to Mal group ($p < 0.001$). Furthermore, the results of Mal/NFW group showed significantly lower values of the weight of the intestinal segments and cecum than for Mal/FWH group ($p < 0.05$). In Mal/C group, a significant increase of the jejunum weight has been obtained compared to Mal group ($p < 0.05$), as well of the cecum and colon weight ($p < 0.01$, $p < 0.001$).

Table 3: Morphometric analysis in different groups; Weight of the small bowel (duodenum, jejunum and ileum), cecum and colon; Lengths of small bowel and colon

	C	MaL	MaL/FWH	MaL/NFW	MaL/C
Small bowel;					
Duodenum weight (g)	+ content 3.25 ± 0.12	2.32 ± 0.10 ##	3.10 ± 0.06**	2.77 ± 0.38 a	2.84 ± 0.09
	- content 2.38 ± 0.13	1.42 ± 0.09 ##	2.28 ± 0.11**	1.69 ± 0.04 a	1.87 ± 0.08 a
Jejunum weight (g)	+ content 3.43 ± 0.21	2.39 ± 0.30 ##	3.29 ± 0.18**	2.89 ± 0.09 a	2.93 ± 0.15 a
	- content 2.98 ± 0.15	1.78 ± 0.05 ##	2.76 ± 0.16**	1.93 ± 0.10 a	2.13 ± 0.19*,a
Ileum weight (g)	+ content 3.67 ± 0.27	2.50 ± 0.11 ##	3.51 ± 0.15**	2.71 ± 0.31 a	2.90 ± 0.19 a
	- content 3.12 ± 0.06	2.13 ± 0.13 ##	2.95 ± 0.11**	2.16 ± 0.16 a	2.27 ± 0.08 a
Cecum weight (g)	+ content 3.27 ± 0.20	1.96 ± 0.17 ##	3.01 ± 0.23**	2.19 ± 0.26 a	2.72 ± 0.34*
	- content 2.52 ± 0.23	1.43 ± 0.20 ##	2.20 ± 0.11*	1.82 ± 0.38	2.02 ± 0.33*
Colon weight (g)	+ content 2.46 ± 0.24	1.03 ± 0.08 ###	2.28 ± 0.16***	1.89 ± 0.15**	2.07 ± 0.06**
	- content 1.78 ± 0.10	0.86 ± 0.04 ###	1.69 ± 0.07***	1.43 ± 0.13**	1.56 ± 0.18 **
Small bowel; Duodenum length (cm)	28.30 ± 0.29	20.08 ± 0.46 ##	26.07 ± 0.70**	22.20 ± 0.60 a	24.56 ± 0.43*
Jejunum length (cm)	33.70 ± 0.73	24.30 ± 0.77 ##	30.20 ± 0.55**	26.68 ± 0.80*,a	28.50 ± 0.63*
Ileum length (cm)	32.30 ± 0.12	23.40 ± 0.36 ##	31.48 ± 0.38**	27.15 ± 0.16*,a	29.20 ± 0.24**
Colon length (cm)	12.20 ± 0.12	8.10 ± 0.36 ###	11.84 ± 0.33**	9.00 ± 0.16*,a	9.80 ± 0.24*,a

The values reported are Means ± Standard Errors; N = 6 rats per group; + content: with content; - content: without content; ###$p < 0.0001$ ##$p < 0.001$ Vs C, ***$p < 0.0001$ **$p < 0.001$ *$p < 0.01$ *$p < 0.05$ Vs Mal, [a]$p < 0.05$ Vs Mal/FWH. #*[a]Mean value was significantly different from those of the other groups ($p < 0.05$); statistical analysis was performed using one-way ANOVA and Student test t.

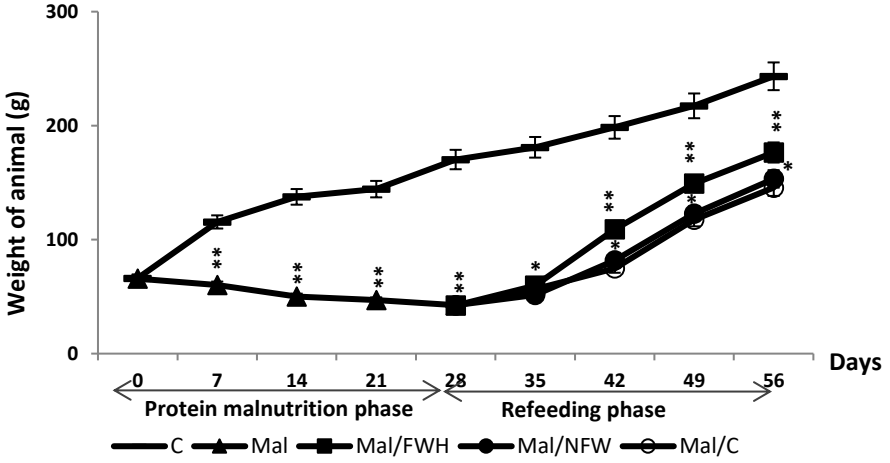

Figure 1: Body weight of control, malnourished and refeeding groups with FWH, NFW, and control diet during 56 days

The values reported are Means ± Standard Errors; N = 6 rats per group; Protein malnutrition phase: from 0 to 28 days; Refeeding phase: from 0 to 56 days; C: Control; receiving the experimental isocaloric control diet, Mal: Malnourished; receiving protein deficient diet 2% **$p < 0.001$ Vs C, Mal/FWH; Malnourished refed with fermented wheat Hamoum **$p < 0.001$ *$p < 0.01$ Vs Mal, Mal/NFW: Malnourished refed with non-fermented wheat *$p < 0.01$ Vs Mal; Mal/FWH, Mal/C: Malnourished refed with control diet *$p < 0.01$ Vs Mal. *Mean value was significantly different from those of the other groups ($p < 0.05$); statistical analysis was performed using one-way ANOVA and Student test t.

In contrast, the improvement of the intestinal organs weight in Mal/C group remaining lower than for Mal/FWH group ($p < 0.05$). In addition, the results of the refeeding period showed that the administration of FWH-based diet (Mal/FWH) promoted the restoration of the intestinal segments length, particularly of the ileum with a very significant difference compared with Mal group ($p < 0.001$). The FWH diet has also contributed to the restoration of the colon length ($p < 0.001$). In group refed with NFW (Mal/NFW), data showed a significant increase of the intestinal segments length, mainly of the ileum compared to Mal group ($p < 0.01$), furthermore, a slight increase of the colon length was observed ($p < 0.01$). This increase has been relatively lower compared with Mal/FWH group ($p < 0.05$). Likewise, the same results were observed in Mal/C group. Except that the values obtained were lower than for Mal/FWH group ($p < 0.05$). The FWH diet presented very satisfactory results in the recovery of the intestinal organs morphometry compared to the other diet namely NFW and control diet.

Measurements of pH in the intestinal Tract

The pH was measured in the intestinal lumen of duodenum, jejunum and ileum as well in the cecal cavity and colonic lumen (Table 4). The results showed a higher pH particularly in ileum of Mal group, compared to C ($p < 0.001$). However, a slight increase of pH was observed in the cecal cavity ($p < 0.01$), as well in colon ($p < 0.01$). In group refed with FWH (Mal/FWH), the pH values decreased, especially in duodenum ($p < 0.001$) and jejunum ($p < 0.001$), a moderate decrease of pH was noted in ileum ($p < 0.01$), as well in the cecal cavity ($p < 0.01$) and colonic lumen ($p < 0.01$) compared to Mal group. Indeed, in Mal/NFW group, a slight decrease of pH was noted, mainly in jejunum ($p < 0.01$), which was also noted in cecum ($p < 0.01$). The same data were obtained in Mal/C group. Whereas, the decrease of pH values in the intestinal and colonic lumen of Mal/NFW and Mal/C groups was less significant than for Mal/FWH group ($p < 0.01$).

Production of short-chain fatty acids (SCFAs)

In the different segments of the small intestine (duodenum, jejunum and ileum) and colon, volatile fatty acids content (acetic, butyric and propionic acid) was measured (Fig.2a, b, c, and d). The results showed that protein malnutrition (Mal) decreased the total SCFA content in all intestinal compartments, particularly in duodenum compared to C group (0.75 ± 0.12 µmole/g *Vs* 3.34 ± 0.29 µmole/g; $p < 0.001$) (Fig.2a). Furthermore, a very significant increase of total SCFA levels was observed in FWH group (Mal/FWH) compared to Mal group, mainly in duodenum ($p < 0.001$) and ileum ($p < 0.001$) as well in colon ($p < 0.001$) (Fig.2a, c, d). In Mal/NFW group, total SCFA content increased slightly in ileum compared to Mal group ($p < 0.01$) (Fig.2c), whereas, the total content of SCFA in jejunum was not affected ($p < 0.05$) (Fig.2b). In Mal/C group, data also showed a slight increase of total SCFA content in ileum compared to Mal group ($p < 0.01$) which was also obtained in colon ($p < 0.05$) (Fig.2c, d).

Table 4: pH levels in the small bowel, cecum and colon of malnourished and refed groups

	C	MaL	Mal/FWH	Mal/NFW	Mal/C
Small bowel ;					
Duodenum	6.11 ± 0.13	8.07 ± 0.22 ##	6.72 ± 0.07**	7.90 ± 0.25 a	7.72 ± 0.30 a
Jejunum	6.42 ± 0.29	8.15 ± 0.26 ##	6.89 ± 0.31**	7.64 ± 0.22*,a	7.49 ± 0.20*,a
Ileum	6.96 ± 0.31	9.95 ± 0.12 ##	7.21 ± 0.19*	8.66 ± 0.20 a	8.36 ± 0.04 a
Cecum	7.10 ± 0.18	8.91 ± 0.12 #	7.48 ± 0.06*	7.97 ± 0.07*	7.79 ± 0.10*
Colon	7.24 ± 0.14	8.84 ± 0.11#	7.64 ± 0.19*	8.57 ± 0.09 a	8.14 ± 0.08*,a

The values reported are Means ± Standard Errors; N = 6 rats per group. #$p < 0.01$ #$p < 0.001$ Vs C, *$p < 0.01$ **$p < 0.001$ Vs Mal, a$p < 0.01$ Vs Mal/FWH. #*aMean value was significantly different from those of the other groups ($p < 0.05$); statistical analysis was performed using one-way ANOVA and Student test t.

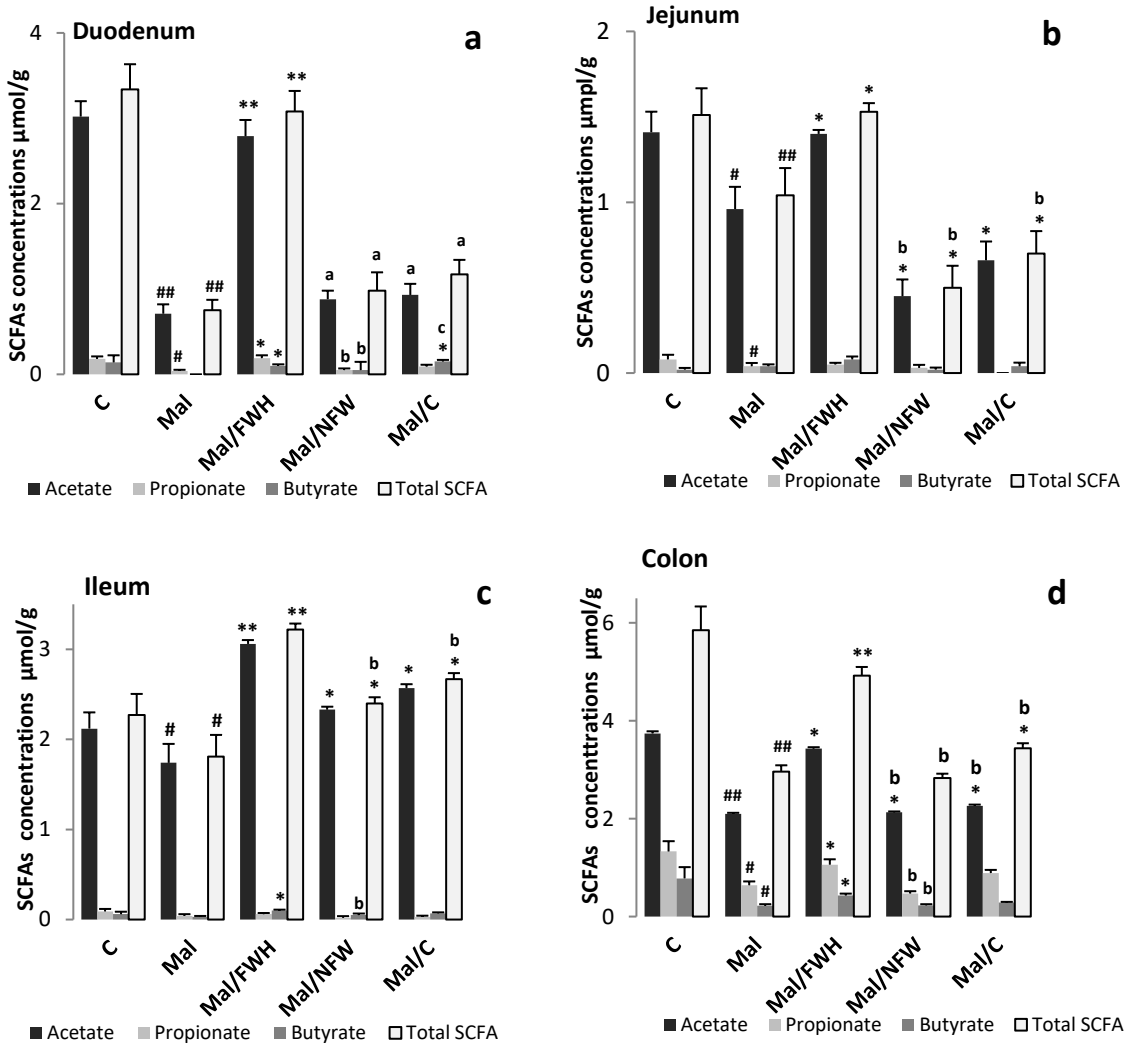

Figure 2: Short-chain fatty acids (SCFAs) concentrations in duodenum (a), jejunum (b), ileum (c) and colon (d) content of malnourished and refeeding groups

Results are expressed as µmole/g of intestinal content and are average values ± Standard errors; $^{\#}p < 0.05$ $^{\#}p < 0.01$ $^{\#\#}p < 0.001$ Vs C, $^{*}p < 0.05$ $^{*}p < 0.01$ $^{**}p < 0.001$ Vs Mal, $^{a}p < 0.001$ $^{b}p < 0.05$ Vs Mal/FWH, $^{c}p < 0.05$ Vs Mal/NFW. $^{\#*abc}$Mean value was significantly different from those of the other groups ($p < 0.05$); statistical analysis was performed using one-way ANOVA and Student test t.

In contrast, the total SCFA content in all intestinal compartments was significantly decreased in Mal/NFW and Mal/C group compared to Mal/FWH group ($p < 0.05$; $p < 0.001$). In addition, results showed that acetic acid content was higher in Mal/FWH group, mainly in duodenum (2.79 ± 0.19 µmole/g *Vs* 0.71 ± 0.11 µmole/g; $p < 0.001$) (Fig.2a) and ileum (3.06 ± 0.04 µmole/g *Vs* 1.74 ± 0.21 µmole/g; $p < 0.001$) (Fig.2c). In Mal/NFW group, we noted a more or less high content of acetic acid in ileum compared to Mal group ($p < 0.05$), while in jejunum, the acetic acid content was reduced ($p < 0.01$) (Fig.2b, c). However, a slight increase of the acetic acid level was observed in Mal/C group, especially in ileum ($p < 0.05$). The acetic acid level was significantly lower in duodenum ($p < 0.001$), jejunum ($p < 0.05$), and colon ($p < 0.05$) in Mal/NFW and Mal/C group than for Mal/FWH group (Fig.2a, b, d). Furthermore, the propenoic acid content was increased in Mal/FWH group, particularly in colon ($p < 0.01$) as well, the butyric

acid content was higher in colon compared to Mal group ($p < 0.05$) (Fig.2d). In Mal/NFW group, no significant difference was observed in propionic and butyric acid content in all intestinal compartments. In Mal/C group, butyric acid level was lower in duodenum ($p < 0.05$) and propionic acid level was reduced in colon, except that no significant difference was observed (Fig.2a, d).

DISCUSSION

The results obtained show that protein malnutrition induced a significant loss of the animal's total weight. The experimental data are in agreement with a number of studies that have confirmed the impact of protein deficient diet on weight growth (Crenn, 2001; Gourine et al., 2018). Fontenla et al., (2007) showed that protein malnutrition causes weight loss over a period of 28 days. The administration of diet with 4% protein to weanling rats resulted in a significant loss of body weight compared to controls with normal growth (Franco et al., 2010). During the refeeding phase we have observed a significant increase of body weight for 28 days. This increase was significantly more important in group refeeding with FWH than with the other diets, namely NFW and control diet. However, the refeeding period does not perfectly correct the body growth of malnourished rats compared to controls. In pediatric nutrition, Sun et al., (2013) reported that the body weight of children subjected to low-protein diet for 48 days followed by 63 days of refeeding was not comparable to that of the control children. Under similar experimental conditions, Mohamed-Benkada et al., (1993) demonstrated that total body weight recovery has been obtained only after 3 months of feeding a balanced diet. To date, limited data are available on effects of fermented diets on the weight gowth recovery of malnourished children.

At the morphometric scale, during the protein malnutrition phase we observed a significant decrease in the weight and length of the intestinal organs (small intestine, colon and cecum). These data are consistent with the study developed by Benackriche et al., (2014) which showed that protein malnutrition induces a decrease in intestinal morphometry of wistar rats. Natali et al., (2005) also demonstrated that feeding a deficient diet with 8% protein to rats causes atrophy of the duodenal and ileal wall. Moreover, the refeeding with FWH diet presented very satisfactory results in the recovery of the intestinal organs morphometry compared to NFW and control diet. Dock et al., (2004) recorded a structural enhancement in intestinal morphometry of malnourished rats after administration of *Streptococcus thermophilus* and *Lactobacillus helveticus*. Similarly, the administration of oligosaccharide probiotics to malnourished rats improved the restructuring of intestinal morphometry induced by protein malnutrition (Benackriche et al., 2014). The FWH used in our experimental diet contains an endogenous bacterial flora endowed with a high richness in lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus pentossus, Lactobacillus brevis, Lactobacillus Lactis, Lactococcus Lactis subsp cremoris, pediococcus acidilactici, pediococcus pentosueus, Streptococcus Bovis, streptococcus thermophilus, Lactococcus raffinolactis*) (Benackriche et al., 2016). This flora of lactic bacteria undoubtedly exerts a protective and beneficial regulatory effect on the intestinal microbiota, in this context on the intestinal barrier of the different intestinal segments and particularly the colonic segments. Therefore, the restructuring of intestinal atrophy caused by protein malnutrition is being treated by the FWH diet.

Our results reveal that protein malnutrition induces a very significant increase of pH in the small intestine, which was slightly observed in the cecum and colon. It also involves a decrease of the total SCFA levels. Although, refeeding with FWH for 28 days resulted in a decrease of pH, especially in duodenum and jejunum lumen, whereas no significant differences in the pH values were found between the cecum and colon. Refeeding with FWH increased the production of SCFA, particularly acetic acid, mainly in the ileum and colon compared to NFW and control diet. This improvement is probably due to the metabolism of FWH lactic acid bacteria. To date, limited data are available on effects of fermented diets on pH value and SCFA levels in the intestinal and colonic lumen of weanling rats. It has been noted that some probiotic bacteria decrease intra-luminal pH and increase the production of volatile fatty acids (Kruis et al., 1997) and could even change in a reproducible way certain endogenous enzymatic activities (Marteau et al., 1993). FWH is characterized by a low pH and very high acidity compared to NFW (Gourchala et al., 2014). During fermentation, the pH decreases with a simultaneous increase in acidity. Organic and lactic acids act in synergy in bacterial metabolic activity (Kohajdova and Karovicova, 2007). SCFAs and mainly acetic acid are the metabolic substrate of lactic acid bacteria in the endogenous flora of FWH during the refeeding phase, this

complementary production of SCFA is more significant than that of NFW (control group). Acetic acid was the main SCFA produced, followed by propionic and butyric acid. Englyst et al. (1987) revealed that the proportions of SCFA produced vary according to the nature of colonic bacterial flora, intestinal transit time and available substrates. However, fermentation due to the action of bacteria and enzymes and probably yeasts seems to be the cause of changes in biochemical composition and nutritional value of wheat stored in granary type of Matmora in the ground (Gourchala et al., 2014). Indeed, the fermentation of wheat during the storage period affects the peripheral layer closely in contact with the land in the ground. Ground flora and fauna contribute to the emergence of lactic acid bacteria during the wheat fermentation process under very specific physical and chemical conditions (Benakriche et al., 2016).

CONCLUSION

The present study demonstrated that refeeding with FWH diet provides satisfactory results compared to NFW and control diet. FWH contributes to improving the pH in the intestinal organs and increasing the production of SCFAs, particularly acetic acid. However, short-chain fatty acids derived directly from the natural fermentation of FWH, especially in colonic microbiota, can prevent the undesirable disorders in the intestinal architecture. However, more knowledge is needed to understand more the different mechanisms involved. This study indicates that natural FWH diet, a food ingredient rich in probiotic lactic acid bacteria and yeasts, could be considered as a nutritional and dietary adjuvant for preventive or curative purposes after weaning in situations of protein malnutrition.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This work was supported by PNR program provided by the Algerian ministry of higher education.

AUTHOR CONTRIBUTIONS

DY designed and performed the experiments, data analysis and also wrote the manuscript. BMB and PG performed experiments, tissue collection and reviewed the manuscript. KO and PP reviewed manuscript. All authors read and approved the final version.

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