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# Effect of a Guar Meal Protein Concentrate in Replacement of Conventional Feedstuffs on Productive Performances and Gut Health of Rainbow Trout (*Oncorhynchus mykiss*)

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**Abstract:** A trial was performed to investigate the effect of a proprietary guar protein concentrate, as a partial replacement of conventional protein sources, on the productive performances of rainbow trout through the growing phase. A total of 2700 rainbow trout were reared in 3 $m^3$, 12 concrete tanks for 90 days. Three diets were formulated to contain a protein level of 43% and a lipid content of 25.3% by replacing 0% (CD), 5% (D5), and 15% (D15) of conventional protein sources with guar protein concentrate. The final mean weight was similar between D5 and CD, significantly higher than D15. The same trend was observed in weight gain and specific growth rate. The feed conversion rate had the most favourable performances in D5 and CD. Feed palatability was higher in CD and D5 than in D15. Histological intestinal score showed significant differences ($p < 0.05$) between the groups with the highest values in CD and the lowest in D15. Significant differences were observed for goblet cell hyperplasia with higher values in the CD group. Based on this trial, the 5% guar protein concentrate inclusion gave the best zootechnical results.

**Keywords:** guar protein concentrate; rainbow trout; growing phase; productive performances; histology; gut health

**Key Contribution:** The present study confirmed that the tested proprietary guar protein concentrate can be used in the feed formulation for rainbow trout in the growing phase. Including this feedstuff was satisfactory at a moderate rate, mainly 5% of the main protein sources.

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**Citation:** Roncarati, A.; Galosi, L.; Di Cerbo, A.; Quagliardi, M.; Marchetti, F.; Fiordelmondo, E.; Mariotti, F.; Magi, G.E. Effect of a Guar Meal Protein Concentrate in Replacement of Conventional Feedstuffs on Productive Performances and Gut Health of Rainbow Trout (*Oncorhynchus mykiss*). *Fishes* **2024**, *9*, 295. https://doi.org/10.3390/fishes9080295

Academic Editor: Changan Wang

Received: 28 June 2024
Revised: 15 July 2024
Accepted: 16 July 2024
Published: 25 July 2024



## 1. Introduction

According to the most recent statistics referred to 2022, rainbow trout (*Oncorhynchus mykiss*) is widely farmed in Europe with 248,106 tonnes, mostly produced in Denmark (28,460 t), Italy (27,800 t), and France (19,619 t) [1]. From the perspective of this important coldwater fish species, genomic selection and sustainable diets are going to be searched and improved as the most targeted actions to support the sector [2–4]. At the same time, particular attention was devoted to maintain the good health status of fish [5]. The use of sustainable protein sources, rich in highly available essential amino acids, to produce fish feed in place of fish meal has important repercussions for all feed manufacturers and aquaculture operators. It is considered a very important practice to obtain a sustainable and responsible production process to reduce the withdrawal of marine resources and mitigate the environmental impact of farming practices. Fish farmers are going to search for feedstuffs with a high protein content that are able to supply high biological value amino acids throughout the year. Many protein sources can be obtained from vegetables. Thanks

to their high protein digestibility, soybean, rapeseed, wheat gluten, and corn gluten are among the most widely common plant protein concentrates used in aquafeeds [6].

Guar meal, deriving from the endosperm of an Indian cluster bean and identified as the galactomannan polysaccharide Guar gum (*Cyamopsis tetragonalobus*), seems to show interesting characteristics. It was initially cultivated in India and Pakistan for cattle feeding and is now re-evaluated as an additive in animal feeding due to its properties and organoleptic characteristics [7]. In recent years, guar meal has been submitted to processing systems and purification technology that can reduce saponin, tannin, phytates, and protease inhibitor concentrations that are considered to negatively affect the growth of salmonids [8]. This confirmed the capacity of guar to increase faecal stability in water, as observed by other authors studying its main properties [9]. That study showed the ability of this indigestible binder to reduce the breakdown of faeces, preventing the dispersion of nitrogen and phosphorus sources into the wastewater.

Recently, cultivating guar in the Mediterranean environment has also been considered to obtain degummed guar seeds favourably employed in animal feeding [10]. In chicken feeding, guar has been known as a potential protein source that can replace soybean meals satisfactorily [11]. In growing pigs, guar powder was added to the feed and did not negatively affect the feed efficiency, although lower average daily feed intake and weight gain were observed [12]. In aquafeeds, an inclusion of 20% guar gum provided a good protein source in the substitution of dietary soybean meal for Nile tilapia fingerlings [13], and affected the quality trait of the final product.

A few papers have investigated the effects of guar meal on rainbow trout's productive performances as an alternative protein source [8,14] and on faecal stability as a guar gum additive [15,16]. However, no studies have been performed on the effects of guar meal on gut health.

Based on these considerations, a trial was performed to investigate the possible use of a commercially available guar meal submitted to a proprietary protein concentration process. The guar meal was used not only as a binder to improve the cohesivity of the feed but also as an alternative protein source to conventional ones in the growing phase of rainbow trout; effects on zootechnical performances and histological gut status were evaluated.

## 2. Materials and Methods

### *2.1. Experimental Diets and Analysis*

The guar protein concentrate (MYCOPRIME®, Panghea spa, Milan, Italy), submitted to a proprietary protein concentration process, was obtained for free from the producer. The technology used to produce this ingredient aims to obtain a fermented guar meal suitable for coping with the high protein requirement levels in animal feeding. Before starting the trial, a sample of this guar protein concentrate was analysed regarding the proximate composition, main fatty acids content, and amino acid profile (Table 1).

A control diet (CD) was used as a growing feed characterised by a protein level of 43% and a lipid content of 25.3% and compared with two experimental feeds (D5; D15) formulated as isoprotein and isolipid feeds with CD. A partial replacement of fish meal, chicken meal, and soybean meal at 5% (D5) and 15% (D15) with guar protein concentrate was considered; in terms of percentage, the soybean meal was the most replaced with the guar concentrate.

All the feed analyses (Table 2) were performed according to the international procedure outlined by the Association of Official Analytical Chemists (AOAC 1990). The essential amino acids in the three feeds were determined by acid hydrolysis (6 N HCl for 24 h at 110 °C), which was followed by ion exchange chromatography utilising an amino acid analyser (L-8800 Auto-analyzer, Hitachi, Tokyo, Japan). The total lipid content was determined using the procedure described by Folch et al. [17]. The fatty acid profile was converted to methyl esters following the method described by Christopherson and Glass [18]. The separation of fatty acids was carried out using a GC 3800 gas chromatograph (Varian Strumentazione, Cernusco sul Naviglio, Italy) with a WP-4 Shimadzu integration

system (Shimadzu Corporation, Tokyo, Japan), which was equipped with a Supelco SPTM-2340 capillary column (30 m × 0.25 mm internal diameter; 0.25 µm film thickness; Supelco, Bellefonte, PA, USA) and a flame ionisation detector.

**Table 1.** Proximate composition (%), fatty acid (%), and amino acid profile (g/100 g) of guar protein concentrate used in the trial.

| **Proximate Composition (% as Fed)** | |
|---|---|
| Moisture | 6.0 |
| Crude protein | 66.0 |
| Crude lipid | 10.0 |
| Fibre | 6.0 |
| Ash | 4.0 |
| **Fatty acid content (% total FA)** | |
| Linolenic acid | 39.34 |
| Oleic acid | 29.98 |
| Palmitic acid | 15.34 |
| Stearic acid | 6.93 |
| Linolenic acid | 2.95 |
| **Amino acid profile (g/100 g)** | |
| Aspartic acid | 6.51 |
| Glutamic acid | 13.1 |
| Alanine | 2.30 |
| Arginine | 9.23 |
| Phenylalanine | 2.61 |
| Glycine | 3.34 |
| Isoleucine | 1.92 |
| Histidine | 1.71 |
| Leucine | 3.56 |
| Lysine | 3.30 |
| Proline | 2.35 |
| Tyrosine | 2.00 |
| Threonine | 1.94 |
| Cysteine | 0.75 |
| Valine | 2.22 |
| Tryptophan | 1.00 |
| Methionine | 0.70 |

**Table 2.** Formulation (g/kg) and proximate composition (% as fed) of CD, D5, and D15 diets.

| | **CD** | **D5** | **D15** |
|---|---|---|---|
| Fish meal | 16 | 15.5 | 14.5 |
| Chicken meal | 16.31 | 15.9 | 11.1 |
| Soybean meal | 13.99 | 11.9 | 7.6 |
| Guar protein concentrate | 0 | 5 | 15 |
| Porcine haemoglobin meal | 12.6 | 12.6 | 12.6 |
| Wheat meal | 12.53 | 12.53 | 12.53 |
| Wheat distillers | 3.2 | 3.2 | 3.2 |
| Wheat gluten | 2.5 | 2.5 | 2.5 |
| Soybean lecithin | 0.5 | 0.5 | 0.5 |
| Fish oil | 10 | 10 | 10 |
| Soybean oil | 8.5 | 8.5 | 8.6 |
| L-lysine | 0.3 | 0.3 | 0.3 |
| DL-methionine | 0.22 | 0.22 | 0.22 |
| Mineral–vitamin premix | 1 | 1 | 1 |
| Choline liquid 75% | 0.35 | 0.35 | 0.35 |

**Table 2.** *Cont.*

| | CD | D5 | D15 |
|---|---|---|---|
| **Proximate composition (%):** | | | |
| Moisture | 7.75 | 7.5 | 7.12 |
| Crude protein | 43.04 | 43.5 | 43.01 |
| Crude fat | 25.31 | 25.01 | 25.01 |
| Fibre | 1.4 | 1.5 | 2.01 |
| Ash | 8.66 | 8.7 | 7.74 |
| Gross energy (MJ/kg) | 22.83 | 22.83 | 22.71 |

CD, D5, and D15 were manufactured in one of the productive feed mill plants of the trout company, in one line of extruders where all the ingredients were mixed according to the target formulation. The temperature reached 110 °C for 5–20 s. Then, a vacuum coating was applied at 0.2 bar for 1 min. The feeds were 4.5 mm in size. After the coating, the three diets were stocked in buckets and maintained in an aerated room. Samples of each diet were taken for proximate composition analysis following the same analytical methods available to determine guar protein concentrate. The feeds were transported to the trout farm, and the growth trial was performed.

### *2.2. Growth Trial*

The experiment was performed in a hatchery of a rainbow trout company located in the north of Italy; at this farm, the water supply came from the adjacent river after treatment with a mechanical and biological filtration system.

A total of 2700 rainbow trout (mean body weight 50 ± 1.4 g) were randomly distributed among nine tanks (6 × 1 × 0.5 m) with 3 $m^3$ volume each. Each feed was administered to tanks in triplicate assigned to that specific treatment (CD, D5, D15). Fish were fed by hand twice daily (8 a.m. and 3 p.m.) until apparent visual satiety. The feed administration occurred in three feeding rounds, taking place at 15 min intervals until the complete stop of the feeding activity. The unconsumed feeds were removed and not included in feed daily administration. During the experiment, the main water physical–chemical parameters (temperature, dissolved oxygen, and pH) were recorded daily in every tank using portable electronic devices (YSI mod. 55 and 60, Yellow Springs, OH, USA). TAN (total ammonia nitrogen), $NO_2$-N (Nitrites), and $NO_3$-N (Nitrates) were analysed every week following APHA standard methods [19] using a spectrophotometer (HACH mod. DR 6000 UV–VIS, HACH Lange Gmbh, Düsseldorf, Germany). The water-cleaning activities of the different groups of tanks were always carried out by siphoning. Fish dead were removed and recorded daily.

At the end of the experiment (90 days), all the fish were weighed using an electronic scale (model WLC 20/A2, ±0.1 g, RADWAG, Radom, Poland), and their final length was recorded by an ichthyometer (Scubla Srl, Remanzacco, UD, Italy). The palatability of the feeds was calculated according to the formula: [(ingested feed/administered feed) × 100] based on the index reported in previous studies [20,21]. The condition factor (KI), weight gain (WG), specific growth rate (SGR), feed conversion rate (FCR), and survivor rate (SR) were calculated according to the following formulas [22]:

$$KI = (\text{fish weight}/\text{fish length}^3) \times 100;$$

$$WG\ (g) = (\text{final weight} - \text{initial weight});$$

$$SGR\ (\%/\text{day}) = \{\ln(\text{final weight}) - \ln(\text{initial weight})/\text{days}\} \times 100;$$

$$FCR = \text{feed delivered (g)}/\text{fish biomass gain (g)};$$

$$SR\ (\%) = \text{final number of fish}/\text{initial number of fish} \times 100$$

An economic comparison was reported based on the production cost of all feedstuffs per each diet essayed in the trial.

### *2.3. Histological Analysis*

At the end of the experiment, to investigate the hypothetical differences in the intestinal morphologies among the groups, the fish were slaughtered in an authorised slaughterhouse, and the proximal intestine of 15 rainbow trout per diet was sampled, immediately fixed in 10% buffered formalin, and then processed for histological examination [23]. Paraffin blocks were serially sectioned at 4 µm using a rotary microtome (Leica RM2235, Leica Microsystems, Wetzlar, Germany). Histological sections were stained with haematoxylin and eosin (HE) and evaluated under a light microscope by a blinded pathologist. An optical microscope (Olympus BX50, Olympus Italia, Segrate, Italy) was used to evaluate the samples. Every section was entirely analysed at low (10×) and medium (20×) magnification considering the following pathologic traits: goblet cell hyperplasia; inflammatory changes characterised by infiltration of macrophages, eosinophilic granular cells, and lymphocytes within lamina propria; the presence of IEL (intraepithelial lymphocytes) and the presence of steatosis in enterocytes. For each of these parameters, a score related to the severity level of modification has been assigned: 0 = not observed; 1 = mild and/or focal; 2 = moderate and/or multifocal; 3 = severe and/or diffuse.

Sections were also stained with Alcian Blue kit (Bio-Optica, Milan, Italy) to study goblet cells. For each sample, four microscopic fields were randomly selected using the above-mentioned optic microscope at 20× magnification, and pictures were captured with a camera (Olympus BX50F4, Tokyo, Japan) provided by Pylon Viewer software 6.2.4.9387 (Basler, Milan, Italy). A quantitative evaluation of goblet cells was performed using an open-source software ImageJ v. 2.9.0 (Fiji, USA version, plug in "Trainable Weka Segmentation"). The algorithm was trained to recognise Alcian blue-stained vacuoles, excluding areas >20 pixel2, as a blue-stained area would have been too small to be a real vacuole.

### *2.4. Statistical Data Analyses*

Growth performances were subjected to one-way analysis of variance (ANOVA) using SPSS 25 (IBM Corp. IBM SPSS, Armonk, NY, USA, v. 25.0, 2017) to check for differences in the productive performances and composition of rainbow trout fillet fed with different experimental diets. Means and standard deviations were calculated. Means were considered significant with a value of $p < 0.05$ and compared using the Student–Newman–Keuls (SNK) test. Histological data were analysed using GraphPad Prism 9 software (GraphPad Software Inc., La Jolla, CA, USA). Data are expressed as median values by using box and whisker plots. A Kruskal–Wallis test followed by a Dunn's multiple comparisons test was used to analyse the differences among the three groups for the overall histological score and each single histological parameter. A $p < 0.05$ was considered significant.

## 3. Results

### *3.1. Growth Trial*

The water chemical parameters showed a very similar trend in terms of temperature, dissolved oxygen, and pH during the entire trial. The nitrogen compounds showed significant differences in TAN, showing an increasing trend passing from CD tanks ($0.24 \pm 0.09$ mg/L) and D5 tanks ($0.22 \pm 0.06$ mg/L) to D15 ($0.44 \pm 0.01$ mg/L). Nitrites and Nitrates did not show notable differences (Table 3).

The productive parameters of rainbow trout receiving the three diets are reported in Table 4. The final mean weight was lower in D15 (−14.4%, $p < 0.05$) than that obtained in CD and D5 (199.9 g, on average). Feeds did not affect the final length (24 cm, on average), WG (140.3 g), and SGR (1.6%/day). FCR had the most favourable performances in CD and D5 (1.035, on average) with respect to D15 (+5.3%). KI was significantly higher (+24.2%) than that recorded in D5 and D15 (1.28, on average). SR was similar without notable

differences (97.8%, on average). Feed palatability resulted higher in CD and D5 diets (100%) with respect to D15 (−2%).

**Table 3.** The main physico-chemical parameters of water sampled in the three groups of tanks used for the trial. Different letters (a, b) on the same line show statistically significant differences ($p < 0.05$).

| | CD | D5 | D15 |
|---|---|---|---|
| Temperature °C | 13.5 ± 0.4 | 13.5 ± 0.6 | 13.5 ± 0.7 |
| Dissolved oxygen | 8.7 ± 0.5 | 8.7 ± 0.6 | 8.7 ± 0.4 |
| pH | 7.86 ± 0.4 | 7.82 ± 0.6 | 7.84 ± 0.5 |
| TAN | 0.24 ± 0.09 $^{b}$ | 0.22 ± 0.06 $^{b}$ | 0.44 ± 0.01 $^{a}$ |
| $NO_2$-N | 0.02 ± 0.002 | 0.02 ± 0.01 | 0.01 ± 0.001 |
| $NO_3$-N | 1.1 ± 0.4 | 1 ± 0.2 | 0.9 ± 0.1 |

**Table 4.** The productive performances of rainbow trout fed with different experimental diets (mean ± standard deviation). Different letters (a, b) on the same line show statistically significant differences ($p < 0.05$).

| Parameters | CD | D5 | D15 |
|---|---|---|---|
| Initial mean weight (g) | 50.0 ± 1.4 | 50.0 ± 1.4 | 50.0 ± 1.4 |
| Final mean weight (g) | 198.8 ± 3.8 $^{a}$ | 201.0 ± 3.7 $^{a}$ | 171.2 ± 5.1 $^{b}$ |
| Final mean length (cm) | 23.2 ± 3.5 | 25.1 ± 0.9 | 23.7 ± 1.1 |
| KI | 1.59 ± 0.11 $^{a}$ | 1.27 ± 0.08 $^{b}$ | 1.29 ± 0.1 $^{b}$ |
| WG (g) | 148.8 ± 26 | 151.0 ± 24 | 121.2 ± 18 |
| SGR (%/day) | 1.65 ± 0.18 | 1.68 ± 0.11 | 1.35 ± 0.30 |
| FCR | 1.03 ± 0.02 $^{b}$ | 1.04 ± 0.01 $^{b}$ | 1.09 ± 0.02 $^{a}$ |
| SR (%) | 97.33 ± 2.89 | 98.07 ± 1.29 | 98.00 ± 1.70 |
| Palatability | 100 ± 0.0 $^{a}$ | 100 ± 0.0 $^{a}$ | 98 ± 0.1 $^{b}$ |

Evaluating the cost of production, the 5% replacement showed the same convenience (0.924 EUR/kg) as the other experimental diet at 15% guar protein concentrate added (0.92 EUR/kg), and both were more affordable than the CD (0.94 EUR/kg).

### *3.2. Gut Histology*

The histological score showed significant differences among the three groups, with the highest values in the CD group and the lowest in the D15 group (Figure 1A). As concerns the single histological parameters, significant differences were observed only for goblet cell hyperplasia, with significant differences between the CD group and D15 group (Figure 1B). The goblet cell count evidenced statistical differences among the CD and D15 groups (Figure 2). A histology of the proximal intestine of the three groups is presented in Figure 3.

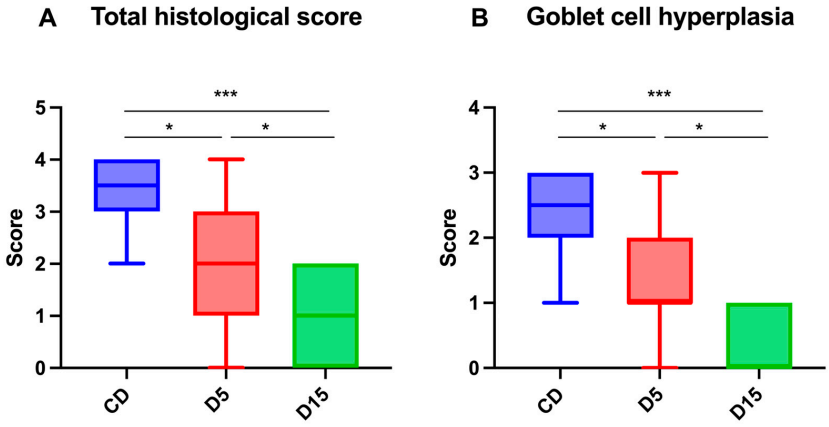


**Figure 1.** Histological analysis. Schematic representation of (**A**) the overall histological score in the CD group, D5 group, and D15 group; (**B**) goblet cell hyperplasia in the CD group, D5 group, and D15 group. * $p < 0.05$; *** $p < 0.001$.

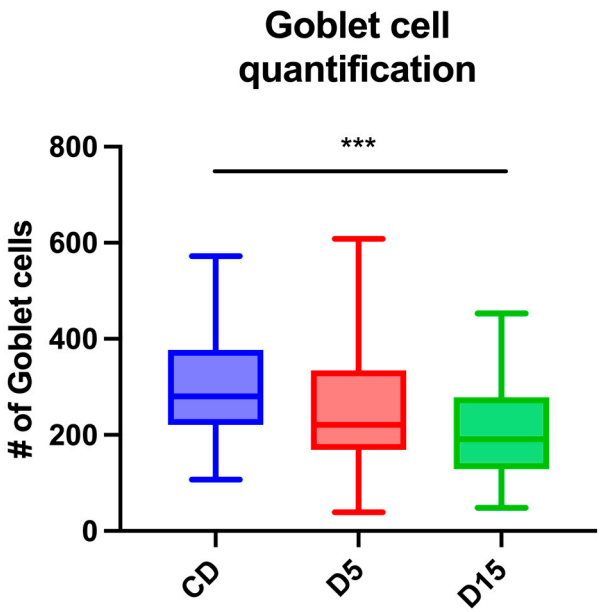


**Figure 2.** Goblet cell count in the proximal intestine. *** $p < 0.001$.

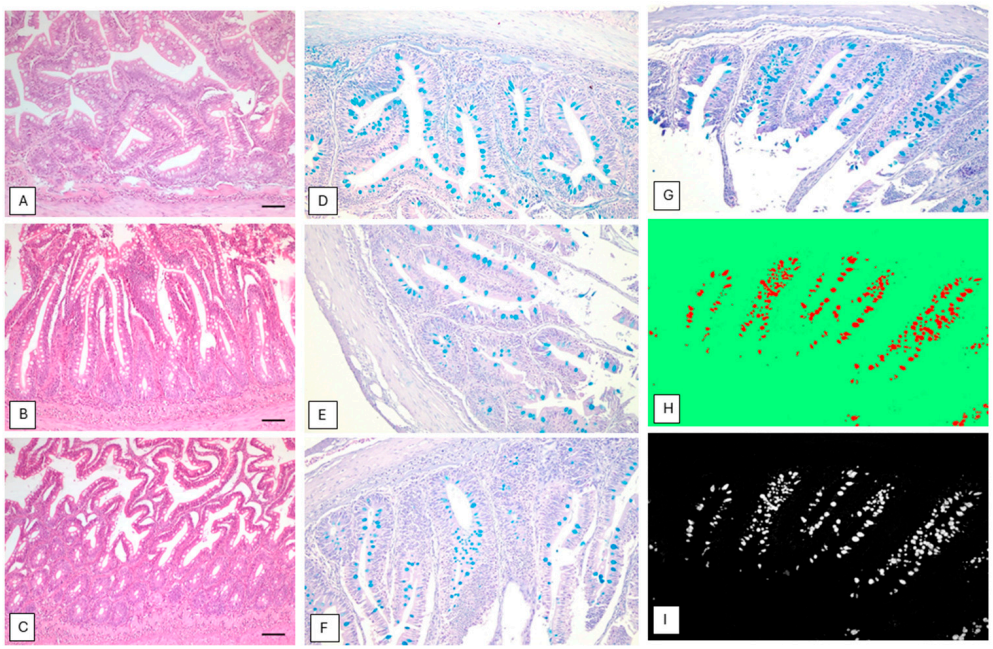


**Figure 3.** Histology of the proximal intestine of rainbow trout from the three different groups: control diet group (CD group), group fed with 5% of guar protein (D5 group), and group fed with 15% of guar protein (D15 group). (**A**): CD group, numerous, and hypertrophic goblet cells are diffusely present within crypts and villi enterocytes. (**B**): D5 group, intestinal epithelium with focal mild goblet cells hyperplasia. (**C**): D15 group, few goblet cells are visible within the mucosa epithelium. (HE, scale bar: 100 µm). (**D**): CD group, numerous Alcian-blue positive goblet cells within epithelium; (**E**,**F**): D5 and D15 group, scattered goblet cells containing acid mucins within the intestinal epithelium (Alcian blue stain). (**G**): example of imaging elaboration of a section of proximal intestine (D5 group) stained with Alcian-blue. (**H**): same figure of (**G**) with algorithm functioning. (**I**): same figure of (**G**,**H**) in greyscale (software ImageJ v. 2.9.0—Fiji, USA version, plug in "Trainable Weka Segmentation").

## 4. Discussion

The current study essayed a protein-rich feedstuff with a valuable amino acid profile, such as guar protein concentrate, to substitute the main three protein sources, fish meal, soybean meal, and chicken meal, in the feed formulation. All three feeds responded to the urgency of adopting a more efficient use of natural resources from the ocean and land for a sustainable manufacturing process. If saving the use of fish meal represents a current practice to mitigate the environmental impact and the reduction in soybean meal is known to limit enteritis in salmonids [24], the substitution of chicken meal aims to reduce a protein source of animal origin but also maximise the economic efficiency. These traits are among the sustainability targets in aquafeed formulation. Today, the research investigates new protein sources to achieve a sustainable supply of feedstuffs for aquafeed production characterised by a low-fish meal inclusion [25,26]. The replacement with guar protein concentrate was considered suitable to provide a good source of essential amino acids, counting on a non-seasonal feedstuff [27]. Two levels of guar protein concentrate were included in the rainbow trout feeding to investigate the effects on the zootechnical performances during the growing phase.

With regard to the water quality, in the D15 group, the TAN showed levels higher than the other two groups. Other researchers [28] working with different techniques (recirculating aquaculture system) focused their attention on the effects of the use of dietary guar meal on the mechanical quality of resulting faecal wastes observing the negative effects of fine solid particles, where clogged biofilters, the increase in oxygen demand, and ammonia production in the waters are among main negative effects [29]. The authors underlined that this situation could favour the onset of pathogens and directly cause fish diseases. The same result was observed replacing soybean meal with guar meal in rainbow trout [15], in which the lowest level of the fish condition factor was recorded in fish fed with 100% of the protein replacement with respect to the fish that received other diets. Comparing the above-mentioned works with the current trial, the highest inclusion of guar protein concentrate gave a higher nitrogen ammonia water. The grids of the tanks of this group (D15) were very often found to be clogged by faeces; at the same time, faeces on the bottom of the tanks were observed. In the tanks where CD and D5 were utilised, the dirtiness and accumulation of faeces were not observed. Presumably, the cleaning activity of the grids of D15 tanks should have been more frequent than that carried out through the trial. This could explain that, in the literature, the effect of guar content to give more stability on faecal particles in water had been ascertained by authors [30,31] that showed a positive resistance of faeces to the hydro-mechanical manipulations of the flow-through systems. Also, another paper focalised the effect of this protein source on the faecal particle size [32], appreciated the solids removal efficiency, and reduced the impact of effluents thanks to guar meal use. In rearing conditions, water faeces are the main sources of organic nitrogen and phosphorus, which are transformed by microorganisms into toxic compounds such as ammonia, nitrites, and nitrates [33].

The increase in the guar-added pellet stability also showed a delay in the feed gastric transition, as reported in the literature by other authors [34] in rainbow trout. In a study by Brinker et al. [35], guar gum showed high viscosity, and its addition to fish feed appeared to not be digestible and unabsorbable by fish. Frequently, trout fed D15 reached satiety before fish receiving CD and D5. This condition forced the feeding administration to skip one day every 3–4 days in the D15 group. This was presumably related to the low palatability exhibited by the diet with the highest guar concentrate inclusion. This condition could have affected the productive performances in D15, as shown by the lowest final mean body weight with respect to the others. As a consequence, the same trend was noted in KI. This unfavourable output was in agreement with a previous study where the apparent digestibility of dry matter, crude protein, and crude lipid were reduced when the guar source was included [36].

As previously introduced, the guar protein concentrate exhibited a very high protein content (66%), significantly higher than those reported in the literature for guar meal before,

which referred to a protein level of the guar meal ranging between 45% [11] and 59.6% [8] and similar to the protein concentration (68%) reported in a paper [15] that was carried out to overcome the inconveniently high viscosity. The guar concentrate employed had been submitted to a fermentation process that should have avoided the inconvenience of inadequate water quality. At the same time, a sufficiently good aminoacidic profile was available in the guar concentrate to cover the requirements of fish, as reported in a study showing the successful effect of dietary guar meal at different rates and the total replacement of soybean meal in feed for rainbow trout [15]. In the experimental feed D5, a moderate inclusion of the guar protein concentrate obtained the most favourable results, with respect to the highest at 15% of feed, and the productive performances were like the control group.

From the histological point of view, fish receiving D15 feed showed a lower histological score than CD and D5, and no pathological changes were observed in the gut histology of fish receiving the guar meal. The use of open-source software (ImageJ), equipped with the plug in "Trainable Weka Segmentation" to evaluate intestinal goblet cells in rainbow trout fed including guar protein concentrate, gave satisfactory results and permitted a precise count of stained vacuoles, separating the background from the foreground (blue-stained vacuoles). The D15 group had a lower number of goblet cells compared to the CD group. A recent study showed that the goblet cells are significantly more abundant and their vacuoles significantly bigger in the proximal intestine of healthy rainbow trout [37], revealing a possible role in gut health. In the absence of other pathological changes, the lower quantity of goblet cells in the D15 group could be correlated with the lower zootechnical performances found in these animals. The secreted mucus lubricates the internal surface of the intestine, facilitating the progression of intestinal contents, prevents the passage of bacteria through the intestinal barrier, and regulates the microbiota [38]; therefore, the observed reduction in the mucus secretion in trout fed with Guar protein concentrate inclusion at 15% possibly negatively affected the gut health and intestinal function. A recent study [36] performed on juvenile largemouth bass demonstrated that the guar diet adversely affected the intestinal morphology and decreased the intestinal digestive and absorptive enzyme activities; additionally, it caused poor nutrient digestibility and low growth performances.

Further research evidenced no difference in goblet cells in rainbow trout challenged with a plant protein-rich diet [39], enforcing the hypothesis of the feasibility of reducing the use of fish meals and oils in favour of more sustainable protein and lipid ingredients, often of vegetal origin. In the current study, rainbow trout fed a conventional diet showed an increase in goblet cells, a condition that may be induced in response to pathogens (in particular, helminths), oxidants, and toxins [40]. This could be due to the major rate content of soybean meal, that is, the documented favouring intestinal inflammation in salmonids [41].

## 5. Conclusions

The present study confirmed that the tested proprietary guar protein concentrate can be used in the feed formulation for rainbow trout in the growing phase. The inclusion of this feedstuff was satisfactory at a moderate rate, mainly 5% of the main protein sources. Considering the most important productive performances, no difference was observed between the feed with a moderate inclusion of the guar meal and the conventional diet. In this situation, fish farmers must pay attention and evaluate every decision to take in relation to the productive performances and the cost of production of rainbow trout size-portion. Considering the zootechnical results obtained with the 5% guar gum inclusion, this study represents a challenge for further studies to perform trials with intermediate guar protein concentrate inclusion, hypothetically between >5% and less than 15%.

**Author Contributions:** Conceptualisation, A.R. and G.E.M.; methodology, A.R., F.M. (Francesco Marchetti) and L.G.; formal analysis, A.D.C. and E.F.; investigation, G.E.M., A.R. and E.F.; data curation, M.Q.; writing—original draft preparation, A.R. and G.E.M.; writing—review and editing, G.E.M., A.R. and L.G.; software, F.M. (Francesco Marchetti); visualisation, G.E.M. and F.M. (Francesca Mariotti); supervision, A.R.; project administration, A.R. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Eureka Project 2019, funding No. 7.

**Institutional Review Board Statement:** The animal study protocol was approved by the Animal Welfare Body of the University of Camerino (protocol n. 9/2023).

**Data Availability Statement:** The data presented in this study are available upon request from the corresponding author.

**Acknowledgments:** The authors would like to thank Panghea (Milan) for providing the proprietary guar protein concentrate and the team of Erede Rossi Group for the valuable collaboration during the field activities.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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