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EFFECT OF DIETARY SUPPLEMENTATION WITH FERMENTED COWS RUMEN CONTENTS AND PROBIOTIC ON SOME BLOOD PARAMETERS OF GRASS CARP (*Ctenopharyngodon idella)*

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ABSTRACT

This study evaluated the effects of dietary supplementation with fermented cow rumen contents, with or without probiotics, on the hematological and biochemical blood parameters of grass carp (*Ctenopharyngodon idella*). Rumen contents underwent anaerobic fermentation, followed by the application of a commercial probiotic (Anova-PROBIO FISH).

A total of 147 fish (initial weight: 30.99 ± 0.04 g) were randomly distributed into seven experimental groups with three replicates each (7 fish per replicate) and fed at 2% of their body weight for 12 weeks.

The treatments consisted of: a control group (T1, no additives), groups T2, T3, and T4 supplemented with 10%, 15%, and 20% fermented rumen contents plus probiotics, respectively; and groups T5, T6, and T7 supplemented with 10%, 15%, and 20% fermented rumen contents without probiotics, respectively.

Dietary supplementation with 20% fermented rumen contents enriched with probiotics (T4) significantly decreased ($P \leq 0.05$) serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) to 59.7 and 67.7 IU L^{-1}, respectively. T4 also demonstrated the most effective reduction in blood glucose (70.7 mg dL^{-1}), cholesterol, triglycerides, and low-density lipoprotein (LDL) levels.

Concurrently, T4 led to a significant increase in high-density lipoprotein (HDL, 48.0 mg dL^{-1}) and total protein (3.4 g dL^{-1}) levels. Furthermore, T4 recorded the highest hematological values, with hemoglobin reaching 11.17 g dL^{-1}, red blood cell (RBC) count at 1.06×10^6 cells μL^{-1}, and packed cell volume (PCV) at 31.7% compared to all other groups.

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In conclusion, incorporating 20% fermented cow rumen contents enriched with probiotics into grass carp diets enhances fish health by improving hematological profiles, reducing stress-related biomarkers, and optimizing lipid metabolism.

Keywords: Grass carp, rumen content, rumen fermentation, probiotic.

INTRODUCTION

Globally, aquaculture is becoming a sustainable solution to meet the demand for high-quality animal protein, especially as food security and reliance on conventional resources grow [3, 4].

Many economic studies have explored the concept of elasticity of substitution among production factors, which reflects producers' ability to substitute one input for another in response to changes in prices or resource availability. This flexibility aims to minimize production costs without compromising output levels [13]. Within this approach, research has focused on the utilization of alternative and non-traditional feed ingredients aimed at reducing production costs while enhancing fish health and growth performance. Animal by-products, such as rumen contents, have shown considerable promise due to their rich nutrient profile and residual undigested fiber, which can positively influence growth performance and feed efficiency [11]. Despite the nutritional value of rumen contents, their direct use in fish feed may face challenges related to their low digestibility and complex composition, which necessitate biological processing. Biofermentation is an effective method for improving nutritional value by breaking down cellulose walls and converting complex compounds into simpler, more digestible ones, thanks to the vital microbial activity within the rumen [20]. Furthermore, adding probiotics to fish feed may enhance digestion efficiency and balance the intestinal microbial environment. It also contributes to strengthening immunity and reducing oxidative stress, which positively impacts the overall health of fish [5, 28].

Several studies [1, 6, 12] have investigated the utilization of cow rumen contents in fish feed through feeding experiments. These experiments have shown positive results, including improved growth and physiological performance in various fish species, such as tilapia, common and grass carp. Studies have shown that dietary modification by adding nutritional supplements such as probiotics or fermented substances can induce significant changes in physiological indicators in various fish species [14, 34]. This study aims to investigate the effect of adding fermented bovine rumen contents, with or without probiotics, to grass carp diets and evaluate their impact on some important physiological traits.

MATERIALS AND METHODS

Fermentation of cow rumen contents

The rumen contents of cows were collected from a local slaughterhouse in Baghdad. The fermentation process was conducted in the Animal Production Farm at the College of Agriculture, University of Diyala, according to the method described by [29], then, it is dried after the fermentation process and added to the fodder components. The probiotics were Vietnamese brand Anova-PROBIO FISH Obtained from local markets in Baghdad, three grams of probiotics per kilogram rumen contents, which contains *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, and *Aspergillus oryzae*.

Experimental Feed

After preparing the feed materials, seven different rations were formulated, with each ration balanced accordingly upon the inclusion of additives (Table 1). The feed ingredients were thoroughly mixed and moistened by adding 400 mL of water per kilogram of feed. The moistened mixtures were then pelleted using a feed compression machine equipped with a 2 mm die. The pellets were air-dried for three days with regular stirring to ensure uniform drying, and subsequently stored in nylon bags under laboratory conditions.

Table 1 :Composition of experimental diets of grass carp.

No.	Feed Ingredient	Control (T1)	T2 (10%)	T3 (15%)	T4 (20%)	T5 (10%)	T6 (15%)	T3 (20%)
1	Fish meal	5	5	5	5	5	5	5
2	Animal protein concentrate	20	20	20	20	20	20	20
3	Soybean meal	33	33	33	33	33	33	33
4	Yellow corn	23	20	15	13	20	15	13
5	Wheat bran	8	4	5	3	4	5	3
6	Barley	9	6	5	4	6	5	4
7	Rumen content + probiotic	--	10	15	20	--	--	--
8	Rumen content	--	--	--	--	10	15	20
9	Fish oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5
10	Vitamins and minerals	1	1	1	1	1	1	1
11	Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Total	100	100	100	100	100	100	100

Blood sampling and assay

Two ml of blood was collected from the caudal vein of the fish and transfered to the 5 ml gel tube. The serum was harvested after centrifugation at 3,000 cycles for 15 minutes. The serum was then transferred into sterile tubes for blood biochemical assay. Serum analysis included measurement of liver enzymes (AST and ALT), glucose, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total proteins. The analyses were conducted using a Mindray BC-30S device, manufactured in China.

Statistical analysis

The data was analyzed statistically using the Statistical Analysis System [26] using a complete randomized design (CRD) to evaluate the effect of treatment on the studied traits, according to the following mathematical model: $Yij=\mu+Ti+eij$ where Y_{ij} is the observed value, μ is the overall mean, T_i is the effect of the i-th treatment, and e_{ij} is the random error associated with the experimental unit.

RESULTS AND DISCUSSION

Blood parameters

Serum AST and ALT activities

The results revealed significant differences among the treatments ($P \leq 0.05$). Treatment T4 exhibited a reduction in the concentrations of liver enzymes AST and ALT, reaching 55.7 ± 0.33 and 67.7 ± 0.88 $IU.L^{-1}$, respectively. However, no significant differences were observed between T4 and treatments T3 and T5 in ALT concentration (Table 2). The results showed a decrease in liver enzyme activities, AST and ALT, with increasing addition of fermented cow rumen contents supported with probiotics. Low serum AST and ALT activities are an indicator of healthy liver cells and the absence of liver damage or inflammation [8]. Therefore, the lower activities recorded in the experimental treatments compared to the control group reflect the effectiveness of these additives in maintaining liver function and reducing metabolic stress.

The fermentation process of cow rumen contents improves protein quality and increases its digestibility and absorption through the secretion of digestive enzymes, as well as reducing or removing anti-nutritional substances, which limits the accumulation of toxic or difficult-to-digest compounds that may reach the liver and cause metabolic stress [18, 23]. This is reflected in lower AST and ALT activities, leading to positive effects on metabolism and liver health [18, 23, 25]. In addition, probiotics promote the growth of beneficial gut bacteria while suppressing harmful microbial populations, thereby reducing endotoxin production and alleviating liver stress. Beneficial bacteria also enhance the absorption of amino acids and vitamins, which supports metabolic efficiency and reduces the synthesis of inflammatory compounds [28].

Probiotics activate the function of the intestinal epithelial barrier, preventing the transfer of toxic molecules and bacteria into the bloodstream, thus reducing the transfer of toxins to the liver and reducing the chances of hepatitis or metabolic stress [24] and decreasing ALT and AST activity as indicators. The current results are consistent with those reported by Peng *et al.* [21], who revealed that using feeds containing fermented ingredients led to decreased levels of AST and ALT in the liver of Nile tilapia (*Oreochromis niloticus*) [34]. Studies on largemouth bass have shown that the use of fermented feeds supplemented with probiotics leads to a reduction in blood levels of liver enzymes AST and ALT. Gao *et al.* [14] and Zhao *et al.* [35] showed that adding probiotics to a high-fat diet reduced fat accumulation in the liver of fish and decreased AST and ALT activities.

Serum glucose concentration

The results of the serum glucose concentration in fish (Table 2) showed significant differences ($P \leq 0.05$) among treatments. Treatments T7, T6, and T5 recorded the highest values compared to the control group and the other treatments, with no significant differences observed among them. However, no significant differences were found between the control treatment and T3, T2, and T4 treatments, reaching 73.7, 73, 72.33, and 70.7 mg/L, respectively (Table 2). These results indicate that probiotics play a role in regulating blood glucose levels, as they can enhance digestive efficiency and improve the absorption of nutrients, including carbohydrates, which are the main source of glucose in the diet [25, 37]. Benoit [32] reported that adding probiotics to grass carp diets can improve the balance of intestinal microbiota and increase the concentration of short-chain fatty acids, such as acetate. This provides a substrate for the tricarboxylic acid (TCA) cycle pathway for energy generation, thus regulating metabolism. Activating this pathway can enhance the tolerance of grass carp to glucose, promote its breakdown, and inhibit glucose formation. Zhu *et al.* [37] demonstrated that the use of a probiotic in grass carp diets, represented by *Bacillus subtilis*, improved the feed conversion rate, which indirectly affected glucose metabolism.

Table 2: Serum AST and ALT activities and glucose concentration in grass carp fish fed different levels of fermented rumen contents and probiotic (Mean ± SE)

Treatment	ALT (IU/L)	AST (IU/L)	Glucose (mg/dL)
T1	95.7 ± 0.33[a]	77.7 ± 0.33[a]	73.7 ± 0.88[b]
T2	72.7 ± 1.45[d]	78.0 ± 0.58[a]	70.7 ± 0.67[b]
T3	70.0 ± 0.58[de]	59.7 ± 0.33[d]	73.0 ± 0.58[b]
T4	67.7 ± 0.88[e]	55.7 ± 0.33[e]	72.3 ± 0.33[b]
T5	69.0 ± 0.58[e]	65.7 ± 0.88[c]	81.0 ± 1.00[a]
T6	75.7 ± 1.45[c]	70.0 ± 0.58[b]	81.7 ± 1.45[a]
T7	80.7 ± 0.88[b]	70.7 ± 0.33[b]	83.7 ± 1.45[a]
Different letters within each column differ significantly ($P \leq 0.05$)			

T1: Control treatment without any addition.
T2: Treatment with fermented rumen content and probiotics at 10% inclusion in the diet.
T3: Treatment with fermented rumen content and probiotics at 15% inclusion in the diet.
T4: Treatment with fermented rumen content and probiotics at 20% inclusion in the diet.
T5: Treatment with fermented rumen content at 10% inclusion in the diet.
T6: Treatment with fermented rumen content at 15% inclusion in the diet.
T7: Treatment with fermented rumen content at 20% inclusion in the diet.

Serum cholesterol, triglycerides, HDL, LDL, and total protein concentrations

The results showed significant differences ($P \leq 0.05$) among all treatments (Table 3). The results showed a clear physiological improvement in treatment T4, represented by a significant decrease in the levels of total cholesterol, triglycerides and LDL, reaching 158.7, 196.7 and 79.3mg/dL, respectively, in addition to a significant increase in the levels of HDL and total proteins (48 and 3.4 mg/dL, respectively; Table 3). These results indicate that the use of fermented cow rumen contents supported with probiotics contributed to improved lipid and protein metabolism in fish. This effect is attributed to the dual role of both fermentation and probiotics. Fermentation breaks down complex molecules, improving the biological value of the additive, enhancing nutrient digestibility and bioavailability, and promoting the growth of beneficial gut bacteria [27, 36]. Probiotics, on the other hand, enhance the balance of intestinal microbiota and stimulate the production of short-chain fatty acids (SCFAs), which contribute to reducing hepatic cholesterol synthesis and improving fat absorption [33]. They also improve the biological value of protein compounds by reducing anti-nutritional compounds [23]. Several recent studies indicate that fermentation of oligosaccharides by probiotics leads to increased production of SCFAs, which regulate lipid metabolism and lower serum total cholesterol and triglyceride levels [14, 19].

A decrease in the pH of the gastrointestinal tract due to the fermentation of sugars by probiotics leads to a decrease in the solubility of bile acids, which contributes to a decrease in the absorption of bile acids and an increase in their excretion from the body. This process increases the hepatic utilization of cholesterol for the synthesis of new bile acids, resulting in a reduction of LDL and a subsequent decrease in the concentrations of accumulated cholesterol and triglycerides [17, 22]. Supporting the current results, Tian *et al.* [30] indicated that using the probiotic *Lactobacillus plantarum* at a rate of 300-600 mg/kg led to a decrease in serum triglyceride, cholesterol, and LDL contents and an improvement in HDL contents in grass carp. The use of probiotics, along with a reduced reliance on antibiotics for treating fish diseases, has been associated with enhanced growth performance, improved immune responses, and elevated total protein levels in fish serum [2].

Table 3: Serum cholesterol, triglycerides, low-density lipoproteins (LDL), high-density lipoproteins (HDL), and total proteins in grass carp fed different levels of fermented rumen contents and probiotic (Mean ± SE)

Treatment	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Total Protein (mg/dL)
T1	168.7 ± 0.88^{d}	270.0 ± 0.33^{a}	32.0 ± 0.6^{e}	89.0 ± 0.6^{c}	2.60 ± 0.06^{d}
T2	169.3 ± 0.33^{d}	206.3 ± 0.88^{e}	44.7 ± 0.33^{b}	87.0 ± 0.6^{d}	2.87 ± 0.08^{c}
T3	174.3 ± 1.45^{c}	204.3 ± 0.33^{f}	45.3 ± 0.33^{b}	85.7 ± 0.33^{d}	3.20 ± 0.06^{b}
T4	158.7 ± 1.45^{e}	196.7 ± 0.33^{g}	48.0 ± 0.6^{a}	79.3 ± 0.33^{e}	3.40 ± 0.08^{a}
T5	198.7 ± 1.33^{a}	226.3 ± 0.33^{d}	41.0 ± 0.6^{d}	92.0 ± 1.15^{b}	2.97 ± 0.03^{c}
T6	188.3 ± 0.33^{b}	245.0 ± 0.58^{bcd}	42.0 ± 1.15^{a}	94.3 ± 0.33^{a}	2.87 ± 0.07^{c}
T7	188.3 ± 0.88^{b}	229.7 ± 0.33^{c}	44.0 ± 1.15^{bc}	90.3 ± 0.33^{c}	2.97 ± 0.03^{c}
Different letters within a single column differ significantly ($P \leq 0.05$).					

T1: Control treatment without any addition.
T2: Treatment with fermented rumen content and probiotics at 10% inclusion in the diet.
T3: Treatment with fermented rumen content and probiotics at 15% inclusion in the diet.
T4: Treatment with fermented rumen content and probiotics at 20% inclusion in the diet.
T5: Treatment with fermented rumen content at 10% inclusion in the diet.
T6: Treatment with fermented rumen content at 15% inclusion in the diet.
T7: Treatment with fermented rumen content at 20% inclusion in the diet.

Hematological parameters

The current results in Table 4 shows significant differences ($P \leq 0.05$) among the treatments in some hematological parameters including hemoglobin (Hb) levels, red blood cell count (RBC), and packed cell volume (PVC). Treatment T4 recorded the highest values in all parameters, reaching 11.17 IU/L, 3.3 IU/L, and 31.7%, respectively, compared to the control treatment and the remaining treatments, followed by treatment T3. The results showed that fed fermented cow rumen contents supported with probiotics and increasing their addition percentage in the T4 and T3 treatments led to an increase in certain hematological parameters, indicating that the addition of probiotics improved the immune response. This is attributed to the positive health effects of probiotics, especially lactic acid bacteria, due to their significant role in stimulating the immune system in fish [12].

Previous studies have confirmed that it is possible to increase the number of RBCs and hemoglobin by enhancing iron absorption due to the release of organic acids in the intestine caused by probiotics, which increases the availability of iron for the production of more red blood cells and hemoglobin [9]. Numerous studies have reported that the addition and increased inclusion of probiotics in carp diets lead to improvements in hematological parameters, including elevated RBCs counts, hemoglobin concentration, and PCV [16, 31]. Improving nutritional balance and increasing metabolic efficiency through the dual role of the fermentation process and probiotics in the feed components also helped stimulate the function of the organs responsible for producing blood components—the liver and spleen [7, 10]. This explains the increased hemoglobin, RBCs count, and compressed cell volume in the T3 and T4 treatments.

Table 4: Some hematological parameters of grass carp fed different levels of fermented rumen contents and probiotic (Mean ± SE).

Treatment	Hb (mg/dL)	RBC ($\times10^6$ / mm^3)	PVC (%)
T1	7.73 ± 0.03[e]	1.77 ± 0.03[d]	14.7 ± 0.2[e]
T2	9.00 ± 0.06[c]	2.10 ± 0.06[c]	25.13 ± 0.2[c]
T3	9.90 ± 0.06[b]	2.80 ± 0.09[b]	28.5 ± 0.8[b]
T4	11.17 ± 0.12[a]	3.30 ± 0.17[a]	31.7 ± 0.7[a]
T5	9.00 ± 0.06[c]	2.13 ± 0.07[c]	24.4 ± 0.4[c]
T6	7.97 ± 0.09[d]	2.23 ± 0.12[c]	20.4 ± 0.2[d]
T7	6.90 ± 0.06[f]	1.57 ± 0.03[d]	14.57 ± 0.3[e]
Different letters within a single column differ significantly ($P \leq 0.05$).			

T1: Control treatment without any addition.
T2: Treatment with fermented rumen content and probiotics at 10% inclusion in the diet.
T3: Treatment with fermented rumen content and probiotics at 15% inclusion in the diet.
T4: Treatment with fermented rumen content and probiotics at 20% inclusion in the diet.
T5: Treatment with fermented rumen content at 10% inclusion in the diet.
T6: Treatment with fermented rumen content at 15% inclusion in the diet.
T7: Treatment with fermented rumen content at 20% inclusion in the diet.

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تأثير اضافة محتويات كرش الابقار المخمرة والمعزز الحيوي الى العليقة في بعض معايير الدم لأسماك الكارب العشبي (*Ctenopharyngodon idella*)

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الملخص

أُجريت الدراسة لتقييم تأثير استعمال محتويات كرش الأبقار المخمرة مع وبدون إضافة المعززات الحيوية في علائق أسماك الكارب العشبي *Ctenopharyngodon idella* في بعض معايير الدم. تم إعداد محتويات الكرش المخمرة بتقنية التخمير اللاهوائي وأستعمل المعززات الحيوية ذات العلامة التجارية Anova- PROBIO FISH، تضمنت الدراسة سبع معاملات تجريبية هي معاملة السيطرة خالية من الاضافات، والمعاملات T2 وT3 وT4 الحاوية على نسبة 10% و15% و20% محتويات كرش الأبقار المخمرة والمدعمة بالمعززات الحيوية على التوالي، والمعاملات T5 وT6 وT7 الحاوية على نسبة 10% و15%و20% محتويات كرش الأبقار المخمرة على التوالي. وزعت 147 سمكة على 21 حوض زجاجي على 7 معاملات بواقع ثلاث مكررات لكل معاملة بمعدل وزن ابتدائي فردي 30.99± 0.04 غم. غذيت الاسماك بنسبة 2% من وزن الجسم. بينت النتائج وجود فروق معنوية بين المعاملات ($P \leq 0.05$).

وقد سجلت المعاملة T4 انخفاض في فعالية انزيمات الكبد ASTوALT بلغتا 59.7و67.7 وحدة دولية.لتر$^{-1}$ ولم تظهر بينها وبين المعاملتين T3 وT5 فروق معنوية في فعالية انزيم ALT. من ناحية أخرى، سجلت معاملة السيطرة والمعاملات T3 وT2 وT4 انخفاض في تركيز الكلوكوز بلغت 73.7 و 73 و72.33 و 70.7 ملغم.لتر$^{-1}$ على التوالي.

اظهرت نتائج المعاملة T4 انخفاض مستويات الكولسترول والدهون الثلاثية والبروتينات الدهنية واطئة الكثافة إلى جانب ارتفاع معنوي في مستويات البروتينات الدهنية عالية الكثافة والبروتينات الكلية (158.7 و196.7 و79.3 و48، 3.4 ملغم.ديسيلتر$^{-1}$ على التوالي)، وسجلت المعاملة T4 اعلى تركيز في الهيموغلوبين وعدد كريات الدم الحمراء وحجم خلايا الدم المضغوطة بلغت 11.17 10^6.مل$^{-1}$ و3.3 ملغم/ديسيلتر$^{-1}$ و731.% على التوالي مقارنة مع كافة المعاملات.

يمكن الاستنتاج أن إضافة 20% من محتويات كرش الأبقار المخمّرة والمدعّمة بالمعزز الحيوي (المعاملة T4) إلى علائق أسماك الكارب العشبي قد حسنت من صحة الأسماك، من خلال زيادة أعداد خلايا الدم، وخفض مستويات الكلوكوز والكوليسترول والدهون الثلاثية والدهون البروتينية منخفضة الكثافة، فضلاً عن رفع مستويات البروتينات الكلية والدهون البروتينية عالية الكثافة في مصل الدم.

الكلمات الدالة: الكارب العشبي، محتويات الكرش المجترات، تخمير محتويات الكرش، المعزز الحيوي

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