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Full Length Article

Replacement of Vegetable Protein Sources with Marine By-Product on Nutrient Utilization, Protein Digestibility, Meat Quality and Economics in Ross-308 Broilers

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Abstract

The objective of this experiment to study the effect of replacement of vegetable protein with marine by-product (fish meal) sources on nutrient utilization and economics in broilers fed on low protein diet. Five diets (CP 18%; ME 2950 kcal/kg); (R1) control: vegetable protein ingredients whereas in R2, R3, R4 and R5, 25, 50, 75 and 100% of total dietary protein was replaced with a Hi-Pro fish meal on a protein-equivalent basis. Four hundred and twenty-five (n=425) day-old Ross-308 broiler chicks were divided into 5 treatments having 5 replicates (17 birds in each). Feed intake, weight gain, FCR and dressing percentage were improved ($P < 0.05$) in birds fed diet R3 in which fish meal contributed 50% of the dietary protein compared to those on all-vegetable protein (Control-diet R1) and those in which 25% (diet R2), 75% (diet R4) or 100% (diet R5) of the dietary protein was replaced with fish meal till day 21. Digestibility of CP was the highest ($P < 0.05$) in birds fed diet R3 in which fish meal replaced 50% of the dietary protein. Production cost/kg live weight was lesser in birds fed diet containing 25 and 50% replacement of fish meal with vegetable protein sources. In conclusion, vegetable protein ingredients can be replaced with fish meal at 50% in broiler diet.

Keywords: Fish meal, Soybean meal, Protein efficiency ratio, Protein utilization, Economics

Introduction

Vegetable protein sources like SBM, canola and sunflower meal have been traditionally used in poultry diets. These ingredients have high protein content and high digestibility of essential amino acids. Several factors including the physical nature and presence of anti-nutritional factors affect the utilization of plant protein sources by poultry. Vegetable protein ingredients (oilseed meals) have low digestibility and are in general, deficient in Lysine, methionine and cystine (Cyrino *et al.* 2010). Raw Full fat soybean causes a higher weight of the pancreas and higher trypsin activity that leads to pancreas hypertrophy, due to an overstimulation of the pancreas to secrete protease (Rada *et al.* 2017). Animal protein sources naturally contain biogenic amines, which are synthesized from amino acids. Polyamines have the potential to support growth, particularly when intestinal integrity is compromised, which may be of importance immediately post-hatch when rapid growth and development are taking place and the birds are particularly susceptible to pathogens and bacteria. This is likely true during times of disease challenge (Smith 2001). These compounds are typically created in feed ingredients due to the action of microorganisms. Bioactive compounds obtained from synthesis within the body or from the consumption of animal protein appear to have a positive impact on poultry production at low levels (Michiels *et al.* 2012).

Rendered animal products for example, feather meal, fish meal, poultry by-product meal are alternative protein sources (Meeker and Hamilton 2006). Fish meal (FM) is reported to have a high nutritional profile but has some other associate quality issues. It is high in Lysine, methionine and cystine which are often deficient in plant-based protein sources (Hall 1992). Fish meal contains 60–70% CP (NRC 1994; Smith 2001). It has high nutrient digestibility and biological value (Médale and Kaushik 2009) and is rich in water-soluble vitamins (B_{12}, choline, niacin, pantothenic acid and riboflavin). The protein of fish meal has over 90% digestibility (Zhou *et al.* 2004). Fish meal is low in fiber. Macrominerals and trace minerals are present in an excellent amount in FM. Diets containing 2.5% fish meal to broiler starter and grower rations have a significant linear increase in intake of feed and body weight gain (Karimi 2009). Bhuiyan *et al*. (2012) reported a 10% reduction in

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feed intake of broilers fed vegetable protein compared to animal protein diets. Vieira and Lima (2005) reported that broiler birds fed diets containing animal or vegetable protein sources did not affect production performance.

Fish meal is scarce in most developing countries, thus expensive for use in poultry feeds (Smith 2001). Fish meal is produced from non-edible oily fish or inedible portion of edible fish. Research that establishes the threshold levels of biogenic amines that cause negative effects in poultry is not available. Guidelines are available for maximum histamine levels acceptable in fish for human consumption, with the Canadian food inspection agency (CFIA) and the European Union stating a maximum of 100 mg/100 kg of fish (Karovicova and Kohajdova 2003). It is a useful feed ingredient for replacing vegetable protein sources especially SBM in the broiler diet. This study determined the response of commercial Ross-308 broilers to dietary protein contributed by fish meal and vegetable protein sources in different proportions. The response of birds was measured as growth performance, nutrient digestibility, efficiency of protein utilization and carcass characteristics.

Materials and Methods

Trial location

The experimental trial was conducted at R&D house at Institute of Animal and Dairy Sciences, University of Agriculture, Faisalabad, Pakistan.

House preparation and management

One week before, the shed was ready for chicks the arrival of chicks. Wood shaving was used as litter material that was raked on daily basis. The experimental birds were raised under standard environmental conditions. Drinkers were washed on daily basis.

Experimental birds and diets

Four hundred and twenty-five (*n = 425*) day-old Ross-308 chicks were procured from Jadeed Farms & Hatchery Ross 308 breeder. Chicks were randomly distributed into 25 experimental units of 17 birds each. Pelleted diets were prepared in separate batches for each treatment. Five diets (CP 18%; ME 2950 kcal/kg) were formulated. Diet R1 control (FM 0) was formulated with all vegetable protein sources (SBM, canola meal and sunflower meal) whereas in diet R2 (FM 25), R3 (FM 50), R4 (FM 75) and R5 (FM 100) marine by-product (Hi-pro Fish meal) replaced 25, 50, 75 and 100% of the dietary protein on protein equivalent basis. The control diet (R1) contains all-vegetable protein-based sources without any contribution from fish meal, the diet R2 having a protein share of 25% from fish meal and 75% from vegetable meals, diet R3 having an equal share of protein that is 50:50 while the R4 diet having 75% contribution of protein from fish meal and only 25% from vegetable sources. Diet R5 of the experiment accounts total share of protein from fish meals which means 100% protein coming from fish meals (Table 1–2).

Vaccination schedule

Vaccines for ND + IB, IBD, IBD and ND were administrated on day 3rd, 8, 18 and 25th, respectively.

Data for production performance

Data on growth performance using formulas were recorded (Kamran *et al.* 2008; Marcu *et al.* 2013).

Feed Intake = Feed Offered – Feed Refusal

FCR = Feed Intake (g) / Weight gain (g)

Protein Efficiency ratio (PER) = Weight gain / Protein intake

European Production Efficiency Factors (EPEF) = Livability/FCR × live weight (kg)/Age (days) × 100

Protein utilization= Total body protein/protein consumed × 100

Whole-body composition on day 1st, 7th, 14th and 21st

On day 1, four birds were picked randomly, fasted for 24 h and killed by cervical dislocation for baseline whole body composition. On day 7, 14 and 21, two birds from each replicate were kept off-feed for 6 h and killed by cervical dislocation. Dead birds were stored in air-tight (polythene zipper) plastic bags at -20°C. Thawing and drying of stored birds were done in a hot air oven at 65°C.

Nutrient digestibility

Digestibility trial was conducted on day 21st through indirect marker method (Dourado *et al.* 2010). Celite® (a source of acid-insoluble ash) was added to the diet @ 1.0% in feed and fed from day 1 to day 21st. An adaptation period of 48 h was allocated before collection of excreta. Polythene sheets were spread over the bedding to prevent contamination and contact of fecal material with litter. Fresh fecal samples were collected into zipper bags. Two representative samples from every replicate were collected and dried at 65ºC. Coefficient of digestibility was determined using the following relationship (AOAC 2000).

Digestibility (%) = 100 - (100 × % maker in feed / % marker in feces × % nutrient in feces / % nutrient in feed)

Carcass response

On day 21, two birds from each pen/replicate were randomly selected and weighed. The birds were slaughtered, de-feathered, eviscerated and processed for carcass response. Dressing percentage (%) and organs weight were measured.

Table 1: Ingredient composition of experimental diets when fish meal replaced vegetable proteins

Ingredients (%)	Experimental diets[1]				
	R1: FM 0 (Control) VM100	R2: FM 25 VM75	R3: FM 50 VM50	R4: FM 75 VM25	R5: FM 100 VM0
Rice hulls, ground	0	2.7	4.3	9.7	11.1
Corn starch*	41.1	42.7	46.9	49.4	52.7
Soybean meal (44%)	25.9 (11.31)[1]	19.2 (8.4)	13.9 (6.1)	9.9 (4.3)	0
Canola meal (35.5%)	9.0 (3.1)	7.1 (2.5)	0	0	0
Sunflower meal (25.5%)	11.0 (2.8)	8.0 (2.0)	10 (2.5)	0	0
Fish meal (61%)	0	7.2 (4.3)	14.3 (8.7)	21.5 (13.1)	28.6 (17.4)
Molasses	1.0	3.0	3.0	3.0	3.0
Vegetable oil	5.1	4.5	2.8	2.6	1.6
Limestone	1.7	1.4	1.1	0.8	0.4
Mono-Calcium phosphate	2.5	1.8	1.1	0.4	0
Salt	0.3	0.2	0.2	0.3	0.3
Sodium bicarbonate	0.18	0.22	0.24	0.26	0.13
Potassium carbonate	0	0	0	0.16	0.41
L-Lysine sulphate, 56%	0.54	0.46	0.38	0.24	0.20
DL-Methionine	0.35	0.35	0.35	0.37	0.34
L-Threonine	0.17	0.16	0.15	0.15	0.14
Celite®	1.0	1.0	1.0	1.0	1.0
Premix	0.2	0.2	0.2	0.2	0.2
Total	100	100	100	100	100

"*Corn starch was supplied by the courtesy of Rafhan Maize products, Faisalabad.
[1]Numbers in brackets shows actual contribution of protein (CP) by vegetable and fish meal source"

Table 2: Nutrient composition of experimental diets when fish meal replaced vegetable proteins

Nutrients (%)	Experimental diets				
	R1: FM 0 (Control) VM100	R2: FM 25 VM75	R3: FM 50 VM50	R4: FM 75 VM25	R5: FM 100 VM0
Calculated					
CP	18	18	18	18	18
ME, Kcal/kg	2950	2950	2950	2950	2950
EE	5.99	6.0	5.0	5.4	5.0
CF	5.0	4.95	5.12	4.85	4.97
Calcium	1.3	1.3	1.3	1.3	1.3
Phosphorus, available	0.65	0.65	0.65	0.65	0.66
Sodium	0.2	0.21	0.25	0.3	0.3
Potassium	0.75	0.67	0.58	0.5	0.5
Chloride	0.25	0.2	0.2	0.2	0.2
DEB	202.6	202.9	200	200.5	201.4
Dig. Lysine	1.2	1.2	1.2	1.2	1.2
Dig Meth + Cys	0.88	0.88	0.88	0.88	0.88
Dig. Threonine	0.78	0.78	0.78	0.78	0.78
Dig. Tryptophan	0.22	0.2	0.19	0.17	0.15
Analyzed					
DM	89.5	90.2	88.5	89.5	89.7
CP	17.4	17.5	17.1	17.3	17.4
EE	4.9	5.1	5.1	4.9	5.3
Ash	7.3	7.5	8.2	7.3	7.1

Meat quality parameters

The pH of ground breast (1 g) blend in 10 mL distilled water was measured (Jeacocke 1977). About 15 gm chopped breast sample was centrifuged at 4ºC for 10 to 15 min at 5000 rpm to measure water holding capacity (Pearson and Dutson 1995). Cooking loss is the loss in weight of breast portion boiled at 80ºC for half hour (Ahmed *et al.* 2015).

Proximate composition

Meat samples from breast fillet were prepared for chemical analysis to determine DM, CP, EE and crude ash according to AOAC (2000). Dry matter was performed by drying the sample in a hot air oven. Crude protein was done by the micro Kjeldahl method. Ether extract was analyzed by n-hexane extraction and crude ash by burning the sample in a Muffle furnace.

Economics

Cost of production per live weight was recorded on the basis of feed cost and live bird weight.

Statistical analysis

"Data collected were analyzed using analysis of variance technique under CRD and Tukey's test was used to compare treatment means (Steel *et al.* 1997)".

Table 3: Growth performance of broiler birds fed different ratio of fish meal to vegetable protein sources

Parameters	Fish meal/vegetable meal ratio					SEM	*P value*
	R1: FM 0 (Control) VM100	R2: FM 25 VM75	R3: FM 50 VM50	R4: FM 75 VM25	R5: FM 100 VM0		
Weight gain (g)	709.38[d]	808.19[ab]	833.50[a]	769.66[bc]	727.73[cd]	13.5	0.0001
Feed intake (g)	1142.01[b]	1210.29[ab]	1229.25[a]	1207.62[ab]	1147.44[b]	17.3	0.004
FCR	1.62[a]	1.50[bc]	1.47[c]	1.57[ab]	1.58[ab]	0.02	0.001
Protein intake (g)	161.85[b]	208.54[a]	226.67[a]	228.46[a]	221.61[a]	9.94	0.001
Protein efficiency ratio	3.00[b]	3.44[ab]	3.62[a]	3.67[a]	3.48[ab]	0.15	0.034
Protein utilization	40.65[ab]	44.79[a]	46.50[a]	44.99[a]	38.22[b]	1.42	0.003
Livability (%)	96.47	97.65	96.47	92.94	91.76	2.00	0.208
EPEF	220.81[ab]	264.80[a]	270.54[a]	230.01[ab]	194.93[b]	13.3	0.003

"SEM: Standard error of mean; $P > 0.05$ (Non-Significant), $P < 0.05$ (Significant)
FM: Fish meal, VM: Vegetable Meals, FCR: Feed conversion ratio, EPEF: European production efficiency factor
* Treatments: Replacement of FM with VM at 0, 25, 50, 75 and 100% on a protein equivalent basis"

Table 4: Carcass response of broiler birds fed different ratio of fish meal to vegetable protein sources

Parameters	Ratios of Fish meal Vegetable meal					SEM	*P value*
	R1: FM 0 (Control) VM100	R2: FM 25 VM75	R3: FM 50 VM50	R4: FM 75 VM25	R5: FM 100 VM0		
Dressing percentage (%)	53.5[ab]	55.7[ab]	56.4[a]	54.7[ab]	52.7[b]	0.711	0.039
Breast meat yield (%)	31.0	30.1	30.2	29.7	29.1	0.554	0.090
Thigh meat yield (%)	19.9[b]	23.2[ab]	23.8[a]	22.5[ab]	21.2[ab]	0.814	0.043
Neck weight (%)	2.49	2.39	2.40	2.53	2.40	0.094	0.085
Liver weight (%)	2.67	2.78	2.89	3.16	2.92	0.145	0.124
Heart weight (%)	0.75	0.66	0.60	0.63	0.63	0.045	0.083
Gizzard weight (%)	1.53	1.36	1.56	1.58	1.60	0.082	0.253
Abdominal fat (%)	1.50	1.32	1.67	1.40	1.73	0.136	0.094

"SEM: Standard error of mean; $P > 0.05$ (Non-Significant), $P < 0.05$ (Significant)
FM: Fish meal, VM: Vegetable Meals
* Treatments: Replacement of FM with VM at 0, 25, 50, 75 and 100% on a protein equivalent basis"

Results

Growth performance

Data on weekly weight gain, weekly feed intake, weekly FCR and EPEF is shown in Table 3. Higher weight gain ($P < 0.05$) was recorded in birds fed diet containing blend of 50% fish meal as an animal protein source and 50% vegetable protein (SBM, CM and SFM) sources on protein equivalent basis while lower weight gain was noted in birds fed diet having 0% fish meal as an animal protein source. Birds fed diet containing blend of 50% fish meal as protein source and 50% vegetable protein sources on protein equivalent basis had higher feed intake ($P < 0.05$) and those fed 0 and 100% fish meal-based diet had lower feed intake. Further, better FCR ($P < 0.05$) was recorded in birds fed diet having 50% share of protein from fish meal and 50% from vegetable protein sources; however, it was poor in birds fed diet formulated solely from vegetable protein sources. Higher ($P < 0.05$) European production efficiency factor was recorded in birds fed diet containing 25 and 50% protein share from fish meal on protein equivalent basis while lower was noted in group fed diet containing 100% protein from fish meal on protein equivalent basis.

Nutrients utilization

Protein intake and PER were not affected ($P > 0.05$) by various ratios of animal to vegetable protein sources. However, higher ($P < 0.05$) protein utilization was found in birds fed containing blend of 50% vegetable and 50 fish meal as protein source of protein equivalent basis in broiler diet. When the protein share of fish meal exceeded 50%, the protein utilization was reduced significantly in 75 and 100% treatment group (Table 3).

Carcass characteristics

Highest ($P < 0.05$) dressing percentage (%) of live weight and thigh meat was observed in birds fed diet having blend of 50% fish meal and 50% vegetable protein source (50FM) on protein equivalent basis. However, breast meat yield, neck weight, liver weight, gizzard weight, heart weight and abdominal fat pad were similar ($P > 0.05$) affected by dietary treatments (Table 4).

Meat quality and proximate composition

Water holding capacity, pH and cooking loss were measured. Results showed that different ratios of animal to vegetable protein sources caused no significant ($P > 0.05$) effects on meat quality parameters. A similar finding was noted in breast meat proximate with no significant effect ($P > 0.05$) on CP, EE, Ash and Moisture percentages due to different ratios of FM to vegetable sources (Table 5).

Crude protein digestibility

Crude protein digestibility was significantly ($P < 0.05$) higher in broilers fed having a blend of 50% fish meal and

Table 5: Meat quality and breast proximate of broiler birds fed different ratio of fish meal to vegetable protein sources

Parameters	Ratios of Fish meal Vegetable meal					SEM	*P value*
	R1: FM 0 (Control) VM100	R2: FM 25 VM75	R3: FM 50 VM50	R4: FM 75 VM25	R5: FM 100 VM0		
Meat quality parameters							
WHC (%)	58.8	59.0	61.2	56.7	60.9	2.38	0.69
PH	6.1	6.1	6.1	6.2	6.1	0.05	0.72
Cooking loss (%)	27.0	27.2	25.3	27.7	33.1	2.12	0.18
Breast meat proximate (%)							
Moisture	74.4	74.0	74.3	73.8	73.6	0.92	0.969
Ash	3.7	3.6	3.3	3.6	3.1	0.26	0.529
CP	18.3	19.2	20.3	20.1	20.1	0.66	0.227
EE	1.6	1.9	1.9	2.0	1.9	0.12	0.33

"SEM: Standard error of mean; $P > 0.05$ (Non-Significant), $P < 0.05$ (Significant)
FM: Fish meal, VM: Vegetable Meals
* Treatments: Replacement of FM with VM at 0, 25, 50, 75 and 100% on a protein equivalent basis"

Table 6: Production cost per kg live weight of broiler birds fed different ratio of fish meal to vegetable protein sources

Production Cost (Rs.)	Ratios of Fish meal Vegetable meal					SEM	*P value*
	R1: FM 0 (Control) VM100	R2: FM 25 VM75	R3: FM 50 VM50	R4: FM 75 VM25	R5: FM 100 VM0		
Bird cost	25	25	25	25	25	-	-
Feed cost / bird	75.04	80.86	81.07	79.72	84.95	4.23	0.599
Miscellaneous[1]	20	20	20	20	20	-	-
Production cost / bird[2]	120.04	125.86	126.07	124.72	129.95	4.23	0.599
Av. body weight (g)	749.94[b]	843.38[a]	864.78[a]	803.60[ab]	765.94[b]	16.4	0.0001
Production cost / kg	160.07[ab]	149.23[b]	145.78[b]	155.21[ab]	169.66[a]	4.79	0.017

"[1] Miscellaneous cost include vaccination cost, farm preparation and brooding expenditures
[2] Production cost per bird = Bird cost + Feed cost per bird + Miscellaneous
a-b values of superscript different in row differ significantly"

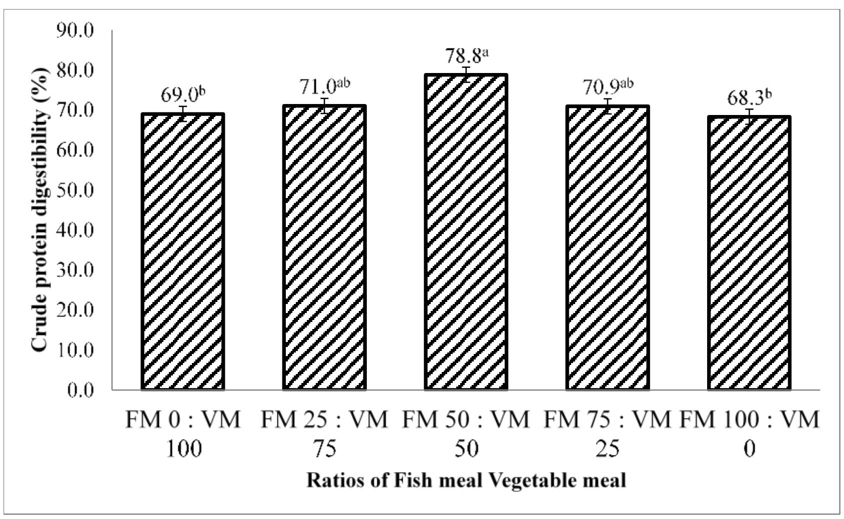

Fig. 1: Effect of different vegetable to animal protein ratios on crude protein digestibility in broiler

50% vegetable protein source diet (50FM) on protein equivalent basis. A lower ($P < 0.05$) CP digestibility was noted in birds fed diet containing no fish meal (Control) and 100% fish meal (Fig. 1).

Economics efficiency

Production cost per kg live weight was lower in birds fed diet containing 25 and 50% replacement of fish meal with vegetable protein sources (Table 6).

Discussion

When fish meal contributed 50% of the dietary protein in broilers' diet, it resulted in higher feed intake, higher BWG and showed the best FCR. Fish meal has been demonstrated to have high biological value, high CP content, high amino acid quality (Médale and Kaushik 2009) and is reported to contain unidentified growth factors. Results are in according with Frempong *et al.* (2019) who showed that replacing FM with SBM resulted in higher BW and FI. Shabani *et al.*

(2018) demonstrated the similar results who reported that the use of fish byproduct meal improved BW and FCR. Findings of Mikulec *et al*. (2004) showed that birds of control group fed FM had higher weight gain and better FCR than those fed SBM. Birds can be shifted from animal sources to vegetable sources or vegetable source to animal sources without compromising their performance (Hossain *et al.* 2014). Results support a study that reported animal and plant protein (50:50) were the most effective in improving live body weight and FCR (Abro *et al.* 2012). It was further demonstrated that feed intake and BWG increased as fish meal level increased from 0 to 2.5% (Karimi 2009). Higher body weight gain and better FCR was reported for birds fed diet containing 50:50 ratios of blood meal and cassava leaf meal (Adeyemi *et al.* 2012). Wang *et al*. (2012) reported that feed cost was decreased and FCR improved by replacing high-protein ingredients like FM, corn gluten meal and SMB with fermented SBM in diet of broiler birds.

Carcass response was higher in birds fed diet containing 50% protein from fish meal and 50% from that vegetable protein sources. Breast yield, neck weight, liver, gizzard, heart and relative abdominal fat weights were not influenced by different ratio of animal to vegetable protein sources. Results are in according with Abro *et al*. (2012) who showed that carcass weight was higher in birds when fed diet containing animal and plant protein (50:50), however, weight of edible and non-edible organ was non-significant. Shifting from animal to vegetable protein sources or *vice versa* reported no effect on carcass response or individual organ weights (Mikulec *et al.* 2004; Hossain *et al.* 2014).

Crude protein digestibility was higher in birds fed diet containing higher fish meal content. This might be due to higher digestibility coefficient of fish meal that is over 90% (Zhou *et al.* 2004). Results are in according with the findings of Sahar *et al.* (2021) who reported that addition of protease in fish meal based diet had improved protein digestibility. Results are consistent with the outcome of Agbede and Aletor (2003) who showed that 50% ratio of fish meal and Glyricidia leaf protein concentrate showed higher nitrogen (N) retention. Improved digestibility of CP and ether extract and reduced excreta ammonia concentration in birds fed fish meal based diets (Shabani *et al*. 2018). The broiler chickens fed PBM based diets improved digestibility for nitrogen compared with corn-soya diets (Mahmood *et al.* 2017).

Conclusion

The dietary protein contributed by vegetable protein ingredients like SBM, canola meal and sunflower meal when substituted on protein equivalent basis with fish meal at 25–75% in broiler starting diets (day 1–21) improved feed intake, BWG, FCR, PER, protein digestibility and net protein utilization. So the blend of protein sources FM/VM was generally better than either vegetable protein or fish meal alone.

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Author Contributions

NS did experimental work, MS write manuscript, MAM, SA and SAB performed experimental designing, SAB and MF performed data analysis, MS and MSR did finalize the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

Data are available on a reasonable request.

Ethics Approval

All the experimental protocols were reviewed and approved by the Departmental Scrutiny Committee of the University of Agriculture, Faisalabad, Pakistan via letter No. 34693-96.

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