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## Haematological parameters of *Cyprinus carpio* with reference to probiotic feed: A machine learning approach

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**Key words:** Haematology; *Lysinibacillus macroides*; Probiotic; R program

### Abstract

The study aims to analyze the haematological parameters of *Cyprinus carpio* with reference to the formulation of the probiotic fortified feeds using a machine learning approach. *C. carpio* fed with pelletized feed, probiotic pelletized feed (5% *Lysinibacillus macroides*), probiotic pearl beads (5% *L. macroides*) and probiotic rice puff (5% *L. macroides*) for 60 days. At the end of the experiments, using blood samples, the haematological indices such as leucocytes, erythrocytes, hemoglobin, hematocrit and packed-cell-volume, were analyzed. Duncan's Multiple Range Test showed that the haematological parameters in control feeding regimes significantly ( $P < 0.05$ ) were low compared with that of the probiotic feeding regimes. The data sets of different feeding regimes were classified using the machine learning method. In the present study, the classifiers like the Random Forest, the Linear Model, and the Decision Tree were employed. To identify the relationship between the features, correlation coefficient and dendrogram were applied. The results of the machine learning method showed high accuracy (98%) in random forest methods followed by the decision tree method. The correlation coefficient between the haematological indices recorded a positive value. But, calculated values of mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular haemoglobin concentration were either low positive or negatively correlated with other haematological indices. Based on the results, the Random Forest, Linear Model and Decision Tree Analysis might be considered for haematological classification of the fish haematological data set.

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## Introduction

Fishes are important organisms in aquaculture industry, because of its rich protein source and omega fatty acids, for maintaining good health (Skonberg and Perkins 2002). Apart from great source of nutrients, fish plays a vital role in human health. Additives in the feed formulation particularly, prebiotic and probiotic, helps in boosting the natural defense of a fish (Gültepe et al., 2011; Amenyogbe et al., 2020). Probiotics are used as immune stimulants in fish farming to overcome the use of antibiotics for fish-related diseases (Ringo 2020). Haematological parameters of fishes are employed as diagnostic tools to study the health status of the fish (Pradhan et al., 2012). In recent years, researchers have been reporting the relationship between biometric parameters and blood parameters of fishes to distinguish the healthy animals from disease-infected animals, and to evaluate the precise health status of the fish (Fazio et al., 2015; 2017). Many authors have reported the influence of haematological parameters of fishes which depends on different types of feed (Dahiya et al., 2012; Nargesi et al., 2020; Ahmadifar et al. 2020). However, very few reports are available related to the haematological indices and immune response of fish health (Ahmed et al. 2020).

Artificial Intelligence (AI) is as of now making tremendous upgrades to the proficiency and sustainability of global aquaculture. Machine learning is the subset of AI, dealing with the design and assessment of algorithms that facilitate pattern recognition, classification and prediction, based on models derived from existing data (Tarca et al., 2007). Machine learning is divided into supervised, unsupervised and reinforcement learning methods (Esteva et al., 2019). Machine Learning, Deep Learning, and Artificial Neural Network (ANN) are used in the medical field for diagnosis of diseases (Nallamuthu and Palanichamy, 2015; Shouval et al., 2021) and clinical trial monitoring was optimized by dynamic programming method (Jennison and Turnbull, 2013). Recently, machine learning methods are applied in taxonomy, environmental modelling and classification of morphometric data in the field of fisheries (Strachan, et al., 1990; Hu et al., 2012a; 2012b). Recently, McGuirl et al. (2020) analyzed the topological data for classification of the zebrafish pattern. Using machine learning method, Gültepe and Gültepe (2020) reported the haematological parameters of the Seabream. The present study classifies the impact of different probiotic formulated feeds on the haematological parameters of *C. carpio* using the machine learning approach.

## Materials and Methods

### Experimental design.

Experiment was conducted using *C. carpio* with mean initial length and weight of  $11.29 \pm 1.24$  cm and  $26.83 \pm 1.33$  g, respectively. Fishes were procured from Tamilnadu fish farm, Kolathur, Chennai. They were immediately transported to the laboratory with proper aeration and acclimatized to the laboratory condition for 24 h. They were divided into 4 groups and fed with pelletized feed without probiotic (Control), probiotic pelletized feed (5% *L. macroides*) (Trial I), probiotic pearl beads (5% *L. macroides*) (Trial II), and probiotic rice puff (5% *L. macroides*) (Trial III). The experiments were conducted for 60 days and they were maintained as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and fed *ad libitum* with respective feed. Culture was maintained in three replicates and stocked with 25 fishes in each replicate. The length and weight of fishes were  $12.34 \pm 1.43$  cm and  $38.57 \pm 4.51$  g respectively.

**Haematological indices.** At the end of the experiments, randomly five fishes were sampled for blood sample collection from three replicates and anaesthetized with Benzocaine (50 mg/L). Blood samples were collected using a heparinized syringe from the caudal vein of the fishes for haematological studies. The haematological parameters, red blood corpuscles (RBC,  $10^6/\text{mm}^3$ ), white blood corpuscles (WBC,  $10^3/\text{mm}^3$ ), haemoglobin (Hb, g/dL), hematocrit (HCT, %), packed-cell-volume (PCV, %), mean corpuscular volume (MCV, fL), mean corpuscular haemoglobin (MCH, pg) and mean corpuscular haemoglobin concentration (MCHC, g/dL) were determined and calculated (Eyiunmi et al. 2018). The haematological parameters were classified using four types of data sets.

**Statistical analysis.** The obtained data sets were first statistically analyzed using SPSS 21.0 ver. Descriptive statistics, mean and standard deviation were calculated. One way ANOVA was analyzed between the different feeding regimes of haematological parameters of the *C. carpio*. Duncan's Multiple Range Test (DMRT) were performed between the mean difference at  $P < 0.05$ . The data is represented as mean  $\pm$  SD and  $P < 0.05$  is considered as significant.

**Machine learning approach.** Data quality was assessed before the analysis with machine-learning methods. The obtained data were subjected to machine learning using the R-project. The data set are represented in **Table 1**. The data was classified employing the Random Forest (RF), Linear Model (LM), and Decision Tree (DT). To identify the relationship between the attributes, correlation coefficient and Heat Map procedures were applied. In the present study, the data sets were efficiently run in machine learning methods with randomly selected 70% training set and 30% test set.

**Table 1** Data set of haematological parameters of *C. carpio* fed with different formulations of probiotic feed

	RBC	WBC	HB	HCT	PCV	MCV	MCH	MCHC
C	2.65	21.55	9.66	26.53	32.21	100.11	36.45	36.41
C	2.86	19.22	9.55	30.89	32.15	108.01	33.39	30.92
C	2.05	24.45	9.28	27.89	28.22	136.05	45.27	33.27
C	2.71	21.11	9.01	27.06	31.07	99.85	33.25	33.30
C	2.66	22.84	9.34	28.17	30.68	105.90	35.11	33.16
T1	4.01	30.11	12.88	38.17	41.24	95.19	32.12	33.74
T1	3.88	36.57	15.49	35.42	39.24	91.29	39.92	43.73
T1	3.78	34.11	14.55	32.55	35.12	86.11	38.49	44.70
T1	4.11	35.18	15.25	36.47	38.14	88.73	37.10	41.82
T1	3.65	35.21	15.67	35.27	39.28	96.63	42.93	44.43
T2	2.88	25.55	11.41	32.55	36.15	113.02	39.62	35.05
T2	3.06	33.22	12.85	32.44	34.25	106.01	41.99	39.61
T2	3.55	30.45	11.77	36.11	30.78	101.72	33.15	32.59
T2	3.18	34.22	12.98	37.28	34.88	117.23	40.82	34.82
T2	3.21	35.24	13.04	38.01	35.17	118.41	40.62	34.31
T3	2.84	32.14	13.74	36.11	35.66	127.15	48.38	38.05
T3	3.15	30.88	12.74	35.44	36.17	112.51	40.44	35.95
T3	3.88	28.11	14.55	30.74	33.58	79.23	37.50	47.33
T3	3.47	27.45	14.27	32.17	35.11	92.71	41.12	44.36
T3	3.57	28.15	12.14	33.15	33.07	92.86	34.01	36.62

RBC – Red Blood Cell( $10^6$  cells /mm<sup>3</sup>); WBC – White Blood Cell( $10^3$  cells/mm<sup>3</sup>); HB – Haemoglobin(g/dL); HCT – Haematocrit(%); PCV – Packed-Cell-Volume(%); MCV – Mean Corpuscular Volume(fL); MCH – Mean Corpuscular Haemoglobin(pg); MCHC – Mean Corpuscular Haemoglobin Concentration(g/dL)

## Results

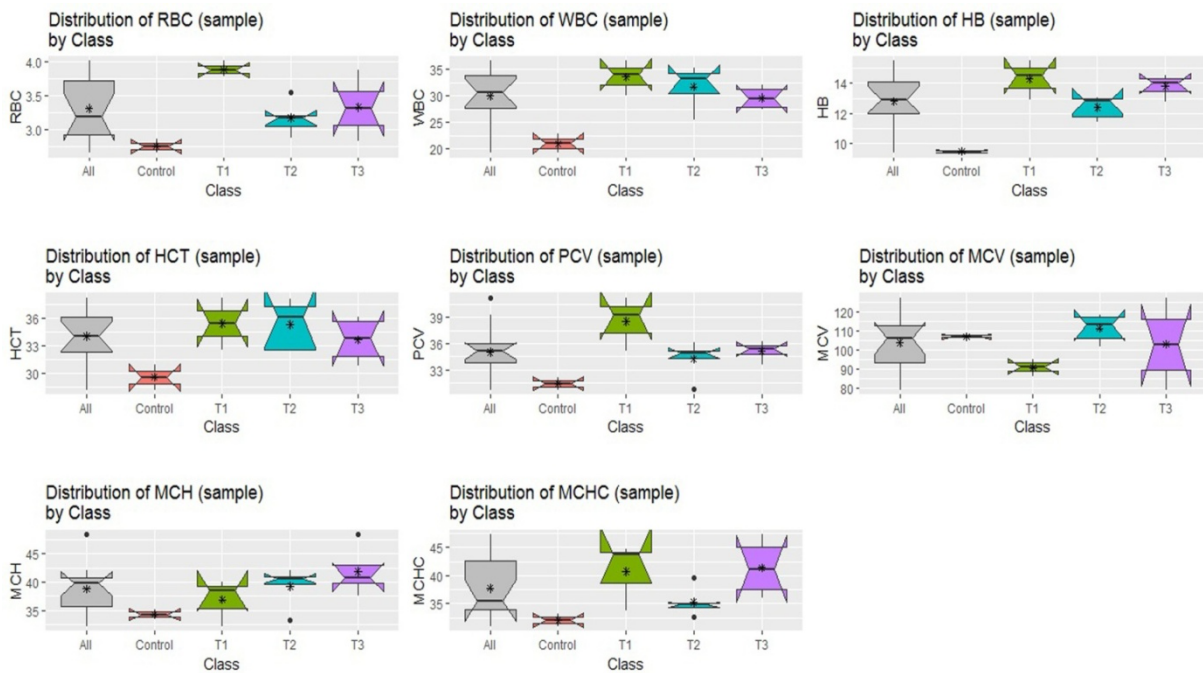
Haematological indices of fish *C. carpio* were fed with different formulations of probiotic feed depicted in **Table 2**. The haematological indices (RBC, HB, HCT, PCV) of *C. carpio* were recorded in the order of Trial 1 (T I) > Trial III (T III) > Trial II (T II) > control (C) feeding regimes. Statistical analysis, ANOVA for blood parameters of *C. carpio* fed in the experimental feeding regimes showed significantly increase ( $P < 0.05$ ) in haematological indices except for MCV ( $P > 0.05$ ). Post-hoc DMRT's indicated significantly low value of haematological parameters in the control feed fed fishes, compared to the fishes fed in the experimental feeds (T1, T2 and T3) ( $P < 0.05$ ) (**Table 2**).

**Table 2** Haematological indices of *C. carpio* fed with different formulations of probiotic feed\*

Blood Parameters	C	T1	T2	T3
RBC ( $10^6$ cells /mm <sup>3</sup> )	2.58 ± 0.31 <sup>a</sup>	3.88 ± 0.18 <sup>c</sup>	3.17 ± 0.24 <sup>b</sup>	3.38 ± 0.39 <sup>b</sup>
WBC ( $10^3$ cells/mm <sup>3</sup> )	21.83 ± 1.95 <sup>a</sup>	34.23 ± 2.46 <sup>c</sup>	31.73 ± 3.89 <sup>bc</sup>	29.34 ± 2.04 <sup>b</sup>
HB (g/dL)	9.36 ± 0.25 <sup>a</sup>	14.76 ± 1.13 <sup>d</sup>	12.41 ± 0.76 <sup>b</sup>	13.48 ± 1.02 <sup>c</sup>
HCT (%)	28.10 ± 1.68 <sup>a</sup>	35.57 ± 2.04 <sup>b</sup>	35.27 ± 2.62 <sup>b</sup>	33.52 ± 2.24 <sup>b</sup>
PCV (%)	30.86 ± 1.36 <sup>a</sup>	38.60 ± 2.24 <sup>c</sup>	34.24 ± 2.05 <sup>b</sup>	34.71 ± 1.33 <sup>b</sup>
MCV (fL)	109.98 ± 15.01 <sup>b</sup>	91.59 ± 4.37 <sup>a</sup>	111.28 ± 7.28 <sup>b</sup>	100.89 ± 1.89 <sup>ab</sup>
MCH (pg)	36.69 ± 4.97 <sup>a,b</sup>	38.11 ± 3.98 <sup>ab</sup>	39.24 ± 3.50 <sup>b</sup>	40.29 ± 5.32 <sup>b</sup>
MCHC (g/dL)	33.41 ± 1.95 <sup>a</sup>	41.68 ± 4.58 <sup>b</sup>	35.57 ± 2.60 <sup>ab</sup>	40.46 ± 5.08 <sup>b</sup>

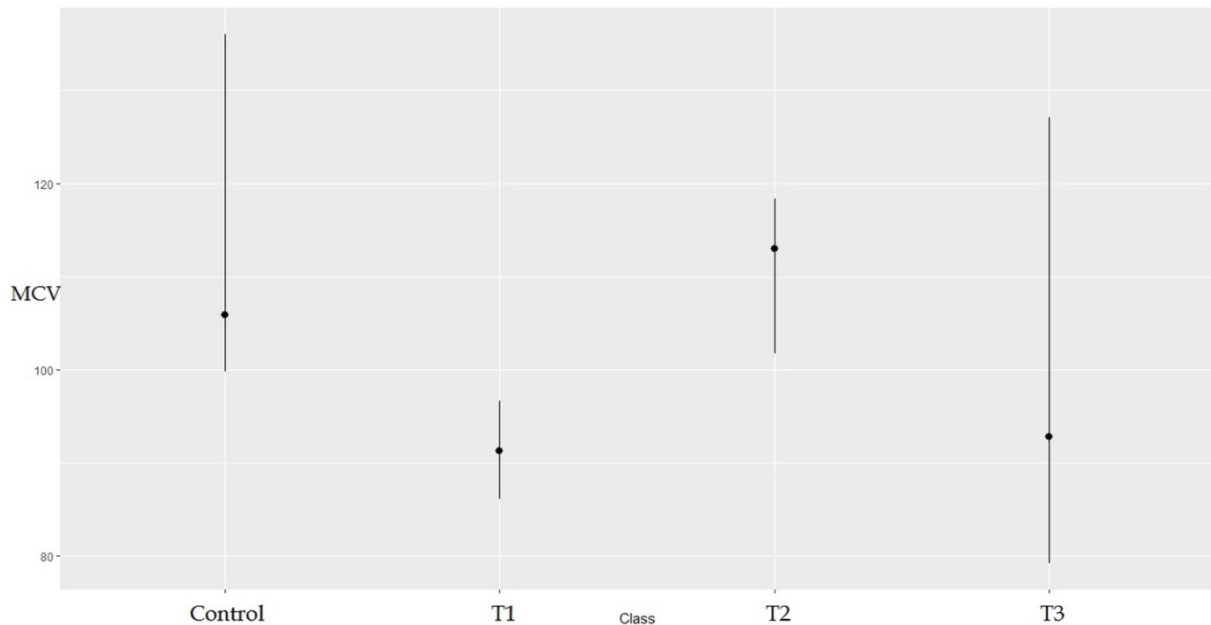
\*The values are represents as Mean±SD; Anova followed by DMRT's performed; Different superscripts in rows shows significantly different at P < 0.05 level

**Figure 1** shows the features with respect to the class label, it is deduced that the distribution of control class in MCV was overlapping with other classes T1, T2, T3 and for the remaining, attributes control was separated from T1, T2 and T3.



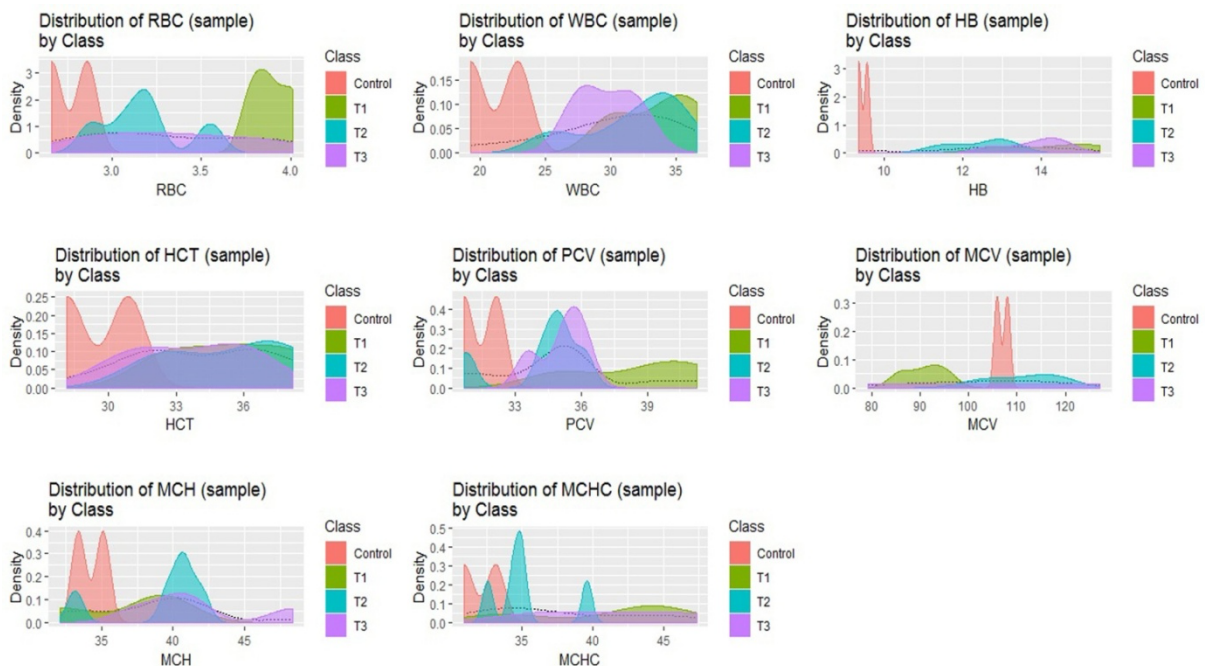
**Figure 1** Distribution of haematological parameters of *C. carpio* by different feeding regimes using box plot.

In **Figure 2**, the statistical summary of MCV for each unique Class label Control, T1, T2 and T3, and the inference was that the values of MCV for individual class labels are overlapping, and it is difficult to identify the class labels using MCV.



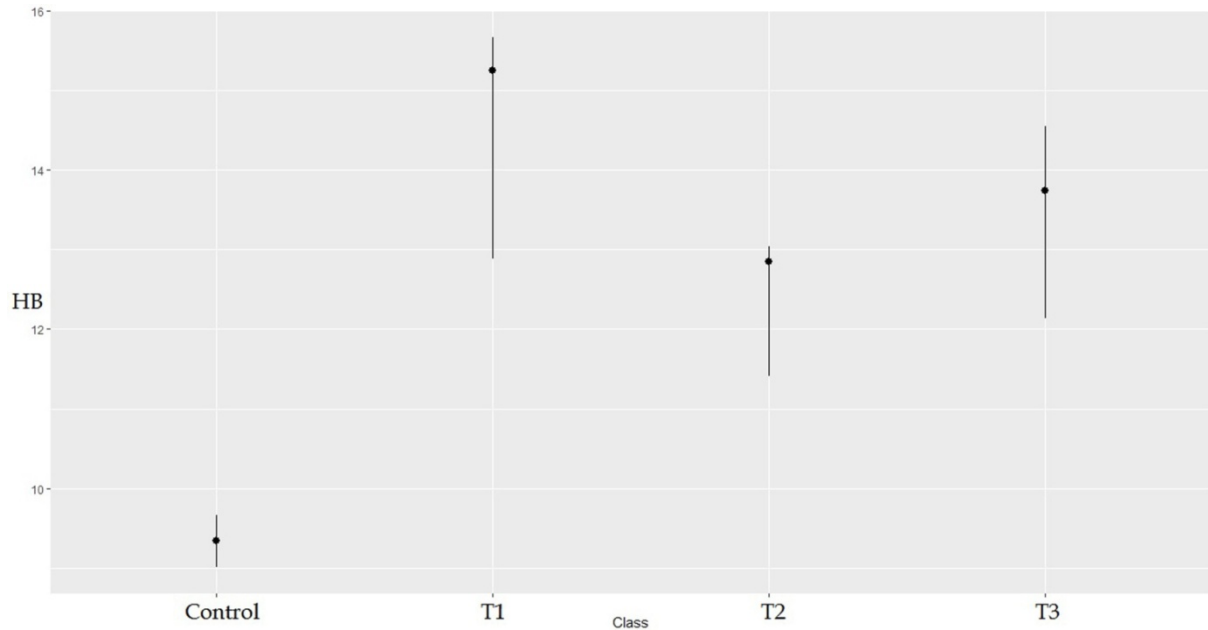
**Figure 2** Summary plot of MCV of *C. carpio* respect to different feeding regimes

In **Figure 3** shows the distribution of features by class labels using histograms. From the figure, it is observed that the value of HB in the Control class was within 10 and there was no overlapping with other class labels T1, T2 and T3.



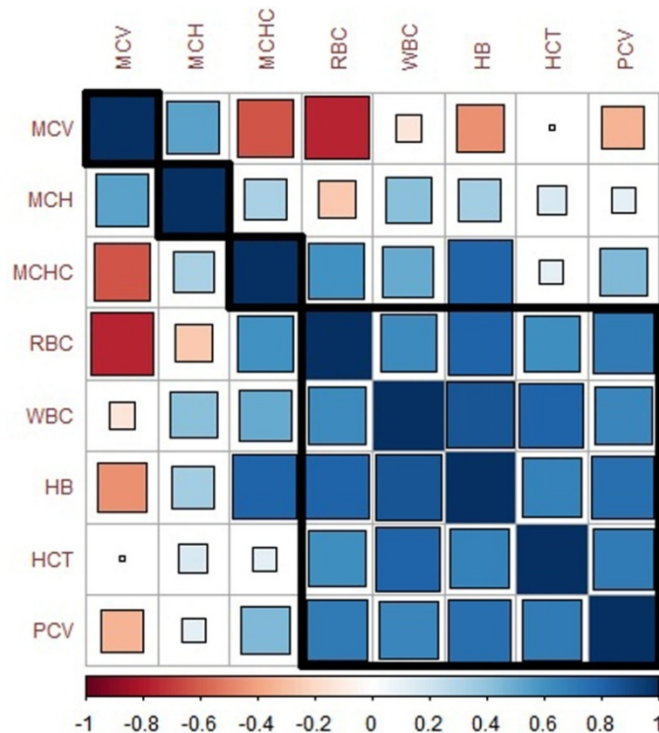
**Figure 3** Distribution of haematological parameters of *C. carpio* by different feeding regimes using histogram density plot

**Figure 4** represents the summary plot of HB with respect to the class labels. When the value of HB was less than 10, it was labelled as control.



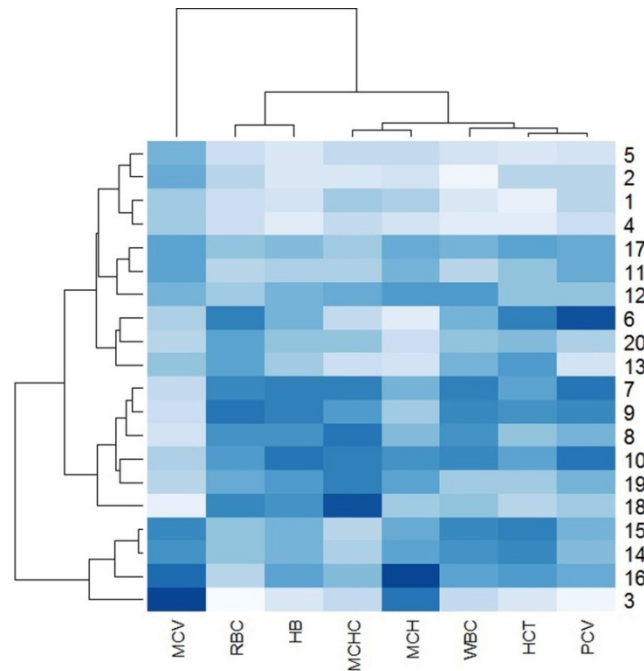
**Figure 4** Summary plot for HB of *C. carpio* with respect to different feeding regimes

The correlation between the parameters shows that, there was a high positive correlation between the features of haematological parameters, whereas calculated values (MCV, MCH and MCHC) were negatively correlated with other haematological parameters (**Figure 5**).



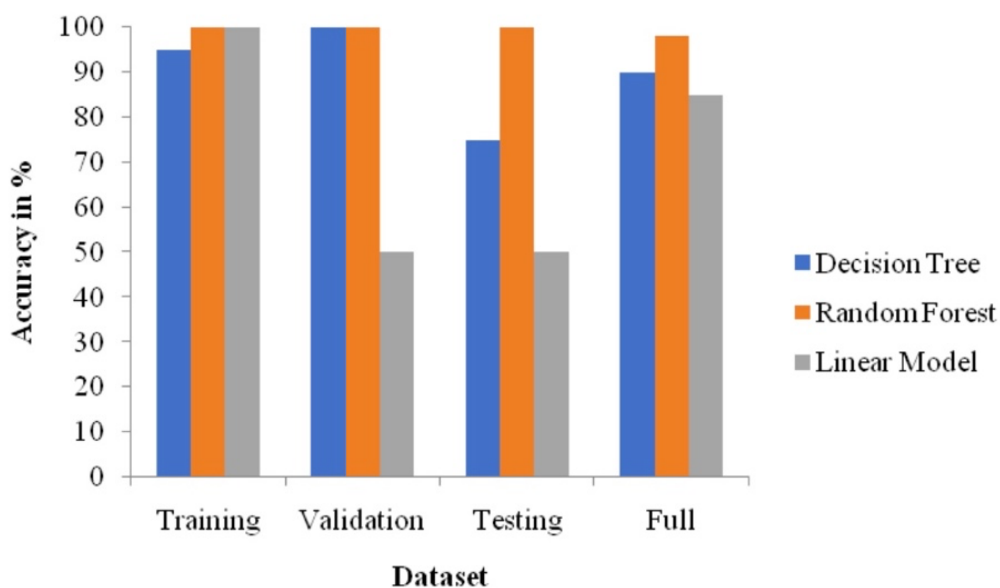
**Figure 5** Correlation co-efficient between the *C. carpio* haematological parameter

The heat map obtained for a given dataset is presented in **Figure 6**. It reorders both features and observations using a hierarchical agglomerative clustering algorithm. It computes the distance between each pair of rows and columns and tries to arrange them by similarity. The corresponding dendrogram is provided beside the heatmap. The x-axis represents the haematological parameters and the y-axis represents the observations of the sample. It deduces the distance between HCT and PCV which is small and the similarity between the observations 14 and 15 is high.



**Figure 6** Heat map of *C. carpio* haematological parameters

The decision tree root node represents the 100% of observations that were used at the node and the probability of occurrence is 14%, 21%, 36% and 29% for the class labels of Control, T1, T2 and T3, respectively, for the given condition  $MCV \geq 98$ . The bottom level leaves show 64% of observations which are classified using  $WBC < 24$  and 36% observations are classified using  $WBC \geq 29$ . In this work, three different classifier models Decision Tree, Random Forest and Linear Model were applied and 70% of the available data is allocated for training. The remaining 30% of the data are equally partitioned as validation and test data sets. The accuracy obtained from the classifier models using training, validation, testing and entire dataset is shown in Figure 8. The Random Forest gives 100% accuracy for training, validation, testing and 98% in the entire dataset. However, validation, testing and the entire dataset was 50%, 50%, and 85% respectively, in the Linear Model (**Figure 7**)



**Figure 7** Accuracy obtained from the classifier models

### Discussion

In fisheries study, only a few reports are available related to the machine learning approach. In general, recognition of pattern data (Mendoza et al., 2011), population (Trifonova 2016), morphological pattern (Cui et al., 2020; Güney and Atasoy 2015) and environmental (Michael et al., 2019) studies were reported in fishery science.

In the present study, classification of haematological indices using machine learning approach in fish with reference to probiotic feed was attempted. Gultepe and Gultepe (2020) analyzed the haematological parameters of the sea bream, by the machine learning methods and reported that the hematocrit value that there was a difference in the manooligosaccharides feed, compared to the control feed. Coz-Rakovac et al (2008) classified the biochemistry profile of three different fishes affected during aquaculture activities. Rahman and D'Este (2015) stated that the decision tree method assessed and attained the best balancing algorithm for predicting the fish location.

The machine learning approaches consist of Decision Tree, Random Forest and Linear Model, which were applied to the haematological parameters of *C. carpio*. Descriptive statistics is an important step before machine learning for looking at the observations from the dataset. Boxplot and histogram density plot are applied to all the features with respect to the class labels to know the distribution of the features. The correlation coefficient and heat map are employed to identify the relationships between the features in a high dimensional space.

The machine learning approach used in the current study gives high accuracy for Random Forest. The machine learning approach and the descriptive statistical models are developed using the R statistical package, provides a systematic prediction of assumed cases, present state, future consequences and possible remedies of any problem in current biology. This study substantiates, haematological parameters of *C. carpio* fed with probiotics added feed are better than the control feed. The results of the present study were encouraging but there is a need to investigate this approach in greater depth. More research should be conducted to focus light on this novel tool and its application in haematological indices of fishes. As this result provides better knowledge about machine learning and its application, in future, we aim to extend this R based machine learning approach for predicting fish-related diseases accurately.



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