Journal of Animal Research: v.7 n.5, p. 841-848. October 2017
DOI: 10.5958/2277-940X.2017.00129.2

Impact analysis of Feeding Fermented Karonda (*Carissa carandas*) Whey Beverage on Growth Performance and Haematological Parameters of Albino Rats

Pushkraj J. Sawant*, Dinesh C. Rai, Vinod K. Paswan and Dilip Kumar

Department of Animal Husbandry & Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, INDIA

**Corresponding author: PJ Sawant; Email: sawantpushkraj@gmail.com*

Received: 10 May, 2017 **Revised:** 07 Aug., 2017 **Accepted:** 12 Aug., 2017

ABSTRACT

The present study was conducted to evaluate the impact of feeding karonda whey beverage (FKWB) on growth performance and haematological parameters of albino rats. A total of 24 albino rats (male) of age 28±3 days and weighing 80 to 95 gm were randomly selected and divided into four groups (n=6) and assigned to experimental dietary treatment. Experimental groups consisted of control group (G_1), while treatment groups fed with basal ration along with 10, 20 and 30% FKWB in groups G_2, G_3 and G_4, respectively. Results showed that the FKWB fed groups had significantly ($P<0.05$) higher feed intake as compared to control, However, the body weight gain was comparable and did not differ significantly in all the experimental groups. Feed efficiency ratio was lower ($P<0.05$) in FKWB fed groups compared to control. In treatment groups there were significantly increased in haemoglobin level ($P<0.05$), whereas, RBC and WBC were comparable in all the groups. The supplemented groups had significantly ($P<0.05$) higher HCT, MCV, MCH and MCHC values, However, the platelet count was statistically similar in all the groups.

Keywords: Fermented whey beverage, Karonda (*Carissa carandas*), growth performance, albino rats

In recent times, there is growing trend for use of herbal medicines for curing various ailments and for maintenance of good health (Kone *et al.*, 2007). Nearly 80% of world's population makes use of herbal medicines for health care delivery (WHO, 2002). Herbal medicines are prepared using plant extracts or their bioactive compounds derived from medicinal plant parts like leaves, fruits, barks, roots, seeds, etc. Karonda (*Carissa carandas*) is common herb of Apocynaceae family, which is hardy, drought tolerant and mainly grows in semi-arid regions of India. It bears berry sized fruits that are rich in various phytochemical constituents, iron and vitamin C content (Verma *et al.*, 2011). The fruits are therefore used ethno-medically to cure anemia, scurvy, allergies, tumors, ulcers, platelet aggregation and hypertension, etc. (Malik *et al.*, 2010; Arif *et al.*, 2016). Edible portion of 100 gm of karonda fruits provides 42.5 kcal energy, 0.39-1.1 g protein, 2.5-4.63 g fat, 0.51-2.9 g carbohydrate, 0.62-1.81 g fibre, 21 mg calcium, 28 mg phosphorous, 1619 IU vitamin A and 9-11 mg ascorbic acid (Malik *et al.*, 2010).

Whey is highly nutritious by product of milk, which possess half of milk solids. It is obtained during manufacturing of casein, channa, paneer, cheese and shrikhand and is rich in lactose, water soluble vitamins, minerals and proteins (Durham *et al.*, 1997). Whey proteins is a mixture of globular proteins which compromises of α-lactoalbumin, β-lactglobulin, glycomacropeptides, immunoglobulins, bovine serum albumin and lactoferrin (Foegeding *et al.*, 2011). Whey proteins are nutritionally valuable proteins as they are rich in essential amino acids (EAAs) notably lysine, cysteine, methionine and cystine (Jelicic *et al.*, 2008) and branched-chain amino acids (BCAAs) *viz.*, leucine, isoleucine and valine. The whey is not only rich in EAAs and BCAAs as compared other plant and animal based protein sources but also more efficiently absorbed and utilized (Harper, 2000). Whey is even useful in curing ailments such as arthritis, anemia and liver complaints (Jelen, 1992).

Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus* are typical bacteria's of yoghurt culture. The yoghurt culture is confirmed as probiotic due its ability to survive in intestinal ecosystem and to impart health benefits to person consuming it (Guarner *et al.*, 2005). The consumption of yoghurt culture aid in preventing gastrointestinal conditions such as lactose intolerance, constipation, diarrheal diseases, colon cancer, inflammatory bowel disease, *Helicobacter pylori* infection, and allergies (Mokoena *et al.,* 2016; Balamurugan *et al.,* 2014). Yoghurt culture even has controlling influence on immune system of individuals consuming it (Van de Water *et al.*, 1999).

Thus, the aim of present study was to determine the effect of supplementation of fermented karonda whey beverage on growth performance and haematological parameters of albino rats to further consolidate the above mentioned facts and to access potential nutritive and therapeutic effect of their supplementation in combination.

MATERIALS AND METHODS

The entire experiment was conducted in the laboratory of Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P., India. The study was approved by Central Ethical Committee of University (No. Dean/2016/CAEC/46).

Preparation of fermented karonda whey beverage

The fermented karonda whey beverage (FKWB) was prepared using standardized and established processing parameter. The ingredients used for preparation of FKWB were procured from local market of Varanasi. The FKWB mixture composed of whey and karonda juice in ratio of 79.68:20.32, respectively, to which 9.77% sugar and 0.4% carboxy methyl cellulose (CMC) stabilizer was added. The mixture was pasteurized at 85ºC for 5 minutes and allowed to cool to 37ºC, which was then inoculated with 2% yoghurt culture (Code no. NCDC-144 procured from NDRI, Karnal (Haryana), India) composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The mixture was incubated for 15 hours at 37±1ºC and then stored at 4±1ºC till further use.

Preparation of basal ration

The ingredients of basal ration i.e. milk, maize, soyabean meal and bengal gram was procured from Dairy Farm of University, while soyabean oil and mineral mixture was procured from local market of Varanasi. The basal ration was formulated as per NRC (1995) to meet the nutritional requirement of rats, which composed of 54% maize, 18% bengal gram, 20% soyabean meal, 2% soyabean oil, 4% milk and 2% mineral mixture. The fresh homogenous mixture of basal ration was prepared twice a day prior feeding.

Experimental animals

Out of 100 albino rats, 24 albino rats of similar sex (male) and body conformation were selected at 28±3 days of age for the study from Animal House of University. The rats having body weight ranging from 80 to 95 gm were randomly divided in four groups (n= 6). The rats were caged in polycarbonate cages, with free access to feeder bowl and plastic bottle filled with fresh and purified water.

Study design

The study to access the impact of dietary treatment was carried out for 28 days (4 weeks). The feeding schedule of different treatment groups was as follows:

- ❒ Group 1 (G_1) - Control group with basal ration only
- ❒ Group 2 (G_2) - Basal ration + 10% FKWB
- ❒ Group 3 (G_3) - Basal ration + 20% FKWB
- ❒ Group 4 (G_4) - Basal ration + 30% FKWB.

The rats were fed twice daily at 8.00 am and 4.00 pm and the feed residue left was measured daily. The behaviour of the rats was observed daily, and their weights were recorded weekly. At the end of experiment, three rats (off feed for 24 hours) of each group were transferred to the box possessing cotton pads treated with anesthetic ether and were allowed to lose their conscious. Anesthetized rats were pinned up on the dissection tray and blood was sucked from the heart by disposable syringe. Blood from each rat was collected separately. Blood was collected with addition of EDTA for heamatological parameters estimation.

Haematological parameter measurement

Haemoglobin and WBC were measured by using automated Coulter HMX haematology analyzer. Red blood cells count (RBC) was determined by using a neubauer chamber and an optical microscope (400 x magnifications). Haematocrit (HCT) of whole blood was estimated with the help of microhaematocrit capillary centrifuge. It is measured as the height of the red cell column in a microhaemotocrit tube after centrifugation. Platelets (PLT) in blood were determined by cone and platelet analyzer (CPA) method (Harrison, 2005). MCH, MCV, MCHC was calculated using standard formulae.

Statistical analysis

The data were statistically evaluated by one way analysis of variance (ANOVA) using SPSS software (Version: IBM SPSS Statistic 23). Duncan multiple range tests (Montgomery, 1977) were used to detect differences among mean values at level of 0.05. Data are presented as mean ± standard error of the mean (Mean±SE).

RESULTS AND DISCUSSION

Growth performance

The growth performance parameters accessed during study of 28 days are presented in Table 1, 2 and 3. It can be seen from Table 1 that feed intake was significantly ($P<0.05$) higher in groups fed on fermented karonda whey beverage (FKWB) as compared to control group. The highest feed intake was observed in group G_2, which was at par with groups G_3 and G_4. Similar result was reported by Halaby *et al.* (2015) in rats fed on cow milk yoghurt. Irrespective to groups, the feed intake increased significantly ($P<0.05$) with every passing week as the age of rats increased. This was only due to biological demand for physiological functions of body of the growing rats. The similar finding was reported by Patel *et al.* (2010) in rats fed on yoghurt and by Stefanut *et al.* (2015); Seifi (2013) and Ghaemi *et al.* (2014) in broiler chicks fed on probiotic supplementation and apple pulp, respectively.

The body weight gain was significantly ($P<0.05$) highest in group G_2, but there was gradual decrease in body weight gain with increase in level of FKWB (G_3 and G_4), which was lower than control group. Although the differences in between the groups body weight gain was not that significant. The similar result was observed in rats fed on *Carissa carandas* fruit extract (Dhodi *et al.,* 2015). Apata *et al.* (2008) reported no significant differences in body weight gain among groups of broiler chicks fed on *lactobacillus bulgaricus* supplemented diet. The amount of feed intake was seen to have no significant effect on the body weight gained. Halaby *et al.* (2015) observed no significant effect of feed intake on body weight gain of albino rats fed on milk and yoghurt. Irrespective of groups, the body weight gain per rat per week increased significantly ($P<0.05$) with every passing week as the age of the rats increased. This may be due physiological changes taking place in body with increase in age.

Table 1: Impact of feeding fermented karonda whey beverage on the feed intake (gm)/rat/day

	Weeks					
Groups	**W_1**	**W_2**	**W_3**	**W_4**	**Total**	**Average**
G_1	42.26^{dD}	48.37^{dC}	54.72^{dB}	57.53^{dA}	202.88	50.72 ± 1.231^{b}
G_2	49.15^{aD}	59.29^{aC}	62.00^{aB}	77.15^{aA}	247.59	61.90 ± 2.090^{a}
G_3	48.88^{bD}	59.00^{bC}	59.70^{bB}	76.81^{bA}	244.39	61.10 ± 2.092^{a}
G_4	43.33^{cD}	52.79^{cC}	57.46^{cB}	76.48^{cA}	230.06	57.52 ± 2.518^{a}
Total	183.62	219.45	233.88	287.97		
Average	45.91 ± 0.654^{d}	54.86 ± 0.951^{c}	58.47 ± 0.562^{b}	71.99 ± 1.742^{a}		

Means in the same column with different small superscript are significantly different at 5% level, Means in the same row with different capital superscript are significantly different at 5% level (Duncan test, $P<0.05$)

Table 2: Impact of feeding fermented karonda whey beverage on the body weight gain (gm)/rat/day

	Weeks					
Groups	W_1	W_2	W_3	W_4	Total	Average
G_1	17.18 ^{bcD} ± 0.265	18.42 ^{bC} ± 0.250	19.33 ^{aB} ± 0.238	20.26 ^{bA} ± 0.224	75.19	18.80 ^{b} ± 0.264
G_2	18.52 ^{aD} ± 0.292	20.36 ^{aC} ± 0.242	19.44 ^{aB} ± 0.258	21.40 ^{aA} ± 0.209	79.72	19.93 ^{a} ± 0.252
G_3	16.43 ^{cD} ± 0.262	17.32 ^{cC} ± 0.252	18.47 ^{bB} ± 0.230	19.37 ^{cA} ± 0.214	71.59	17.90 ^{c} ± 0.258
G_4	17.28 ^{bB} ± 0.253	17.41 ^{cB} ± 0.228	17.47 ^{cB} ± 0.211	19.32 ^{cA} ± 0.245	71.48	17.87 ^{c} ± 0.207
Total	69.41	73.51	74.71	80.35		
Average	17.35 ^{c} ± 0.200	18.38 ^{b} ± 0.279	18.68 ^{b} ± 0.198	20.09 ^{a} ± 0.204		

Values are mean± standard error of six replicates per treatment. Means in the same column with different small superscript are significantly different at 5% level, Means in the same row with different capital superscript are significantly different at 5% level (Duncan test, P<0.05)

Table 3: Impact of feeding fermented karonda whey beverage on the feed efficiency ratio/rat/day

	Weeks				
Groups	W_1	W_2	W_3	W_4	Average
G_1	0.407 ^{aA} ±0.006	0.381 ^{aB} ±0.005	0.353 ^{aC} ± 0.004	0.352 ^{aC} ± 0.032	0.373 ^{a} ± 0.005
G_2	0.377 ^{bA} ± 0.006	0.343 ^{bB} ± 0.004	0.313 ^{bC} ± 0.004	0.277 ^{bD} ± 0.003	0.328 ^{b} ± 0.008
G_3	0.336 ^{cA} ± 0.005	0.294 ^{dC} ± 0.004	0.310 ^{bB} ± 0.004	0.252 ^{cD} ± 0.003	0.298 ^{c} ± 0.007
G_4	0.399 ^{aA} ± 0.006	0.330 ^{cB} ± 0.004	0.304 ^{bC} ± 0.004	0.253 ^{cD} ± 0.003	0.321 ^{b} ± 0.011
Average	0.380 ^{a} ± 0.006	0.337 ^{b} ± 0.007	0.320 ^{b} ± 0.004	0.284 ^{c} ± 0.009	

Values are mean± standard error of six replicates per treatment. Means in the same column with different small superscript are significantly different at 5% level, Means in the same row with different capital superscript are significantly different at 5% level (Duncan test, P<0.05)

Feed efficiency ratio (FER) is the mass of the output divided by input, which signifies animal's efficiency in converting feed mass into increases of the desired output. The FER was significantly (P<0.05) higher in control group as compared to groups fed on FKWB. Whereas, FER was significantly (P<0.05) higher in first week, it was lowest in last week. The reason for this lower FER might be lower body weight gain at higher rate of feed intake. The similar results were reported by authors, who fed broiler chicks (Seifi, 2013; Khan *et al.,* 2011), mices (Stefanut *et al.,* 2015), rats (Halaby *et al.,* 2015) and calves (Noori *et al.,* 2016) with probiotic supplemented diets. On contrary to present investigation Foo *et al.* (2003) reported greater feed efficiency ratio in rats fed on fermented fruits.

Haematological parameters

The effect of dietary treatments on haematological parameters of rats is presented in table 4. Haemoglobin is iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (Maton *et al.,* 1993). Haemoglobin content of rats was significantly (P<0.05) higher and at par with each other in groups fed on FKWB as compared to control group. The reason for higher haemoglobin content in FKWB fed groups may be due to higher iron content in karonda fruit and presence of yoghurt bacteria in beverage. The iron content in karonda fruit is 39.1 mg/100 gm of edible portion (Anonymous, 1950). Schoorl *et al.* (2012) reported increase in haemoglobin content on iron supplementation. Karonda fruit is useful in curing anemia, a condition in which blood lacks enough healthy red blood cells or haemoglobin (Bhaskar *et al.,* 2009). Lactic acid bacteria produce acetic acid which acts a precursor to formation of haemoglobin (Guyton and Hall, 2006). The similar results for increase in haemoglobin content were observed on feeding fruit juices of fruits such as phalsa cherry (Ali, 2009), lemon (Riaz *et al.,* 2014), Indian mulberry (Saminathan *et al.,* 2014) and wild orange (Uddin *et al.,* 2014) to rats and other animals. While increase in haemoglobin content on feeding formulations containing yoghurt bacteria was

Table 4: Impact of dietary treatment on haematological parameters of albino rats

Groups	Haemoglobin (gm/dl)	WBC (10^3/mm^3)	RBC (million/mm^3)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Platelets (lacs/mm^3)
	Haematological parameters							
G_1	9.67 ^{b} ± 0.84	4.43 ^{b} ± 0.318	5.81 ^{b} ± 0.491	33.17 ^{b} ± 3.048	57.03 ^{b} ± 0.784	16.63 ^{c} ± 0.120	29.20 ^{b} ± 0.586	3.69 * ± 0.450
G_2	12.17 ^{a} ± 0.467	3.97 ^{b} ± 0.186	6.89 ^{a} ± 0.208	41.30 ^{a} ± 1.419	59.90 ^{a} ± 0.709	17.63 ^{b} ± 0.167	29.47 ^{b} ± 0.233	3.87 * ± 0.410
G_3	11.93 ^{a} ± 0.376	4.63 ^{b} ± 0.694	6.62 ^{ab} ± 0.147	39.70 ^{a} ± 1.222	59.97 ^{a} ± 0.669	18.00 ^{b} ± 0.173	30.07 ^{b} ± 0.373	3.21 * ± 0.026
G_4	12.33 ^{a} ± 0.088	6.73 ^{a} ± 0.145	6.07 ^{ab} ± 0.090	35.27 ^{ab} ± 0.498	58.23 ^{ab} ± 0.348	20.37 ^{a} ± 0.219	34.97 ^{a} ± 0.260	2.98 * ± 0.251

Values are mean± standard error of three replicates per treatment. Means in the same column with different letters are significantly different at 5% level (Duncan test, P<0.05)

reported by Hossian *et al.* (2005), Islam *et al.* (2004) in broiler chicks and by Salahuddin *et al.* (2013) in mice.

The RBC count was significantly (P<0.05) higher in FKWB fed groups as compared to control. The reason for the higher RBC count may be due to presence of yoghurt bacteria and due to proteins, vitamins and minerals presence in whey and karonda juice. Lactic acid bacteria reduce deformation of erythrocyte via improved membrane fluidity or elasticity, in turn improving ability to maintain erythrocyte integrity (Ganong, 1985). Robin *et al.* (1998) proposed that RBC count decreases in harmful environment, while in present study the RBC count was higher in FKWB treated groups, confirming its safety for consumption. The findings of present investigation corroborate well with El-Ishaq *et al.* (2014), Marc *et al.* (2013), Valentova *et al.* (2007), Uddin *et al.* (2014) and Riaz *et al.* (2014) who fed fruit juices of blood plum, cashew apple, cranberry, wild orange and lemon, respectively to the rats. Lollo *et al.* (2013) and Khan *et al.* (2011) reported similar results on feeding yoghurt bacteria incorporated whey and probiotic supplementation to rats and broiler chicks, respectively.

WBC count was significantly (P<0.05) highest in rats fed on 30% FKWB (G_4), while it was at significantly (P<0.05) low and at par with each other in remaining groups. Although differences in between WBC count in groups was not that significant, suggesting that overall immune function has not been compromised on feeding FKWB. The similar results for increase in WBC count but not that significantly different among treatments was reported by other authors on feeding juice of fruits such as watermelon juice (Oyesola *et al.,* 2015), pomegranate (Bhandary *et al.,* 2013), Indian mulberry or noni (Mhatre and Marar, 2016 and Saminathan *et al.,* 2014) and lemon (Riaz *et al.,* 2014) to rats, while increase in WBC count in laying hen by feeding apple pulp was reported by Ghaemi *et al.* (2014). On contrary, El-Ishaq *et al.* (2014) reported decrease in WBC count on feeding blood plum juice to albino rats. Apata (2008) reported increase in WBC count in broiler chicks from 9.34 to 13.49 (10^9 L^{-1}) on feeding diet supplemented with 80 mg/kg *lactobacillus bulgaricus* (LB) and attributed this increase to presence of LB in diet stimulating the production of lymphocytes, particularly B types, which are responsible for forming antibodies that provides humoral immunity (Guyton and Hull, 2006). The present results for WBC count corroborated with findings reported by Khan *et al.* (2011), Khan *et al.* (2013) and Stefanut *et al.* (2015) on feeding probiotic formulations to broiler chicks, crossbred cockerels and mice, respectively.

Haematocrit (HCT) is the volume percentage of RBC's in blood. HCT is considered as a point of reference for RBC's capability of delivering oxygen (Prange *et al.,* 2001). HCT was significantly (P<0.05) higher in FKWB fed groups as compared to control group. The higher HCT in FKWB fed groups may be due to higher haemoglobin and RBC in these groups, as these three components have positive correlation (Swenson, 1977) and due to consumption of mineral and protein rich product. Similar results were reported by authors feeding fruit juices (Marc *et al.,* 2013; Uddin *et al.,* 2014 and Riaz *et al.,* 2014) and yoghurt bacteria incorporated whey beverage (Lollo *et al.,* 2013) to rats.

Mean corpuscular volume (MCV), also known as mean cell volume is a measure of average volume of RBC. Mean corpuscular haemoglobin (MCH) is average mass of haemoglobin per RBC, while mean corpuscular haemoglobin concentration (MCHC), is a measure of concentration of haemoglobin in given volume of packed red blood cells. MCV values were significantly ($P<0.05$) higher in FKWB fed groups as compared to control. The MCH and MCHC values increased with increase in FKWB level administered to rats, and it was significantly ($P<0.05$) higher in groups fed with FKWB then control. The higher MCH and MCHC values in FKWB fed groups can be attributed to high haemoglobin content in these groups. Although not that significant differences in between groups were observed for MCV, MCH and MCHC. The findings in present investigation for MCV, MCH and MCHC are in corroboration with findings reported by authors on feeding fruit juices (El-Ishaq *et al.,* 2014; Marc *et al.,* 2013; Uddin *et al.,* 2014; Abdollahi *et al.,* 2014; Riaz *et al.,* 2014) and yoghurt bacteria formulations (Lollo *et al.,* 2013) to rats.

The dietary treatments had no significant ($P<0.05$) effect on platelets count of rats. The non-significant differences in between groups for platelets count confirms absence of marker diseases such as microangiopathy and macroangiopathy, thus assuring FKWB won't contribute to risk for cardiovascular diseases (Brown *et al.,* 1998). The present findings corroborates with findings reported by Oyesola *et al.* (2015), El-Ishaq *et al.* (2014) and Riaz *et al.* (2014), who fed fruit juices of watermelon, blood plum and lemon to rats. Lollo *et al.* (2013) reported non-significant differences in platelet count of rats of control group and groups fed on plain yoghurt and whey beverage incorporated with yoghurt bacteria.

CONCLUSION

Groups fed on fermented karonda whey beverage (FKWB) had significantly higher feed intake, whereas there was no significant effect on body weight gain by FKWB consumption. The feed intake and body weight gain increased with increase in age. Feed efficiency ratio was lower in FKWB fed groups as compared to control group. Haemoglobin level in blood was significantly increased by consumption of FKWB. RBC and WBC count was not that significantly affected by FKWB consumption, confirming safety and no compromise of immune system of individual on its consumption. FKWB consumption not had that significant effect on HCT, MCV, MCH, MCHC values and platelet count of blood.

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