Indian J. Anim. Nutr. 2021. 38 (3): 240-245
doi: 10.5958/2231-6744.2021.00035.9

Indian Journal of Animal Nutrition

Combined Effect of Bel and Curry Leaves Supplementation on the Performance of Anestrous Cattle

P. Priyadarshinee, Kamdev Sethy*, R.K. Swain, S.K. Mishra, K. Behera, S.K. Pradhan and S. Tripathy

Department of Animal Nutrition, Odisha University of Agriculture and Technology Bhubaneshwar, 751 003, India

ABSTRACT

To assess the effect of bel and curry leaves supplementation on the performances of anestrous cattle, 40 cross bred cattle (24 heifers and 16 cows) were divided into four groups of 10 animals each (containing 06 heifers and 04 cows) following completely randomised design. Dietary treatments were as follows: Group T_1: control (concentrate mixture and paddy straw), group T_2: group control diet + dried bel leaves at50 g/animal/day, group T_3: control diet + dried curry leaves at 50 g/animal/day, and group T_4: control diet + dried bel leaves at 50 g/animal/day and dried curry leaves at 50 g/animal/day. The experiment was continued for 60 days. The growth performances were measured in terms of body weight and average daily gain (ADG). Blood collection was done at the end of the experiment for assessment of blood biochemical and antioxidant status of the animals. Results revealed significant ($P<0.05$) improvement in antioxidant status and reproductive performances and decreased ($P<0.01$) total cholesterol and triglycerides concentration in treatment group as compared to control group. Higher percentage of conception was achieved in group T_4 (50%) followed by group T_3 (30%) and group T_2 (20%), the least percentage was in group I (0%). Therefore, it was concluded that supplementation of bel and curry leaves prevent stress and improved the reproductive status of anestrous cattle.

Key words: Anestrous, Bel, Curry, Stress, Reproduction

INTRODUCTION

Reproductive disorders are the major bottleneck in exploiting the fullest production potential of livestock. Anestrous is one of the most common functional reproductive disorders occurring almost in all species of livestock that adversely affect the economics of production. It lowers the reproductive rate of heifers by delaying the entry into the breeding herds, decreases the number of total calves and milk yield per cow resulting in significant economic loss to the small and marginal farmers as well as dairy industry as a whole (Chaudhari *et al.,* 2012). Now-a-days, various medicinal plants have brought future promise to treat reproductive disorders in livestock as being cheaper, efficacious and safer alternatives to costlier hormones. In early human civilizations, the problem of infertility was treated using herbs and other traditional ways. *Murraya koenigii* (Curry leaf) and *Aegle marmelos* (Bel) leaves have been documented in traditional Indian practice to promote fertility in animals. Leaves of these plants promoted hypocholesterolemic effects and helpful against oxidative stress and lipid peroxidation action (Mitra *et al.,* 2012). Bel and curry leaves have been demonstrated individually to augment the reproductive function in laboratory rats, anestrous goats and buffaloes (Kumar, 2008; Jondhale *et al.,* 2009). Thus, it was hypothesized that a combination of these two leaves would further improve the reproductive performance. Keeping in view the magnitude and complexity of the problem, the present experiment was designed to asses the combined effect of bel and curry leaves supplementation on the growth, blood metabolites, oxidant/antioxidant status and reproductive performance in cross bred cattle.

MATERIALS AND METHODS

Forty anestrous cattle (24 heifers above 3 years of age and 16 post-partum anestrous cows from 2nd to 3rd parity) with no physiological and anatomical abnormality were selected from Kakatpur block in Puri district of Odisha. These animals were maintained on

*Corresponding Author: E-mail: babuivri@gmail.com

diet comprising of concentrate mixture and paddy straw. Animals were dewormed with broad spectrum anthelmintic (Fenbendazole at 10 mg/kg body weight). All the animals were kept in a well-ventilated sheds with individual feeding and watering arrangements. The concentrate mixture consisted of 25% crushed maize, 30% soya bean meal, 42% wheat bran, 2% mineral mixture and 1% common salt. Animals were divided into four groups of 10 animals each (containing 06 heifers and 04 cows) following completely randomised design. Dietary treatments were Group T_1: control (concentrate mixture and paddy straw), group T_2: control diet supplemented with dried bel leaves at50 g/animal/day, group T_3: control diet supplemented with dried curry leaves at 50 g/animal/day, and group T_4: control diet supplemented with dried bel leaves at 50 g/animal/day and dried curry leaves at 50 g/animal/day. The experiment was continued for 60 days. All the animals were maintained as per the standard management practices. The green bel and curry leaves were collected and washed thoroughly and sun dried for 48 h. The dried leaves were grinded into powder with the help of vertical grinder and stored in 50 g plastic bags at room temperature. The grinded leaves were fed to the animals by mixing with jaggery. The chemical composition of concentrate mixture, paddy straw, dried bel and curry leaves were estimated as per AOAC(1995).The body weight of the animals was recorded in monthly interval during the experimental period using Johnson's formula (1940).

After 60 days of the experimental feeding, blood samples (10 ml) were collected aseptically from each animal by jugular vein puncture in the morning (before watering and feeding), into clean and dry test tubes and kept in slanting position for 45 minutes at room temperature. The blood samples were then centrifuged at 3000 rpm for 15 minutes to harvest serum and stored at -20°C for further analysis. The serum biochemical parameters like glucose, total protein, urea, triglycerides and total cholesterol were estimated in automated biochemistry analyser (Turbo Chem 100, CPC Diagnostic Pvt. Ltd., Chennai, India) by following standard procedure. Likewise, whole blood (2 ml) was collected from each animal into sterilized micro-centrifuge tube containing 0.30 ml of acid citrate dextrose (ACD, citric acid 8.0 g: sodium citrate 22.0 g and dextrose 25.0 g and volume made to 1 litre in distilled water) as anticoagulant. The blood samples were centrifuged at 3000 rpm for 10 min at 4°C and plasma and buffy coat were separated. The resulting erythrocyte pellet was washed thrice with phosphate buffer saline (PBS; disodium hydrogen phosphate 13.65 g, sodium dihydrogen phosphate 2.43 g and sodium chloride 10 g dissolved in 800 ml distilled water, pH adjusted to 7.4 and volume made to 1litre) (Yagi *et al.,* 1989). RBC diluted to 1:1 in PBS was used for the estimation of haemoglobin. For the estimation of catalase, super oxide dismutase (SOD) and lipid peroxidation (LPO), 1 ml of the 1: 1 diluted RBCs in PBS were mixed with 9 ml distilled water to prepare RBC haemolyzate of 1:20 dilution. Lipid peroxidation in RBC hemolyzate was determined following method of Placer *et al.* (1966); wherein, the concentration of malonaldialdehyde (MDA) in nmol of MDA/mg haemoglobin was calculated using the extinction coefficient of 1.56×10^8 (Utley *et al.,* 1967). Catalase (CAT) was assayed in erythrocytes by the method of Bergmeyer (1983). Super oxide dismutase (SOD) activity of RBC haemolyzate samples was measured using nitro blue tetrazolium as a substrate after suitable dilution according to Marklund and Marklund (1974) with certain modifications as suggested by Minami and Yoshikawa (1979).

All animals were regularly monitored for the onset of heat. The heat was detected by behavioural symptoms (Layek *et al.,* 2011). Animals exhibiting the sign of heat were per-rectally inseminated artificially by the local veterinary assistant surgeon. Pregnancy diagnosis was conducted routinely per rectum at 45 days post-insemination. Data obtained were subjected to one-way analysis of variance using Software Package for Social Sciences (SPSS) version 17.0 (2008) and comparison among treatment means was made by Duncan's multiple range test (Duncan, 1955) with

Table 1. Chemical composition of feeds offered to anestrous cattle (%DM basis)

Attributes	Concentrate mixture	Paddy straw	Bel leaves	Curry leaves
Organic matter	90.10	89.40	90.86	86.43
Crude Protein	19.40	2.01	5.82	7.35
Crude fibre	12.77	38.90	15.56	12.80
Nitrogen Free extract	55.13	46.39	67.58	61.67
Ether extract	2.80	2.10	1.90	4.61
Total Ash	9.90	10.60	9.14	13.57

significance level of P<0.05.

RESULTS AND DISCUSSION

Data on the chemical composition of concentrate mixture, paddy straw, bel and curry leaves are presented in Table 1. The crude protein (CP) content of the concentrate mixture and paddy straw was 19.40 and 2.01 per cent respectively (Table 1). The bel leaves contained 5.82% CP; 15.56% crude fiber (CF) and 9.14% total ash. Curry leaves contain 7.35% CP, 12.80% CF and 13.57% total ash respectively (Table 1). Jain *et al.* (2017) also observed that *Murraya koenigi* contain 2.1-12.5% protein, 9.70-13.06% total ash and 1.35-1.82% of acid insoluble ash. Average daily gain was similar (P>0.05) in all the treatment groups (Table 2) indicating that supplementation of bel and curry leaves have no effect on body weight gain of anestrous cattle. This finding was in agreement with the observations of significant decrease in body weight of rats fed bel leaves powder (Asghar *et al.,* 2018). Sahoo *et al.* (2001) reported that feeding of polyherbal preparation containing *Withania somnifera, Sphaeranthus indicus, Loranthus falcata, Scrophularia lielzzi, Panax ginseng, Nyctanthes arbor-tristis, Phyllanthus emblica, Mimosa tenuiflora, Ocimum tenuiflorum* and *Tinospora cordifolia* to Black Bengal goat in the last month of the pregnancy resulted in significantly higher

Table 2. Body weight changes in experimental animals

Days	Treatments				P value
	T_1	T_2	T_3	T_4	
Initial (0 day), kg	321.7±17.35	324.8±17.56	325. 7±19.73	327.0±19.76	0.998
Final (60 days), kg	332.8±17.75	336.3±17.55	336.5±20.28	338.7±19.9	0.901
Gain (kg)	11.16±1.54	11.50±1.23	10.83±0.76	11.67±1.09	0.762
ADG (g)	186.0±24.31	191.7±19.65	180.5±12.47	194.5±17.56	0.743

Table 3. Serum biochemical profile of cross bred animals

Parameter	Treatments				P value
	T_1	T_2	T_3	T_4	
Glucose(mg/dl)	54.14±4.09	53.61±3.17	55.01±3.53	52.06±3.48	0.937
Total Protein (g/dl)	6.81±0.03	7.05±0.22	6.80±0.02	6.79±0.03	0.311
Urea (mg/dl)	34.40±7.12	28.11±3.12	33.20±5.62	38.90±3.08	0.441
Triglyceride (mg/dl)	81.42^{a}±1.81	68.60^{b}±3.53	76.06^{b}±1.74	70.72^{b}±0.85	0.002
Total cholesterol (mg/dl)	154.34^{a}±10.28	131.82^{b}±5.22	114.75^{b}±8.10	125.01^{b}±5.61	0.009

[a,b]Means bearing different superscripts in a row differ significantly (P<0.05)

Table 4. Antioxidant enzyme status of anestous cattle supplemented with bel and curry leaves

Parameter	Treatments				P value
	T_1	T_2	T_3	T_4	
Catalase (U/mg Hb)	1.57^{a}±0.08	1.76^{b}±0.12	1.68^{b}±0.11	1.92^{c}±0.20	0.035
SOD (U/mg Hb)	19.16^{a}±2.12	22.11^{b}±4.05	23.20^{b}±3.77	23.90^{b}±1.68	0.013
LPO (µmol MDA formed / mg Hb)	3.41^{c}±0.11	3.11±0.24^{b}	2.96^{a}±0.12	3.04^{a}±0.19	0.030

^{a,b}Means bearing different superscripts in a row differ significantly (P<0.05)

birth weight and absence of kid mortality.

The data regarding blood glucose, total protein, urea, triglycerides and total cholesterol at 60 days of experiment are presented in Table 3. Among the different serum biochemical parameters, the triglycerides and total cholesterol were significantly (P<0.01) lower in treatment groups than control. Higher concentration of genistein, diphenolic phytoestrogens present in *Murraya koenigii* might be responsible for this effect (Ramadan *et al.,* 2015). Thomas *et al.* (2017) suggested that bio active compound present in the plant was responsible for depleting total cholesterol levels by reducing fat absorption in the digestive system and increasing fat excretion into faeces. Krupanidhi *et al.* (2016) observed that plant secondary metabolites like flavonoids present in A. *marmelos and M. koenigii* are responsible to maintain the normal lipid profile in rats through high lecithin acetyl transferase activity, which regulates blood lipids concentration.

Supplementation of bel and curry leaves significantly (P<0.05) increased erythrocytic catalase, SOD levels and decreased (P<0.05) lipid peroxidation as indicated by fall in MDA levels in all the treatments than control animals (Table 4). Bel and curry leaves have potential to protect against oxidative stress.

These plants has been chemically characterised to be rich in alkaloids, polyphenols, flavonoids and chlorophyll. The antioxidant properties of alkaloids, poly phenols, flavonoids and phytols (breakdown of chlorophyll) from different herbal sources are used as nutritional supplements against oxidative stress. (Tachibana *et al.,* 2001; Ningappa *et al.,* 2008).

The overall conception rates in different treated groups were found to be 20, 30 and 50% respectively (Table 5). Supplementation of either bel or curry leaves improved the conception rate that corroborated well with the finding of Kumar *et al.* (2009) in anoestrus buffaloes and Das *et al.* (2016) in delayed pubertal heifers. The maximum conception rate was found in combined bel and curry treated group than other treatments. Pharmacological active compounds like koenimbine, murrayacine, girinimbine, koenigine, koenine, mahanimbine, murrayanol and mahanine present in these leaves have higher antimicrobial, anti-inflammatory, and antioxidant properties (Handral *et al.,* 2012), which may be responsible for increased conception in treated animals. Presence of genistein in the plant may be another contributing factor which bind with estrogen receptor and thus stimulating development and maintenance of reproductive organs with estrous

Table 5. Distribution of conceived animals in different treatment at the end of experiment

Parameters	Treatments			
	T_1	T_2	T_3	T_4
Total number of animal	10	10	10	10
Number of animal comes to estrus	1	4	4	6
Number of animal conceive	0	2	3	5
Conception rate (%)	0	20.00	30.00	50.00

induction (Kumi-Diaka *et al.,* 1998).

CONCLUSION

Supplementation of dried bel (50 g) and curry leaves (50 g) per animal/per day improved the antioxidant status and reproduction rate and reduced the blood cholesterol and triglycerides concentration of anoestrous cattle.

ACKNOWLEDGMENT

The authors are thankful to the Vice Chancellor, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India, for providing the necessary facilities to carry out this work.

REFERENCES

AOAC. 1995. *Official Methods of Analysis*, 16thed., Association of Official Analytical Chemist, Washington, D.C. USA.

Asghar, N., Mushtaq, Z., Arshad, M.U., Imran, M., Ahmad, R.S. and Hussain, S.M. 2018. Phytochemical composition, antilipidemic and antihypercholestrolemic perspectives of Bael leaf extracts. *Lipids Health Dis.* 17:1-9.

Bergmeyer, H U. 1983. *U.V. Method of Catalase Assay*. In: Methods of Enzymatic Analysis, Wenheim Deer Field Beach, Florida, 3: 273-286.

Chaudhari, C.F., Suthar, B.N., Sharma, V.K, Dabas, V.S., Chaudhari, N.F. and Panchsara, H. H. 2012. Estrus induction and fertility response in delayed pubertal Kankrej heifers treated with norgestomet ear implant, *Vet. World*. 5: 453-458.

Das, G.K., Mehrotra, S., Narayanan, K., Kumawat, B.L., De Kumar, U. and Khan, T.A. 2016. Estrus induction response and fertility performance in delayed pubertal heifers treated with *Aegle marmelos* and *Murraya koenigii*. *J. Anim. Res.* 6: 151-156.

Duncan, D. B. 1955. Multiple range and multiple "F" tests. *Biometrics* 11: 1-42.

Handral, H.K., Pandith, A. and Shruthi, S.D. 2012. A review on Murraya koenigii: multipotential medicinal plant. *Asian J. Pharm. Clin. Res.* 5: 5-14.

Jain, M., Gilhotra, R., Singh, R.P. and Mittal, J. 2017. Curry leaf (*Murraya koenigii*): A spice with medicinal property. *MOJ. Biol. Med.* 2 : 236-256.

Johnson, D.W. 1940. Livestock Weights from Measurements. *Minnesota Agricultural Experiment Station* Folder 70.

Jondhale, N.S., Uma, S., Mehrotra, S., Agarwal, S.K., Singh, S.K., Das, G.K. and Deori, S. 2009. Effect of Aegle marmelos and Ficus religiosa leaves extracts on the ovarian function in rats. *Indian Vet. J.* 86:1141-1144.

Krupanidhi, A.M., Kalleshappa, C.M. and Chanchi, A.R. Antihyperlipidemic Activities of Isolated bio Compounds of *Aegle Marmelos*. J. *Pharm. Biol. Sci.* 11: 42-45.

Kumar, R., Mehrotra, S., Shanker, U., Barshney, V.P., Dutt, R., Agarwal, S.K., Das, G.K. and Singh, S.K. 2009. Effect of *Aegle marmelos* and *Ficus religiosa* on endocrine profile and reproductive performance in anoestrus buffaloes. XXV Annual Convention and National Symposium of ISSAR, Namakkal, Tamil Nadu, India, 10-12, Compendium and Souvenir, pp:55.

Kumar, R. 2008. *Effect of Aegle marmelos (Bel) and Ficus religiosa (Pipal) on the reproductive performance in anoestrus goat and buffalo*. M.V.Sc. Thesis, Indian Veterinary Research Institute, Izatnagar (Deemed University), India.

Kumi-Diaka, J., Rodriguez, R. and Goudaze, G. 1998. Influence of genistein (42,5,7 trihydroxyisoflavone) on the growth and proliferation of testicular cell lines. *Biol. Cell.* 4: 349-354.

Layek, S.S., Mohanty, T.K., Kumaresan, A., Behera, K. and Chand, S. 2011. Behavioural signs of estrus and their relationship to time of ovulation in Zebu (Sahiwal) cattle. *Anim. Reprod. Sci.* 129: 140-145.

Marklund, S. and Marklund, G. 1974. Involvement of superoxide anion radicals in autoxidation of phyrogallol and convenient assay for superoxide dismutase. *Eur. J. Biochem.* 47: 469-474.

Minami, M. and Yoshikawa, H. 1979. Simplified assay method of super oxide dismutase activity of clinical use. *Clin. Chim. Acta.* 92: 337-342.

Mitra, E., Ghosh, A.K., Ghosh, D., Mukherjee, D., Chattopadhyay, A., Dutta, S., Pattari, S.K. and Bandyopadhyay, D. 2012. Protective effect of aqueous curry leaf (*Murraya koenigii*) extract against cadmium induced oxidative stress in rat heart. *Food Chem. Toxicol.* 50: 340-353.

Ningappa, M.B., Dinesh, R. and Srinivas, L. 2008. Antioxidant and free radical scavenging activities of polyphenol enriched curry leaves (*Murraya koenigii*) extracts, *Food Chem.* 106: 720-728.

Placer, Z.A, Cushman, L.L and Johnson, B. 1966. Estimation of product of lipid peroxidation (malonaldehyde) in biochemical system. *Anal. Biochem.* 16: 359-364

Ramadan, A.A., Afifi, N.A., Erian, E.Y, Saleh, D.O and Sedik, A.A. 2015. Beneficial Effect of Trigonelline on the

metabolic changes associated with insulin resistance in rats. *World J. Pharm. Pharm. Sci.* 5: 1238-50.

Sahoo, N., Behura, N.C and Mishra, J. 2001. Effects of Immu-21 on certain blood biochemicals, milk, colostrum, body weight gain and livability in goats, *Phytomed.* 2: 69-76.

SPSS. 2008. *Statistical Packages for Social Sciences*, Version 17.0, SPSS Inc., Illinois, USA.

Tachibana, Y., Kikuzaki, H., Lajis, N. H. and Nakatani, N. 2001. Antioxidative activity of carbazoles from *Murraya koenigii* leaves. *J. Agric. Food Chem.* 49: 5589-5594.

Thomas, S.Z., Pertiwi, D., Bagus, S.A., Nuri, S., Jelita, B.E.M. and Alfiza, N.S. 2017. Effect of tender coconut water on blood lipid levels in height fat diet fed male rats, *J. Krishna Inst. Med. Sci. Univ.* 6: 63-68.

Utley, H. G., Bernheim, F. and Hochsein, P. 1967. Effect of sulphydril reagents on per-oxidation of microsomes. *Arch. Biochem. Biophys.* 118: 29-32.

Yagi, Y., Furunchi, S., Takashi, H. and Koyama, H. 1989. Abnormality of osmotic fragility and morphological disorder of bovine erythrocytes infected with *Theileria sergenti. Jpn. J. Vet. Res.* 51*:* 389-395.

Received on 02.10.2021 and accepted on 01.12.2021