

Research Article

EFFECT OF BAEI AND CURRY LEAVES ON THE REPRODUCTIVE PERFORMANCE OF ANESTROUS BINJHARPURI CATTLE

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ABSTRACT: Medicinal plants have shown promising potential in treating reproductive disorders in livestock. Bael and curry leaves are an excellent source of calcium, phosphorus, iron and other minerals, which sensitizes the activity of reproductive hormones enhancing fertility in animals. 32 Anestrous Binjharपुरi heifers were randomly assigned into four dietary treatment groups, with 8 cattle in each group. The treatments were T1: basal diet, T2: basal diet + bael leaves (50g/ day / animal), T3: basal diet + curry leaves (50g/ day/ animal), T4: basal diet + bael leaves (50g/ day/ animal) + curry leaves (50g/ day/ animal). The trial continued for 60 days, body weight was measured on the first and last day of the experiment. Blood samples were collected at the end to assess the blood chemistry, antioxidants and serum minerals concentration of the animals. Onset of oestrous and conception rate was assessed throughout the experiment. Results revealed that supplementation of bael and curry leaves significantly ($P<0.05$) improved body weight gain, antioxidants, mineral status and conception rate in Binjharपुरi anestrous cattle. Serum triglycerides and total cholesterol were significantly ($P<0.05$) reduced in treated animals compared to control animals.

Keywords: Anestrous, Bael, Binjharपुरi cattle, Body weight, Curry, Stress, Reproduction.

INTRODUCTION

Binjharपुरi cattle is one of the important indigenous cattle breeds of the state of Odisha serving to the farming community as dual-purpose cattle. It still exists in its purest form after so long use of artificial insemination. It is also known as “Deshi”, in its native tract of the Jajpur district of Odisha state [1]. This breed of cattle is mainly reared by marginal farmers and poorer sections of the people for production of the milk and agricultural operations like ploughing. Reproductive health issues pose significant challenges to the Binjharपुरi cattle affecting the economic status of poor farmers [2]. Reproductive health concerns negatively impact the reproductive rate of heifers, causing delays in their integration into breeding herds and reducing the overall number of calves and milk production per cow. This results in substantial financial losses for small and marginal farmers [3]. Anestrous is the one of important reproductive cycle functional abnormality characterized by the lack of an apparent sign of estrus, either expressed or not recognized. A variety of treatment options, including both hormonal

and non-hormonal approaches, are utilized to effectively manage this problem. However, the majority of synthetic medications have been met with reluctance from Binjharपुरi dairy farmers due to substantial financial outlay and inconsistent outcomes. Alternative, more affordable, and safer options are available instead of more expensive hormones. Various medicinal plants have shown promising potential in treating reproductive disorders in livestock [4]. The plants curry (*Murraya koenigii*) and bael (*Aegle marmelos*) leaf have been individually studied for their potential to enhance fertility in laboratory animals, and anoestrous goats [5,6]. There is limited research on Binjharपुरi cattle. This research intends to improve our understanding of this field, so contributing to the preservation and advancement of the Binjharपुरi breed’s productivity and sustainability. Using the aforementioned information into consideration, a planned study has been designed to evaluate the effectiveness of *Murraya koenigii* and *Aegle marmelos*, separately and in combination, in inducing estrus in Anestrous Binjharपुरi cattle in field conditions.

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MATERIALS AND METHODS

Selection of animals and dietary treatments

For the trial, 32 anoestrous Binjharपुरi heifers with age more than 3 years with no physiological or anatomical abnormalities and normal genital tracts were selected from the Binjharपुर block of Jajpur district, Odisha.

The study was carried out as per the guidelines laid down by the Institutional Animal Ethics Committee (433/GO/Re/S/01CCSEA). The animals were dewormed prior to the trial with fenbendazole, a broad-spectrum anthelmintic, at a dose of 10 mg/kg body weight. For the trial, the animals were randomly assigned to four treatment groups, each comprising 8 heifers following a completely randomized design. Treatment groups were T1: basal diet, T2: basal diet + bael leaves (50g/ day / animal), T3: basal diet + curry leaves (50g/ day/ animal), T4: basal diet + bael leaves (50g/ day/ animal) + curry leaves (50g/ day/ animal). The animals were offered a diet with concentrate + paddy straw (basal diet) and were managed in accordance with standard husbandry practices. 32% crushed maize, 26% soybean, 39% wheat bran, 2% mineral mixture, and 1% salt were used to make the concentrate mixture. The animals were given individual feed and clean water on a regular basis, two to three times daily, without access to outdoor grazing. The trial was carried out over a span of 60 days.

Preparation of bael and curry powder

Fresh, mature green leaves of *Aegle marmelos* and *Murraya koenigii* were collected in and around Bhubaneswar. The leaves underwent a thorough washing process and were subsequently left to dry in the shade for 48 hours at ambient temperature. Following the drying process, the leaves were separated from their stems and ground into a fine powder using a vertical grinder. Subsequently, 50 grams of each powdered sample were carefully stored in sealed plastic bags under ambient temperature conditions. Prior to administration to the animals, the powdered leaves were combined with jaggery. The proximate composition of the basal diet along with *Aegle marmelos* and *Murraya koenigii* samples were determined in accordance with [7] guidelines.

Body weight gain

The body weight was measured using Johnson's formula [8] at 0 and 60 days of the experiment. The BW gain was assessed by subtracting initial from final weight and average daily gain (ADG) was calculated.

Estimation of blood chemistry

Blood samples from each animal were collected at 60 days of the experiment through jugular vein puncture in a 20 ml test tube. The tubes were then placed for 3-4 hours in a slanted position to ease the serum separation. Subsequently, the blood clot was carefully detached from the tube walls using an applicator stick, ensuring the prevention of haemolysis. The serum was then carefully extracted using an auto-pipette and transferred to labelled, capped glass vials. These serum samples were then placed in a thermocol container with ice for transportation and subsequently stored at -40°C for future analysis. An automated biochemistry analyser was used to assess the serum glucose, total protein, urea, triglycerides, and total cholesterol.

Estimation of antioxidant enzymes

According to [9], lipid peroxidation in RBC hemolysate was measured. The extinction coefficient of 1.56×10^8 was used to compute malondialdehyde (MDA) concentration in haemoglobin [10]. The Bergmeyer [11] method was used to measure the amount of catalase (CAT) in erythrocytes. Nitro blue tetrazolium was used as a substrate to evaluate the superoxide dismutase (SOD) activity following appropriate dilution in accordance with Marklund and Marklund [12], with some adjustments recommended by Minami and Yoshikawa [13].

Estimation of serum minerals

Triple acid mixture (nitric acid, sulphuric acid and perchloric acid at a ratio of 4:2:1) was used to digest the serum samples and the concentration of Ca, P, Fe, Zn, and Cu was measured using ICP-OES (Toplab India, Mumbai).

Reproductive status

For the onset of heat, the animals were observed on a regular basis. Behavioural signs were used to identify the heat [14]. The local veterinary assistant surgeon artificially inseminated animals showing signs of heat using the semen of a Binjharपुरi bull. After 45 days of insemination, a routine pregnancy diagnosis was performed per rectally.

Statistical analysis

Software Package for Social Sciences (SPSS) version 17.0 [15] was used to do a one-way analysis of variance on the collected data, and Duncan [16] was used to compare the means at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION**Chemical composition of basal diet**

The concentrate mixture and paddy straw had crude protein (CP) contents of 19.35% and 2.15 percent, respectively (Table 1). The CP, crude fiber (CF) and total ash content of bael leaves were 5.70%, 18.70%, 9.50% respectively, whereas curry leaves had CP 7.20 %, CF 12.80 %, and TA 10.60 %. (Table 1).

Similar to our result [17] observed 5.90 % CP, 1.80 % ether extract and 9.20 % total ash in bael leaf

powder. It was also found that *Murraya koenigii* have CP content between 2.1–12.5%, TA content in the range of 9.70–13.06%, and acid insoluble ash (AIA) ranging from 1.35–1.82% [18].

Body weight and average daily gain

BW gain and ADG were significantly ($P < 0.05$) higher in all the treated groups than control group (Table 2) indicated that addition of leaves of bael and curry improved the BW gain of anoestrous Binjharपुरi

Table 1. Chemical composition of the basal diet (% DM basis)

Attributes	Concentrate mixture	Paddy straw	Bael leaves	Curry leaves
Organic matter	89.90	88.50	90.50	89.40
Crude Protein	19.35	2.15	5.70	7.20
Crude fibre	12.20	39.20	18.70	12.80
Nitrogen Free extract*	55.45	45.10	64.25	64.90
Ether extract	2.90	2.05	1.85	4.50
Total Ash	10.10	11.50	9.50	10.60

*Calculated value

Table 2. Body weight (Kg) Changes in Binjharपुरi cattle.

Days	Treatments				P value
	T1	T2	T3	T4	
Initial (0 day)	199.50± 3.90	199.90± 4.60	199.50± 3.50	200.00± 4.00	0.930
Final (60 days)	205.70± 3.70	208.50± 3.00	208.00± 4.01	210.20± 4.10	0.068
Gain (Kg)	6.20 ^a ±0.60	8.60 ^b ±0.55	8.50 ^b ±0.49	10.20 ^c ±0.58	0.031
ADG (g)	103.33 ^a ±12.16	143.33 ^b ±12.50	141.67 ^b ±11.90	170.00 ^c ±10.20	0.017

^{ab}Values bearing different superscripts in a row differ significantly ($P < 0.05$)

Table 3. Serum biochemical profile of Binjharपुरi cattle

Parameter	Treatments				P value
	T1	T2	T3	T4	
Glucose(mg/dl)	50.52±3.90	52.60±3.07	53.05±3.10	52.94±3.50	0.885
Total Protein (g/dl)	6.60±0.27	6.85±0.05	6.80±0.18	6.69±0.16	0.601
Urea (mg/dl)	33.55±5.11	32.05±3.12	32.20±5.20	31.90±3.64	0.147
Triglyceride (mg/dl)	80.20 ^a ±1.25	74.90 ^b ±1.50	75.10 ^b ±1.74	71.57 ^b ±1.02	<0.01
Total cholesterol (mg/dl)	160.30 ^a ±7.28	140.50 ^b ±5.00	134.45 ^b ±6.30	121.03 ^b ±4.70	<0.01

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

Table 4. Antioxidant enzyme status (U/ mg haemoglobin) of Anestrous Binjharपुरi cattle.

Parameter	Treatments				P value
	T1	T2	T3	T4	
Catalase	1.80±0.05	2.18 ^b ±0.04	2.05 ^b ±0.08	2.42 ^c ±0.20	0.012
SOD	19.50 ^a ±1.90	25.90 ^c ±2.05	22.70 ^b ±1.70	26.88 ^c ±2.10	<0.01
LPO	3.57 ^c ±0.21	2.85 ^{ab} ±0.10	3.00 ^b ±0.12	2.47 ^a ±0.05	0.020

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

Table 5. Mineral status of experimental animals

Minerals	Treatments				P value
	T1	T2	T3	T4	
Ca (mg/dl)	7.60 ^a ±0.31	8.90 ^b ±0.52	8.60 ^b ±0.57	9.15 ^{bc} ±0.49	<0.01
P(mg/dl)	4.17 ^a ±0.15	5.03 ^b ±0.17	5.11 ^b ±0.10	5.48 ^c ±0.23	0.005
Fe (ppm)	19.15 ^a ±1.05	23.52 ^b ±0.98	28.51 ^c ±1.57	29.56 ^c ±1.06	<0.01
Zn (ppm)	4.40 ^a ±0.31	5.67 ^b ±0.26	5.80 ^b ±0.32	5.92 ^b ±0.24	0.013

^{abc}Values bearing different superscripts in a row differ significantly (P<0.05).

cattle. Combined supplementation has shown better response compared to individual supplementation.

This result corresponds with the findings where rats' body weight significantly increased upon feeding of powdered bael leaves [19]. Significantly (P<0.05) higher BW was observed in kids supplemented with polyherbal preparation containing *Withania somnifera*, *Ocimum tenuiflorum*, *Tinospora cordifolia* and *Aegle marmelos* to Black Bengal goats [20]. Contrary to this, [21] noticed non-significant difference in body weight gain in crossbred heifers supplemented with bael and curry leaves. This may be due to a different breed of cattle compared to the present experiment. Increased gain might be due to better nutrient digestion and availability in supplemented animals compared to the control [22].

Blood biochemicals

The values of blood glucose, total protein, and urea at 2-month experimental period showed non-significant (P>0.05) difference among the treatments, whereas significant (P<0.05) decreased in serum triglycerides and total cholesterol was seen in treated animals than the control (Table 3).

Similar to our observation, Priyadarshinee *et al.* [21] observed similar serum glucose, total protein concentration and decreased serum total cholesterol and triglycerides in cross bred cows supplemented with bael and curry leaves. *Murraya koenigii* is rich in genistein, diphenolic phytoestrogens which reduces the cholesterol and triglycerides in animals [23]. [24] also proposed that bioactive compounds found in a variety of plants were in charge of lowering total cholesterol levels by boosting fat excretion into faeces and decreasing fat absorption in the digestive tract. According to [25], *A. marmelos* and *M. koenigii* contain secondary plant metabolites such as flavonoids, which are responsible for monitoring the normal lipid profile in animals due to strong lecithin acetyl transferase activity.

Antioxidant status

Supplementing with bael and curry leaves significantly (P<0.05) raised catalase and SOD levels and lowered (P<0.05) LPO, as seen by a drop in MDA levels (Table 4). Similarly, [26] observed increased catalase and SOD activity in rats supplemented with curry leaves extract. Curry and bael leaves give protection against oxidative damage to the living cells. Various chemical analysis suggest that these plants are abundant in flavonoids, alkaloids, polyphenols, and chlorophyll [17]. These substances acted as antioxidants to combat oxidative stress in animals [27, 28].

Serum minerals

The serum concentration of Ca, P and Fe and Zn was significantly (P<0.05) improved due to bael and curry supplementation in experimental animals (Table 5). The bael and curry leaves are very good sources of Ca, P and Fe and other minerals [29]. Presence of these minerals enhances the utilization of other microminerals and sensitizes various reproductive hormones for better fertility in animals [30]. Similarly, [31] and [32] also observed higher serum Ca and P concentration in animals supplemented with *Murraya koenigii* and *Aegle marmelos*

Reproductive status

The percentage of animals that came into oestrus (oestrus %) and overall conception rates in various treatments were significantly higher in combined bael and curry supplemented animals compared to other treatments. It was also observed that bael and curry supplementation alone improved the reproductive performance of animals compared to non-supplemented control animals (Fig 1).

The results of [33] in delayed pubertal heifers and [34] in Anestrous buffaloes support the result that the conception rate was improved when either

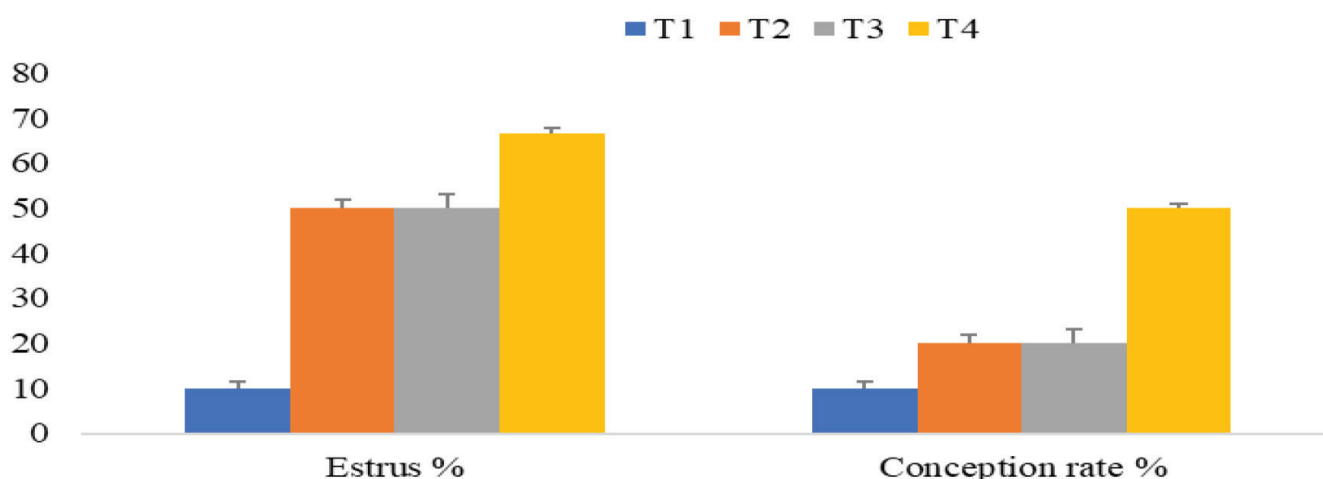


Fig. 1. Reproductive performance in experimental animals.

curry or bael was supplemented. The plant *Murraya koenigii* plays an important role as it may enhance follicular growth and steroidogenesis, thereby leading to induce estrous in Anestrous heifers [31]. Higher antibacterial, anti-inflammatory, and antioxidant qualities of pharmacologically active substances such as koenimbine, murrayacine, girinimbine, koenigine, koenine, mahanimbine, murrayanol, and mahanine may be the cause of treated animals' enhanced conception rates [35]. The plant's genistein content may bind to the estrogen receptor, promoting the growth and development of reproductive organs through the induction of estrous [36].

CONCLUSION

Combined supplementation of dried bael and curry leaves enhanced the body weight gain, antioxidant, mineral status, conception rate and decreased the blood cholesterol and triglyceride levels of anoestrous Binjharपुरi cattle.

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