



Conch Shell (*Turbinella pyrum*) Powder: A Potential Marine Biological Source of Calcium and Some Trace Minerals for Growing Crossbred Calves

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Abstract

The present study was conducted to evaluate the effect of feeding conch shell (*Turbinella pyrum*) powder (either fresh or calcined) as a marine organic source of calcium (Ca) supplemented in the diet of crossbred calves on voluntary intake, growth performance, and blood biochemistry in growing crossbred Jersey calves. A growth trial of 90 days was conducted on 15 Jersey crossbred female calves (Av. weight, 70.68 ± 2.90 kg; Av. age, 197.73 ± 12.40 days), equally divided into three groups of 5 animals each, i.e., control (T_0), treatment 1 (T_1), and treatment 2 (T_2). All animals were fed total mixed ration (TMR) prepared with a concentrate mixture, chaffed paddy straw, and green fodder at the ratio of 40:30:30 on DM basis. Calves under the control group were fed with TMR containing a standard mineral mixture having dicalcium phosphate (DCP) as a Ca source. Calves under T_1 group were supplemented with TMR containing fresh conch shell powder (FCSP), and T_2 calves were fed with TMR containing conch shell calcined powder (CSCP) as Ca source. We observed 11.66% increase ($p < 0.01$) in Ca concentration in CSCP compared to FCSP. The concentration of minerals like Mg, Co, Mn, and Fe was enhanced in CSCP compared to the FCSP. However, the calcination process of fresh conch shell powder (FCSP) reduced the concentration of Cu, and Zn. The Ca/P ratio was estimated as 2.11, 2.06, and 2.10 in T_0 , T_1 , and T_2 diets, which could be considered ideal for calf ration. Calves under T_1 , and T_2 groups consumed significantly ($p < 0.001$) greater amounts ($\text{g/kg W}^{0.75}$) of DM and CP compared to T_0 . However, increased voluntary intake did not translate into increased body weight gain (kg), and feed conversion ratio (kg DMI/kg gain) in T_1 and T_2 groups in comparison to T_0 . We observed similar blood glucose, urea, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine transaminase (ALT) concentration among the three treatments. Ca, and P levels in blood plasma were also identical among the three groups. The digestibility of Ca was increased significantly ($p = 0.01$) in FCSP (T_1)- and CSCP (T_2)-treated calves compared to control (T_0) calves. Similarly, T_1 and T_2 enhanced P digestibility compared to T_0 . This first report with short-term experimentation depicted some promising scope for the use of locally available conch shell powder (fresh or calcined form) as a potential source of Ca for feeding to livestock, because these new sources of Ca did not affect intake, digestibility of Ca and P, growth performance, blood chemistry, and liver enzymes negatively in weaned crossbred calves.

Keywords Blood chemistry · Conch shell · Calcium · Calves · Calcined · Growth · Powder

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Introduction

The total coastal length in India (including Andaman and Nicobar Islands and Lakshadweep) is 7516.6 km [1] which has plenty of sea/marine shells [2]. Bay of Bengal, Arabian Sea, and Indian Ocean, surrounding Indian subcontinent, are wealthier in biological resources of calcium (Ca) and other important minerals. The major sources of Ca include crustacean shells, fish bones, coral, shell fish, and seaweed. Sometimes, marine processing leftovers are considered unusable; however, it is a copious and cheaper source of Ca [3]. “*Turbinella pyrum*,” a marine animal of “mollusc” group under the family Turbinellidae [4], is used as conch shells or Shankha (Chart 1) in India. Molluscs are a class of animals that have no bones and have a hard outer shell [5]. Hindu religious groups in India use Shankha and ladies’ Bangles, commercially produced from conch shells (Chart 1) which are fished in southern coastal lines in India and Sri Lanka. Fished conch shells are processed in several small-scale industries located in the state of West Bengal, India. The conch shell powder is the by-product produced during the process of hand-crafting of Shankha, Bangles, and other valued products (Chart 1). The calcined product of conch shell powder is known as Shankha Bhasma which is traditionally used in Ayurvedic medicines to treat many ailments in human beings [6] like peptic ulcers, piles, cough, and certain gastrointestinal disorders [7]. Minerals accomplish various basic physiological functions within the tissues and organs; so, insufficiency or surplus of one or more of them may result into metabolic dysfunctions that may cause low productive and reproductive efficiency [8]. Ca is an essential macro-mineral required for appropriate bone health, integrity, and metabolism in livestock and human beings. Ca enters the circulation through the feed/food chain or Ca supplemented through the mineral mixture, and a dynamic homeostatic balance is maintained between blood and bone Ca [9]. Growing calves need to be supplied with adequate amounts of Ca in order to accomplish acceptable performance, as well as sufficient bone mineralization. Lack of information about Ca availability in different feed resources may impair the accuracy of ruminant’s ration formulation [10]. In recent years, Ca supplements of marine origins have gained considerable attention due to their copious reserves, higher biological safety, and activity [11, 12].

The production of meat (chicken, pork, chevon, mutton, and beef) and milk will accelerate to meet the increasing demand of human population. Hence, the requirements for different nutrients, including minerals, will also increase in the future. Animals can never fully utilize their dietary Ca and P, as some of them are inaccessible or lost during normal

digestion and metabolic processes or have an unfavorable impact on their absorption due to a number of dietary or non-dietary variables [13]. The utilization of Ca and P in animals depends upon their bioavailability in feed resources [14]. However, an inappropriate diet of livestock and poultry birds may limit the bioavailability of Ca and P. For example, the presence of phytic acid in cereal grains and oxalic acid in some green fodder can cause Ca to precipitate as calcium phytate and calcium oxalate, which are insoluble compounds [15, 16]. An adequate supply of Ca in the diet could entirely be provided by animal-based and inorganic mineral salts, the most common sources of which include ground limestone, calcium chloride, mono- and dibasic calcium phosphate, and oyster shells [17]. However, the cost of mineral mixtures is a concern these days due to the increased cost of various mineral salts. Instead of purchasing expensive mineral salt, we may use locally available mineral resources of natural origin. For example, Ca from CaCO_2 can be obtained from marine shells or wastes such as crustaceans and bivalve shells. The seashells are composed of more than 95% inorganic minerals and a small quantity of organic matrix [18, 19]. Xu et al. [3] reported that the marine shell, an organic source of Ca, contained about 95% CaCO_2 . CaCO_3 in marine shells is commonly found as calcite and aragonite in adult individuals and often amorphous CaCO_3 (ACC) in young animals [20].

Conch shell powder, along with another P source, can replace the commonly used dicalcium phosphate (DCP) in the mineral mixture for feeding to livestock. Since no published reports are available on the use of conch shell (*Turbinella pyrum*) powder (either fresh or calcined) in the mineral mixture supplemented in the diet of livestock, the present study was conducted to assess the effect of replacing DCP with fresh conch shell powder or conch shell calcined powder (a marine organic source of Ca) on voluntary intake, growth performance, blood chemistry, and liver enzymes in growing crossbred Jersey calves.

Materials and Methods

Site of the Experiment

The experiment was conducted in the Animal Nutrition Laboratory and Cattle Yard Complex of ICAR-National Dairy Research Institute, Eastern Regional Station, Kalyani, West Bengal (located in the lower Gangetic region of India) as per the approval of the University (20-M-AN-01 of 2020). The latitude and longitude position of the study site is $22^{\circ}56'30''\text{N}$ and $88^{\circ}32'04''\text{E}$, respectively. The experiment was divided into two phases, i.e., I and II.



Chart 1 Processing of conch shells to different commercial products and generated by-products

Phase-I: Physico-chemical Evaluation of Fresh Conch Shell Powder and Conch Shell Calcined Powder

The sufficient quantity of fresh conch shell powder (FCSP) was procured from Rupam Sankha Silpalaya (registered Industry), Kolmijore, Paschim Midnapur, West Bengal,

India, for conducting the experiment. Conch shell calcined powder (CSCP) was prepared by ashing part of FCSP in the muffle furnace at 650–700 °C for 7–8 h. FCSP and CSCP were subjected to solubility test using different inorganic acids and water.

Solubility Test

The weighed samples (5 g) of FCSP and CSCP were allowed to dissolve in 25 ml of distilled water, normal tap water (source deep tube well), and concentrated acids like H_2SO_4 , HCl, HNO_3 , and $HClO_4$. The samples were kept

for dissolution for 18 h. The residual samples were filtered through repeated washing with double-distilled water on pre-weighed filter paper no. 1 until the sample present in the beaker was completely filtered. Then, the filter paper, along with undissolved part of the sample, was kept in a hot air oven until complete drying at 80.0 ± 5.0 °C.

$$\text{Solubility \%} = 100 - \frac{(\text{wt. of filter paper} + \text{wt. of undissolved sample}) - \text{wt. of filter paper}}{\text{wt. of sample}} \times 100$$

Estimation of Minerals

Minerals such as Ca, Mg, Cu, Zn, Mn, Fe, and Co in FCSP, CSCP, feeds, fodders, fecal samples, and water samples were analyzed by using Atomic Absorption Spectrophotometer (Agilent 240AA model). P content in these feeds and mineral resources was evaluated as per the method described by O'Dell [21].

Evaluation of Density and pH

The density of both FCSP and CSCP was evaluated by using the following formula,

$$\text{Density of powder (g/cm}^3\text{)} = \text{weight (g)}/\text{volume (cm}^3\text{)}.$$

The pH of each powder was estimated by dissolving 10 g of each powdered sample in 100 ml of double distilled water. A digital pH meter (Elico, India) was used for the purpose.

Phase-II: Assessment of Fresh Conch Shell Powder and Conch Shell Calcined Powder in Crossbred Calves

Experimental Animals

The experiment was conducted in 2022 on 15 healthy female Jersey crossbred calves (av. weight, 70.68 ± 2.90 kg; av. age, 197.73 ± 12.40 days) divided into 3 equal treatments (5 calves in each group), i.e., T_0 as control, T_1 , and T_2 . The average initial body weight and age of calves were statistically similar among the three treatments. All calves were housed individually in the well-ventilated experimental shed under similar management conditions. Ad libitum clean and fresh drinking water was offered twice daily to all animals at around 10.00 AM and 4.00 PM. The experimental shed and calves were cleaned regularly throughout the trial period.

Experimental Feeding

The growth trial in crossbred calves continued for 90 days. Total mixed rations (TMRs) (calculated CP 12% and TDN 65%) were prepared separately for each group using concentrate mixture containing separate mineral mixture (Table 1), chaffed paddy straw, and green fodder at the ratio of 40:30:30 on DM basis. We used seasonal oat fodder during the initial 2 months of the experiment, followed by maize fodder, to prepare TMRs for the last month of the experiment.

Control group (T_0): Calves under the control group were fed ad libitum TMR (CP 12% and TDN 65%) with a mineral mixture containing dicalcium phosphate (DCP) as a source of Ca and P.

Treatment group (T_1): Ad libitum TMR (CP 12% and TDN 65%) with a mineral mixture containing fresh conch

Table 1 Composition (%) of mineral mixture and total mixed rations (TMRs) for different experimental groups

Composition	T_0	T_1	T_2
Composition of mineral mixture* (%)			
Dicalcium phosphate	97.852	-	-
Fresh conch shell powder	-	42.945	-
Conch shell calcined powder	-	-	39.480
Diammonium phosphate	-	55.829	59.220
$CuSO_4$	0.682	0.389	0.413
$ZnSO_4$	1.355	0.773	0.820
$MnSO_4$	0.070	0.040	0.043
$CoSO_4$	0.040	0.023	0.024
Total	100.00	100.00	100.00
Composition of total mixed rations (% on DM basis)			
Concentrate mixture**	40	40	40
Green fodder	30	30	30
Paddy straw	30	30	30
Total	100	100	100

**Concentrate mixture (CP 20.79%, TDN 74.56%) prepared with maize grain 35%, wheat bran 24%, groundnut cake 14%, mustard oil cake 24%, *respective mineral mixture 2%, and common salt 1%

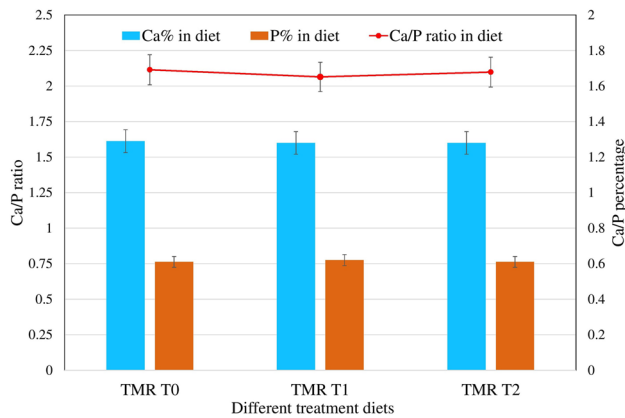


Fig. 1 Ca and P levels as well as Ca/P ratio in the diets as affected by different sources of calcium

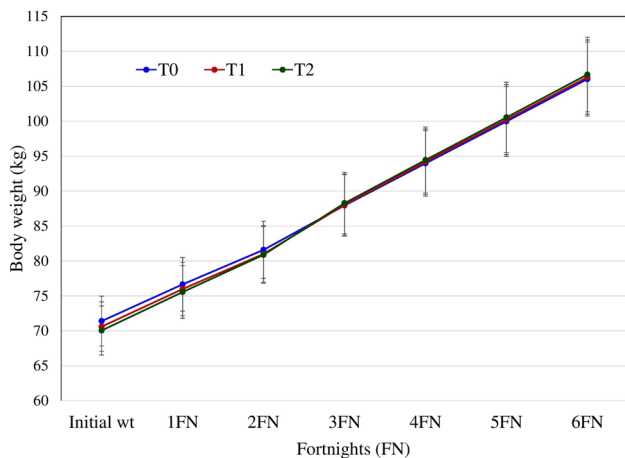


Fig. 2 Growth of crossbred calves affected by different sources of calcium

shell powder (FCSP) as a source of Ca and diammonium phosphate as a source of P.

Treatment group (T₂): Ad libitum TMR (CP 12% and TDN 65%) with a mineral mixture containing conch shell calcined powder (CSCP) as a source of Ca and diammonium phosphate as a source of P.

Three experimental mineral mixtures were prepared using the ingredients mentioned in Table 1. The percentage of calcium and phosphorus was kept similar among all mineral mixtures (Fig. 1).

Observations

Chemical Composition of Feeds

Throughout the experimental period, representative samples of feeds and residues were taken and analyzed in triplicate

in the Animal Nutrition Laboratory for Proximate Principles [22] and cell wall fractionations [23].

Voluntary Intake of Nutrients, Body Weight, and Feed Conversion Efficiency

The body weight of experimental calves was recorded before the start of the experiment and during different phases (fortnightly) of the trial period by using the ISI-branded digital platform balance (Fig. 2). Fortnightly dry matter intake (DMI) by individual calf was measured by deducting the DM of the residue from the DM of the total feed offered. Similarly, organic matter intake (OMI) and crude protein intake (CPI) patterns were recorded in all experimental animals on fortnightly basis. Average daily body weight gain (ADG), feed conversion efficiency (FCE), and feed conversion ratio (FCR) were calculated based on the following formulae.

$$\text{Average daily body weight gain (ADG)} = \frac{\text{weight gain (kg)}}{\text{Number of days of observation}}$$

$$\text{Feed conversion efficiency (FCE)} = \frac{\text{weight gain (kg)}}{\text{dry matter intake (kg DMI)}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{average daily dry matter intake (kg DMI)}}{\text{average daily body weight gain (kgADG)}}$$

Similarly, FCE and FCR were also calculated based on the total crude protein intake (CPI) by the calves under different treatment groups.

Blood Metabolites and Enzymes

Blood samples were collected from the jugular vein of all experimental calves at 0, 45, and 90 days of feeding under aseptic conditions. Some relevant blood metabolites (urea, glucose), minerals (Ca, P), and liver enzymes (alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT)) were analyzed. Blood glucose was estimated using the commercially available glucose kit (GOD-POD) method (Span Diagnostic Ltd., India). Blood urea was estimated using the commercially available Urea Test Kit (urease Berthelot, endpoint assay) method (Span Arkray Healthcare Pvt. Ltd., India). Blood Ca and P were estimated using the commercially available Liquixx Calcium and Phosphorus test kit of Trans Asia Bio Medicals Ltd., India. Blood ALT was evaluated using the commercially available ALT Test Kit of Coral Clinical Systems (P) Ltd., India. We evaluated Blood AST using the commercially available AST Test Kit of Coral Clinical Systems (P) Ltd., India. Blood ALP activity was also measured by the method of pNPP Kinetic method using Diagnostic Kit manufactured by Arkray Healthcare Pvt. India.

Digestibility Trial

A digestion trial of 6-day collection period was conducted on 15 animals (5 experimental calves under each group) by total collection method after the completion of 90 days of the growth trial to study Ca and P intake, outgo through feces, and digestibility. The digestion trial was conducted following the detailed method described by Anil et al. [24]. Ca and P content of feeds offered, residues left, and fecal samples were evaluated as per the methods described in the earlier section.

Statistical Analysis

Data related to the chemical and mineral composition of different samples were statistically analyzed by using one-way analysis of variance (ANOVA) [25]. The intake of nutrients, growth performance, feed conversion efficiency, and blood parameters were analyzed by a mixed ANOVA with repeated measures, where treatment was a fixed factor (between subject effect), time period (within-subject) was a random factor having subject (calf) nested within it, and their (period × treatment) interaction was evaluated. A computerized IBM SPSS 26.0 software program was used for ANOVA. Tukey's HSD test was used to measure the differences of means. The levels of significance were considered at $p < 0.05$, and $p < 0.01$.

Results

Evaluation of Physicochemical Properties of FCSP and CSCP

The FCSP is white colored, while the CSCP is slightly grayish (Chart 1). The mean organic matter, total ash, and acid insoluble ash (AIA) of FCSP were estimated 10.62 ± 16.16 , 89.17 ± 1.79 , and 0.96 ± 0.01 , respectively. The density (g/cm^3) of FCSP and CSCP was measured as 0.25 ± 0.0004 and 0.40 ± 0.0004 , respectively. The pH of FCSP and CSCP, suspended in doubled distilled water,

Table 3 Mineral composition of fresh conch shell powder (FCSP) and conch shell calcined powder (CSCP)

Minerals	FCSP	CSCP	Pooled mean \pm S.E.M	<i>p</i> value
Ca (%)	34.89 ^a	38.96 ^b	37.04 \pm 0.84	$p = 0.005$
P (%)	0.23 ^b	0.006 ^a	0.08 \pm 0.05	$p < 0.001$
Mg (ppm)	108.53 ^a	175.53 ^b	144.95 \pm 7.51	$p < 0.001$
Co (ppm)	22.74 ^a	23.53 ^b	23.01 \pm 0.17	$p = 0.035$
Mn (ppm)	7.41 ^a	10.31 ^b	8.99 \pm 0.33	$p < 0.001$
Fe (ppm)	101.83 ^a	196.23 ^b	153.48 \pm 10.58	$p < 0.001$
Cu (ppm)	12.57 ^b	4.22 ^a	7.57 \pm 0.95	$p < 0.001$
Zn (ppm)	779.46 ^b	307.18 ^a	506.89 \pm 52.00	$p < 0.001$

The values with different superscripts (a, b) between two Ca sources differ significantly

was 9.80 and 12.02, respectively. The solubility of Ca-rich powders (FCSP, and CSCP) was found to be highest ($p < 0.001$) in concentrated HCl followed by HNO_3 , HClO_4 , H_2SO_4 , tap water, and distilled water (Table 2). However, the overall solubility of FCSP was higher ($p < 0.001$) than CSCP. The interaction of treatments × solvents was also significant ($p < 0.001$).

The concentration (%) of Ca was found to be significantly greater ($p = 0.005$) in CSCP compared to FCSP, wherein the P content was depressed ($p < 0.001$) in CSCP due to the calcination of FCSP (Table 3). We observed an 11.66% increase in Ca concentration in CSCP compared to FCSP. The concentration of minerals like Mg, Co, Mn, and Fe was enhanced in the CSCP compared to the FCSP (Table 3). However, the calcination process of FCSP reduced the concentration of Cu and Zn, which could be due to the partial vaporization of these essential minerals during the calcination process at high temperatures ($> 650^\circ\text{C}$).

Evaluation of Chemical and Mineral Composition in Different Feeds

All feed ingredients (oat fodder, paddy straw, maize grain, wheat bran, groundnut cake (GNC), and mustard oil cake

Table 2 Solubility % of fresh conch shell powder and conch shell calcined powder in different solvents

Solvents	Solubility % FCSP	Solubility% CSCP	Pooled mean \pm S.E.M	Significance
Distilled water	5.99	3.98	4.99 ^b \pm 0.45	Treatment effect (T), $p < 0.001$ Solvent effect (S), $p < 0.001$ T × S effect, $p < 0.001$
Tap water	5.47	3.42	4.44 ^a \pm 0.46	
HCl	99.21	99.11	99.16 ^f \pm 0.03	
HNO_3	97.41	98.14	97.77 ^e \pm 0.19	
H_2SO_4	40.64	43.95	42.29 ^c \pm 0.76	
HClO_4	98.10	94.46	96.28 ^d \pm 0.82	
Pooled mean \pm S.E.M	57.80 ^B \pm 10.21	57.18 ^A \pm 10.25	57.49 \pm 7.13	

The values with different superscripts (A, B; and a, b, c, d, e, f) among different treatments and between two Ca sources differ significantly

Table 4 Chemical composition (On % DM basis) of different fodders and concentrate mixtures

Parameter	Oat fodder	Paddy straw	Maize grain	Wheat bran	GNC	MOC
Chemical composition (%)						
DM	24.30 ± 0.002	90.18 ± 0.11	94.93 ± 0.25	96.38 ± 0.02	92.98 ± 0.29	91.85 ± 0.13
CP	7.47 ± 0.07	3.66 ± 0.06	8.28 ± 0.15	15.52 ± 0.26	43.22 ± 0.02	33.09 ± 0.31
OM	92.15 ± 0.02	85.13 ± 0.03	96.86 ± 0.01	96.28 ± 0.13	95.58 ± 0.05	92.10 ± 0.03
EE	1.80 ± 0.01	1.23 ± 0.02	4.20 ± 0.05	3.71 ± 0.03	7.23 ± 0.03	7.17 ± 0.03
TCHO	82.77 ± 0.07	80.23 ± 0.02	83.84 ± 0.19	77.06 ± 0.24	45.13 ± 0.07	51.85 ± 0.33
Total ash	7.85 ± 0.02	14.87 ± 0.02	3.14 ± 0.01	3.71 ± 0.13	4.41 ± 0.05	7.89 ± 0.028
NDF	66.23 ± 0.13	78.28 ± 0.02	22.87 ± 0.003	30.33 ± 0.13	27.39 ± 0.04	25.41 ± 0.009
ADF	38.17 ± 0.02	53.35 ± 0.31	5.85 ± 0.10	9.60 ± 0.01	14.28 ± 0.12	16.27 ± 0.08
Hemicellulose	28.05 ± 0.14	24.92 ± 0.31	17.01 ± 0.10	20.71 ± 0.12	13.11 ± 0.14	9.14 ± 0.08
Cellulose	31.46 ± 0.02	44.43 ± 0.06	4.61 ± 0.13	4.74 ± 0.04	11.2 ± 0.01	11.40 ± 0.03
ADL	6.76 ± 0.02	8.58 ± 0.07	1.25 ± 0.21	4.86 ± 0.05	3.08 ± 0.11	4.87 ± 0.06
Mineral composition						
Ca (%)	0.89 ± 0.001	0.20 ± 0.001	0.12 ± 0.001	0.18 ± 0.001	0.18 ± 0.005	0.58 ± 0.004
P (%)	0.29 ± 0.002	0.05 ± 0.002	0.32 ± 0.006	0.53 ± 0.004	0.54 ± 0.005	0.68 ± 0.001
Mg (%)	0.23 ± 0.003	0.17 ± 0.001	0.13 ± 0.01	0.51 ± 0.01	0.60 ± 0.002	0.54 ± 0.001
Cu (ppm)	13.45 ± 0.02	2.75 ± 0.08	1.03 ± 0.004	10.26 ± 0.02	12.86 ± 0.05	6.93 ± 0.05
Zn (ppm)	38.71 ± 0.003	11.81 ± 0.01	20.27 ± 0.13	48.44 ± 0.06	52.32 ± 0.001	47.24 ± 0.14
Mn (ppm)	56.33 ± 0.01	62.33 ± 0.01	6.85 ± 0.08	40.61 ± 0.17	31.29 ± 0.04	58.37 ± 0.02
Fe (ppm)	154.62 ± 0.15	256.11 ± 6.17	58.42 ± 0.28	200.99 ± 7.24	120.41 ± 0.04	178.24 ± 0.003
Co (ppm)	0.06 ± 0.001	0.43 ± 0.002	0.004 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.23 ± 0.001

OM organic matter, CP crude protein, EE ether extract, TCHO total carbohydrate, NDF neutral detergent fiber, ADF acid detergent fiber, ADL acid detergent lignin, GNC groundnut cake, MOC mustard oil cake

(MOC) used for the preparation of total mixed rations (TMRs) were analyzed for chemical composition, such as dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), total carbohydrates (TCHO), total ash (TA), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and acid detergent lignin (ADL). Table 4 displays the estimated values of different feed ingredients. Oil cakes (GNC, and MOC) contained greater amounts of CP compared to other feed resources, whereas fodder resources (paddy straw, and oats fodder) had higher amounts of NDF and ADF. Ca concentration in oats fodder, and MOC was estimated to be higher than other feed ingredients (Table 4). However, P content was greater in MOC, GNC, and wheat bran than in other feed resources. Table 4 presents the detailed values of micro-minerals (Cu, Zn, Mn, Fe, and Co) of these feed resources.

The TMRs prepared as per the composition mentioned in Table 1 were analyzed for chemical and mineral composition (Table 5). We estimated similar DM, OM, CP, EE, TCHO, total ash, ADF, hemicellulose, and lignin among three TMRs, wherein NDF and cellulose concentration were greater in TMRs under T₁ and T₂ than T₀. Three TMRs had a similar concentration of Ca, and P. TMR T₁ and TMR T₂ contained greater amounts of Mg, Cu, Zn, Mn, and Fe

compared to TMR T₀. Cobalt concentration was found identical among the three TMRs.

Voluntary Intake of Nutrients and Utilization of Ca and P

Throughout six fortnights, the total dry matter intake (kg/day/calf) varied from 2.21 to 3.20 in the control (T₀) group, 2.36 to 3.56 in T₁, and 2.33 to 2.97 in T₂, respectively. The average DM intake was significantly higher ($p < 0.05$) in T₁ and T₂ compared to T₀ (Table 6). There was a significant ($p < 0.001$) difference in total DMI (kg/d/calf) among different fortnights indicating the periodic effect, which was mainly due to gain in body weight (BW) by growing calves over different fortnights. DMI (kg/100 kg body weight and g/kg W^{0.75}) was significantly ($p < 0.05$) greater in T₁ and T₂ groups compared to T₀ group, with no significant difference between T₁ and T₂ (Table 6). Similarly, the period effect (Fortnights) was also significant for DMI per unit of body weight (kg/100 kg BW and g/kg W^{0.75}), whereas the interaction between treatment, and period (T × P) showed ($p > 0.05$) non-significant effect for DMI. Calves under T₁ (containing FCSP), and T₂ (containing CSCP) consumed a greater ($p = 0.012$) amount of total OM compared to the control

Table 5 Chemical composition (On % DM basis) and mineral profile of different total mixed rations (TMRs)

Parameter	TMR T ₀	TMR T ₁	TMR T ₂	Pooled mean ± S.E.M	<i>p</i> value
Chemical composition (%)					
DM	43.70	47.03	43.28	44.67 ± 1.26	0.469
OM	89.29	89.43	89.29	89.34 ± 0.04	0.231
CP	12.04	12.13	12.13	12.10 ± 0.05	0.765
EE	2.83	2.85	2.83	2.83 ± 0.01	0.610
TCHO	74.13	74.24	74.12	74.40 ± 0.06	0.766
Total ash	10.71	10.57	10.71	10.66 ± 0.04	0.231
NDF	57.04 ^a	57.21 ^b	57.32 ^c	57.19 ± 0.04	<i>p</i> < 0.001
ADF	33.29	33.65	33.56	33.60 ± 0.12	0.539
Hemicellulose	23.75	23.55	23.76	23.69 ± 0.12	0.770
Cellulose	28.29 ^a	28.83 ^b	28.85 ^b	28.66 ± 0.09	<i>p</i> < 0.001
Lignin	5.00	4.82	4.71	4.84 ± 0.12	0.689
Mineral composition					
Ca (%)	1.29	1.28	1.28	1.28 ± 0.002	0.583
P (%)	0.61	0.62	0.61	0.61 ± 0.009	0.929
Mg (%)	0.25 ^a	0.26 ^b	0.26 ^c	0.22 ± 0.001	<i>p</i> < 0.001
Cu (ppm)	16.36 ^a	20.17 ^b	21.44 ^c	18.51 ± 0.795	<i>p</i> < 0.001
Zn (ppm)	86.72 ^a	89.90 ^c	88.09 ^b	88.24 ± 0.485	0.001
Mn (ppm)	46.56 ^a	51.47 ^b	51.85 ^b	49.96 ± 0.863	<i>p</i> < 0.001
Fe (ppm)	59.26 ^a	63.78 ^b	65.85 ^c	62.96 ± 0.978	<i>p</i> < 0.001
Co (ppm)	0.51	0.59	0.61	0.57 ± 0.029	0.402

The values with different superscripts (a, b, c) among different treatments differ significantly

(T₀) (Table 6). The OMI (kg)/100 kg BW and OMI (g)/kg W^{0.75} ranged from 2.59 (T₀) to 2.92 (T₂) and 79.77 (T₀) to 89.78 (T₂). The values were statistically greater in T₁ and T₂ than in T₀. However, similar to DMI, the periodic effect on OM intake (kg/d/calf) was also statistically significant (*p* < 0.001), whereas OM intake per unit BW increased significantly (*p* < 0.001) as the experiment progressed, indicating a periodic effect. However, the T × P effect was found to be non-significant (*p* > 0.05) for these parameters. We estimated significantly greater (*p* < 0.001) crude protein intake per unit body weight (CPI, g/100 kg BW and g/W^{0.75}) in the treatments supplemented with FCSP (T₁) and CSCP (T₂) compared to the control (T₀) treatment, wherein calves under T₂ consumed a higher (*p* = 0.016) amount of total CP than control T₀. Similar to DM intake, the periodic effect showed statistical significance (*p* < 0.001) only for total CPI, and CPI/kg W^{0.75}, whereas the T × P effect was non-significant.

Calves under different treatments consumed (g/day) similar total Ca through TMR diets (Fig. 3) offered during the digestion trial conducted at the last phase of the growth trial. However, Ca intake (g)/kg W^{0.75} was greater (*p* = 0.021) in CSCP-treated calves (T₂) compared to DCP-treated control calves (T₀). Calves under T₁ had similar values (Ca intake (g)/kg W^{0.75}) with T₀ and T₂. Ca outgo (g/day) through feces was identical among three treatments, whereas Ca digestibility increased significantly (*p* = 0.01) in FCSP (T₁) and

CSCP (T₂) treated calves compared to control T₀. During the same digestion trial, we observed similar total P intake among different treatments (Fig. 4). However, P intake (g)/kg W^{0.75} enhanced (*p* < 0.001) in CSCP-treated calves (T₂) compared to FCSP (T₁) and control calves (T₀). Fecal outgo of P reduced significantly in T₂ in comparison to T₀; however, the value of T₁ was similar to T₀ and T₂. The addition of fresh and calcined powder of conch shell increased (*p* < 0.001) P digestibility in T₁ and T₂ compared to T₀ in the present study (Fig. 4).

Growth Performance and Feed Conversion Efficiency

The initial body weight (kg) was similar among T₀, T₁, and T₂ groups (Table 6). At the end of the 90-day trial, the corresponding final body weight (kg) was 106.03, 106.31, and 106.68 in the T₀, T₁, and T₂ groups, respectively. The statistical difference among the groups was non-significant (*p* > 0.05). Calves under T₁ and T₂ treatments gained 3.03, and 5.78% higher compared to T₀ during the feeding period; however, the difference was non-significant. The pattern of fortnightly body weight of calves is expressed graphically in Fig. 2, which indicated no difference among different treatments. The average daily body weight gain (g/d/animal) ranged from 366.60 (T₀) to 392.24 (T₂); the difference was observed similar among the treatment groups. Furthermore,

Table 6 Body weight change, voluntary intake of different nutrients, and feed conversion efficiency affected by different dietary treatments

Attributes	Treatments			Pooled Mean ± S.E.M	p value		
	T ₀	T ₁	T ₂		Treatment effect (T)	Period effect (P)	Interaction effect (T×P)
Voluntary intake of nutrients							
Av. DM intake (kg/d)	2.71 ^a	2.95 ^b	2.97 ^b	2.88 ± 0.06	0.044	<0.001	0.981
Av. DM intake kg/100 kg BW	2.99 ^a	3.25 ^b	3.27 ^b	3.17 ± 0.03	<0.001	0.019	0.394
Av. DM intake (g/kg W ^{0.75})	91.85 ^a	100.07 ^b	100.54 ^b	97.49 ± 0.91	<0.001	<0.001	0.095
Av. OM intake (kg/d)	2.36 ^a	2.64 ^b	2.65 ^b	2.55 ± 0.06	0.012	<0.001	0.992
Av. OM intake kg/100 kg BW	2.59 ^a	2.91 ^b	2.92 ^b	2.81 ± 0.03	<0.001	0.013	0.626
Av. OM intake (g/kg W ^{0.75})	79.77 ^a	89.47 ^b	89.78 ^b	86.34 ± 0.92	<0.001	<0.001	0.394
Av. CP intake (kg/d)	336.30 ^a	370.56 ^{ab}	376.29 ^b	361.05 ± 7.89	0.016	<0.001	0.986
Av. CP intake kg/100 kg BW	369.81 ^a	408.40 ^b	414.14 ^b	397.45 ± 3.77	<0.001	0.072	0.594
Av. CP intake (g/kg W ^{0.75})	11.37 ^a	12.56 ^b	12.74 ^b	12.23 ± 0.12	<0.001	<0.001	0.295
Body weight gain							
Initial body weight (kg)	71.40	70.60	70.05	70.68 ± 2.90	0.984	-	-
Final body weight (kg)	106.03	106.31	106.68	106.34 ± 4.22	0.998	-	-
Total body weight gain (kg)	34.63	35.71	36.63	35.64 ± 1.70	0.905	-	-
Fortnightly body weight gain (kg)	5.77	5.95	6.10	5.94 ± 0.19	0.786	0.124	1.000
Av. daily body weight gain (g)	366.60	379.57	392.24	379.47 ± 11.25	0.695	0.806	1.000
Feed conversion efficiency (FCE) and feed conversion ratio (FCR)							
FCE (ADG as % of DMI)	14.08	13.66	14.12	13.95 ± 0.44	0.893	0.017	0.999
FCR (kg DMI/kg gain)	8.99	8.03	7.54	8.19 ± 0.60	0.621	0.500	0.613
FCE (ADG as % of CPI)	0.11	0.11	0.11	0.11 ± 0.003	0.840	0.020	0.999
FCR (kg CPI/kg gain)	47.77	49.81	51.59	49.72 ± 1.60	0.644	0.129	1.000

Values with different superscripts (a, b) in a row are significantly different

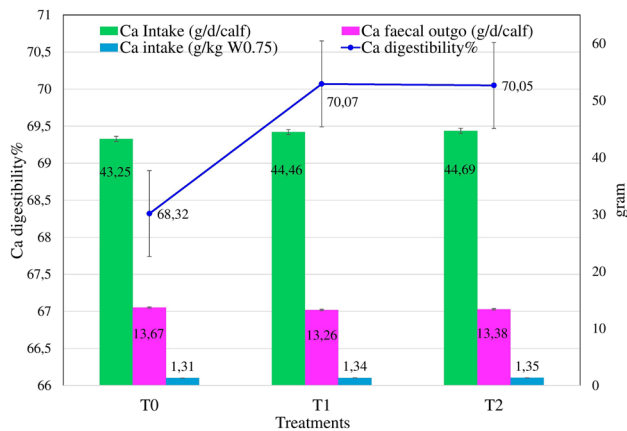


Fig. 3 Effect of different Ca sources on Ca utilization pattern in calves under different treatments

periodic (P), and interaction (P×T) effects were also non-significant ($p > 0.05$). Similarly, fortnightly body weight gain was also statistically similar among the three treatments.

Different sources of Ca in the present study did not alter the feed conversion efficiency (ADG as percent of DM intake), and feed conversion ratio (kg DMI/kg weight gain)

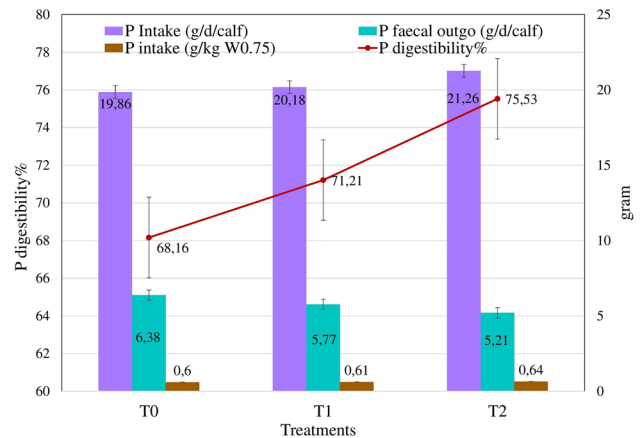


Fig. 4 Effect of different Ca sources on P utilization pattern in calves under different treatments

in calves under three treatment groups (Table 6). Similarly, FCE (ADG as percentage of CPI) and FCR (kg CPI/kg weight gain) were also statistically identical among different treatments. However, the periodic effect was found significant ($p < 0.05$) for FCE (based on DMI and CPI), indicating

Table 7 Blood glucose, urea, Ca and P, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine transaminase (ALT) concentration in animals fed with different TMRs

Days of feeding	T ₀	T ₁	T ₂	Pooled mean ± S.E.M	Significance
Blood glucose (mg/dl plasma)					
0th days	49.06	51.59	51.30	50.65 ± 0.68	Treatment effect (T), <i>p</i> = 0.190 Period effect (P), <i>p</i> = 0.190 T × P, <i>p</i> = 0.224
45th days	49.86	47.17	51.67	49.57 ± 0.85	
90th days	50.81	51.88	52.14	51.61 ± 0.73	
Mean ± S.E.M	49.91 ± 0.79	50.22 ± 0.80	51.70 ± 0.68	50.61 ± 0.45	
Urea (mg/dl of plasma)					
0th days	16.90	16.36	17.49	16.92 ^c ± 0.25	Treatment effect (T), <i>p</i> = 0.867 Period effect (P), <i>p</i> < 0.001 T × P, <i>p</i> = 0.088
45th days	14.11	13.71	13.99	13.94 ^a ± 0.14	
90th days	15.94	16.49	15.52	15.98 ^b ± 0.25	
Mean ± S.E.M	15.65 ± 0.34	15.52 ± 0.37	15.67 ± 0.47	15.61 ± 0.22	
Calcium (mg/dl plasma)					
0th days	10.28	10.16	10.38	10.27 ^a ± 0.12	Treatment effect (T), <i>p</i> = 0.598 Period effect (P), <i>p</i> < 0.001 T × P, <i>p</i> = 0.084
45th days	10.82	11.32	11.38	11.17 ^b ± 0.09	
90th days	11.41	11.18	11.14	11.24 ^b ± 0.07	
Mean ± S.E.M	10.84 ± 0.17	10.88 ± 0.15	10.97 ± 0.13	10.90 ± 0.09	
Phosphorus (mg/dl of plasma)					
0th days	5.49	5.61	5.56	5.56 ± 0.08	Treatment effect (T), <i>p</i> = 0.088 Period effect (P), <i>p</i> = 0.313 T × P, <i>p</i> = 0.041
45th days	5.14	5.70	5.71	5.52 ± 0.09	
90th days	5.72	5.62	5.64	5.66 ± 0.05	
Mean ± S.E.M	5.45 ± 0.08	5.65 ± 0.08	5.64 ± 0.05	5.58 ± 0.04	
ALP (IU/l serum)					
0th days	275.76	275.83	275.96	275.85 ± 3.72	Treatment effect (T), <i>p</i> = 0.994 Period effect (P), <i>p</i> = 0.778 T × P, <i>p</i> = 1.000
45th days	278.25	278.25	279.34	278.61 ± 3.27	
90th days	279.26	279.55	279.66	279.49 ± 3.46	
Mean ± S.E.M	277.76 ± 3.00	277.88 ± 5.09	278.32 ± 1.45	277.98 ± 1.98	
AST (IU/l serum)					
0th days	98.30	98.65	98.30	98.42 ± 1.11	Treatment effect (T), <i>p</i> = 0.906 Period effect (P), <i>p</i> = 0.944 T × P, <i>p</i> = 0.999
45th days	99.36	99.01	98.30	98.89 ± 1.64	
90th days	98.65	98.63	97.24	98.18 ± 1.40	
Mean ± S.E.M	98.77 ± 1.25	98.77 ± 0.96	97.95 ± 1.84	98.49 ± 0.79	
ALT (IU/l serum)					
0th days	20.86	21.57	21.04	21.16 ± 0.35	Treatment effect (T), <i>p</i> = 0.181 Period effect (P), <i>p</i> = 0.507 T × P, <i>p</i> = 0.991
45th days	20.29	21.30	21.06	20.89 ± 0.31	
90th days	20.96	21.97	21.43	21.45 ± 0.34	
Mean ± S.E.M	20.70 ± 0.34	21.61 ± 0.34	21.18 ± 0.28	21.17 ± 0.19	

The values with different superscripts (a, b, c) among different treatments/period differ significantly

calves fed at younger age had greater FCE compared to older age. However, the interaction between treatment × period (T × P) was similar for FCE, and FCR.

Blood Metabolites and Enzymes

The blood glucose concentration (mg/dl of plasma) ranged from 49.06 to 50.81 in T₀, 47.88 to 51.88 in T₁, and 51.30 to 52.14 in T₂ groups, respectively (Table 7). Ca from FCSP and CSCP did not alter the blood glucose concentration. Additionally, no statistically significant difference was

recorded among different periods of blood sampling. Similarly, the interaction effect between treatment, and period (T × P) was also observed to be non-significant. The average blood urea concentration was 15.65, 15.52, and 15.67 (mg/dl) in the T₀, T₁, and T₂ groups, indicating no significant difference among different treatments. However, the periodic effect was significant (*p* < 0.001). Furthermore, the interaction effect (T × P) was non-significant.

The Ca concentration (mg/dl of plasma) at the start of the experiment was estimated 10.28, 10.16, and 10.38 (mg/dl) in T₀, T₁, and T₂ groups, and it was 11.41, 11.18, and 11.14

(mg/dl of plasma) at the end of the experiment (Table 7). The average plasma Ca concentration (mg/dl of plasma) in calves of the present study ranged from 10.88 (T_0) to 10.97 (T_2). However, the difference ($p > 0.05$) was non-significant among the treated groups. A significant rise ($p < 0.001$) in blood Ca concentration was observed as the experiment progressed due to the feeding of dicalcium phosphate in T_0 and conch shell powders (fresh and calcined) in T_1 and T_2 as a source of Ca. However, the interaction effect ($T \times P$) was similar for plasma Ca concentration. We observed average plasma P concentration (mg/dl plasma) ranging from 5.45 in T_0 to 5.65 in T_2 . The treatment effect (T), periodic effect (P), and interaction effect ($T \times P$) were indistinguishable among the three treatments.

The average concentration (IU/l of plasma) of alkaline phosphatase (ALP) ranged from 275.76 (T_0) to 279.66 (T_2) in the study (Table 7). We observed no difference among the three treatments. The values for alkaline phosphatase level in blood were within the normal physiological range which indicated no adverse effect of new Ca source on the liver. The treatment effect (T), periodic effect (P), and interaction ($T \times P$) effect were comparable among the three treatments. The average concentration (IU/l plasma) of aspartate aminotransferase (AST) was observed to be non-significant ($p > 0.05$) among the different groups (T_0 98.77 IU/l, T_1 98.77 IU/l, and T_2 97.95 IU/l). In addition, the periodic effect, and interaction ($T \times P$) effect were also statistically non-significant. We observed similar average concentrations (IU/l plasma) of alanine transaminase (ALT) in different treatment groups; the values were within the normal physiological range. Moreover, the periodic effect and interaction effect ($T \times P$) were also identical.

Discussion

Physico-chemical Properties of FCSP and CSCP

The calcination process of fresh conch shell powder (FCSP) in the present study transformed the most stable polymorphs of CaCO_3 of conch shell (shankha) aragonite form into calcite form, along with the formation of portlandite and lime as the majority component [26]. Similar to our findings, properly ground ocean quahog clamp shells (OQCS), and surf clam shells (SCS) contain a higher percentage of Ca ($> 36\%$) [27]. Both FCSP and conch shell calcined powder (CSCP) had greater solubility in HCl, which is advantageous to livestock due to the acidic environment in the stomach (including abomasum in ruminants). Similarly, Rasheed et al. [7] concluded that conch shell powder was soluble in diluted HCl. Similar to our finding, Savita et al. [26] also reported pH values of 12.80 and 9.05 for conch shell calcined powder, and fresh conch shell powder. The acid-insoluble ash (AIA) content of FCSP corroborated the findings of Savita et al. [26] who observed 0.96% AIA in

fresh conch shell powder. The calcination process increased the Ca concentration of conch shell powder; whereas it depressed the P concentration in the present study. High temperature ($> 650^\circ\text{C}$) during the calcination process in muffle furnace could have resulted in the reduction of P, Cu, and Zn in conch shell powder, whereas the level of Mg, Co, Mn, and Fe increased in this powder along with Ca due to such calcination process. The elevated levels of these essential minerals might be advantageous to the livestock to fulfill their nutritional requirements.

Voluntary Intake of Nutrients and Utilization of Ca and P

The incorporation of FCSP and CSCP in the total mixed ration (TMR) of T_1 and T_2 resulted in similar Ca, and P concentrations with that of control T_0 TMR. The Ca/P ratio was estimated at 2.11, 2.06, and 2.10 in T_0 , T_1 , and T_2 , which could be considered ideal for calf ration (Fig. 1). The Ca:P ratio of 2:1 or greater was recommended by NRC [28] to avoid the formation of urinary calculi in sheep. Similarly, it was investigated that the Ca/P ratio in the complete ration plays an imperative role in plummeting the occurrence of mineral calculi in beef cattle, and that a good balanced diet could have an essential protective role in the development of this pathology [29]. The Ca/P ratio should be maintained 2:1 in cattle diet; any Ca/P imbalance may result in higher phosphate elimination through urine which is an imperative factor in the genesis of calculi [30]. Antonio Esteves et al. [16] also explained that the ratio of Ca/P has a great impact on the degree of absorption of both elements in animals. An excess of either causes amplification in fecal voidance by the development of insoluble Ca that is not obtainable for absorption. However, Mg, Cu, Zn, Mn, and Fe increased significantly due to the addition of FCSP and CSCP during the formulation of TMR in T_1 and T_2 in the present study. Voluntary intake of nutrients (DM, OM, and CP) as expressed per unit of body weight (per 100 kg body weight or $\text{kg W}^{0.75}$) enhanced due to the replacement of DCP with FCSP, and CSCP in the diets of crossbred calves. Calves under three treatments fulfilled their nutritional requirements in terms of DM, and CP intake as recommended by NRC [31]. Anil et al. [24] also reported a similar DM intake pattern per 100 kg body weight of finisher female calf fed with TMR (DCP 7.85%, TDN 57.51%) under an intensive system of management.

We have not found any published reports on the effect of feeding conch shell powder or conch shell calcined powder in ruminants (calves or heifers or dairy cows) and non-ruminants (including poultry birds) to compare our present results. However, some reports are available on the use of different marine shells as a source of Ca. Similar to our findings, Finkelstein et al. [27] reported that the DMI

was lowest for cows fed aragonite and greatest for cows fed ocean quahog clam shell (*Arctica islandica*), and surf clam shell (*Spisula solidissima*). However, some researchers reported that the oceanic source of oyster shells could be used effectively in poultry birds as a source of Ca. Similar to our findings, Lana [32] discovered that broiler hens fed diets with 0.42% calcium from Charru Mussel Shell meal consumed more feed than the other groups fed different organic calcium sources. Similarly, Oso et al. [33] also reported that broilers fed a diet containing oyster shells as the Ca source had greater feed consumption, and body weight gain than broilers fed a diet having limestone. Daily intake of Ca significantly increased in poultry birds fed with oyster shells, and limestone compared to control [34]. Cambra-Lopez et al. [35] evaluated the P digestibility of the three commercial sources of DCP, and found that the digestibility of P was 80.1% (DCP1), 77.4% (DCP2), and 71.4% (DCP3), respectively. We have also recorded similar digestibility of P in the present study. Omole et al. [36] discovered that replacing dicalcium phosphate with oyster shell did not reduce feed intake but increased growth rate in finishing broiler. Mako et al. [37] observed that the amount of feed consumed by the poultry birds on diet 1 (oyster shells 54% and bone meal 46%) was greater than other treatment combinations. Similarly, the report by Olgun [38] showed that the supplementation of oyster shells up to one-third of the Ca requirement increased eggshell weight; however, it had an antagonistic effect on feed consumption and egg weight. This finding, however, conflicts with a study by Ahmed et al. [39] that claimed 24-week-old Bovan pullets treated with oyster shells had reduced feed intake than those treated with limestone. Badejo et al. [40] observed no significant ($p < 0.05$) difference between the oyster shell group, and the periwinkle shell group concerning feed consumption in the spent layer. CaCO_3 supplementation improved apparent starch digestibility, whereas the digestibility of other nutrients (DM, OM, CP, EE, and ADF) was not affected by CaCO_3 [41]. Santana et al. [42] investigated that Ca sources did not affect the digestibility coefficients in pigs.

Growth Performance and Feed Conversion Efficiency

Despite increased voluntary intake, the new marine source of Ca, either in fresh conch shell powder (FCSP) form or conch shell calcined powder (CSCP) form, did not alter the growth pattern in weaned crossbred calves compared with control calves fed with DCP. We did not find any published literature to compare the works on the usage of conch shell powder (fresh or calcined) as a potential source of Ca in ruminants. However, our findings are in close agreement with the finding of Santana et al. [42] who investigated that calcium supplied in the form of calcitic limestone,

monocalcium phosphate, calcinated bone flour, and oyster flour had no difference in growth performance of pigs. Also, similar results were obtained in brown laying hens; different Ca sources (100% fine limestone; 60% fine limestone and 40% large limestone; and 60% fine limestone and 40% oyster shell) had identical effects on body weight gain [43]. The FCE and FCR remained unaltered due to FCSP and CSCP treatments in calves. The results corroborated the findings of Olgun et al. [38], who reported no significant difference between the control (limestone) and the treatments (replaced with 1/3 eggshell, 1/3 oyster shell, 1/3 eggshell + 1/3 oyster shell, 2/3 eggshell, and 2/3 oyster shell) to modify FCR. The study by Badejo et al. [40] revealed that feed consumption and feed conversion ratio in the spent layer did not differ significantly among different Ca sources (bone meal, oyster shell, periwinkle shell, and limestone).

Blood Metabolites and Enzymes

Ca from three sources (DCP, FCSP, and CSCP) did not affect blood glucose, and blood urea concentration in growing calves. Similarly, the concentration of plasma Ca, and P was also similar among the three treatments. The results clearly indicated that marine sources of Ca in different forms (fresh and calcined) had no adverse effects on the concentration of glucose, urea, Ca, and P in blood plasma. The concentration of ALP, AST, and ALT did not differ among the three treatments, indicating no adverse effects on liver enzymes due to the feeding of new Ca sources. All these parameters were within the normal physiological range. Manowari and Singh [44] estimated almost similar blood glucose levels in crossbred heifers and cows. The mean blood urea value in normal healthy control livestock was reported as 26.76 mg/dl [45]. Reece and Hotchkiss [46] measured similar Ca, and P levels in calves fed with different feeding modules. Gading et al. [47] also observed identical blood Ca, and P concentrations in weaner calves. The phosphorus concentration in the present study corroborated the finding of Ramana et al. [48] who revealed that plasma P (mg%) concentrations in stall-fed heifers and cattle calves were from 5.03 to 5.18 mg% and 5.24 to 6.60 mg%, respectively. Brazilian male sheep fed a basal ration supplemented with different sources of Ca, namely limestone (L), alfalfa hay (AH), oyster shell meal (OSM), and citrus pulp (CTP) utilized inorganic sources of Ca in a greater way compared to other sources [10]. The average ALT values reported in this study were within the normal physiological range of cattle [49]. The typical ALT range is 11–40 IU/l of plasma [50]. Similarly, the normal activity of AST in the blood is 78 to 132 IU/l in healthy cattle [50]. Mamun et al. [51] reported a similar pattern of blood ALP, AST, ALT, and P levels in cattle reared in the tropical zone of Bangladesh. However, we estimated greater plasma Ca concentration in the present study compared to the study of Mamun et al. [51].

Conclusions

Both fresh conch shell powder, and conch shell calcined powder are rich in Ca, Zn, Fe, and Co; however, CSCP is richer in Ca, Mg, Mn, and Fe, whereas FCSP contained a greater amount of Cu, and Zn. Despite enhanced voluntary intake of different nutrients, the new marine organic source of Ca, either in fresh or conch shell calcined powder form, did not modify the growth performance in weaned crossbred calves compared to control dicalcium phosphate-fed calves. Blood metabolites and liver enzyme profiles remained unaffected on supplementation of fresh or calcined conch shell powder. Short-term experimentation depicted some promising scope for the use of locally available conch shell powder (fresh/calcined) as a potential source of Ca for feeding to the livestock; however, long-term experimentation can ascertain the positive response of this marine-based organic Ca source in growing, pregnant, and lactating livestock as well as in poultry birds.

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Author Contribution TKD, and AC conceived and designed the experiment; JB, TKD, AC, and SR conducted experiment and formal analysis; AM, and SR analyzed the data; JB, TKD, and AC wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability The data will be made available from the corresponding author on reasonable request.

Declarations

Ethical Approval The animal feeding experiment was conducted as per committee approval of ICAR-National Dairy Research Institute (NDRI Deemed University), Karnal, Haryana, India (NDRI Reg. No. 20-M-AN-01, 2020), and accordingly, all standard Institutional and Government of India ethical protocols including animal right/welfare issues were followed during the entire animal trial period.

Competing Interests The authors declare no competing interests.

References

- NCERT (2015) India – size and location. National Council of Education, Research and Training. <https://ncert.nic.in/ncerts//iess101.pdf> (assessed on 08.11.2023)
- Silva HT, Mesquita-Guimaraes J, Henriques B, Silva FS, Fredel MC (2019) The potential use of oyster shell waste in new value-added by-product. *Resources* 8:13. <https://doi.org/10.3390/resources8010013>
- Xu Y, Ye J, Zhou DJ, Su L (2020) Research progress on applications of calcium derived from marine organisms. *Scientific Rep* 10:18425. <https://doi.org/10.1038/s41598-020-75575-8>
- Wikipedia. Conch. Available online: <https://en.wikipedia.org/wiki/Conch> (accessed on 11 Nov. 2023)
- Babafakruddin DH, Doddamani MS (2017) Marine products and their therapeutic importance. *Internat Ayurveda Pub* 2:527–533
- Gupta LN (2020) Physicochemical evaluation of Shankha Bhasma: a calcium-enriched ayurvedic preparation. *World J Pharmacol Res Technol* 9:1–9
- Rasheed SP, Shivashankar M (2017) Synthesis and characterization of Shanku Bhasma - an anti-ulcer herbomineral formulation. In IOP Conference Series: Materials Sci Eng 263:022026. <https://doi.org/10.1088/1757-899X/263/2/022026>
- Ammerman CB, Goodrich D (1983) Advances in mineral nutrition in ruminants. *J Anim Sci* 57 (suppl 2):519–533. <https://pubmed.ncbi.nlm.nih.gov/6352594> (assessed on 09.11.2023)
- Shojaeian Z, Sadeghi R, Roudsari RL (2019) Calcium and vitamin D supplementation effects on metabolic factors, menstrual cycles and follicular responses in women with polycystic ovary syndrome: a systematic review and meta-analysis. *PubMed* 10:359–369. <https://doi.org/10.22088/cjim.10.4.359>
- Dias RS, Kebreab E, Vitti DMSS, Roque AP (2008) Application and comparison of two models to study effects of calcium sources in sheep. *Anim Feed Sci Technol* 143:89–103. <https://doi.org/10.1016/j.anifeedsci.2007.05.006>
- Jm L, Lamotte C, Boukandoura B, Cayzele A, Libersa C, Delanoy CW, Borgies B (2007) Effects of two marine dietary supplements with high calcium content on calcium metabolism and biochemical marker of bone resorption. *Eur J Clin Nutr* 62:879–884. <https://doi.org/10.1038/sj.ejcn.1602797>
- Flammini L, Martuzzi F, Vivo V, Ghirri A, Salomi E, Bignetti E, Barocelli E (2016) Hake fish bone as a calcium source for efficient bone mineralization. *Internat J Food Sci Nutri* 67:265–273. <https://doi.org/10.3109/09637486.2016.1150434>
- Kiarie E, Nyachoti CM (2010) Bioavailability of calcium and phosphorus in feedstuffs for farm animals. In: Vitti DMSS, Kebreab E (eds) Phosphorus and calcium utilization and requirements in farm animals, Chapter 6. Publisher CABI, pp 76–93. <https://doi.org/10.1079/9781845936266.0076>
- Coffey RD (1994) The biological availability of phosphorus in various feed ingredients for pigs and chicks. PhD thesis submitted to University of Kentucky, Princeton, Kentucky, USA. <http://ci.nii.ac.jp/ncid/BA47748061>
- Kim S, Ravichandran YD, Kong C (2012) Applications of calcium and its supplement derived from marine organisms. *Critical Rev Food Sci Nutr* 52:469–474. <https://doi.org/10.1080/10408391003753910>
- Antonio ELM, Roldan STMC, Avendaño RL, Minor PF, González-Reyna A, Javier Trejo Meza F, Cesar CRJ (2023) Importance of minerals in the diet of cattle in the tropical climate of Mexico. In: Book - Landraces - its productive conservation in animals and plants. Editors: González-Reyna. A. and Kaushik P. pp 1–136. IntechOpen. <https://doi.org/10.5772/intechopen.110491>
- NRC (2005) Mineral tolerance of animals, National Research Council, 2nd edn. National Academy of Sciences National Academy Press, Washington, DC
- Marin F, Roy NL, Marie B (2012) The formation and mineralization of mollusk shell. *Front Biosci* S4:1099–1125. <https://doi.org/10.2741/321>
- Checa AG (2018) Physical and biological determinants of the fabrication of molluscan shell microstructures. *Front Mar Sci* 5:353. <https://doi.org/10.3389/fmars.2018.00353>
- McDougall C, Degnan BM (2018) The evolution of mollusk shells. *Wires Dev Biol* e313. <https://doi.org/10.1002/wdev.313>
- O'Dell JW (1993) Determination of phosphorus by semi-automated colorimetry. *Method* 365. 1 Environmental Monitoring

- Systems Laboratory Office of Research and Development. U.S. Environmental Protection Agency Cincinnati, Ohio 45268. In Elsevier eBooks (pp. 479–495). <https://doi.org/10.1016/b978-0-8155-1398-8.50027-6>
22. AOAC (2012) Official methods of analysis of AOAC International, 19th ed. Gaithersburg, Maryland, USA
 23. Van Soest PJ, Robertson JB, Levis BA (1991) Method of dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci* 74:3583–3587
 24. Anil Dutta TK, Chatterjee A, Yadav SK, Mandal DK, Mohammad A (2023) Effect of exogenous fibrolytic enzymes supplementation to improve voluntary intake, availability of nutrients and growth performance in weaned crossbred calves. *Indian J Anim Sci* 93:896–902. <https://doi.org/10.56093/ijans.v93i9.131419>
 25. Snedecor GW, Cochran WG (1994) Statistical methods. Oxford and IBH Publishing Co., New Delhi
 26. Savita S, Yadevedra Y, Usha S, Sushma R, Khemchand S (2020) Characterization of conch shell nanoparticles (Shanka Bhasma) synthesized by the classical method. *Scholars Inter J Trad Compl Med* 3:90–99. <https://doi.org/10.36348/sijctm.2020.v03i05.002>
 27. Finkelstein AD, Wohlt JE, Emanuele SM, Tweed SM (1993) Composition and nutritive value of ground sea clam shells as calcium supplements for lactating Holstein cows. *J Dairy Sci* 76(2):582–589. [https://doi.org/10.3168/jds.S0022-0302\(93\)77378-6](https://doi.org/10.3168/jds.S0022-0302(93)77378-6)
 28. NRC (1985) Nutrient requirements of sheep. National Research Council, 6th Edition, National Academy of Sciences, National Academy Press, Washington, D.C
 29. Gianesella M, Giudice E, Messina V, Cannizzo C, Florian E, Piccione G, Morgante M (2010) Effect of an unbalanced Ca/P diet on blood parameters and urolithiasis in growing calves. *Veterinari-jaIr Zootechnika* 32–36. <https://vetzoo.lsmuni.lt/data/vols/2010/49/pdf/gianes> (assessed on 13.11.2023)
 30. Rosol TJ, Capen CC (1997) Calcium-regulating hormones and diseases of abnormal mineral (calcium, phosphorus, magnesium) metabolism. In: Elsevier eBooks, 619–702. <https://doi.org/10.1016/b978-012396305-5/50024-5>
 31. NRC (2001) Nutrient requirements of dairy cattle. 7th, Revised. National Academy Press, National Research Council, Washington, DC
 32. Lana GRQ (2017) Calcium sources in the coastal region of Alagoas in diets for broiler chickens. Doctoral thesis submitted to Federal University of Alagoas, Rio Largo, AL, Brazil
 33. Oso AO, Idowu AA, Niameh OT (2011) Growth response, nutrient and mineral retention, bone mineralisation and walking ability of broiler chickens fed with dietary inclusion of various unconventional mineral sources. *J Anim Physiol Anim Nutri* 95:461–467. <https://doi.org/10.1111/j.1439-0396.2010.01073.x>
 34. Rathnayaka RMD, Mutucumarana RK, Andrew MS (2020) Free-choice feeding of three different dietary calcium sources and their influence on egg quality parameters of commercial layers. *J Agricul Sci* 15:50–62. <https://doi.org/10.4038/jas.v15i1.8671>
 35. Cambra-Lopez M, Moset V, del Carmen LM, Sebastian Mesa J, Carpintero L, Donadeu A, Pascual JJ (2021) Evaluation of phosphorus digestibility from monocalcium and dicalcium phosphate sources and comparison between total tract and prececal digestibility standard methods in broilers. *Anim* 11:3427. <https://doi.org/10.3390/ani11123427>
 36. Omole AJ, Ogbosuka GE, Salako RA, Ajayi OO (2005) Effect of replacing oyster shell with gypsum in broiler finisher diet. *J Appl Sci Res* 1:245–248
 37. Mako AA, Mosur AO, Adedeji BS, Jemiseye FO, Abokede T (2017) Comparative use of oyster shell and limestone as sources of calcium in the diet of laying chickens. *Nigerian J Anim Prod* 44:275–281
 38. Olgun O, Yildiz AO, Cufadar Y (2015) The effects of eggshell and oyster shell supplemental as calcium sources on performance, eggshell quality and mineral excretion in laying hens. *Indian J Anim Res* 49:205–209. <https://doi.org/10.5958/0976-0555.2015.00056.4>
 39. Ahmed NM, Atti AKA, Elamin KM, Dafalla KY, Malik HEE, Dousa BM (2013) Effect of dietary calcium sources on laying hens performance and egg quality. *J Anim Prod Adv* 3:226–231
 40. Badejo HA, Dilala MA, Potiskum SB, Doma UD (2019) The effect of various calcium and phosphorus sources on productive and egg quality performances of spent layers. *IOSR J24:69–75*
 41. Clark JH, Plegge AW, Davis CL, McCoy GC (1989) Effects of calcium carbonate on ruminal fermentation, nutrient digestibility, and cow performance. *J Dairy Sci* 72:493–500. [https://doi.org/10.3168/jds.S0022-0302\(89\)79131-1](https://doi.org/10.3168/jds.S0022-0302(89)79131-1)
 42. Santana ALA, de Oliveira Carvalho PL, Cristofori EC, da Silva Chambo PC, Barbizan M, Nunes RV, Genova JL (2018) Supplementation of pig diets in the growth and termination phases with different calcium sources. *Trop Anim Health Prod* 50:477–484. <https://doi.org/10.1007/s11250-017-1456-8>
 43. Safaa H, Serrano MP, Valencia DG, Frikha M, Jiménez-Moreno E, Mateos GG (2008) Productive performance and egg quality of brown egg-laying hens in the late phase of production as influenced by level and source of calcium in the diet. *Poultry Sci* 87:2043–2051. <https://doi.org/10.3382/ps.2008-00110>
 44. Manowari, Singh C (2001) Blood glucose concentration in cross-bred (Friesian x Harijana) heifers and cows. *Indian J Anim Sci* 71: 848–849. <http://epubs.icar.org.in/ejournal/index.php/IJAnS/arti> (assessed on 10.11.2023)
 45. Hagawane SD, Shinde SB Rajguru DN (2009) Haematological and blood biochemical profile in lactating buffaloes in and around Parbhani city. *Vet World* 2:467–469. <http://www.veterinaryworld.org/Vol.2/December/Haem> (assessed on 10.11.2023)
 46. Reece WO, Hotchkiss DK (1987) Blood studies and performance among calves reared by different methods. *J Dairy Sci* 70:1601–1611. [https://doi.org/10.3168/jds.S0022-0302\(87\)80188-1](https://doi.org/10.3168/jds.S0022-0302(87)80188-1)
 47. Gading BMWT, Agus A, Irawan A, Panjono P (2020) Growth performance, hematological and mineral profile of post-weaning calves as influenced by inclusion of pelleted-concentrate supplement containing essential oils and probiotics. *Iranian J Applied Anim Sci* 10:461–468. http://ijas.iaurasht.ac.ir/article_675343_885d26155ceb8c4190bf285af6c538b6.pdf (assessed 13.11.2023)
 48. Ramana DBV, Nirmala G, Kumar AV (2011) Mineral profile of feeds, fodders and blood plasma of dairy animals in KVK adopted villages of Ranga Reddy district in Andhra Pradesh. *Indian J Dryland Agricul Res Dev* 26:26–31. <http://www.indianjournals.com/ijor.aspx?target=ijor:ijda> (assessed on 05.11.2023)
 49. Stojević Z, Piršljjin J, Milinković-Tur S, Zdelar-Tuk M, Ljubić BB (2005) Activities of AST, ALT and GGT in clinically healthy dairy cows during lactation and in the dry period. *Veterinarski Arhiv* 75:67–73 (http://ufgrs.br/lacvet/restrito/pdf/stojevic_liver_enzym)
 50. Ingvarsten KL (2006) Feeding and management-related diseases in the transition cow: physiological adaptations around calving and strategies to reduce feeding-related diseases. *Anim Feed Sci and Technol* 126:175–213
 51. Mamun MdAA, Hassan MM, Shaikat AH, Islam S, Hoque MdA, Uddin H, Hossain MB (2014) Biochemical analysis of blood of native cattle in the hilly area of Bangladesh. *Bangladesh J Vet Med* 11:51–56. <https://doi.org/10.3329/bjvm.v11i1.16513>

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