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Blood profile of grazing Bunaji bulls supplemented with diets containing cooked mucuna

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Target Audience: Livestock farmers, Animal scientists

Abstract

A total of 12 yearling Bunaji bulls weighing between 146.00 kg – 147.67 kg were used to evaluate the blood profile of grazing white Bunaji bulls supplemented with diets containing cooked mucuna seed meal (CMSM) in a completely randomized design. Mucuna seeds were bought from farmers within Makurdi metropolis and its environs and used for the study. Collected seeds were poured into boiling water in a pot set over an open fire and allowed to boil for 60 minutes. Thereafter the cooked seeds were removed from the fire, drained using local baskets and sun-dried on concrete slabs for 7 days. The sun-dried seeds were then packed and crushed into a meal using a cereal grinding mill and bagged in synthetic sacks for use. Four (4) experimental diets were compounded to contain 0%, 25%, 50% and 75% CMSM and the diets were tagged diets A, B, C and D respectively. Results showed that cooking improved the crude protein (CP) and ether extract (EE) values of the seeds. Boiling the mucuna seeds reduced all anti-nutrients except flavonoids. There was no treatment effect (P>0.05) in the mean values for all the hematological parameters measured. The serum biochemical parameters however, showed treatment effect (P<0.05) in the mean values for all the parameters measured except in urea and total cholesterol values. Although almost all the serum biochemical parameters showed treatment effect (P<0.05) values were within normal ranges. Cooked mucuna seed meal can be used to replace soybean meal in the diets of Bunaji bulls without compromising health of the animals.

Key words*: Anti-nutrients, Cooked mucuna seed meal, Haematology, Serum biochemistry, Bunaji bulls*

Description of Problem

The population of Nigeria is increasing rapidly and with the increase in demography comes the need for increase in livestock production in order to meet the protein needs of the populace. The level of animal protein consumption has a direct influence on the general wellbeing and health of the populace (1). In Nigeria, like many other developing countries of the world, the daily dietary intake of animal protein falls short of the recommended 27g /caput/day (2). This short fall in protein intake is orchestrated by the high cost of livestock products brought about by the cost of conventional feed ingredients, which consequently leads to high production cost (3). Among all the livestock that makes up the farm animals in Nigeria, ruminants constitute the farm animals largely reared by farm families (4). However, there is an age long problem of feed unavailability for these livestock particularly during the long dry season, hence the need for concentrate feed supplementation to ameliorate this challenge. Poppi and McLennan (5) reported that supplementation to provide essential nutrients has been found to be the most feasible, economic and preferred method of improving the utilization of poor quality forage materials by ruminant animals in the tropics. Ruminants do better when energy and protein rich diets are strategically combined for feeding (6). Nevertheless, most of the times, this is not feasible because conventional feed stuffs are generally costly as a result of the competition between man and livestock (7) for their use either as food for man, feeds for animals or for production of industrial chemicals like starch and glues. Therefore, utilization of unconventional feed stuff which are viable, available, and cheap and not in high demand by man and other animals for use are being sought for. This search has led to the identification and evaluation of many agro-industrial by-products, agricultural by-products and lesser known plants as a means of addressing the seasonal shortages in the quality and quantity of natural pasture (8).

The Velvet bean (*Mucuna pruriens*) seed is one such unconventional feedstuff. The Velvet bean (*Mucuna pruriens*) is a vigorous annual climbing tropical legume, originally from Southern China and Eastern India, (9, 10). It thrives well where others fail due to its excellent adaptability to extreme climatic conditions (11). *Mucuna pruriens* seeds contain 22-350% crude protein (CP) and 4600-5400 kcal metabolizable energy (ME) and about 48% carbohydrates (11, 12, 13). Although it is comparable to soya bean in amino acids and mineral profile (14, 15, 16), the utilization of *Mucuna* as feed ingredient is limited by the presence of anti-nutritional factors like protease inhibitors, haemogglutinin, phytic acids, hydrocyanic acid and tannins (14). Babatunde *et al.* (17) reported that it is imperative to evaluate blood parameters particularly when unconventional feeds are fed to animals in order to determine the performance of the experimental animals as well as the suitability of such feed on the specie of livestock that is been used. This study was therefore designed to evaluate the blood profile of white Fulani bulls grazing natural pasture and supplemented with diets containing graded levels of cooked *Mucuna pruriens* seed meal.

Materials and Methods

Experimental site

The experiment was conducted at the Cattle Unit of the Livestock Teaching and Research Farm of the University of Agriculture Makurdi. Makurdi is located at latitude $7^0 43^1$N and longitude $8^0 3^1$E and lies within the Southern guinea savanna region of Nigeria. It has a temperature range of 17.3^{o}C to 35^{o}C with an annual rainfall of about 508 mm and 1016 mm. The relative humidity varies from 47% to 85% (18).

Preparation of test ingredient

The mucuna seeds were bought from farmers within Makurdi metropolis and its environs. The seeds were cleaned, poured into boiling water over an open fire and allowed to cook for 40 minutes, thereafter, it was drained using local baskets and sun-dried on concrete slabs for 7 days. After the seeds were properly dried, it was packed and crushed into a meal and stored in synthetic bags until needed.

Experimental diets

Four experimental diets were compounded using the cooked mucuna seed meal (CMSM). The CMSM replaced maize offal at 0%, 25%, 50% and 75% levels, and the diets were designated A, B, C and D, respectively.

Proximate analysis

The CMSM and the raw samples were ground and sub-samples were taken, packaged in air tight containers and sent to the laboratory for anti-nutrients, proximate constituents and crude fibre fractions were determined by the methods outlined by (19).

Experimental animals, housing and management

Twelve (12) Bunaji yearling bulls were bought from the cattle market in Lafia, Nasarawa State and used for the feeding trial. The animals were housed in individual stalls and the floor of each stall was cemented. Each compartment was equipped with a feed trough for serving the concentrate and a drinking trough for supply of fresh clean water. Daily, each animal was fed 1.5 kg of the concentrate supplement at about 8.00 hours and at about 10.00 hour, the animals were watered and allowed to go grazing on natural pasture till 14.00 hour when they were herded back to the stalls and shut in till the next morning. The experiment lasted for 98 days (14 weeks)

Collection of blood samples

On the last day of the study, blood samples were collected from all the experimental animals, using needles and syringes via the jugular vein. The blood was put in two sets of sample bottles. The first set of bottles contained EDTA (ethylene diamine tetra acetic acid), this was to help prevent the clotting of blood so that the hematological parameters could be evaluated. Hematological parameters measured were packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), platelets, lymphocytes, monocytes, eosinophils and neutrophils.

Serum biochemistry

The second set of sample bottles used did not contain EDTA, this was to enable the harvest of the serum for the biochemical assay. Serum biochemical parameters determined were total protein, albumin, urea, creatinine, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), total cholesterol, alkaline phosphate (ALP), total bilirubin (TBIL), fasting blood sugar (FBS), low density lipoprotein (LDL), high density lipoprotein (HDL) direct bilirubin (DBIL), triglycerides and chloride.

Experimental design

The experimental design for this study was completely randomized design (CRD).

Statistical analysis

All data obtained from this study were subjected to analysis of variance (ANOVA) using Minitab 16 (20) statistical software. Where significant differences occur, it was separated using fisher's least square difference (LSD) using the same statistical software.

Results and Discussion

The result for the proximate composition of the raw and cooked mucuna seed meal is presented in Table 1. The dry matter (DM) of the raw mucuna seeds was 93.24%, while that of the cooked seeds was 93.58% implying that both the raw and cooked seeds would have long shelf life. The crude protein value for the raw seeds was 22.75%, while that of the cooked seeds was 28.80%, cooking seemed to improve the CP value of the seeds. The CP values of the cooked mucuna seed can be compared with the crude protein values of legumes such as pigeon pea, [25% CP] jack bean, [25.6% CP] snap bean, [16% CP] and lima bean [23.6% CP] (21, 22). Ether extract values for raw seeds was 2.95% while that of the cooked seeds was 7.20%, like the CP, ether extract values were improved with cooking.

Table 1: Proximate analyses of raw and cooked mucuna seed meal

Proximate composition	Raw mucuna seeds	Cooked mucuna seeds
Dry matter	93.24	93.58
Crude protein	22.75	26.80
Crude fibre	5.46	5.00
Ether extract	2.95	7.20
Ash	3.94	3.60
Nitrogen free extract	64.90	57.40

Table 2 presents the anti-nutrient composition of the test ingredient. The values for the raw seeds were 1.67%, 4.18%, 0.00%, 5.60%, 2.84%, 4.32%, 0.06% and 0.65% while those of the cooked seeds were 1.64%, 3.64%, 0.00% 1.70%, 1.96%, 5.02%, 0.03% and 0.43% for oxalate, phytate, tannin, saponin, alkaloid, flavonoid, phenolic and cyanides respectively. All the parameters of the anti-nutrients' values were higher for the raw mucuna seeds than the cooked seeds, except for flavonoid values which were slightly higher in the cooked mucuna seed than the raw seeds. Flavonoids have anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties (23), therefore, the cooked seed value in this study which was slightly higher than the raw seeds will exert beneficial and not adverse effect. Anti-nutrients are generally toxic and have the capacity to negatively affect the nutrient value of seeds by impairing protein digestibility and mineral availability (24), especially in monogastric animals. However, (25) reported that anti-nutrients are liable to heat, thus they can be, to a large extent inactivated by processing methods involving heat generation. The reduction trend in the anti-nutritional factors in this study can be attributed to leaching of the anti-nutrients and mallard reaction due to heat (25, 26). The results in this study agrees with those of (27, 28) who reported that processing mucuna seeds by boiling reduced the concentrations of the anti-nutrients in them. Essig (29) also reported that though anti-nutrients occur in mucuna seeds, processing the seeds reduced the anti-nutrients in them to tolerable levels safe for ruminant to consume. Other processed legume seeds like pigeon pea (*Cajanus cajan*) and cowpea (*Vigina unguiculata*) all showed decreased in anti-nutrients with processing (30).

Table 2: Anti-nutrient composition of test ingredient

Anti-nutrients (%)	Raw mucuna seeds	Cooked Mucuna seed meal
Oxalate	1.67	1.64
Phytate	4.18	3.64
Tannin	0.00	0.00
Saponin	5.60	1.70
Alkaloid	2.84	1.96
Flavonoid	4.32	5.02
Phenolics	0.057	0.034
Cyanide	0.65	0.43

The dietary and proximate composition of the experimental diets is presented in Table 3. The dry matter varied from 93.44-94.78%, indicating that the experimental diets will have long shelf life. The CP values were between 15.68-20.54 %, values decreased with increasing levels of the CMSM in the diets however, CP levels were adequate and sufficient for optimal microbial activities. The ether extract values were between 4.74-5.86%, like the CP, ether extract values increased slightly from diet A-D, which means extra energy for the animals. Nitrogen free extract had similar trend like ether extract, values increased from treatment A-D with increasing levels of the CMSM in the diets. This implies that there were appreciable fermentable carbohydrates for energy in the diets. The neutral detergent fibre (NDF) values (36.20-39.90%) reflect the good quality of the diets since very high levels of NDF in a feed will limit feed intake and utilization (31).

Table 3: Dietary and proximate composition of experimental diets fed to the Bunaji bulls

Ingredients (%)	A	B	C	D
Rice	10.00	10.00	10.00	10.00
Maize offal	54.55	54.55	54.55	54.55
Soya bean meal	30.45	22.84	15.23	7.61
CMSM	0.00	7.61	15.23	7.61
Bone meal	3.00	2.00	2.00	2.00
Common salt	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00
Determined analysis (%)				
Dry matter	93.44	94.78	93.60	93.50
Crude protein	20.54	18.92	17.30	15.68
Crude fibre	10.25	10.08	9.92	9.75
Ether extract	4.74	5.11	5.48	5.86
Ash	5.76	5.50	5.24	4.97
Nitrogen free extract	58.71	60.39	62.06	63.74
Neutral detergent fibre	36.20	36.30	38.80	39.90
Acid detergent fibre	19.00	20.80	22.40	24.70
Acid detergen lignin	13.50	12.10	13.50	12.80
Hemicellulose	17.20	15.50	16.40	15.90
Cellulose	5.30	8.70	8.90	11.90
Energy (kcal/kg)	2876.96	2905.010	2915.75	3050.72

CMSM= cooked mucuna seed meal, A = 0% CMSM, 25% CMSM, C = 50%, D = 75% CMSM

The result for the haematology of the white Fulani bulls fed the experimental diets is presented in Table 4. There were no treatment effects (P>0.05) in all the haematological parameters measured. Packed cell volume (PCV) values which were between 29.30-32.00% were normal and within the reference range of 24-48% reported by (32) for cattle. The PCV is involved in the transport of oxygen and absorbed nutrient, if an animal is anaemic, it will usually be indicated by a decrease in the PCV value which will be usually lower than the normal range. This was not the case in this study showing that the bulls were not anaemic. The RBC values ranged between were 6.90-7.39 ($\times 10^6/\mu l$), this was normal and within the reference range of 5-10 ($x10^6/\mu l$) reported by (33) for cattle. Red blood cells serve as a carrier of haemoglobin, it is also involved in the transport of oxygen and CO_2 in the body. A reduced RBC will mean a reduction in the level of oxygen that would be carried to the tissues as well as a reduction in the amount carbon dioxide that would be returned to the lungs (34, 35). Since RBC values were within normal range it implies that oxygen circulation within the body, and removal of carbon dioxide was not impaired as a result of the presence of CMSM up to 75% level in the diets of the experimental bulls.

The WBC values were 9.73-12.27 ($\times 10^3$/µl) and it was normal and similar to 4-12 ($x10^3$µ/l) reported by (32) for cattle. The main function of the white blood cells are to fight infections, defend the body against invasion of foreign organisms and to produce, transport, and distribute antibodies in immune response (35). This implies that if the WBC values are lower than normal the animals would be susceptible to disease attacks. Observed WBC values were comparable with 10.30-12.47($x10^3$/µl) reported by (36) for Bunaji bulls fed agro-industrial by-products based diets. Wuanor *et al*. (37) however, reported higher WBC values of 12.16-14.20 ($x10^3$/µl) for Bunaji bulls fed *Pleurotus tuber regium* biodegraded rice offal diets. The MCV, MCH and MCHC were all within normal reference range of 40-60 (fl), 11-17 (pg) and 30-36 (g/dl) reported by (32) for healthy cattle. This further shows that the experimental animals were not anaemic by any means. Platelets are involved in clotting of blood, if the platelet concentration is low, it means the process of blood clotting will be prolonged and in the event of injury the animal will loss excessive blood (37). Values in this study were normal suggesting that the CMSM in the diets of the bulls was not injurious to the platelet number as to cause a decrease that would be hazardous to the bulls. Observed Platelet value were within 300-800 ($x10^3$µL) reported by (33) for healthy cattle.

Table 4: Haematological indices of Bunaji bulls fed the experimental on diets

Parameters	Experimental Diets				SEM
	A	B	C	D	
Packed cell volume (%)	29.33	31.00	32.00	31.00	1 0.90^{ns}
Red blood cells ($\times 10^6$/µl)	6.90	6.92	7.16	7.39	0.42^{ns}
Haemoglobin (g/dl)	10.35	10.83	11.03	10.90	0.61^{ns}
White Blood Cell ($\times 10^3$/µl)	12.27	9.73	10.70	11.73	1.07^{ns}
MCV (fl)	42.77	44.87	44.67	41.67	1.90^{ns}
MCH (pg)	15.20	15.63	15.47	14.73	0.48^{ns}
MCHC (g/dL)	35.57	34.90	34.60	35.43	0.76^{ns}
Platelets ($\times 10^3$/µl)	361.00	364.00	447.70	476.30	79.74^{ns}
Lymphocytes (%)	63.67	64.70	70.33	61.67	7.26^{ns}
Monocytes (%)	3.67	3.33	4.00	6.67	7.26^{ns}
Eosinophils (%)	0.67	1.00	1.33	1.33	1.71^{ns}
Neutrophils (%)	32.00	31.00	24.33	30.33	6.38^{ns}

MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, ns = not significant (P>0.05), SEM = Standard error of mean, A = 0% CMSM, B= 25% CMSM, C = 50%, D = 75% CMSM

The result for the serum biochemistry of the white Fulani bulls fed the experimental diets is presented in Table 5. All the parameters measured were significantly different (P<0.05) among the treatment means except the values for urea, high density lipoprotein

(HDL) and total cholesterol (Tchol). The total protein values were between 47.33-75.00 g/l and treatment A (47.33g/l) value was significantly lower (P<0.05) than the rest treatments, while C (75.00 g/l) was higher than all the other treatments, but between treatment B (65.67 g/l) and D (66.33 g/l) there was no difference. Although total protein values showed significant differences (P<0.05) among the treatment means, the values were within normal reference range for all the treatment containing the CMSM. This implies that the test ingredient (CMSM) did not exert adverse effects that caused undue elevation or decrease in the total protein values which would have resulted to a disease condition. Observed values of total protein for all the treatments containing the CMSM were within 52.00-87.00 g/dl reported by (40) for healthy cattle, but higher than 53.00-58.33 g/dl reported by (41) for Bunaji bulls on natural pasture fed diets containing graded levels of sweet orange peels. Albumin values ranged between 28.67-34.00 g/l, treatment A (28.67 g/l) value was the lowest (P<0.05), while B (34.00 g/l) was the highest.

Albumins which are very strong health indicators are the most abundant circulating protein found in the plasma. It has numerous functions in the body and the most important function is that of maintaining intracellular colloid osmotic pressure (COP). COP helps fluid stay within the vasculature instead of leaking into the tissue (42), although albumin values showed significant differences among the treatments, but values were normal and within 29-38 g/l reported by (40) for cattle. This indicates that replacement of soybeans meal with CMSM up to 75% level in the diets of the bulls was safe. Observed albumin values were comparable with 27-63 g/l reported by (36) for Bunaji bulls fed varying levels of agro industrial by-products based diets, but lower than 36.33-37.00 g/l reported by (44) for Bunaji bulls fed diets containing graded levels of sweet orange peels, and 43.50-48.50 g/l reported by (45) for cattle naturally infected with *Theileria Annulata*. Creatinine values were between 0.67 mg/dl-1.17 mg/dl, and values seemed to decrease significantly (P<0.05) from treatment A-D with increasing levels of the CMSM, nonetheless this was normal. Treatment A (1.17 mg/dl) and B (1.11 mg/dl) were significantly higher (P<0.05) than C (0.85 mg/dl) and D (0.67 mg/dl). Creatinine values were normal indicating there was no emaciation of the lean tissues of the experimental bulls.

The SGOT values were between 56.33-80.00 IU/L and treatment B (80.00 IU/L) was higher (P<0.05) than the rest of the treatments. Although SGOT values showed significant differences (P>0.05) among the treatments, the observed values of 56.33-80.00 IU/L were within normal value of 25-194 reported by (45) for cattle. SGPT values were between 24.3-32.33 IU/L and treatment A (24.33 IU/L) was lower (P<0.05) than the rest treatments, while between B, C and D there was no difference. The SGPT values were also within normal reference range of 10-17 IU/L reported by (45) for cattle. Observed SGPT values were also within 26.37-37.47 IU/L reported by (44) for native cattle in a hilly area of Bangladash. The ALP values were between 421.00-721.33 IU/L and it was normal and within the range of 13-953 IU/L reported by (45) for cattle. The ALP, SGPT and SGOT are all liver enzymes and since their values were within the normal reference ranges, it means that the presence of CMSM in the diets of the bulls did not cause hepatic tissue damage such as coagulation, necrosis and distortion of hepatic cords (46). The total bilirubin values were between 1.83-2.27 mg/dl, and treatment B (1.83 mg/dl) was significantly lower than the rest treatments, but between treatment A, C and D, there was no difference. The direct bilirubin values on the other hand were between 1.53-1.83 mg/dl and the values followed a similar trend as the total bilirubin values. The total bilirubin and direct bilirubin were all within the normal reference range of 1.71-8.55 and 0.68-24 mg/dl respectively as reported by (47) indicating that the CMSM did not cause hyper bilirubinemia.

The triglycerol values were between 0.37-0.45 g/l and treatment A (0.45 g/l) and D (0.45 g/l) were similar (P>0.05) but higher than B (0.37 g/l) and C (0.38 g/l), but between B and C there was no difference. The LDL varied between 1.19-1.70 mg/dl, treatment B (1.70 mg/dl) and C (1.64 mg/dl) were similar (P>0.05) but higher than A (1.43 mg/dl) and D (1.19 mg/dl). Abnormal lipid and lipoprotein concentrations are often associated with liver disorders (48). Lipoproteins are complex molecules that are heterogeneous in composition, size and biological activity (48). The triglycerol, high density lipoprotein and the low density lipoprotein in this study were normal. This implies the CMSM did not exert negative influence on the normal lipids levels as to endanger the wellness of the liver. Chloride values ranged between 90.00-96.00 mEq/l. and treatment C (96.00 mEq/l) was higher than B (90.00 mEq/l) (P>0.05) but similar (P>0.05) with A and D, B was similar (P>0.05) with A and D. The chloride values were improved for treatments with higher levels of CMSM (50% and 75%). Although significant differences (P<0.05) were observed in chloride values, values in this study were normal and within the normal range of 95-105 mEq/l reported by (48) for cattle. Chloride is one of the most important electrolytes in the blood, it helps in maintaining intracellular and extracellular fluid balance. It also helps to maintain proper blood volume, blood pressure and pH of the body fluids (49). This means that the normal chloride functions were not altered by the presence of CMSM in the diets of the bulls, which would have compromised the health of the animal grossly.

Table 5: Serum biochemical indices of the Bunaji bulls fed the experimental diets

Parameters	Experimental diets				SEM
	A	B	C	D	
Total protein (g/L)	47.33^{c}	5.67^{b}	75.00^{a}	66.33^{b}	1.2*
Albumin (g/L)	28.67^{c}	34.00^{a}	30.00^{bc}	31.67^{b}	0.55*
Urea (mg/dl)	6.33	6.77	5.33	5.43	0.40^{ns}
Creatinine (mg/l)	1.17^{a}	1.11^{a}	0.85^{b}	0.67^{c}	1.75*
Fasted blood sugar	2.60^{b}	3.10^{a}	2.80^{ab}	2.67^{b}	0.07*
SGOT (IU/L)	69.33^{b}	80.00^{a}	71.33^{b}	56.33^{c}	1.47*
SGPT (IU/L)	24.33^{b}	32.00^{a}	32.33^{a}	31.00^{a}	1.03*
Alkaline phosphate (IU/L)	421.00^{b}	721.33^{a}	479.70^{b}	454.00^{b}	31.48*
Total bilirubin (µmol/c)	2.40^{a}	1.53^{a}	2.27^{a}	2.23^{a}	1.10*
Direct bilirubun (µmol/c)	1.60^{ab}	1.53^{b}	1.83^{ab}	1.70^{b}	0.06*
Triglycerol (g/l)	0.45^{a}	0.37^{b}	0.38^{b}	0.45^{a}	0.01*
HDL (mg/dl)	2.94^{a}	2.64^{b}	2.73^{ab}	2.82^{ab}	0.06*
LDL (mg/dl)	1.43^{b}	1.70^{a}	1.64^{a}	1.19^{c}	0.03**
Total cholesterol (g/l)	4.58	4.61	4.39	4.28	0.10^{ns}
Chloride (mEq/L)	91.33^{ab}	90.00^{b}	96.00^{a}	93. 33^{ab}	1.25*

FBS= fasted blood sugar, HDL= High density lipoprotein, LDL= Low density lipoprotein, SGOT= Serum glutamic oxaloacetic transaminase, SGPT= Serum glutamic pyruvic transaminase, *= Significant difference (P< 0.05), ns=not significant (P> 0.05), SEM=Standard error of mean, a,b,c= mean values with the same letters along the same row are not significant (P>0.05), A = 0% CMSM, B= 25% CMSM, C = 50%, D = 75% CMSM

Conclusion and Applications

1. Cooking of mucuna seeds increased the crude protein value and lowered the anti-nutritional factor values.
2. Farmers can incorporate of up to 75% of CMSM in the diets of bulls/cattle without deleterious effects on the health of the animals to enhance productivity at minimal cost.

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