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# Impact of Waste Bread on the Nutrient Utilization and Performance of Buffalo Calves

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## ABSTRACT

This study was undertaken to detoxify waste bread (WB), assess its impact on the nutrient utilization, rumen fluid outflow rate and productive performance of buffalo calves. The WB was detoxified by sundrying for 16h. Isonitrogenous concentrate mixtures (CMs) were prepared, in which wheat was replaced with WB at 0, 25, 50, 75 and 100% levels on nitrogen basis. *In-vitro* and *in-sacco* studies were followed by a 130 days growth trial on 12 male buffalo calves (BW 175 ± 2.0 kg), divided into three equal groups, offered total mixed ration (TMR) containing 0, 75 and 100% WB based CMs. The aflatoxin B1 content in the WB before and after detoxification was 63 and 6.8 ppb. The *in vitro* net gas production (NGP), digestibility of nutrients, ME and feed conversion ratio was comparable in all the CMs. The total VFAs production was highest (P<0.05) in CM containing 75% WB. The effective degradability of CMs containing 75 and 100% WB was comparable but higher (P<0.05) than other CMs.The daily DM intake, digestibility of nutrients, and N-retention in buffalo calves was comparable in all the groups. The rumen outflow rate and rumen volume were lowest (P<0.05) in animals fed TMR containing 100% WB as compared to other groups. The daily live weight gain improved in 100% WB group. It was concluded that waste bread could replace wheat grains completely on nitrogen basis without any adverse effect on nutrient utilization, health or productive performance of buffalo calves.

**KEYWORDS:** Biochemical changes, Buffalo calves, Digestion kinetics,Nutritional evaluation, Performance, Waste bread

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## INTRODUCTION

India ranks first in the world with regards to milk production. To sustain this status and to achieve the future targets in milk production, the animals must be fed quality feedstuffs as per their production potential. About 978.7 MT of green fodder is produced/annum against the requirement of 1325.7MT/annum to support the existing livestock population. At present, the country is facing a net shortage of 44% concentrate feeds, 10.9% dry roughages and 35.6% green fodder (IGFRI, 2015). Therefore, to meet the nutrient requirements of such a vast animal population, either efficiency of utilization of existing feed ingredients should be improved or new non-conventional feed resources, which do not compete with human food should be explored.

Cereal grains and molasses are the major resources of energy in the ration of the livestock. However, only 2-5% of fine cereal grains and 5-50% of coarse grains are available for livestock feeding, rest are used for human consumption. Likewise, only 8% of the molasses produced in the country is used for livestock feeding rest is used by ethanol and paint industry. Therefore, new non-conventional energy resources need to be tapped.

In bread manufacturing industry two types of wastes are produced, bread waste and waste bread. The bread waste is generated while manufacturing bread, it includes low quality products, default size or texture, burnt products, bread shreddings and dough waste etc. (Heuze et al., 2018). The bread waste is used as energy source in the ration of ruminants (Almahdawi and Altalib, 2020), swine (Kumar et al., 2014) and poultry (Ayanrinde et al., 2014) and economize the feeding cost. Whereas the waste bread available in plentyis the one which is stale, molds infested, may contain mycotoxins and is collected from the retail counters/super markets at the end of its shelf life by the manufacturers or their contractors for recycling. It may serve as potential energy source in the ration of livestock. However, hardly any report is available on the nutritional value of WB and its utilization in ruminants. This study was therefore, taken up to detoxify the WB and assess the optimum level of its incorporation in the diet, nutrients utilization and growth performance of buffalo calves.

## MATERIALS AND METHODS

### Detoxification of waste bread

The batch of WB infested with mold and fungi was procured from Cremica industries, Phillaur. It was tested for aflatoxin content.It was spread on concrete floor under direct sun light for 16 to 20h for complete elimination of aflatoxin (Gowda et al., 2007).

### Nutritional evaluation

Iso-nitrogenous and iso-caloric concentrate mixtures were prepared, in which wheat grains were replaced at 0, 25, 50, 75 and 100% by WB on nitrogen basis (Table 1). The effect of different levels of WB on the nutritive value of concentrate mixture (CM) was assessed by *in vitro* gas production technique (IVGPT; Menke et al., 1979; Menke and Steingass, 1988) and *in sacco* method (Mehraz and Ørskov, 1977).

### *In vitro* gas production studies

Rumen contents were collected from three buffalo calves fitted with permanent rumen fistulae. The rumen fistulated buffalo calves were offered 2 kg concentrate mixture (wheat 25, mustard cake 10, de-oiled mustard cake 20, paddy bran 15, de-oiled paddy bran 15, wheat bran 12, mineral mixture 2, common salt 1% each), 2 kg green fodder with*ad lib* wheat straw.The rumen contents collected before feeding were blended for 2-3 min in a blender and strained through four-layers of muslin cloth. About 375 ± 5mggroundconcentrate mixture (DM basis) was incubated with buffered rumen liquor at 39°C for 24h in triplicate in 100 ml calibrated glass syringes (Haberle Labortechnik, Germany) for assessing the net gas production, digestibility of nutrients, VFA production and ME availability. After 24 h, the volume of gas produced in each syringe was recorded and the contents of syringes were transferred to spout-less beaker, boiled with neutral detergent solution for assessing the true OM & NDF digestibility andammoniacal-N (AOAC, 2007). After 24 h of incubation, a 5 ml aliquot of fluid from each syringe was mixed with 1 ml of 25% meta-phosphoric and kept for 1 h at ambient temperature. Thereafter, it was centrifuged at 5500 rpm for 10 min and clear supernatant was collected and stored at -20°C until analyzed. The volatile fatty acids were estimated (Cottyn and Boucque, 1968) using Netchrom 9100 gas chromatograph.

### *In sacco* degradability

The *in-sacco* degradability and digestion kinetic parameters were determined by using rumen fistulated buffalo calves. About 5g of finely ground (1mm) sample was placed in the nylon bag (8×17cm) stitched with monofilament polyester thread with pore size of 50±10$\mu$. The bags were incubated in the rumen for 3, 6, 9, 12, 24, 36, and 48h in triplicate. The bags were removed after the stipulated period, washed until rinsing water became colourless, bags were dried in a forced air oven at 60°C for 48h. The 'zero' h samples were not incubated in the rumen but the bags containing samples as such were washed in the same manner as incubated bags. The disappearance of DM was assessed as the loss in weight of the bag content. The residue was analyzed for DM and CP content. The different physical constants characterizing extent and rate of ruminal degradation, i.e., rapidly soluble fraction (a),insoluble but potentially degradable fraction (b) and degradation rate (c) constant of 'b' were worked out for DM and CP. The effective degradability (ED) was calculated by using the equation of McDonald (1981).

### *In vivo* studies

Twelve male buffalo calves with average live weight of 175.0±2.0 kg were randomly distributed into three equal groups. The animals in each group were fed as per NRC (2001) feeding standards for 130 days. The animals in the control group were fed total mixed ration (TMR) containing wheat grain based concentrate mixture (CM). The animals in the experimental group were fed TMRs containing CM in which wheat grains were replaced by WB at 75 and 100% on N-basis (Table 1). The TMR also contained green fodder and wheat straw. The roughage to concentrate ratio was 60:40 in all groups.The animals were weighed for 3 consecutive days at 15 days interval before feeding and the feeding schedule was adjusted accordingly.Before the termination of growth study, a 7 days metabolic trial was conducted on all the animals kept in individual metabolic cages as per the standard procedure.

Table 1. Ingredient composition of concentrate mixtures containing waste bread

| Ingredient, % | Concentrate mixtures, % | | | | |
|---|---|---|---|---|---|
| | WB-0 | WB-25 | WB-50 | WB-75 | WB-100 |
| Wheat | 30.0 | 22.5 | 15.0 | 7.5 | 0.0 |
| Waste bread | 0.0 | 8.75 | 17.5 | 26.2 | 35.0 |
| Mustard cake | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Deoiled mustard cake | 10.0 | 11.0 | 12.0 | 12.8 | 13.8 |
| Rice bran | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| Deoiled rice bran | 22.0 | 19.75 | 17.5 | 15.5 | 13.3 |
| Mineral mixture | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Common salt | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

WB-Waste bread

### Rumen studies

The effect of above TMRs containing CM or CM in which WB replaced wheat grain at 75 and 100% on N-basis was assessed on the rumen metabolites. Three rumen fistulated male buffaloes were offered one diet at a time for 30 days. Thereafter, rumen contents were collected for 3 consecutive days at 0, 2, 4, 6, 8 and 12 h post feeding. The samples collected at different intervals were pooled for respective animal. The samples were strained through 4 layers muslin cloth and preserved with few drops of saturated mercuric chloride and refrigerated at 4°C till analyzed for total VFAs and different N-fractions (AOAC, 2007). On 3rd day, 50g polyethylene glycol (PEG) molecular weight 4000 dissolved in 100ml water was introduced in the rumen and rumen liquor was collected as per above schedule. The SRL samples were analyzed for PEG (Hyden, 1955; Russell, 1982) to measure liquid outflow parameters.

The weighed mixed rumen contents (as per the requirement) were squeezed through muslin cloth to separate solid (particulate material) and liquid fractions. The liquid fraction was centrifuged at 450g at 37°C for 5 minutes to separate protozoa. The supernatant was centrifuged at 27,000g at 4°C for 20 minutes to separate bacteria (Kamra et al., 1991).

### Chemical and statistical analysis

Samples of feeds, orts and faeces were pooled for each animal separately and thoroughly mixed. These were then ground to pass through 1mm sieve and analyzed in duplicate for nitrogen and total ash (AOAC,2007), cellulose (Campton and Maynard, 1938) and other cell wall constituents (VanSoest et al., 1991). The urine samples were analyzed for nitrogen content only (AOAC,2007).The ME was determined from the apparent digestible OM using the equation given by Broster and Oldham (1981). The data were analyzed by completely randomized design (Snedecor and Cochran, 1994) using SPSS

(2009) version 16 and the differences in means by using Tukey's b test.

## RESULTS AND DISCUSSION

The aflatoxin B1 content in the WB (procured for this experiment) before and after detoxification was 63 and 6.8 ppb respectively. Earlier studies revealed that feed dried either at 80°C for 6h or under direct sunlight for 14h resulted in significant reduction in aflatoxin B1 (Gowda et al., 2007). The detoxified waste bread contained 96.4% OM, 13.3% CP, 11.4% NDF, 2.4% cellulose and 62.6% starch.

### *In vitro/in sacco* evaluation of concentrate mixtures

The net gas production (NGP), digestibility of NDF and OM and ME availability was comparable in all the CMs. These parameters were the highest from concentrate mixture in which 75% of wheat grains were replaced with WB. However, the differences were statistically non-significant. The total VFAs production was higher (P<0.05) in CM, in which 75% of wheat grains were replaced with WB than those containing 0 and 25% WB,but comparable with those containing50 and 100% WB (Table 2).

Table 2. Effect of level of waste bread on the *in vitro* utilization of nutrients.

| Parameter | Concentrate mixtures, % | | | | | PSE |
|---|---|---|---|---|---|---|
| | WB-0 | WB-25 | WB-50 | WB-75 | WB-100 | |
| NGP, ml/g DM/24h | 202.6 | 206.8 | 215.5 | 213.8 | 188.2 | 4.25 |
| OMD, % | 65.1 | 69.1 | 70.0 | 68.4 | 67.7 | 0.78 |
| NDFD, % | 31.7 | 29.6 | 31.9 | 33.4 | 35.8 | 2.09 |
| $NH_3$-N, % | 0.024 | 0.023 | 0.022 | 0.023 | 0.019 | 0.001 |
| TVFA, mM/DL | 9.65[a] | 9.78[a] | 10.40[ab] | 10.90[b] | 9.90[ab] | 0.17 |
| ME, MJ/kg DM | 9.76 | 9.49 | 9.96 | 10.06 | 8.72 | 0.98 |

Figures with different superscripts in a row differ significantly (P<0.05).

The digestion kinetic parameters for DM revealed that CM containing 75%WB had the highest (P<0.05) rapidly soluble fraction which was comparable with the one containing 100%WB (Table 3). The CM containing 75% WB had the lowest (P<0.05) potentially degradable fraction, but it was statistically comparable with that of CMs in which 100% of wheat were replaced by WB. The rate of degradation of potentially degradable fraction of all the CMs containing WB was comparable to that of conventional CM. The rumen UDF was highest (P<0.05) in conventional CM and lowest in CM containing 100% WB. The effective degradability of CMs containing WB from75 to 100% was comparable and higher (P<0.05) than other CMs. The true digestibility and apparent extent of digestion of DM of the CMs containing graded levels of WB was statistically comparable with that of control CM. However, 48h degradability was highest (P<0.05) in CM containing 100% WB in comparison to CM or mixtures containing other levels WB.

The digestion kinetic parameters for CP in the CM revealed that rapidly soluble fraction was higher (P<0.05) inCM containing 75 and 100% WB and the lowest was observed in 50% WB group (Table 3). The insoluble but potentially degradable fraction was comparable in all the groups except at 50% level of replacement, in which it was highest (P<0.05). The effective degradability of CP of CMs containing 100% WB was higher (P<0.05) and comparable to that containing 50 and 75%WB. The true digestibility and apparent extent of degradation were highest (P<0.05) in CM containing 50%WB. But the48h degradability, degradation rate and undegradable fraction of CP was not affected by the level of WB in the CM.

Table 3. Effect of level of waste bread, in the concentrate mixture, on the digestion kinetics parameters for DM

| Fraction, % | Concentrate mixtures | | | | | PSE |
|---|---|---|---|---|---|---|
| | WB-0 | WB-25 | WB-50 | WB-75 | WB-100 | |
| Digestion kinetics parameters for DM, % | | | | | | |
| Rapidly soluble (a) | 35.9[ab] | 36.5[ab] | 34.3[a] | 42.1[c] | 40.6[bc] | 0.90 |
| Potentially degradable (b) | 54.0[ab] | 55.0[ab] | 57.9[b] | 50.6[a] | 53.3[ab] | 0.75 |
| Degradation rate (c), %/h | 0.052 | 0.049 | 0.054 | 0.048 | 0.051 | 0.001 |
| Un-degradable (UDF) | 10.1[c] | 8.5[b] | 7.9[b] | 7.3[b] | 6.1[a] | 0.36 |
| Effective degradability (ED) | 63.6[a] | 63.7[a] | 64.1[a] | 67.0[b] | 67.5[b] | 0.47 |
| True digestibility (TD) | 51.2 | 49.4 | 51.6 | 49.2 | 50.5 | 0.38 |
| Apparent extent of digestion (AED) | 48.4 | 47.5 | 50.0 | 47.9 | 49.8 | 0.37 |
| 48h degradability | 89.9[a] | 91.5[b] | 92.1[b] | 92.7[b] | 93.9[c] | 0.36 |
| Digestion kinetics parameters for CP, % | | | | | | |
| Rapidly soluble | 36.0[ab] | 36.0[ab] | 33.1[a] | 40.8[b] | 39.8[b] | 0.89 |
| Potentially degradable | 53.6[a] | 53.9[a] | 57.8[b] | 50.8[a] | 52.8[a] | 0.71 |
| Degradation rate, %/h | 0.049 | 0.044 | 0.051 | 0.044 | 0.046 | 0.001 |
| Un-degradable | 10.4 | 10.1 | 9.1 | 8.4 | 7.5 | 0.41 |
| Effective degradability | 62.6[ab] | 61.3[a] | 62.2[ab] | 64.5[ab] | 65.0[b] | 0.47 |
| True digestibility | 49.6[ab] | 47.0[ab] | 50.3[b] | 46.6[a] | 47.8[ab] | 0.49 |
| Apparent extent of digestion | 46.7[ab] | 44.4[a] | 48.1[b] | 44.9[ab] | 46.5[ab] | 0.45 |
| 48h degradability | 89.6 | 89.9 | 90.9 | 91.6 | 92.5 | 0.41 |

Figures with different superscripts in a row differ significantly ($P<0.05$).

The *in vitro* gas production and *in sacco* studies conclusively revealed that wheat grains in the CM could be replaced completely and safely by WB. Therefore, for *in vivo* studies on buffalo calves, the CMs containing 75 and 100% of WB in place of wheat grains were selected.

**Impact of waste bread (WB) on nutrient utilization in buffalo calves**

Twelve male buffalo calves divided into 3 equal groups were offered TMR containing iso-nitrogenous and iso-caloric CMs containing 75 or 100% WB on N-basis supplemented with green fodder and wheat straw for 130 days. The chemical composition of all the CMs (Table 4) was quite comparable indicating that the diets were isonitrogenous and isocaloric.The concentrate to roughage ratio varied between 37: 63 to 38: 62, and green fodder to wheat straw ratio varied from 34:66 to 36:64.

Table 4. Chemical composition of feedstuffs used for *in vivo* study, % DM basis

| Nutrient | Concentrate mixtures | | | Wheat straw | Green |
|---|---|---|---|---|---|
| | WB-0 | WB-75 | WB-100 | | |
| OM | 91.3 | 92.3 | 93.0 | 92.2 | 89.8 |
| CP | 21.5 | 21.6 | 21.6 | 3.0 | 18.2 |
| NDF | 38.5 | 35.0 | 34.0 | 79.5 | 47.5 |
| ADF | 17.8 | 14.3 | 12.0 | 47.5 | 24.8 |
| Cellulose | 10.0 | 8.0 | 6.0 | 36.5 | 17.5 |
| Hemi-cellulose | 20.7 | 20.7 | 22.0 | 32.0 | 22.7 |

The level of WB in the TMR did not show any significant (P>0.05) effect on the DM intake (either as kg/d or as per cent of live weight) in buffalo calves (Table 5).

Table 5. Effect of level of waste bread on the DM intake, digestibility of nutrients and N-retention in buffalo calves

| Parameters | WB-0 | WB-75 | WB-100 | PSE |
|---|---|---|---|---|
| DM intake, kg/d | 7.62 | 7.61 | 7.60 | 0.19 |
| Digestibility of nutrients, % | | | | |
| DM | 58.7 | 62.7 | 61.3 | 1.45 |
| OM | 61.8 | 65.4 | 63.7 | 1.41 |
| CP | 70.8 | 74.0 | 74.2 | 1.31 |
| NDF | 49.3 | 53.6 | 50.1 | 1.53 |
| ADF | 36.1 | 44.1 | 35.6 | 2.62 |
| Cellulose | 51.3 | 56.5 | 53.8 | 1.87 |
| Hemi-cellulose | 65.3 | 56.9 | 58.9 | 1.66 |
| Nitrogen balance, g/d | | | | |
| N- intake | 160.0 | 160.3 | 159.5 | 4.09 |
| Faecal-N | 46.7 | 41.9 | 41.5 | 2.83 |
| Urinary-N | 28.3 | 32.8 | 29.1 | 1.32 |
| N-retained | 84.9 | 85.6 | 88.9 | 2.86 |
| Apparent BV, % | 52.9 | 53.3 | 55.9 | 1.25 |

The similar DM intake recorded in all the groups revealed that WB did not have any deleterious effect on the palatability of diets. The digestibility of nutrients (both proximate as well as cell wall constituents, except for hemicellulose) was numerically higher (P>0.05) in both the groups containing WB as compare to control group.The level of WB in the diets did not have any significant (P>0.05) effect on the daily nitrogen intake, because the diets were iso-nitrogenous and animals had comparable intake. No statistically significant differences were observed in faecal and urinary-N excretion in different groups. Though statistically non-significant, the N-retained and apparent biological value was highest at 100% replacement. Ayandiran et al. (2019) revealed that bread waste, a by-product of bakery industry wasrich in energy and low in fiber. Further, inclusion of bread waste and

*Moringa oleifera* in the diet of West African Dwarf goats led to significant improvement in growth performance, nutrient digestibility and N-retention.

## Rumen studies of complete feeds containing different levels of WB

The total VFAs concentration in the rumen was comparablein control and 75% WB groups, but the complete replacement of wheat grains with WBincreased ($P<0.05$) the total VFAs concentration (Table 6).The total-N and the TCA-N concentration in the rumen was higher ($P<0.05$) in diet having 75% WB than that of 100% WB group, but was comparable with that of control group.As compared to control and 75% WB groups, the lower ($P<0.05$) rumen outflow rate and rumen volume in animals fed diet containing 100%WB, suggested higher retention time in the rumen, resulting in better exposure to rumen microbes and in return higher digestibility of nutrients. Significantly higher ($P<0.05$) dilution rate was observed in group which was fed diet containing 100%WB in CM.

Table 6. Effect of level of waste bread on the rumen profile of animal fed concentrate mixture containing waste bread.

| Parameters | WB-0 | WB-75 | WB-100 | PSE |
|---|---|---|---|---|
| TVFAs, meq/dl | 12.6[a] | 12.7[a] | 13.4[b] | 0.13 |
| N-fractions, mg/dL | | | | |
| Total-N | 94.3[ab] | 99.6[b] | 88.0[a] | 2.10 |
| NPN | 22.5 | 24.6 | 22.9 | 0.47 |
| TCA-N | 71.8[ab] | 75.0[b] | 65.1[a] | 1.09 |
| $NH_3$-N | 9.43 | 10.1 | 8.47 | 0.42 |
| Rumen outflow parameters | | | | |
| Rumen volume, l | 275.8[b] | 297.2[b] | 157.1[a] | 22.84 |
| Dilution rate, l/h | 0.19[a] | 0.21[a] | 0.28[b] | 0.02 |
| Rumen outflow rate | 52.2[ab] | 61.7[b] | 44.0[a] | 2.90 |

Figures with different superscripts in a row differ significantly ($P<0.05$).

## Microbial profile of rumen liquor of animals

The ruminal microbial profile of buffaloes revealed that the number of bacteria and protozoa were higher in the animals at 4h post feeding as compared to those before feeding. Moreover, the population of both bacteria and protozoa (Table 7) was increased when animals were fed on diet containing 100 percent WB as compare to other groups.

Table 7. Effect of level of waste bread on the changes in microbial population in animal fed concentrate mixture containing waste bread.

| Diet | Bacteria, $10^{10}$/ml | | Protozoa, $10^4$/ml | |
|---|---|---|---|---|
| | Pre- prandial | Post-prandial(4 h) | Pre-prandial(4h) | Post - prandial(4 h) |
| $BW_0$ | 2.72 | 4.57 | 2.25 | 3.70 |
| $BW_{75}$ | 3.68 | 6.96 | 3.28 | 5.25 |
| $BW_{100}$ | 7.30 | 12.0 | 3.90 | 8.50 |

**Effect of diets containing WB on the performance of buffalo calves**

The WB did not have any adverse effect on the health of animals as indicated by live weight changes (Table 8). Though statistically non-significant, the animals fed diet containing WB gained more weight as compared to those offered conventional control diet. The feed conversion ratio was also similar in all three groups.

Table 8. Effect of level of waste bread on body weight (BW) changes in buffalo calves, kg

| Parameters | WB-0 | WB-75 | WB-100 | PSE |
|---|---|---|---|---|
| Initial BW | 173.6 | 177.9 | 180.4 | 6.58 |
| Final BW | 246.6 | 251.9 | 254.3 | 8.59 |
| Gain in BW | 73.0 | 74.0 | 73.98 | 2.79 |
| ADG, g | 597.5 | 605.7 | 605.7 | 22.8 |
| Feed conversion ratio | 11.3 | 11.3 | 11.7 | 0.34 |

ADG, Average daily gain

No supporting reference was available in the literature on the effect of waste bread on daily live weight gain. Passini et al. (2001) and Afzalzadeh et al. (2007) observed that feeding 30% bakery residues in the diet of fattening Aberdeen Angus calves had no adverse effects on their health and performance. Guiroy et al. (2000) found that when steers given rations containing corn and bread by-product, the differences in average daily gain in weight were not significant. Animals fed bread by-product diet required 8.1% less DM/kg gain than those fed the corn diet indicating higher feed efficiency of bread by-product than corn diet. Studies on dried bread waste revealed significant improvement in daily and total weight gain and most blood traits of lambs as compared to control group (Hindiyeh et al., 2011; Tayeb and Yassin, 2018;Almahdawi and Altalib, 2020).

## CONCLUSION

From the results obtained in the study, it was concluded that cereal grains like wheat could be replaced completely by waste bread on nitrogen basis without any deleterious effect on the digestibility of nutrients, N-utilization, nutritive value or growth of animals. Moreover, keeping in view the cost of wheat (Rs 19-20/kg) and WB (Rs 7-9/kg), the feeding of animals could be economized through WB incorporated rations.

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